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Density- and trait-mediated effects of a parasite and a predator in a tri-trophic food web

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Summary

1. Despite growing interest in ecological consequences of parasitism in food webs, relatively little is known about effects of parasites on long-term population dynamics of non-host species or about whether such effects are density or trait mediated.

2. We studied a tri-trophic food chain comprised of (i) a bacterial basal resource (*Serratia fonticola*), (ii) an intermediate consumer (*Paramecium caudatum*), (iii) a top predator (*Didinium nasutum*) and (iv) a parasite of the intermediate consumer (*Holospora undulata*). A fully factorial experimental manipulation of predator and parasite presence/absence was combined with analyses of population dynamics, modelling and analyses of host (*Paramecium*) morphology and behaviour.

3. Predation and parasitism each reduced the abundance of the intermediate consumer (*Paramecium*), and parasitism indirectly reduced the abundance of the basal resource (*Serratia*). However, in combination, predation and parasitism had non-additive effects on the abundance of the intermediate consumer, as well as on that of the basal resource. In both cases, the negative effect of parasitism seemed to be effaced by predation.

4. Infection of the intermediate consumer reduced predator abundance. Modelling and additional experimentation revealed that this was most likely due to parasite reduction of intermediate host abundance (a density-mediated effect), as opposed to changes in predator functional or numerical response.

5. Parasitism altered morphological and behavioural traits, by reducing host cell length and increasing the swimming speed of cells with moderate parasite loads. Additional tests showed no significant difference in *Didinium* feeding rate on infected and uninfected hosts, suggesting that the combination of these modifications does not affect host vulnerability to predation. However, estimated rates of encounter with *Serratia* based on these modifications were higher for infected *Paramecium* than for uninfected *Paramecium*.

6. A mixture of density-mediated and trait-mediated indirect effects of parasitism on non-host species creates rich and complex possibilities for effects of parasites in food webs that should be included in assessments of possible impacts of parasite eradication or introduction.

Key-words: density-mediated indirect interaction, *Didinium*, *Holospora*, *Paramecium*, trait-mediated indirect interaction

Introduction

Parasitism is ubiquitous. Parasites infect hosts across all trophic positions and can drastically alter host behaviour, morphology and life-history patterns (Hatcher, Dick & Dunn 2006; Lafferty *et al.* 2008; Sukhdeo 2010). In so doing, they affect food web properties such as stability, species interaction strengths and energy flow (Lafferty, Dobson & Kuris 2006; Lafferty & Kuris 2009; Hatcher & Dunn 2011). Some of these food web level effects of

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parasitism are likely caused by effects of parasites on non-host species. Such indirect effects of parasites can occur via density and/or trait mediation (Hudson, Dobson & Lafferty 2006; Hatcher, Dick & Dunn 2012, 2014). Density-mediated effects are likely pervasive and large, since parasitism commonly has negative effects at the population level that are at least as large as the effects of predation (Lefèvre *et al*. 2009; Watson 2013). Trait-mediated effects of parasitism can also be important. For example, parasitism can change the behaviour of hosts, affecting their likelihood of being consumed (Lagrue *et al*. 2007; Yanoviak *et al*. 2008), their feeding rates (Crompton 1984; Dick *et al*. 2010), and their activity as ecosystem engineers (Mouritsen & Poulin 2005). Understanding the roles of parasites in food webs therefore requires knowledge about the density- and trait-mediated indirect effects that parasite species can have via direct effects on host species. This knowledge would also aid assessments of possible impacts of parasite introductions or removals on non-host species (Torchin *et al*. 2002; Koop *et al*. 2011; Barry *et al*. 2014).

While trait-mediated effects of parasites can manifest quickly (i.e. faster than a generation), density-mediated effects (caused by reduced host reproductive rate, for example), will manifest over longer time-scales (i.e. time-scales equivalent to multiple host generations). Consequently, studying trait- and density-mediated effects empirically often entails long-term investigation to obtain long-term data. Such data are scarce for naturally occurring food webs (Williams 2009) and also present difficulty when assigning observed patterns to processes. One approach to circumventing these obstacles is to employ manipulative experiments with rapidly reproducing organisms such as protozoa and bacteria. In their review of microcosm studies, Jessup *et al*. (2004) highlight important contributions that such experiments have recently made to our understanding of ecological/evolutionary processes and note that microcosm studies have also played a historical role in the shaping of ecology.

To allow for diverse effects of parasitism on non-host species, our experimental community was a tri-trophic food web, with the host (*Paramecium caudatum* Ehrenberg) occupying the intermediate trophic level. Hence, the host can be both a consumer and a resource. The base of the food chain was the bacterium *Serratia fonticola*, and the top of the food chain was the predator *Didinium nasutum* Stein. The parasite was the *Paramecium*-specific *Holospora undulata* (Fig. 1). *Didinium nasutum* and *P. caudatum* predator–prey dynamics in the absence of parasites are well studied (Li *et al*. 2013) and have been simulated using recent mathematical models (Harrison 1995; Kozlova, Singh & Easton 2002). Both species are cosmopolitan freshwater ciliates that reproduce via binary fission. *Didinium* feeds exclusively on other ciliates and mainly on *Paramecium*, while *Paramecium* is primarily bacterivorous (Berger 1979).

Harrison (1995) and others obtained quantitative agreement between observed and predicted dynamics by assuming that *Didinium* exhibits a sigmoidal functional response and a delayed numerical response on *Paramecium* (Kozlova, Singh & Easton 2002). We found, however, that a more recent model developed by Li *et al*. (2013) was the most conducive for the purposes of the present study. Though less parsimonious than its predecessors, the Li *et al*. model is structured in a way that ensures that all parameter estimates are biologically realistic (i.e. within ranges that have been empirically derived). Moreover, the culture methods used in the experiment to which the model had originally been tailored were very similar to those employed in the present study. This model therefore provided an ideal baseline of comparison for quantifying the effects of parasitism of *Paramecium* by the bacterium *H. undulata*.

Holospora undulata is a sessile, single-host, bacterial parasite (Gromov & Ossipov 1981) with two developmental stages: a long (*c*. 20 μm), tilde-shaped infectious form and a smaller (5 μm), round reproductive form. While foraging, *Paramecium* ingests the infectious form, which – if it survives – ends up in the micronucleus and differentiates into the reproductive form. The reproductive form multiplies, fills the nucleus and begins to differentiate again. Reproductive forms are vertically transmitted to the daughter cells of *Paramecium* during mitosis. Infectious forms are released for horizontal transmission during mitosis or when *Paramecium* dies (Fokin 2004). *Holospora* has been observed in multiple locations throughout the range of its host, indicating that it, too, is cosmopolitan (Fujishima 2009).

Holospora affects *Paramecium* morphology, reproduction and behaviour – causing – for example, earlier onset of clonal decline, reduced division rates, shrunken buccal ('mouth') cavity and shortened cell length (Table S1, Supporting information). Finding that *Holospora* reduces *Paramecium's* dispersal capacity, Fellous *et al*. (2011) speculated that *Holospora* may also reduce *Paramecium's* per capita mobility. Via these direct effects on *Paramecium*, *Holospora* can theoretically have indirect effects on the long-term abundances of *Didinium* and *Serratia*. For example, by reducing *Paramecium* abundance, *Holospora* may indirectly cause *Didinium* to grow slower and consume fewer *Paramecium* cells. This, in turn, may allow both *Paramecium* and *Didinium* to persist, with *Didinium* failing to achieve sufficient abundance to eliminate all of its prey (Salt 1979). Since *Paramecium* consumes *Serratia*, *Holospora's* reduction of *Paramecium* abundance may also indirectly cause an increase in *Serratia* abundance. These would be density-mediated indirect effects (Abrams 2007). Trait-mediated indirect effects of *Holospora* could yield similar results.

The behaviour and morphology of *Paramecium* affect both its vulnerability to predators (Salt 1979; Hewett 1988) and its foraging ability (Hall *et al*. 1976; Fenchel 1980). *Paramecium* responds to certain physical and chemical predator cues with sudden changes in direction and bursts of speed (Knoll, Haacke-Bell & Plattner 1991; Hamel *et al*. 2011). *Paramecium* size determines its rate of

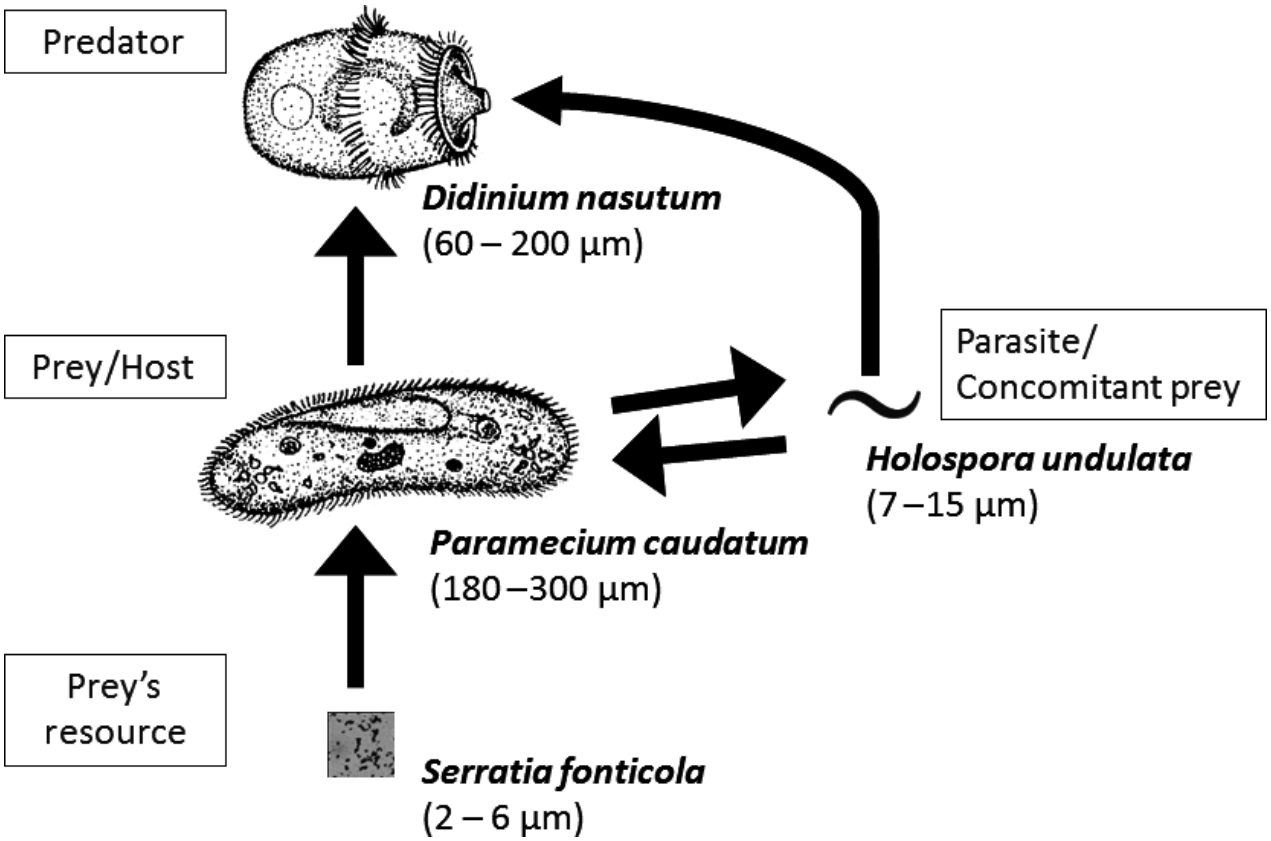

Fig. 1. Food web depiction of the study system, a four-species assemblage of freshwater protists and bacteria. Arrows denote trophic transfers of biomass/energy from one species to another.

capture and handling by *Didinium* (Hewett 1980) and very likely its encounter rate with bacterial prey (Fenchel 1980; Shimeta & Jumars 1991; Verity 1991). To summarize, *Paramecium* has a suite of traits that influence and are influenced by both parasitism and predation, and this creates the potential for its predator, parasite and prey to each have trait-mediated indirect effects on one another.

The objectives of the present study were to (i) quantify the effects of predation and parasitism with respect to the population dynamics of the intermediate consumer (*Paramecium*) and its resource (*Serratia*), (ii) determine whether effects of predation and parasitism function additively or synergistically, (iii) assess whether these effects are predominantly density or trait mediated and (iv) evaluate potential density- and trait-mediated indirect interactions between the predator (*Didinium*) and parasite (*Holospora*). Controlled predation experiments, mathematical modelling, and semi-automated image analysis of *Paramecium* behaviour and morphology were used to achieve these objectives.

Materials and methods

CULTURING OF MICROBIAL SPECIES

Species were cultured in growth medium consisting of 0·55 g Carolina Biological Supply protozoan pellets, 0·5 mL concentrated Chalkley's medium and 1 L reverse osmosis-purified water. Methyl cellulose (0·2041 g L^{-1}) was added to the medium to increase its viscosity to *c.* 5 cP at 20 °C, a technique shown to prolong coexistence between *Didinium* and *Paramecium* by reducing the swimming speed of both species equally without poisoning either species or providing refuge space for *Paramecium* (Luckinbill 1973; Veilleux 1979). Although studies have also specified the importance of using low-nutrient growth medium to prolong *Didinium-Paramecium* coexistence (Harrison 1995), nutrient concentrations below that which was used in the present study were not enough to sustain our infected *Paramecium* cultures (data not shown). Once autoclaved and allowed to cool to *c.* 20 °C, the medium was inoculated with *S. fonticola*.

Isogenic stock cultures were prepared from clonal lines of parasite-free and *Holospora*-infected *Paramecium* by adding one uninfected or one infected individual to the growth medium. We used a *Paramecium* strain originally collected near Venice, Italy and infected in the laboratory (see Duncan *et al.* 2010). *Didinium* was obtained from Sciento (strain P220), and an isogenic stock culture was prepared by rearing a *Didinium* cell and its subsequent offspring in petri dishes containing growth medium inoculated with *c.* 150 cells mL^{-1} of uninfected *Paramecium* (of a separate strain of uncertain origin).

EXPERIMENTAL MANIPULATION OF PARASITISM AND PREDATION

To quantify the separate and combined effects of predation (by *Didinium*) and parasitism (by *Holospora*) on *Paramecium* and *Ser-*

ratia abundance and the indirect effect of parasitism (of *Paramecium* by *Holospora*) on *Didinium* abundance, a two-way fully factorial design was used, with one factor being the presence/absence of *Didinium* and the other being the presence/absence of *Holospora*. As a control for changes in *Serratia* abundance independent of the effects of other species in the system, *Serratia* was also grown alone. For brevity, we use the following treatment codes: the letter S if *Serratia* was present (which was the case in all microcosms), P if *Paramecium* was present, D if *Didinium* was present, H if *Holospora* was present, and a hyphen (–) in place of D or H if either or both species were absent. The letter S by itself denotes the *Serratia*-only control.

The four treatment combinations (SP-, SPD-, SP-H, SPDH) and S were thrice replicated for a total of 15 experimental units. Each replicate was in a 50-mL Falcon tube (™Becton, Dickinson and Company – BD Biosciences © 2013, 1 Becton Dr., Franklin Lakes, NJ, USA) containing 30 mL of growth medium that had been inoculated with *Serratia* 2 days prior to Day 0 of the experiment. On Day 0 of the experiment, 1 mL was extracted from each replicate tube to estimate the starting abundances of *Serratia*. Manipulation of the presence/absence of *Holospora* was performed by replacing this 1 mL with either 1 mL from the uninfected *Paramecium* stock culture or 1 mL from the *Holospora*-infected *Paramecium* stock culture, as appropriate to the treatment. S replicates were inoculated with 1 mL from the same *Serratia*-containing growth medium that had been used to establish the *Paramecium* stock cultures, so that they remained the same volume and experienced the same handling conditions as the treatment replicates.

Manipulation of the presence/absence of *Didinium* was performed by adding 30 *Didinium* cells via micropipette to each replicate of the SPD- and SPDH treatments after *Paramecium* had exited its exponential growth phase (Day 11). Subsequently, 1 mL from each tube was sampled once a day, for 3 days a week (every second day, followed by a 2-day break), and replaced with fresh bacterized medium at the end of each week. Upon sampling and upon replacement, microcosms were mixed thoroughly. These procedures created semi-continuous culture conditions that permitted long-term observation of dynamics (McGrady-Steed, Harris & Morin 1997; Banerji & Morin 2009). All tubes were stored in the same compartment of the same incubator, which kept a constant temperature of 20 °C and a 12-h light/dark cycle.

The abundances of healthy and infected *Paramecium* and of *Didinium* were estimated via direct counts using a light microscope. If individuals of either species were not detected in the 1-mL sample of a replicate, a systematic search was performed of the entire volume of the replicate. *Serratia* abundance was estimated via serial dilution and plating. Once a week, 5–20 cells from the infected populations of *Paramecium* were isolated, stained with lacto-aceto-orcein (1%; Görtz & Dieckmann 1980) and examined under the microscope at 400× magnification to verify that these populations remained infected throughout the experiment.

Variation in species abundances was analysed using repeated-measures generalized linear mixed models, with time as a continuous linear covariate and replicate identity as a random factor. Note, however, that treating time as a factor gives the same ecological interpretation. We used a logistic regression approach, with population size of *Paramecium*, *Didinium* or *Serratia* as response variables, and an underlying Poisson error and log link function. We fitted fully factorial models, containing all possible interactions between experimental treatments and the day covariate. Analyses were restricted to the time interval between Day 13 (2 days after the introduction of *Didinium*) to Day 43 (last date before global *Didinium* extinction). Complementary analyses that allowed for auto-regressive error structure and therefore accounted for temporal autocorrelation in the data did not significantly improve model fits (not shown).

MODELLING OF PREDATOR–PREY DYNAMICS

To evaluate which of the processes underlying the predator–prey interaction of *Didinium* and *Paramecium* are less likely to be altered by the presence of *Holospora*, we fit to the data the deterministic model developed by Li *et al.* (2013). This approach also enabled us to explicitly account for observed variation in abundances of *Paramecium* at the time that *Didinium* was introduced to the *Didinium*-containing microcosms. The Li *et al.* model was specifically designed for interpreting the predator–prey dynamics of *Didinium* and *Paramecium* in growth medium thickened with methyl cellulose. We assumed that four parameters of the model could be affected by parasitism: the growth rate of *Paramecium*, the carrying capacity of *Paramecium*, the maximum attack rate of *Didinium* on *Paramecium* and the maximum birth rate of *Didinium* feeding on *Paramecium*. Details of the model, including the methods that were used to incorporate the potential effects of *Holospora,* are given in Appendix S1 (Supporting information). The model was fit to the population dynamics via maximum-likelihood estimation, fit was assessed using AIC, and estimates of effects of parasitism on parameter estimates were tested with *z*-tests.

EFFECTS OF PARASITISM ON PARAMECIUM BEHAVIOUR AND MORPHOLOGY

Movement pattern, swimming speed, cell shape and cell size were extracted from video recordings of *Paramecium* cells with differing levels of infection – 'overtly infected', 'covertly infected' or 'uninfected'. Cells categorized as overtly infected were drawn from the infected stock culture and were conspicuous due to the presence of massively inflated micronuclei (loaded with high numbers of infectious forms) that could already be identified as opaque spots in the cytoplasm at low magnification under the microscope. Covertly infected cells did not exhibit obvious outward symptoms of infection, and their less inflated micronuclei carried fewer, if any, infectious forms (Fig. S1, Supporting information; see also Kaltz & Koella 2003).

Recordings were acquired, analysed and processed in accordance with the workflow proposed by Pennekamp & Schtickzelle (2013), using a stereomicroscope (Leica M205 C; Leica Mikrosysteme Vertrieb GmbH, Ernst-Leitz-Strasse 17-37, 35578 Wetzlar, Germany) and mounted digital CMOS camera (Hamamatsu C11440) in combination with the software programs ImageJ (Abramoff *et al.* 2004) and R (R Development Core Team 2008). *Paramecium* cells were transferred via micropipette into 1 mL of growth medium spread across a glass Sedgewick–Rafter counting cell. Videos were recorded for 5 s with a 40-ms field delay and 10-ms exposure (giving 25 frames per second) at low (7·8×) magnification. To minimize blur and achieve highest optical resolution and contrast, the image was set to grey scale, and high-intensity external illumination was placed around the stage plate (Schott VisiLED MC 1500; SCHOTT AG, Hattenbergstrasse 10, 55122 Mainz, Germany). So that the software could visually sep-

arate *Paramecium* from artefacts, the video recordings were converted to 8-bit format, and a size threshold of 10–255 pixel lengths was specified for *Paramecium*. Videos in which *Paramecium* ceased swimming, swam vertically or exited the field of view were discarded. ImageJ's Particle Analyzer and Particle Tracker returned *x*, *y* coordinates (pixel locations within the field of view) for cells within each frame of each video – along with estimates of length and width in pixels, aspect ratio (dimensionless ratio of length to width), and cross-sectional area in square pixels.

Mobility was quantified in terms of the frequency of turns made by *Paramecium* and the average swimming speed of *Paramecium* in each video. Turns were defined as movements that caused *Paramecium* to deviate at least 45 degrees from its original trajectory. If, throughout the video, *Paramecium* deviated <45 degrees from its original trajectory, its movement pattern was defined as being linear (having no turns).

Swimming speed was defined as spatial displacement over time. This was calculated by taking the square root of the sum of the squared change in position along the *X*-axis and the squared change in position along the *Y*-axis (Pythagorean Theorem). The resulting measure was converted from pixels per frame to millimetres per second and then averaged for each *Paramecium* cell. To ensure that this measure of swimming speed was not confounded by how often *Paramecium* made turns and how much it slowed down or sped up at the beginning or end of a turn, only linear parts of trajectories were used to make the calculation. Also omitted were videos in which *Paramecium* was undetected or misidentified by ImageJ's Particle Tracker (e.g. due to quick turns and loss of frames resulting from deletion of background artefacts), leaving a total of 55 videos upon which to base the calculation. Estimates of cross-sectional length, width, area and aspect ratio were calculated for these same remaining videos by taking the mean of the output of ImageJ's Particle Analyzer across frames for each cell. Effects of infection level on swimming speed, aspect ratio, total per capita turns and cross-sectional area were assessed using one-way analysis of variance (ANOVA).

To assess whether differences in swimming speed and cell length among infected and uninfected *Paramecium* were enough to create differences in *Paramecium's* predicted rate of encounter of *Serratia*, we employed two separate methods of estimating encounter rates: one developed by Fenchel (described in Shimeta & Jumars 1991), the other by Verity (1991). Details regarding the terms used in these equations are given in Appendix S2 (Supporting information).

Results

PROTIST AND BACTERIAL ABUNDANCES

Paramecium–Didinium Dynamics

Despite the addition of methyl cellulose to the growth medium, the population dynamics of *Didinium* and *Paramecium* did not exhibit sustained oscillations in any of the replicates. In one replicate of the SPDH treatment, *Didinium* went extinct immediately after its introduction to the system. This replicate was therefore excluded from analyses.

After *c*. 1 week, *Paramecium* abundance reached carrying capacity. Abundances in the predator- and parasite-free control populations (- -) remained high for *c*. 4 weeks and then declined. Infection with *Holospora* (SP-H populations) reduced abundance by about one order of magnitude (Fig. 2). Statistical analysis of the temporal dynamics revealed a significant day*predator*parasite interaction ($F_{1,139} = 9{\cdot}71$, $P = 0{\cdot}0022$), indicating different trajectories of infected and uninfected populations after the introduction of *Didinium* on day 11. Initially, predation by *Didinium* led to similar rates of decline in *Paramecium* abundance in both infected and uninfected populations. After Day 22, the decline stabilized in two uninfected (SPD-) populations, while the third went extinct. In contrast, at the same time, abundance began to increase in the two infected (SPDH) populations to levels observed in corresponding uninfected populations with the predator, and even exceeding abundance levels of infected (SP-H) populations in the predator-free treatment (Fig. 2).

Didinium abundance increased initially, reaching peak levels 10–12 days after introduction. However, all populations went extinct over the following 20 days. *Didinium* generally reached higher abundances feeding on uninfected (SPD-) *Paramecium* populations than on infected (SPDH) *Paramecium* populations ($F_{1,3} = 28{\cdot}46$, $P = 0{\cdot}0129$). This difference was particularly pronounced during the initial period after introduction (Days 11–22).

Serratia Dynamics

The abundance of the bacterial resource *Serratia* was generally lower in the presence of the intermediate consumer *Paramecium* than in the *Serratia*-only (S) controls ($F_{1,13} = 792$, $P < 0{\cdot}0001$). This reduction was almost 100-fold, when *Paramecium* populations were at their peak density (Fig. 2). We further detected a significant day*predator*parasite interaction ($F_{1,139} = 8{\cdot}39$, $P = 0{\cdot}0044$): in the absence of *Didinium*, *Serratia* abundance was mostly lower when *Paramecium* was infected (SP-H) than when *Paramecium* was uninfected (SP-). However, this difference disappeared *c*. 10 days after the addition of the *Didinium* predator, such that equivalent *Serratia* abundances were observed with infected (SPDH) and uninfected (SPD-) *Paramecium* populations (Fig. 2).

In other words, the combined action of parasite infection (*Holospora*) and predation (*Didinium*) on the abundance of the intermediate consumer (*Paramecium*), as well as that of its resource (*Serratia*), was non-additive. Specifically, predation by *Didinium* reduced the negative effect of *Holospora* infection not only on *Paramecium* abundance, but also on *Serratia* abundance – such that the presence of the predator removed the population size advantage observed in uninfected *Paramecium* populations.

MODELLING

Maximum-likelihood estimation produced the parameter estimates listed in Table 1. The best fit of the model to the SPD replicates yielded a negative log likelihood of 104·434. Without *Holospora's* potential effects on *Didini-*

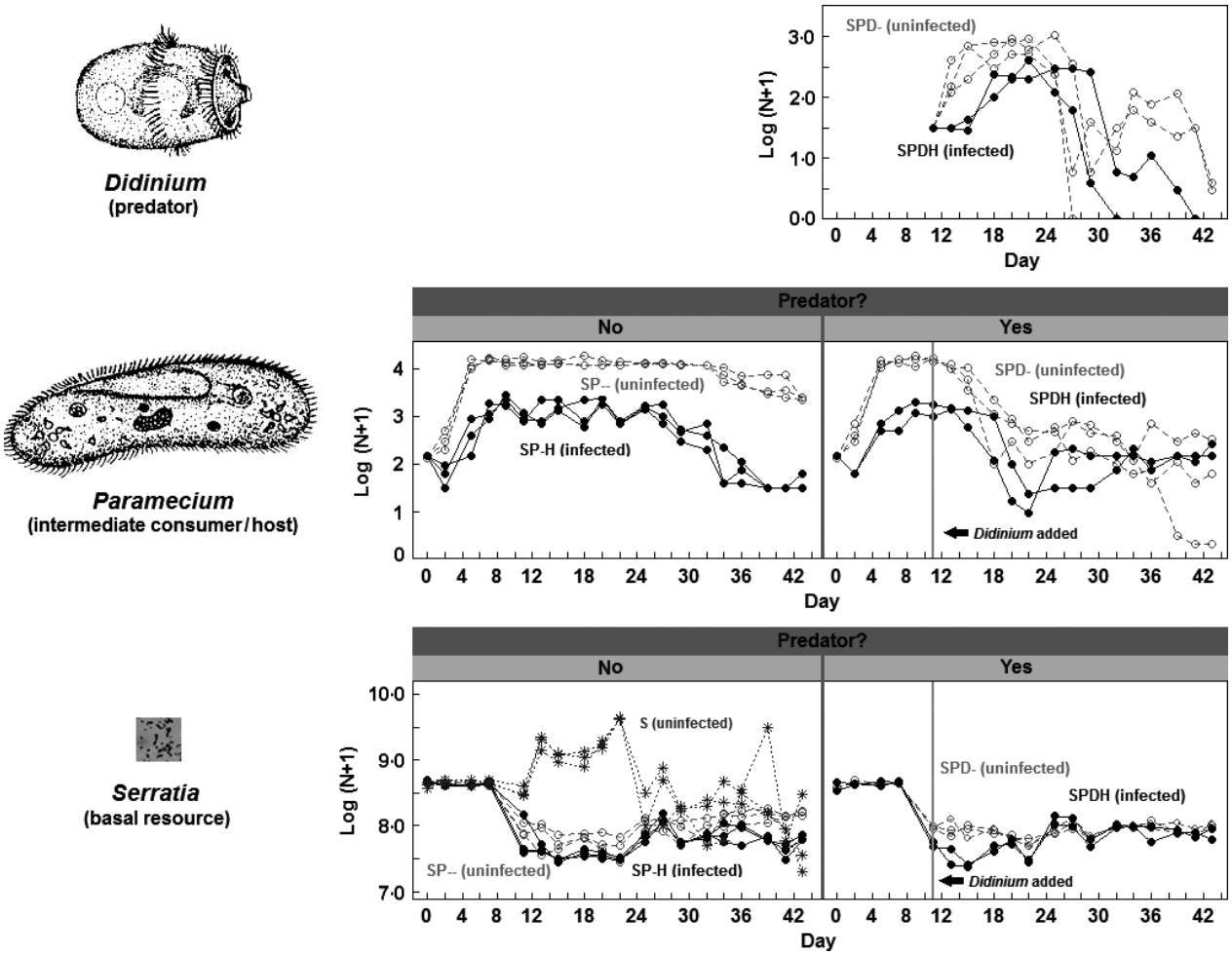

Fig. 2. Population growth curves showing abundances (N) of *Didinium*, *Paramecium* and *Serratia* in each microcosm. N was measured as no. cells per 30 mL in the case of the ciliates and as no. colony-forming units per plate volume in the case of *Serratia*. Treatment codes: 'SP-' = no antagonist of *Paramecium* present; 'SPD-' = *Didinium* present; 'SP-H' = *Holospora* present (infected *Paramecium*); 'SPDH' = *Didinium* and *Holospora* both present; 'S' = only *Serratia* present.

um's functional response, the best fit of the model to the SPDH replicates yielded a negative log likelihood of 73·196 (Fig. 3). Inclusion of effects of parasitism produced a best fit with a negative log likelihood of 71·599. Only the effects of parasitism on r and K were statistically distinguishable from 0 at the 0·05 level. Based on AIC, the model with effects on *Didinium's* functional and numerical responses was less parsimonious than the model with only density-related effects (dAICc = 4·9, d.f. = 5, weight = 0·078).

PARAMECIUM BEHAVIOUR AND MORPHOLOGY

Overtly infected, covertly infected and uninfected *Paramecium* cells did not significantly differ in terms of their total per capita turning frequency ($F_{2,77} = 2{\cdot}634$, $P = 0{\cdot}078$) or cross-sectional area ($F_{2,63} = 1{\cdot}091$, $P = 0{\cdot}342$). Swimming speed ($F_{2,52} = 4{\cdot}459$, $P = 0{\cdot}016$) and aspect ratio ($F_{2,63} = 95{\cdot}95$, $P < 0{\cdot}01$), however, did differ among the infectious groups. Swimming speed was higher in covertly infected *Paramecium* cells than overtly or uninfected cells. Aspect ratio was lowest in overtly infected *Paramecium*, moderate among covertly infected *Paramecium* and highest among uninfected *Paramecium* – meaning that – as is consistent with the literature (Fokin 1985), *Paramecium* cells became shorter and fatter with increasing parasite load (Fig. 4).

Given the differences in swimming speed and cell length among infected and uninfected *Paramecium*, both Fenchel's method and Verity's method of estimation predict higher encounter rates with *Serratia* for infected *Paramecium* than for uninfected *Paramecium* (Fig. S2, Supporting information).

Discussion

We investigated the mechanisms and consequences of parasite-mediated effects in experimental food webs, containing two protozoans (predator and prey), a bacterial parasite and a bacterial prey species. The observed population dynamics of the different protagonists revealed a mix of direct and indirect effects along the food web,

Table 1. Maximum-likelihood estimates of parameters and initial state variables used in the model

Parameter	Definition	Value	95% Confidence Interval [lower bound, upper bound]
r	*Paramecium* per capita growth rate (day^{-1})	2·079	[0·012, 3·623]
K	*Paramecium* carrying capacity (*Paramecium* * mL^{-1})	463·464	[173·259, 1239·755]
ω	Maximum per capita rate of consumption of *Paramecium* by *Didinium* (mL^{-1} * *Didinium*$^{-1}$ * day^{-1})	3·869	[2·171, 5·672]
β	Maximum half-saturation abundance of *Paramecium* for *Didinium* (*Paramecium* * mL^{-1})	24·864	[10·193, 60·888]
q	Dimensionless constant	−3·899	[3·333, 4·000]
λ	Dimensionless constant	−5·586	[−6·000, −4·999]
a	Maximum per capita birth rate of *Didinium* feeding on *Paramecium* (day^{-1})	1·110	[1·951, 3·078]
b	Dimensionless constant	60·001	[49·84, 71·76]
c	Dimensionless constant	−0·755	[−0·809, −0·591]
d	Dimensionless constant	41·5	[33·819, 47·542]
f	Dimensionless constant	−0·498	[−0·541, −0·346]
ε_r	*Holospora's* effect on r [ln(mL^{-1} * day^{-1})]	−0·946	[−6·163, 4·436]
ε_K	*Holospora's* effect on K (ln(*Paramecium* * mL^{-1}))	−2·277	[−3·730, −0·825]
ε_ω	*Holospora's* effect on ω (ln(mL^{-1} * *Didinium*$^{-1}$ * day^{-1}))	2·332e-7	[1·897e-8, 2·442e-7]
ε_a	*Holospora's* effect on a [ln(day^{-1})]	1·777e-7	[1·766e-7, 1·783e-7]
Initial state variables			
N (cells mL^{-1})	SPD- | Replicate 1: 346·667	SPDH | Replicate 2: 46·7	
	SPD- | Replicate 2: 340	SPDH | Replicate 3: 50	
	SPD- | Replicate 3: 426·667		
P (cells mL^{-1})	SPD- | Replicate 1: 4	SPDH | Replicate 2: 1	
	SPD- | Replicate 2: 13·333	SPDH | Replicate 3: 1	
	SPD- | Replicate 3: 5		

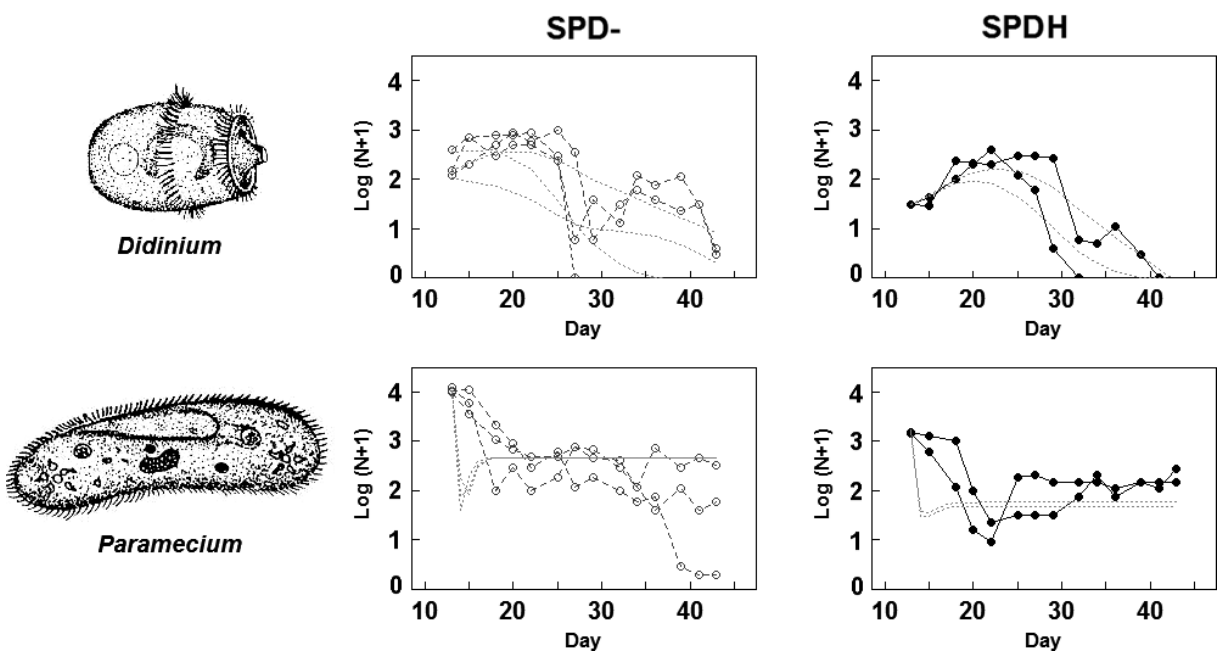

Fig. 3. Best fits of the model to the population dynamics observed in all microcosms containing *Didinium* (treatment codes and symbols same as in Fig. 2; see Table 1 for initial state variables and parameter estimates). Dotted lines denote model predictions.

including a modulation of parasitic effects in the presence of a predator. Below, we explore some potential explanations for and implications of the observed results, involving density-mediated and trait-mediated mechanisms.

paramecium–didinium DYNAMICS

As expected, both parasitic infection by *Holospora* and predation by *Didinium* had a strong negative impact on

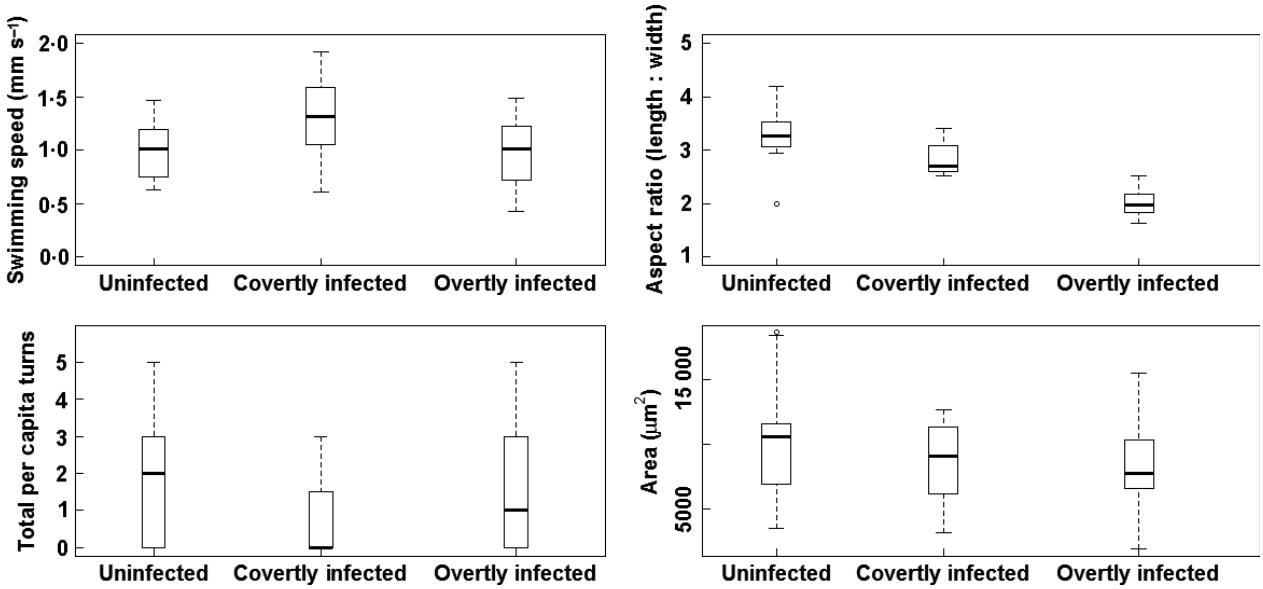

Fig. 4. Differences in behaviour and morphology among *Paramecium* with differing levels of *Holospora* infection.

Paramecium abundance. Initially, the introduction of the predator led to a massive decrease in abundance of both infected and uninfected *Paramecium*, and a concomitant increase in *Didinium*. *Didinium* grew to lower peak density and tended to die out earlier when preying on infected *Paramecium*. Since *Didinium* does not feed on *Holospora* independently of *Paramecium*, and *Holospora* cannot infect *Didinium*, one can infer that *Holospora's* effect on *Didinium* abundance was mediated via *Paramecium*. Most likely, this was due to the abundance of infected *Paramecium* being generally lower than that of uninfected *Paramecium*. However, the methods employed in our study do not entirely rule out the possibility of infection reducing the nutritional quality of *Paramecium*. Butzel & Bolten (1968) demonstrated that there is a link between prey nutritive status and *Didinium* population dynamics in a study that involved *D. nasutum* and a species of *Paramecium* in the *aurelia* complex. They found that *Didinium* exhibits decreased fission rates, abnormal cell formation and inability to encyst when fed *Paramecium aurelia* that have been progressively starved or malnourished. Future studies should explore the likelihood of *Holospora*-infected *Paramecium* being less nutritious to *Didinium* than uninfected *Paramecium* and of this being the basis of *Holospora's* effects on *Didinium* population dynamics.

There were signs of synergism between the effects of parasitism and predation during the decline phase of the *Didinium*. In particular, the infected *Paramecium* populations recovered and achieved the same if not higher abundance than their uninfected counterparts. This suggests some kind of predator-related buffering or overcompensation for parasitic effects on the part of *Paramecium*. A similar effect has been observed in another study involving *Paramecium* and *Holospora*, wherein a certain type of stochastic environmental fluctuation allowed infected populations to maintain the same density as uninfected populations (Duncan *et al.* 2013). This phenomenon may be generalizable and therefore warrants further investigation.

Image analysis revealed significant effects of infection on *Paramecium* aspect ratio and swimming speed. Cell length decreased with increasing parasite load, meaning that heavily infested *Paramecium* cells become smaller and fatter. Theoretically, this could increase vulnerability to predation by *Didinium*, given that *Didinium* feeds more readily on smaller cells (Hewett 1980). On the other hand, we found that infection also tended to increase swimming speed, at least in covertly infected individuals with more moderate parasite loads. This may counter the disadvantage of being smaller, as faster prey have better chances of escaping encounters with predators like *Didinium* (Knoll, Haacke-Bell & Plattner 1991; Hamel *et al.* 2011). This enhanced activity is intriguing in that it contrasts with previous findings of reduced short-distance dispersal of infected *Paramecium* (Fellous *et al.* 2011). Moreover, the modified aspect ratio it is associated with should make the energetic cost of locomotion higher for infected *Paramecium* (Roberts 1981), which (in combination with infected *Paramecium* having higher rates of encounter with *Serratia*) could explain *Paramecium's* enhanced negative effect on *Serratia* abundance in the presence of *Holospora* (discussed further below).

In a follow-up experiment, we evaluated the potential net effect of these parasite-induced modifications on predation risk. To this end, the feeding rate of individual *Didinium* cells facing 10 infected or uninfected *Paramecium* cells was measured over the course of several hours (further details provided in Fig. S3 caption, Supporting information). This experiment revealed no significant effects of infection status on *Didinium* feeding rate (Fig.

S3, Supporting information), suggesting that the above trait modifications either played no role in terms of predation risk or cancelled each other out. Future studies might re-evaluate this conclusion based on results of a rigorous functional response experiment that systematically varies the level of *Paramecium* abundance to which *Didinium* is exposed.

The modelling results were in line with the above observations. We found that *Holospora's* effects on *Didinium* abundance and on *Didinium* and *Paramecium's* predator–prey dynamics were more likely due to density-mediated indirect effects than to a combination of density- and trait-mediated indirect effects. Altogether, reducing *Paramecium's* growth rate and carrying capacity was sufficient to produce a close fit of the model to the dynamics of *Didinium* and *Holospora*-infected *Paramecium*, implying that, under these circumstances, *Holospora's* density-mediated indirect effects were the most important.

serratia DYNAMICS

While *Serratia* abundance was indeed highest in the complete absence of *Paramecium*, it was lower in the presence of infected *Paramecium* than in the presence of uninfected *Paramecium*. This suggests that infection with *Holospora* may increase *Paramecium's* per capita feeding rate, despite *Holospora's* generally negative effect on *Paramecium* fitness. If so, this may represent a compensatory response to *Holospora's* depletion of energy/nutrients, but may also reflect an adaptive parasite strategy. *Holospora* may, for example, actively induce feeding in *Paramecium* to obtain more resources for its own reproduction (Lefèvre *et al.* 2008).

Similar to what it did in the case of *Paramecium* abundance, the presence of the predator also reduced the negative effect of infection on *Serratia* abundance. Indeed, a week after introduction of the *Didinium*, *Serratia* abundances in infected *Paramecium* populations had caught up with those in uninfected populations, which was not the case in predator–free populations. The reduction in abundance due to *Didinium* may have had greater weight in the case of the infected *Paramecium* populations due to *Holospora's* enhancement of *Paramecium's* reduction of *Serratia*.

IMPLICATIONS FOR PARASITE DYNAMICS

One important finding regarding parasite (*Holospora*) dynamics is the buffering effect of predation. Predation by *Didinium* ultimately had a net beneficial effect on infected *Paramecium* populations, allowing them to recover from critically low abundances reached towards the end of the experiment. The present experiment did not address the consequences for parasite (horizontal) transmission and epidemic spread. However, it is clear that maintaining relatively higher population density may further increase the force of infection, as the number of infected hosts is directly related to the frequency of new infections. Moreover, a second follow-up experiment provided evidence for a direct impact of predation on *Holospora* transmission. Twenty-four hours after the introduction of 10 *Didinium* to high-density infected populations, we observed a significant increase in the concentration of free infectious forms of *Holospora* in the medium which remained in effect 48 h later ($F = 10{\cdot}32$, $P > 0{\cdot}01$; Fig. S4, Supporting information; further details provided in Fig. S4 caption, Supporting information). A possible explanation is that these parasite transmission stages are released while *Didinium* devours and digests infected *Paramecium*. This may directly enhance the chance of transmission in a population under predator attack. Similar results have been reported in systems comprising the predator *Chaoborus* and its prey *Daphnia* (Cáceres, Knight & Hall 2009; Duffy *et al.* 2011).

PERSPECTIVES

Parasite effects in multitrophic communities are poorly understood and sometimes greatly underappreciated (Morand & Gonzalez 1997; Lafferty *et al.* 2008; Poulin 2010). Our study illustrates how the addition of a single additional antagonist (here *Didinium*) can have complex, and partly unexpected, demographic feedbacks on host–parasite interactions and *vice versa*. We also found that such effects can be mirrored at lower levels of a food chain. The broader significance of these results is that they point to possible complications for pest management in agriculture and conservation, when additional players in the natural community are certain to come into play. For example, the use of certain parasites as biological control agents may be inadvisable, if another species (here *Didinium*) interacting with the target obstructs the expected effects due to the target's behavioural and physiological responses.

Here, we described the impact of a single episode of proliferation and prey reduction by a predator which subsequently went extinct. Building on this simple framework, future work may address more complex scenarios, such as the spread and maintenance of an epidemic or the occurrence of co-evolution, when all interacting species are maintained over longer time-scales.

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Data accessibility

Data available from the Dryad Digital Repository: http://dx.doi.org/10.5061/dryad.3kb72 (Banerji *et al.* 2014).

References

Abramoff, M.D., Magalhaes, P.J. & Ram, S.J. (2004) Image processing with ImageJ. *Biophotonics International*, **11**, 36–42.

Abrams, P.A. (2007) Defining and measuring the impact of dynamic traits on interspecific interactions. *Ecology*, **88**, 2555–2562.

Banerji, A. & Morin, P.J. (2009) Phenotypic plasticity, intraguild predation and anti-cannibal defences in an enigmatic polymorphic ciliate. *Functional Ecology*, **23**, 427–434.

Banerji, A., Duncan, A.B., Griffin, J.S., Humphries, S., Petchey, O.L. & Kaltz, O. (2014) Data from: Density- and trait-mediated effects of a parasite and a 1 predator in a tri2 trophic food web. *Dryad Digital Repository*, http://dx.doi.org/10.5061/dryad.3kb72.

Barry, J., McLeish, J., Dodd, J.A., Turnbull, J.F., Boylan, P. & Adams, C.E. (2014) Introduced parasite *Anguillicola crassus* infection significantly impedes swim bladder function in the European eel *Anguilla anguilla* (L.). *Journal of Fish Diseases*, **37**, 921–924.

Berger, J. (1979) The feeding behavior of *Didinium nasutum* on an atypical prey ciliate (*Colpidium campylum*). *Transactions of the American Microscopical Society*, **98**, 487–494.

Butzel, H.M. Jr & Bolten, A.B. (1968) The relationship of the nutritive state of the prey organism *Paramecium aurelia* to the growth and encystment of *Didinium nasutum*. *The Journal of Protozoology*, **15**, 256–258.

Cáceres, C.E., Knight, C.J. & Hall, S.R. (2009) Predator–spreaders: predation can enhance parasite success in a planktonic host–parasite system. *Ecology*, **90**, 2850–2858.

Crompton, D.W. (1984) Influence of parasitic infection on food intake. *Federation Proceedings*, **43**, 239–245.

Dick, J.T.A., Armstrong, M., Clarke, H.C., Farnsworth, K.D., Hatcher, M.J., Ennis, M. *et al.* (2010) Parasitism may enhance rather than reduce the predatory impact of an invader. *Biology Letters*, **6**, 636–638.

Duffy, M.A., Housley, J.M., Penczykowski, R.M. & Cáceres, C.E. (2011) Unhealthy herds: indirect effects of predators enhance two drivers of disease spread. *Functional Ecology*, **25**, 945–953.

Duncan, A.B., Fellous, S., Accot, R., Alart, M., Sobandi, K.C., Cosiaux, A. *et al.* (2010) Parasite-mediated protection against osmotic stress for *Paramecium caudatum* infected by *Holospora undulata* is host genotype specific. *FEMS Microbiology Ecology*, **74**, 353–360.

Duncan, A.B., Gonzalez, A. & Kaltz, O. (2013) Stochastic environmental fluctuations drive epidemiology in experimental host-parasite metapopulations. *Proceedings of the Royal Society B*, **280**, 20131747.

Fellous, S., Quillery, E., Duncan, A.B. & Kaltz, O. (2011) Parasitic infection reduces dispersal of ciliate host. *Biology Letters*, **7**, 327–329.

Fenchel, T. (1980) Suspension feeding in ciliated protozoa: functional response and particle size selection. *Microbial Ecology*, **6**, 1–11.

Fokin, S.I. (1985) Vegetative functions of the micronucleus and cell morphogenesis of *Paramecium caudatum*. I. Changes induced by nucleolar symbionts. *Tsitologiya*, **27**, 322–328.

Fokin, S.I. (2004) Bacterial endocytobionts of ciliophora and their interactions with the host cell. *International Review of Cytology*, **236**, 181–249.

Fujishima, M. (2009) *Endosymbionts in Paramecium*. Springer, Tokyo, Japan.

Görtz, H.-D. & Dieckmann, J. (1980) Life cycle and infectivity of *Holospora elegans* Haffkine, a micronucleus-specific symbiont of *Paramecium caudatum* (Ehrenberg). *Protistologica*, **16**, 591–603.

Gromov, B.V. & Ossipov, D.V. (1981) *Holospora* (*ex* Hafkine 1890) nom. rev., a genus of bacteria inhabiting the nuclei of paramecia. *International Journal of Systematic and Evolutionary Microbiology*, **31**, 348–352.

Hall, D.J., Threlkeld, S.T., Burns, C.W. & Crowley, P.H. (1976) The size-efficiency hypothesis and the size structure of zooplankton communities. *Annual Review of Ecology and Systematics*, **7**, 177–208.

Hamel, A., Fisch, C., Combettes, L., Dupuis-Williams, P. & Baroud, C.N. (2011) Transitions between three swimming gaits in *Paramecium* escape. *Proceedings of the National Academy of Sciences of the United States of America*, **108**, 7290–7295.

Harrison, G.W. (1995) Comparing predator-prey models to Luckinbill's experiment with *Didinium* and *Paramecium*. *Ecology*, **76**, 357–374.

Hatcher, M.J., Dick, J.T.A. & Dunn, A.M. (2006) How parasites affect interactions between competitors and predators. *Ecology Letters*, **9**, 1253–1271.

Hatcher, M.J., Dick, J.T.A. & Dunn, A.M. (2012) Diverse effects of parasites in ecosystems: linking interdependent processes. *Frontiers in Ecology and the Environment*, **10**, 186–194.

Hatcher, M.J., Dick, J.T.A. & Dunn, A.M. (2014) Parasites that change predator or prey behaviour can have keystone effects on community composition. *Biology Letters*, **10**, 20130879.

Hatcher, M.J. & Dunn, A.M. (2011) *Parasites in Ecological Communities: From Interactions to Ecosystems*. Cambridge University Press, New York, NY, USA.

Hewett, S.W. (1980) The effect of prey size on the functional and numerical responses of a protozoan predator to its prey. *Ecology*, **61**, 1075–1081.

Hewett, S.W. (1988) Predation by *Didinium nasutum*: effects of predator and prey size. *Ecology*, **69**, 135–145.

Hudson, P.J., Dobson, A.P. & Lafferty, K.D. (2006) Is a healthy ecosystem one that is rich in parasites? *Trends in Ecology & Evolution*, **21**, 381–385.

Jessup, C.M., Kassen, R., Forde, S.E., Kerr, B., Buckling, A., Rainey, P.B. *et al.* (2004) Big questions, small worlds: microbial model systems in ecology. *Trends in Ecology & Evolution*, **19**, 189–197.

Kaltz, O. & Koella, J.C. (2003) Host growth conditions regulate the plasticity of horizontal and vertical transmission in *Holospora undulata*, a bacterial parasite of the protozoan *Paramecium caudatum*. *Evolution*, **57**, 1535–1542.

Knoll, G., Haacke-Bell, B. & Plattner, H. (1991) Local trichocyst exocytosis provides an efficient escape mechanism for *Paramecium* cells. *European Journal of Protistology*, **27**, 381–385.

Koop, J.A.H., Huber, S.K., Laverty, S.M. & Clayton, D.H. (2011) Experimental demonstration of the fitness consequences of an introduced parasite of Darwin's Finches. *PLoS ONE*, **6**, e19706. DOI: 10.1371/journal.pone.0019706.

Kozlova, I., Singh, M. & Easton, A. (2002) Predator-prey models with diffusion based on Luckinbill's experiment with *Didinium* and *Paramecium*. *Mathematical and Computer Modelling*, **36**, 83–102.

Lafferty, K.D., Dobson, A.P. & Kuris, A.M. (2006) Parasites dominate food web links. *Proceedings of the National Academy of Sciences of the United States of America*, **103**, 11211–11216.

Lafferty, K.D. & Kuris, A.M. (2009) Parasites reduce food web robustness because they are sensitive to secondary extinction as illustrated by an invasive estuarine snail. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, **364**, 1659–1663.

Lafferty, K.D., Allesina, S., Arim, M., Briggs, C.J., De Leo, G., Dobson, A.P. *et al.* (2008) Parasites in food webs: the ultimate missing links. *Ecology Letters*, **11**, 533–546.

Lagrue, C., Kaldonski, N., Perrot-Minnot, M.J., Motreuil, S. & Bollache, L. (2007) Modification of hosts' behavior by a parasite: field evidence for adaptive manipulation. *Ecology*, **88**, 2839–2847.

Lefèvre, T., Roche, B., Poulin, R., Hurd, H., Renaud, F. & Thomas, F. (2008) Exploiting host compensatory responses: the 'must' of manipulation? *Trends in Parasitology*, **24**, 435–439.

Lefèvre, T., Lebarbenchon, C., Gauthier-Clerc, M., Missé, D., Poulin, R. & Thomas, F. (2009) The ecological significance of manipulative parasites. *Trends in Ecology and Evolution*, **24**, 41–48.

Li, J., Fenton, A., Kettley, L., Roberts, P. & Montagnes, D.J. (2013) Reconsidering the importance of the past in predator-prey models: both numerical and functional responses depend on delayed prey densities. *Proceedings of the Royal Society B*, **280**, 20131389.

Luckinbill, L.S. (1973) Coexistence in laboratory populations of *Paramecium aurelia* and its predator *Didinium nasutum*. *Ecology*, **54**, 1320–1327.

McGrady-Steed, J., Harris, P.M. & Morin, P.J. (1997) Biodiversity regulates ecosystem predictability. *Nature*, **390**, 162–165.

Morand, S. & Gonzalez, E.A. (1997) Is parasitism a missing ingredient in model ecosystems? *Ecological Modelling*, **95**, 61–74.

Mouritsen, K.N. & Poulin, R. (2005) Parasites boost biodiversity and change animal community structure by trait-mediated indirect effects. *Oikos*, **108**, 344–350.

Pennekamp, F. & Schtickzelle, N. (2013) Implementing image analysis in laboratory-based experimental systems for ecology and evolution: a hands-on guide. *Methods in Ecology and Evolution*, **4**, 483–492.

Poulin, R. (2010) Network analysis shining light on parasite ecology and diversity. *Trends in Parasitology*, **26**, 492–498.

R Development Core Team (2008) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org.

Roberts, A.M. (1981) Hydrodynamics of protozoan swimming. *Biochemistry and Physiology of Protozoa*, Vol. 4, 2nd edn (eds M. Levandowsky & S.H. Hutner), pp. 6–66. Academic Press, New York, NY, USA.

Salt, G.W. (1979) Density, starvation, and swimming rate in *Didinium* populations. *The American Naturalist*, **113**, 135–143.

Shimeta, J. & Jumars, P.A. (1991) Physical mechanisms and rates of particle capture by suspension-feeders. *Oceanography and Marine Biology: An Annual Review*, **29**, 191–257.

Soetaert, K., Petzoldt, T. & Setzer, R.W. (2010) Solving differential equations in R: package deSolve. *Journal of Statistical Software*, **33**, 1–25.

Sukhdeo, M.V.K. (2010) Food webs for parasitologists: a review. *Journal of Parasitology*, **96**, 273–284.
Torchin, M.E., Lafferty, K.D., Dobson, A.P., McKenzie, V.J. & Kuris, A.M. (2002) Introduced species and their missing parasites. *Nature*, **421**, 628–630.
Veilleux, B.G. (1979) An analysis of the predatory interaction between *Paramecium* and *Didinium*. *Journal of Animal Ecology*, **48**, 787–803.
Verity, P.G. (1991) Measurement and simulation of prey uptake by marine planktonic ciliates fed plastidic and aplastidic nanoplankton. *Limnology and Oceanography*, **36**, 729–750.
Watson, M.J. (2013) What drives population-level effects of parasites? Meta-analysis meets life-history. *International Journal for Parasitology: Parasites and Wildlife*, **2**, 190–196.
Williams, N. (2009) Tangled banks. *Current Biology*, **19**, R390–R392.
Yanoviak, S.P., Kaspari, M., Dudley, R. & Poinar, G. Jr (2008) Parasite-induced fruit mimicry in a tropical canopy ant. *The American Naturalist*, **171**, 536–544.

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Supporting Information

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

Appendix S1. Population dynamic modelling.

Appendix S2. Encounter rate estimation.

Fig. S1. Photographs of *Paramecium* taken at 400× magnification.

Fig. S2. Estimated rates of encounter between *Paramecium* and *Serratia*.

Fig. S3. *Paramecium* abundance over time with and without *Didinium* in the first follow-up predation experiment.

Fig. S4. *Holospora* infectious form density with and without *Didinium* in the second follow-up predation experiment.

Table S1. Summary of reported effects of *Holospora undulata* on *Paramecium caudatum*.

