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PRIMARY RESEARCH PAPER

Effects of *Chaetogaster limnaei limnaei* (Oligochaeta, Tubificidae) on freshwater snail communities

Stefan Stoll · Nico Hormel · Denise Früh · Jonathan D. Tonkin

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Abstract From laboratory studies, the relationship between the oligochaete *Chaetogaster limnaei limnaei* (*CL*) and its freshwater snail hosts is known to be context-dependent, ranging from mutualistic to parasitic. We monitored snail communities of seven streams in Germany during three seasons of a year and investigated infestation by *CL*. Some snail species never were infested. In snail species that were infested, size, substratum type, oxygen concentration and species identity were the most important variables explaining the variance in *CL* infestation. Independent of individual snail size, *Bithynia tentaculata*, *Ancylus fluviatilis* and *Acroloxus lacustris* showed the highest *CL* abundances. Across species, *CL* abundances were highest in large individuals on silty substratum at well-oxygenated sites. Reproductive success of snail populations was estimated from proportion of juveniles in populations. This measure of reproductive success of snail populations was inversely related with *CL* infestation level. These results suggest that *CL* infestation affects aquatic snails at the population and community level in the field. Differential infestation levels and different impacts of *CL* infestation between species lead to an asymmetric distribution of positive and negative effects among all snail species present in a habitat. Thus, *CL* may be an overlooked agent in structuring snail communities.

Keywords Aquatic molluscs · Oligochaete · Prevalence · Infestation intensity · Density-dependent effect · Reproductive success

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S. Stoll (✉) · N. Hormel · D. Früh · J. D. Tonkin
Department of River Ecology and Conservation, Senckenberg Research Institute and Natural History Museum Frankfurt, 63571 Gelnhausen, Germany
e-mail: stoll@uni-landau.de

S. Stoll
Department of Ecotoxicology and Environment, University of Koblenz-Landau, 76829 Landau, Germany

D. Früh
Environment and Consumer Protection, North Rhine-Westphalia State Agency for Nature, 40221 Düsseldorf, Germany

J. D. Tonkin
Department of Integrative Biology, Oregon State University, Corvallis, OR 97331, USA

Introduction

The biology of the common and widespread naidid oligochaete *Chaetogaster limnaei limnaei* von Baer, 1827 (CL) (Vaghin, 1946) and its relationship with its various pulmonate and prosobranch freshwater snail hosts has received significant scientific attention (e.g.

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Michelson, 1964; Gruffydd, 1965b; Buse, 1974; Streit, 1977; Fernandez et al., 1991; Rodgers et al., 2005; Ibrahim, 2007). Much of this research was stimulated by the finding that this epizoic subspecies of *C. limnaei* [in contrast to the endoparasitic subspecies *Chaetogaster limnaei vaghini* Gruffydd, 1965 (Gruffydd, 1965a)] ingests trematode cercariae and miracidia (Mrazek, 1917; Wagin, 1941; Backlund, 1949). These latter studies concluded that *CL* may be an important regulator of snail trematode infections, giving rise to a raft of parasitological studies (Khalil, 1961; Michelson, 1964; e.g. Fernandez et al., 1991; Rodgers et al., 2005; Ibrahim, 2007). However, even though an inhibitory effect of *CL* on the infestation success of trematode larvae in snails was found (Sankurathri & Holmes, 1976, Rodgers et al., 2005, Ibrahim, 2007), this has so far not led to concrete measures for a biotic control of these important human diseases. *CL* is most commonly regarded as a commensal of its snail hosts, because it profits from its association with the host in terms of shelter from predators and increased food availability, without feeding directly on the host or indirectly restricting its food sources (Gruffydd, 1965b; Stoll et al., 2013). However, recent laboratory experiments revealed that the nature of the relationship between *CL* and the pulmonate snail *Physa acuta* Draparnaud, 1805 is density dependent (Stoll et al., 2013). At high infestation levels (10–20 *CL*/snail), the oligochaete caused behavioural changes of their host snails, along with a significant reduction of the fitness-relevant life history traits, growth and reproductive output (Stoll et al., 2013). Furthermore, laboratory experiments have demonstrated that the effects of *CL* on their hosts vary between different host species and are temperature dependent as well (Buse, 1974; Höckendorff et al., 2015). These findings led to the hypothesis that *CL* may differentially affect snail species within mixed species communities in the field and therefore be a more important factor in structuring snail communities than previously assumed (Stoll et al., 2013).

Moreover, very little is known about the ecological niche of *CL*, especially regarding their ecological amplitude, host choice and dispersal between hosts. From the nearly worldwide distribution, including Europe (Gruffydd, 1965b), Asia and Australia (Brinkhurst & Jamieson, 1971; Rajasekariah, 1978), Africa (Fashuyi & Williams, 1977), North America (Hoover & Lodes, 1986) and South America (Martins & Alves, 2010), it may be concluded that *CL* is rather euryoecious, but tolerances towards physico-chemical water properties as well as the influence of other biotic controls, such as host availability, have never been tested.

Host choice and dispersal experiments with *CL* conducted by Buse (1974) provided some evidence that *CL* tend to re-infest the same host species, but this result differed when *CL* were presented with either actual snails of different species, or indirect species cues only (i.e. mucus trails or chemical cues). Most field-based studies on the population dynamics of *CL* in natural populations of freshwater snails have focused on a single host species and did not include alternative host species (e.g. Gruffydd, 1965b; Streit, 1974; Hopkins et al., 2013). In the most comprehensive study including multiple host species so far, Ibrahim (2007) monitored prevalence and infestation intensity of five infested snail species at seven sites over a one year period, demonstrating that *CL* infestation of all species had similar seasonal patterns, with peaks in May or June. Ibrahim (2007) found differences in *CL* infestation patterns (i.e. infestation intensity and prevalence) between different host species, as well as a correlation of infestation patterns with host size. However, it remains unclear if this is because different snail species had different sizes, or if different infestation levels occurred independently of host size. Furthermore, it is unknown whether *CL* infestation changes life history parameters of the host snails, leading to long-term effects in snail populations.

Two previous experimental studies in the laboratory manipulating *CL* infestation levels measured fitness-relevant life history parameters of host snails. In their study on *Biomphalaria glabrata* Say, 1818 as host snails which they infested with 8 *CL* per snail as well as miracidia of *Schistosoma mansoni* Sambon, 1907, Rodgers et al. (2005) found that *CL* infestation overall increased growth rates of the snails. Their reproductive output, however, was not affected. Stoll et al. (2013) used *P. acuta* as the host snail and varied *CL* infestation levels, finding that at infestation levels of 10 *CL* per snail and higher, both growth rates and reproductive output were significantly reduced. We are not aware of any study that tried to assess the effects of *CL* infestation on fitness-relevant life history traits such as reproductive output in situ.

To investigate the potential of *CL* to structure snail populations and communities in the field, we examined populations of *CL* in snail communities at seven stream sites in central Germany during three seasons of one year. The following specific questions were addressed: (1) Do snail species, independent of their size, get differentially infested by *CL* and are these patterns stable throughout the year? (2) Can we identify environmental variables that regulate *CL* infestation? (3) Is there an effect of *CL* infestation on host snail reproduction, suggesting that *CL* infestation has a long-term effect on snail populations?

Methods

Sampling procedure

A total of seven sites in the area of Frankfurt, Germany were chosen for sampling. Three sites were situated in the Long-Term Ecological Research (LTER) area "Rhine-Main-Observatory", which comprises the River Kinzig catchment (S1, S3, S4; Appendix Table S1). Three more sites were situated in the neighbouring River Nidda catchment (S5, S6, S7) as well as one site in their main stem, River Main (S2). We selected sites that were (1) at least 5 km apart from each other to ensure complete independence of the ongoing community processes, for which (2) high snail diversity was known from a previous sampling campaign and (3) *CL* was found in at least one snail species in a test sampling campaign before the start of this study. For this study, each of the sites was sampled three times in different seasons: winter (November 2011), spring (April 2012) and summer (June 2012). Each sampling site consisted of a 30-m stretch of water that was characterized using the AQEM (Development and Testing of an Integrated Assessment System for the Ecological Quality of Streams and Rivers throughout Europe using Benthic Macroinvertebrates) site protocol (AQEM, 2002). Before each sampling occasion, the coverage of mineral and organic substrata was estimated according to Haase et al. (2004). All substrata with a coverage $\geq$ 10% were sampled for freshwater snails, with three 0.25 m^2 subsamples taken from each of these substratum categories. Fine-grained hard substrata (microlithal, argyllal and psammal) were sampled by dragging a net (aperture: 25 cm $\times$ 25 cm; mesh size: 0.5 mm), 1 m along the substratum. Subsequently, the sample was rinsed carefully with water and transferred into a tray for sorting. On coarse hard substrata (mesolithal and macrolithal), samples were collected by hand by carefully lifting appropriate stones out of the water and collecting the snails with forceps. Afterwards, the upper surface area of each stone was estimated from the length and width of the stone and sampling was continued until a total area of 0.25 m^2 was collected. Organic substrata (CPOM and macrophytes) were collected from the same area, carefully rinsed in a net and subsequently transferred into a tray for species sorting. Emergent macrophytes (e.g. reeds) were cut at ground level and snails were collected from the shoots. In the field, all snails were sorted according to species and then placed in plastic zip-lock bags in a way that they did not touch each other, with not more than 10 individuals per bag. All bags were immediately put on ice in a cooling box and subsequently frozen at -18°C in the laboratory.

Additionally, the following hydrological and physico-chemical variables were measured at the level of microhabitats (defined by substratum types) at each site. Current velocity was measured using a hand-held Schiltknecht MiniWater flow-meter (Schiltknecht, Gossau, Switzerland), oxygen concentration, water temperature, conductivity and pH were measured using a hand-held WTW Multi 340i probe (WTW, Weilheim, Germany), and water samples were taken to titrate nitrate concentration and ammonium concentration. Each measurement was taken in duplicates, and values were averaged. Throughout the study period, we also deployed a temperature logger at each site, recording at an hourly resolution.

For further processing in the laboratory, the snails were placed individually in small plastic dishes filled with tap water and defrosted for 5 min. Subsequently, the soft body of the snails was extracted from the shell with a fine forceps, and the number of *CL* individuals on each snail was counted under a stereomicroscope (Olympus SZX 12; Olympus, Hamburg, Germany). Subsequently, the shell length of the snails was measured with the software cell^A (Olympus, Hamburg, Germany). We tested the effects of freezing and thawing procedure on the detectability of *CL* in a preliminary experiment, and did not find any negative effects. We also rinsed the emptied zip-log bags in the lab and did not find any remaining *CL*.

Data analysis

Host species and size dependence of CL infestation

For data analysis, data were filtered in two steps. First, all data from snail species that never got infested by *CL* were removed. Second, in species that got infested by *CL*, all individuals smaller than the minimum size at which the respective species got infested were removed. Following Ibrahim (2007), we calculated the mean abundance (average number of *CL* per snail considering all snails at infestable sizes in a population), mean intensity (average number of *CL* per snail considering only snails that are infested) and prevalence (fraction of individuals in a population that is infested) of *CL* infestation for each species in each of the subsamples. All the following statistical analyses were performed using *R* software version 2.13.1 (R Development Core Team, 2011). We used linear mixed models to explain mean abundances of *CL* on host snails in each of the subsamples using the independent variables host species identity, sampling season and mean shell length of all snails in a subsample. Visual inspection of the regressions between shell length and *CL* abundance suggested a non-linear relationship, and thus, quadratic and cubic terms of mean shell lengths were added to the model. To explore the differences in *CL* infestation between snail species, the interaction terms host species x mean shell length and host species x season were added to the model. Sampling site was used as a random factor to account for potential differences in *CL* abundances between the sampling sites.

Ecological control of CL infestation

To explore the environmental variables that affect *CL* infestation, mean *CL* abundances, infestation intensities and prevalences were related to two sets of environmental variables. The first set comprised abiotic site and substrata characteristics, including the hydrological and physico-chemical variables that were assessed. From the logged temperatures over the entire study period, mean, minima, maxima and standard deviations of all hourly water temperature measurements were used as additional variables. The second set of variables described the identity, densities and sizes of suitable hosts. These variables were host species identity, average shell length of the species population, density of the infested host species per microhabitat and per site as well as combined density of all snails per microhabitat and per site.

The main biotic and abiotic influences driving mean *CL* density, infestation intensity and prevalence were examined separately using boosted regression trees (BRT) (Friedman et al., 2000; Hastie et al., 2009) in *R* the package "dismo" (Elith et al., 2008; Hijmans et al., 2013). BRT is an advanced form of regression, combining machine learning with traditional regression, enhancing their predictive ability. Rather than building complex trees as per other regression tree approaches based on a single tree, boosting combines large numbers of simple trees (Elith et al., 2008; Buston & Elith, 2011). For this reason, BRT is particularly useful for identifying influential variables with complex non-linear relationships.

BRTs were performed taking a stage-wise model selection approach, using the gbm.step procedure and the Gaussian family loss function. Ten-fold cross-validation was performed to select the optimal number of trees. By testing the developing model on held-out data, generality is insured in the non-training data predictive ability of the final model. The bag fraction, which randomly selects the proportion of training data for each successive tree, was set to 0.5. Trees had five splits and, to ensure at least 1,000 trees were built, the learning rate was set to 0.01.

The relative influence of abiotic and biotic variables in the models, which is calculated based on how often a variable is selected, and how its selection improves the model, was examined as a percentage. The specific role that each variable had in the model was examined using partial dependence plots. To assess the overall performance of the BRT models, two key values were examined: the cross-validated percent deviance explained and the cross-validated correlation coefficient between observed and fitted values.

Effects of CL on snail reproduction

The reproduction of all snail species considered in this study start in late spring. The spring sampling was carried out before the onset of the reproductive period, whereas at the summer sampling, juveniles were already present. All individuals present at the summer sampling that were smaller than the smallest individual of each species at the spring sampling were

considered juveniles. Cut-off points of the five most abundant infested species were 2.803 mm in *Acroloxus lacustris* L., 1758, 2.041 mm in *Ancylus fluviatilis* O.F. Müller 1774, 2.057 mm in *Bithynia tentaculata* Leach, 1818, 3.436 mm in *P. acuta* and 5.182 mm in *Radix balthica* L., 1758. These cut-off points for each species were fairly constant between sites. To investigate the relationship between *CL* infestation and reproductive success of snails, an indirect measure of reproductive success of snails at the population level was used: the proportion of juveniles in a population, which was determined for each population in the summer sampling data. The advantage of this approach is that snails did not require transport to the lab and individual housing there to quantify egg output. The disadvantage is that, as in many snail species, adult snails die throughout summer. Only samples collected within a narrow spatio-temporal window can be safely compared, and thus, this measure does not lend itself for comparisons across studies.

This measure of reproductive success was related to the mean *CL* abundances of snail populations sampled before the start of the reproduction season. Only populations that were infested by *CL* were considered in this analysis. We used multiple linear regression models to analyse the relationship between this estimate of reproductive success and a suite of variables describing host species populations, inclsuding host species identity, mean host size, mean *CL* abundance and adult snail density. Starting from a full model, the model was backward selected to a minimum Akaike Information Criterion (AIC). To stabilize variances, the proportions of juveniles in the populations were arcsine-square root transformed and mean *CL* abundance and adult snail density were log transformed.

Results

CL infestation patterns in host snails

Five of 15 species of freshwater snails collected and examined were found to be regularly infested with *CL* (Appendix Table S1), namely *A. lacustris*, *A. fluviatilis*, *B. tentaculata*, *P. acuta* and *R. balthica*. The overall most abundant snail species, the invasive and small-bodied *Potamopyrgus antipodarum* J.E. Gray, 1843, was never infested with *CL*. The other non-infested species occurred only at a few sites and were often not detected in every season.

All five regularly infested host species showed seasonal variation in the mean abundance, mean intensity and prevalence of *CL* infestation (Fig. 1). The patterns were not consistent between all the host species (Table 1), but two main types of seasonal patterns occurred. *A. lacustris* and *B. tentaculata* featured a low mean abundance, mean intensity and prevalence in winter and spring and infestation parameters increased in summer. In contrast, *A. fluviatilis*, *P. acuta* and *R. balthica* showed a peak of infestation in spring, while the lowest infestation was in summer.

All five host species showed a positive relationship between host size and *CL* mean abundance (Fig. 2); however, large individuals showed a low infestation level in *A. lacustris*. The closest relationships were found in *R. balthica* and *A. fluviatilis*, while these relationships in *P. acuta, B. tentaculata* and *A. lacustris* were weaker. Minimum sizes for infestation varied considerably between the snail species. Minimum infestation size was the lowest in *A. fluviatilis* (1.62 mm), followed by *B. tentaculata* and *P. acuta* (both 2.62 mm), while in *A. lacustris* no individuals smaller than 3.09 mm were found to be infected. *R. balthica* had the highest minimum size of infestation (4.35 mm) (Fig. 2).

Infestation levels principally varied between snail species, and also the effect of size on infestation levels varied between the snail species (Table 1; Fig. 2). *Radix balthica* reached the overall highest infestation levels with the largest snails of approximately 20 mm shell length supporting average infestation intensities of 40 *CL* per snail. However, at size of 6–7 mm, the maximum size that the smallest species (*A. lacustris*) commonly reached, *A. fluviatilis* was on average infested most heavily, with approximately 10 *CL* per snail. The other four species showed only infestation intensities of approximately 1–2 *CL* per snail at that size.

Ecological control of CL infestation

With high percentages of cross-validated deviance explained, BRT analyses performed well in explaining *CL* infestation of snails (Table 2). With combined relative influences of 54 and 46%, biotic and abiotic

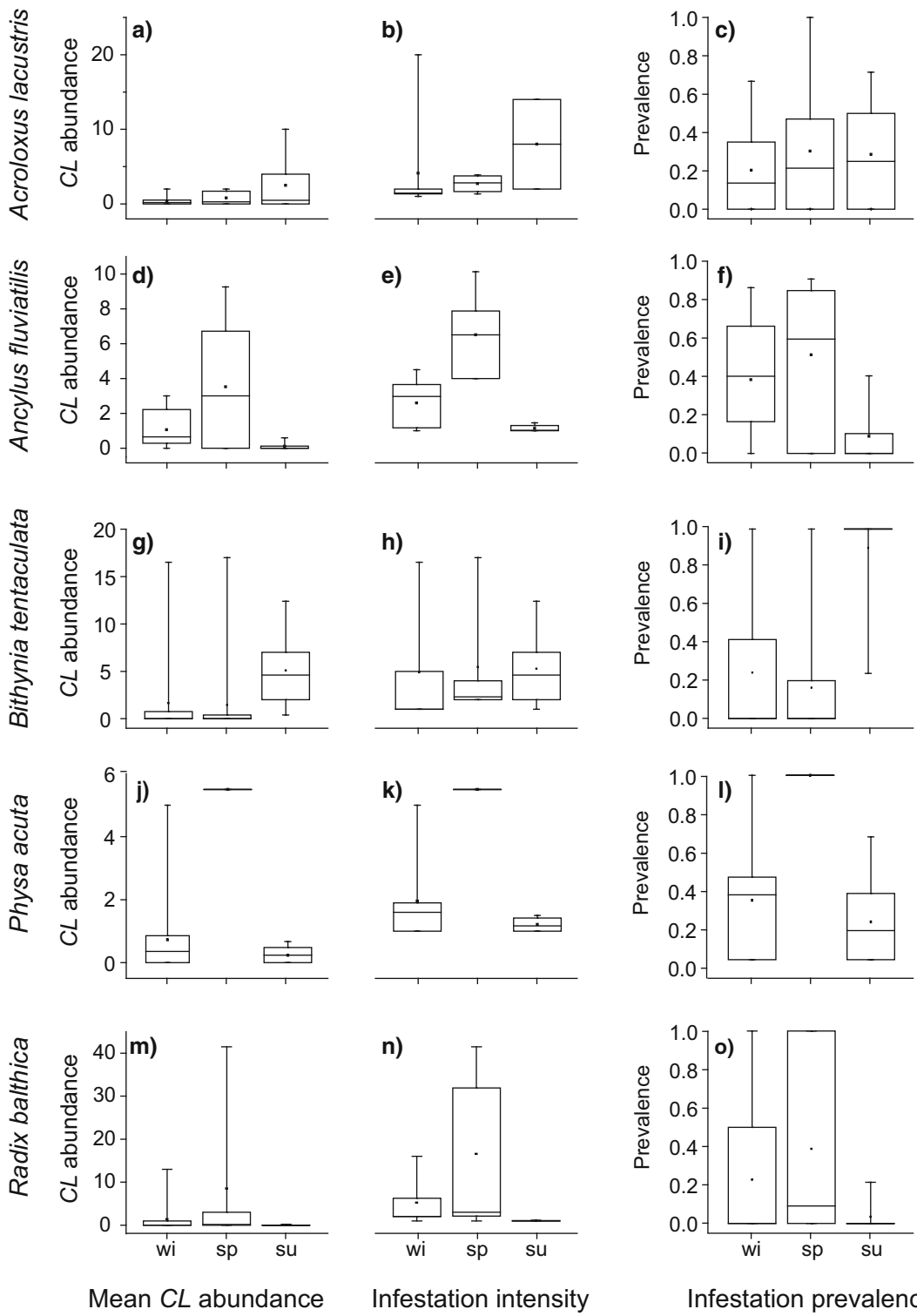

Fig. 1 Seasonal variability of mean abundance, infestation intensity and prevalence of *Chaetogaster limnaei limnaei* (*CL*) infestation in the subsamples of the five snail species *Acroloxus lacustris* (**a–c**), *Ancylus fluviatilis* (**d–f**), *Bithynia tentaculata* (**g–i**), *Physa acuta* (**j–l**) and *Radix balthica* (**m–o**). *Centre lines* in the boxes are medians, *dots* are means, *boxes* indicate the interquartile range, *whiskers* indicate 5/95th percentiles. *wi* winter; *sp* spring; *su* summer

Table 1 Results from linear mixed models on variables explaining the mean abundance of *Chaetogaster limnaei limnaei* per snail population subsample

Groups	Variance		SD
Site (intercept)	0.009		0.094
Residual	0.320		0.566
Fixed variables	Est. ± SE	z value	Pr(>|z|)
(Intercept)	−2.56 ± 0.99	−2.58	**0.010***
Spec_Ancflu	1.56 ± 1.13	1.38	0.17
Spec_Bitten	1.63 ± 1.02	1.59	0.11
Spec_Phyacu	1.94 ± 1.04	1.87	0.062
Spec_Radbal	2.33 ± 1.17	2.00	**0.046***
Length	8.2E−4 ± 3.0E−4	2.75	**0.006****
I(Length^2)	−4.2E−8 ± 3.2E−13	−1.34	0.18
I(Length^3)	2.2E−12 ± 9.5E−13	2.28	**0.022***
Date_spring	−0.08 ± 0.29	−0.28	0.78
Date_summer	0.71 ± 0.29	2.45	**0.014***
Spec_Ancflu:Length	−2.9E−4 ± 2.7E−4	−1.06	0.29
Spec_Bitten:Length	−4.0E−4 ± 2.3E−4	−1.74	0.082
Spec_Phyacu:Length	−4.6E−4 ± 2.4E−4	−1.95	0.051
Spec_Radbal:Length	−5.8E−4 ± 2.5E−4	−2.34	**0.019***
Spec_Ancflu:Date_spring	0.43 ± 0.41	1.05	0.3
Spec_Bitten:Date_spring	8.8E−3 ± 0.37	0.02	0.98
Spec_Phyacu:Date_spring	1.27 ± 0.72	1.76	0.078
Spec_Radbal:Date_spring	0.21 ± 0.34	0.60	0.55
Spec_Ancflu:Date_summer	−0.95 ± 0.39	−2.47	**0.014***
Spec_Bitten:Date_summer	0.21 ± 0.37	0.56	0.58
Spec_Phyacu:Date_summer	−0.98 ± 0.40	−2.48	**0.013***
Spec_Radbal:Date_summer	−0.90 ± 0.34	−2.66	**0.008****

Significant values are highlighted in bold and marked with asterisks. * $P < 0.05$, ** $P < 0.01$

Random effects; Number of obs. 193; groups: Site, 7

variables, respectively, were almost equally important in determining *CL* infestation patterns. Twelve biotic and abiotic environmental variables affecting *CL* infestation were found to explain more than 1% of the *CL* infestation patterns each. The order of importance and relative contribution of each variable was very similar for mean abundance of *CL* and infestation intensity; however, oxygen concentration was not a high ranking variable for *CL* prevalence whereas host species identity, in particular, played a stronger role (Fig. 3). Nevertheless, the directions of the effects were always the same; therefore, individual variable partial dependence plots are only shown for infestation intensity (Fig. 4). The size of the host species was most important for all infestation measures. Also substratum type, oxygen concentration and host species identity were consistently among the highest ranking variables. Independent of all other environmental variables, *B. tentaculata* was the most infested among the different host species, followed by *A. fluviatilis*, *A. lacustris*, *P. acuta* and *R. balthica*. For mean worm abundance and infestation intensity, the most important host population descriptors were both total snail density and individual host species density at the site level. Density measures at the individual substratum level contributed much less to the model (Fig. 3). For the prevalence of *CL*, host species density at the individual substratum level was more important.

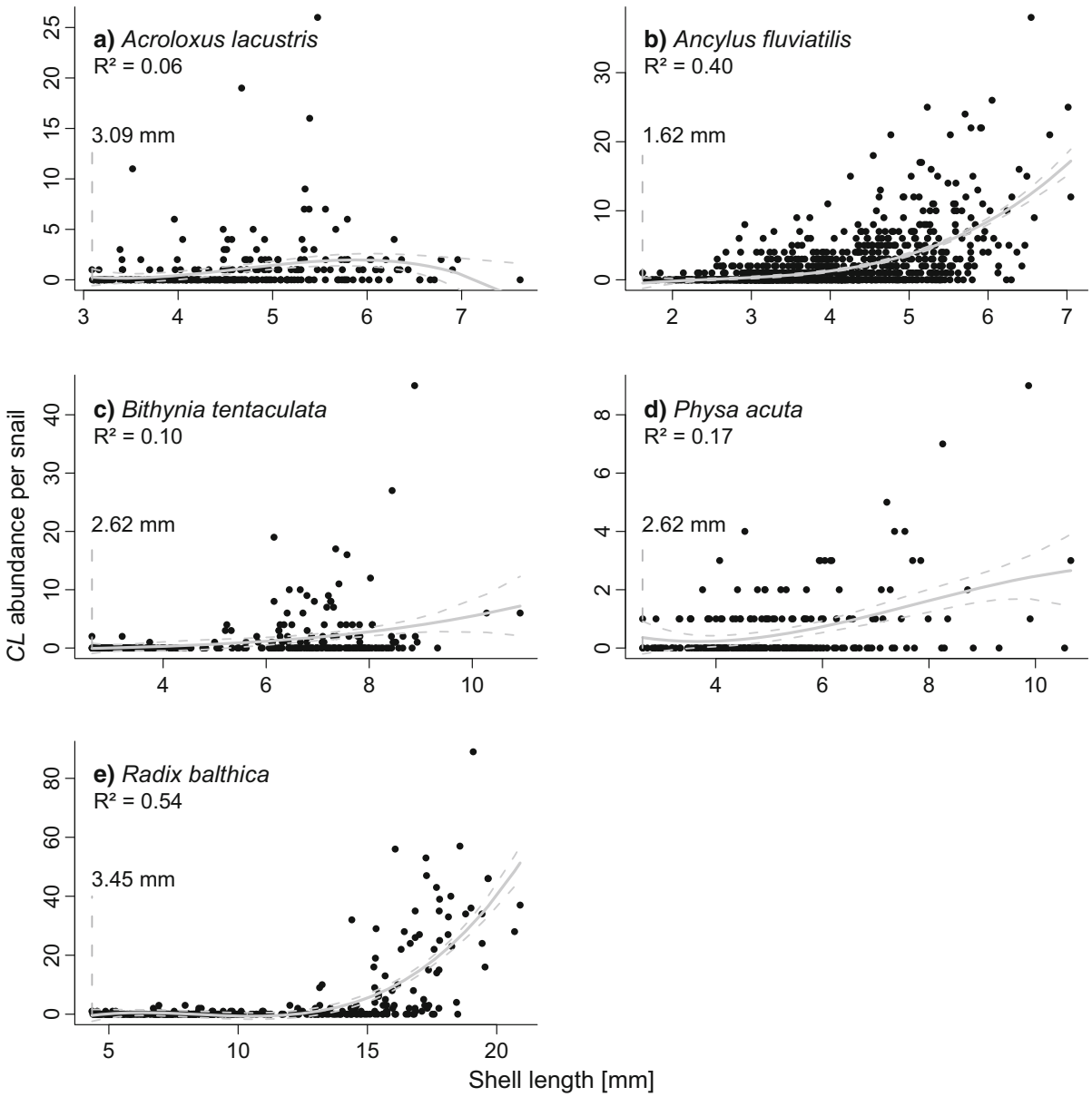

Fig. 2 Relationship between mean abundance of *Chaetogaster limnaei limnaei* (*CL*) and size of the host snail species **a** *Acroloxus lacustris*, **b** *Ancylus fluviatilis*, **c** *Bithynia tentaculata*, **d** *Physa acuta*, **e** *Radix balthica*. Polynomial fits were applied, dashed lines represent 95% confidence intervals. Coefficients and statistical specifications of the fits are presented in Appendix Table S2. Grey vertical lines indicate the species-specific minimum size of *CL* infestation. Note different scaling of *x*- and *y*-axes

Interestingly, infestation levels were positively related to host species densities at the substratum level, i.e. in the near surroundings (Fig. 4). At the site level, infestation levels were negatively related to host species density. High variability in water temperature and high oxygen concentration were favourable for *CL* (Fig. 4).

Effects of CL on snail reproduction

Backward selection of the multiple linear regression model eliminated the variables host species identity and mean host size, retaining only mean *CL* abundance and adult snail density (Table 3). Snail reproductive success, as determined by the proportion of

Table 2 Statistical results of boosted regression tree (BRT) analysis on environmental variables affecting infestation patterns of snails by *Chaetogaster limnaei limnaei* (*CL*)

Data		Mean *CL* abundance	Infestation prevalence	Infestation intensity
	Number of trees	2,550	3,350	2,500
Training	% deviance explained	92.1	65.2	91.2
	Correlation	0.96	0.85	0.96
	Mean null deviance	20.42	29.08	22.12
	Mean residual deviance	1.68	10.11	1.94
CV	% deviance explained	67.3	35.1	64.4
	Correlation	0.60 ± 0.11	0.61 ± 0.07	0.56 ± 0.11
	Estimated deviance	7.01 ± 1.18	18.86 ± 0.61	7.88 ± 1.32

juveniles in populations, decreased with increasing mean *CL* abundance in a population (Fig. 5). No significant relationship with adult snail density was detected, but as a trend, snail reproductive success increased with population density.

Discussion

This study demonstrated that under field conditions, the proportion of juveniles in snail populations, which we took as a proxy for reproductive success, was inversely related with *CL* infestation levels. Effects of *CL* infestation on snails were so far predominantly examined at the level of individuals. Positive effects of *CL* infestations on host snails were reported from experiments, in which snails were additionally parasitized by trematodes (Rodgers et al., 2005; Zimmermann et al., 2011). Experiments without trematode infections found either no effect of *CL* infestation (8 *CL* per snail on *B. glabrata*, Rodgers et al., 2005) or even negative effects of *CL* infestation on snail reproduction (10 *CL* per snail on *P. acuta*, Stoll et al., 2013). Unfortunately, trematode infestation of the host snails was not assessed in this study as snails were immediately frozen in the field to avoid post-sampling alterations in *CL* infestation levels, which can occur rather quickly (Hopkins et al., 2013). However, trematode infestations are common in aquatic snail populations in Europe (Faltynkova & Haas, 2006). It was shown in laboratory studies that changes in trematode infestation can alter host profitability, which then is reflected in *CL* abundances on individual host snails (Hopkins et al., 2013). We can therefore not rule out that decreasing reproductive success in snail populations with high *CL* infestation levels were actually caused by higher levels of trematode infections, with *CL* only responding to the trematodes. Further experimental studies are needed to clarify causality of the relationships that we found. In the present study, no species-specific differences in the effects of *CL* infestation on reproductive output of host snail populations could be discerned, while previous studies found clear evidence for differential effects of *CL* infestations on important life history parameter in different host snail species (Rodgers et al., 2005; Höckendorff et al., 2015). The actual snail species that were found to be preferentially infested by *CL* varied between different studies. In controlled host choice experiments conducted in the laboratory by Höckendorff et al. (2015), the physids *P. acuta* and *P. fontinalis* were preferred to *R. balthica* and *B. tentaculata*. Also in Buse (1974), studying a partly overlapping set of species, *P. fontinalis* showed a higher prevalence of *CL* infestation than *B. tentaculata*, while in Ibrahim (2007), *P. acuta* was among the least preferred snail species. In the present field study, as the net difference between host species, taking into account all environmental variables, *P. acuta* was among the least infested species, while *B. tentaculata* showed the highest levels of infestation. Nevertheless, even though being least infested as a net species effect, *R. balthica* reached the highest individual infestation levels, as this was the species that reached the largest body sizes. These results emphasize that host species preference is always context dependent, and general conclusions are difficult to draw. Interacting effects like host size and population densities, as well as

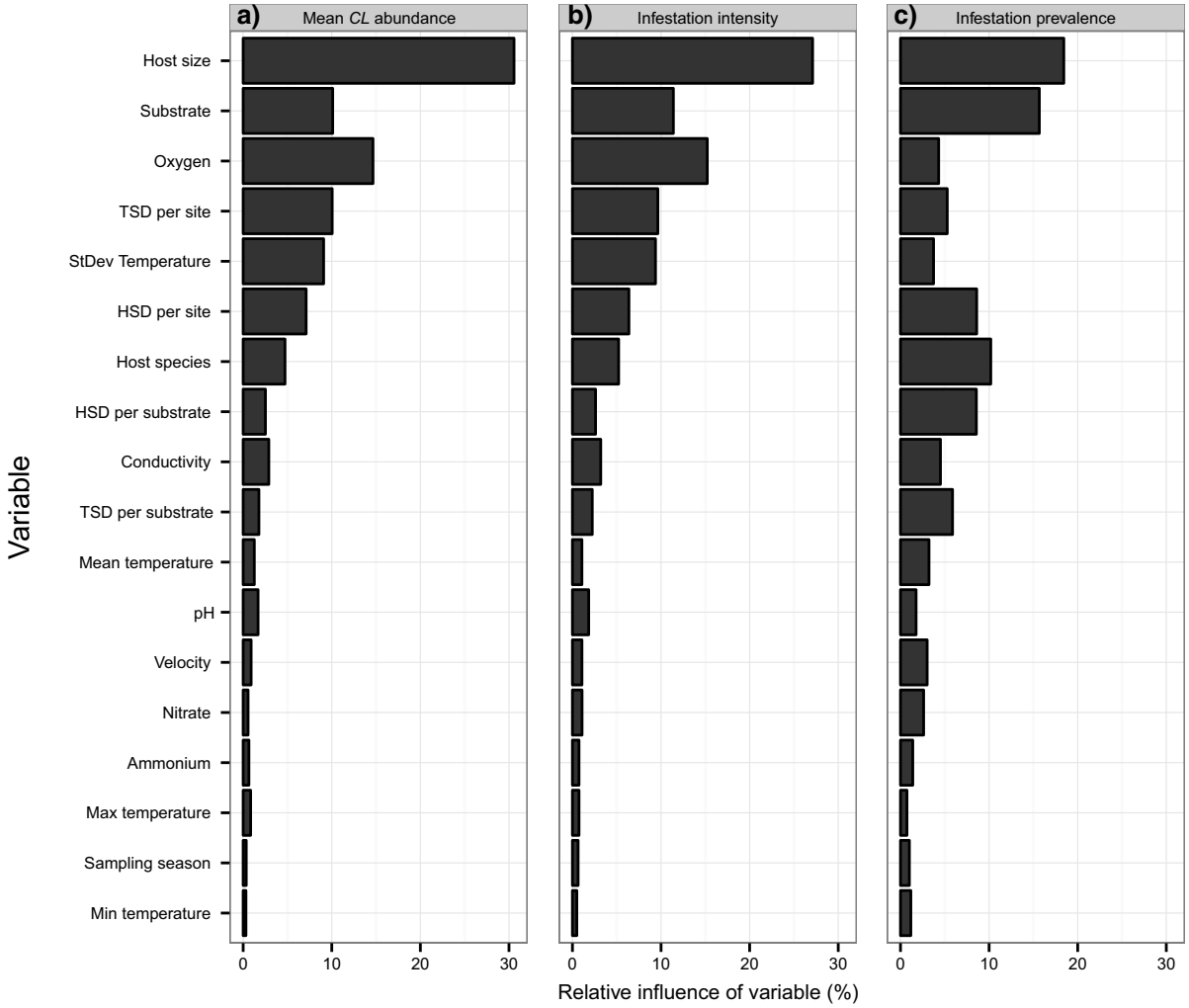

Fig. 3 Importance of individual variables in explaining **a** mean *Chaetogaster limnaei limnaei* abundance of host snails, **b** infestation intensity and **c** infestation prevalence of host snails. Variables used in the analyses were host species, host size, total snail density (TSD) per site, TSD per substratum, host species density (HSD) per site, HSD per substratum, substratum type, sampling season, oxygen concentration, water velocity, pH, conductivity, nitrate concentration, ammonium concentration as well as mean, maximum, minimum and standard deviation of water temperature during the study period

environmental variables can obscure infestation patterns, and additionally, relative infestation patterns of the snail species were shown to change between different seasons in the present study. This context dependency of species interactions in general makes this important aspect of ecology so difficult to include into predictive modelling approaches (Domisch et al., 2015; Früh et al., 2015).

Our findings of seasonal changes in *CL* infestation levels are generally consistent with the results of other studies on the population dynamics of *CL* (Gruffydd, 1965b; Streit, 1974; Young, 1974; Ibrahim, 2007) with peak *CL* abundance in spring or summer, and lower abundances during other seasons of the year. This strong increase of asexual *CL* reproduction coincides with the reproduction season of their snail hosts and may aid the colonization of the next host generation. Hopkins et al. (2015) showed that direct contact between host individuals is necessary for efficient transmission of *CL*. This dispersal hypothesis to explain seasonal abundance peaks was already put forward by Buse (1971) for the endoparasitic congener

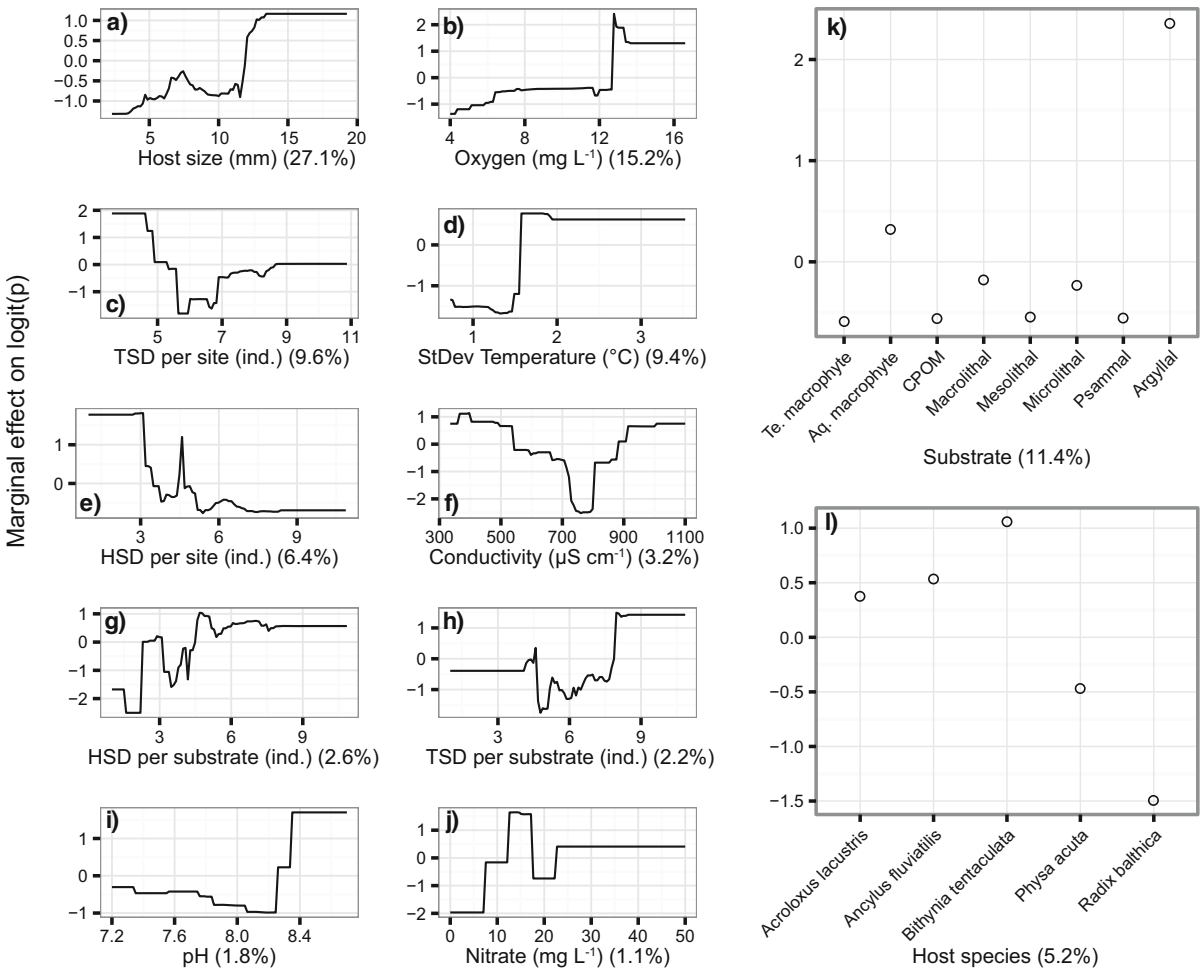

Fig. 4 Individual effects of 12 most important variables explaining infestation intensity by *Chaetogaster limnaei limnaei*. *Values* in brackets indicate the relative importance of each variable. Additional variables that cover less than 1% of the explained variance are not shown. Compare with Fig. 3 for variable names

Table 3 Results from multiple linear regression model on snail reproductive success ($F_{2,13} = 7.02$, $P = 0.009$, $R^2_{adj} = 0.45$)

	Estimate ± SE	*T* value	Pr(>|*t*|)
Intercept	0.59 ± 0.21	2.8	0.016
Log (mean *CL* abundance)	−0.25 ± 0.07	−3.6	0.003
Log (adult snail density)	0.11 ± 0.08	1.4	0.182

Models were backward selected until the minimal Akaike Information Criterion was reached ($AIC_{min} = -31.1$)

of *CL*, *C. limnaei vaghini*. He also suggested that high abundances of *CL* infestation outside this critical phase of dispersal would lead to increased intraspecific competition. An alternative hypothesis for *CL* abundance peaks in summer put forward by Learner et al. (1978) simply points to the higher temperatures and greater food availability during this time of the year.

The profitability of host snails also varies with their body size (Buse, 1971; Ibrahim, 2007). Interestingly,

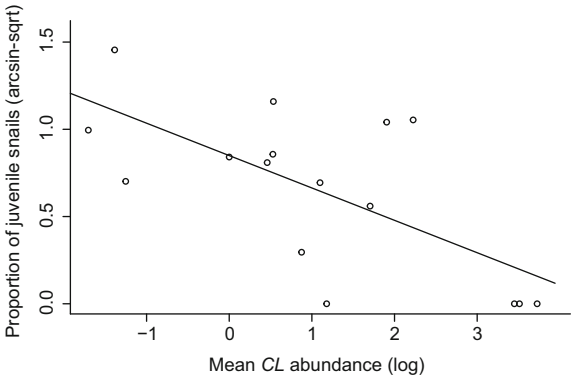

Fig. 5 Relationship between reproductive success of snail populations, indicated by the proportion of juveniles, and mean *CL* abundance in this population before the onset of the reproductive season

the minimum sizes at which hosts get infested by *CL* vary considerably between species. It may be speculated that these differences reflect shell and soft body morphology of the host species, leading to varying space limitations to ectosymbiotic *CL*.

In accordance with the hypothesis that dispersal and colonization of new hosts is critical for *CL*, we found that higher densities of snails, both of the respective host species and all snail species combined, at the substratum level, were favourable for *CL* abundances, possibly as transmission of *CL* between hosts is facilitated by frequent contacts of snails (Hopkins et al., 2015). The role of host density as a determinant of parasite abundance is well known from parasitological studies (Arneberg et al., 1998), especially when parasites are directly transmitted (Arneberg, 2001). At the same time, higher densities of the respective snail host species and the combined densities of all snail species at the site level were connected with lower *CL* abundances. This finding suggests that ultimately total *CL* densities are not controlled by snail population size, but by other environmental variables. Among the physico-chemical variables that were assessed in this study, especially low oxygen concentration and stable temperatures throughout the year seemed to be comparatively unfavourable for *CL*. Nevertheless, it has to be noted that this analysis included only a small number of sites and the gradients in the variables covered by the set of sampling sites and substratum types were very limited.

Conclusion

In summary, this study presents the first evidence that *CL* can play a significant role in structuring snail populations in the field. Previous laboratory studies and studies at the level of individual snails demonstrated that the effects of *CL* on snails can be positive or negative. These relationships depended on the ecological context, particularly infestation intensity and co-occurrence with parasitic trematodes and nematodes. In the present study, we demonstrated in field conditions, and at the population level, that reproductive success of snail populations is inversely related with mean *CL* infestation. As species within snail communities are differentially infested, *CL* infestation effects are asymmetrically distributed among all snail species present in a habitat, which may affect the structure of entire snail communities.

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References

AQEM, 2002. Manual for the application of the AQEM system. http://www.aqem.de/index.php.

Arneberg, P., 2001. An ecological law and its macroecological consequences as revealed by studies of relationships between host densities and parasite prevalence. Ecography 24: 352–358.

Arneberg, P., A. Skorping, B. Grenfell & A. F. Read, 1998. Host densities as determinants of abundance in parasite communities. Proceedings of the Royal Society B 265: 1283–1289.

Backlund, H. O., 1949. En kommensal som äter sitt värddjurs parasiter. Fauna och flora: populär tidskrift för biologi 44: 38–41.

Brinkhurst, R. O. & B. G. M. Jamieson, 1971. Aquatic Oligochaeta of the World. Oliver and Boyd, Edinburgh.

Buse, A., 1971. Population dynamics of *Chaetogaster limnaei vaghini* Gruffydd (Oligochaeta) in a field population of *Lymnaea stagnalis* L. Oikos 22: 50–55.

Buse, A., 1974. The relationship of *Chaetogaster limnaei* (Oligochaeta: Naididae) with a variety of gastropod species. Journal of Animal Ecology 43: 821–837.

Buston, P. M. & J. Elith, 2011. Determinants of reproductive success in dominant pairs of clownfish: a boosted regression tree analysis. Journal of Animal Ecology 80: 528–538.

Domisch, S., S. Jähnig, J. P. Simaika, M. Kuemmerlen & S. Stoll, 2015. Application of species distribution models in stream ecosystems: the challenges of spatial and temporal

scale, environmental predictors and species occurrence data. Fundamental and Applied Limnology 186: 45–61.
Elith, J., J. R. Leathwick & T. Hastie, 2008. Boosted regression trees - a new technique for modelling ecological data. Journal of Animal Ecology 77: 802–813.
Faltynkova, A. & W. Haas, 2006. Larval trematodes in freshwater molluscs from the Elbe to Danube rivers (Southeast Germany): before and today. Parasitology Research 99: 572–582.
Fashuyi, S. A. & M. O. Williams, 1977. The role of *Chaetogaster limnaei* in the dynamics of trematode transmission in natural populations of freshwater snails. Zeitschrift für Parasitenkunde 54: 55–60.
Fernandez, J., T. M. Goater & G. W. Esch, 1991. Population dynamics of *Chaetogaster limnaei limnaei* (Oligochaeta) as affected by a trematode parasite in *Helisoma anceps* (Gastropoda). American Midland Naturalist 125: 195–205.
Friedman, J., T. Hastie & R. Tibshirani, 2000. Additive logistic regression: a statistical way of boosting. Annals of Statistics 28: 1189–1232.
Früh, D., P. Haase, & S. Stoll, 2015. Temperature drives asymmetric competition between alien and indigenous freshwater snails (*Physa acuta* vs. *Physa fontinalis*). PeerJ PrePrints 3: e1227.
Gruffydd, L. D., 1965a. Evidence for the existence of a new subspecies of *Chaetogaster limnaei* (Oligochaete) in Britain. Journal of Zoology London 146: 175–196.
Gruffydd, L. D., 1965b. The population biology of *Chaetogaster limnaei limnaei* and *Chaetogaster limnaei vaghini* (Oligochaeta). Journal of Animal Ecology 34: 667–690.
Haase, P., S. Lohse, S. Pauls, K. Schindehütte, A. Sundermann, P. Rolauffs & D. Hering, 2004. Assessing streams in Germany with benthic invertebrates: development of a practical standardized protocol for macroinvertebrate sampling and sorting. Limnologica 34: 349–365.
Hastie, T., R. Tibshirani & J. Friedman, 2009. The elements of statistical learning: Data mining, inference and prediction. Springer, New York.
Hijmans, R. J., S. Phillips, J. R. Leathwick, & J. Elith, 2013. Dismo: species distribution modelling. R package version 08-11. http://CRAN.R-project.org/package=dismo.
Höckendorff, S., D. Früh, N. Hormel, P. Haase & S. Stoll, 2015. Biotic interactions under climate warming: temperature-dependent and species-specific effects of the oligochaete *Chaetogaster limnaei* on snails. Freshwater Science 34: 1304–1311.
Hoover, J. J. & M. J. Lodes, 1986. *Chaetogaster limnaei* (Oligochaeta: Naididae) in Oklahoma. The Southwestern Naturalist 31: 542–544.
Hopkins, S. R., J. A. Wyderko, R. R. Sheehy, L. K. Belden & J. M. Wojdak, 2013. Parasite predators exhibit a rapid numerical response to increased parasite abundance and reduce transmission to hosts. Ecology and Evolution 3(13): 4427–4438.
Hopkins, S. R., L. J. Boyle, R. R. Sheehy, L. K. Belden & J. M. Wojdak, 2015. Dispersal of a defensive symbiont depends on contact between hosts, host health, and host size. Oecologia 179(2): 307–318.
Ibrahim, M. M., 2007. Population dynamics of *Chaetogaster limnaei* (Oligochaeta: Naididae) in the field populations of freshwater snails and its implications as a potential regulator of trematode larvae community. Parasitology Research 101: 25–33.
Khalil, L. F., 1961. On the capture and destruction of miracidia by *Chaetogaster limnaei* (Oligochaeta). Journal of Helminthology 35: 269–274.
Learner, M. A., G. Glochhead & B. D. Hughes, 1978. A review of the biology of British Naididae (Oligochaeta) with emphasis on the lotic environment. Freshwater Biology 8(4): 357–375.
Martins, R. T. & R. G. Alves, 2010. Occurrence of *Chaetogaster limnaei* K. von Baer, 1927 (Oligochaeta, Naididae) associated with Gastropoda mollusks in horticultural channels in Southeastern Brazil. Brazilian Journal of Biology 70: 1055–1057.
Michelson, E. H., 1964. The protective action of *Chaetogaster limnaei* on snails exposed to *Schistosoma mansoni*. Journal of Parasitology 50: 441–444.
Mrazek, A., 1917. The feeding habits of *Chaetogaster limnaei*. Sbornik Zoologicky 1: 22–23.
R Development Core Team, 2011. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna.
Rajasekariah, G. R., 1978. *Chaetogaster limnaei* K von Baer 1872 on *Lymnaea tomentosa*: Ingestion of *Faciola hepatica* cercariae. Experientia 34: 1458–1459.
Rodgers, J. K., G. J. Sandland, S. R. Joyce & D. J. Minchella, 2005. Multi-species interactions among a commensal (*Chaetogaster limnaei limnaei*), a parasite (*Schistosoma mansoni*), and an aquatic snail host (*Biomphalaria glabrata*). Journal of Parasitology 91: 709–712.
Sankurathri, C. S. & J. C. Holmes, 1976. Effects of thermal effluents on parasites and commensals of *Physa gyrina* Say (Mollusca: Gastropoda) and their interactions at Lake Wabamun, Alberta. Canadian Journal of Zoology 54: 1742–1753.
Stoll, S., D. Früh, B. Westerwald, N. Hormel & P. Haase, 2013. Density-dependent relationship between *Chaetogaster limnaei limnaei* (Oligochaeta) and the freshwater snail *Physa acuta* (Pulmonata). Freshwater Science 32: 642–649.
Streit, B., 1974. Populationsdynamik von *Chaetogaster limnaei limnaei* in einer Population von *Ancylus fluviatilis*. Archiv für Hydrobiologie Supplement 47: 106–118.
Streit, B., 1977. Morphometric relationships and feeding habits of two species of *Chaetogaster*, *Ch. limnaei* and *Ch. diastrophus* (Oligochaeta). Archiv für Hydrobiologie Supplement 48: 424–437.
Vaghin, V., 1946. On the biological species of *Chaetogaster limnaei* k. Baer. Doklady Akademii Nauk SSSR 51: 481–484.
Wagin, V. W. L., 1941. *Chaetogaster limnaei* K. Baer als Cercarienvertilger. Zoologischer Anzeiger 95: 55–59.
Young, M. R., 1974. Seasonal variation in the occurrance of *Chaetogaster limnaei limnaei* Gruffydd (Oligochaeta) in two of its molluscan hosts in the Worcester-Birmingham canal and its relationship with the digenean parasites of these molluscs. Journal of Natural History 8: 529–535.
Zimmermann, M. R., K. E. Luth & G. W. Esch, 2011. Complex interactions among a nematode parasite (*Daubaylia potomaca*), a commensalistic annelid (*Chaetogaster limnaei limnaei*), and trematode parasites in a snail host (*Helisoma anceps*). Journal of Parasitology 97: 788–791.