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► To cite this version:

Andrea Bertolo, Marco A. Rodriguez, Gérard Lacroix. Control mechanisms of photosynthetic epibionts on zooplankton: an experimental approach. *Ecosphere*, 2015, 6 (11), 10.1890/ES14-00451.1. hal-02634868

HAL Id: hal-02634868

<https://hal.inrae.fr/hal-02634868>

Submitted on 27 May 2020

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Control mechanisms of photosynthetic epibionts on zooplankton: an experimental approach

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Citation: Bertolo, A., M. A. Rodríguez, and G. Lacroix. 2015. Control mechanisms of photosynthetic epibionts on zooplankton: an experimental approach. *Ecosphere* 6(11):219. <http://dx.doi.org/10.1890/ES14-00451.1>

Abstract. Several top-down and bottom-up forces have been put forward to explain variable infestation rates of zooplankton by epibionts. Among top-down forces, fish predation affects epibiont prevalence on zooplanktonic organisms, either by eliminating more conspicuous, heavily burdened individuals, or by reducing population size of zooplankton hosts, with consequences for substrate availability for epibionts. However, detailed experimental-based information on the effects of top-down forces is still lacking. Among bottom-up forces, light can potentially control populations of photosynthetic epibionts. Therefore, both changes in light penetration in the water column and the vertical position of hosts in the water column could affect the photic conditions in which epibionts live and could thus control their population growth. We tested experimentally the hypothesis that both light limitation and fish predation affect epibiont burden on zooplankton. Moreover, we also tested the hypothesis that zooplanktivorous fish affect the prevalence and burden of the epibiotic alga *Colacium* sp. (Euglenida) on zooplankton not only by direct predation, but also by affecting the vertical distribution of zooplankton. We analyzed *Colacium* burden on two zooplankton genera that responded differently to the presence of zooplanktivorous fish by altering their daytime vertical distributions, thus exposing photosynthetic epibionts to different light conditions. *Colacium* burden on the two zooplankton genera was also compared between enclosures with different degrees of light limitation. Our results suggest that (1) ambient light limitation has the potential to reduce the burden of photosynthetic epibionts on zooplankton in natural conditions, and (2) zooplankton behavior (e.g., daytime refuge use to escape fish predation) can reduce the burden by exposing photosynthetic epibionts to suboptimal light conditions.

Key words: Bayesian framework; *Ceriodaphnia*; *Colacium*; *Diaphanosoma*; epibiosis; fish predation; light limitation; non-consumptive effects; shared doom hypothesis; zooplankton vertical distribution.

Received 19 November 2014; revised 4 March 2015; accepted 18 March 2015; **published** 12 November 2015. Corresponding Editor: E. García-Berthou.

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INTRODUCTION

Epibiosis is a form of inter-specific interaction that is commonly found in aquatic ecosystems (e.g., Cattaneo and Kalff 1980, Bickel et al. 2012).

It could result in parasitism, mutualism or commensalism (e.g., Decaestecker et al. 2005) and could have important direct community-wide effects, such as the collapse of host populations due to shading (Sand-Jensen 1977).

Indirect population and community-wide effects are also possible because epibionts have the potential to alter the interactions between the host and its environment (Wahl 2008a, b). Epibionts can, for example, alter host vulnerability to predators which can, in turn, have an impact on food-web dynamics (Wahl et al. 1997, Wahl 2008a). Despite these potential effects, epibiosis has attracted relatively little interest in ecology and much of our knowledge on this form of parasitism still stems from case studies (e.g., Hanamura 2000, Regali-Selegnim and Godinho 2004). Moreover, although our knowledge of the effects of epibionts on hosts is expanding (e.g., Stirnadel and Ebert 1997, Barea-Arco et al. 2001), very little is known about the effects of hosts (e.g., in terms of abundance or behavior) on epibiont populations.

Zooplanktonic organisms are known to be used as substrates by an array of different epibiont taxa such as bacteria (Grossart et al. 2010, Tang et al. 2010), ciliates (Bickel et al. 2012) and euglenoids (Al-Dharheri and Willey 1996). Epibionts attached to zooplanktonic organisms have been shown to have great potential to affect the functioning of aquatic systems, for example, by controlling whole-lake bacterial respiration (Tang et al. 2010) or remineralization (Bickel et al. 2012).

Hosts are not inert substrate for epibionts. For example, molting in crustacean zooplankton drives a constant renewal of substrate that has both positive and negative effects for the epibionts. Although molting reduces competition for the substrate, during inter-molt phases the epibionts must become mobile and be therefore exposed to zooplankton grazing (Barea-Arco et al. 2001). Factors usually postulated to potentially benefit zooplankton epibionts include the use of zooplankton excretion products and increased dispersal due to hitchhiking on mobile zooplankton (Grossart et al. 2010). Whereas some positive effects of epibionts on host have been observed (Barea-Arco et al. 2001), most studies have focused on the costs of epibiont burden on zooplankton hosts (e.g., increased drag and reduction of fecundity) (Threlkeld and Willey 1993, Gilbert and Schroder 2003). Increased exposure to fish predation because of greater visibility is a potential cost for both host and epibionts (Willey et al. 1993). This latter phe-

nomenon (a “shared doom” effect, sensu Wahl 1997, 2008a), demonstrated to date only on epibiont prevalence (Willey et al. 1993), might be essentially an indirect effect of fish size-selectivity. Fish might in fact reduce epibiont prevalence simply by eliminating larger zooplankton, which are more likely to carry epibionts (Threlkeld and Willey 1993). It is therefore necessary to control for host body size to confirm that a shared doom effect exists in this kind of epibiosis. Non-consumptive effects of fish on host behavior, such as diel vertical migration, (Lampert 1989) could also expose epibiont organisms to sub-optimal conditions (Threlkeld et al. 1993). It is therefore necessary to take into account both consumptive and non-consumptive effects of predators to understand their overall impact on epibiont populations.

In this paper, we analyzed the interactive effects of predation and light limitation in determining the burden of *Colacium* sp. (Euglenophyceae), a photosynthetic epibiont, on *Ceriodaphnia* and *Diaphanosoma*, two cladoceran genera that show different responses to fish predation, both in terms of consumptive and non-consumptive effects (Bertolo et al. 1999b). Our aim is to analyze epibiosis in a trophic context and demonstrate experimentally that: (1) there is a shared doom effect in the presence of fish, i.e., that the negative effects of fish on epibiont burden is not only a by-product of fish size selectivity; (2) light limitation has the potential to reduce photosynthetic epibiont burden; and (3) non-consumptive effects on host behaviour have the potential to modulate light limitation for epibionts.

To reach these objectives, we used zooplankton samples from an in situ mesocosm experiment in which we already demonstrated that predation and light limitation have clear additive effects on lower trophic levels (Bertolo et al. 1999a). Moreover, we also showed that both consumptive and non-consumptive effects of fish differ between the two selected cladocerans (Bertolo et al. 1999a). More specifically, *Ceriodaphnia* responds more heavily to predation than *Diaphanosoma* in terms of reduction of individual size and total population biomass, and also in terms of daytime vertical distribution. Bertolo et al. (1999b) showed that only *Ceriodaphnia* shifted from a relatively homogeneous distribution in

the water column (either with or without fish), to a relatively huge concentration in the bottom layers if the deeper part of the water column was protected from the fish predators presents. As this system has been shown to be light-limited (Bertolo et al. 1999a), we expect to find lower epibiont burdens for a given host size when the hosts use deepwater (i.e., dark) refuges. This provides an ideal setup to analyze the non-consumptive effects of fish on epibiont burden.

The three main hypotheses were tested as follows: (1) the shared doom effect hypothesis was tested by comparing the *Colacium* burden (corrected for host body size) on the two host genera under different predation pressures; (2) the light limitation hypothesis was tested by comparing *Colacium* burden on host collected from enclosures of different depth, that differed in their level of light limitation; and (3) the non-consumptive effects hypothesis was tested by comparing *Colacium* burden in host that differ in their use of light-limited strata when exposed to fish predation. Here, controlling for the consumptive effects of fish was key to properly analyze their non-consumptive effects on epibiont burden, together with the effects of light limitation.

METHODS

Experimental design

The experiment was conducted in Lake Créteil (48°46'37" N, 2°26'47" E), a small (42 ha), shallow (mean depth: 4 m, maximum depth: 6 m) sand-pit lake situated 15 km SE of Paris, France. Fifteen experimental enclosures made of translucent polyethylene (300 μ m thickness) were reached 25 cm above the lake surface, inside a rectangular pontoon. Each enclosure was open to the atmosphere and completely sealed at the bottom. Enclosures of two different sizes were used, with a constant wall area/volume ratio: deep enclosures (1.5 $\times$ 1.5, 4.5 m depth; volume 9.5 m³) and shallow ones (1.5 $\times$ 1.5, 2.5 m depth; volume 5 m³). We explicitly chose the shape of the enclosures to reduce differences in optical condition related to wall reflectance. It has been demonstrated that using enclosures with a constant cross-section should homogenize patterns of periphyton growth and help to reduce the bias tied to this wall artifact (Chen et al.

1997). The enclosures were filled with lake water, taken at 1.5 m depth with a gasoline-powered pump. In order to minimize seston heterogeneity, bags were filled randomly and in several steps between 29 June and 3 July 1995. Taking into account differences in enclosure size, we filled each day equal portions of total enclosure volume. Enclosures were thereafter enriched, proportionally to their volume, with living zooplankton netted in the pelagic zone of the lake. To allow for initial development of the planktonic community, fish enclosures were stocked with roach (*Rutilus rutilus*) only on 13 July (hereafter called day 0). At day 0, zooplankton biomass in enclosures was >200 mg DW L⁻¹. Five different treatments, each having three replicates, were randomly applied to the 15 enclosures (NF = no fish, F = fish, S = shallow, D = deep, R = refuge):

1. NFS: shallow enclosures without fish
2. FS: shallow enclosures with 6 fish; 20.6 g fresh weight FW m⁻²
3. NFD: deep enclosures without fish
4. FD: deep enclosures with 6 fish; 20.6 g FW m⁻²
5. FDR: similar to FD, but with fish confined in the surface layer (0–2 m) of the enclosure in a net-bag (1.5 $\times$ 1.5; 2 m depth; 23 mm² maximum mesh size) that allowed zooplankton passage.

Fish length measurements were taken to the nearest millimetre. To reduce manipulation of fish used for the treatments, the initial body mass of roach was estimated from a sample of fish of the same origin as those stocked in the enclosures. Average roach fork length (FL) was 8.1 $\pm$ 0.4 cm (mean $\pm$ SD) and fresh weight was 7.5 $\pm$ 0.4 g (mean $\pm$ SD). To avoid nutrient limitation of phytoplankton, enclosures were enriched with inorganic fertilizers. Because phytoplankton experience nutrient limitation on a volumetric basis (Petersen et al. 1997), we used volume to scale nutrient load. Nutrients were added twice a week as a liquid mixture of potassium phosphate (KH₂PO₄) and ammonium nitrate (NH₄NO₃) with an N:P ratio of 20:1 by weight, for a load of phosphorus equal to 3.16 μ g L⁻¹ d⁻¹. Epibiont development was followed weekly during 21 days after day 0 by collecting vertically integrat-

ed zooplankton samples. To analyze separately epibiont burden on hosts having different positions in the water column (deep enclosures only), on the second sampling occasion we separated samples from surface (0–2 m) and bottom layers (2–4 m). Zooplankton were counted under a stereoscopic microscope in subsamples at different dilutions in Dolfuss chambers (four to five subsamples per sample). When individuals were rare, the whole sample was analyzed. Crustacean body length and epibiont burden were measured with an ocular micrometer for 40 randomly chosen individuals per taxon in each sample. Epibiont burden was measured for each individual host on an ordinal 0–4 scale (0 = absence; 1 = 1–3 ind.; 2 = 4–10 ind.; 3 = 11–50 ind.; 4 = more than 50 ind.). While losing precision relative to full sample counts, this method allowed to estimate epibiont burden on a large number of hosts and in a relatively large number of experimental units. Zooplankton biomass was calculated by genus as in Bertolo et al. (1999a). Four cladoceran genera were present in the enclosures: *Ceriodaphnia*, *Bosmina*, *Diaphanosoma*, and *Daphnia*. Three copepod genera were also present: *Acanthocyclops*, *Thermocyclops* and *Eudiaptomus*. With the exclusion of *Bosmina*, all cladocerans commonly hosted *Colacium* sp. with a variable burden. *Daphnia* epibiont burden was not analyzed here because this genus was rare in the fish enclosures. Among copepods, only the cyclopoid *Acanthocyclops* and the calanoid *Eudiaptomus* showed occasionally a light epibiont burden. *Colacium* sp. was only observed on the former, whereas the latter hosted an unidentified stalked ciliate.

In addition to individual body length and mass of hosts, we calculated total host biomass as an index of potential sources of epibiont recolonization. Total host biomass was calculated as the sum of biomass over all the taxa observed to host epibionts in our experimental enclosures (i.e., *Daphnia*, *Ceriodaphnia*, *Diaphanosoma*, and *Acanthocyclops*). Analyses were run with and without *Acanthocyclops* in the pool of hosts because overall epibiont prevalence was low for this genus. Additional details on the experimental setup and on zooplankton counts can be found in Bertolo et al. (1999a, b).

Background information:

predation refuges and host vertical distribution

Experimental treatments had a clear impact on the individual body size and abundance of both host genera, with clear consequences on their biomass (Table 1). Fish predation had a clear negative effect on the body size of both genera, whereas the presence of refuge somewhat reduced this effect (Table 1). Whereas *Diaphanosoma* abundance and biomass were not strongly affected by fish predation, *Ceriodaphnia* showed a clear reduction of numbers in the presence of fish (Table 1). However, the availability of physical refuge (FDR) allowed *Ceriodaphnia* to maintain a population biomass comparable to that of the control (NFD) even in the presence of fish.

Previous analyses of the daily vertical distribution of zooplankton in our experimental enclosures showed that use of the water column by *Ceriodaphnia* was clearly affected by the presence of fish and of physical refuge (Bertolo et al. 1999b). In the absence of fish (NFD treatment), *Ceriodaphnia* was slightly more abundant in the lower than in the upper enclosure, whereas the opposite pattern held in the presence of fish (Table 1). The presence of the refuge dramatically changed the vertical distribution of *Ceriodaphnia* in the presence of fish (FDR vs FD treatment), with most individuals concentrating during the day in the bottom, predator-free, layer (Table 1). In contrast, *Diaphanosoma* vertical distribution was rather homogeneous among the treatments (Bertolo et al. 1999b), with individuals slightly more abundant in the lower (60–70% ca.) than in the surface layer (Table 1).

In absence of light limitation, we should expect that *Ceriodaphnia* size-corrected epibiont burden in the presence of the refuge would be intermediate between epibiont burden in fish and fishless enclosures without refugia. In fact, both mean *Ceriodaphnia* body size and total biomass resulted to be intermediate relatively to fish (without refugia) and fish enclosures in the presence of deepwater refugia. However, since the vertical distribution of *Ceriodaphnia* was strongly affected by the presence of refugia, we expect that epibiont burden would be strongly reduced because of light limitation, once controlled for host size. We expect to find no difference in *Diaphanosoma* epibiont burden between fish enclosures (with or without refugia)

Table 1. Abundance, biomass, mean size and vertical distribution (percentage of individuals in the upper layer of the enclosures) of *Ceriodaphnia* and *Diaphanosoma* in the experimental enclosures. Values are medians calculated over three weeks, with the exception of vertical distribution, which was assessed only at the second week. The 25% and 75% quantiles are shown within brackets. NFS: fishless shallow enclosures; FS: fish in shallow enclosures; NFD: fishless deep enclosures; FD: fish in deep enclosures; FDR: fish in deep enclosures with a physical refuge for zooplankton. Modified from Bertolo et al. (1999a, b).

Genus	NFS	FS	NFD	FD	FDR
Abundance (ind L ⁻¹)					
<i>Ceriodaphnia</i>	16.8 (12.8–40.8)	6.1 (4.5–6.2)	30.2 (26.3–43.5)	12.4 (9.1–12.5)	21.3 (20.8–27.8)
<i>Diaphanosoma</i>	10.7 (9.3–11.5)	7.4 (6.2–15.0)	2.9 (1.9–6.7)	6.7 (5.3–8.1)	2.5 (1.5–4.6)
Biomass (µg L ⁻¹)					
<i>Ceriodaphnia</i>	19.8 (14.7–43.2)	3.9 (2.7–4.5)	36.2 (30.2–49.6)	8.7 (7.5–9.2)	19.5 (19.3–23.6)
<i>Diaphanosoma</i>	19.3 (18.7–23.2)	4.4 (3.7–8.9)	9.3 (5.8–14.5)	7.3 (5.5–7.7)	3.3 (2.1–4.8)
Body size (µm)					
<i>Ceriodaphnia</i>	443.3 (440.4–455.9)	371.0 (355.9–375.8)	471.3 (469.3–472.0)	403.8 (397.6–411.7)	427.4 (417.8–434.7)
<i>Diaphanosoma</i>	638.6 (622.6–653.9)	480.7 (475.5–484.0)	726.0 (704.3–729.7)	591.7 (565.6–616.4)	617.3 (603.5–640.6)
Percentage in the upper layer					
<i>Ceriodaphnia</i>	100	100	33.0 (27.8–39.9)	66.7 (63.3–70.2)	2.6 (2.0–19.7)
<i>Diaphanosoma</i>	100	100	58.1 (29.1–59.9)	20.0 (15.5–37.8)	41.7 (20.8–53.1)

because *Diaphanosoma* was relatively more abundant in the bottom layers of the experimental enclosure independently of the presence of fish or refugia.

Background information: effects of depth on light limitation

We had previously shown that our deep enclosures were light-limited (Bertolo et al. 1999a). These results suggested that, by increasing light limitation, depth was associated to relatively low phytoplankton biomass, independently of the grazing pressure by zooplankton.

Therefore, we expect that epibiont burden for a given host size would be higher in shallow than in deep enclosures.

Data analysis

The biology of crustacean molting and the hierarchical structure of the data in this study system pose some challenges for the analysis of epibiont burden. First, in the absence of molting, epibiont burden could be expected to increase linearly with host body size. However, epibiont burdens are reduced to zero at regular intervals by molting, which occurs at different sizes in different individuals (Fig. 1a). This pattern of molting therefore introduces considerable variation in the relationship between burden and host body size at the population level (Fig. 1b, c and

Appendix: Fig. A1). Furthermore, intra-group correlations arise from the nested sampling design because measurements made on individuals from a given enclosure are not independent; these dependencies must be considered in the analysis. Finally, the analysis must take into account that the measurements of burden are on an ordered categorical scale.

To characterize the relationships between epibiont burden and experimental treatments, we used a hierarchical version of an ordered multinomial logit model (Johnson and Albert, 1999). This model for ordered outcomes is a generalized linear model based on a cumulative link function, which typically is assumed to be the cumulative distribution function from a normal or a logistic probability density. The model was formulated in terms of a continuous unobserved, or latent, variable ω the values of which are associated with the five possible categorical outcomes as follows:

$$Pr(y_i \geq j) = F(\tau_j - \omega_i), \quad j = 0, \dots, J - 1$$

$$Pr(y_i = J) = 1 - F(\tau_{J-1} - \omega_i)$$

$$\omega_i = \mathbf{x}_i \boldsymbol{\beta} + \psi_k$$

$$\psi_k \sim N(0, \sigma^2)$$

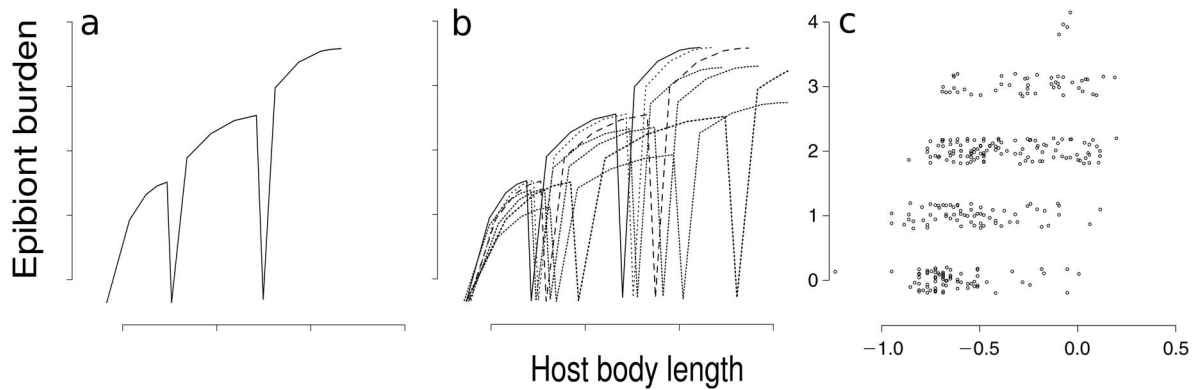


Fig. 1. Effects of host molting on epibiont burden. Theoretical relationship between host body length and epibiont burden (counts): (a) single zooplankton host and (b) multiple hosts with asynchronous molting. Asynchronous molting events associated with a sudden drop of epibiont burden introduces substantial variation in the relationship: (c) example of noisy data from our experiment with *Colacium* burden (five categories) vs. *Diaphanosoma* body size [$\log_e(\mu\text{m})$].

where k indexes the 12 enclosures, i indexes individual hosts, j indexes the $J = 5$ ordinal response categories, $N(\mu, \sigma^2)$ is the normal distribution with mean μ and variance σ^2 , and F is the cumulative distribution function of the logistic density. The y_i variable denotes the observed outcomes (abundance category for individual i), whereas ω_i is a latent continuous variable whose value is determined by a set of fixed effects and a normally distributed random effect (ψ_k) for the enclosure. The τ_i parameters represent cutoff or threshold points that separate the outcomes (categories of abundance) (Appendix: Fig. A2). The x_i represents a design matrix for the p treatment variables: $\log_e(\text{cladoceran length})$ (continuous), time (week number, categorical), enclosure size (deep or shallow, categorical), fish (presence or absence, categorical), refuge (presence or absence, categorical), and experiment-specific interactions between these variables. Effects coding was used for all categorical variables. The components of $\beta = (\beta_1, \dots, \beta_p)'$ are the regression coefficients associated with the treatment variables.

Inference was conducted in a Bayesian framework, which requires the specification of a set of prior distributions. We used diffuse priors for all parameters:

$$\beta \sim N_p(\mathbf{0}, 10^8 \mathbf{I}_p)$$

$$\tau_0 \sim N(0, 10^2) I(\tau_0 < \tau_1)$$

$$\tau_j \sim N(0, 10^2) I(\tau_{j-1} < \tau_j < \tau_{j+1}), j = 1, \dots, 2$$

$$\tau_3 \sim N(0, 10^2) I(\tau_2 < \tau_3)$$

$$\sigma \sim U(0, 5),$$

where $N_p(\mu, \Sigma)$ is the multivariate normal distribution with mean vector μ and covariance matrix Σ , $\mathbf{I}_p$ is the p -dimensional identity matrix, and $U_{(a,b)}$ is the uniform distribution with lower limit a and upper limit b . The indicator function $I(\cdot)$ is notational shorthand to indicate that the cutoff points must follow the constraint: $\tau_0 < \tau_1 < \tau_2 < \tau_3$.

All models were implemented in the R environment using the *rjags* package to access the JAGS sampling engine (Plummer 2014). We ran two parallel MCMC chains of 100,000 samples each. The first 50,000 iterations of each chain were discarded (burn-in) and the following 50,000 iterations were thinned (1 in 50) to yield 2000 samples from the posterior distribution. The Brooks-Gelman-Rubin criterion implemented in package CODA was used to check for convergence of the chains. Software code and data are

Table 2. Coefficients (mean and upper and lower limits of the 95% credibility interval) of models for size-adjusted epibiont burden as a function of time and the treatments enclosure size (shallow or deep) and fish (present or absent), for *Ceriodaphnia* and *Diaphanosoma*. Bold text indicates factors with credibility intervals excluding zero.

Model term	Mean	Upper CI	Lower CI
<i>Ceriodaphnia</i>			
$\log_e(\text{length})$	0.397	-0.162	0.937
week₂	0.066	-0.125	0.255
week₃	1.136	0.945	1.331
enclosure size	-0.057	-0.443	0.315
fish	-0.456	-0.811	-0.101
enclosure size $\times$ fish	0.128	-0.249	0.498
fish $\times$ week ₂	0.131	-0.053	0.327
fish $\times$ week₃	-0.351	-0.543	-0.168
<i>Diaphanosoma</i>			
$\log_e(\text{length})$	1.403	0.926	1.890
week₂	0.441	0.262	0.619
week₃	1.159	0.961	1.361
enclosure size	0.074	-0.388	0.543
fish	-0.756	-1.207	-0.330
enclosure size $\times$ fish	0.098	-0.294	0.520
enclosure size $\times$ week ₂	-0.139	-0.32	0.035
enclosure size $\times$ week₃	-0.427	-0.616	-0.244
fish $\times$ week ₂	0.064	-0.118	0.250
fish $\times$ week ₃	-0.171	-0.364	0.020

Table 3. Coefficients (mean and upper and lower limits of the 95% credibility interval) of models for size-adjusted epibiont burden as a function of time and the treatments refuge (presence or absence) and fish (present or absent), for *Ceriodaphnia* and *Diaphanosoma* in deep enclosures. Bold text indicates factors with credibility intervals excluding zero.

Model term	Mean	Upper CI	Lower CI
<i>Ceriodaphnia</i>			
$\log_e(\text{length})$	0.469	-0.060	0.997
week₂	0.683	0.485	0.882
Week₃	-0.193	-0.485	0.077
Refuge	-0.220	-0.703	0.322
fish	-0.572	-1.216	-0.037
week₂ $\times$ refuge	0.985	0.710	1.268
week₃ $\times$ refuge	-0.690	-1.066	-0.305
week₂ $\times$ fish	0.465	0.191	0.756
week₃ $\times$ fish	-0.872	-1.333	-0.468
<i>Diaphanosoma</i>			
$\log_e(\text{length})$	0.791	0.224	1.370
week₂	0.756	0.553	0.966
week ₃	-0.026	-0.257	0.222
refuge	-0.631	-1.320	0.089
Fish	-0.545	-1.391	0.220
week₂ $\times$ refuge	0.937	0.634	1.233
week₃ $\times$ refuge	-1.197	-1.589	-0.809
week ₂ $\times$ fish	0.128	-0.136	0.401
week ₃ $\times$ fish	-0.033	-0.383	0.318

provided in the Supplement.

RESULTS

Effects of host body size and fish on epibiont burden

We found a positive relationship between host body length and epibiont load for both host genera when analyzing the three-week series (Tables 2 and 3). Whereas for *Ceriodaphnia* this relationship seems to be weak (CI including the zero value), the evidence is much stronger for *Diaphanosoma*, whose coefficients for body length differ from zero (Tables 2 and 3). Host body length had a positive effect on epibiont burden for both host genera in the analysis of epibiont vertical distribution (Appendix: Table A1). After controlling for host body size, we found clear negative effects of fish on epibiont burden of both cladoceran genera, as shown by the coefficients for the “fish” factor in three out of four models built (Tables 2 and 3; Figs. 2 and 3). Total host biomass was not included in the models because this variable was strongly correlated with host body length.

Effects of depth on light limitation

The analyses of the three-week time series on both deep and shallow enclosures with and without fish showed that epibiont load monotonically increased with time on both host genera (Fig. 2, Table 2). After controlling for the effect of body size, we observed a clear effect of fish in both shallow and deep enclosures for both host genera. Epibiont load was clearly higher in control enclosures (NFD and NFS), relatively to fish (FD and FS) (Table 2: coefficients involving the “fish” factor), and this negative effect of fish further increased on both host genera at the third week (Table 2: coefficients for “week₃ $\times$ fish” factor). *Ceriodaphnia* epibiont burden did not respond to the variations in enclosure depth (Table 2: coefficients including the “enclosure size” factor). In contrast, epibiont burden on *Diaphanosoma* was higher in shallow enclosures than in deep ones at the third week (Table 2: coefficient for the “enclosure size $\times$ week₃” factor).

Predation refuges and host vertical distribution

Whereas no clear differences among the treatments NFD, FD and FDR were observed

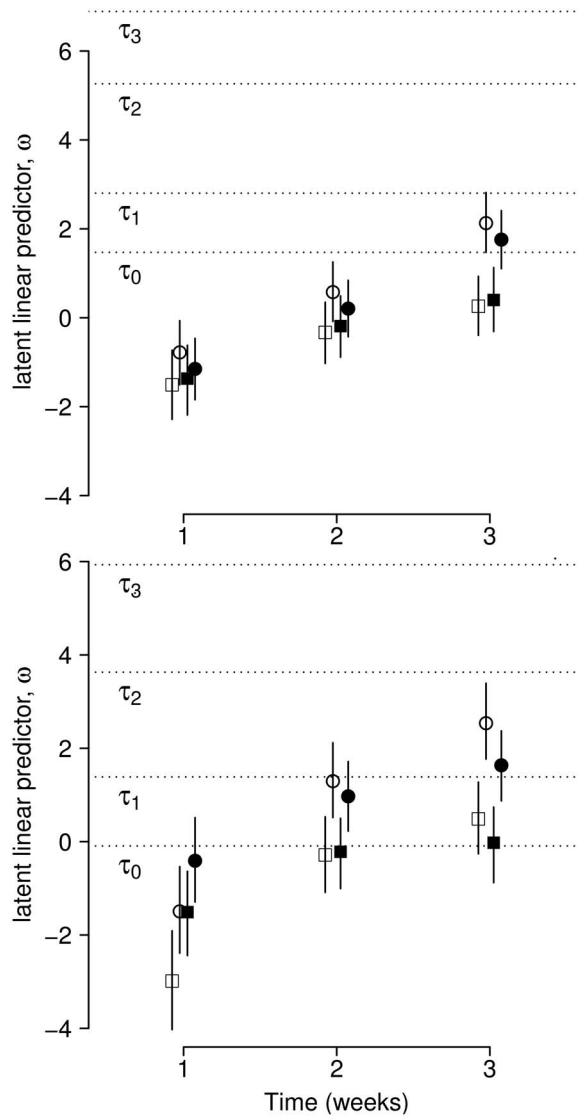


Fig. 2. Fish predation vs. light limitation effects. Variation over time (weeks 1–3) of a proxy for individual epibiont burden (latent variable, $\omega = \beta X$, with the random effect ψ set to its mean value, zero) in shallow (open symbols) and deep (dark symbols) enclosures in the presence (squares) or absence (circles) of fish. Data are adjusted for host body length. Horizontal dashed lines represent cutoffs or thresholds (τ values) associated with changes in epibiont burden categories (Appendix: Fig. A2). Upper panel: *Ceriodaphnia*; lower panel: *Diaphanosoma*.

during the first two weeks, a clear pattern emerged for both host genera at week three (Fig. 3). Compared to the control (NFD), the treatment with fish and a refuge for zooplankton

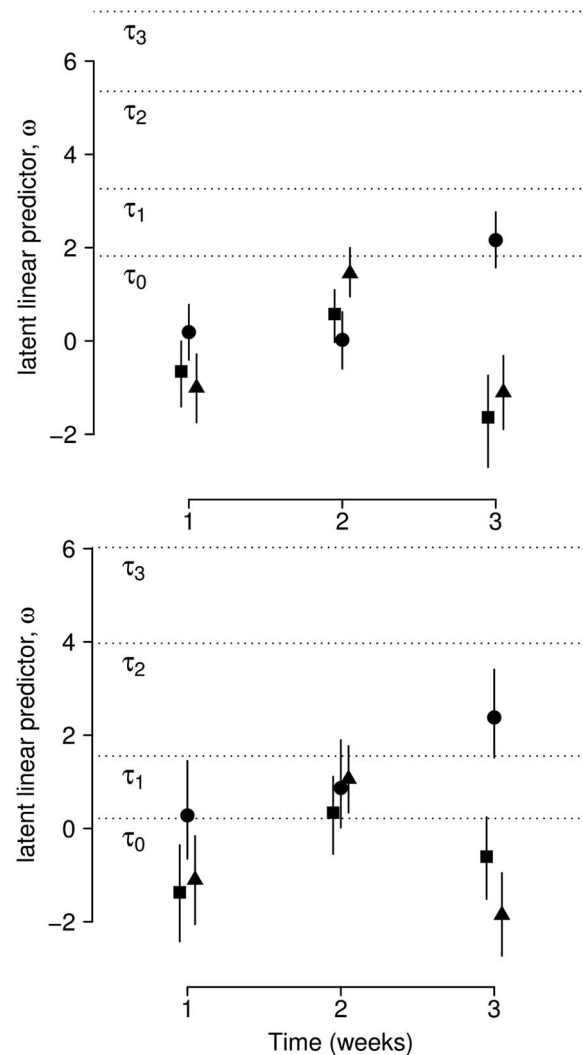


Fig. 3. Zooplankton refuge use vs. epibiont burden. Variation over time (weeks 1–3) of a proxy for individual epibiont burden (latent variable, $\omega = \beta X$, with the random effect ψ set to its mean value, zero) in deep enclosures. Results are shown for three treatments: absence of fish (circles); fish and refuge present (triangles); fish present, no refuge (squares). Data are adjusted for host body length. Horizontal dashed lines represent cutoffs or thresholds (τ values) associated with changes in epibiont burden categories (Appendix: Fig. A2). Upper panel: *Ceriodaphnia*; lower panel: *Diaphanosoma*.

(FDR) showed a clear reduction in epibiont burden for both *Ceriodaphnia* and *Diaphanosoma* (Fig. 3, Table 3). Epibiont burden in fish enclosures (FD) showed a similar pattern for

both genera, but resulted to be significant only for *Ceriodaphnia* (coefficients for “fish”, “week₂ × fish” and “week₃ × fish” in Table 3).

The analysis of the vertical distribution of zooplankton, which was conducted on data from week 2 only, allowed us to examine more closely the differences in vertical distribution of epibionts in the deep enclosures. This analysis revealed that *Diaphanosoma*, but not *Ceriodaphnia*, carried more epibionts in the surface layers (0–2 m depth) than in the bottom layers (2–4 m depth). In contrast, only *Ceriodaphnia* showed a positive effect of the presence of refuge on its epibiont burden (Appendix: Fig. A3 and Table A1). However, this effect was small when compared with the overall effect observed on *Ceriodaphnia* epibiont burden at the end of the experiment (Fig. 3), when the presence of the refuge was clearly associated to a reduction of *Colacium* burden compared to the control.

DISCUSSION

Our results clearly confirm the existence of a shared doom effect induced by fish predation on both zooplankton host genera and their epibionts. Our experiments also show that light penetration in the water column and host behavior have the potential to control the photosynthetic epibiont burden in semi-natural conditions.

Effects of host body size and fish on epibiont burden

The existence of a clear negative effect of fish after controlling for host body size highlights the fact that fish not only selected larger host individuals, but also those with heavier epibiont burdens. Previous results on the effects of zooplanktivorous fish on epibiont prevalence suggested a similar mechanism (Willey et al. 1990), but could not demonstrate it directly because host body size was not controlled for in the analyses. We avoided this shortcoming since in our models size-selectivity and the shared doom effect were explicitly taken into account and treated separately (i.e., “log_e(length)” and “fish” terms in Tables 2–3, and Appendix: Table A1). Therefore, our results clearly suggest that epibiosis can strengthen trophic interactions between fish and zooplankton, with potential effects

for both host and epibiont population dynamics. Previous work has demonstrated that by selecting heavily infected hosts, predators have the potential to shape prey demographics, and, in turn, to control the spread of parasites (Pulkkinen and Ebert 2006). In *Daphnia*, size-selective removal of individuals has the potential to control the persistence of parasites in the population (Bittner et al. 2002).

Other examples of how epibionts can modulate trophic interactions can be found in the literature (e.g., Wahl 2008a). As in our case, epibiosis has been shown to increase predation risk in a number of taxa, but the opposite effect is also possible (Wahl 2008a), as shown by epibiotic limpets that lower the vulnerability of their mussel host to starfish predation (Thieltges 2005), and by epibiotic sponges that reduce the vulnerability of the marine bivalve *Arco noae* against predators (Marin and Belluga 2005). In consequence, by either strengthening or weakening trophic interactions, epibiosis has the potential to generate community-wide effects, as suggested by Wahl (2008a). In our model system, epibionts have thus the potential to enhance the top-down effects of planktivorous fish on zooplankton, with potential structuring effects at the community level as well as cascading effects on lower trophic levels.

Effects of depth on light limitation

Our results also support our initial hypothesis on light limitation of photosynthetic epibiont burden in our experimental enclosures. We observed a higher epibiont burden on *Diaphanosoma* in shallow enclosures than in deep ones (i.e., lower vs. higher light limitation conditions, respectively), independently of fish predation and host body size. However, this effect was not observed in *Ceriodaphnia*. The observed difference in response to light limitation between the two host genera could probably be explained in terms of host behavior. In fact, *Diaphanosoma* used the part of the water column below 2 m when possible (i.e., in deep enclosures only), independently from the presence or absence of fish. This created a contrast in host vertical distribution between shallow and deep enclosures and increased the likelihood of detecting the effect of light limitation for this host genera. *Ceriodaphnia*, in contrast, had a slightly greater

epibiont burden in shallow enclosures, but only in the absence of fish. The complete lack of response of *Ceriodaphnia* to light limitation in fish enclosures might be explained by a shift towards the shallower part of deep enclosures when fish were present, thus reducing light limitation.

Predation refuges and host vertical distribution

In agreement with our initial hypothesis, the refuge against fish predation did not allow epibionts on *Ceriodaphnia* to maintain a burden intermediate between those of fish and fishless enclosures, despite the potential for a reduced shared doom effect. Although the refuge was effective in maintaining a relatively high abundance of potential hosts for *Colacium* even in the presence of fish, it did not seem to benefit the epibionts. The epibiont burden on *Ceriodaphnia* was in fact similar in the two fish treatments (deep enclosures), independently of the presence of refuge. Therefore, an overall weaker predation pressure in the enclosures with refuge did not appear to result in a smaller shared doom effect. Although it is possible that the shared doom effect was in fact similar with or without the refuge because of a threshold effect, it seems more likely that this similarity was related to the shift in vertical distribution of *Ceriodaphnia* in the presence of refuge. The vertical distribution of *Ceriodaphnia* in the presence of fish was clearly affected by the presence of the refuge: aggregating in the lower and safer part of the enclosures probably increased light limitation for *Colacium* on *Ceriodaphnia*.

Alternatively, these results might be partly due to a weaker response to light limitation in *Ceriodaphnia* than in *Diaphanosoma*, which would hinder the detection of non-consumptive effects. However, the observed difference between epibiont burden responses to light limitation in the two host genera is interesting per se. After controlling for body size, differences in key biological traits of the host genera, such as behavior, are likely to explain the differences in epibiont burden among hosts and deserve more attention in the future. A similar overall effect of the refuge on *Colacium* burden was found for *Diaphanosoma*, but in this case we did not expect any non-consumptive effect due to host behaviour. The vertical position of *Diaphanosoma*, and thus its average exposure to light, were in fact

similar in the two fish treatments in the deep enclosures, and no major differences between treatments in either predation pressure or light limitation should be expected. Unfortunately, the interpretation of results on the vertical distribution of *Colacium* burden (Appendix: Fig. A3 and Table A1) has limited scope in the comparison among treatments because, in contrast to week 3, on week 2 there were no marked differences among the treatments (see Figs. 2 and 3). Nevertheless, these results give some extra support to the light limitation hypothesis at the within-treatment level since the *Colacium* burden was higher in surface compared to bottom samples, at least in *Diaphanosoma*.

Conclusions

Beside explicitly confirming the existence of a shared doom effect and the role of environmental factors in the control of epibiont populations, our study showed that host behavior has the potential to control the epibiont burden in semi-natural conditions. To our knowledge, this is the first demonstration of epibiont control by host behavior since a similar effect was suggested by Threlkeld et al. (1993). These authors suggested that host behaviours such as modulation of vertical distribution might control the epibiont burden via light limitation. Such non-consumptive effects are often underestimated, but might have profound implications for community and food-web dynamics (Kéfi et al. 2012). Understanding the potential consequences of this result for the host in terms of control of the costs related to the epibiont burden is a potentially rewarding research avenue that merits further exploration.

Similar questions to those explored in our work have been raised concerning *Daphnia*-parasite interactions (Cáceres et al. 2014). For example, Decaestecker et al. (2002) suggested that *Daphnia* aggregation caused by vertical migration can affect parasite transmission dynamics. By concentrating near pond sediments to avoid fish predators, *Daphnia* might in fact increase their encounter rate with parasite spores (the “deep trouble hypothesis”; Decaestecker et al. 2002). As a consequence of the presence of multiple enemies (fish and parasites), habitat selection behavior in *Daphnia* could be driven by a tradeoff between predator and parasite avoidance. However, host-epibiont interactions are

often complex and predicting their outcome is not always straightforward (Wahl 2008a). Our study system may not conform to the deep trouble hypothesis because in our experiments deeper layers are associated to both lower photosynthetic epibiont burden and predation risk. Taken together, these examples show how host-epibiont interactions can be complex and depend on the specific host-epibiont/parasite association examined. More generally, these and previous (Bertolo et al. 2010) experimental results confirm that fish predation has much more complex effects on primary producers than the classically observed increase in phytoplankton biomass (see Hulot et al. 2014) through a trophic cascade via zooplankton.

Epibionts are common on zooplankton, which are a unique and generally neglected habitat. Given the potential impact of interactions between epibionts and their zooplankton hosts on ecosystem processes such as respiration (Tang et al. 2010) and carbon recycling (Tang et al. 2010, Bickel et al. 2012), it is important to frame these interactions within a wider perspective. Future work should help to better understand the effects of predation and competition on the spatial distribution of hosts and their parasites and epibionts within lakes and to reveal potential feedback mechanisms.

ACKNOWLEDGMENTS

We thank Alexandra M. Schmidt for discussion on an earlier draft of this work. A. Bertolo acknowledges support by the “Fonds d’Animation de la Recherche” of UQTR (FAR Project “Ecologie et évolution des réseaux d’interaction en milieux aquatiques peu profonds”). G. Lacroix was supported by the French National Research Agency (ANR BLANC, PHYTBAC project 10-BLAN-1709-01). The preparation of the International Programs For Scientific Cooperation (project PICS06763), supported by the French National Center of Scientific Research, stimulated the publication of the paper. M. A. Rodríguez acknowledges the support of the Natural Sciences and Engineering Research Council of Canada. A. Bertolo and G. Lacroix conceived and conducted the experiments. M. A. Rodríguez conducted the statistical analysis. A. Bertolo, G. Lacroix and M. A. Rodríguez contributed in writing the paper.

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SUPPLEMENTAL MATERIAL

ECOLOGICAL ARCHIVES

The Appendix and the Supplement are available online: <http://dx.doi.org/10.1890/ES14-00451.1.sm>