

# A nematomorph parasite explains variation in terrestrial subsidies to trout streams in Japan

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Nematomorph parasites alter the behavior of their orthopteran hosts, driving them to water and creating a source of food for stream salmonids. We investigated whether nematomorphs could explain variation in terrestrial subsidies across several streams. In nine study streams, orthopterans comprise much of the stomach contents of trout ( $46 \pm 31\%$  on average). Total mass of ingested prey per trout biomass positively correlated with the mass of orthopterans ingested, suggesting that the orthopterans enhanced absolute mass of prey consumption by the trout population. The orthopterans ingested per trout biomass positively correlated with the abundance of nematomorphs in the stream, but not with the abundance of camel crickets (the dominant hosts) around the streams. Streams in conifer plantations had fewer nematomorphs than streams in natural deciduous forests. These results provide the first quantitative evidence that a manipulative parasite can explain variation in the allochthonous energy flow through and across ecosystems.

In riparian ecosystems, terrestrial and aquatic food webs are tightly linked by reciprocal energy subsidies (Nakano and Murakami 2001). For instance, adults of aquatic insects can become prey for terrestrial predators (Nakano and Murakami 2001, Sabo and Power 2002), and terrestrial invertebrate input into streams constitutes an important energy subsidy to stream salmonids (Wipfli 1997, Kawaguchi and Nakano 2001, Sato et al. 2011) and can alter trophic cascades within streams (Nakano et al. 1999).

In a Japanese stream, orthopterans (grasshoppers and camel crickets) infected by nematomorphs ('horsehair worm', Phylum Nematomorpha: Family Gordiidae; *Gordionous* spp.) account for 60% of the annual energy intake of an endangered trout population of *Salvelinus leucomaenis japonicus* (Sato et al. 2011). This occurs because nematomorphs manipulate their orthopteran host to enter streams so the adult worm can emerge and mate (Thomas et al. 2002). The worms resist ingestion by aquatic predators, but leave their abandoned host a helpless prey for the predator (Ponton et al. 2006, Sato et al. 2008). By moving crickets from the forest to the stream, the parasite diverts substantial energy across habitats, benefiting the trout population (Sato et al. 2011). Furthermore, satiated trout eat fewer benthic invertebrates, potentially leading to multiple indirect effects in the stream and forest ecosystem (Sato et al. 2011).

To explain variation in terrestrial subsidies across streams, we asked whether the abundance of orthopterans, the abundance of nematomorphs, or both, explained variation in the diet of trout among riparian ecosystems in Japan. We also considered whether nematomorph abundance varied among streams with different amounts of conifer plantation, a major forest land use in the area.

## Material and methods

### Field observations

We studied nine headwater streams and their surrounding terrestrial habitats within four geographically distant river basins around the Kii Peninsula, central Honshu, Japan. The nine streams were historically in deciduous forest, but this has been replaced to various degrees with conifer plantations (Table 1). In each river basin, we chose two (or three in Totsu River basin) study sites that differed markedly in their relative percent cover of conifer plantation habitat. The relative percent plantation habitat was defined as the ratio of plantation to the catchment area of each study stream.

Sampling occurred from late August to early October 2008, the season when nematomorphs *Gordionous* spp. manipu-

Table 1. Site characteristics.

Study sites	Catchment area (km <sup>2</sup> )	Artificial forest <sup>a</sup> (%)	Stream gradient (%)	Discharge (m <sup>3</sup> s <sup>-1</sup> )	Fish species <sup>b</sup>	Fish density (n m <sup>-2</sup> )	Fish size (mm)	Nematomorphs <sup>c</sup> (n)	Crickets (n)	Prevalence (%)
Totsu River basin										
Nadani stream	1.11	62.9	14.0	0.04	C	0.10 ± 0.03	158 ± 27	90	47	46.9
Fudo stream	0.16	71.4	19.3	0.02	C/S	0.08 ± 0.02	149 ± 29	65	46	42.3
Sono stream	0.80	81.5	8.4	0.04	C/S	0.07 ± 0.02	165 ± 32	9	34	21.2
Arida River basin										
Ninomata stream	0.70	14.3	15.3	0.06	S	0.14 ± 0.05	167 ± 27	18	14	35.7
Hachiman stream	0.84	57.1	16.5	0.06	S	0.12 ± 0.05	155 ± 25	9	18	33.3
Miya River basin										
Mizutani stream	1.16	58.2	26.1	0.06	S	0.09 ± 0.03	155 ± 48	20	14	71.4
Yachiyama stream	1.76	97.8	12.6	0.04	S	0.21 ± 0.03	157 ± 34	1	12	18.2
Kumozu River basin										
Higashimata stream	0.75	5.3	17.0	0.02	S	0.22 ± 0.08	180 ± 33	17	35	38.2
Nishimata stream	1.13	24.6	26.0	0.02	S	0.11 ± 0.04	153 ± 21	19	5	20.0

<sup>a</sup>Plantations consisted of *Cryptomeria japonica* and *Chamaecyparis obtusa*. Common native, but second-growth, deciduous trees across sites were *Fraxinus spaethiana*, *Pterocarya rhoifolia*, and some species of the genus *Acer*. <sup>b</sup>C and S are Japanese charr *Salvelinus leucomaenis* and red-spotted masu salmon *Onchorhynchus masou ishikawae*, respectively. <sup>c</sup>This count data was standardized by the stream discharge. Estimated fish density and average body size (FL) are shown with 95% confidence interval and standard deviation (SD), respectively.

late orthopteran hosts to enter streams (Sato et al. 2011). To examine the relative abundance of orthopteran hosts and the prevalence of nematomorph infection in orthopterans, we sampled camel crickets (Rhopidophoridae; *Diestrammena* spp., *Neotachycines furukawai* and *Anoplophilus* sp.), the dominant hosts of the nematomorph, using six baited traps (Sato et al. 2011) set in the forest. Traps were set for three days near the stream and separated by at least 20 m. Captured crickets were preserved in 99% ethanol for later determination of nematomorph infections. To examine the relative abundance of adult nematomorphs during the peak month that nematomorphs mature (September), we set six underwater traps [commercial netting fence (L × W: 300 × 600 mm) with 50 × 50 mm mesh size] perpendicular to stream flow, with at least 20 m between individual traps. After 30 days, we counted the worms captured in the trap. The traps appeared efficient because captured nematomorphs (45–302 mm in body length) were entwined very tightly in the trap, and there were no obvious decreases in the number of trapped worms with time (Sato unpubl.), suggesting that trapped individuals did not escape. The relative abundance of nematomorphs at a site was calculated as the total number of nematomorphs captured by six traps during 30 days standardized by the stream discharge (flow × cross-sectional area), which was measured once a sampling period in each stream during average water flow.

To determine trout diet, we captured fish (*Salvelinus leucomaenis* or *Onchorhynchus masou ishikawae* ≥ 110 mm in fork length (FL), n = 8–14) in a 100 m-long section in each stream twice per study period, with at least a 14-day interval between captures, using a backpack electrofishing unit. Fish were measured for fork length (FL) and body mass to the nearest 1 mm and 0.1 g, respectively; stomach contents were collected by stomach pumping (Giles 1980), which were sorted (aquatic adult insects, benthic invertebrates, orthopterans and terrestrial non-host taxa) and measured for dry mass to the nearest 0.01 mg.

## Statistical analysis

All statistical analyses were conducted in R 2.10.1 (R Development Core Team 2005). We used generalized linear mixed models (GLMM), with basin treated as a random effect, to test: 1) whether the amount of orthopterans ingested by a trout was associated with trout species, trout size, sampling day, and/or stream; and 2) whether the among-stream variation in the orthopterans ingested per trout biomass was associated with the relative abundances of crickets, nematomorphs, or both. We included all first-order interaction terms in initial models. The significance of individual factors was tested by means of stepwise backward elimination by starting with a full model and obtaining a reduced model containing only significant effects. We first assessed the GLMMs with a Gaussian distribution and log-link function under the assumption of a log-normal distribution of error. However, the model performances evaluated by the residual distribution and/or AIC were poor. Therefore, we log-transformed all continuous variates of both response and predictor variables and confirmed the asymptotic normality of error by a Q-Q plot. For these reasons, we used GLMMs with normal error distribution and identity link function. The pseudo R<sup>2</sup> was calculated to show the extent to which the best GLMM models explained the deviance in the data (i.e. pseudo R<sup>2</sup> = 1 - residual deviance / null deviance) in each analysis. A Spearman rank correlation test was used to examine whether the total mass of ingested prey per trout biomass was associated with the mass of orthopterans, terrestrial non-hosts, or aquatic invertebrates ingested.

To test whether the relative abundances of nematomorphs or their hosts (camel crickets in this study) were associated with the relative percent cover of plantation habitat, we used GLMMs with a Poisson error distribution and log-link function. In the GLMMs, basin was treated as a random effect. For the analysis of nematomorphs, the GLMM was standardized using an offset to account for the number of nematomorphs per discharge passing through the traps in each stream.

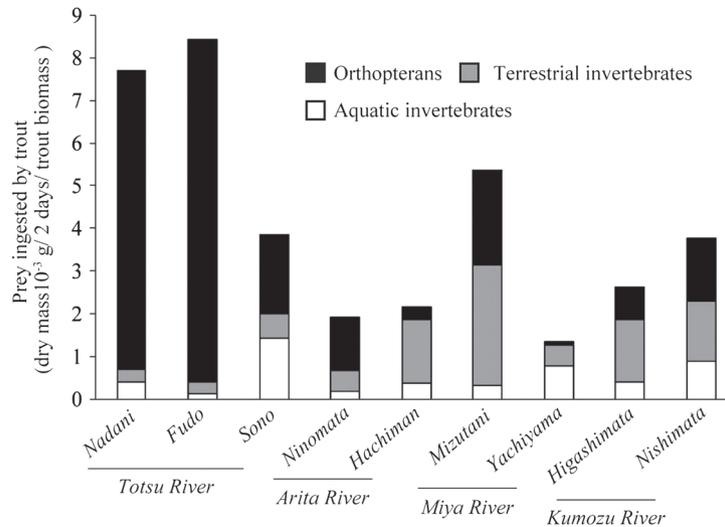


Figure 1. The mass of different prey categories ingested per trout biomass over the study period in each study stream.

## Results

Infected orthopterans occurred at all study sites, but the number of camel crickets, number of nematomorphs captured by the traps, and the prevalence of the nematomorph infection in the crickets varied among study sites (Table 1).

From late August to early October, we examined 184 trout, with at least 14 trout per stream. Orthopterans were a common part of the diet (by weight) of trout in the study streams (mean  $\pm$  SD:  $46 \pm 31\%$ ; range: 1–95%). Other terrestrial invertebrates and aquatic invertebrates occupied  $33 \pm 24\%$  (3–69%) and  $19 \pm 19\%$  (2–60%) of the diet, respectively (Fig. 1). Camel crickets were the most common orthopterans by mass ( $89 \pm 14\%$ , 58–100%). Other hosts were relatively rare: 10.1 and 1.2% on average for grasshoppers (e.g. *Kinkiconocephalopsis koyasanensis* and *Psyrana japonica*) and *Nippancistroger testaceus* (Gryllacrididae), respectively.

The optimal GLMM model (pseudo  $R^2 = 0.52$ ,  $p < 0.0001$ ) revealed that the mass of orthopterans preyed on by a trout differed significantly among streams ( $F_{8,173} = 14.36$ ,  $p < 0.0001$ )

and increased with trout body size (coefficient =  $0.53 \pm 0.11$  SE;  $F_{1,182} = 17.27$ ,  $p < 0.0001$ ). Species, day, and all first-order interactions were excluded in the process of the model selection. The mass of ingested prey per trout biomass increased with the mass of orthopterans ingested (Spearman's  $r = 0.93$ ,  $p = 0.0007$ ), but not with other prey categories (terrestrial non-hosts:  $r = -0.23$ ,  $p = 0.55$ ; aquatics:  $r = -0.18$ ,  $p = 0.64$ ).

The optimal GLMM model (pseudo  $R^2 = 0.37$ ,  $p = 0.0006$ ) revealed that the amount of orthopterans ingested per trout biomass was only explained by the relative abundance of adult nematomorphs in a stream (coefficient =  $1.05 \pm 0.17$  SE;  $F_{1,7} = 36.93$ ,  $p < 0.0001$ ; Fig. 2). The abundance of orthopterans collected in the forest and the interaction term between the two variables were excluded in the process of the model selection.

The relative abundance of camel crickets, the dominant host of nematomorphs, in each site was not correlated with the relative percent of conifer plantation habitat (GLMM:  $p = 0.60$ ). On the other hand, the relative abundance of nematomorphs in each stream was negatively correlated with

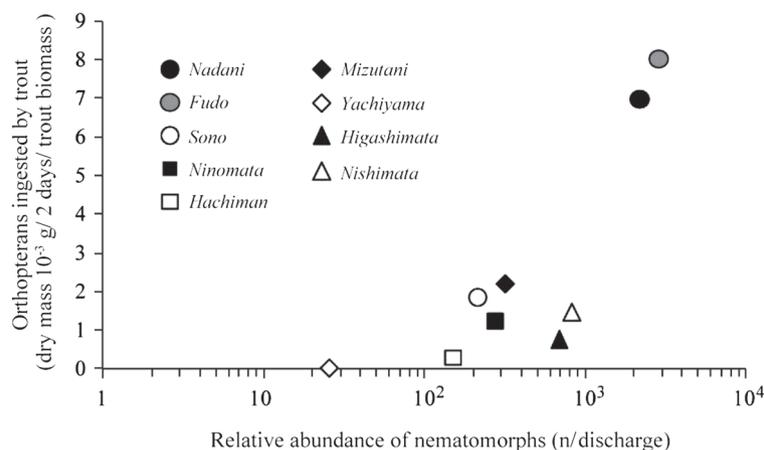


Figure 2. Inter-stream variation in the orthopterans ingested per trout biomass as a function of the abundance of the nematomorph *Gordionous* spp. Different symbols show different river basins. Symbols with different colors show the individual sites (see also Table 1).

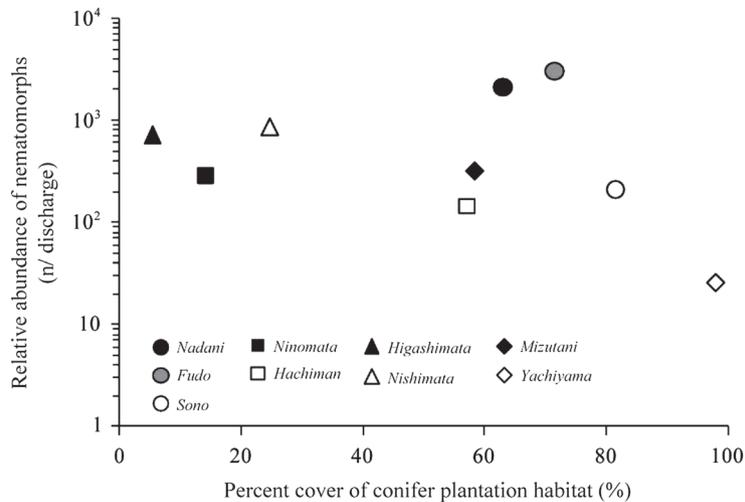


Figure 3. Inter-stream variation in the relative abundances of nematomorphs as a function of the relative percent of conifer plantation habitat. Different symbols show different river basins. Symbols with different colors show the individual sites (see also Table 1).

the relative percent of conifer plantation habitat (GLMM: pseudo  $R^2 = 0.18$ ,  $p < 0.0001$ ; coefficient =  $-3.12 \pm 0.65$  SE; Fig. 3).

## Discussion

Parasites are suspected of affecting ecosystem scale processes (Kuris et al. 2008, Lafferty et al. 2008), but nothing of this magnitude and scale has been previously documented. Nematomorphs are pervasive across our study region, generalizing our earlier finding that this parasite strongly increases the terrestrial subsidy for trout (Sato et al. 2011). This was underscored by the fact that parasite abundance, not orthopteran abundance, determined trout diet. Other studies report frequent manipulation of orthopteran hosts by nematomorphs (Thomas et al. 2002) and subsequent predation by aquatic predators on those hosts (Ponton et al. 2006). Nematomorphs occur throughout the world (Poinar 2008) and can be surprisingly abundant (Hanelt et al. 2001), suggesting that this host-parasite interaction could have strong and generalized effects in riparian food webs. Parasitized orthopterans enter streams in the fall when the allochthonous prey supply and in situ prey resources for trout have declined (Nakano and Murakami 2001, Sato et al. 2011). Therefore, in riparian ecosystems, nematomorphs can indirectly modify the seasonality of spatial energy subsidies, which can be important for the dynamics of the recipient community (Takimoto et al. 2009).

We do not know why nematomorph abundance declined in conifer plantations. Forest type can affect parasites in other systems for unknown reasons. For instance, stomach nematodes in rats are more abundant in coconut plantations than native forests (Lafferty et al. 2010). Previous studies have not found an effect of landscape type on nematomorphs (Hanelt et al. 2001). However, parasites can be less abundant in disturbed habitats if disturbance interrupts parasite life cycles (Hudson et al. 2006). Intensive forest management in conifer plantations might interrupt the complex life cycle of nematomorphs. Although host orthopterans were not affected by

habitat type, the community of benthic invertebrates that include the paratenic hosts for nematomorphs is often modified by conifer-forestation (Yoshimura 2007). Future work is required to elucidate how forest management, coupled with the other basin-wide factors (e.g. potential natural vegetation and stream network structure), affects the life cycle and abundance of nematomorphs.

Our study suggests that conversion of natural forests to conifer plantations could have unanticipated effects on stream ecosystems. One might normally assume that a reduction in parasites would have positive benefits for an ecosystem. However, in the case of Japanese streams, a reduction in parasitism of crickets would substantially reduce the food supply of endangered trout, with important implications for conservation.

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