

# Biomass and productivity of trematode parasites in pond ecosystems

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## Summary

1. Ecologists often measure the biomass and productivity of organisms to understand the importance of populations and communities in the flow of energy through ecosystems. Despite the central role of such studies in the advancement of freshwater ecology, there has been little effort to incorporate parasites into studies of freshwater energy flow. This omission is particularly important considering the roles that parasites sometimes play in shaping community structure and ecosystem processes.

2. Using quantitative surveys and dissections of over 1600 aquatic invertebrate and amphibian hosts, we calculated the ecosystem-level biomass and productivity of trematode parasites alongside the biomass of free-living aquatic organisms in three freshwater ponds in California, USA.

3. Snails and amphibian larvae, which are both important intermediate trematode hosts, dominated the dry biomass of free-living organisms across ponds (snails =  $3.2 \text{ g m}^{-2}$ ; amphibians =  $3.1 \text{ g m}^{-2}$ ). An average of 33.5% of mature snails were infected with one of six trematode taxa, amounting to a density of 13 infected snails  $\text{m}^{-2}$  of pond substrate. Between 18% and 33% of the combined host and parasite biomass within each infected snail consisted of larval trematode tissue, which collectively accounted for 87% of the total trematode biomass within the three ponds. Mid-summer trematode dry biomass averaged  $0.10 \text{ g m}^{-2}$ , which was equal to or greater than that of the most abundant insect orders (coleoptera =  $0.10 \text{ g m}^{-2}$ , odonata =  $0.08 \text{ g m}^{-2}$ , hemiptera =  $0.07 \text{ g m}^{-2}$  and ephemeroptera =  $0.03 \text{ g m}^{-2}$ ).

4. On average, each trematode taxon produced between 14 and 1660 free-swimming larvae (cercariae) infected snail $^{-1}$   $24 \text{ h}^{-1}$  in mid-summer. Given that infected snails release cercariae for 3–4 months a year, the pond trematode communities produced an average of  $153 \text{ mg m}^{-2} \text{ yr}^{-1}$  of dry cercarial biomass (range =  $70\text{--}220 \text{ mg m}^{-2} \text{ yr}^{-1}$ ).

5. Our results suggest that a significant amount of energy moves through trematode parasites in freshwater pond ecosystems, and that their contributions to ecosystem energetics may exceed those of many free-living taxa known to play key roles in structuring aquatic communities.

**Key-words:** complex life cycle, disease ecology, ecosystem energetics, ecosystem process, freshwater food web, macroparasite, pathogen, secondary production, wetland

## Introduction

Quantifying the biomass and production of populations, communities and entire trophic levels has been a central tool in understanding how energy moves through ecosystems (Odum 1971). The early use of such methods led to

fundamental ecological concepts including the designation of trophic levels, the corresponding decrease in trophic-level biomass with increasing trophic position, and the efficiency of energy transfer between consumers and resources (Elton 1927; Lindeman 1942). More recently, biomass and production measurements have proven useful in studies of biomass turnover (i.e. ratios of production to biomass [P to B]; Waters 1977), in constructing food webs with quantified interaction strengths (e.g. Hall, Wallace & Eggert

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2000) and as metrics of population success (Benke & Huryn 2010). Throughout the development of the field of production ecology, freshwater lakes, ponds and streams have proven especially useful as model systems (e.g. Lindeman 1942; Odum 1957) and most methods of quantifying secondary production have been pioneered with aquatic macroinvertebrates (Benke 1984; Downing & Rigler 1984).

The rich literature on freshwater ecosystem energetics has largely omitted the roles of infectious agents, despite the increasingly recognized importance of parasites in ecosystem structure and function (Loreau, Roy & Tilman 2005; Tompkins *et al.* 2010). Despite being cryptic and small in size, parasites are involved in a disproportionately high number of species interactions in some ecosystems (Lafferty, Dobson & Kuris 2006; Lafferty *et al.* 2008) and comprise a large component of biodiversity (Hudson, Dobson & Lafferty 2006). These findings suggest that parasites can play important roles in trophic interactions and energy flow and several studies have shown that parasites can alter primary and secondary production through population-level effects on their hosts. For example, plant foliar fungal pathogens can exert strong top-down control on primary production, sometimes surpassing the impacts of herbivores in certain grassland ecosystems (Mitchell 2003). Animal parasites can also alter ecosystem energetics through a variety of indirect mechanisms including population reduction of herbivores that control primary production (Sinclair 1979), alteration of secondary production through reduced fecundity or growth of hosts (Hurd 2001), behavioural modification of habitat-forming species leading to changes in local production (Mouritsen & Poulin 2005, 2006), and manipulation of host behaviour resulting in increased energetic subsidies from terrestrial to aquatic ecosystems (Sato *et al.* 2011, 2012). Until recently, however, the direct contributions of parasites to ecosystem energetics have either been ignored or assumed to be minimal due to their small size (Marcogliese & Cone 1997).

The first study to empirically assess the direct role of parasites alongside free-living organisms in ecosystem energetics found that parasite biomass in three Pacific Coast estuaries was surprisingly high; indeed, parasite biomass exceeded that of the top bird predators and was comparable to fishes and many invertebrate groups (Kuris *et al.* 2008). Trematodes were particularly important, with a biomass density that was one to five times larger than the other parasite groups and an annual production of free-swimming larval stages (cercariae) that was three to ten times greater than the winter bird biomass. These striking results indicate that a large amount of energy can move through parasites, and they may have important direct effects on ecosystem dynamics. Nonetheless, the generality of these findings remains an open question. If estuaries are exceptionally rich in parasites relative to other ecosystems, the findings of Kuris *et al.* (2008) may represent a 'special case,' where parasites play an uncommon role in ecosystem energetics.

In this study, we focus on trematode parasites within freshwater pond ecosystems. Ponds are common model systems in studies of food webs and energetics (Warren 1989; Wilbur 1997), providing an extensive ecological framework for the integration of parasites. Small lakes and ponds also represent the most abundant type of inland waters (Downing *et al.* 2006) and are of importance to the surrounding terrestrial landscape due to the movement of nutrients and biomass between water and land (Polis, Power & Huxel 2004). We focus our estimates of parasite biomass and productivity on trematodes, which are known to play important ecological roles; by virtue of their complex life cycles, trematodes influence interactions between a large number of organisms from multiple trophic levels (Fried & Graczyk 1997). Furthermore, trematodes can alter the growth, reproduction, feeding behaviour, predation risk, morphology and survival of their hosts, leading to significant consequences for host populations and communities (Lafferty & Morris 1996; Poulin *et al.* 2005; Hatcher, Dick & Dunn 2006). We also suspected that trematodes would play the largest direct role of all parasites in pond ecosystem energetics because infection prevalence can be high in many host species and because trematodes consume a large proportion of host body mass in intermediate hosts such as snails (Esch & Fernandez 1994; Hechinger *et al.* 2009).

Our specific aims were to quantify the biomass and productivity of trematode parasites alongside the biomass of the most abundant free-living taxa in three ponds in California, USA. We utilized quantitative field surveys to characterize the composition and density of free-living aquatic organisms >1 mm (excluding some inadequately sampled invertebrates living within the pond substrate) and combined measurements of body size with length-to-mass regressions to calculate mid-summer biomass of the most abundant taxa. To quantify trematode biomass, we targeted macroinvertebrate and amphibian hosts of aquatic trematode life stages for dissections (> 1600 individuals) and measured the biomass of trematode life stages in the laboratory. Lastly, we measured the *in situ* release of free-swimming larval trematode stages and used data on infection prevalence and host densities to estimate annual cercarial productivity. We made several predictions about the importance of trematodes in the flow of energy in pond ecosystems relative to previously studied marine systems. First, we predicted that the biomass of trematodes would vary significantly between the pond ecosystems as a result of changes in the prevalence of trematode infection and the density of suitable hosts, which in turn are linked to environmental characteristics (e.g. pond hydroperiod; Hoverman *et al.* 2011). Second, we predicted that the standing biomass of trematodes in the pond ecosystems would be comparable or greater than the biomass of trematodes in estuarine systems because snail hosts are able to utilize the entire pond habitat and the infection prevalence of snail hosts can attain exceptionally high levels in ponds (Esch & Fernandez 1994). We suspected that this

effect would outweigh the fact that estuarine snail hosts (e.g. *Cerithidea californica*) are larger at maturity and can attain higher densities than freshwater pond snails (Fingerhut, Zimmer & Zimmer 2003). Lastly, we predicted that environmental characteristics that differ between freshwater ponds and estuaries would lead to changes in the timing of trematode productivity between the two ecosystems. Two of our three study sites become completely dry in late summer, presenting a challenging environment in which most organisms (including trematode hosts) are highly productive in the spring and summer when they can grow and reproduce rapidly prior to the onset of unfavourable conditions (Plante & Downing 1989). We predicted that this environment would lead pond trematodes to be highly productive over a short-time period when conditions are favourable for parasite transmission.

## Materials and methods

### STUDY SYSTEM

We estimated free-living and trematode parasite biomass in three ponds (Quick, North and Sheep) within Alameda County in the San Francisco Bay Area of California, USA. The local ecoregion surrounding our study sites consists of oak woodland and grassland. All three ponds lie between 360 and 440 m in elevation and have surface areas in early summer of 2234 m<sup>2</sup> (Quick), 404 m<sup>2</sup> (North) and 145 m<sup>2</sup> (Sheep). In 2009, Sheep Pond dried on July 1st, North Pond dried in mid-August, while Quick Pond has a permanent hydroperiod. The pond shorelines are completely vegetated by emergent plants including *Juncus*, *Typha* and *Scirpus* and none of the ponds support fish. At the beginning of the summer, total dissolved nitrogen in the three ponds ranged between 686 and 1150 µg L<sup>-1</sup> and total dissolved phosphorus ranged between 64 and 81 µg L<sup>-1</sup>.

In these study systems, many trematodes utilize ram's horn snails (*Helisoma trivolvis*) as first intermediate hosts, larval amphibians or aquatic invertebrates as second intermediate hosts, and birds, mammals or adult amphibians as definitive hosts (Fried & Graczyk 1997). Trematode eggs hatch into miracidia, which seek suitable snail hosts wherein they develop into rediae or sporocysts, which in turn reproduce asexually and generate free-swimming cercariae. Cercariae are short lived (< 24 h) and either actively penetrate, or are consumed by, downstream hosts. Cercariae form metacercariae or mesocercariae within second intermediate hosts, which are trophically transmitted into definitive hosts, wherein they develop into mature worms, reproduce sexually and lay eggs. Eggs are passed through the faeces of definitive hosts into water bodies, completing the life cycle.

### BIOMASS ESTIMATES FOR FREE-LIVING TAXA

Between May and July of 2009, we sampled Quick, North and Sheep Ponds biweekly using a stovepipe sampler (53 cm diameter × 74 cm height) to characterize the macroinvertebrate and larval amphibian communities. Stovepipe samplers are effective at quantifying invertebrate densities and performed well when compared with seven other macroinvertebrate sampling methods in heavily vegetated wetlands (Turner & Trexler 1997). The total number of samples per pond was adjusted to pond size (Sheep:

$n = 10$ ; North:  $n = 15$ ; Quick:  $n = 23$ ) and the location of samples was randomly selected using a number generator and a diagram of each pond with an overlaid numbered grid. This method allowed us to capture potential spatial variability in invertebrate communities by sampling all vegetation types within each pond. After pushing the stovepipe sampler through the water column into the pond substrate, we used a D-net (1.4 mm mesh, 2600 cm<sup>2</sup> opening) to remove larval amphibians and macroinvertebrates until five consecutive sweeps yielded zero additional organisms. All of the organisms removed from each sample were transferred to a sorting tray, identified, counted, measured (nearest mm) and released. When identification in the field was not possible, macroinvertebrates were preserved and identified using Merritt, Cummins & Berg (2008). Most taxa were identified to genus or species; however, some were identified only to family (Table S1). In addition, several invertebrate groups were detected but not sampled thoroughly enough to provide an accurate biomass estimate, including benthic invertebrates that were found within the substrate (i.e. some chironomid midges and annelid worms) and zooplankton < 1 mm (see Table S1 for a full list of free-living aquatic taxa and whether each was included in biomass estimates). Given that the biomass of aquatic organisms changes considerably over the course of a year due to growth, reproduction, mortality and emergence, our estimates should be considered 'snap-shots' of mid-summer biomass.

The dry biomass of free-living invertebrates was estimated by converting the density and size distribution of each taxon into a biomass density (g m<sup>-2</sup>) using published length-to-dry mass regressions for the same or closely related species (Anderson, Darring & Benke 1998; Benke *et al.* 1999; Hall, Dybdahl & Vanderloop 2006). We developed our own regressions for *H. trivolvis* snails and the amphibian larvae (Table S2). For snails, we report the dry tissue mass excluding the shell in all results and figures (as per Kuris *et al.* 2008).

### TREMATODE QUANTIFICATION

We identified the trematode community (six taxa) infecting the obligate first intermediate snail hosts (*H. trivolvis*) within our study sites using keys based on morphological characters (Gibson, Jones & Bray 2002; Jones, Bray & Gibson 2005; Bray, Gibson & Jones 2008), followed by additional molecular analyses (S.A. Orloffoske, R.C. Jadin & P.T.J. Johnson unpublished data). We targeted downstream hosts for dissection by determining the life cycle of each trematode taxon from the literature (Bosma 1934; Macy, Cook & DeMott 1960; Lang 1968; Kanev *et al.* 1995; Johnson *et al.* 2004; Bolek & Janovy 2008). The aquatic free-living taxa we identified as trematode hosts included *H. trivolvis* snails, and ten potential second intermediate hosts that included four species of pond-breeding amphibians (*Rana draytonii*, *Pseudacris regilla*, *Anaxyrus* [= *Bufo*] *boreas* and *Taricha torosa*), two dragonflies (*Anax junius* and *Tramea* sp.), two damselflies (*Lestes* and *Coenagrion* spp.) and California clam shrimp (*Cyzicus californicus*). To determine snail infection prevalence, we dissected 414 *H. trivolvis* from Quick Pond, 395 from North Pond and 327 from Sheep Pond, which ranged in size from 2 to 16 mm shell length. We dissected a subset of amphibian and invertebrate second intermediate hosts from each pond to quantify metacercariae and mesocercariae ( $n > 30$  per amphibian species;  $n > 20$  per invertebrate species). One amphibian host, the California red-legged frog (*R. draytonii*) was omitted from parasite quantification because it is listed under the US Endangered

Species Act. Adult trematodes, which use mammals, birds and adult amphibians as definitive hosts in our study systems, were not quantified due to the ethical and logistical challenges associated with dissecting these taxa and because the definitive hosts are primarily terrestrial, thus representing transient members of the pond food web.

#### BIOMASS AND PRODUCTIVITY ESTIMATES FOR TREMATODES

We dissected infected *H. trivolvis* snails in the laboratory to obtain estimates of the average proportion of the total snail host and trematode biomass that consisted of trematode rediae or sporocysts (5–9 field-collected snails for each trematode taxon; Table S3). The trematode tissue and snail tissue were separated under a dissecting microscope and individually dried (60 °C for 24 h) and weighed on a microbalance using pre-weighed aluminium tins, and compared against 'blank' tins without tissue to ensure accuracy between measurements. We obtained estimates of the mass of cercariae from each trematode taxa by directly measuring the dry mass and volume of cercariae from two species and calculating a general cercaria density ( $\mu\text{g } \mu\text{m}^{-3}$ ) that was applied to volume measurements of cercariae from the other four trematode taxa (see Appendix S1 for details). We directly measured the dry mass of metacercariae or mesocercariae from five of the trematode taxa and extrapolated the mass of the sixth taxon in the same manner as with cercariae (Appendix S1).

To quantify trematode productivity (the change in biomass over time), we measured output of cercariae for all six trematode taxa at four times during the summer and combined these data with the estimates of taxon-specific cercariae mass. Cercarial output was obtained by isolating snails (150 per time point) inside of capped 50 mL conical vials maintained *in situ* within Quick Pond to ensure a normal range of environmental conditions during cercarial release. Vials were filled with pond water and suspended at a depth of 30 cm for 24 h, after which we counted the number of cercariae released from infected snails under a dissecting microscope. To ensure that pond water in the vials did not contain cercariae prior to the addition of snails, we deployed five extra vials on each occasion to which we did not add snails. Cercariae were never found in these vials.

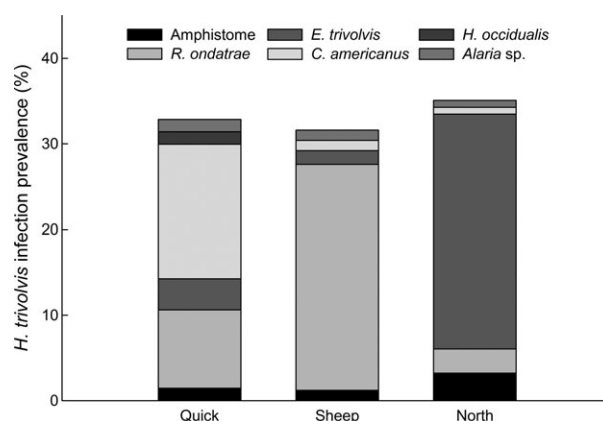
Our estimates of total trematode standing biomass were calculated as the sum of the rediae/sporocyst biomass in snails, the short-lived daily biomass of cercariae released from snails, and the metacercariae/mesocercariae biomass in second intermediate hosts. To calculate the rediae/sporocyst biomass in snails, we multiplied species-specific snail infection prevalence by the density of snails  $> 8$  mm and the average species-specific proportional trematode biomass within each infected snail. Our snail infection data indicated that snails  $< 8$  mm were rarely infected ( $< 1\%$  prevalence), so we omitted snails under this size from our infection prevalence estimates and applied an average prevalence for all snails  $> 8$  mm (this size class was generally 8–12 mm, as very few snails were  $> 12$  mm). The standing biomass of cercariae was calculated as the species-specific density of infected snails multiplied by the species-specific average daily output of cercariae and the biomass of each cercaria. The metacercariae/mesocercariae biomass in second intermediate hosts (amphibians and invertebrates) was calculated as the density of infected hosts multiplied by the estimated biomass of metacercariae or mesocercariae.

Our equation for cercarial productivity included the species-specific density of infected snails multiplied by the species-specific average daily output of cercariae, the estimated biomass of each cercaria, and the duration of the year over which we assumed infected snails were releasing cercariae. Because previous research suggests that infected snails from our study sites are not releasing cercariae during the winter, after most second intermediate hosts have emerged (D.L. Preston unpublished data), we made the conservative assumption that trematodes only released cercariae for 4 months  $\text{yr}^{-1}$  in the ponds with the longest hydroperiods (Quick and North) and for 3 months  $\text{yr}^{-1}$  in Sheep Pond, which has a shorter hydroperiod. We also assumed that cercarial release rates were relatively constant during this time period, which is an assumption supported by data on the numbers of cercariae released at three time points (May, June and July) for two trematode taxa within our study systems (S.H. Paull unpublished data).

## Results

### INFECTION PREVALENCE

We detected six trematode taxa within *H. trivolvis* snails at Quick Pond, including *Ribeiroia ondatrae*, *Echinostoma trivolvis*, *Halipegus occidualis*, *Cephalogonimus americanus*, one trematode identified to the genus *Alaria* and another identified only as an amphistome. At Sheep and North Ponds we detected the same taxa, minus *H. occidualis*. Total infection prevalence in first intermediate snails hosts (summed among trematode species) was similar between the three ponds, with infections detected in 33% of mature *H. trivolvis* from Quick Pond, 32% from Sheep Pond and 35% from North Pond (Fig. 1). Although the species composition of trematodes infecting snails was generally similar across ponds, the most prevalent trematode taxa varied: 48% of infections at Quick were *C. americanus*, 84% of infections at Sheep were *R. ondatrae* and 78% of infections at North were *E. trivolvis*

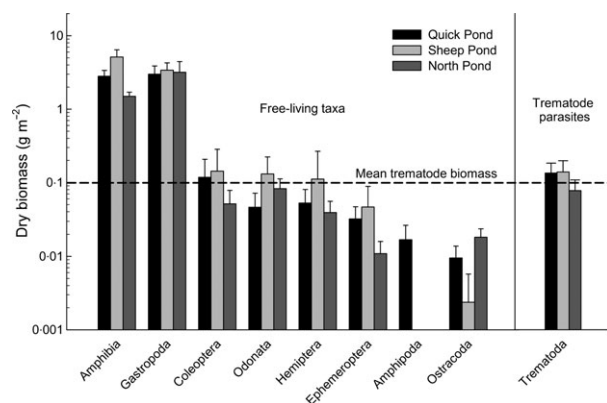


**Fig. 1.** Prevalence of trematode infections in *Helisoma trivolvis* snails ( $n > 325$  per pond) from three California ponds. *H. trivolvis* is an obligate first intermediate host of each trematode taxon. The legend shows six trematode taxa that were detected, which correspond to stacks in the bars. Infection prevalence is for snails  $> 8$  mm in shell length only, as smaller snails were rarely infected ( $< 1\%$  prevalence).

(Fig. 1). We detected four of these trematodes (*R. ondatrae*, *E. trivolvis*, *Alaria* sp. and *C. americanus*) in larval amphibian second intermediate hosts (*P. regilla*, *A. boreas* and *T. torosa*; see Table S4 for a summary of infection prevalence of trematodes in second intermediate hosts from all three ponds). One trematode (*H. occidualis*) was detected in invertebrate second intermediate hosts (damselfly and dragonfly larvae [*Anax*, *Tramea*, *Coenagrion* and *Lestes* spp.] and California clam shrimp [*Cyzicus californicus*]; Table S2). The mean infection prevalence was 98% (SE = 0.9%) for *R. ondatrae* and 73% (SE = 10.7%) for *E. trivolvis* in the three amphibian taxa from all three ponds (Table S4). *Alaria* sp. and *C. americanus* only infected the anuran tadpoles (*A. boreas* and *P. regilla*), at average prevalences of 43% (SE = 18%) and 68% (SE = 12%), respectively. The mean infection intensity in second intermediate hosts ranged from a low of five mesocercariae (*Alaria* sp.) per host to 35 metacercariae (*R. ondatrae*) per host in the three ponds. Trematode infections occurred in 53% of odonates and 3% of clam shrimp dissected from Quick, 10% of odonates from Sheep and 8% of odonates from North (Table S4).

#### FREE-LIVING AND TREMATODE BIOMASS

We detected 31 taxa of free-living aquatic macroinvertebrates and amphibians, of which 24 were sampled adequately to obtain reliable biomass estimates (Table S1). Amphibian larvae (primarily *P. regilla*, *A. boreas* and *T. torosa*) and snails (*H. trivolvis*) dominated the standing crop animal biomass in all three ponds, with dry biomass densities of one to two orders of magnitude above the other macroinvertebrate groups (Fig. 2). Larval amphibian biomass ranged between 1.5 g m<sup>-2</sup> (North) and 5.1 g m<sup>-2</sup> (Sheep) with a mean across ponds of 3.1 g m<sup>-2</sup> (± 1.1 SE). Snail biomass was remarkably consistent among the three ponds and ranged between 3.0 g m<sup>-2</sup>



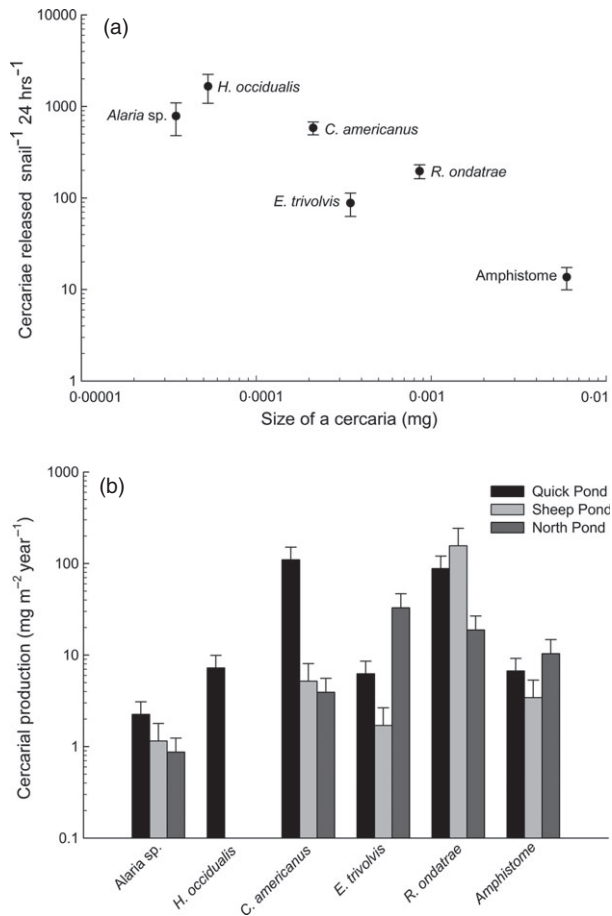
**Fig. 2.** Biomass of free-living taxa and aquatic life stages of trematode parasites from three ponds in California. Biomass of free-living taxa are on the left side of the figure and the combined trematode parasite community is on the right. The dashed line shows the mean trematode parasite biomass across all three ponds. Error bars show upper 95% confidence intervals.

(Quick) and 3.4 g m<sup>-2</sup> (Sheep), with a mean of 3.2 g m<sup>-2</sup> (± 0.1 SE). The insect orders with the largest biomass densities (Fig. 2) included coleoptera (0.10 g m<sup>-2</sup> ± 0.03 SE), odonata (0.09 g m<sup>-2</sup> ± 0.02 SE), hemiptera (0.07 g m<sup>-2</sup> ± 0.02 SE) and ephemeroptera 0.03 g m<sup>-2</sup> (± 0.01 SE). The biomass of the aquatic life stages of trematode parasites was comparable to, or greater than the insect orders (Fig. 2). Estimates of trematode biomass (all trematode life stages excluding adult worms) from within each pond were 0.07 g m<sup>-2</sup> for North Pond (95% CI = 0.05–0.10), 0.12 g m<sup>-2</sup> for Quick Pond (95% CI = 0.08–0.16) and 0.13 g m<sup>-2</sup> for Sheep Pond (95% CI = 0.08–0.18).

Of the total trematode biomass, the majority (87%) consisted of redial or sporocyst life stages within snail first intermediate hosts. Our laboratory dissections indicated that an average of 25% of the total host and parasite biomass of infected *H. trivolvis* snails consisted of larval trematode tissue (range = 18–33% for the six trematode taxa; see Table S3 for sample sizes and variances). Within the three ponds, the biomass of larval trematodes within snails was 3.6× the combined biomass of cercariae and metacercariae in Sheep Pond, 8.6× in North Pond and 17.1× in Quick Pond. The trematode biomass within snails comprised 3.9% of the total snail tissue biomass in Quick Pond, 3.5% of the total snail biomass in Sheep Pond and 2.1% of the total snail biomass in North Pond (excluding snail shell mass). If we follow Kuris *et al.* (2008) in considering the entire infected snail (i.e. trematode plus castrated snail host tissue) as the parasite ‘extended phenotype’, the proportion of extended phenotype biomass relative to total snail biomass (infected and uninfected) increased to 17% for Quick Pond, 16% for Sheep Pond and 11% for North Pond, which is 3× greater than the biomass of the largest macroinvertebrate group in each pond.

#### TREMATODE PRODUCTIVITY

The cercarial density of the two trematode taxa for which we directly measured dry biomass were similar (*R. ondatrae* cercariae =  $1.38 \times 10^{-7} \mu\text{g } \mu\text{m}^{-3}$ ; *E. trivolvis* cercariae =  $1.54 \times 10^{-7} \mu\text{g } \mu\text{m}^{-3}$ ). Applying an average tissue density of  $1.46 \times 10^{-7} \mu\text{g } \mu\text{m}^{-3}$  to volume measurements of the other four trematode taxa resulted in individual cercaria dry masses (Fig. 3a) that ranged between  $3.5 \times 10^{-5}$  mg (*Alaria* sp.) and 0.006 mg (amphistome). The average number of cercariae released 24 h<sup>-1</sup> was inversely related to cercarial size (Fig. 3a) and ranged between 14 (amphistome) and 1660 (*H. occidualis*). Assuming cercarial release for 3–4 mos and constant snail density and infection prevalence, the total annual production of cercariae (Fig. 3b) was estimated at 0.22 g m<sup>-2</sup> yr<sup>-1</sup> for Quick Pond (95% CI = 0.14–0.30), 0.17 g m<sup>-2</sup> yr<sup>-1</sup> for Sheep Pond (95% CI = 0.08–0.26) and 0.07 g m<sup>-2</sup> yr<sup>-1</sup> for North Pond (95% CI = 0.04–0.10). The largest annual cercarial production was for *R. ondatrae* in Sheep Pond (156 mg m<sup>-2</sup> yr<sup>-1</sup>) and *C. americanus* in Quick Pond (110 mg m<sup>-2</sup> yr<sup>-1</sup>). Annual cercarial production estimates for the



**Fig. 3.** (a) The mean number of cercariae released infected snail<sup>-1</sup> 24 h<sup>-1</sup> plotted against the estimated dry biomass of a single cercaria of each trematode taxon. Error bars for the number of cercariae released represent one standard error. (b) Yearly cercarial production of six trematode taxa in three California ponds. Error bars show upper 95% confidence intervals.

other trematodes ranged between 0.9 and 37 mg m<sup>-2</sup> yr<sup>-1</sup> (Fig. 3b).

## Discussion

Our results indicate that trematode parasites can be prevalent members of pond communities with a large cumulative biomass. The aquatic life stages of trematodes had an ecosystem-level biomass that was comparable to the most abundant insect orders, including the beetles, damselflies and dragonflies, hemipterans and mayflies. Across the three ponds, 87% of the total trematode biomass was composed of larval stages within snail first intermediate hosts. This result was due primarily to the high density of snail hosts (mean = 106 snails m<sup>-2</sup>), the high trematode infection prevalence (33% of mature snails), and the large proportional trematode tissue mass per infected snail (17–33% across the six trematode taxa). Contrary to our prediction, the three ponds displayed remarkably similar densities of snail hosts and similar infection prevalence between sites. These results suggest that trematode

biomass is linked to the biomass of suitable snail hosts, but may be less dependent on downstream hosts such as amphibians and larval insects, despite their high abundance and infection intensity. Furthermore, trematodes produced large numbers of short-lived, free-swimming larval stages, which amounted to an annual mean biomass across the three ponds of 153 mg m<sup>-2</sup>. Collectively, our results underscore the potential roles of parasites in freshwater ecosystem energetics, and provide further evidence that parasites have the potential to exert effects on ecosystem processes that are comparable to their free-living counterparts.

Our findings highlight both similarities and differences in the role of trematodes in the flow of energy through some freshwater and marine ecosystems. In the Pacific estuaries studied by Kuris *et al.* (2008), the biomass of parasites was dominated by trematodes, which use *Cerithidea* spp. snails as first intermediate hosts. Consistent with our results, trematode biomass in the three estuaries was comparable to, or exceeded that of many free-living groups. Although Kuris *et al.* (2008) reported trematode biomass in wet mass, if we are to assume a wet-to-dry mass conversion factor of 0.1 (Benke 1984), the dry biomass of trematodes in the three estuaries would average close to 0.1 g m<sup>-2</sup>, which is similar to the estimates from our ponds (0.07–0.13 g m<sup>-2</sup>). Similarly, converting the yearly cercarial productivity for the combined trematode communities in the three estuaries into dry mass yields estimates of about 0.1–0.4 g m<sup>-2</sup> yr<sup>-1</sup>, which are slightly higher than our pond estimates (0.07–0.22 g m<sup>-2</sup> yr<sup>-1</sup>). The ratio of annual cercarial production to biomass of trematode life stages within snail hosts was about three in the estuaries, whereas in our pond systems it averaged two. The relative similarity of the production to biomass ratios of trematodes between the two different aquatic ecosystems is interesting considering the fact that in our study systems trematodes only produce cercariae for a few months during the summer. If the pond trematodes released cercariae for 12 mos yr<sup>-1</sup>, our estimated ratios of cercarial production to biomass would increase to approximately 6.7, which is more than double the estimate from the estuaries. In addition, the individual cercarial production per trematode taxon within our study systems ranged between  $8 \times 10^{-4}$  g m<sup>-2</sup> yr<sup>-1</sup> and 0.1 g m<sup>-2</sup> yr<sup>-1</sup>, which are within the range of values reported for 18 marine trematode taxa (Thieltges *et al.* 2008).

The high biomass and productivity of trematodes suggest that parasites could play an important role as a prey resource in ponds. The fate of trematode cercariae biomass is not well known, but some (presumably small) fraction successfully infects downstream hosts, whereas the rest are eaten by predators or die within a short time and enter detrital food webs. The diversion of resources from snail reproduction into trematode reproduction presents a unique pathway of energy flow; in the absence of trematodes, snail secondary production remains embed-

ded within benthic food webs, whereas the presence of trematodes producing free-swimming cercariae diverts some benthic production into the planktonic food web (Morley 2012). Studies from marine systems suggest that many predators feed on cercariae, which are nutrient-rich and poorly defended from predation (Kaplan *et al.* 2009). Our own laboratory studies indicate that a variety of organisms present in the study ponds, including damselflies and clam shrimp, feed readily on cercariae, potentially reducing disease risk to downstream hosts (Orlofske *et al.* 2012). Evidence from lake ecosystems also suggests an important role of parasites as prey. The free-living zoospores of chytrid fungi that infect phytoplankton are an abundant prey resource, perhaps shunting energy from inedible host phytoplankton species to planktivores via the consumption of zoospores (Kagami *et al.* 2007; Gleason *et al.* 2008). Food web analyses further support the prominent role of parasites as prey; a food web including parasites from one of our study sites (Quick Pond) included 1088 total links, of which 36% represented predation on parasites (Preston *et al.* 2012). This large number of predator–parasite links includes predation on free-living parasite stages (e.g. cercariae) as well as concomitant predation, when predators consume parasites alongside their infected hosts (Johnson & Thielges 2010; Johnson *et al.* 2010). Considering that every free-living host can become infected with multiple parasite species (Price 1980), it is not surprising that predator–parasite links can be as prevalent as traditional predator–prey links in some food webs (e.g. Lafferty, Dobson & Kuris 2006; Amundsen *et al.* 2009). Measurements of parasite production in nature, alongside laboratory feeding studies and food web analyses, all suggest that parasites have the potential to function as important prey resources in diverse ecosystems.

The indirect consequences of parasitism on energy flow may be equally important as the direct roles of parasites as prey in pond ecosystems. For example, several of the trematodes within our study system reduce the fitness of amphibian hosts, which may make amphibian larvae more susceptible to predation, thereby strengthening trophic links between hosts and their free-living predators. *Ribeiroia ondatrae*, which was found in nearly 100% of the amphibian larvae at our study sites, causes limb abnormalities which reduce amphibian performance and likely increase predation susceptibility (Johnson *et al.* 1999; Goodman & Johnson 2011a,b). Similarly, *Echinostoma trivolvis*, which were found in close to 75% of all amphibian larvae, can cause oedema and lethargy in amphibians when infection intensities are high, which again may lead to higher predation rates and reduced amphibian survival (Johnson & McKenzie 2008; Rohr, Raffel & Sessions 2010). Whether such morphological and behavioural alterations, lead to community-level shifts in secondary production or the strength of trophic interactions remains to be tested. Nonetheless, parasite-induced mortality and host manipulation suggest that

trematodes might play important indirect roles in trophic interactions, especially considering the significant role of amphibians in linking aquatic and terrestrial food webs (Gibbons *et al.* 2006; Regester, Lips & Whiles 2006). Currently, one compelling example exists of parasites indirectly altering the strength of subsidies between aquatic and terrestrial ecosystems. In Japan, nematomorph worms cause cricket and grasshopper hosts to jump into streams, dramatically increasing the flow of energy from the forest to the stream, where the insects provide prey to trout (Sato *et al.* 2011, 2012).

Our results, alongside findings in marine ecosystems, provide the first steps towards a broader understanding of the direct contributions of parasites to ecosystem energetics. Ponds and small lakes are the most abundant type of inland waters globally, and more research is needed to evaluate the generality (or rarity) of our findings across other sites. Furthermore, the roles of parasites in energy flow within most terrestrial ecosystems, and many additional freshwater (e.g. streams, lakes) and marine systems (e.g. coral reefs, open ocean, sea floor) remains poorly understood. By showing that trematodes play significant roles in the energetics of pond ecosystems, our results help to advance the integration of parasitism into community- and ecosystem ecology. Future efforts aimed at understanding how parasite biomass alters the fate of nutrients and energy both within and across ecosystems are warranted and will provide valuable insights into the ecosystem-level roles of disease.

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## Supporting Information

Additional Supporting Information may be found in the online version of this article.

**Appendix S1.** Trematode biomass measurements.

**Table S1.** List of free-living taxa from pond sampling.

**Table S2.** Length-to-dry mass regression equations for snails and amphibian larvae.

**Table S3.** Proportional trematode biomass within snail hosts.

**Table S4.** Infection prevalence of trematodes in second intermediate hosts.