ECOLOGY LETTERS

*Ecology Letters*, (2012) **15**: 786–793 doi: 10.1111/j.1461-0248.2012.01798.x

LETTER

# Nematomorph parasites indirectly alter the food web and ecosystem function of streams through behavioural manipulation of their cricket hosts

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## Abstract

Nematomorph parasites manipulate crickets to enter streams where the parasites reproduce. These manipulated crickets become a substantial food subsidy for stream fishes. We used a field experiment to investigate how this subsidy affects the stream community and ecosystem function. When crickets were available, predatory fish ate fewer benthic invertebrates. The resulting release of the benthic invertebrate community from fish predation indirectly decreased the biomass of benthic algae and slightly increased leaf break-down rate. This is the first experimental demonstration that host manipulation by a parasite can reorganise a community and alter ecosystem function. Nematomorphs are common, and many other parasites have dramatic effects on host phenotypes, suggesting that similar effects of parasites on ecosystems might be widespread.

## Keywords

Ecosystem function, decomposition, manipulative parasite, nematomorph, resource subsidy, riparian, stream salmonid.

*Ecology Letters* (2012) **15**: 786–793

## INTRODUCTION

Parasites account for a large proportion of living species and even biomass on Earth (Dobson *et al.* 2008; Kuris *et al.* 2008). As consumers, parasites modify food-web topology by increasing food-chain length, number of links and connectance, which may affect the stability of food webs (Lafferty *et al.* 2006, 2008). In addition to being consumers in food webs, parasites can modify the morphology, behaviour or physiology of their hosts in ways that enhance transmission (Moore 2002; Libersat *et al.* 2009). Such host manipulations are known from many phyla (Moore 2002) and might have indirect consequences for food webs and ecosystems (Lefèvre *et al.* 2009). The best example of indirect consequences is from a larval trematode parasite that encysts in the foot of the cockle, *Austrovenus stutchburyi*. The parasite reduces the burrowing ability of the cockles, which makes them easier prey for host birds (Thomas & Poulin 1998), and the shells of stranded cockles create a habitat for a rich and distinctive epibiont community (Thomas *et al.* 1998). Herein, we ask how another manipulative parasite, a nematomorph worm, affects a stream community, including ecosystem function.

Nematomorphs (known as horsehair or Gordian worms; Phylum: Nematomorpha) are common parasites in terrestrial-aquatic ecotones throughout the world (Poinar 2008). They have a complex life cycle with parasitic larval stages and a free-living adult stage (Hanelt *et al.* 2005). The final stage of the larval parasite manipulates its cricket host to enter streams, where the adult parasite emerges and seeks a mate (Thomas *et al.* 2002). At the water's surface, the worms, together with their cricket hosts, are vulnerable to aquatic predators, such as fishes and frogs, although the worms usually survive attacks on the crickets by squirming out the mouth, gills or anus (Ponton *et al.* 2006). Trout rise to the surface to rapidly eat crickets and other drift insects as they fall (Sato *et al.* 2011a). Infected crickets can be an important seasonal prey subsidy for stream trout, accounting for 60% of the annual energy intake of an endangered Kirikuchi char population *Salvelinus leucomaenis japonicus* in a temperate Japanese stream (Sato *et al.* 2011a). Trout satiated by infected crickets eat fewer benthic invertebrates, leaving open the potential for indirect effects on the stream community (Sato *et al.* 2011a). However, the abundance of nematomorphs varies from stream to stream. For instance, intensive forest management appears to disrupt the nematomorph's life cycle, which reduces the terrestrial subsidy to trout streams (Sato *et al.* 2011b).

Ecosystems are often tightly linked to each other through resource subsidies (Polis *et al.* 1997). The linkages among ecosystems will be strongest when the ratio of the resource subsidy to equivalent resources in the recipient systems is high (Takimoto *et al.* 2002; Marczak *et al.* 2007; Leroux & Loreau 2012). Pulsed resource subsidies temporarily increase this ratio and hence can alter tempo-

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ral aspects of communities and ecosystem functions in recipient systems (Yang *et al.* 2010). Subsidies like litter fall and terrestrial invertebrates have pronounced effects on stream communities, including basal resources like benthic algae (Wallace *et al.* 1997; Nakano *et al.* 1999; Baxter *et al.* 2004). The biomass of algae can enhance in-stream nutrient uptake (Cardinale 2011), but this finding has never been tested in the context of ecosystem linkages. As a pulsed subsidy, infected crickets enter streams when other sources of prey (benthic and terrestrial invertebrates) are scarce (Fig. 1).

We hypothesise that the cricket subsidy in Japanese streams might indirectly alter stream community and ecosystem functions like leaf break-down rate and nutrient uptake. Specifically, we predict that experimentally adding crickets to a stream increases the growth and reproduction of trout. Then, if the subsidy is sufficient to satiate fish, we expect to see an increase in the biomass of invertebrate prey that are released from predation. If a simple trophic cascade occurs in our system (cf. Nakano *et al.* 1999; Baxter *et al.* 2004), we predict that a release of shredders and grazers increase leaf break-down and reduce the biomass of benthic algae, leading to decreased nutrient uptake.

## MATERIALS AND METHODS

### Study design

We conducted a large-scale field experiment in which we manipulated the inputs of either crickets or of all terrestrial invertebrates into the stream. To make it easier to manipulate the system, we chose a site with low nematomorph density (see Method 1 in Appendix S1 for rational and more detailed methods). For the first treatment, crickets were added (cricket addition) at levels approaching (*c.* 75%) natural inputs known from previous studies of Kirikuchi char (Sato *et al.* 2011a,b). This treatment was the 'control' we used to approximate what we think happens in nature. For the second treatment (cricket removal), we prevented infected crickets at the site from entering streams to mimic the effects of removing the parasite from the system. Comparing aspects of the stream in the cricket addition and cricket removal treatments allowed us to evaluate the effects of parasite behavioural manipulation on the stream community and ecosystem function. To broaden our interpretations, we added a third treatment (terrestrial removal) where all terrestrial invertebrates (including crickets) were prevented from entering the stream. Comparing cricket removal and terrestrial removal treatments indicated the importance of the cricket subsidy relative to other terrestrial inputs. We did not include an unmanipulated control because our experiment required choosing a site with few crickets (i.e. we were attempting to compare no subsidy with average subsidy, not no subsidy with low subsidy).

### Study site and experimental preparations

The field experiment was conducted from 20 August to 20 October, 2010 in two small streams in the upper drainage of the Arida River system, which drains a watershed within the Wakayama Research Forest Station (WRFS), Field Science Education and Research Center (FSERC), Kyoto University. The experiment coincided with the season when nematomorphs manipulate their cricket hosts (Sato *et al.* 2011a,b). The two study streams (Hachiman and Ninomata streams) have similar in-stream characteristics and riparian vegetation (see Table S1 in Appendix S1). Each stream had two blocks of three treatments, resulting in 12 experimental reaches. Within a block, the three treatments were randomly assigned to three 26-m (± 4 m) long reaches with gravel-dominated stream beds that were 2–5 m width, and had 5–8 pools. The experimental reaches were separated by > 30-m intervals (except a 10-m interval in one case) and 10-mm mesh wire netting that prevented the passage of fish larger than 70 mm in fork length, but did not impede benthic invertebrates (although the biomass of insects that drifted

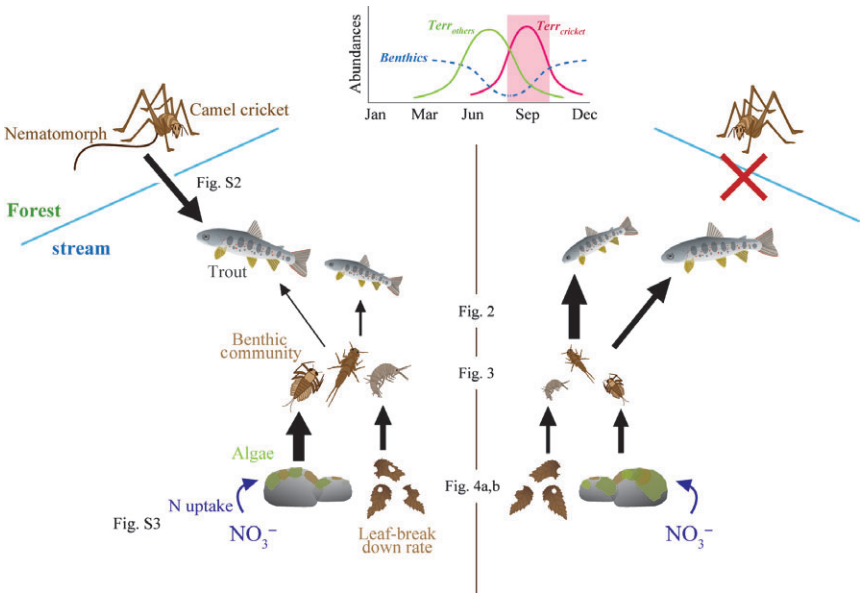


**Figure 1** Trophic interactions and a ecosystem function modified by a prey subsidy mediated by parasites. Black arrows, except for the cricket-trout link, denote direct consumptive interactions; the thinness of arrows shows the strengths of each interaction. Figure numbers represent which figures demonstrate data testing each of the hypothesised effects. The upper graph shows the seasonal dynamics of potential prey abundances for trout around the study site (Sato *et al.* 2011a). $Terr_{cricket}$ and $Terr_{others}$ represent cricket hosts and other terrestrial invertebrates, respectively.

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between treatments was very low). In the cricket addition treatment reaches, we added 9–15 dead camel crickets *Diestrammena* spp., a dominant host of nematomorphs, every night (except during rain) to approximate natural levels of the parasite-mediated terrestrial subsidy (Method 1 in Appendix S1). We collected crickets around the study site (un-dissected), stocked them in a freezer, and added them to the treatment reaches after thawing. Based on past experience, we assumed that crickets added to a reach would be unlikely to escape predation by trout in the experimental treatment or downstream reach that acted as a buffer between treatments. In the cricket removal treatment reaches, cricket input was prevented by the combination of 4-mm nylon mesh net fence (1.5 m height) built parallel to the stream, and 10 large water-filled pan traps on both forest sides of the fence (see Figure S1). In the terrestrial removal treatment reaches, greenhouse covers made of transparent 1-mm nylon mesh excluded all terrestrial invertebrate subsidies. Consistent with previous studies (Nakano *et al.* 1999; Baxter *et al.* 2004), average air temperatures under the greenhouse covers were < 1.1 °C higher than those without the covers, but water temperatures did not differ. There was no significant difference in light intensity among treatments ($F_{2,9} = 0.262$, $P = 0.775$ by one-way ANOVA), probably because high forest canopy covers (81 ± 3%, 71–86%) masked the effect of the greenhouse covers.

We determined the above treatments were effective; that is, the cricket removal treatment reduced the input of crickets 11-fold, while the input rate of other terrestrial invertebrates was not affected; in the terrestrial removal treatment, cricket and other terrestrial invertebrate inputs were reduced to almost zero (see Appendix S2). We did not install mesh nets in the cricket addition treatments, which might affect input rate of leaf litter, another important terrestrial subsidy (Wallace *et al.* 1997). However, the experiment occurred before the defoliation period around the study site. Thus, there was little leaf litter moving from the forest to the stream in covered or uncovered treatments.

The fish community was similar among treatments. Before the experiment, all fish [red-spotted masu salmon *Oncorhynchus masou ishikawae* (hereinafter, trout) and very few minnows, *Phoxinus oxycephalus jouyi*] were removed from the reaches by three passes of electrofishing (LR-24; Smith-Root, Inc.). After fish removal, we released trout [2:1 of small (107 ± 6 mm in fork length; $n$ = 13–26) and large (162 ± 6 mm; $n$ = 7–13) fishes; 0.35 fish/m$^2$ in density] into each reach. This population structure and density were like natural populations in the study site (0.2–0.4 fish/m$^2$). These fish were anaesthetised, measured (fork length to the nearest 1 mm and weighed to the nearest 0.1 g) and individually marked by visible implant elastomer (North West Marine Technology, Inc.) for growth calculation.

To examine the biomass stock of benthic algae in each treatment, seven bricks (20 × 10 × 5 cm high) with an anchored tile (3.5 × 3.5 cm) were randomly placed in 5–6 pools of each study reach for 1 month prior to the experiment. This allowed benthic algae and invertebrates to colonise at the beginning of the experiment.

To examine the leaf break-down rate in each treatment, we placed 10 leaf packs (mean ± SE: 1.42 ± 0.26 g air-dried beech leaves), which were each directly anchored to a brick (20 × 10 × 5 cm high), in 5–6 pools of each replicate at the beginning of the experiment. Using leaf packs instead of leaf bags let fish directly attack shredder invertebrates feeding on the leaves. The duration of leaf breakdown was 55 days in this study.

### Sampling of community components

To evaluate the contribution of each prey category (cricket and other terrestrial invertebrates, and benthic invertebrates) to fish diets, fish were captured by electrofishing during the last week of the experiment. After capture, fish were measured as described above, and their stomach contents quickly pumped (Giles 1980). We examined 114 fish diets (9–12 fish in each reach) (see Appendix S3 for sample sizes). We measured the growth rate of each trout by comparing weights before and after the experiment (Wootton 1998). We also captured fish just before their spawning season (around 1 week after the experiment was concluded) and judged maturity if fish released sperm or eggs when we softly pressed the abdomen. Females brooding eggs were regarded as mature even if they did not release eggs. We collected at least 10 eggs/fish from five to eight females in each treatment to test the effect of treatment on egg size.

Benthic stream invertebrates were captured using a Surber sampler (30 × 30 cm in quadrat area, 1 m net length, 225-$\mu$m-mesh) from seven randomly selected streambeds per reach in the last week of the experiment. All of the invertebrate samples, including falling/drifting and fish diet samples, were preserved in 70% ethanol, identified to at least order (terrestrial and aquatic adult invertebrates) or the taxonomic level required to determine functional feeding groups for benthic invertebrates (shredders, predators, scrapers, collectors, filter feeders). Then, the mass of each invertebrate was calculated by using a wet-dry mass or length-mass regression (see Method 2 in Appendix S1). At the end of the experiment, we collected periphyton samples from 5 to 7 tiles (see Appendix S3) in each reach to estimate the biomass of benthic algae. Within a week of collection, chlorophyll *a* content was measured using a spectrophotometer (Steinman *et al.* 2006).

### Measurements of ecosystem function

Leaf packs (3–9 packs in each replicate; see Appendix S3) were collected from the bricks, dried for 72 h at 30 °C and weighed in the laboratory. We did not measure ash-free dry mass (AFDM) because leaf packs were well rinsed in the field and very little inorganic material was expected. Leaf break-down rate ($L_R$) was then calculated by comparing changes in mass (Benfield 2006).

In one of the two experimental blocks (where block is a subset of cricket addition, cricket removal and terrestrial removal treatment reaches) in each stream, we conducted a nutrient addition experiment (Stream Solute Workshop 1990) before and after the experiment to test whether or not the changes in the standing crop biomasses of benthic algae among treatments affected the in-stream uptakes of $NO_3^-$ (Cardinale 2011). The nutrient addition experiment consisted of a short-term injection of a nutrient ($NaNO_3$) and a conservative tracer (NaCl) and measurement of $NO_3$-N concentration and electrical conductivity (EC) at six stations in each treatment reach downstream from the injection point after steady state was achieved. Then, $NO_3^-$ uptake length is calculated as the downstream decrease of nutrient concentration standardised with EC. We confirmed that we were able to detect uptake even though ambient levels of $NO_3^-$ were high (see details in Method 3 in Appendix S1).

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### Statistical analysis

All statistical analyses were conducted in R 2.10.1 (R Development Team 2008). We used generalised linear mixed models (GLMM; lme4 in R), with streams and blocks within each stream as random effects, to test: (1) whether or not treatment, as well as fish body size, affected the mass of each prey category (cricket, other terrestrial, and benthic invertebrates) ingested by trout, and growth rate and egg size of trout; (2) whether or not treatment, as well as fish body size, affected the maturity of trout; (3) whether or not treatment affected the biomass of benthic algae and leaf break-down rate. We included all first-order interaction terms in initial models and selected the optimal models by means of stepwise backward elimination (Method 3 in Appendix S1). When the treatment effect was included in the optimal model, it could be that only one treatment was different from the others. Therefore, we tested if combining some treatments further maximised the log-likelihood of the optimal model by using the log-likelihood ratio test in lmer (Crawley 2007). Specifically, we compared the optimal model [cricket addition (CA) vs. cricket removal (CR) vs. terrestrial removal (TR)] with three combined models ('CA' + 'CR' vs. TR; 'CA' + 'TR' vs. CR; or 'CA' + 'TR' vs. CA).

We extended the delta-lognormal linear model (Lo *et al.* 1992) to evaluate the treatment effects on three elements of community structure of benthic invertebrates: total biomass, proportional compositions of functional feeding groups, and families within each functional group. In our approach, the total biomass and the two proportional compositions were modelled separately with a log-normal GLMM with a Gaussian distribution and identity-link function respectively(more details are in Method 4 in Appendix S1). When a significant treatment effect was found, we conducted a *post hoc*, log-likelihood ratio test as described above. Another GLMM model, with streams, blocks within each stream and treatments within each block as random effects, was used to test whether or not the biomass of invertebrate predators was associated with those of scrapers and shredders respectively.

The effect size of the cricket addition treatment was calculated as the natural log of the response ratios of grazing (biomasses of scrapers and benthic algae) and detritus (biomass of shredders and leaf break-down rate) chains where the response ratios were the mean values in the cricket addition (CA) treatments divided by the mean values in the cricket removal (CR) and the mean values in the terrestrial removal (TR) treatments respectively; for example, $R_{CR} = \ln(CA/CR)$, where $R_{CR}$ is log response ratio of CA to CR for a given variable (Marczak *et al.* 2007). Finally, to see if effects were attenuated through the food chain, we compared the effect sizes of the treatment from one trophic level to the next. For instance, we calculated the absolute ratio ($R$) of the effect of the treatment on the scraper/shredder guild to the effect of the treatment on the benthic algae/leaf break-down rate; for example, for the grazing chain in the cricket removal, $|R| = R_{CR\ (scraper)}/R_{CR\ (algae)}$, where $R_{CR\ (scraper)}$ and $R_{CR\ (algae)}$ are log response ratios of CA to CR for scraper and algae biomasses respectively (Leroux & Loreau 2008). Note that $|R| = 1$ represents equivalent treatment effects on the two trophic levels, whereas $|R| > 1$ ($|R| < 1$) indicates attenuation (amplification) of the treatment effects.

## RESULTS

The experimental manipulation of cricket inputs altered trout diets (Fig. 2 and Figure S2 in Appendix S2). Crickets made up a large proportion of trout diets in the cricket addition treatments; 67% in large fish and 30% in small fish (Fig. 2). When crickets were available, small trout ate far fewer benthic invertebrates (GLMM: $F_{2,74} = 7.42$, $P = 0.0009$), whereas large trout did not reduce their consumption ($F_{2,28} = 0.044$, $P = 0.95$; Fig. 2 and Table S3 in Appendix S4).

We did not detect a strong effect of crickets on the performance of trout. However, the specific growth rate (SGR) of small trout was lower in the terrestrial removal treatment than in the other treatments ($F_{2,88} = 13.20$, $P < 0.0001$; cricket addition ≈ cricket removal < terrestrial removal in the *post hoc*, log-likelihood ratio tests; Table S4-1 in Appendix S4), indicating that small trout benefited from non-cricket terrestrial inputs. The SGR of larger trout was generally low irrespective of the treatments (Table S4-2 in Appendix S4), but tended to be higher in the cricket addition treatment ($F_{2,35} = 2.88$, $P = 0.059$). The treatment effect on the SGR of large fish was marginally non-significant in a two-tailed test, but we applied the *post hoc* test, cricket addition ≈ cricket removal, cricket addition > terrestrial removal and found cricket removal ≈ terrestrial removal. Larger trout had higher maturation rates and larger egg sizes, but no treatment effect was found for maturation and egg size (Table S4-3, 4 in Appendix S4).

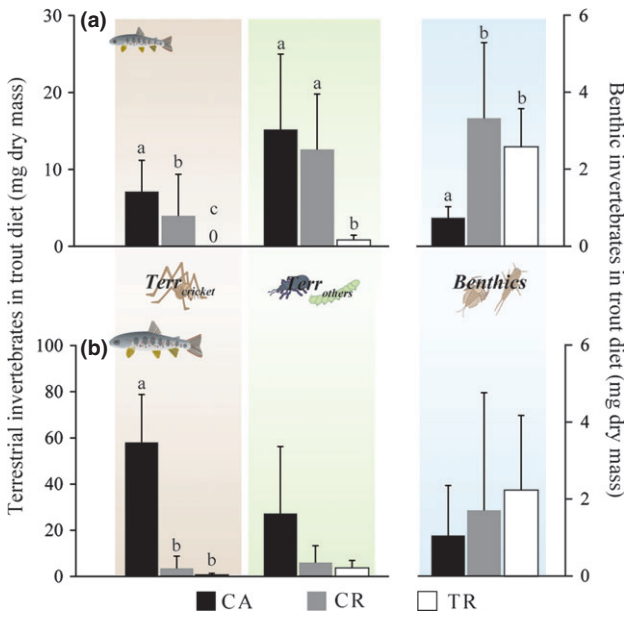


**Figure 2** The effects of treatments on the mass (mean ± SE) of each invertebrate category ingested by trout. Small (a) and large (b) trout size classes. $Terr_{cricket}$ and $Terr_{others}$ are same with Fig. 1. By convention, letters indicate statistically different responses (see Supplementary method and Table S4). Fish size classes affected the response to the treatments. Large trout ate disproportionately more crickets in the cricket addition treatment (CA) than in other treatments (cricket removal, CR and terrestrial removal, TR) ($F_{2,33} = 22.95$, $P < 0.0001$; CA > CR ≈ TR). Small trout ate disproportionately fewer benthic prey in the cricket addition treatment than in other treatments ($F_{2,79} = 7.42$, $P = 0.0009$; CA < CR ≈ TR).

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Reduced predation pressure on benthic invertebrates led to increases in invertebrate biomass and changes to community structure. The mean estimate of the total biomass of benthic invertebrates in the cricket addition treatment was more than three times that in the other treatments ($F_{2,71}$ = 17.36, $P$ < 0.0001; Fig. 3a and Table S5-1 in Appendix S4). This effect was greater for predators and shredders than for scrapers ($F_{8,367}$ = 2.89, $P$ = 0.011; Fig. 3b and Table S5-2 in Appendix S4) (Fig. 3). Furthermore, the relative abundances of invertebrate families also changed within scraper and shredder functional feeding groups respectively (Fig. 3c and Table S5-3 in Appendix S4). No evidence of strong predatory interactions among benthic invertebrates was found; i.e., the biomass of invertebrate predators was positively associated with both scraper and shredder biomasses respectively (GLMMs: $P$ < 0.001; Table S6-1, 2 in Appendix S4).

Increases in the benthic invertebrates led to reductions in their resources. The mean standing crop biomass of benthic algae was *c.* halved in the cricket addition treatment compared with the other treatments ($F_{2,67}$ = 34.38, $P$ < 0.0001; Fig. 4a and Table S7-1 in Appendix S4). However, despite the large differences in algal biomass (and counter to our preliminary prediction), we did not detect effective $NO_3^-$ uptake in any treatment reaches (see Figure S3 in Appendix S5). Leaf break-down rate was *c.* 30% faster in the cricket addition treatment compared with the other treatments, but the treatment effect was marginally non-significant in a two-tailed test ($F_{2,57}$ = 3.56, $P$ = 0.059; Fig. 4b and Table S6-2 in Appendix S4). However, if we combined the cricket removal and terrestrial removal treatments, then a significant treatment effect was found (Table S7-2 in Appendix S4). Effect sizes were equivalent for adjacent (scraper invertebrates) and non-adjacent (algae) trophic levels in the grazing chain; that is, the ratio ($|R|$) of absolute effect size value of scrapers to that of algae was near 1 both for cricket removal and terrestrial removal treatments (Fig. 5). However, the effect size was greater for shredders than for leaf break-down rate ($|R|$ > 3) both for cricket removal and terrestrial removal treatments (Fig. 5).

## DISCUSSION

Nematomorph parasites create a resource subsidy for trout in the form of manipulated crickets (Sato *et al.* 2011a,b) that might have broad implications. Our experiment suggests that this subsidy was enough to alter a trophic cascade that indirectly increased grazers and shredders, which, in turn, decreased benthic algae and, to a lesser extent, increased leaf break-down rate. Recent studies have suggested potential community- and ecosystem-wide roles of parasites in natural ecosystems (Dobson *et al.* 2008; Lafferty *et al.* 2008), but the magnitude and scale documented by our experiment is unprecedented. Because cricket subsidies occur over large areas (Sato 2011; Sato *et al.* 2011b), these results can apply to whole streams, not just reaches. Furthermore, although terrestrial-aquatic linkages are most obvious in the headwater regions of streams (Vannote *et al.* 1980), the cricket subsidy might influence downstream reaches of a river network via the export of invertebrates or particulate organic matter (Wipfli & Gregovich 2002). We succeeded in significantly manipulating the system, but, on average, the mass of crickets ingested by

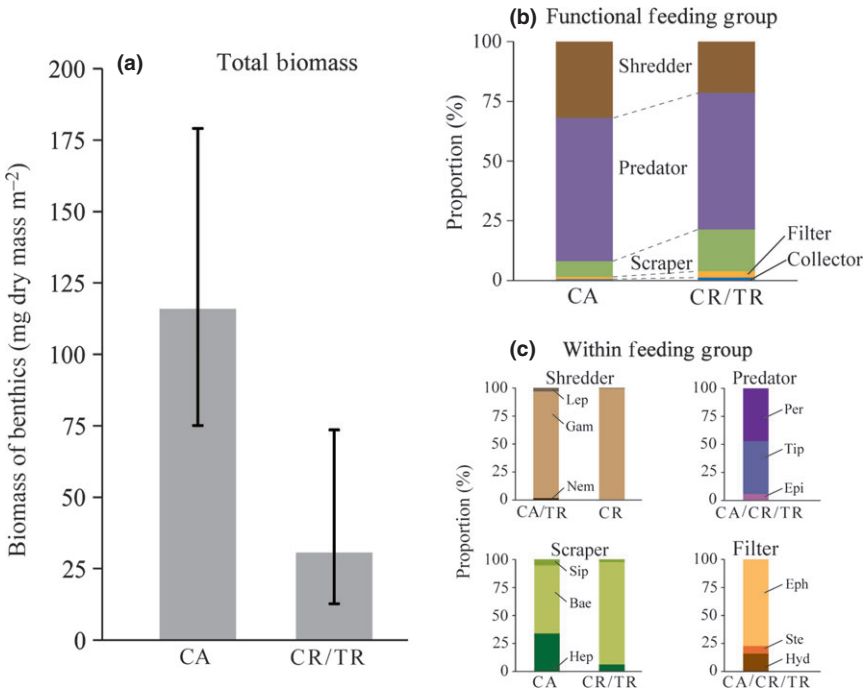


**Figure 3** The effects of treatments on the community structure of benthic invertebrates. Total biomass (a), functional feeding groups (b) and families (c) expressed as biomass (error bars for 95% confidence interval) and proportions calculated from the delta-lognormal GLMM model. Multiple bars show that significant differences were detected among the treatments in the log-likelihood ratio test. A single bar in panel (c) indicates no significant treatment effects among the categories. A biomass estimate of each category in each treatment can be obtained by the product of total biomass and the proportions of the categories (see Method 4 in Appendix S1). Abbreviations of family names are respectively Lepidostomatidae, Gammaridae, Nemouridae, Perlidae, Tipulidae, Epiophlebiidae, Siphlonuridae, Baetidae, Heptageniidae, Ephemeridae, Stenopsychidae, Hydropsychidae.

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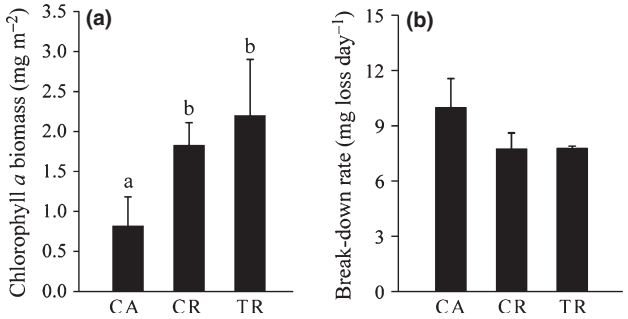


**Figure 4** The effects of treatments on ecosystem function measures. Mean ± SE of algal biomass (a) and leaf break-down rate (b). Different letters indicate that significant difference was detected among the treatment in the log-likelihood ratio test (see Table S6).

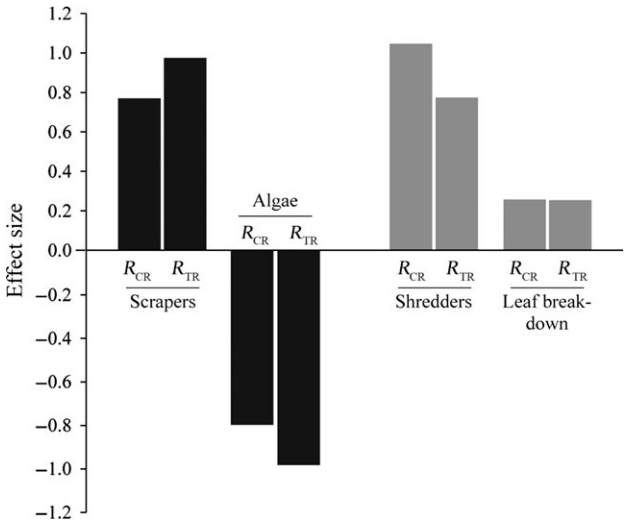


**Figure 5** Effect sizes of treatments on different trophic levels and ecosystem function. The ratios of the mean outcomes in cricket addition treatment (CA) to those in cricket removal (CR) and terrestrial removal (TR) treatments were used to calculate the effect sizes (i.e. $R_{CR}$ and $R_{TR}$). The effect sizes of the treatment from one trophic level to the next were compared by calculating their ratio: e.g. $|R| = R_{CR(scraper)}/R_{CR(algae)}$.

trout is even higher in nature (60 and 8 mg in large and small trout respectively) (Sato *et al.* 2011b), suggesting that our experimental results are conservative.

An interpretation of the experimental results is that cricket input would saturate trout, which indirectly released benthic invertebrates from predation (Nakano *et al.* 1999; Baxter *et al.* 2004; Zhang & Richardson 2011). Smaller trout drove this change in diet, even though small trout ate fewer crickets than large trout. In part, this was because when large trout ingested many crickets, they did not force small trout to feed in the benthos, releasing small trout to eat other drifting terrestrial invertebrates. We plan to analyse these intraspecific interactions in more detail (T. Sato & K. Watanabe, unpublished data). Simple three-level food chains (fish-benthics-algae), rather than more complex four-level chains (fish-predator invertebrates-scrapers-algae) (Power 1990), appear to dominate this *in situ* stream community. Both scrapers and invertebrate predators increased after cricket input, which altered the trophic cascade. The alteration of nutrient availability for benthic algae, such as by fish egestion (faeces production) and excretion (urine production) (Vanni & Layne 1997; Vanni 2002) and/or non-consumptive effects of fish on herbivore behaviour (Schmitz *et al.* 2010) might also mediate the trophic cascade. However, subsidy effects on nutrient availability for algae seem unlikely in this system because (1) the cricket subsidy would probably not be enough to significantly alter the amounts of biologically available nitrogen and/or phosphorous for benthic algae in these steep streams, and (2) algal biomass was lowest in the cricket addition treatment where we would expect that available nutrients for algae would be the highest. Although algal biomass affects $NO_3^-$ uptake in streams (Cardinale 2011), we did not detect effective $NO_3^-$ uptake in any treatment reaches at the end of our experiment. This might be because algal biomass was not sufficiently high in these shaded streams (3.8 mg chlorophyll *a*/$m^2$ in maximum) to significantly reduce nutrient concentrations or because during the period of measurement, ambient $NO_3^-$ levels were too high for a small amount of algae to deplete (Mulholland *et al.* 2002). Algal production, not biomass, could also drive nitrogen uptake (Arango *et al.* 2009), but this does not fully explain our results (i.e. no nutrient uptake in any treatment reaches).

In contrast to the strong alteration of the trophic cascade through the grazing food chain, the cascading effect in the detritus chain was attenuated, so that we found only marginally significant differences in leaf break-down rate among treatments. As occurred in the grazing chain, fish affected both invertebrate predators and shredders similarly, and predation among the invertebrates did not appear to increase the detritus chain (i.e. three-level food chain). Trout predation does not directly affect fungi and bacteria, and the ability of these decomposers to break-down leaf litter might have compensated for the reduction of invertebrate shredders (Hieber & Gessner 2002).

Our findings raise a more general question about how pulsed resource subsidies can indirectly affect ecosystem functions. It is not well known how subsidies that affect top-down and bottom-up processes (Polis *et al.* 1997, 2004; Yang *et al.* 2010) link to ecosystem functions like biogeochemical cycling and decomposition rate. Our results suggest the subsidy attenuated through the food chain. In other words, even though the cricket subsidy strongly altered the trophic cascade, we found fairly weak effects on an ecosystem function. Future studies might investigate how properties of recipient systems, such as food-web complexity and physical/chemical habitat structures, determine the extent that subsidies indirectly affect ecosystem functions.

Our experiment did not measure long-term effects of the parasite-driven subsidy. For instance, we suspect that the cricket subsidy would eventually result in a higher density of trout, leading to net decreases in benthic invertebrates (Takimoto *et al.* 2009). The potential long-term effects further relate to the complex life cycle of the nematomorphs. First-stage nematomorph larvae are eaten by some benthic invertebrates that emerge from the stream as adults. When these infected insects die in the forest, they are scavenged by crickets, in which the nematomorph larvae develop further (Hanelt *et al.* 2005). Therefore, indirect effects of the cricket subsidy on benthic invertebrate hosts could affect parasite transmission, which could, in return, affect the cricket subsidy for trout. In our experiment, we did not find treatment effects on trout maturation or egg size that might be signs of future reproductive success associated with the subsidy (i.e., a reproductive numerical response). Furthermore, our comparative field data indicate that there is not a

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significant association between the ingestion rate of trout on infected crickets and trout biomass/abundance among natural streams around the study region (Sato *et al.* 2011b). However, targeted and longer term studies will be required to elucidate how the cricket subsidy mediated by the nematomorphs affects dynamics of trout populations, as well as whole communities, including nematomorph populations themselves.

In conclusion, the present study demonstrates that nematomorph parasites have indirect, but strong cascading effects on both grazing and detrital stream communities, resulting in slightly increased leaf break-down rates. Nematomorphs are common parasites in terrestrial-aquatic ecotones, with presumably > 2000 species (Poinar 2008). Thus, the effects we observed potentially occur throughout the world. Many other parasites have comparably dramatic effects on host behaviour (Lafferty & Morris 1996; Moore 2002; Lefèvre *et al.* 2009; Libersat *et al.* 2009), which can cause or alter trophic cascades; for example, the trematode parasite *Cryptocotyle lingua*–periwinkle *Littorina littorea*–macroalgae system in rocky intertidal shores (Wood *et al.* 2007), and the trematode parasite *Posthodiplostomum minimum*–freshwater snail *Physa acuta*–filamentous algae *Cladophora glomerata* system in streams (Bernot & Lamberti 2008). Furthermore, sticklebacks *Gasterosteus aculeatus* manipulated by tapeworm *Schistocephalus solidus* and isopods manipulated by acanthocephalan parasite *Acanthocephalus tahlequahensis* become more susceptible to fish or avian predations, which may result in alteration of energy flow, as well as their roles in aquatic ecosystems (Bergersen 1996; Hernandez & Sukhdeo 2008). By altering energy flow within and across ecosystems, manipulative parasites might have greater effects on community and ecosystem functions than previously thought.

## ACKNOWLEDGEMENTS

We thank H. S. Greig for comments that improved the manuscript. K. Iguchi and A. Sakai provided the experimental equipment. T. Fukushige, Y. Horii, other student helpers and staff of WRFS are acknowledged for their assistance in setting up and running the experiment. This work was funded by Grant-in-Aid for Young Scientists (B) (21770023) and Grant-in-Aid for JSPS Fellows (10J02194). Any use of trade, product or firm names in this publication is for descriptive purposes only and does not imply endorsement by the US government.

## AUTHOR CONTRIBUTION

TS designed the study, collected data, analysed the data and wrote the paper; TE, KF, TO, NO and NT contributed data for nutrient addition experiment; KW discussed study design and results with TS; MK managed all statistical analyses; IM contributed data for benthic invertebrate community; KL discussed data representation and wrote the paper with TS.

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Editor, David Post
Manuscript received 11 January 2012
First decision made 03 February 2012
Second decision made 28 March 2012
Manuscript accepted 16 April 2012

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