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Healthy herds in the phytoplankton: The benefit of selective parasitism

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Abstract

The impact of selective predation of weaker individuals on the general health of prey populations is well-established in animal ecology. Analogous processes have not been considered at microbial scales despite the ubiquity of microbe-microbe interactions, such as parasitism. Here we present insights into the biotic interactions between a widespread marine thraustochytrid and a diatom from the ecologically important genus *Chaetoceros*. Physiological experiments show the thraustochytrid targets senescent diatom cells in a similar way to selective animal predation on weaker prey individuals. This physiology-selective targeting of 'unhealthy' cells appears to improve the overall health (i.e. increased photosynthetic quantum yield) of the diatom population without impacting density, providing support for 'healthy herd' dynamics in a protist-protist interaction, a phenomenon typically

associated with animal predators and their prey. Thus, our study suggests caution against the assumption that protist-protist parasitism is always detrimental to the host population and highlights the complexity of microbial interactions.

Animal predators can exert overall positive effects on the health of prey populations by removing individuals with suboptimal health [1, 2] in a manner that has been termed 'healthy herd' dynamics [3]. While such top-down processes are well-established in animal ecology [1–3], they have largely been unconsidered in microbe-microbe interactions.

Protist-protist parasitism is widespread in the marine environment [4] and is generally considered to be detrimental to host populations [5, 6]. However, despite their ubiquity, the ecophysiological impact of protist-protist parasitism remains poorly understood. An important case that necessitates investigation is protist parasitism of diatoms, which have limited representation with culture-dependent model systems despite the significance of diatoms in marine ecosystem functioning and global primary production [7].

We observed and isolated a heterotrophic protist growing epibiotically on moribund and dead *Chaetoceros* sp. diatoms from a summer bloom at Station L4 in the Western English Channel off Plymouth (UK) (Figure 1A-B; Supplementary Figures 1-2; Supplementary Methods). Single cell picking achieved diatom and parasite co-cultures and uninfected host diatoms. The 18S rRNA gene V4 region of the protist (termed 'ThrauL4') identified the epibiont as a novel thraustochytrid (Stramenopila; Labyrinthulomycota; Thraustochytrida) (Supplementary Figure 3). Searching for ThrauL4 18S rRNA gene homologs in the Ocean Sampling Day dataset revealed that the parasite has a wide distribution in coastal temperate regions (Supplementary Figure 4).

Stable *Chaetoceros*-ThrauL4 co-cultures permitted the characterisation of ThrauL4 internal structures (Supplementary Figures 5-6), epibiotic growth (Figure 1A-B; Supplementary Figures 7-8) and infection dynamics (Figure 1C-D). ThrauL4 also attached to other diatoms (*Odontella sinensis*, *Ditylum brightwellii* and *Coscindodiscus* sp.) in a similar manner to *Chaetoceros* sp. but not dinoflagellates (Figure 1C; Supplementary Figure 9).

The proportion of diatom cells with ThrauL4 attached increased when *Chaetoceros* sp. cells entered the stationary growth phase (Figure 1D). Time lapse microscopy revealed the dynamic nature of the ThrauL4-diatom interaction (Figure 1E, Supplementary Movie 1-6), with the motile ThrauL4 apparently targeting physiologically ‘unhealthy’ cells identified by cytoplasmic blebbing prior to colonisation (Figure 1E).

We set out to test the hypothesis that ThrauL4 targeted unhealthy diatoms using population-level ecophysiology experiments. When introduced to heat-stressed diatom populations, ThrauL4 had a higher fitness (i.e. became more abundant) and infected more *Chaetoceros* sp. cells than when exposed to healthy un-stressed diatoms (Figure 1F-G), confirming more optimal growth of the parasite amongst unhealthy diatom populations. Furthermore, selective targeting was also demonstrated at the single-cell level using laser-damaged individual cells and time-lapse microscopy (Figure 1H-I). 80% of stressed cells and 60% of dead cells were colonised by ThrauL4 during the 30 min experimental period, whereas diatoms in healthy control populations were un-colonised.

These results led us to investigate the physiological impact of thraustochytrid parasitism on host diatom populations by comparing the dynamics and health of parasite exposed and non-exposed *Chaetoceros* sp. populations (Figure 2 A-C). Based on the previous growth experiments showing ThrauL4 proliferation during the diatom stationary phase (Figure 1D), *Chaetoceros* sp. cultures grown to their stationary phase after 7 d were chosen to mimic environmental bloom decline. Using the photosynthetic quantum yield (Fv/Fm) as a proxy for overall diatom health [8], after 8 d, the parasitized *Chaetoceros* sp. populations were consistently healthier than those in the control non-exposed populations (Figure 2A). Diatom population density was similar in both treatments (Figure 2B) and parasite prevalence peaked after 8 d (Figure 2C). In a separate experiment to investigate the role of genotype specificity in ThrauL4 parasitism, we generated a clonal *Chaetoceros* sp. population by single-cell picking and exposed the population to ThrauL4 cultures growing independently from diatoms. Although the clonal population declined in health more rapidly overall, ThrauL4 parasitism also resulted in healthier populations (Figure 2 D-F) suggesting that these results are a not an artefact of genotype specificity and succession.

By removing physiologically weaker individuals from the population, the remaining cells will constitute an overall healthier population. However, other mechanisms may also promote an overall healthier diatom population. It may be that selective parasitism relieves nutrient competition between unhealthy and healthy individuals. In the natural environment, diatom-diatom competition is a major growth limiting factor [9, 10] and removing the pressure exerted by weaker cells may allow the population to be more robust. It is also possible that the thraustochytrid could be 'cleaning' the population by preventing the build-up of toxic waste products or the proliferation of detrimental co-culture bacteria in an analogous way to how carrion removal by vultures prevents the spread of diseases to mammals [11]. Additionally, thraustochytid parasitism could accelerate nutrient recycling by releasing nutrients from dying cells. The consequences of physiology-selective diatom parasitism should be assessed in the marine environment, including impacts at the community scale and in the context of ecosystem functioning.

The proposed influence of thraustochytrid parasitism on diatom population health is summarised in Figure 2G. We suggest that this thraustochytrid-diatom interaction provides evidence of 'healthy herd' dynamics in a protist-protist interaction, an ecological phenomenon typically associated with animal predator-prey interactions [3]. As we show here with ThrauL4, animal predators such as lions [12], cougars [13], African wild dogs [14], and wolves [15] have been shown to target prey with suboptimal health. The 'healthy herd' hypothesis states that by selective predation on unhealthy prey, predators increase the overall health of the prey population by increasing resource availability or by removing potential carriers of disease [3]. Evidence for 'healthy herd' dynamics where predation generates healthier prey populations has also been demonstrated in lobster-sea urchin [16], fish-*Daphnia* [17], and fox-grouse [18] predator-prey systems. Here, we provide analogous supportive evidence from a marine protist-protist system.

‘Heathy herd’ dynamics between protists challenges the assumption that protist-protist parasitism is always detrimental to the host population and raises caution in this assumption in ecosystem modelling or inference from molecular ecology surveys (e.g. metabarcoding). Our results have demonstrated the potential complexity of protist-protist symbioses, highlighting the value of culture-based experimentation and the importance of developing model co-culture systems in resolving complex ecological interactions. The underpinning biology and ecological importance *in natura* of such interactions now require further investigation.

CONFLICT OF INTEREST

The authors declare no conflict of interest

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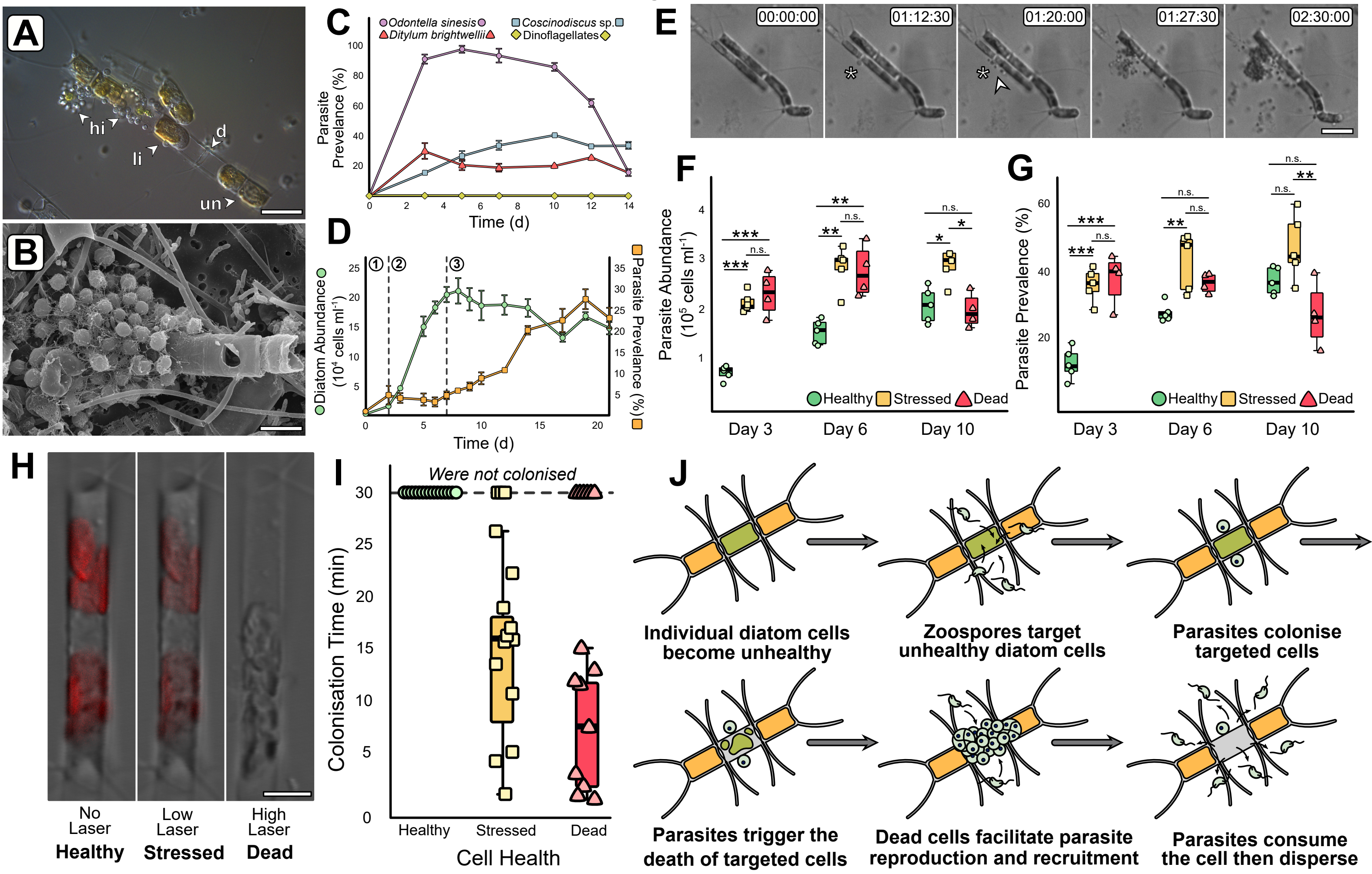
FIGURE LEGENDS

Figure 1. Growth experiments demonstrate that thraustochytrids preferentially target and grow on unhealthy diatom cells. (A) Differential interference contrast (DIC) image of *Chaetoceros* chain exhibiting different degrees of infection by ThrauL4. Uninfected cell (un), a lightly infected cell (li), heavily infected cells (hi) and a dead, empty frustule (d). Scale bar = 20 µm. (B) Scanning Electron Micrograph (SEM) of a *Chaetoceros* diatom swarmed by ThrauL4. Scale bar = 5 µm. (C) ThrauL4 growth dynamics on a selected range of diatoms and dinoflagellates (*Alexandrium minutum* and *Prorocentrum minimum*) (±SEM, $n = 3$). (D) *Chaetoceros* growth with ThrauL4 (±SEM, $n = 5$). Dashed lines demarcate the lag (1), exponential (2) and stationary (3) phases of *Chaetoceros* growth. (E) Time-lapse of *Chaetoceros*-ThrauL4 showing ThrauL4 colonising unhealthy cells. Asterisk = cytoplasmic bleb from unhealthy diatom. Arrowhead = initial thraustochytrid colonisation. Timestamp = HH:MM:SS. (F-G) Difference in the abundance (F) and prevalence (G) of parasites in healthy (control), stressed and dead *Chaetoceros* populations ($n = 5$) inoculated with ThrauL4 following heat stress exposure. ANOVA Tukey's HSD n.s $p > 0.05$ (not significant), *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. (H) Example diatom exposed to different laser powers used to generate individual *Chaetoceros* cells of varying health. Red channel overlay

demarks chlorophyll autofluorescence. Scale bar = 5 µm. (I) Time taken for individual diatom cells (*n* = 15) exposed to varying laser treatments to be colonised by ThrauL4. (J) Diagrammatic representation of the proposed diatom-thraustochytrid interaction cycle based on time-lapse microscopy observations (see Supplemenarty Videos).

Figure 2. Selective targeting of unhealthy diatom cells by thraustochytrids improves the overall health of the diatom population. (A-C) Population dynamics of the Fv/Fm (A) and total number (B) of stationary *Chaetoceros* diatoms for control and parasitized diatom populations over the experimental period (±SEM, $n = 5$). Welch's t-test *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. The parasite prevalence did not exceed about a third of the total population (C) (±SEM, $n = 5$). Parasites added at 0 d. In a separate experiment (D-F), a clonal *Chaetoceros* population was generated. Population dynamics of the Fv/Fm (D), total number (E) and infection prevalence (F) of stationary *Chaetoceros* diatoms for control and parasitized populations made clonal by single cell picking (±SEM, $n = 5$). Significance values as above. Parasites added at 0 d. Taken together these results indicate that preferential thraustochytrid parasitism of unhealthy diatoms strengthens the overall health of the population therefore providing evidence for the 'healthy herd' hypothesis in a phytoplankton population, which is summarised diagrammatically in (G).

Figure 1


A
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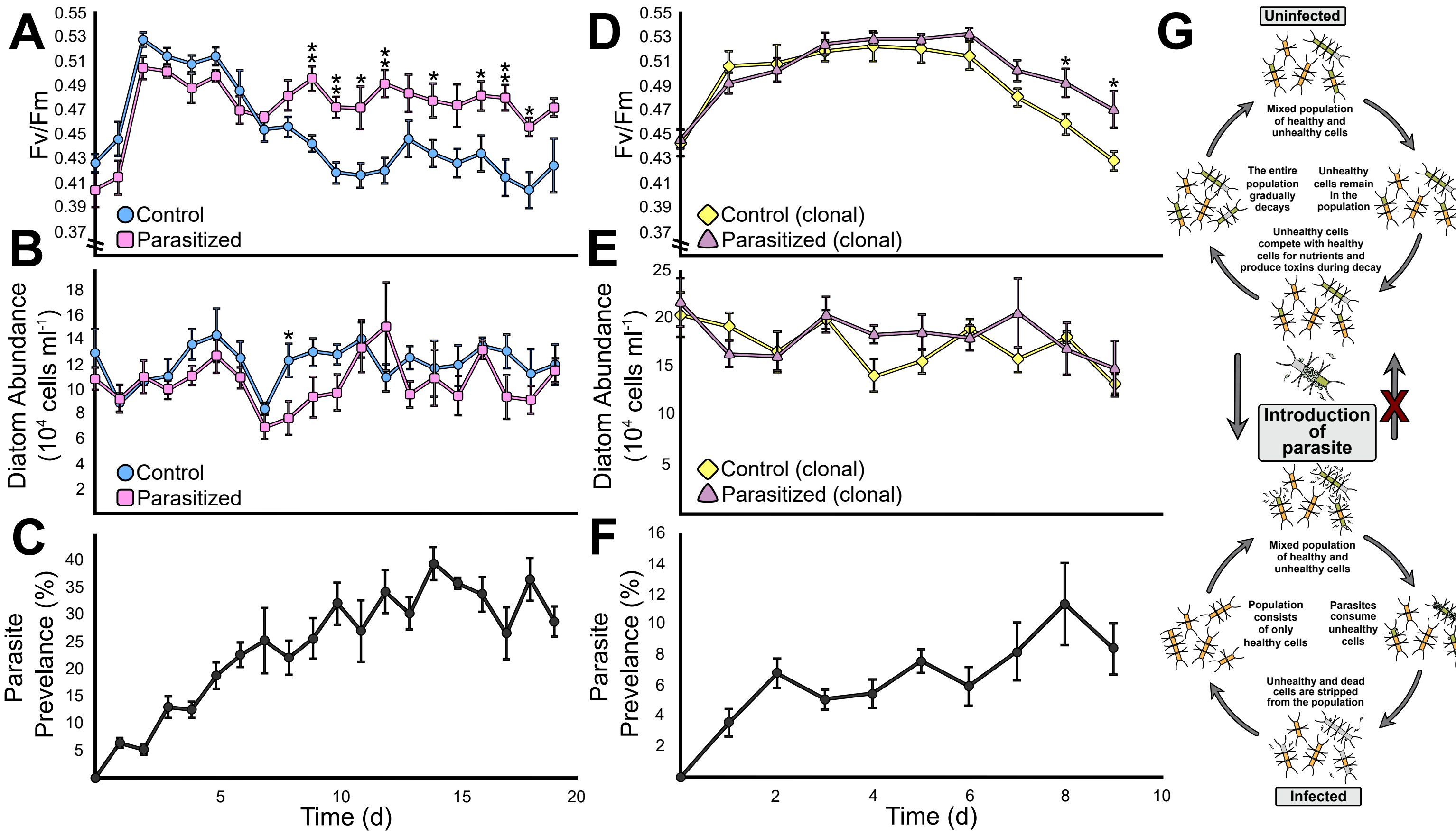
li
d
un
B
C
Odontella sinesis
Coscinodiscus sp.
Ditylum brightwellii
Dinoflagellates
Parasite Prevelance (%)
Time (d)
D
Diatom Abundance (10⁴ cells ml⁻¹)
Parasite Prevelance (%)
Time (d)
E
00:00:00
01:12:30
01:20:00
01:27:30
02:30:00
F
Parasite Abundance (10⁵ cells ml⁻¹)
Healthy
Stressed
Dead
Day 3
Day 6
Day 10
G
Parasite Prevalence (%)
Healthy
Stressed
Dead
Day 3
Day 6
Day 10
H
No Laser
Low Laser
High Laser
Healthy
Stressed
Dead
I
Were not colonised
Colonisation Time (min)
Healthy
Stressed
Dead
Cell Health
J
Individual diatom cells become unhealthy
Zoospores target unhealthy diatom cells
Parasites colonise targeted cells
Parasites trigger the death of targeted cells
Dead cells facilitate parasite reproduction and recruitment
Parasites consume the cell then disperse

Figure 2



A
B
C
D
E
F
G
Fv/Fm
Control
Parasitized
Diatom Abundance (10^4 cells ml^{-1})
Parasite Prevelance (%)
Time (d)
Control (clonal)
Parasitized (clonal)
Uninfected
Mixed population of healthy and unhealthy cells
The entire population gradually decays
Unhealthy cells remain in the population
Unhealthy cells compete with healthy cells for nutrients and produce toxins during decay
Introduction of parasite
Mixed population of healthy and unhealthy cells
Population consists of only healthy cells
Parasites consume unhealthy cells
Unhealthy and dead cells are stripped from the population
Infected