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Unhealthy herds: indirect effects of predators enhance two drivers of disease spread

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Summary

1. Predators could reduce disease prevalence in prey populations by culling infected hosts and reducing host density. However, recently observed positive correlations between predator density and disease burdens in prey/hosts suggest that predators do not always 'keep the herds healthy'. Several possible mechanisms could explain this 'unhealthy herds' effect, including a predator-induced change in prey/host traits which enhances susceptibility or alters other epidemiologically important traits.

2. Here, we use an invertebrate predator, zooplankton host, yeast parasite system to demonstrate such trait-mediated indirect effects. We exposed ten genotypes of the prey/host *Daphnia dentifera* to infochemicals ('kairomones') produced by the invertebrate predator *Chaoborus* and to a yeast parasite.

3. We found that kairomone exposure induced larger and more susceptible *D. dentifera*. Clones that showed substantial increases in body length also yielded more spores upon death. However, exposure to kairomones did not alter reproduction from uninfected hosts. All of these results were captured with a dynamic energy budget model of parasitism.

4. Overall, our empirical and theoretical results show that predators can have strong indirect effects on host–parasite interactions that could produce positive correlations between predation intensity and disease burden.

Key-words: chemical cues, dynamic energy budget models, *Metschnikowia* nonconsumptive effects, trait-mediated indirect effects, trait-mediated indirect interactions

Introduction

Theory suggests that many predators should 'keep the herds healthy' for at least two reasons. First, predators reduce host density. As disease transmission often increases with host density (Anderson & May 1991), predation on hosts can reduce opportunities for disease spread. Second, predators eat infected prey, sometimes quite preferentially (Hatcher, Dick & Dunn 2006). If predators themselves cannot spread parasites while eating infected prey, predation that removes infected individuals should decrease contact between susceptible and infected hosts and/or free-living parasite propagules, thereby inhibiting disease spread. Indeed, recent theoretical (Packer *et al.* 2003; Ostfeld & Holt 2004; Hall, Duffy & Cáceres 2005) and empirical (Hudson, Dobson & Newborn 1992; Duffy *et al.* 2005; Johnson *et al.* 2006) work supports this 'healthy herds' hypothesis, particularly in cases where predators preferentially select infected prey. This hypothesis suggests that two common management goals – conserving predators and reducing disease – act in concert.

However, some populations suffer both high predation and high rates of parasitism (Woodroffe 1999; Cardinale *et al.* 2003; Duffy 2007; Cáceres, Knight & Hall 2009; Hawlena, Abramsky & Bouskila 2010). Such patterns seem to flout the healthy herds hypothesis. Of course, predators may still control disease in these systems, but weakly; if so, predator removal would further elevate disease. However, several other possibilities implicate predators in spreading or enhancing disease. First, predators may directly disperse parasites through sloppy feeding or defecation (Cáceres, Knight & Hall 2009; Duffy 2009). Second, predators can increase disease in their prey by culling individuals that have recovered from infection and become immune. Such culling enhances

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compensatory births of susceptible individuals, thereby increasing disease spread (Holt & Roy 2007). Third, nonlethal effects of predators may alter host susceptibility and otherwise increase disease through trait-mediated indirect effects (TMIEs; Abrams *et al.* 1996; Raffel *et al.* 2010; Werner & Peacor 2003). For example, predators may alter host behaviour in a manner that increases susceptibility of hosts to parasitism (Thiemann & Wassersug 2000).

In this study, we develop a mechanistic connection between TMIEs of predators and key epidemiological traits. Larvae of the phantom midge, *Chaoborus*, are major predators of the freshwater grazer *Daphnia dentifera* (Fig. 1; Garcia & Mittelbach 2008; González & Tessier 1997). Yet, lakes with higher density of *Chaoborus* but lower intensity of vertebrate (fish) predation tend to have larger epidemics of the virulent yeast parasite *Metschnikowia bicuspidata* (Fig. 1; Cáceres, Knight & Hall 2009; Hall *et al.* 2010b). Based on prior work, we suspected that predator-induced TMIE could help to catalyse this 'unhealthy herds' pattern. Nonlethal exposure of *Daphnia* to infochemicals (kairomones) produced by *Chaoborus* can induce plastic changes in body size of *Daphnia* (reviewed by Tollrian & Dodson 1999; Lass & Spaak 2003). In many cases, *Daphnia* can become larger at an earlier age and then remain larger. This response is mediated through shifts in energy allocation by hosts towards growth rather than reproduction (Stibor & Lüning 1994). More allocation to growth reduces predation risk from *Chaoborus* (a gape-limited predator; Pastorok 1981).

Fig. 1. Top: *Chaoborus* attacking a juvenile *Daphnia*. Bottom: Uninfected (left) and *Metschnikowia*-infected (right) *D. dentifera*. Photograph credits: Alan J. Tessier.

Despite its role in reducing predation risk, this TMIE-based growth response could enhance spread of a virulent yeast (*Metschnikowia*) through its influence on three key traits. Each of these traits influence the parasite's reproductive ratio (R_0) and therefore its ability to invade and spread (Anderson & May 1991; Hall *et al.* 2009a). First, increases in body size could boost susceptibility of host *Daphnia*. Susceptibility increases with body size because larger hosts consume more parasite spores while eating (Hall *et al.* 2007b). Second, larger hosts typically yield more spores once infection kills them (Hall *et al.* 2009a,b, 2010a). Third, larger hosts typically produce more offspring because feeding rate increases with body size (Hall *et al.* 2009a,b, 2010a). Thus, larger individuals acquire more resources which then can be allocated to reproduction. In addition, larger hosts can physically fit larger broods within their carapace (Lynch 1980). Increased reproductive rate should increase disease by increasing the density of susceptible hosts (Anderson & May 1991). All else being equal, then, kairomones might increase fecundity of hosts. However, kairomones may substantially elevate the cost of reproduction and/or age at first reproduction (Stibor & Lüning 1994; Rinke, Hulsmann & Mooij 2008). These factors could depress fecundity, thereby reducing disease spread. We explored these connections between kairomone-induced TMIE, body size and epidemiological traits using experiments and a dynamic energy budget model.

Study system and overview of approaches used

Daphnia dentifera is a dominant grazer in stratified lakes in temperate North America (Tessier & Woodruff 2002). *Metschnikowia* is a common parasite of *Daphnia dentifera* (Duffy *et al.* 2010; Hall *et al.* 2010b). It is highly virulent, reducing fecundity and lifespan (Duffy & Hall 2008), and can strongly influence the ecological and evolutionary dynamics of *D. dentifera* populations (Duffy *et al.* 2008, 2009; Hall *et al.* 2011). *Chaoborus punctipennis* and *C. flavicans* (hereafter *Chaoborus*) are important predators of *D. dentifera* (González & Tessier 1997; Garcia & Mittelbach 2008), but do not prey selectively on *Metschnikowia*-infected hosts (Cáceres, Knight & Hall 2009). *Chaoborus* induce plastic responses in *Daphnia* through waterborne infochemicals (Tollrian & Dodson 1999). Thus, *Chaoborus*-conditioned water can be used to stimulate indirect effects of predators on the zooplankton host–yeast parasite system.

To make the connections between predator-induced trait-mediated effects and disease, we combined multiple experiments with several different quantitative approaches. The experiments quantified key epidemiological parameters estimated in the presence and absence of predator kairomones. The first experiment measured infection prevalence. Then, using infected animals from that assay, we estimated time until death and spore yield in a follow-up experiment. In a

third experiment, we conducted a separate life-table-based assay of fecundity of uninfected hosts, reared or not reared with kairomones. We then made quantitative inferences from these data in three ways. First, we fit standard GLM-based models to the data (treating clones as random effects but the kairomone treatment as a fixed effect). These GLM models permitted frequentist-based inferences from the experimental treatments. Next, while focusing on susceptibility alone, we fitted a suite of biologically informed models to the prevalence data. These models estimated the key susceptibility parameter directly from prevalence data, allowed for mechanistic representations of our body size and genotype-based hypotheses and were compared using information-theoretic-based statistics. Finally, after presenting the data and results from the first two suites of statistical models, we used simulations of a dynamic energy budget model (Hall *et al.* 2009a,b) to better understand why predator kairomones might produce the effects on body size, fecundity and spore yield that we observed.

Empirical materials and methods

To generate *Chaoborus* kairomones, we incubated field-collected *Chaoborus* in Artificial *Daphnia* Medium (ADaM; Klüttgen *et al.* 1994). We added five *Chaoborus* (95% CI for body length: 7·65, 8·18 mm) to beakers filled with 1 L of ADaM. This density falls within realistic ranges for natural populations (Garcia & Mittelbach 2008; Cáceres, Knight & Hall 2009). *Chaoborus* produce the kairomone while feeding on *Daphnia* (Tollrian & Dodson 1999), so we also added 25 juvenile *D. dentifera* to each beaker. After incubating for 48 h at 4 °C, *Chaoborus* were removed from the beakers; the kairomone water was then filtered (Pall A/E), pooled in a large carboy and allocated to 150-mL beakers for use in the experiments.

We used standard susceptibility assays to measure how *Chaoborus* kairomones influenced susceptibility of hosts to infection. Our measure of susceptibility cannot distinguish between changes in contact rate per spore and changes in infectivity per spore. These assays used ten genotypes of *Daphnia dentifera*, all originally collected from lakes in Southwest Michigan located near the Kellogg Biological Station; however, one genotype was excluded from analyses because of high mortality. These genotypes span a wide range of susceptibility to *Metschnikowia* (Duffy & Sivars-Becker 2007; Duffy *et al.* 2008; Hall *et al.* 2010a). We used our standard strain of *Metschnikowia*, which also originates from a lake in Southwest Michigan. We have found that strains of *Metschnikowia* collected from different lakes and in different years do not vary in their infectivity or virulence to *Daphnia dentifera* (Duffy & Sivars-Becker 2007).

To conduct the susceptibility assays, we first reared hosts in kairomone or control ADaM and then exposed them to spores. To generate animals for the experiment, *D. dentifera* of each genotype were reared in 150-mL beakers, with six *D. dentifera* per beaker and fed 20 000 cells mL^{-1} *Ankistrodesmus falcatus* (a green alga) every day. After 2 days, *D. dentifera* (1–2 days old) were harvested and placed in 150-mL beakers containing either control or *Chaoborus*-treated ADaM. Individuals were transferred to new beakers (maintaining *Chaoborus* kairomone treatments) every other day until individuals were 7–8 days old (but still juveniles). Then, we placed six animals of each combination of a given genotype**Chaoborus* treatment into 150-mL beakers (eight replicate beakers) filled with 100 mL of control or *Chaoborus*-treated ADaM. We measured up to 10 additional individuals in each of the genotype**Chaoborus* treatments (at 40× magnification; top of head to base of tail, measured with Olympus DP2-BSW software). We added 190 spores mL^{-1} and 10 000 cells mL^{-1} *A. falcatus* to each beaker. Spores were generated for the experiment by exposing *D. dentifera* from a single, highly susceptible genotype to spores from our standard strain of *Metschnikowia*. Infected animals were then harvested and ground to release spores; the resulting spore slurry was used in the experiment. After exposure to the parasite for 24 h, hosts were transferred to fresh medium. For the remainder of the experiment, individuals were transferred to new beakers every other day (maintaining kairomone treatments), fed with 20 000 cells mL^{-1} *A. falcatus* every day, and kept at 20 °C and 16:8 h light : dark. We visually screened individuals for infections at 25–50× magnification 10 days after exposure (Duffy & Sivars-Becker 2007). Beakers in which more than three animals died during the experiment (37 total) were excluded from analyses of infection prevalence. Individuals that died during the experiment most likely did not die as a result of the infection, as hosts infected with *Metschnikowia* generally live approximately 20 days postinfection (Ebert, Lipsitch & Mangin 2000; Duffy & Sivars-Becker 2007).

We conducted two additional experiments to examine kairomone-induced changes in spore yield from infected hosts and fecundity of uninfected hosts. Both involved placing individual animals in 150-mL beakers containing 80 mL of control or kairomone-treated ADaM containing 15 000 cells mL^{-1} of *A. falcatus* as food. The first was a continuation of the susceptibility assay. For this experiment, infected individuals from the susceptibility assays were transferred to new beakers filled with the appropriate medium. Upon death from infection, individuals were photographed for length measurements. Then, they were placed in 250 μL fresh ADaM in a plastic microcentrifuge tube and gently smashed using a pestle. Spores in the resulting slurry were counted using a hemocytometer at 200× magnification. Second, in a separate 45-day life table experiment, we determined whether *Chaoborus* kairomones altered age at maturity and total reproduction in *D. dentifera*. We collected 1-day-old *D. dentifera* from each of 12 genotypes (the ten used in the susceptibility assay, plus two additional genotypes) and placed them in individual beakers. For most genotypes, we had 11 replicates (range: 6–11, mean = 10 replicates). We put animals individually into 150-mL beakers filled with 80 mL of either regular ADaM or *Chaoborus*-treated ADaM and 15 000 cells mL^{-1} of *A. falcatus* as food. Reproduction and survivorship were monitored as individuals were transferred to fresh medium every other day.

In our first set of quantitative analyses, we used the same underlying statistical model to analyse data from these three experiments, using Proc Mixed in SAS 9.1 (Littell *et al.* 2006). The model included *Chaoborus* treatment as a fixed effect, *D. dentifera* genotype as a random effect, and their interaction. We tested for significance of random effects by using differences in the −2 restricted log likelihood, which are χ^2 distributed with 1 degree of freedom, between models with and without the particular random effect included (Littell *et al.* 2006). Because we did not assume equal variances for fixed effects, we used a Satterthwaite procedure that yielded noninteger degrees of freedom. For the susceptibility assay, we analysed the arcsine square-root-transformed infection prevalence data, as well as data on size at exposure to the parasite. For the continuation of this life table, we analysed data on size at death and spore yield at death. For one highly resistant genotype, there were not enough infected individuals to accurately estimate spore yield. Therefore, we have only eight genotypes in our analysis of spore production. Finally, for the life table, we analysed data on age at first reproduction, fecundity and survivorship over the course of the 45-day experiment. We also calculated an instantaneous population growth rate, r, for each kairomone-genotype combination using the Euler–Lotka equation. We compared values of r between treatments using a t-test.

In a second quantitative analysis, we competed six biologically informed statistical models. In this competition, we sought to connect variation in size-specific susceptibility among genotypes, body size and prevalence of infection in the assays (Table 1; see Supporting Information for details). Models 1–4 assumed that genotypes differ in underlying susceptibility (as previously observed; Duffy & Sivars-Becker 2007; Duffy *et al.* 2008; Hall *et al.* 2010a), while models 5–7 assume that all genotypes share the same underlying susceptibility. Models 1 and 5 also incorporated a (body length)^4 term (based on Hall *et al.* 2007b); for a given genotype, any difference in body length between the treatments was driven by *Chaoborus* kairomones. Models 2 and 6 assume no relationship existed between body length and susceptibility (and, therefore, that the observed *Chaoborus*-driven increase in size did not affect infection prevalence). Model 3 incorporated body length as well as an 'additional *Chaoborus* effect' beyond that promoting larger body size; the related model 4 assumes 'additional Chaoborus effects' but does not include body length in the parameter estimates. (This model produces the same AIC-based results as model 3 but different parameter estimates for susceptibility.) These additional parameters for the effects of *Chaoborus* in models 3 and 4 represented the possibility that *Chaoborus* increased susceptibility through both body length and/or some other factors. Finally, the null model 7 assumed that neither host genotype nor kairomone-induced changes in body length nor other kairomone effects were important. We fit these models using the binomial distribution as the likelihood function and evaluated them based on standard information theoretic approaches (as summarized in Table 1; Burnham & Anderson 2002).

Empirical and statistical results

Daphnia dentifera that were exposed to *Chaoborus* kairomones were significantly larger ($F_{1,\ 151} = 21{\cdot}9$, $P < 0{\cdot}0001$, Fig. 2a) and more susceptible to *Metschnikowia* ($F_{1,\ 97{\cdot}9} = 5{\cdot}1$, $P = 0{\cdot}03$, Fig. 2b). As expected, we also observed significant differences in size ($\chi^2 = 4{\cdot}5$, $P = 0{\cdot}03$, Fig. 2a) and susceptibility ($\chi^2 = 5{\cdot}2$, $P = 0{\cdot}02$, Fig. 2b) among *D. dentifera* genotypes, but no clonal genotype*kairomone interaction was found for either size or susceptibility. Model comparison suggests that this kairomone-induced increase in body size in the + *Chaoborus* treatment can explain the increased susceptibility: the best-performing model (model 1) incorporated both host genotype and *Chaoborus*-induced changes in host body length (Table 1, Fig. 3). The second best-performing model (model 2), which did not account for body length but did incorporate differences among genotypes, garnered considerably less support (ΔAIC = 4, Table 1; Burnham & Anderson 2002). The remaining models received virtually no support.

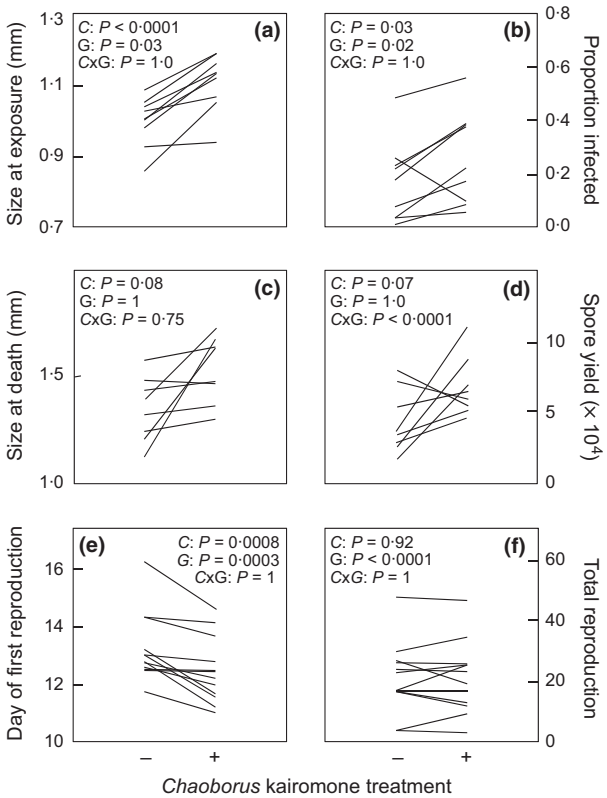

Fig. 2. Response to *Chaoborus* kairomones, shown as reaction norms. *Host susceptibility*: (a) length of *Daphnia* (top of head to base of tail spine) at the time of exposure to yeast spores and (b) disease susceptibility (measured as proportion infected). *Parasite production*: (c) size at death from infection, and (d) spore yield from dead, infected hosts. *Reproduction of uninfected hosts*: (e) day of first reproduction, and (f) total reproduction. Each line links mean values of different genotypes of *D. dentifera*; '+' indicates exposure to kairomones, '–' denotes control. *P*-values of ANOVA results are shown in the insets, with '*C*' indicating *Chaoborus* kairomone effects, 'G' indicating *Daphnia* Genotype effects and '*C* × G' indicating their interaction.

Table 1. Results from a competition among models that estimated host susceptibility (β) from susceptibility assays, sorted from best performer to worst. The various models incorporated different aspects of the experimental design. (1) The 'genotype' term involved estimating a separate, size-independent β for each genotype. (2) The 'length' column means that body length (mm)^4 was included. Any difference in body length between the treatments for a given genotype is driven by *Chaoborus* kairomones. (3) 'ACE' represents 'additional *Chaoborus* effects' that were estimated for both kairomone and nonkairomone treatments. This factor incorporated the potential for effects of *Chaoborus* on susceptibility not involving body length. For each model, standard information theoretic parameters are reported.* See Appendix S1 for more details on the models

H	Genotype	Length^4	ACE	NLL	*K*	AICc	Δ	*w*
1	Y	Y	N	128·1	9	276·1	0	0·88
2	Y	N	N	130·2	9	280·2	4·1	0·12
3	Y	Y	Y	125·5	18	294·8	18·7	8×10^{-5}
4	Y	N	Y	125·5	18	294·8	18·7	8×10^{-5}
5	N	Y	N	158·7	1	319·4	45·6	1×10^{-10}
6	N	Y	Y	158·6	2	321·3	47·6	4×10^{-11}
7	N	N	N	164·7	1	331·5	57·7	1×10^{-12}

*NLL, negative log-likelihood; *K*, number of parameters; AICc, small sample-corrected Akaike Information Criterion; Δ, AIC delta; *w*, AIC weights (i.e. likelihood of model, given the data).

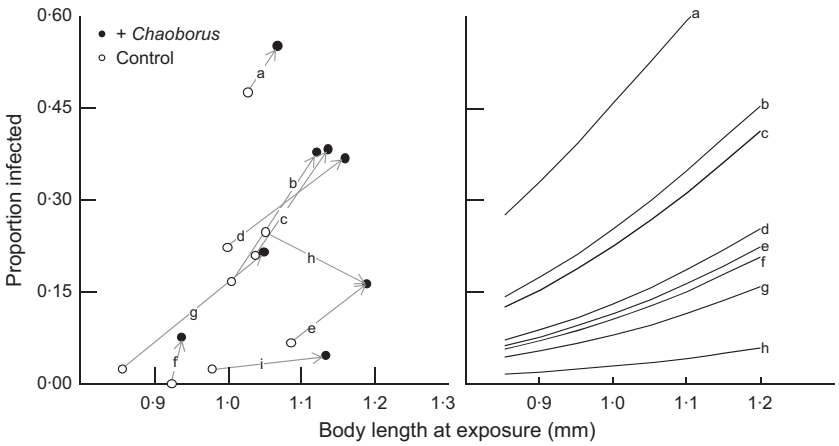

Fig. 3. Relationship between body length (head to tail) and host susceptibility in the two *Chaoborus* kairomone treatments. Left panel: Proportion infected data from the susceptibility assays. Arrows connect mean values of the control treatment (open symbols) to the + *Chaoborus* kairomone means (closed symbols) for nine genotypes. Right panel: Model predictions for changes in susceptibility (proportion infected) with increasing body size. The curves use size-specific susceptibility parameters estimated from each of the genotypes as fit by the winning model (Table 1). The letters identify clonal genotypes.

Infected hosts were not significantly larger at death when exposed to kairomones ($F_{1,\ 5\cdot87} = 4\cdot7$, $P = 0\cdot08$; Fig. 2c). However, spore yield was greater in the + *Chaoborus* treatment in some genotypes but not all, as indicated by a significant *Chaoborus* treatment × *D. dentifera* genotype interaction ($\chi^2 = 10\cdot5$, $P = 0\cdot001$, Fig 2d). Genotypes that were substantially larger at death in the + *Chaoborus* treatment (as compared to the control treatment) yielded more spores at death than in the controls ($r = 0\cdot81$, $P = 0\cdot01$; Fig. 4). Individuals in the *Chaoborus* treatment reproduced significantly earlier ($F_{1,\ 169} = 11\cdot7$, $P = 0\cdot0008$; Fig. 2e). However, they did not have higher overall reproduction ($F_{1,\ 225} = 0\cdot01$, $P = 0\cdot9$; Fig. 2f) than animals in the control treatment, despite being larger. Therefore, *Chaoborus* kairomones did not substantially alter the overall reproductive output of *D. dentifera*. Kairomones also did not alter fecundity rate (offspring per day; $F = 0\cdot01$, $P = 0\cdot93$) or instantaneous rate of increase, r ($t = 1\cdot4$, $P = 0\cdot194$; not shown).

Explanation using a dynamic energy budget model

In our third quantitative approach, we explored the epidemiological implications of the kairomone-induced size increases using a dynamic energy budget (DEB) model of parasitism. We have used variations on this model to explain how resource quantity, quality and genetic variation in feeding rates of hosts influence susceptibility, fecundity and spore yield – our focal epidemiological traits (Hall, Becker & Cáceres 2007a; Hall *et al.* 2009b, 2010a). This model (see Appendix S1 in the Supporting Information for details and parameter values) tracks flow of energy from ingestion and assimilation to storage in a reserve pool (Fig. 5; Kooijman 1993). That reserve energy is then used for growth, reproduction in adults and development in juveniles, and metabolic costs associated with maintenance of body structure, reproduction and growth. Allocation of reserves is governed by the kappa (κ) parameter. Parasites consume energy from the reserves before the host can use it and replicate within the host. The parasite eventually kills its host once parasite mass reaches a certain threshold, a proportion of structural mass of the host. Before killing it, the parasite inflicts energetic stress on its host by depleting its internal energy reserves. The consumption of reserves by parasites causes virulent reductions in fecundity and growth of the host.

To incorporate the trait-mediated indirect effect into this DEB model, we assumed that kairomones elevated the kappa parameter, thereby increasing energy flow to growth

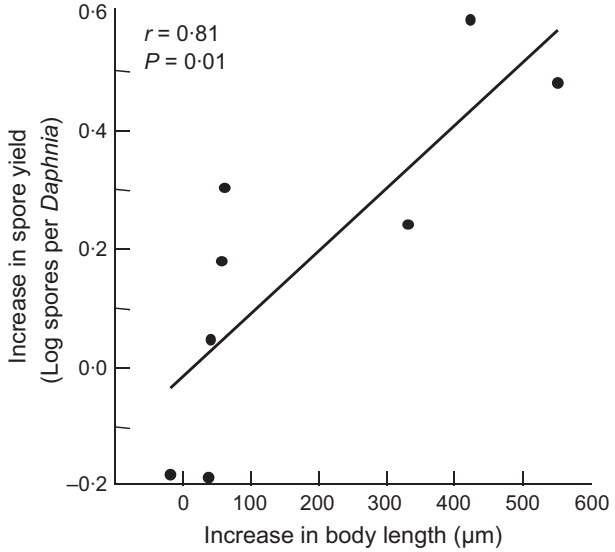

Fig. 4. Increase in body size vs. increase in spore yield in + *Chaoborus* kairomone treatments. The axes show the difference in body length (*x*-axis) and spore yield (*y*-axis) between the + *Chaoborus* kairomone treatment and the control treatment. Each point represents the mean of one of the *D. dentifera* genotypes. Pearson correlation statistics are provided.

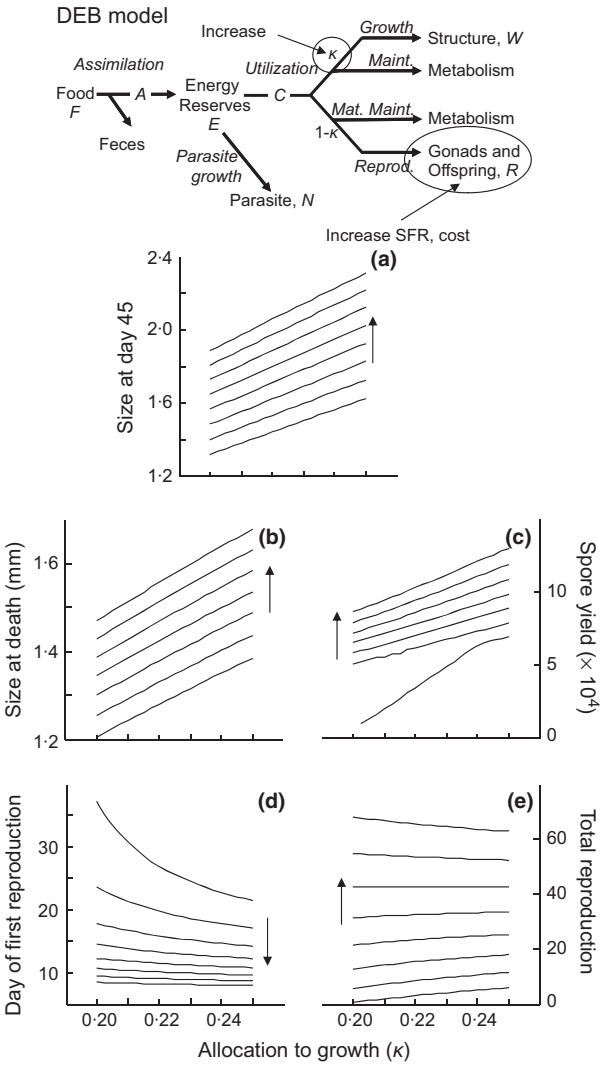

Fig. 5. Results from a dynamic energy budget (DEB) model of parasitism. Top: This model tracks energy flow through hosts and parasites replicating within hosts. *Chaoborus* kairomones increase (i) allocation of utilized energy towards growth (i.e. increase κ, the *x*-axis), (ii) size at first reproduction (SFR) and (iii) cost of creating offspring. The consequences of these assumptions produce changes in (a) size of uninfected hosts at day 45 (i.e. the length of the life table experiment; (b) size and (c) spore yield of infected at death; (d) age at first reproduction and (e) total reproductive output of uninfected hosts. Arrows point in the direction of increasing maximal size-specific feeding rate, from 7·0 to 11·0 at 0·5 increments (mg C L^{-1} mm^{-2} day^{-1} × 10^{-3}). This range is meant to mimic variation in feeding rate among clonal genotypes.

rather than reproduction (Fig. 5; Stibor & Lüning 1994; Rinke, Hulsmann & Mooij 2008). Additionally, we assumed that hosts exposed to kairomones first reproduced at a larger size (see Appendix S1 for details; Stibor & Lüning 1994). Finally, we built in an increased cost of offspring production under exposure to kairomones (again, see Appendix S1 for details; Rinke, Hulsmann & Mooij 2008). We simulated the model over the length of the life table experiment (45 days), assuming that hosts varied in maximal size-specific feeding rates. Given strong links between feeding rate and host susceptibility (Hall *et al.* 2007b, 2010a), this assumption mimics an array of genotypes that vary in susceptibility, with or without kairomones (i.e. as in our experiment).

The DEB model qualitatively captured the trait-mediated indirect effects seen in the experiment. Given higher allocation to growth (κ), at the end of 45 days, hosts were larger when exposed to kairomones (Fig. 5a). Indeed, kairomone-exposed hosts always had larger body size, at any day of the simulations (not shown). Once infected with the parasite, hosts exposed to kairomones (higher κ) reached a larger size at death (Fig. 5b) and yielded more spores (Fig. 5c), despite dying at an earlier age (Appendix S1). Although we assumed that they first reproduced at larger size, hosts with higher κ reproduced earlier because they grew faster (as seen in the data; Fig. 5d; see Appendix S1 for more discussion of this point). However, given the parameter values that we used, the kairomone-exposed, larger hosts produced roughly the same amount of offspring overall (Fig. 5e). This relatively flat fecundity response reflects the assumption of elevated cost of offspring production because of kairomone exposure. Otherwise, with no or lower costs, these larger, kairomone-exposed hosts would have produced more offspring. (Furthermore, if kairomone-induced costs were higher than illustrated, the DEB model indicated that fecundity of hosts exposed to kairomones would have been lower than that of unexposed hosts; results not shown.) Host genotypes with higher feeding rate (and therefore higher susceptibility; *x*-axis) also grew to larger size, first reproduced at an earlier age, yielded more offspring when uninfected and more spores when infected, and grew to larger size despite dying faster because of infection (Fig. 5a–e).

Discussion

A common predator, *Chaoborus*, induced trait-mediated indirect effects on two key epidemiological factors, susceptibility of *Daphnia* hosts and yield of parasite spores. Both effects could promote spread of disease. *Daphnia dentifera* that were exposed to *Chaoborus* kairomones grew significantly larger than control animals. To grow larger, these kairomone-exposed hosts likely allocated more internal energy resources towards growth rather than reproduction (based on Stibor & Lüning 1994; implemented in the dynamic energy budget [DEB] model; see also Rinke, Hulsmann & Mooij 2008). The growth response to kairomones enhanced susceptibility of clonal genotypes to infection by a virulent yeast parasite. This size-susceptibility link was anticipated because larger hosts contact more infectious spores while feeding (Hall *et al.* 2007b). All else being equal, any plastic response inducing changes in allocation to growth should change susceptibility by altering the rate at which hosts contact infectious spores. For example, we would predict that fish kairomones, which induce smaller body size (Stibor & Lüning 1994; Rinke, Hulsmann & Mooij 2008), should decrease susceptibility (all else equal). In addition, *D. dentifera* genotypes that yielded more spores in the + *Chaoborus* treatment were larger when they died from infection, a result predicted by the DEB model. Larger hosts consume more food resources per unit time that can then support higher rates of parasite replication within hosts. Additionally, larger hosts can physically house more spores (Hall, Becker & Cáceres 2007a; Hall *et al.* 2009b, 2010a).

A third component of disease spread did not respond to the kairomone treatment. In principle, *Chaoborus* kairomones could either increase or decrease host reproduction (Kooijman 1993; Tollrian & Dodson 1999). An increase in reproduction would increase the spread of disease (Anderson & May 1991), all else being equal. This increase in reproduction could be promoted by kairomones if increased body size elevated food intake and/or drove earlier age at reproduction. However, even though they reproduced at an earlier age, kairomone-exposed hosts produced essentially equivalent numbers of offspring as control hosts. The DEB model predicted similar results, assuming that kairomones boosted costs of reproduction (scaled per mass of offspring: this study) and/or size of individual offspring (Rinke, Hulsmann & Mooij 2008). If the cost of reproduction increased beyond that illustrated because of kairomone exposure, the host would produce fewer offspring. Regardless, our findings here suggest that at least under the experimental conditions used, *Chaoborus*-induced TMIE should influence epidemiology predominantly via effects on susceptibility and spore yield rather than reproduction.

If *Chaoborus* indirectly increase susceptibility and mortality of hosts from virulent parasites, why do *D. dentifera* still respond to *Chaoborus*? One possible answer involves the seasonal and episodic nature of parasitism in lakes. *D. dentifera* is abundant in lakes for months prior to the onset of *Metschnikowia* epidemics in autumn (Duffy & Hall 2008; Duffy *et al.* 2009; Hall *et al.* 2010b). In addition to being seasonal, *Metschnikowia* epidemics do not occur every year in each lake (Cáceres *et al.* 2006; Hall *et al.* 2010b). In contrast, *Chaoborus* are present in these lakes every year, almost year round (Garcia & Mittelbach 2008). Thus, *D. dentifera* populations experience months (or even years) of selection from *Chaoborus* predation but only episodic selection from epidemics of *Metschnikowia*.

The observed trait-mediated indirect effects could work simultaneously with other mechanisms to increase disease in lakes with more *Chaoborus* and lower vertebrate (fish) predation intensity. For instance, *Chaoborus* release spores from infected hosts while feeding (Cáceres, Knight & Hall 2009). As kairomones boost spore yield from infected hosts, both factors – direct spore release and indirect elevation of spore yield – promote disease spread, especially if spore availability limits the spread of epidemics. This combined spore yield–spore release mechanism would work best, from the parasite's perspective, if kairomone exposure did not boost host size too much. This caveat arises because the size effect of kairomones enhances susceptibility but could deter *Chaoborus* predation, and hence spore release, if hosts became too large. In our experiment, hosts remained well within the range of predation by *Chaoborus*. Still, the influence of kairomones on epidemics could depend on the net effect of these various factors (and would be best worked out with a dynamical model). However, because of their size selective behaviour (Swift & Fedorenko 1975; Pastorok 1981), *Chaoborus* can still increase epidemics by enhancing susceptibility because *Chaoborus* predation should select for larger body size of hosts (Spitze 1991). This factor alone could boost epidemics because larger hosts become more susceptible to infection (all else being equal). Regardless, the indirect effects of *Chaoborus* kairomones documented for this system almost certainly play an important role in spreading disease. Hence, they can help to explain why higher densities of *Chaoborus* correlate with larger epidemics (Cáceres, Knight & Hall 2009; Hall *et al.* 2010b).

In this study, we found that exposure to *Chaoborus* kairomones induced larger body size of *Daphnia* hosts. Similar effects of invertebrate predators often arise in other daphniid systems (e.g. Tollrian 1995b; Wolinska, Loffler & Spaak 2007; Coors & De Meester 2008). However, *Chaoborus* do not always induce larger overall body size in their daphniid prey (reviewed by Tollrian & Dodson 1999). In some cases, *Daphnia* defend themselves against *Chaoborus* predation by developing neck teeth or elongated tail spines (e.g. Krueger & Dodson 1981; Dodson 1989; Tollrian 1995a). If those changes did not increase overall body size, then kairomones would likely not affect susceptibility or spore yield via the size- and energy-allocation-based mechanisms hypothesized here. Of course, *Chaoborus* could still spread disease through the sloppy feeding mechanism (Cáceres, Knight & Hall 2009). Finally, in some cases, *Chaoborus* induce changes that may have conflicting effects on disease (e.g. producing fewer but larger individuals: Lüning 1992; Coors, Hammers-Wirtz & Ratte 2004). In these cases, dynamical models are required to

predict the net effects of *Chaoborus* on infection prevalence in *Daphnia*.

More generally, our study provides mechanistic links between trait-mediated indirect effects (TMIE) and epidemiology. Indirect effects of parasites on predation are well known for trophically transmitted epidemiology (Cezilly & Perrot-Minnot 2005; Hatcher, Dick & Dunn 2006). In those cases, the parasite manipulates host traits (such as behaviour) to increase predation on infected hosts, thereby boosting transfer of parasite propagules to the predator host (e.g. *Toxoplasma* increases predation rate of cats on infected rats: Vyas *et al*. 2007). However, as in our example, predators themselves can exert TMIEs on host–parasite systems. Other recent studies show similar phenomena. For instance, aboveground predators make beetles more susceptible to belowground pathogens (for unknown reasons: Ramirez & Snyder 2009). Additionally, predators made *Rana* tadpoles more susceptible to trematode infections, possibly because predators reduced activity levels of the host (allowing for easier infection: Thiemann & Wassersug 2000). Finally, in other *Daphnia* systems, fish predators induce trait-mediated indirect effects on disease. Fish predators can alter habitat use by hosts, thereby increasing their contact with a bacterial parasite (Decaestecker, De Meester & Ebert 2002). Induction of defences against fish predators can increase susceptibility to *Metschnikowia* (Yin *et al*. 2011). However, exposure to fish kairomones can decrease body size of hosts, thereby decreasing spore yield from infected individuals (Coors & De Meester 2011). In the latter two cases, causation might be inferred from a mechanistic approach such as ours, based on body size, feeding rates and energetics.

Trait-mediated indirect effects of predators on disease may be common. Thus, similar mechanisms may operate in a variety of other disease systems if predators cause hosts to change their traits or behaviours in manners that enhance contact with and/or production of parasites (Peckarsky *et al*. 2008; Raffel *et al*. 2010). As theory for community ecology of disease matures, the various indirect roles predators play in disease spread need to receive further mechanistic development (Hatcher, Dick & Dunn 2006; Raffel, Martin & Rohr 2008; Johnson *et al*. 2010).

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Supporting Information

Additional supporting information may be found in the online version of this article.

Appendix S1. Additional information on model selection, the DEB model, and supplemental data.

Figure S1. Time until death for infected animals, as seen in the life table experiment and the dynamic energy budget model.

Table S1. Parameter values and ranges of parameters used in simulations in the text.

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