# Social hosts evade predation but have deadlier parasites

Jason Walsman[a,*], Mary J. Janecka[a], David R. Clark[a], Rachael D. Kramp[a], Faith Rovenolt[a], Regina Patrick[a], Ryan S. Mohammed[b], Mateusz Konczal[c], Clayton E. Cressler[d], Jessica F. Stephenson[a]

[a]*Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260*

[b]*Department of Life Sciences, The University of the West Indies, St. Augustine, Trinidad and Tobago*

[c]*Evolutionary Biology Group, Faculty of Biology, Adam Mickiewicz University, 60-614 Poznań, Poland*

[d]*School of Biological Sciences, University of Nebraska, Lincoln, NE 68588*

[*]*Author for correspondence: Jason Walsman; Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA 15260; walsmanjason@gmail.com*

**Abstract.** Parasites exploit hosts to replicate and transmit, but overexploitation kills both host and parasite[1]: parasite virulence evolves to balance these costs and benefits. Predators can in theory shift this balance by consuming hosts[2–4]. However, the non-consumptive effects of predators may be as important as their consumptive effects[5]. Here, we use an eco-coevolutionary model to show that predators select for host grouping, a common anti-predator, defensive social behaviour[6]. Host grouping simultaneously increases parasite transmission, thus within-host parasite competition, and therefore favours more exploitative, virulent, parasites[7]. When parametrized with data from the guppy-*Gyrodactylus* spp. system, including our experimentally demonstrated trade-off

between virulence and transmission, our model accurately predicted the common garden-assayed virulence of 18 parasite lines collected from four Trinidadian guppy populations under different predation regimes. The quantitative match between theory and data lends credence to the model's insight that the non-consumptive, social behaviour pathway is entirely responsible for the observed increase in virulence with predation pressure. Our results indicate that parasites play an important, underappreciated role in guppy evolutionary ecology. Moreover, group living is a common anti-predator defence[6] and our general model accommodates host-parasite interactions across taxa: its insight into the interactions among predation, sociality, and virulence evolution may apply broadly. Our results additionally suggest that social distancing, by reducing host-host contact, can select for less virulent parasites and pathogens.

**Main.** Unprecedented infectious disease emergence among human and wildlife populations[8,9] demands that we improve our understanding of the evolutionary ecology of parasites and pathogens (hereafter 'parasites'). A key tenet of infectious disease evolutionary theory is that parasites face a trade-off between virulence and transmission[10–12]. Parasites exploit their hosts to replicate and transmit, but increasing this exploitation harms parasite fitness by killing hosts ('virulence'), which reduces the time window for successful transmission, or the 'infectious period'[1]. With appropriate curvature, this trade-off should result in stabilising selection for intermediate virulence[13–16]. While theory predicts various mechanisms can shift the costs and benefits of exploitation, altering virulence evolution[2–4,7] and host disease outcomes[12,15,17,18], empirical evaluation of their relative importance remains scant[12,19,20].

Predation, a ubiquitous ecological interaction, can theoretically select for higher or lower virulence[2–4,12]. Predation directly increases host mortality, selecting for faster exploitation and transmission before the host dies, and hence increased virulence[2,3,19]. Conversely, predation may select for lower virulence through a more complex pathway. By reducing the density of infected hosts, predation reduces the per-host rate at which new infections arise ('force of infection'); lower force of infection reduces infections with multiple parasite genotypes[4] ('multiple infections') and thus the within-host competition between parasite genotypes that selects for higher virulence[20]. In addition to these consumptive effects, predators can also affect host traits[5], shifting how they interact with their parasites[21–24], and probably virulence evolution. One example is host grouping, a common defence[6] which effectively decreases predator-induced mortality[25,26], potentially selecting for lower virulence[7]. However, grouping also increases host-host contact rates, increasing the force of infection, so host grouping should increase multiple infections and therefore select for higher virulence[7,20,27]. Both consumptive and non-consumptive effects of predation act simultaneously in natural communities[23], but their relative importance for selection on parasite virulence lacks empirical and theoretical clarification.

Here, we elucidate how predators affect virulence evolution using theory and data from Trinidadian guppies, *Poecilia reticulata* and their *Gyrodactylus* spp. parasites (Fig. 1). Persistent, natural variation in predation risk drives population-level variation in guppy grouping for defence ('shoaling')[26,28,29], which likely controls the contact rate for directly transmitted, *Gyrodactylus* spp. ectoparasites[30,31]: high-predation populations shoal more and suffer higher *Gyrodactylus* spp. infection prevalence[30,32,33]. First, we experimentally confirm that *Gyrodactylus* spp. parasite lines fall along the commonly assumed virulence-transmission trade-

off (Fig. 2) and show that *Gyrodactylus* species (*G. turnbulli* and *G. bullatarudis*) and genotypes co-occur on individual guppies in the wild (Extended Data [ED] Table 1). These factors make this system ideal for testing how predation shifts virulence evolution. We then used laboratory and field measurements to parametrize a model with selection on host and parasite phenotypes interacting with host and parasite densities ('eco-coevolutionary model'), revealing one dominant pathway (Fig. 3): predators shift coevolution toward higher shoaling rate, increasing multiple infections, and thus also higher virulence. The model accurately predicted that wild-collected, *Gyrodactylus* spp. parasites from high shoaling populations had higher laboratory-assayed virulence (Fig. 4). Because we assayed parasite virulence in common garden conditions, this difference was due to differences in parasite exploitation of hosts, not host defences. Overall, the quantitative model-data match lends credence to the model's inference into parasite evolution in wild communities.

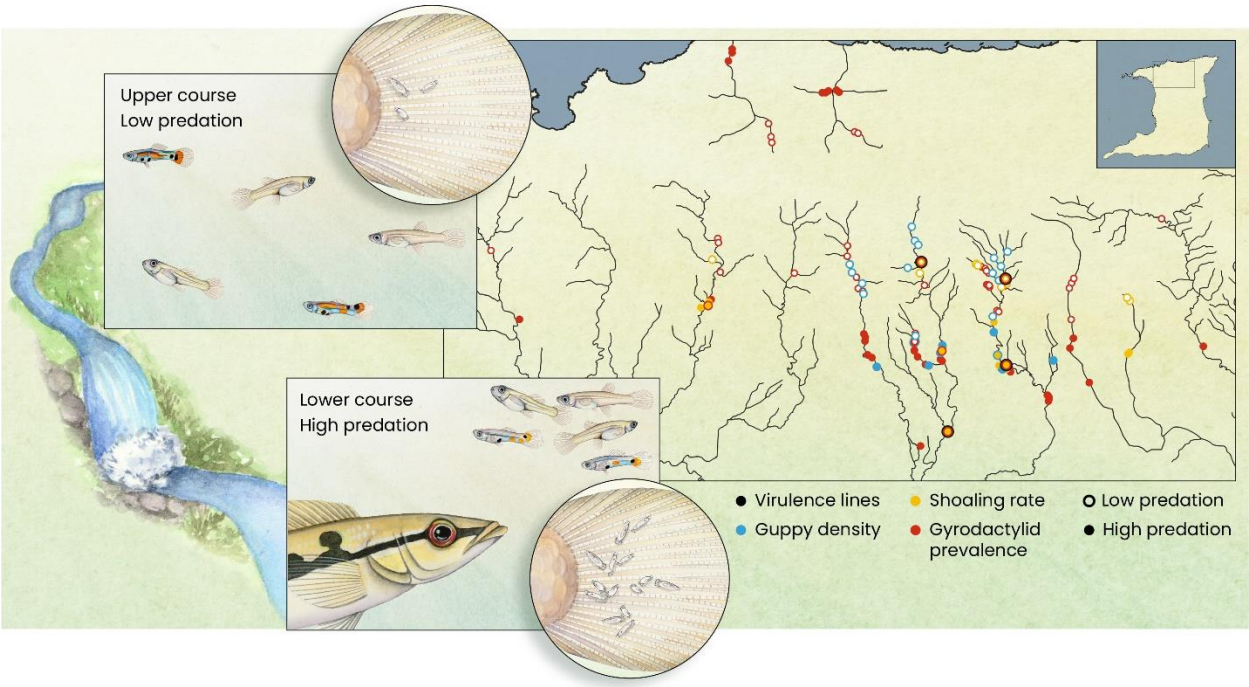



**Figure 1. Natural guppy populations differ in predation, driving evolutionary divergence in shoaling rate.** *Waterfalls divide upper course and lower course guppy populations and prevent the upstream migration of large piscivores. Natural populations have therefore evolved under different predation regimes, replicated across rivers. Shoaling rate differences likely drive population-level differences in transmission rate of*[30,34,35]*, and thus selection on the virulence of, their highly prevalent, directly transmitted monogenean ectoparasites* Gyrodactylus *spp.. The map shows locations and data types that we use.*

***Gyrodactylus* spp. transmission trades off with virulence, mediated by parasite intensity**

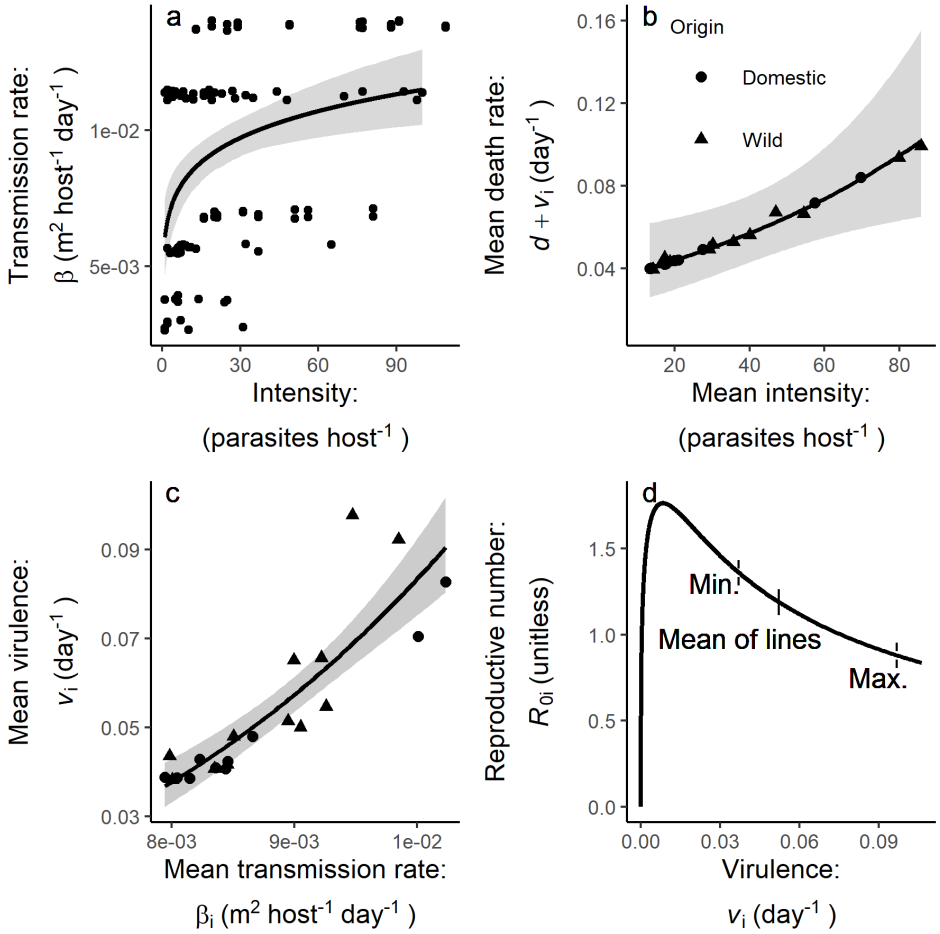



**Figure 2. Infection intensity links transmission rate and virulence with a stabilizing trade-off. *a,*** *Across four parasite lines, transmission rate increased with infection intensity. Points are transmission events jittered vertically.* ***b,*** *Across 22 [‘domestic’ (circles) or ‘wild’ (triangles)] parasite lines, higher intensity (a measure of parasite growth rate) induced higher host death rate ($d+v_i$, a measure of virulence; back-transformed partial residuals).* ***c,*** *(**a**) allows a link between transmission rate and virulence across lines. Points in (**b, c**) are mean line traits. In (**a-c**), bands are 95% C.I.* ***d,*** *The curvature in (**c**) maximizes $R_{0i}$ at intermediate transmission rate/virulence.*

We found a virulence-transmission trade-off among *Gyrodactylus* spp. lines. First, we found that transmission rate, given arena area and time until infection, increased asymptotically as intensity increased (Fig. 2a; $\beta = 6.08\text{x}10^{-3}$ intensity$^{0.138}$; $\chi^2_{(1,\, N = 101)} = 17.1$, $P = 3.48 \text{ x } 10^{-5}$, $r = 0.35$). This pattern did not differ across two experiments using different, domestic parasite lines (isolated from commercial guppies instead of wild-caught; line effect: $\chi^2_{(3,\, N = 101)} = 1.89$, $P = 0.596$).

Second, 22 laboratory-maintained lines (including three of four from Fig. 2a) differed in the mean intensity they attained ($F_{21,\, 1149} = 8.49$, $P = 9.36 \text{ x } 10^{-25}$, $\eta^2 = 0.13$), and higher intensity lines imposed a higher mean death rate on infected hosts (Fig. 2b; $\chi^2_{(1,\, N = 22),} = 6.97$, $P = 0.008$, $r = 0.49$). Across intensities, domestic lines imposed less death ($\chi^2_{(1,\, N = 22)} = 14.73$, $P = 1.24 \text{ x } 10^{-4}$, $r = 0.63$; domestic = 0.016 *vs*. wild = 0.052 at overall mean intensity). We used back-transformed, partial residual death rate to control for non-focal predictors (line origin: Fig. 2b shows the relationship as if all lines were wild) so that we could determine the relationship relevant to wild parasites (all results are very similar if instead we set all lines as domestic, see SI).

Third, we predicted the mean transmission rate for each line, given its intensity measurements and the transmission-intensity relationship in Fig. 2a. We found that the mean transmission rate for each line traded-off with the mean virulence ($d+v$ minus a reasonable background death rate, $d = 1.30 \text{ x } 10^{-3}$ day$^{-1}$, see ED Table 2 for symbols, meanings, values, and sources): parasite lines that attained higher intensity benefitted from higher transmission rate but higher virulence shortened their infections, on average (Fig. 2c). Since *Gyrodactylus* spp. are

capable of sexual and asexual reproduction, we tested whether any of our lines were clones (i.e., identical multi-locus genotypes, MLGs). Genotyping a subset (13/22, based on sample availability) found no identical MLGs (all pairs within populations differed at $\geq$ 16 loci, representing at least half of all variable loci for each source population, see SI).

The curvature of this trade-off indicated stabilizing selection on virulence ($v_i = 1.38 \times 10^6 \beta_i^{3.61}$; $d\ v_i$ and $\beta_i$ are average traits for a line). Bootstrapping parasite lines included in the analysis showed this curvature was significant (exponent > 1 in all $10^4$ bootstrapped samples) and AIC indicates a better fit to the data than for a linear model ($\Delta$AIC = 13.3). Theory predicts that the trade-off curvature leads to optimal parasite fitness at intermediate transmissibility ($\beta_i = cT_i$, $c$ is host shoaling rate, $T_i$ is a line's transmissibility) and virulence [Fig. 2d; $R_{0i} = cT_iS/(d+v_i+y)$ for pure infections where we use reasonable values of $S$ = 10 susceptible host m$^{-2}$ and $\gamma$ = 0.020 day$^{-1}$ $d$, see ED Table 2]. Unsurprisingly, parasite lines fell to the right of this optimum: higher background mortality in wild than laboratory guppies shifts the optimum higher; the mixed-stock guppies in our common garden assay may differ substantially from local, wild hosts; and multiple infections (common in the wild, ED table 1) select for higher virulence than would optimize $R_{0i}$ of pure infections[4].

**Eco-coevolutionary model resolves complexity to show that social hosts have deadlier parasites**

Our model provides two general insights. First, it demonstrates that the net, selective effect of predation, shoaling rate, or other ecological factors on virulence depends only on whether they increase or decrease the infectious period (see SI for proof). Second, the model

shows that shoaling rate evolves to balance predator-induced mortality against parasite-induced mortality. When predation increases, hosts evolve higher shoaling rate, increasing transmission rates. When parasites are more abundant and virulent, hosts evolve lower shoaling rate to prevent infection. For some parameter values, higher virulence can select for higher shoaling rate if parasites become so virulent that the density of infected hosts and force of infection decline strongly (ED Fig. 1).

When parametrized with empirical data from the guppy-*Gyrodactylus* spp. system, the model complexity resolves into one dominant pathway: predation increases coevolutionarily stable contact rate and thus virulence (hereafter 'coevolutionary' traits; Fig. 3). When predators only have consumptive effects on predation (i.e., hosts do not evolve), predation decreases prevalence and virulence (grey curves in Fig. 4a-e; presence/absence of parasite evolution has little effect on grey curves). However, as hosts evolve higher shoaling rate in response to predation (Fig. 4a), predation also increases prevalence (black curve in Fig. 4b) in all tested parameter sets (see sensitivity analysis methods). Increasing predation increases overall host mortality and reduces host density, particularly when hosts evolve (Fig. 4c). Further, most of the increase in mortality (76%) with predation is due to increased parasite-induced mortality (prevalence × virulence), rather than increased predator-induced mortality (ED Fig. 2; robust to parameters: ED Fig. 3). Higher shoaling rate increases the force of infection (and thus multiple infections), selecting for higher parasite intensity and virulence (Figs. 4d, e; note intensity was not modelled directly but back-calculated from transmissibility according to Fig. 2b); in all parameter sets, higher predation increased virulence. Importantly, predators increase virulence through their non-consumptive effects on shoaling rate (Fig. 4e; robust to parameters: ED Fig.

3). The consumptive effects of predation only slightly affect virulence (grey curves in Fig. 4e) because decreasing multiple infections is as important as increasing mortality.

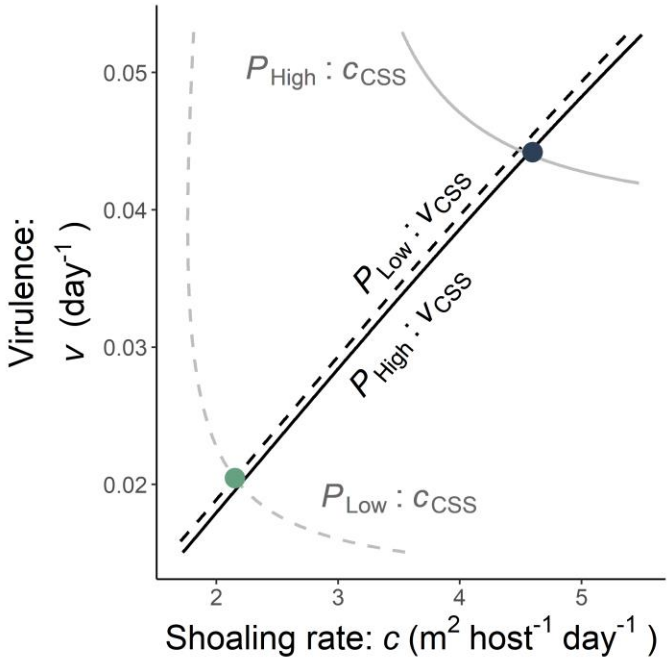



**Figure 3. Predation drives increased shoaling rate and virulence in the eco-coevolutionary model.** *Curves give the continuously stable strategy (CSS) for parasite (*$v_{CSS}$ *black) or host (*$c_{CSS}$ *grey) evolution at predation levels corresponding to natural populations (*$P_{Low}$*; dashed or* $P_{High}$*; solid). Parasites evolve higher virulence with higher shoaling rate. The consumptive effects of predation have little effect on evolved virulence. Hosts evolve lower shoaling rate with increasing virulence (grey curves move toward lower* c *as* v *increases). Predation substantially increases evolved shoaling rate. Host and parasite curves intersect at coevolutionarily stable points (green and blue points). As predation increases (from green point to blue point), coevolutionarily stable shoaling rate and virulence increase.*

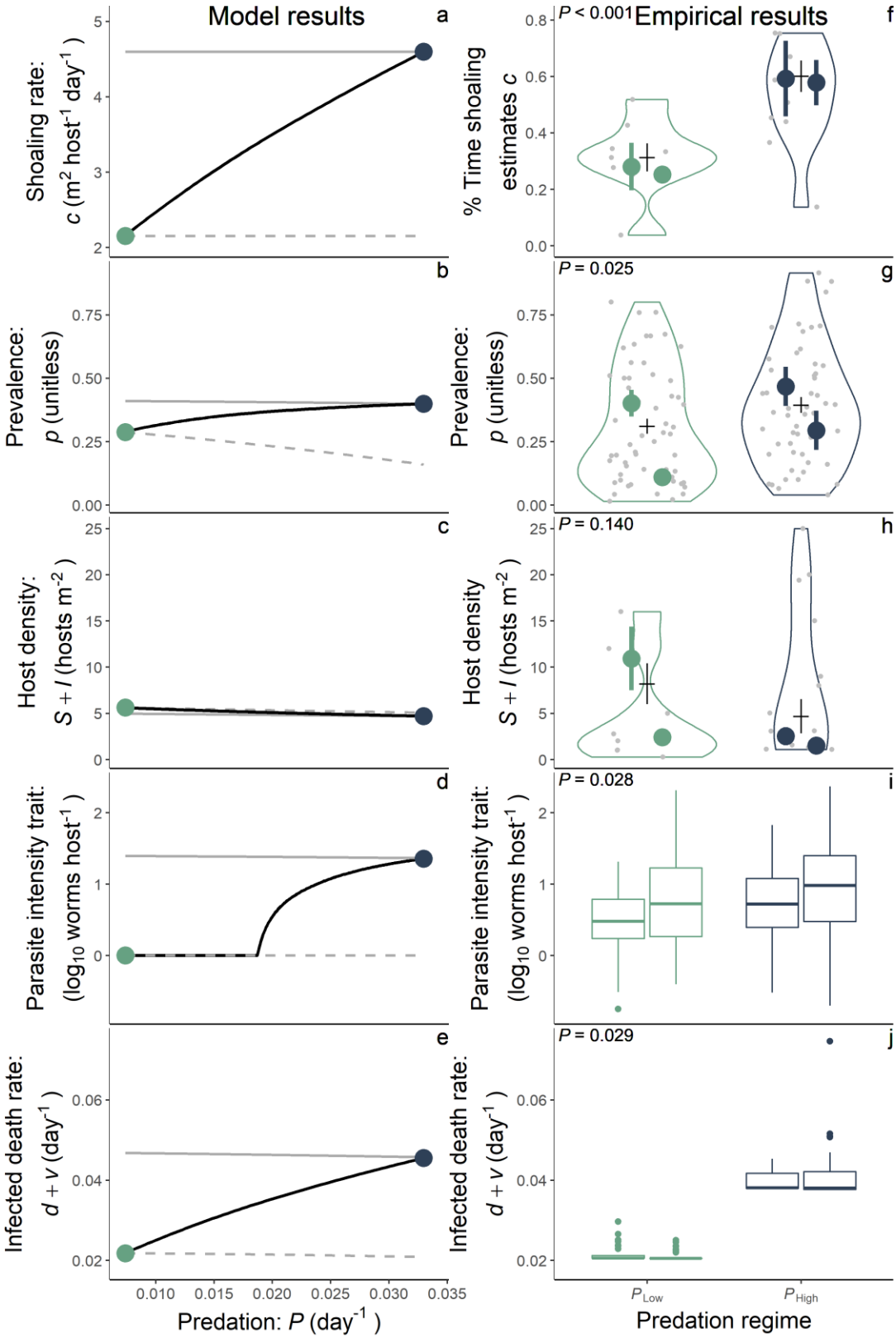
**Empirical results quantitatively match model predictions that social hosts have deadlier parasites**

The model's coevolutionary outcomes successfully fit training data (Fig. 4a-c, f-h; ED table 2). High predation fish spent more time shoaling in our assay ($\chi^2_{(1, N = 68)} = 7.68$, $P = 0.006$, $r = 0.32$) and across population estimates extracted from the literature ($\chi^2_{(1, N = 22)} = 11.17$, $P = 8.31 \times 10^{-4}$, $r = 0.58$; Fig. 4f). While percent time shoaling is unlikely precisely proportional to host-host contact rate (as we use it, see ED Table 2), shoaling is relevant to transmission in this system[34,35]. This helps explain why high predation populations suffered higher parasite prevalence across multiple rivers, sites, and years ($df = 1$, $\chi^2_{(1, N = 107)} = 5.02$, $P = 0.025$, $r = 0.21$; Fig. 4g). Higher levels of predation and parasitism may depress host density (though not significantly so: $df = 1$, $\chi^2_{(1, N = 23)} = 2.18$, $P = 0.140$, $r = -0.29$; Fig. 4h). Overall, the model fit the training data reasonably well, with an average relative error of 10.5% per fitted quantity (shown in Fig. 4f-h and ED table 2).

The trained model predicted higher virulence in high predation populations. We validated this prediction by quantifying the traits of 18 genotypically-distinct parasite lines isolated from wild populations and maintained in the lab under common garden conditions for 65 days. Lines from high predation populations attained higher intensity on ($df = 1$, $\chi^2_{(1, N = 425)} = 4.80$, $P = 0.028$, $r = 0.11$; Fig. 4i) and induced higher death rate for infected, mixed-stock hosts ($df = 1$, $\chi^2_{(1, N = 216)} = 4.75$, $P = 0.029$, $r = 0.15$; Fig. 4j). The two parasite species, *G. turnbulli* (11 known lines) and *G. bullatarudis* (3 known lines) did not differ significantly in intensity ($df = 1$, $\chi^2_{(1, N = 345)} = 2.73$, $P = 0.098$, $r = 0.09$) or virulence ($df = 1$, $\chi^2_{(1, N = 177)} = 0.891$, $P = 0.345$, $r = 0.07$; unlike[36]). Restricting the analysis for Fig. 4j to *G. turnbulli* found marginally higher intensity (*df*

= 1, $\chi^2_{(1, N = 208)}$ = 3.15, $P$ = 0.076, $r$ = 0.12) and significantly higher virulence in high predation populations ($df$ = 1, $\chi^2_{(1, N = 102)}$ = 6.86, $P$ = 0.009, $r$ = 0.25). The overall quantitative match between the model-predicted mortality and our empirical results supports our model's inferences ($d+v$ in the model *vs.* mean of $d+v$ back-transformed partial residuals: low predation 0.020 *vs*. 0.021; high predation: 0.044 *vs*. 0.041). We compared theoretical predictions to partial residuals to control for non-focal predictors in the statistical model, especially duration of parasite maintenance in the laboratory, to obtain the most biologically relevant estimate of virulence. Our theoretical model makes this prediction based on higher shoaling rate increasing multiple infections, which may be more frequent in high predation populations (see ED table 1).

The model-data agreement on evolved virulence aids model-data agreement on how mortality changes across predation regime. In our model, 76% of the increased mortality across predation regime is increased parasite-induced mortality while increased predator-induced mortality accounts for 24%. Prevalence in natural populations and virulence data on laboratory fish ($p_{\mathrm{Hi}}v_{\mathrm{Hi}}$-$p_{\mathrm{Low}}v_{\mathrm{Low}}$) indicated that parasitism would explain 64% of the mortality difference from Reznick *et al*[37] while predation may account for the remaining 36%.


Model results
Empirical results
a
b
c
d
e
f
g
h
i
j
P < 0.001
P = 0.025
P = 0.140
P = 0.028
P = 0.029
Shoaling rate: c (m2 host-1 day-1)
Prevalence: p (unitless)
Host density: S + I (hosts m-2)
Parasite intensity trait: (log10 worms host-1)
Infected death rate: d + v (day-1)
% Time shoaling estimates c
Host density S + I (hosts m-2)
Predation: P (day-1)
Predation regime
PLow
PHigh

230

**Figure 4. Predation increases shoaling rate and thus selects for higher virulence.** ***a-e,*** *Model results. Black curves: eco-coevolution (connecting Fig. 3 coloured points). Grey curves: no host evolution.* ***f-h,*** *empirical results. Horizontal segments: predation regime means. Grey points: one river/regime/year mean. Coloured points: one focal river/predation regime mean (Aripo left of Guanapo). Vertical bars: standard errors. Training data: violins. Non-training data: boxplots.* ***(a, f)*** *Shoaling increased with predation.* ***(b, g)*** *Prevalence increased with predation while* ***(c, h)*** *host density decreased non-significantly.* ***(d, i)*** *Parasite intensity and* ***(e, j)*** *virulence increased with predation (boxplots are back-transformed partial residuals; centre line, median; box limits, first and third quartiles; whiskers, 1.5x interquartile range; points, outliers).*

**Discussion.** Our theoretical-empirical approach clarifies that predation drives the evolution of parasite virulence by increasing host shoaling rate. As a result, shoaling in response to increasing predation pressure leads to parasite-induced mortality rising more than that induced by predators. In contrast to these strong non-consumptive effects, we found that the consumptive effects of predation are small, balance one another out, and barely alter virulence evolution. We discuss key emergent patterns.

How shoaling rate drives virulence evolution depends on multiple infections. Without multiple infections, shoaling decreases predator-induced mortality and thus selects for decreased virulence[38]. With multiple infections, we and others[7] find that increased shoaling rate selects for higher virulence. While we do not know of previous empirical tests of the effect of host-host contact rate on virulence evolution, our result is analogous to more host dispersal[18,39,40] and

parasite founder diversity[17] selecting for higher host exploitation through high local parasite diversity[41].

We assayed parasite traits using infections on mixed-stock, wildtype guppies: this approach allowed us to draw robust conclusions about parasite evolution but leaves untested how host defences besides shoaling rate may affect disease dynamics in natural communities. Thus far, guppy defences against parasites have not been robustly characterised across predation regime, although illustrative data suggest that guppies from low predation regimes are better defended against, and better able to evolve in response to *Gyrodactylus* spp.[30,32,33,42,43]: they need not balance this investment with defence against predators[24], and sexual selection, which acts on gyrodactylid resistance[44,45], is more effective in the absence of predators[46]. If high predation guppies are indeed less well defended against *Gyrodactylus* spp., our common garden assay likely underestimates the difference in virulence that would be expressed in local hosts across predation regimes. How host behavioural and immunological defences may coevolve with parasite virulence in natural communities remains an outstanding and complex theoretical and empirical challenge.

The importance and implications of predation and social parasite transmission, evident from our model and data, may hold across directly transmitted parasites of group-living hosts[47]. Predators drive defensive group-living in animals across taxa[6], which can increase parasitism[48], creating ecological and evolutionary feedbacks between host sociality and parasites[47]. Parasites evolve along virulence-transmission trade-offs in systems ranging from viruses of humans[16], bacterial pathogens of birds[14], protozoan pathogens of insects[15], and our monogenean fish

ectoparasite. Multiple infections are common for many parasites, allowing the simple mechanism of competition for within-host resources to select for higher virulence[20]. Thus, diverse systems likely meet the essential assumptions of our model: predators may frequently shift the antagonistic interplay of host sociality and parasite virulence, driving hosts into the arms of more virulent parasites. Conversely, these results also indicate that social distancing may select for lower virulence when parasites exhibit multiple infections and a virulence-transmission trade-off (e.g., influenza A virus[13,49]). Host behaviour that reduces contact may thus effectively control both the spread and virulence evolution of pathogens and parasites.

**Methods.**

*1. Molecular methods*

Identifying parasites to species. We used two methods of molecular species identification for individual parasites: we sequenced the mitochondrial COII gene for a subset of domestic lines established before March 2020, and we used a newly developed restriction enzyme assay for wild and domestic lines established after March 2020 (see SI for further details). To prevent disrupting data collection, sample collection was restricted to already-dead hosts, stored in 70 % EtOH. This conservative precaution meant we were unable to collect useable samples from some of our parasite lines.

Distinguishing parasite genotypes. We developed panels of single nucleotide polymorphisms (SNPs) for *G. turnbulli* and *G. bullatarudis* to examine the incidence of multi-genotype coinfections from wild collections, and to verify that the lines we established in the lab were genetically distinct. Genotypes were called using Fluidigm SNP genotyping analysis

software. A subset of individuals was re-genotyped to identify and remove error-prone loci and estimate error rates (the proportion of mismatches to matches for re-genotyped loci). Loci which amplified consistently and with score calls greater than 90% were selected for use in the analysis. This resulted in a total of 140 variable loci for *G. turnbulli* (error rate 1.5%) and 83 loci for *G. bullatarudis* (error rate 2 %) across all populations. However, due to significant local variation in informative loci, the total number of loci used for each source population varied (see SI). The resulting multilocus genotypes (MLGs) were assessed using GENALEX 6.502 to estimate the fraction of multi-genotype infections at six sites across Trinidad including our four focal populations (low- and high-predation in the Aripo and Guanapo rivers), and to assess the genotypes of the established lines.

As a conservative estimate, two individuals were considered to have different genotypes if they varied in at least 50% of the total variable loci from a given source population (see SI for further method details). This applied both to determining that our lines were different genotypes and finding the frequency of multiple infections. In finding the frequency of multiple infections, we genotyped worms from fish with at least two worms and accounted for the proportion of fish with only one worm, estimated for each site from our 2020 survey and Stephenson *et al*.[30] (as our 2020 survey lacked power for some sites).

*2. Parasite trait data*

General maintenance. We established parasite lines by transferring a single worm to an uninfected host from our mixed, laboratory-bred stocks descended from wild populations. Each founding worm was obtained from a single guppy either from a commercial supplier ("domestic" lines) or wild-caught in Trinidad ("wild" lines from Caura, Aripo, Guanapo, and Lopinot rivers

in Caroni drainage). Wild-caught adult guppies (~50/population) were shipped from Trinidad to the University of Pittsburgh in March 2020 (see ethics for permit information). We established 43 parasite lines (18 domestic and 25 wild, 10 from low predation populations and 15 from high predation). Different lines were included in different analyses, as explained below. Lines were maintained under common garden conditions for 65 days (some domestic lines were maintained in the lab much longer) on groups of 3-6 guppies. We added uninfected fish to each line as required to replace those that either died or were found parasite-free during twice-weekly screening of all fish in each line under anaesthetic (tricaine methanesulfonate; 4g/L) using a dissecting microscope. During these screens, we recorded the number of parasites infecting each fish. Each line was housed in a single 1.8 L tank on a recirculating system (Aquaneering Inc.; 12L:12D; 24ºC). Recirculated water passes through fine foam, sand, and ultraviolet filters before re-entering other tanks: individual parasites cannot transmit between lines, as supported by our genotyping.

Trait measurements. We estimated transmission rate by re-analysing data from Stephenson *et al.*[50] and conducting a similar new experiment. Donor fish received an infection of one parasite line or multiple infection and were individually housed in 1.8 L tanks (donor fish received line A, B, C, or A and B; no lines in the new experiment were used by Stephenson *et al.*[50]). We added a parasite-naïve recipient fish on day 8 of the donor's infection, and screened both fish for infection every 24 hrs.

We also assayed intensity and death rate. For intensity (a proxy for within-host growth rate), we counted the number of parasites on each host at each date (1 observation). We estimated the per-capita host death rate from the number of fish found dead divided by the

number of infected fish in the tank at the previous observation time point and the days between observations (1 observation; 79% of observations were 3±1 days after the previous one).

Trade-off statistics. We calculated transmission rate from the number of days until successful infection of the recipient and intensity as the number of worms on the donor fish on the day of transmission, following Stephenson *et al.*[50] (total $n$ = 101 transmission events). Worm numbers changed slowly on the scale of the number of days until successful infection. In these assays, dS/dt = $-cT_iIS$ ($c$ is contact rate, $T_i$ is the transmissibility of parasite line i, $I$ is infected host density, and $S$ is susceptible host density) so susceptible hosts follow an exponential decay pattern; thus, mean time until infection is $1/(cT_iI)$. If days until transmission are $D$, then the transmission rate estimated by a given transmission event is $cT_i = 1/(DI)$. Based on the area of enclosures, $I$ = 88.5 hosts m$^{-2}$ for Stephenson *et al.*[50] and $I$ = 72.0 hosts m$^{-2}$ for the follow-up experiment. We fit these estimates of transmission rate to $\log_{10}$ intensity with parasite line (line A, B, C, or coinfection of lines A and B) as a fixed effect using a generalized linear model with a Gamma error family and log link function. Parasite line was non-significant, so we refit the model without parasite line (P-value for intensity reported from this model; all P-values represent two-sided, Type II Wald chi-square or F tests for this and other methods). Effect sizes are $\eta^2$ for a simple ANOVA or partial correlation coefficients, $r$. For all analyses, we provide the code, output, and validation of the model fit in the SI.

When determining average traits for a line, we only included lines maintained in the laboratory for more than 30 days ($n$ = 22; 10 domestic, 12 wild lines, including 1-4 lines from each of the four focal populations). We used an ANOVA to determine whether lines differed significantly in log intensity (1171 observations of intensity for the 22 parasite lines, at least 25 for each line). We modelled mean death rate for each line (344 observations and at least 13 for

each line) as a function of mean intensity and line origin (wild *vs.* domestic) with a beta error family and logit link function. We estimated virulence ($v$) by subtracting background death rate (see ED Table 2) from partial residuals of infected death rate (to get $v$ from $d+v$). We used partial residuals of infected death rate back-transformed onto the response scale to control for non-focal predictors (here, line origin) and find the relationship for wild parasites. We estimated mean transmission rate for a line by mapping the individual intensity measurements for each line onto the relationship observed between intensity and transmission in the experiments described above (Fig. 2a); we then calculated the mean transmission rate for each line. With estimates of transmission rate and virulence for each line, we examined the relationship between them using a generalized linear model of virulence with Gaussian error distribution and log link function as a function of natural log transmission rate. The statistical model was free to fit a positive (exponent > 1), neutral (exponent = 1), or negative curvature (exponent < 1) to these data. We bootstrapped this fit by sampling lines to include in the analysis from the 22 parasite lines with replacement to determine how often the exponent was more than one.

Statistics on predation regime differences in parasite traits. To examine the patterns in Fig. 4i and 4j, we used our estimates of parasite intensity and host death, (calculated as above) as response variables in GLMMs. These traits were assayed from parasites collected from low and high predation populations of our focal rivers (low/high predation; river distances: low Guanapo-high Guanapo = 11.1 km, high Guanapo-high Aripo = 23.9 km, high Aripo-low Aripo = 6.6 km; 3 lines from high-predation Aripo and 5 from each of the other focal populations; 18 lines total). Each observation was of one line at one date but intensity on and death of multiple hosts for each line. This analysis included some lines maintained in the laboratory for less than 30 days but these lines necessarily had fewer observations and thus did not influence the analysis as much as

lines with more observations. For intensity ($n$ = 425 observations), we used predation regime, river, and number of fish in the common garden at time of measurement (since *Gyrodactylus* spp. can move relatively freely among fish) as fixed effects. Parasite line and lineday (days the line had been in lab) nested within parasite line were included as random effects. We used log(intensity) as the response variable. For death rate ($n$ = 216), we used predation regime and river as the fixed effects with parasite line and lineday nested within line as random effects, a Tweedie error family, and a log link function. The same analyses were used when determining the effect of predation regime on intensity ($n$ = 208) and host death rate ($n$ = 102) for known *G. turnbulli* lines. We compare the theoretical model to mean partial residuals of infected host death rate, back-transformed onto the response scale, to control for non-focal predictors, such as lineday, to find the most representative estimate of death rate in each predation regime.

Statistics on species differences in parasite traits. To determine trait differences by species, we considered all wild lines that were identified to species (including 7 from rivers considered non-focal due to lack of high predation *vs.* low predation comparisons). Three of these lines were *G. bullatarudis* and 11 were *G. turnbulli*. Because all three *G. bullatarudis* lines were from high predation populations, we only compared species differences within that regime. Species identity was used as a predictor instead of predation regime but statistical models were otherwise identical to those for the effect of predation regime for intensity ($n$ = 345) and infected host death rate ($n$ = 177).

### 3. *Theoretical modelling*

Model ecology. We wrote a relatively simple, ordinary differential equation model that retains the key biology of the predator-prey/host-parasite system. In the model, predators are not

limited by prey/host density (e.g., because predators are generalists), allowing us to represent predation as a parameter ($P$) for simplicity instead of a state variable. Susceptible hosts ($S$) become infected ($I$) and can recover to an immune state ($R$) with waning immunity (see ED Table 2 for symbols, values, and units):

$$dS/dt = [a - q(S + I + R)](S + I + R) - (d + \frac{Pc_1}{c})S - cTIS + zR \quad (1a)$$

$$dI/dt = cTIS - (d + \frac{Pc_1}{c})I - vI - \gamma I \quad (1b)$$

$$dR/dt = \gamma I - (d + \frac{Pc_1}{c})R - zR \quad (1c)$$

Susceptible hosts are born from all hosts with density dependent, per-capita birth rate $a$-$q(S+I+R)$ (i.e., hosts grow logistically, eq. 1a). Hosts die at background rate $d$ and from predation at rate $Pc_1/c$ that decreases with host shoaling rate ($c$; that $P$ and $c_1$ together determine the strength of predation). Along with parasite transmissibility given contact ($T$), the rate of density-dependent transmission from $I$ to $S$ depends on the host-host contact rate, dictated by shoaling rate. Recovered hosts also move back into the susceptible class as immunity wanes at rate $z$ (average duration of immunity is $1/z$).

Infected hosts (eq. 1b) suffer background mortality and predation (predation is not selective in this model) while suffering additional mortality due to virulence, $v$. If predation was selective, especially to remove the sickest hosts, the consumptive effects of predation would be even more likely to select against virulence[3], strengthening our conclusion that shoaling rate drives virulence (Fig. 4). Infected hosts recover with rate $\gamma$ (eq. 1c). Recovered hosts suffer background mortality and predation while losing immunity over time.

<u>Model evolution:</u> We modelled parasite and host evolution via Adaptive Dynamics[51]. Biologically, parasite evolution is rendered more complex by the presence of multiple lines as

well as two phenotypically similar species; host evolution is also complex because shoaling rate has important genetic and plastic components[31]. For both host and parasite, our model is agnostic regarding the basis of adaptation but simply examines competition between phenotypes based on fitness of the rarer phenotype (i.e., invasion analysis). Each phenotype corresponds to an asexual genotype in the model. Parasite fitness when invading depends on the traits of the mutant (m) and resident genotypes (r):

$$\frac{1}{I_\mathrm{m}}\frac{dI_\mathrm{m}}{dt} = cT_\mathrm{m}S_\mathrm{r}^* - d - \frac{Pc_1}{c} - v_\mathrm{m} - \gamma + \sigma cT_\mathrm{m}I_\mathrm{r}^* - \sigma cT_\mathrm{r}I_\mathrm{r}^* \quad \text{(2a)}$$

$$v_\mathrm{i} = k_1 T_\mathrm{i}^{k_2} \quad \text{(2b)}$$

The resident sets host densities at $S_\mathrm{r}$ and $I_\mathrm{r}$ which depend on its traits, $T_\mathrm{r}$ and $v_\mathrm{r}$. Mutant fitness depends on transmission to susceptible hosts, background death, predation, virulence, recovery, and gains and losses due to superinfection (eq. 2a). In superinfection, parasites of one genotype take over a host already infected by another genotype. We assume that each genotype has the same probability of successful superinfection given transmission, $\sigma$. This assumption is likely conservative as the probability of successful superinfection is often expected to increase with virulence[4], which could amplify the impact of contact rate to select for higher virulence. From parasite fitness when invading, we find the continuously stable strategy[51] (CSS) for parasite virulence; a CSS represents a trait that can be reached by gradual trait changes but that cannot be invaded by rare phenotypes with slightly different traits.

For host fitness, we used the Next Generation Matrix technique[52] that accounts for fitness within each class and the rates of movement between classes (see SI). In the model, host genotypes differ in their contact rates, $c_i$. An invading host genotype (contact rate $c_\mathrm{m}$) contacts the resident genotype ($c_\mathrm{r}$) at rate sqrt($c_\mathrm{m}c_\mathrm{r}$) (for the purposes of transmission and predation), following Bonds et al.[38]. An invading genotype suffers crowding (-$q$ term, eq. 1a) from all hosts.

Only intersections of a host CSS curve and parasite CSS curve (Fig. 3) can be coevolutionary stable points (coCSSs). We confirmed that a potential coCSS is indeed a coCSS with the strong convergence stability criterion[53].

Model parametrization. We have empirical estimates of some, but not all, of the parameters of the eco-coevolutionary model. As such, we used a simple evolutionary algorithm (described below) to estimate all model parameters, using empirical parameter estimates and field estimates of model outputs (e.g., prevalence) to fit the model to the data. Importantly, we did not use empirical data on evolved virulence in the fitting process, i.e., model virulence is free to evolve along the virulence-transmissibility trade-off however best fits the other data; instead, we used virulence data to validate the model.

Many empirical estimates came from the literature (see below and ED table 2). We estimated maximum birth rate ($a$) from lifetime fecundity of guppies with high food[54] in the laboratory. We estimated background mortality in the absence of parasites and predators ($d$) as the inverse of life expectancy in the laboratory[54]. We estimated recovery rate ($\gamma$) as the inverse of the time required for fish to clear infection in the laboratory[55]. We estimated the rate at which immunity wanes ($z$) from the observation that fish were roughly half resistant (averaging across totally resistant, partially resistant, and not resistant) 21 days after initial infection[56]. Our trait measurements provided estimates for the trade-off parameters (Fig. 2c) given an estimate of $d$.

For parameters where we lacked estimates [importantly $Pc_1$ (which acts as one parameter) in low- and high-predation populations and the superinfection parameter, $\sigma$], we also fit the model's eco-coevolutionary outputs at low and high predation to estimates of such outcomes in the field. We converted recapture rates from a mark-recapture experiment[37] into instantaneous mortality rates to determine overall mortality rate in the field ($d+Pc_1/c+pv$) for both a high predation

population and a low predation population. We found a transmission rate reasonable for low predation populations ($cT_{\mathrm{Low}}$) by fitting transmission rate to peak prevalence and timing of peak prevalence found for low predation Aripo guppies and parasites in a stream mesocosm[57]. We also used estimates of shoaling rate, prevalence, and host density (see SI). We were able to fit some outputs to estimates from low predation populations, high predation populations, and the ratio of the two (representing the impact of changing predation regime). For others, we only have estimates of one of these three. This scheme weights the model training toward quantities that are well-characterized. See ED Table 2 for estimates used and sources.

We optimized the model's fit to both these empirical parameter estimates and field-estimated model outputs with the evolutionary algorithm[58]. We began with parameters at their estimated values (for $a$, $d$, $k_1$, $k_2$, $\gamma$, and $z$). Initial values of other parameters were chosen arbitrarily [$q = 1 \times 10^{-2}$, $(Pc_1)_{\mathrm{Low}} = 1 \times 10^{-2}$, $(Pc_1)_{\mathrm{Hi}} = 3 \times 10^{-2}$, $\sigma = 0.5$]. Each parameter was mutated by a factor of 10^$x$ where $x$ is a single sample from the normal distribution $N(0,0.05)$ to create a parameter set; each parameter set formed one strategy in the evolutionary algorithm. One un-mutated strategy and 299 mutated strategies were evaluated in one "generation" of the algorithm by their summed relative error from all inputs and outputs with known estimates: summed relative error of strategy x = $|a_x - a_{\mathrm{data}}|/a_{\mathrm{data}} + \ldots + |cT_{\mathrm{Low},x} - cT_{\mathrm{Low},\mathrm{data}}|/cT_{\mathrm{Low},\mathrm{data}}$. There are four constrained parameters, four unconstrained parameters, and 8 independent scored outputs (e.g., $p_{\mathrm{Low}}$ is independent from $p_{\mathrm{Hi}}$ but not $p_{\mathrm{Hi}}/p_{\mathrm{Low}}$). The strategy with the lowest summed relative error was passed to the next generation of the algorithm along with 299 mutated versions of itself. This process continues for 20 generations of the algorithm, as the model had asymptotically converged to a fit, yielding the parameter values used for the model (see ED Table 2).

Sensitivity analysis. We performed a sensitivity analysis to determine how key outcomes responded to parameter values. We performed this analysis for coCSS parasite prevalence, host density, parasite virulence, host contact rate, overall host mortality, transmission rates, and the ratios of these quantities between high and low predation populations. We also examine two other key 'proportion change metrics' representing how key factors change between the two, focal predation levels. First was the proportion of total host mortality change with predation regime that is caused by increased parasite-induced mortality: $(p_{Hi}v_{Hi}-p_{Low}v_{Low})/[(d+p_{Hi}v_{Hi}+P_{High}c_1/c_{Hi}) - (d+p_{Low}v_{Low}+P_{Low}c_1/c_{Low})]$. Second was the proportion of change in coevolutionary virulence across predation regime that is via predator's non-consumptive effects (evolution of higher shoaling rate). We defined this proportion as 1- (virulence change when predators only have consumptive effects/virulence change when predators have consumptive and non-consumptive effects); the virulence change when predators only have consumptive effects was calculated as mean($v_{CSS\ high\ P,\ low\ c} - v_{CSS\ Plow,\ low\ c}$, $v_{CSS\ Phigh,\ high\ c} - v_{CSS\ Plow,\ high\ c}$) and virulence change when predators have both is $v_{coCSS\ Phigh} - v_{coCSS\ Plow}$ (Fig. 3). To generate random parameter sets, we used Latin Hypercube sampling with the 'lhs' R package[59] and then computed the partial rank correlation coefficients of the parameters with respect to each model outcome of interest (coevolutionary outcomes and key proportion change metrics) for $5 \times 10^3$ runs using the R package 'sensitivity'[60]. We provide the code for the model and sensitivity analysis in the SI.

*4. Analysis of shoaling rate, prevalence, and host density*

We extracted estimates of shoaling rate, prevalence, and host density from the literature and supplemented them with our own, new data on prevalence and shoaling rate (see SI). We used mixed models to test if guppy shoaling behaviour, *Gyrodactylus* spp. prevalence, and guppy density differed between predation regimes. In these models we used predation regime as a fixed

effect along with river and year as random effects. We only included rivers with paired estimates from high and low predation populations because of variation across rivers. Each population average for a given site+year was one data point in these analyses. For % time shoaling (22 estimates, 4 rivers)[61–64] and prevalence (107 estimates, 11 rivers)[30,32,33,43,57,65,66], we used a generalized linear mixed model beta regression with a logit link. For host density (23 estimates, 4 rivers)[67–71], we used a linear mixed model.

### *5. Data availability statement*

The authors declare that all data used in this study are available within this publication and its supplementary files.

### *6. Code availability statement*

Custom computer code used to conduct the theoretical modelling and statistical tests is included in the supporting information.

### *7. Animal use ethics statement*

All collections and fish handling protocols were approved by IACUC protocol 18072155 and 21069471 from the corresponding author's institution. The permit to collect and export guppies was granted by the Director of Fisheries in the Ministry of Agriculture, Land and Fisheries Division, Aquaculture Unit of the Republic of Trinidad and Tobago on 2/26/2020 (copy available on request). The United States Import/Export License for live and preserved fish was issued by the U.S. Fish and Wildlife Service, Office of Law Enforcement Permit number

A107080 on February 21, 2020 for the shipment of live guppies to the corresponding author's institution. Samples were declared and cleared via USFWS form 3-177 on 03/11/2020.

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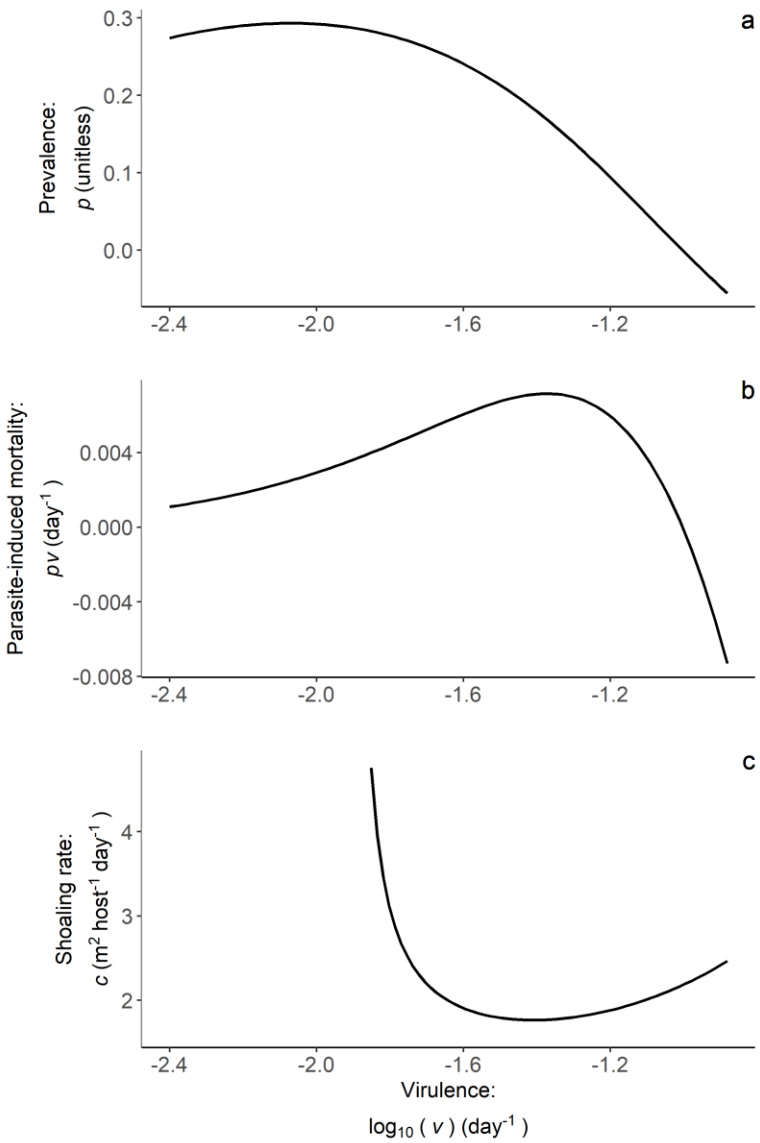
**Extended Data Table 1. Coinfection rates in the wild.** *For each site (river+predation regime), we genotyped multiple worms per a sample of fish hosting more than one worm. Coinfection within species = (no. fish with >1 genotype of species x/no. fish with > 1 worm of species x) × % of infections with >1 worm. Overall coinfection = (no. fish with at least two different worms/no. fish with at least two worms) × % of infections with >1 worm. Infections were diverse overall. Coinfection by identical multilocus genotypes was rare (31 out of the 73 worms possible).* G.t. = G. turnbulli *and* G.b. = G. bullatarudis.

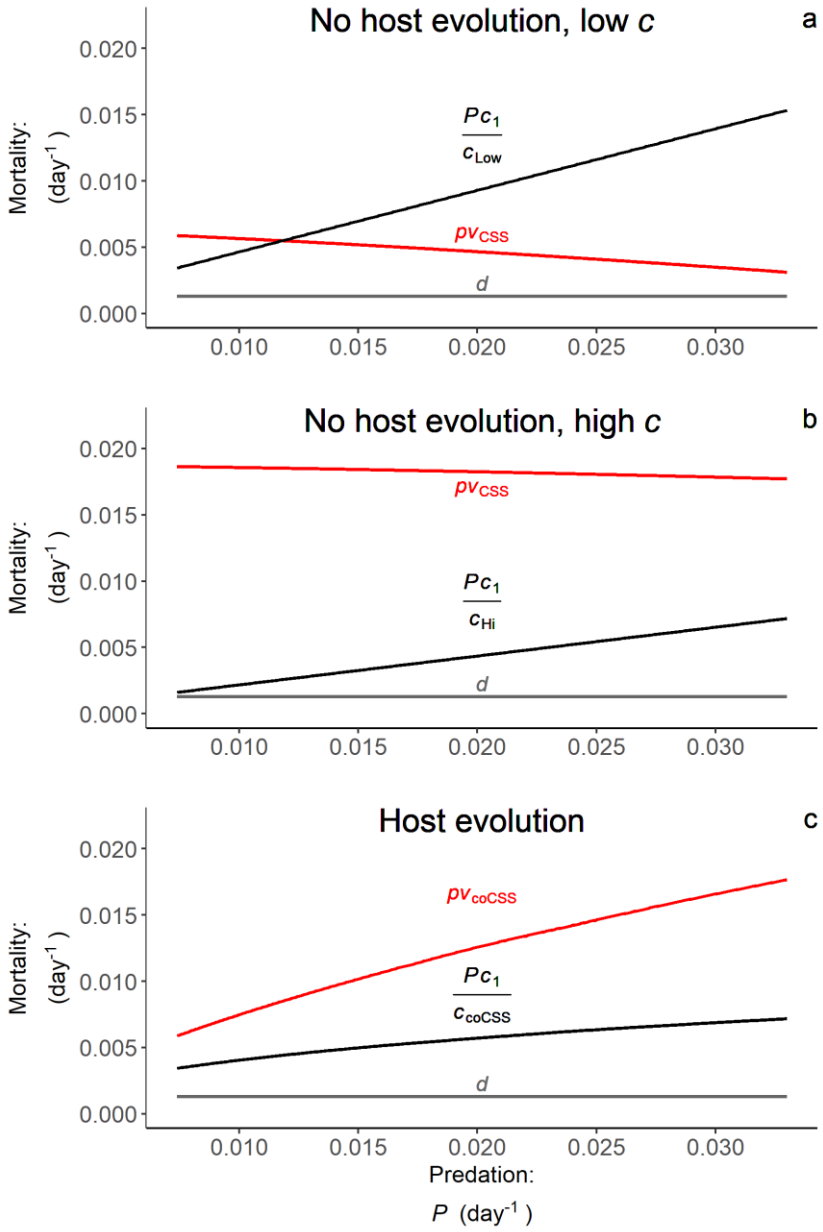
| **Site** | | **Sample size** | | **Species presence on fish** | | | **Infections >1 worm** | **Coinfection within species** | | **Overall coinfection rate** |
|---|---|---|---|---|---|---|---|---|---|---|
| **River** | **Predation** | **Fish** | **Worms/fish (mean±SE)** | **%*G.t.* only** | **%*G.b.* only** | **%Both** | **%** | ***G.t.*** | ***G.b.*** | **%** |
| Aripo | High | 7 | 2.43±0.20 | 14.3 | 85.7 | 0 | 70.8 | 0 | 35.4 | 30.3 |
| Aripo | Low | 4 | 3.25±0.75 | 100 | 0 | 0 | 58.2 | 14.6 | NA | 14.6 |
| Guanapo | High | 7 | 2.29±0.18 | 14.3 | 85.7 | 0 | 62.5 | 62.5 | 52.1 | 53.6 |
| Guanapo | Low | 5 | 2 | 80 | 20 | 0 | 61.9 | 15.5 | 0 | 12.4 |
| Santa Cruz | High | 7 | 4.86±1.30 | 0 | 71.4 | 28.6 | 92.6 | 92.6 | 92.6 | 92.6 |
| Santa Cruz | Low | 3 | 3±1 | 66.7 | 33.3 | 0 | 61.7 | 30.9 | 61.7 | 41.1 |

**Extended Data Table 2. Meaning, value, and units for state variables, parameters, or outputs.** *Training values may be for a parameter, an output, or outputs related to a parameter or output. Training values were used in the model fitting and their sources are provided. Low/hi. pred. indicates predation regime. Values result from the model training. Note that the model was not trained with evolved virulence data but instead validated against it.*

| Symbol | Meaning | Value and/or units | Training value | Source |
|---|---|---|---|---|
| $S$ | Susceptible host density | hosts $m^{-2}$ | | |
| $I$ | Infected host density | hosts $m^{-2}$ | | |
| $R$ | Recovered host density | hosts $m^{-2}$ | | |
| $(S+I+R)_{Low}$ | Total host density, low pred. | 5.61 hosts $m^{-2}$ | 8.19 | 67–71 |
| $(S+I+R)_{Hi}$ | Total host density, hi. pred. | 4.69 hosts $m^{-2}$ | 4.68 | 67–71 |
| $(S+I+R)_{Hi}/(S+I+R)_{Low}$ | Host density ratio | 0.837 hosts $m^{-2}$ | 0.571 | 67–71 |
| $p$ | Prevalence: $p = I/(S+I+R)$ | unitless | | |
| $p_{Low}$ | Prevalence, low pred. | 0.288 | 0.311 | 30,32,33,43,57,66 |
| $p_{Hi}$ | Prevalence, hi. pred. | 0.400 | 0.394 | 30,32,33,43,57,66 |
| $p_{Hi}/p_{Low}$ | Prevalence ratio | 1.39 | 1.27 | 30,32,33,43,57,66 |
| $a$ | Maximum host per-capita fecundity | 0.106 $day^{-1}$ | $a$ = 0.106 | 54 |
| $c$ | Host social contact rate | 1.7-5.5 $m^2$ $host^{-1}$ $day^{-1}$ | | |
| $c_{Hi}/c_{Low}$ | Contact rate ratio | 2.23 | 1.93 | 61–64 |
| $c_1$ | Scaling parameter for contact rate | 1 $m^2$ $host^{-1}$ $day^{-1}$ | | |
| $d$ | Background host death rate | $1.30 \times 10^{-3}$ $day^{-1}$ | $d = 1.41 \times 10^{-3}$ | 54 |
| $k_1$ | Trade-off parameter: Virulence with transmissibility = 1 | $1.38 \times 10^{6}$ $day^{-1}$ | See text | Fig. 2c |
| $k_2$ | Trade-off parameter: Exponent | 3.61 | See text | Fig. 2c |
| $P$ | Predation regime | 7.4-33.0$\times 10^{-3}$ $day^{-1}$ | | |
| $(d+Pc_1/c+pv)_{Low}$ | Overall host mortality, low pred. | 0.011 $day^{-1}$ | 0.011 | 37 |
| $(d+Pc_1/c+pv)_{Hi}$ | Overall host mortality, hi. pred. | 0.026 $day^{-1}$ | 0.026 | 37 |
| $(d+Pc_1/c+pv)_{Hi}/(d+Pc_1/c+pv)_{Low}$ | Overall host mortality ratio | 2.46 | 2.47 | 37 |
| $q$ | Density dependence of host fecundity | 0.017 $m^2$ $host^{-1}$ $day^{-1}$ | | |
| $T$ | Transmissibility given contact | 6.2-8.81$\times 10^{-3}$ | | |
| $(cT)_{Low}$ | Transmission rate, low pred. | 0.015 $m^2$ $host^{-1}$ $day^{-1}$ | 0.014 | 57 |
| $cTI$ | Force of infection | $day^{-1}$ | NA | |
| $v$ | Mortality virulence | 0.015-0.053 $day^{-1}$ | Intentionally not trained | |
| $v_{Low}$ | Virulence, low pred. | 0.020 $day^{-1}$ | Validated: 0.021 | Fig. 4j |
| $v_{Hi}$ | Virulence, hi. pred. | 0.044 $day^{-1}$ | Validated: 0.041 | Fig. 4j |
| $z$ | Rate of waning of host immunity | 0.033 $day^{-1}$ | z = 0.033 | 56 |
| $\gamma$ | Infected host recovery rate | 0.020 $day^{-1}$ | y = 0.033 | 55 |
| $\sigma$ | Probability of successful superinfection given transmission | 1.21 | | |

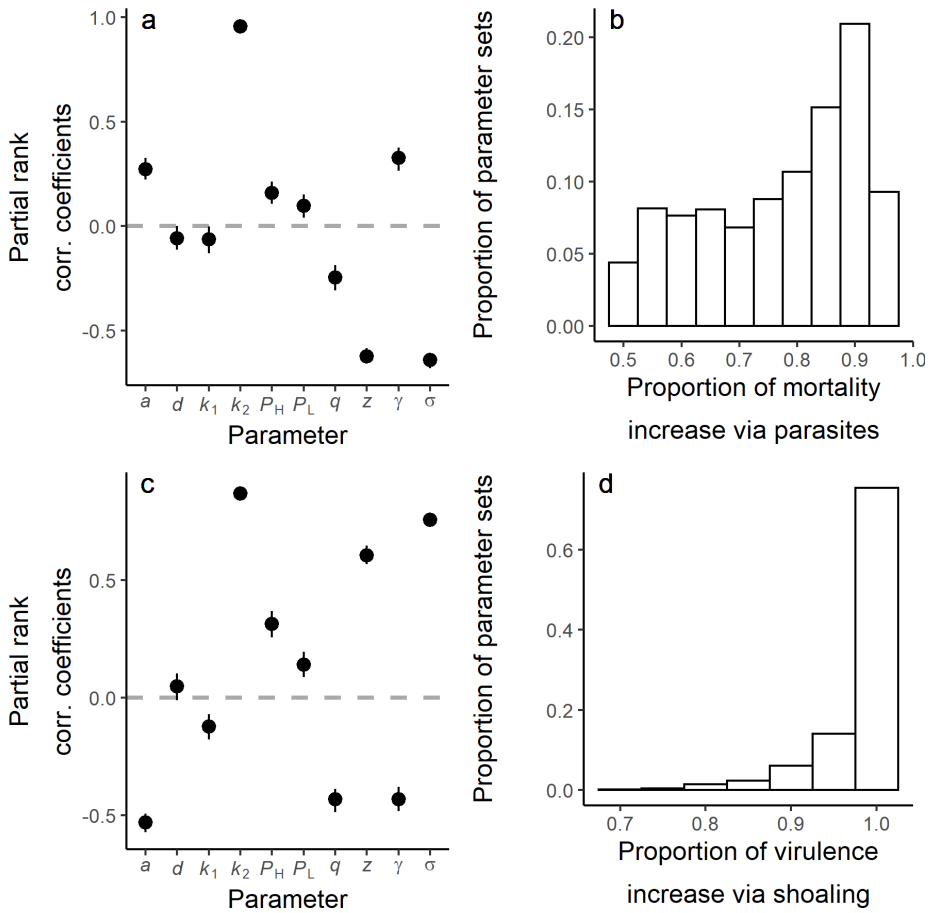



**Extended data figure 1. Virulence can select for increased contact rate.** *Hosts can evolve increasing contact rate in response to increased virulence, especially at very high virulence (beyond range used in main text).* ***a****, Increasing virulence (and transmissibility along the trade-off) can decrease prevalence.* ***b,*** *Overall parasite-induced mortality can decrease if prevalence declines sharply enough. This decrease occurs because, while parasites are very virulent, very few hosts are infected and suffering that virulence.* ***c,*** *At high virulence, increasing virulence can select for higher host contact rates.* c *= 2 and* P *= 0.074 used. All other parameters at default.*




**Extended data Figure 2. Host evolution in response to increasing predation causes parasite-induced mortality (red curves) to increase more than predator-induced mortality (black curves). *a,*** *Without host evolution (contact rate,* c*, set to the green point in Figs. 3 while parasites evolve to some CSS), parasite-induced mortality declines with predation while predator-induced mortality increases. Death from background sources (*d*, grey line) does not*

*change.* ***b,*** *This trend is similar for a higher* c *(set to high, blue point in Fig. 3).* ***c,*** *When hosts evolve increasing* c *with increasing predation (coCSS curve in Fig. 3), parasite-induced mortality increases more than predator-induced mortality. This pattern is due to host evolution and is qualitatively unchanged if hosts evolve but parasites do not.*




**Extended data Figure 3. Sensitivity analysis shows the impact of parameters on key outputs and the range for those outputs.** *Key model outputs: the proportion of the mortality increase from increasing predation that is via increased parasite-induced mortality* ***(a-b)*** *and the proportion of the virulence increase from increasing predation that is via increased contact rate* ***(c-d)****. Vertical lines are 95% confidence intervals.* ***(a & c)****.* ***a,*** *Notable among other parameters, the trade-off exponent,* $k_2$*, strongly increases the proportion of mortality increase due to*

*parasites.* ***b,*** *The mortality increase via parasites is typically well over half (0.5).* ***c,*** *Both* $k_2$ *and* $\sigma$*, the superinfection parameter, strongly increase the proportion of virulence increase via contact rate.* ***d,*** *The proportion of virulence increase via contact rate is typically well over half. Note here that* $P_H = P_{Hi}$ *and* $P_L = P_{Low}$.

**Extended data table 3. Parameter effects based on 95% CIs.** *For each model output (first column), we report which parameters increased (second column) or decreased (third column) that model output in the sensitivity analysis. A positive or negative effect is only reported if the 95% CI for that parameter's effect did not overlap 0. Trait outputs are the coCSS trait values.*

| Model output | Parameters w. + effect | Parameters w. – effect |
|---|---|---|
| $p_{Low}$ | $a$, $k_2$, $P_{Low}$, $z$ | $k_1$, $q$, $\gamma$, $\sigma$ |
| $p_{Hi}$ | $a$, $k_2$, $P_{Hi}$, $z$ | $k_1$, $q$, $\gamma$, $\sigma$ |
| $p_{Hi}/p_{Low}$ | $d$, $k_1$, $P_{Hi}$, $q$, $z$, $\gamma$, $\sigma$ | $a$, $k_2$, $P_{Low}$ |
| $(S+I+R)_{Low}$ | $a$, $k_1$, $\gamma$ | $k_2$, $P_{Low}$, $q$ |
| $(S+I+R)_{Hi}$ | $a$, $k_1$, $\gamma$ | $k_2$, $P_{Hi}$, $q$ |
| $(S+I+R)_{Hi}/(S+I+R)_{Low}$ | $a$, $k_1$, $P_{Low}$, $q$, $\gamma$ | $k_2$, $P_{Hi}$ |
| $v_{Low}$ | $a$, $k_2$, $P_{Low}$, $\gamma$, $\sigma$ | $k_1$, $q$, $z$ |
| $v_{Hi}$ | $a$, $k_2$, $P_{Hi}$, $\gamma$, $\sigma$ | $k_1$, $q$, $z$ |
| $v_{Hi}/v_{Low}$ | $a$, $k_2$, $P_{Hi}$ | $P_{Low}$, $\gamma$, $z$ |
| $c_{Low}$ | $k_1$, $P_{Low}$, $q$, $\gamma$ | $a$, $k_2$, $z$, $\sigma$ |
| $c_{Hi}$ | $k_1$, $P_{Hi}$, $q$, $\gamma$ | $a$, $k_2$, $z$, $\sigma$ |
| $c_{Hi}/c_{Low}$ | $k_2$, $P_{Hi}$ | $P_{Low}$, $q$, $z$, $\sigma$ |
| $(d+Pc_1/c+pv)_{Low}$ | $a$, $d$, $k_2$, $P_{Low}$ | $k_1$, $q$, $\gamma$ |
| $(d+Pc_1/c+pv)_{Hi}$ | $a$, $k_2$, $P_{Hi}$ | $k_1$, $q$, $\gamma$ |
| $(d+Pc_1/c+pv)_{Hi}/(d+Pc_1/c+pv)_{Low}$ | $a$, $k_1$, $P_{Hi}$, $\gamma$ | $d$, $k_2$, $P_{Low}$ |
| Proportion of mortality change via parasites | $a$, $k_2$, $P_{Hi}$, $P_{Low}$, $\gamma$ | $k_1$, $q$, $z$, $\sigma$ |
| $cT_{Low}$ | $k_2$, $P_{Low}$, $q$, $\gamma$ | $a$, $k_1$, $z$, $\sigma$ |
| $cT_{Hi}$ | $k_2$, $P_{Hi}$, $q$, $\gamma$ | $a$, $k_1$, $z$, $\sigma$ |
| $cT_{Hi}/cT_{Low}$ | $k_2$, $P_{Hi}$ | $P_{Low}$, $q$, $z$, $\sigma$ |
| Proportion of virulence change via contact rate | $k_2$, $P_{Hi}$, $P_{Low}$, $z$, $\sigma$ | $a$, $k_1$, $q$, $\gamma$ |