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The effect of host density on parasite infection: a case study of blue mussels *Mytilus edulis* and their trematode parasites

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Abstract

Host density is an important factor determining transmission success of parasites in marine ecosystems. Here we suggest a simple model linking mean abundance (the proportion of the number of parasite individuals found to the number of host individuals examined) and parasite density (parasite population per area) with the density of the host for parasites with a complex life cycle, where an infected host cannot be the source of infection for conspecifics. The model predicts a power-law dependence: negative for mean abundance and positive for parasite density. This means that the increase of the host density simultaneously reduces the average load per host individual and increases the transmission success of the parasite. For the case when host density is estimated "per unit area" (e.g., ind. per m²), our model predicts the exponents of this dependence of -0.5 for mean abundance and 0.5 for parasite density. We tested the model on our data on accumulation of metacercariae of trematodes Himasthla spp. and Cercaria parvicaudata (Renicola sp.) in second intermediate hosts, mussels Mytilus edulis, at the White Sea intertidal and found a good correspondence between the empirical data and the model ($R^2 = 0.7 - 0.9$). A positive correlation between the density of the parasite population and the host density suggests that dense host settlements with a lower infection level (prevalence, mean abundance and mean intensity) should play a greater role in the transmission of the parasite than sparse settlements with a high infection level.

Keywords

Trematoda · Mytilus edulis · Host density effect · Model · Intertidal · Parasite transmission

Introduction

Transmission of parasites depends on many things such as biological features of infective stages, environmental parameters and host-dependent factors. Host density plays an important role among the latter, since as it grows, the probability of the host encounter with the parasite increases (see for review Roberts et al. 2002). In the case of parasites with direct (one-host) life cycle, infected hosts become the source of infection of the conspecifics, ensuring the growth of all infection indices (prevalence, mean intensity, mean abundance and parasite population) with the increasing host density (Dobson and Hudson 1992; Hudson et al. 1992; Côté and Poulin 1995; Arneberg et al. 1998; Arneberg 2001; Rifkin et al. 2012; Patterson and Ruckstuhl 2013).

In the case of parasites with complex life cycles, such as digenetic trematodes (Trematoda, Digenea), which involve several categories of hosts (final host, one or several intermediate hosts), infected host individuals are not infective for conspecifics. Therefore, the parasite supply is determined by the upstream host [the host "from which" the parasite comes (Combes 2001)], which underlies the positive correlation between the indices of infection of downstream (target) hosts [the next host in the life cycle "to which" the parasite goes (Combes 2001)] and abundance and density of upstream hosts. This correlation has been repeatedly noted for parasites with complex life cycles, including trematodes. Some examples are a positive dependence between the infection indices of intertidal gastropods (the first intermediate hosts of trematodes) and the abundance of birds (their final hosts) (e.g. Matthews et al. 1985; Bustnes et al. 2000; Smith 2001; Skírnisson et al. 2004; Hechinger and Lafferty 2005; Fredensborg et al. 2006; Byers et al. 2008; Merlo and Etchegoin 2011; Levakin et al. 2013a; Galaktionov et al. 2015) and a positive dependence between the density of gastropods (the first intermediate host of Himasthla spp. and Renicola roscovita) and the infection mean intensity of cockles *Cerastoderma edule* (the second intermediate hosts) by metacercariae of these trematodes (Thieltges and Reise 2007).

In theory, an increasing density of the downstream host (at the background of the same parasite supply from the upstream host) should increase the probability of the encounter with infective stages and thus the number of parasites successfully infecting the hosts. In this way, the total transmission success and, respectively, the parasite population should increase. On the other hand, the growth of the downstream host density results in the distribution of the limited number of infective stages across a larger number of host individuals, i.e. in the decreasing mean abundance of the parasite and the average load on a host individual. This effect was described by Buck et al. (2017) with the help of the term "safety in numbers" (Turner and Pitcher 1986; Lehtonen and Jaatinen 2016). These apparent effects of increased host density on mean abundance (decrease) and population size (increase) of a parasite with a complex life cycle are sometimes considered as a manifestation of the encounter-dilution effect (Buck and Lutterschmidt 2017; Buck et al. 2017). To note, Mooring and Hart (1992) applied the term "encounter-dilution effect" to "protection when the probability of detection of a group does not increase in proportion to an increase in group size (the encounter effect), provided that the parasites do not offset the encounter effect by attacking more members of the group (the dilution effect)." Thus, the encounter-dilution effect describes a situation involving the infection of grouping hosts (aggregated distribution) but not the infection of host patches with various density. Therefore, the character of the influence of the density of hosts on their infection with infective stages of parasites with complex life cycles requires verification.

Speaking of trematodes, a decreasing mean abundance of the parasites under condition of increasing density of the hosts is clearly manifested in case of infection of the second intermediate hosts (bivalves and fish were considered) with cercariae (Mouritsen et al. 2003; Thieltges and Reise 2007; Prinz et al. 2011; Rohr et al. 2015; Buck and Lutterschmidt 2017). In case of infection of snails (the first intermediate host) with the eggs and free-swimming miracidia, a negative association between trematode prevalence and snail density was found (Ewers 1964; Wilson and Taylor 1978; Johnson et al. 2012; Buck et al. 2017). Several authors

(Buck and Lutterschmidt 2017; Buck et al. 2017) also observed a growth of the parasite population under conditions of increasing host density.

In a number of cases, no statistically significant correlation between infection indices and host density was observed in natural host settlements of various density (Hansen and Poulin 2006; Studer et al. 2013; Galaktionov et al. 2015). However, the comparison of different host settlements can be uninformative because the settlements under comparison may differ as to the parasite supply, associated with the abundance or behaviour of the upstream host (Hansen and Poulin 2006; Galaktionov et al. 2015), and as to the biotopic features essential for the parasite transmission. Therefore, for a reliable assessment of the effect of host density on the indices of its infection with parasites with a complex life cycle experimental studies should be conducted within one and the same biotope. The most suitable experimental design would involve exposure in the chosen marine biotope of several cages containing uninfected individuals of the organism serving as the downstream host of one or several parasites species transmitted in this biotope.

The results of cage experiments of this kind involving marine molluscs *Cerithideopsis californica* and digenean intramolluscan stages parasitising them, the data from field surveys of different populations of these molluscs, and the analysis of materials on fish infection with metacercariae of trematodes *Posthodiplostomum minimumu* revealed a power-law dependence of the parasite abundance on host density (Buck and Lutterschmidt 2017; Buck et al. 2017). However, we failed to find a theoretical justification of these power-law dependences in the literature. In this paper we suggest a simple model describing the dependence of parasite mean abundance and parasite population on the downstream host density in a complex parasite life cycle under conditions of a constant parasite inflow. This model was tested in a case study of infection of the second intermediate hosts (mussels) with cercariae of trematodes (*Himasthla* spp. and *Cercaria parvicaudata*) under field conditions at the White Sea coast.

Materials and Methods

Model

The presumptive dependence of the number of trematode metacercariae in the second intermediate host on the density of this host is based on a simple geometric fact: the number of intersections of a random line segment with randomly placed identical convex figures ($n_{intersection}$) is proportional to the density of these figures in the space (D) in a degree inverse to the dimensionality (d) of this space (1). (Here and below d — minimal dimensionality of the space with the figures.) (See S1 in Supplementary material for details.) In addition, it is obvious that for several line segments the number of intersections would be proportional to the number of these segments ($n_{segment}$).

$$n_{\text{intersection}} \propto n_{\text{segment}} \times D^{\frac{1}{d}}$$
 (1)

Let us assume that the line segment is the track of the dispersive larva of the parasite, e.g., a cercaria, that the figures are host individuals and that an intersection of a line segment with a figure is a necessary condition of the infection, i.e., the host-parasite encounter. [It is easy to see that if we substitute a moving larva infecting an immobile host for a parasite egg ingested by the moving host, the preconditions of the model are not altered in any way, since in the reference frame associated with a motile randomly moving host (e.g., snails) the parasite eggs would also describe random tracks intersecting the figures (hosts).] Introducing a certain proportionality coefficient (*a*), we can write down a theoretically expected dependence of the parasite's population density ($D_p = n_p / V$ where n_p is the number of parasites in a volume V) on host density ($D_h = n_h / V$ where n_h is the number of host individuals in the same volume) as (2).

$$D_p = a D_h^{\frac{1}{d}} \tag{2}$$

Or, if we denote the exponent of the power-law dependence D_p on D_h as b_D :

$$D_p = a D_h^{b_D} \tag{3}$$

Mean abundance (A) of the parasite is the proportion of the number of parasite

individuals found to the number of host individuals examined (Bush et al. 1997) (i.e. $A = n_p / n_h$). It is obvious that *A* equals the proportion of their densities (4). (To note, a two-dimensional volume is the area.)

$$A = \frac{n_p}{n_h} = \frac{D_p V}{D_h V} = \frac{D_p}{D_h} = \frac{a D_h^{b_D}}{D_h}$$
(4)
$$A = a D_h^{(b_D - 1)} = a D_h^{\left(\frac{1}{d} - 1\right)} = a D_h^{b_A}$$
(5)

where b_A – exponent of the power-law dependence A on D_h . It is evident from (5) that the different of the exponents should be equal to 1 (6).

$$b_D - b_A = 1 \tag{6}$$

Though the model proposed here does not describe the dependence of prevalence (*Pr*) (the proportion of infected hosts with a certain parasite species in each sample) and mean intensity (*Int*) (the proportion of the number of parasite individuals found to the number of infected host individuals in a sample) on host density (*D_h*) in a general case, it does describe the product of these infection indices, the mean abundance ($A = Pr \times Int$). It is obvious that if one of the indices changes weakly, the other will be approximately proportional to A and will show a negative power-law dependence on *D_h* (if $Pr \approx$ const then *Int* $\propto A$ or if *Int* \approx const then $Pr \propto A$).

Thus, we may expect a power-law dependence of mean abundance (*A*) and density (D_p) of parasites on host density (D_h) and, correspondingly, a linear dependence of logarithms of these values (ln*A* and ln D_p on ln D_h). Accordingly, we can predict a negative correlation between the mean abundance and the parasite density under conditions of increasing host density (for a parasite with a complex life cycle). Power-law dependences (negative *A* on D_h and positive D_p on D_h), as well as the fulfilment of equation (6) may serve as a confirmation that the model is adequate.

It should be noted that the exponents of the dependences of A and D_p on D_h (b_A and b_D) in this model (3 and 5) depend only on the minimal dimensionality of the space (d) potentially inhabited by the host. If the host is located on the substrate, which may be approximated by the two-dimensional surface (d = 2), as is the case for mussels in natural settlements, then the theoretically expected values of the exponents should be: $b_D = 0.5$, $b_A = -0.5$. Since the exponents of these power-law dependences (3) and (5) reflect the probability of the encounter between the parasite and the host (or the probability of infection for the empirical estimations of these exponents), it can be easily seen that these exponents should be influenced by all the factors affecting the transmission success. For instance, an adaptive behaviour of the parasite's larvae (a non-random behaviour bringing the parasite into the host space) should increase b_D and b_A . The factors decreasing transmission success of dispersive larvae such as hydrodynamism, extremes of water temperature and water mineralisation, and predation should decrease the values of these exponents (b_D and b_A). Thus, we should not expect an exact correspondence of the empirical constants to the expected value (±0.5). However, the deviations of these constants would testify to the impact of factors affecting the transmission success.

Host-parasite association

The study was made on mussels *Mytilus edulis* and their parasites, the larvae (metacercariae) of trematodes from the genera *Himasthla* and *Renicola*. At the intertidal study sites of the White Sea (see below) mussels were found to harbour metacercariae of *Himasthla elongata*, *H. littorinae* and *H. leptosoma* (syn. *Cercaria littorinae obtusata*) (Galaktionov et al. 2021). The larvae of *Renicola* were represented by *Cercaria parvicaudata* (see Prokofiev et al. 2016). Littoral molluscs *Littorina* spp. were the first intermediate hosts for all these trematodes. Parthenitae developing in these periwinkles produce larvae, cercariae, which emerge from the molluscan hosts and swim freely in water. Daily cercarial output (DCO) differs considerably between *Himasthla* spp. and *C. parvicaudata*. At the White Sea DCO of *H. elongata* is on the average 707.9 \pm 89.2 (438–1225) from infected rough periwinkle *Littorina littorea* and 146.8 \pm

19.6 (70–294) from *L. saxatilis* (Prokofiev et al. 2016). Similar values of DCO may be expected for *H. littorinae* and *H. leptosoma* from *L. saxatilis* and *L. obtusata*, whose larvae have a similar size as cercariae of *H. elongata* (Galaktionov and Skírnisson 2000; Galaktionov et al. 2021). DCO of *C. parvicaudata* is much higher, on the average 2276.2 \pm 342.2 (854–3728) from infected *L. littorea* and 763.7 \pm 93.3 (383–1223) from *L. saxatilis* and *L. obtusata* (Prokofiev et al. 2016).

During their short lifespan cercariae have to infect the second intermediate host represented by blue mussels (*Mytilus edulis*) at the White Sea. Cercariae get into the mantle cavity of mussels passively with the water flow via the inhalant siphons. The larvae of *Himasthla* spp. penetrate the foot of the mussel and after a short migration encyst to become metacercariae, the stage infective for the final host. The cercariae of *C. parvicaudata* penetrate the mantle of mussels, migrate across the mollusc and encyst in the hepatopancreas. Final hosts of *Himasthla* spp. and *C. parvicaudata* are sea gulls and shorebirds, which are infected when feeding on mussels containing infective metacercariae.

Sites of the study

Our field experiments were carried out in two sites of the White Sea intertidal – in 2017 at the Kem' Ludy site and in 2020 at the Krasniy site. The sites chosen for the exposure of cages differed considerably in the prevalence of parthenitae of *Himasthla* spp. and *C. parvicaudata* in the first intermediate hosts. One of them was located on a nameless islet in the Kem' Ludy archipelago (Kem' Ludy site) (66° 25' 09" N; 33° 48' 48" E) and the other was located on Cape Krasniy (Krasniy site) (66° 24' 61" N; 33° 42' 17" E). Kem' Ludy site is a small (\approx 350 sq. m) closed bay with a relatively narrow stretch of the intertidal zone continuing into the subtidal, which deepens precipitously with increasing distance from the shore. The microrelief in Kem' Ludy site is weakly expressed, and the bottom surface is relatively smooth. Krasniy site was

represented by a vast intertidal lagoon (\approx 2200 sq. m) with a depth range of 0 — 0.5 m at low tide. The microrelief is pronounced: numerous depressions, which appear to form during ice clearance, persist through the summer owing to a low exposure.

At Kem' Ludy site *L. littorea* were almost absent, and the infection of *L. saxatilis* and *L. obtusata* with trematode intramolluscan stages was low. Prevalence of *L. saxatilis* with *Himasthla littorinae* was, as shown by our long-term data, $0.23 \pm 0.13\%$, and that by *C. parvicaudata*, $0.08 \pm 0.07\%$. In *L. obtusata* prevalence with *Himasthla* spp. (mostly *H. littorinae*, more rarely with *H. leptosoma*) was $0.18 \pm 0.17\%$, and prevalence with *C. parvicaudata* was $0.52 \pm 0.3\%$. At Krasniy site the density of *L. littorea* was relatively high, while *L. saxatilis* and *L. obtusata* were absent. Prevalence of parthenitae of *H. elongata* and *C. parvicaudata* in *L. littorea* was $30.3 \pm 4\%$ and $0.5 \pm 0.4\%$, correspondingly (Nikolaev et al. 2017, 2020).

Experimental design

Cages with mussels were placed in the intertidal in three parallel lines, 6 cages in a line, and exposed for 41 days. The cages were spaced 3 m apart (so that the distance between outermost cages in a line made up 15 m).). Each line modelled 6 densities, which were the multiples $(0.25 \times, 0.5 \times, 1 \times, 2 \times, 4 \times$ and $8 \times$) of the density of the natural mussel settlement at Kem' Ludy site ($\approx 1600 \text{ m}^{-2}$, our data). We placed 10 (390.6 m⁻²), 20 (781.3 m⁻²), 41 (1601.6 m⁻²), 82 (3201.1 m⁻²), 164 (6406.3 m⁻²) and 328 (12812.5 m⁻²) mussels into cages of the same size ($l \times w \times h = 0.16 \times 0.16 \times 0.08 \text{ m}$, $S_{cage} = 0.0256 \text{ m}^2$). Blue mussels were taken from artificial suspended substrates of a sea farm, where they were uninfected with trematode larvae (Nikolaev et al. 2017). The mussels were size-aligned, since the pumping rate in bivalve mussels and thus the probability that they would capture cercariae depends on their size (Winter 1978; Alimov 1981; Jørgensen 1990; Nikolaev et al. 2006). We chose mussels of the same size, the geometric mean of the length, the height and the width of the shell being 9.2 ± 0.2 mm. After exposure, all

experimental mussels were dissected under a stereomicroscope, the species of metacercariae was identified and their number in each mussel was counted. Two storm-damaged cages at Krasniy site (contaning 82 and 164 mussels) were excluded from the analysis.

The combination of mussel size and cage size made it possible to smooth out the influence of aggregated distribution of the host, which can make a considerable impact on the infection indices (Grosholz 1994). Considering the aggregation of the host in the light of possible infection, we should note that the host may be surrounded by a space modified by its presence (Combes 2001). When an infective agent gets into this space, the contact and the infection become highly probable. It is obvious that if these spaces start to overlap with the increasing host density, the group of hosts whose spaces overlap in this way can be considered an aggregation from the viewpoint of infection with the parasite. Since mussels become infected with swimming cercariae as they pump the water through the mantle cavity, the volume of the modified host space would be determined by the pumping activity of the mussel. Based on the dependence of the pumping activity (E, 1/h) of the White Sea mussels on the geometric mean of the mussel size $[L = (l_s \times h_s \times w_s)^{1/3}$, mm; where l_s , h_s and w_s are length, height and width of the shell, respectively] $E = 0.04L^{1.6}$ (Lezin et al. 2006), we estimated that a mussel in our experiments ($L = 9.2 \pm 0.2$ mm) pumped, on the average, the volume of $V = 1.394 \pm 0.049$ l of water per hour. This volume corresponded to a hemisphere with a diameter of 0.1746 m ($V=2\pi r^3/3$), which is comparable with the size of the cage $(0.16 \times 0.16 \times 0.08 \text{ m})$. Taking into account that cercariae of *Himasthla* spp. and C. parvicaudata remain infective for several hours (Prokofiev 2006; Levakin et al. 2013b), we concluded that the modified host spaces in our experiment (spaces where a cercaria, once there, would be get into a mussel with a high probability) overlapped considerably for all the mussels in a cage.

Data treatment

Bootstrap approach (Efron 1979) with resampling parameter N = 100000 was used to determine average values and confidence limits of means of parasite density (D_p) and mean abundance (A). Density of metacercariae (D_p) was calculated as the ratio of the total number of metacercariae to the area from which the molluscs were sampled. D_p values were considered significantly different (P < 0.05), if their confidence intervals for 95% significance level did not overlap. To determine the significance of differences in mean abundance and infection mean intensity, median tests were used. To determine the significance of differences in prevalence Fisher exact test (pairwise) and Chi-test (multiplay) were used.

The values of D_p and A were assessed for each cage. These values were used to describe the dependence of these parameters on the density of mussel population. We used a power-law model of dependence of these parameters (*y*) on host density (D_h): $y = a_y D_h^{b_y}$. Fisher *F*-test (for the proportion of explained variance to random variance) was used to assess the correctness of linear regression model of logarithms of values ($\ln y = \ln a_y + b_y \ln D_h$). Parameters of linear dependence of logarithms of these values were assessed with the help of the Kendall line (Theil 1950; Sen 1968). This robust method was chosen because the line of cages in our study was very long (15 m), and therefore one could expect considerable deviations (outliers of *A* and D_p) due to the uneven distribution of the first intermediate hosts and other microbiotopic differences between the places with the cages. It should be noted that the use of the Kendall line allows a more precise calculation of the slope coefficients (b_D and b_A) under conditions when outliers are present and at the same time skews empirical assessments of coefficient *a* so that they no longer coincide for the dependences of D_p and *A* on D_h for the same data set ($a_D \neq a_A$).

Calculations were performed in R 4.2.1 (<u>https://cran.r-project.org/</u>). All confidence intervals were calculated for 95% significance level. Asymmetrical confidence limits are given in brackets after the values.

Results

No trematode stages except metacercariae of *Himasthla* spp. and *C. parvicaudata* were found in experimental mussels. The numbers of metacercariae of *Himasthla* spp. and *C. parvicaudata* recorded in dissected mussels did not show any statistically significant negative correlations at any cages at any of the sites (see Table S2 in Supplementary material for details).

Mussels exposed at the same density at Kem' Ludy site and at Krasniy site differed in respect of prevalence (Fisher exact test, P < 0.05), mean intensity (median test: P < 0.00001), mean abundance (median test: P < 0.00001) and density (no overlapping of confidence intervals) of metacercariae *Himasthla* spp. and *C. parvicaudata* (See S3 in Supplementary material for details).

Mean abundance (*A*) and metacercariae density (D_p) were negatively correlated (Fig. 1) both at Krasniy site ($r_s = -0.78$, P < 0.001, $t_{14} = 4.655$ for *H. elongata* and $r_s = -0.68$, P < 0.01, $t_{14} = 3.493$ for *C. parvicaudata*) and at Kem' Ludy site ($r_s = -0.85$, P < 0.00001, $t_{16} = 6.425$ for *Himasthla* spp. and $r_s = -0.83$, P < 0.0001, $t_{16} = 5.964$ for *C. parvicaudata*).

As the density of the exposed mussels (D_h) increased from the minimal (390.6 m⁻²) to the maximal (12812.5 m⁻²), the prevalence (Pr) of metacercariae at Krasniy site (Fig. 2a) weakly decreased both for *H. elongata* (χ^2 -test: P < 0.01, $\chi^2_5 = 25.81$) and for *C. parvicaudata* (χ^2 -test: P < 0.01, $\chi^2_5 = 30.9$). At Kem' Ludy site, the same changes in D_h decreased the *Pr* of metacercariae more considerably (Fig. 2c) both for *Himasthla* spp. (χ^2 -test: P < 0.0001, $\chi^2_5 = 170.6$) and for *C. parvicaudata* (χ^2 -test: P < 0.0001, $\chi^2_5 = 133.9$).

A decreasing mean intensity (*Int*) with increasing D_h was more pronounced at Krasniy site (Fig. 2b) both for *H. elongata* (median test: P < 0.0001, $\chi^2_5 = 133.6$) and for *C. parvicaudata* (median test: P < 0.0001, $\chi^2_5 = 117.9$). At Kem' Ludy site, the decrease of *Int* with the growing D_h was somewhat lower (Fig. 2d) both for *Himasthla* spp. (median test: P < 0.001, $\chi^2_5 = 17.51$) and for *C. parvicaudata* (median test: P < 0.0001, $\chi^2_5 = 45.55$). As the density of the exposed mussels (D_h) increased from 390.6 m⁻² to 12812.5 m⁻², mean abundance (A) of metacercariae of *Himasthla* spp. decreased (median test: P < 0.0001, χ^{2}_{5} = 177.8) from 62.13 (50.43 — 75.17) to 6.68 (5.48 — 8.04) at Krasniy site (Fig. 1a) and from 1.28 (0.9 — 1.69) to 0.33 (0.3 — 0.37) at Kem' Ludy site (median test: P < 0.0001, χ^{2}_{5} = 170.6; Fig. 1c). This increase of D_h was also accompanied by a decreasing A of metacercariae of *Cercaria parvicaudata*: from 28 (20 — 37.73) to 5.42 (4.49 — 6.39) at Krasniy site (median test: P < 0.0001, χ^{2}_{5} = 139.8; Fig. 1a) and from 1.66 (1.1 — 2.21) to 0.31 (0.27 — 0.36) at Kem' Ludy site (median test: P < 0.0001, χ^{2}_{5} = 133.9; Fig. 1c).

As D_h increased, all assessments of the density of the metacercarial population (D_p) increased considerably (Fig. 1b, d). At Krasniy site D_p increased from 24309.9 m⁻² (19609.4 — 29153.6) to 85548.1 m⁻² (70271.6 — 102992.8) for *H. elongata* and from 10950.5 m⁻² (7851.6 — 14674.5) to 69384.6 m⁻² (57360.6 — 82098.6) for *C. parvicaudata* (Fig. 1b). At Kem' Ludy site D_p increased from 481.8 m⁻² (338.5 — 638) to 4088.5 m⁻² (3632.8 — 4557.3) for *Himasthla* spp. and from 625 m⁻² (416.7 — 833.3) to 3828.1 m⁻² (3333.3 — 4375) for *C. parvicaudata* (Fig. 1d).

The linear model for the dependence of logarithms of *A* and D_p on D_h was adequate (Fisher test: P < 0.0001) in all the cases (at Krasniy site: F_{1,14} = 94.15 for ln*A H. elongata*, F_{1,14} = 68.43 for ln*A C. parvicaudata*, F_{1,14} = 40.11 for ln D_p *H. elongata*, F_{1,14} = 53.85 for ln D_p *C. parvicaudata*; at Kem' Ludy: F_{1,16} = 105.1 for ln*A Himasthla* spp., F_{1,16} = 151.3 for ln*A C. parvicaudata*, F_{1,16} = 259.4 for ln D_p *Himasthla* spp., F_{1,16} = 107 for ln D_p *C. parvicaudata*). Kendall lines showed high values of the determination coefficient for the linear dependence of ln*A* and ln D_p on D_h (R^2 estimations are given in Fig. 1). Figure 1 shows the exponents (slopes of these Kendall lines) for each site-parasite combination and the values of *A* (Fig. 1a, c) and D_p (Fig. 1b, d) for each cage. Equation (6: $b_D - b_A = 1$) for coefficients *b* was observed in all the cases (Fig. 1).

Discussion

In this paper we proposed for the first time a model for the explanation of the power-law dependence of the mean abundance and population of the parasite with a complex life cycle on the host density. The performance of the model was tested using the results of our field experiments on infection of mussels with trematode cercariae under conditions of the White Sea intertidal.

Model check

The absence of a negative correlation between the infection by *Himasthla* spp. and that by *C*. *parvicaudata* indicates that there was no interference between the infection agents. Therefore, the processes of infection of mussels by the larvae of these trematodes can be considered as independent.

The results of our experiments confirmed the negative power-law dependence (5) of the mean abundance of the parasite on host density (Fig. 1a, c) and the positive power-law dependence (3) of the density of the parasite (D_p) on host density (Fig. 1b, d). Accordingly, for a parasite with a complex life cycle we confirmed a negative correlation between mean abundance (A) and saturation of the environment (D_p) with infective agents under conditions of an increasing density of the downstream host (D_h) and a constant parasite supply provided by the upstream host (Fig. 1). The model's specific prediction that the difference of the power-law exponents should be equal to 1 (6) was experimentally confirmed, too (Fig. 1).

The effect of the downstream host density on the prevalence and mean intensity of the parasite with a complex life cycle seems to depend on the parasite supply provided by the upstream host. In our experiments, a high cercarial supply at Krasniy site, which was due to a relatively high prevalence of *L. littorea* with digenean intramolluscan stages (see Materials and

Methods), smoothed over the dependence of the prevalence of *H. elongata* and *C. parvicaudata* on host density (prevalence $\approx 100\%$ for any D_h — Fig. 2a). For this case (mean intensity \approx mean abundance), our model (5) suggests a negative power-law dependence of mean intensity on D_h , which was indeed observed at that site (Fig. 2b).

A relatively low parasite supply at Kem' Ludy site, which was due to a low prevalence of digenean intramolluscan stages in the periwinkles (see Materials and Methods), made it less probable that a host individual (a mussel) would be infected with several larvae of the parasite. Accordingly, the dependence of Int on D_h was weak (Fig. 2d). For the same reason, the prevalence observed at Kem' Ludy site was lower: only 1004 out of 1853 experimental mussels were infected (prevalence \approx 54%). Apparently, at that site the mean intensity changed rather weakly with an increasing mussel density (Fig. 2d), and so the prevalence was approximately proportional to the mean abundance. In this case (mean intensity = const., prevalence \propto mean abundance), the negative power-law dependence of prevalence on D_h (Fig. 2c) reflected the dependence of mean abundance on D_h . A similar situation has apparently been registered in the field experiments of Buck et al. (2017), where out of 840 snails exposed in the biotope only 170 became infected (prevalence $\approx 20\%$) and where a negative power-law dependence of the prevalence on host density was also observed, regardless of whether the snails were infected actively (by miracidia) or passively (by trematode eggs). Thus, the data of Buck et al. (2017) confirm our idea that the form of correlation between the infection indices (a negative power-law dependence of mean abundance on host density and a positive power-law dependence of parasite density on host density) does not depend on the mode of host infection (see Materials and Methods, Model).

To test our model, we also used the published data on the dependence of *Posthodiplostomum minimum* metacercariae mean abundance on *Lepomis* spp. fish density in natural settlements (creeks) (Buck and Lutterschmidt 2017). This dependence was power-law (logarithms of parasite mean abundance and host density related linearly — Fig. 3) and the

observed exponent (slope b = -0.51) was close to that expected for this case (b = -0.5 for d = 2, Equation 5 in 2.1. Model). (See S4 in Supplementary Material for details.) Thus, the model predictions correspond not only to the results of our experiments but also to the descriptions of the influence of host density on parasite infection in the works of other authors. The preconditions of our model of dependence of the mean abundance and population of the parasite with a complex life cycle on the host density do not rely on any features of the host-parasite association and can be applied *a priori* to any fragments of complex parasite life cycles if the infected host individual is not the source of infection for conspecifics.

Influence of the biotope and parasite species

The exponents of the power-law dependence of mean abundance and metacercarial density on mussel density at Krasniy site, as compared with the corresponding values for Kem' Ludy site, were slightly lower for *Himasthla* spp. ($\Delta b_A = \Delta b_D = -0.07$) but greater for *Cercaria parvicaudata* ($\Delta b_A = \Delta b_D = 0.13$) (Fig. 1). In this case we observed the joint influence of the biotope and the biological features of the parasite on the dependence of infection indices on host density. Considering these exponents as values associated with the transmission success (see Materials and Methods, Model), we should conclude that the transmission of *Himasthla* spp. was somewhat more successful at Kem' Ludy site, whereas the transmission of *C. parvicaudata* was somewhat more successful at Krasniy site.

Immediately after leaving the first intermediate host, the cercariae of both *Himasthla* spp. and *C. parvicaudata* find themselves in the second intermediate host space — the bottom water layer. A possible explanation of the situation observed in our study (Fig. 1) is that the cercariae of *Himasthla* spp. do not leave the host space throughout their lifetime, dispersing in the thin water layer above the substrate, while the cercariae of *C. parvicaudata* rise to the upper water layer after leaving the molluscan host and sink to the host space only several hours later

(Prokofiev 2002; Prokofiev et al. 2016; Nikolaev et al. 2017). A pronounced microrelief at Krasniy site (see Materials and Methods, Study sites) may prevent the transmission of *H. elongata*, since micro-depressions in the sediments may serve as natural traps for the parasite larvae with a strongly expressed negative geotaxis. On the other hand, at Kem' Ludy site the intertidal is rather narrow, and many larvae of *C. parvicaudata* are most likely washed away, losing the chance to infect mussels in intertidal settlements. Thus, microscale biological features such as the behaviour of the parasite's larvae and the features of the biotope were reflected in macroscale indices such as the abundance and the density of the parasite in the biotope.

Abundance and prevalence vs population size (or parasite density)

Besides the decreasing mean abundance of the parasite in denser host settlements (given the same parasite supply), it seems important to bear in mind that in such settlements the saturation of the environment with the parasite (parasite density) would be higher than in less dense ones (Fig. 1). This follows from our model (3, 5), and was also shown empirically in this work and other studies (Mouritsen et al. 2003; Thieltges and Reise 2007; Prinz et al. 2011; Buck and Lutterschmidt 2017; Buck et al. 2017). In case of infection by an environmentally transmitted parasite, this would result in a greater production of parasitic infective stages (eggs or free-living larvae) or pre-adults (in some cases of larval parasitism, e.g. nematomorphs and mermitid nematodes) in a dense host settlement.

For trophically transmitted parasites, dense accumulation of prey such as mussels in our study are more attractive for predators, which play the role of the downstream host (in our case, gulls). A predator, which, in accordance with the optimal foraging theory, strives to save time for food search, would avoid feeding at sites with a low density of prey. Consequently, the potential importance of a biotope for trophically transmitted parasites in case of a relatively small and abundant prey host would directly depend on the saturation of the biotope with infective stages

of the parasite, in other words, its density. Since the density of the parasite may increase considerably with the increasing density of the host, dense settlements of the host prey with a low mean abundance of the parasite should play a greater role in the transmission of the parasite to the host predator than sparse settlements of host prey with a high mean abundance of the parasite. Therefore, assessing the importance of a biotope for the transmission of parasites with complex life cycles it is useful to consider, alongside with traditional infection indices, also the density of parasite component population or the density of the parasite (the measure of the saturation of the environment by the parasite) measured as the number of infective stages in hosts per unit of area/volume.

"Safety in numbers" - encounter-dilution and host density effects

A decrease in the parasite mean abundance with the increasing host density was considered as a manifestation of the "safety in numbers" effect, which is sometimes equated with the encounterdilution effect (Buck and Lutterschmidt 2016; Buck et al. 2017). For the process of host infection by parasites with complex life cycles, the "safety in numbers" notion may be expanded to the host density effect discussed in this paper. Thus, "safety in numbers" may refer to two independent phenomena: the encounter-dilution effect (Mooring and Hart 1992) and the host density effect. These two phenomena both reduce mean abundance of parasites ("safety in numbers") but have an opposite effect on the total infection success.

The encounter-dilution effect (Mooring and Hart 1992) describes the character of infection in aggregations: centrally placed individuals would be infected to a lesser extent than individuals on the periphery. An aggregated host distribution also entails a *decreased* probability of the encounter with the host outside aggregations and hence a decreased total infection success as compared with random or even host distribution. Therefore, given the same parasite supply, in case of an aggregated distribution of the downstream host fewer individuals of the parasite with a

complex life cycle would successfully infect the host than in case of random or even host distribution, i.e. there will be a decrease in the parasite population size. In turn, if host density remains the same and the number of parasite individuals successfully infecting the host decreases, the parasite mean abundance inevitably decreases, too. For instance, in cage experiments of Grosholz (1994) on the infection of bivalves *Transennella tantilla* with cercariae of *Parvatrema borealis* at prevalence = 100% (and, accordingly, mean abundance = mean intensity) the mean intensity of the parasite decreased under conditions of the aggregated distribution of the hosts.

The effect of host density for a parasite with a complex life cycle is that given the same parasite supply the probability of the host-parasite encounter (and thus the parasite density) *grows*, but more slowly (as a positive exponent of host density less than 1 – see Model, equation 2) than host density (Fig. 1b, d). Similarly to the encounter-dilution effect, this results in a decrease in the parasite mean abundance (see Model, equation 5 and Fig. 1a, c) and thus in the parasite load on the host individual, which may be considered as "safety in numbers". However, in contrast to the encounter-dilution effect, the parasite population size increases (Material and Methods, Model, equation 3 and Fig. 1b, d).

It is evident that these two effects can be observed clearly if the host settlements under comparison differ by only one parameter: the degree of aggregation (and then the encounterdilution effect is observed) or the density (and then the host density effect is observed). In practice it may be difficult to differentiate between the host density effect and the encounterdilution effect, because the hosts settlements under comparison may differ both in the density and in the degree of aggregation. Then the effects may overlap (they both reduce mean abundance) and obscure each other (their effect on the parasite population size is opposite). This seems to have been the case in the experiments of Grosholz (1994) (see above), who did not reveal any effect of the host density on the parasite population. At the same time, the knowledge of theoretically expected patterns of the dependence of the mean abundance and the population size of the parasite on the host density may help one differentiate between the impact of these two effects.

Conclusions

In this study we showed that if one deals with fragments of complex life cycles in which an infected host cannot be the source of infection for conspecific individuals, the influence of host density on the population size and the mean abundance of the parasite can be described by a simple and convenient model. According to it, the mean abundance of the parasite and the size of its population are associated with host density by power-law dependences, a negative one for the parasite mean abundance and a positive one for the parasite population. The parameters of these dependencies are influenced by factors acting on the success of the transmission. A positive correlation between the density of the component parasite population [(all the individuals of a given life cycle phase at a particular space and time (Bush et al. 1997)] and the host density suggests that dense host settlements with a lower infection level (prevalence, mean abundance and mean intensity) should play a greater role for the transmission of the parasite than sparse settlements with a high infection level. The host density effect simultaneously reduces the average load per host individual and increases parasite transmission success in contrast to the encounter-dilution effect, which reduces total transmission success. A theoretical description (model) of the host density effect may allow the encounter-dilution effect to be isolated and possibly modeled in future studies.

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Author contributions All authors contributed to the study conception, design and data analysis. KN and IL carried out the field experiments and sampling at the White Sea and the primary treatment of collected material. IL developed the model and worked up the primary data. IL and KG wrote the manuscript with input from KN. All the authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare no conflict of interest.

Ethics approval No ethical approval was required for this study.

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Figure captions

Fig. 1. Mean abundance (A — a, c) and density (D_p — b, d) of metacercariae of *Himasthla* spp. and *Cercaria parvicaudata* at various mussel densities (D_h) at Krasniy site (a, b) and at Kem' Ludy site (c, d). Curves — exponents of Kendall lines for the dependence of the logarithms of values (A and D_p on D_h).

Fig. 2. Prevalence (a, c) and mean intensity (b, d) of metacercariae *Himasthla* spp. and *C*. *parvicaudata* in mussels exposed at Krasniy site (high cercariae supply — a, b) and at Kem' Ludy site (low cercariae supply — c, d) at various host densities (D_h). Confidence intervals are given for 95% significance level.

Fig. 3. Dependence of logarithms of metacercarial abundance of a trematode parasite (*Posthodiplostomum minimum*) on logarithms of centrarchid fish host (*Lepomis* spp.) density (ln*D*) from eight small creeks. The data on the association between total *P. minimum* population abundance and *Lepomis* spp. density are taken from the graph (fig. 2, p. 209) in Buck and Lutterschmidt (2017) (See S4 in Supplementary material for details).

Figures



Figure 1

Mean abundance (A - a, c) and density $(D_p - b, d)$ of metacercariae of *Himasthla* spp. and *Cercaria parvicaudata* at various mussel densities (D_h) at Krasniy site (a, b) and at Kem' Ludy site (c, d). Curves – exponents of Kendall lines for the dependence of the logarithms of values (A and D_p on D_h).



Figure 2

Prevalence (a, c) and mean intensity (b, d) of metacercariae *Himasthla* spp. and *C. parvicaudata* in mussels exposed at Krasniy site (high cercariae supply -a, b) and at Kem' Ludy site (low cercariae supply -c, d) at various host densities (D_h). Confidence intervals are given for 95% significance level.



Figure 3

Dependence of logarithms of metacercarial abundance of a trematode parasite (*Posthodiplostomum minimum*) on logarithms of centrarchid fish host (*Lepomis* spp.) density (ln*D*) from eight small creeks. The data on the association between total *P. minimum* population abundance and *Lepomis* spp. density are taken from the graph (fig. 2, p. 209) in Buck and Lutterschmidt (2017) (See S4 in Supplementary materials for details).

Supplementary Files

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