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Cercarial behaviour alters the consumer functional response of three-spined sticklebacks

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Abstract

1. Free-living parasite life stages may contribute substantially to ecosystem biomass and thus represent a significant source of energy flow when consumed by non-host organisms. However, ambient temperature and the predator's own infection status may modulate consumption rates towards parasite prey.
2. We investigated the combined effects of temperature and predator infection status on the consumer functional response of three-spined sticklebacks towards the free-living cercariae stages of two common freshwater trematode parasites (*Plagiorchis* spp., *Trichobilharzia franki*).
3. Our results revealed genera-specific functional responses and consumption rates towards each parasite prey: Type II for *Plagiorchis* spp. and Type III for *T. franki*, with an overall higher consumption rate on *T. franki*. Elevated temperature (13 °C) increased the consumption rate on *Plagiorchis* spp. prey for sticklebacks with mild cestode infections (<5% fish body weight) only.
4. High consumption of cercarial prey by sticklebacks may impact parasite population dynamics by severely reducing or even functionally eliminating free-living parasite life stages from the environment. This supports the potential role of fish as biocontrol agents for cercariae with similar dispersion strategies, in instances where functional response relationships have been established.
5. Our study demonstrates how parasite consumption by non-host organisms may be shaped by traits inherent to parasite transmission and dispersal, and emphasizes the need to consider free-living parasite life stages as integral energy resources in aquatic food webs.

Introduction

Parasites are ubiquitous and functionally important organisms in aquatic ecosystems (Poulin & Morand, 2004), contributing significantly to ecosystem biomass (Kuris et al., 2008; Preston et al., 2013; Thieltges et al., 2013), and food web topology and stability (Amundsen et al., 2009; Dunne et al., 2013; Lafferty et al., 2008). However, parasites remain largely ignored in food web ecology (e.g. Jephcott et al., 2016; Marcogliese & Cone, 1997). Whilst parasites have the potential to alter both food web linkage density (number of links per species) and connectance (proportion of possible links actually occurring) (Johnson et al., 2010; Thieltges et al., 2013), little is known about the importance of free-living parasite life stages as prey resources for non-host species in aquatic food webs (Kaplan et al., 2009; Kuris et al., 2008; Mironova et al., 2019).

Cercariae, one of the free-living infectious stages of trematode parasites, emerge from their first intermediate molluscan hosts in considerable biomasses (e.g. Kuris et al., 2008; Soldánová et al., 2016) prior to infecting their second intermediate or final hosts in either a two- or three-host life cycle (Combes et al., 1994; Morley, 2012). However, cercariae may also encounter non-host organisms, whereby the parasite's functional role may change to that of a prey resource (Goedknecht et al., 2012; Johnson et al., 2010; Thieltges et al., 2008). Cercariae typically fall into the meso-zooplankton size range (0.2–2 mm), and given their abundance, they thus represent a noteworthy and easily digestible glycogen and lipid nutritional resource for aquatic predators akin to zooplankton (Johnson et al., 2010; McKee et al., 2020). Potential consumers of cercariae encompass a variety of non-host organisms including fish, insect larvae, rotifers, bivalves or arthropods (e.g. Kaplan et al., 2009; Orlofske et al., 2015; Welsh et al., 2014, 2019), in which consumption does not lead to successful parasite transmission (Johnson et al., 2010; Thieltges et al., 2013).

Trematode infection levels, and thus cercarial density, are naturally heterogeneous in aquatic ecosystems, due to the combined influences of spatial (e.g. distribution of infected molluscan hosts; Thieltges & Reise, 2007) and temporal factors (e.g. species-specific diurnal and/or temperature-dependent cercarial emergence patterns; Pietrock & Marcogliese, 2003; Born-Torrijos et al., 2014), in addition to species-specific traits (e.g. density: size trade-off of released cercariae; Koehler et al., 2012; McCarthy et al., 2002). Studies examining the potential impact of predators on cercariae populations have largely focused on identifying potential predator species (e.g. Kaplan et al., 2009; Welsh et al., 2014), or quantifying consumption rates at a few selected prey densities ($n = 1-3$ densities; Mironova et al., 2019; Orlofske et al., 2015; Welsh et al., 2017),

rather than understanding predator-prey dynamics across naturally occurring prey densities (e.g. Barrios-O'Neill et al., 2015; Paterson et al., 2015).

Increasingly, consumer functional responses (i.e. the relationship between the consumption rate of a predator and the density of its prey, hereafter 'FR') have been shown to offer a robust measure to assess the impact of predators on prey populations across ecologically relevant prey densities (e.g. Holling, 1959; Jeschke et al., 2004). Previous studies evaluating the impact of predators on cercariae excluded low prey densities, which are important for distinguishing between the potentially destabilizing or stabilizing effects of Type II and Type III responses on prey populations (Juliano, 2001). Until recently, the only study using a FR approach to understand the potential impact of aquatic predators on cercariae populations was confounded by the predator species also being a host of the focal parasite (Anderson et al., 1978). However new studies now demonstrate that non-host invertebrate predators may display contrasting FR types on cercariae populations (Born-Torrijos et al., 2020; Mironova et al., 2020).

In order for consumer FR approaches to provide a realistic insight into predator-prey interactions, it is essential to account for how other ecologically relevant factors, in addition to prey availability, alter resource consumption dynamics. For instance, parasitic infections in the predators themselves are capable of altering consumption rates, and thus the impact on prey populations (Larsen et al., 2013; O'Shaughnessy et al., 2014; Toscano et al., 2014). Coupled with seasonally- and/or climate change-induced temperature variation, the added metabolic costs associated with parasite infections may also have the capability of amplifying the effects that increasing temperature alone has on prey consumption (Larsen & Mouritsen, 2009; Laverty et al., 2017). Hence, FRs may exhibit both temperature and predator infection status driven effects (e.g. Daugaard et al., 2019; Iltis et al., 2018), though stability of FRs across different temperatures and infection status has also been noted elsewhere (e.g. Laverty et al., 2017; Wasserman et al., 2016). The assessment of consumer FRs in relation to the combined effects of temperature and predator infection status has the potential to reveal circumstances that can modulate (constrain or facilitate) a predator's ability to control parasite-induced diseases, which are particularly important in the context of identifying biocontrol agents suited to future climate change scenarios.

Three-spined stickleback *Gasterosteus aculeatus* L. (Actinopterygii, Gasterosteiformes; hereafter 'stickleback') is an ideal model predator species for investigating how the combined effects of temperature and infection status alter the FR of a non-host vertebrate on parasite prey. This widely distributed freshwater fish species is host to a variety of parasites (Scharsack et al.,

2016), including the cestodes *Dibothriocephalus* spp. (formerly *Diphyllobothrium* spp., Waeschenbach et al., 2017) and *Schistocephalus solidus* (Müller, 1776) Steenstrup, 1857 (Kuhn et al., 2015), and has been shown to display Type II responses to invertebrate prey (see review Jeschke et al., 2004). Whilst temperature may alter stickleback prey consumption (Wootton et al., 1980), the characteristic behavioural and physiological changes (reduced fright response, visible bulge and restricted stomach capacity) caused by severe *S. solidus* infections may also alter the foraging activity of this fish (Milinski, 1985; Tierney et al., 1993). Furthermore, parasite-specific morphological and behavioural traits inherent to dispersal and transmission also alter its predation risk (Born-Torrijos et al., 2020; Catania et al., 2016; Kaplan et al., 2009; Orlofske et al., 2015; Selbach et al., 2019).

In this study, we utilise a FR approach to test the ability of three-spined sticklebacks to consume two common trematode genera, by assessing their predatory impact on a naturally occurring range of prey densities. Furthermore, we evaluate if trematode genera-specific traits linked to their dispersion strategies influence the rate they are consumed, and we explore whether ambient temperature and the predator's cestode infection severity influence observed predator-prey interactions.

Materials and methods

Study system and organisms

The study was carried out in an oligotrophic, dimictic sub-Arctic freshwater ecosystem, Takvatn (northern Norway, 69°07'N, 19°05'E), which represents one of the world's best explored lake food webs (Klemetsen et al., 2002; Amundsen et al., 2009, 2019). The lymnaeid snail *Radix balthica* (L.) (Gastropoda, Lymnaeidae) serves as first intermediate host for the majority of larval trematodes of this lake (prevalence \approx 30%; Soldánová et al., 2017). Sticklebacks occupy shallow benthic areas in Takvatn to shelter from predators and to access diverse small prey (ostracods, cladocerans, rotifers; Jørgensen & Klemetsen, 1995; Amundsen et al., 2009), and are frequently infected with *Dibothriocephalus* spp. and *S. solidus* (prevalence 60% and 25% respectively; Kuhn et al., 2015). Two trematodes (identified *in vivo* according to previous research, Soldánová et al., 2017) were selected as focal prey, which i) exhibited genera-specific morphological and behavioural traits of dispersion strategies common among cercariae (i.e. light-oriented aggregate swimming vs benthic horizontal dwelling), and ii) did not use fish as their next intermediate host.

Trichobilharzia franki haplotype “peregra” (Schistosomatidae) (hereafter *Trichobilharzia*) has a two-host (snail, birds) life cycle (Soldánová et al., 2017). Its furcocercariae (i.e. bifurcated tail) exhibit diurnal emergence patterns from their snail host (Soldánová et al., 2016) and show negative geotaxy and positive phototaxy (Horák et al., 2015). In contrast, *Plagiorchis* spp. (Plagiorchiidae) (hereafter *Plagiorchis*) use a three-host life cycle, with aquatic invertebrates and usually birds as second intermediate or definitive hosts, respectively (Soldánová et al., 2017). Monocercous xiphocercariae of *Plagiorchis* (i.e. body with stylet and single tail) are released during the night (McCarthy, 1999), exhibit both negative phototaxy and geotaxy, and are benthic dwelling, swimming with continuous but slow movements (Lowenberger & Rau, 1994). Of the seven *Plagiorchis* species known to infect *R. balthica* in this lake (Soldánová et al., 2017), we selected large-sized *Plagiorchis* cercariae based on their high abundance at the time of the experiment, thus being the one most likely encountered by sticklebacks (as opposed to small-sized cercariae, ~480 vs ~410 μm ; based on our previous experience and Zikmundová et al., 2014). Cercariae of *Trichobilharzia* and *Plagiorchis* used in this study were ~940 μm (body, tail and furcae; mean length \pm SD: 267.0 \pm 29.1, 410.0 \pm 58.8, 263.3 \pm 25.7) and 480 μm long (body and simple tail: 312.1 \pm 62.7, 164.8 \pm 53.2), respectively.

Sampling and maintenance of study organisms

Sticklebacks were collected during August and October 2018 by setting baited traps (30 \times 70 cm, mesh size of 0.5 cm) overnight at 2–5 m depth. Infection status (mild or severe) of captured individuals (over 310) was determined by the presence of cestode-induced abdomen bulge (see Suppl. Video S1 and S2 for example). Sticklebacks were maintained for 3–10 days prior to the FR experiments in aquaria (12 L) containing aerated lake water under natural light (light:dark photoperiod: 4:20, 10:14 h, data loggers Onset HOBO UA-002-64 Pendant 64K, MA, US) and temperature conditions (epilimnetic temperature – 13 and 6 $^{\circ}\text{C}$) representative of Takvatn in August or October, respectively, and were provided *ad libitum* with natural prey (e.g. cladocerans, ostracods).

Naturally infected snails were collected by hand from the littoral zone of Takvatn and individually screened for trematode infections in beakers containing 20 mL lake water which were held under a light source for 24 h to stimulate cercarial emergence. Infected snails were individually maintained at ambient temperature in 30 mL of filtered lake water (replaced daily) and fed lettuce (*Lactuca sativa*). A pool of freshly emitted cercariae (*Trichobilharzia*-infected

snails were incubated under a light source, whereas *Plagiorchis*-infected snails were covered with aluminium foil to imitate night time) was prepared by combining emerged cercariae from several single infected snails, to minimise potential inter-snail variability in cercariae. Thereafter, cercariae were individually pipetted and placed into a plastic beaker in the required density with a small volume of filtered lake water (10 mL) for later use in the FR experiment. At the start of each FR experiment, all cercariae were alive, active and of same age (<4 h).

Experimental design

We employed a randomised, fully factorial design to test the combined effects of temperature (13 and 6 °C, average water temperatures in August and October, respectively) and cestode infection severity (expressed as percentage of total fish weight, <5%: mild, >5%: severe) on cercarial prey consumption. By setting the limit at 5% parasite:host biomass ratio to differentiate between mild- and severe-infected sticklebacks, we explored changes in FRs of sticklebacks with recognizable physical alterations due to cestode infections in the fish's coelomic cavity. Although the parasite weight is mostly due to *S. solidus*, the high prevalence of *Dibothriocephalus* spp. could not be ignored despite its small weight compared to *S. solidus*, and both parasites were therefore included in the infection severity index. Each stickleback was supplied with one of nine prey densities (2, 4, 6, 10, 20, 50, 70, 100 or 200 cercariae) at random from one of the two trematode genera, *Plagiorchis* and *Trichobilharzia*, with each treatment combination replicated four times. Prey densities were chosen based on the density of cercariae released from naturally infected snails and estimated emergence rates (0.01–35.04 cercariae m⁻², Suppl. Table S1; unpublished data, Soldánová M.). Whilst intermediate prey densities were chosen to cover the entire range of selected prey densities, the highest densities were those at which satiation was reached, thus prey densities encompass the range of ecologically relevant cercariae densities sticklebacks likely encounter. Stickleback size and age was standardised to avoid their confounding effects on the observed consumption rates (1+ and 2+ fish, standard length mean ± SD (mm); *Plagiorchis* prey: fish mild infection at 6 °C, 44.9 ± 7.9; at 13 °C, 47.0 ± 5.7; severe infection at 6 °C, 50.1 ± 8.5; and at 13 °C, 44.9 ± 5.9; *Trichobilharzia* prey: fish mild infection at 6 °C, 45.2 ± 7.6; at 13 °C, 47.1 ± 7.1; severe infection at 6 °C, 48.5 ± 6.1; and at 13 °C 50.0 ± 8.1).

Prior to each experimental trial, sticklebacks were acclimatized for 48 h to the selected temperature and light:dark photoperiod, and held without food for 24 h to standardise satiation levels (Croy & Hughes, 1991). Each stickleback was randomly assigned to an individual

experimental arena containing filtered lake water to allow natural movements (container diameter 150 mm, 500 mL), which was placed on a surface covered by green coloured paper to mimic their natural habitat.

Cercariae were added to the experimental arena containing a single predator by carefully pouring them from the plastic beakers, which were rinsed twice with filtered lake water. Sticklebacks were allowed 30 min to consume cercariae without replacement of consumed individuals, with the experiment duration determined by pilot trials using times suggested in previous research (Heinclová, 2018; Orlofske et al., 2012; 2015). Sticklebacks were then carefully removed and the number of unconsumed cercariae was assessed by thoroughly rinsing the experimental arena with filtered lake water through a 12 µm-pore filter membrane (Whatman, Nuclepore Track-Etch Membrane, Sigma-Aldrich). Each filter was placed into a petri dish and remaining cercariae were fixed and stained in a 70% ethanol and carmine solution, and counted under the stereomicroscope. To account for the potential loss of cercariae due to human error or the filtration procedure, control replicates were run for each trematode genus following the same procedure in the absence of sticklebacks. The number of cercariae consumed by each fish was adjusted by the mean loss of cercariae specific to each trematode genus and cercariae density. Furthermore, to verify the consumption of both trematode genera by severe- and mild-infected sticklebacks, the foraging behaviour was video-recorded for a subset of individuals (Suppl. Video S1, S2).

Individual sticklebacks were used only once, with the exception of the October *Plagiorchis* experiment, where three individuals were used in two trials due to low sticklebacks availability. In these cases, each stickleback was maintained for 24 h in lake water containing natural prey between trials, and then starved as usual before the assay. After the experiment, all sticklebacks were euthanized by concussion (percussive blow to the head) and subsequent destruction of the brain, according to directive 2010/63/EU, measured and weighed, prior to cestode examination and confirmation of cestode infection severity. *Dibothriocephalus* spp. and *S. solidus* were counted and weighed (mean ± SD cestode weight per fish, 0.02 ± 0.06 mg, 0.12 ± 0.22 mg, respectively). When the proportion for considering the infection as mild or severe differed from the initial visual inspection, the replicate was reallocated to the correct infection severity status, and the experiment was repeated where necessary (correct infection severity detection for 83.5% of experiments).

Statistical analysis

All statistical analyses were undertaken using R (version 3.6.1, R Core Team, 2017). Prior to analysis, all raw data was visually inspected to assess whether differences in the experimental timeframe (e.g. between months, August/October) influenced the number of prey consumed, with no outliers detected.

Logistic regressions were fitted to each predator-prey combination using `frair::frair_test` (version 0.5.100, Pritchard et al., 2017) to determine whether a Type II or III FR best described the relationship between cercariae density and the number of cercariae consumed. Type II responses are described by a negative first-order term (decreasing proportional consumption with increasing prey density), whereas Type III responses are indicated by a positive first-order term followed by a negative second-order term (initial increase and subsequent decrease in proportional consumption; Juliano, 2001). While the parameters a (capture/attack rate), h (handling time, i.e. time spent subjugating, ingesting and digesting each prey item) and T (experimental duration) describe a Type II response; a Type III response is characterized by h , q (scaling component), b (search coefficient, related to the foraging time of a predator) and T . The scaling component q is a critical determinant of FR shape as it shows the extent to which FR changes from a decelerating hyperbola to a sigmoidal form, being thus $q = 0$ in Type II and $q > 0$ in Type III. Plotting of raw data indicated that a Type I response (linear relationship only reported in filter-feeding species; Jeschke et al., 2004) was not appropriate for our data. The fixed parameter T (experimental time) was set to 0.5/24 (30 min) and the FR models were fitted using maximum likelihood estimation (MLE) by using `frair::frair_fit` (Pritchard et al., 2017). A single model was fitted for each stickleback-trematode combination to test for differences in cercariae consumption between infection severity and temperature. The prey density of 200 *Plagiorchis* cercariae was excluded from the analyses as estimates became unstable due to the high variability in consumed prey, especially at 6 °C (Suppl. Table S2). Functional response models could be applied to both stickleback-trematode combinations, yielding estimates for all parameters. Bootstrapping ($n = 1,500$) was used to construct 95% confidence intervals to visualize the uncertainty around the fitted FRs.

Results

Prey loss was somewhat higher at some densities (e.g. in the 50- and 200-prey density of the *Trichobilharzia*-stickleback combination, Suppl. Table S3), whereas both prey survival (in

terms of vital/mobile cercariae) in the absence of sticklebacks, and the survival of sticklebacks themselves was 100%. The overall prevalence and mean intensity of cestode-infections in sticklebacks were 51.8% and 2.38 for *S. solidus*, and 54.9% and 8.28 for *Dibothriocephalus* spp. (n = 257). The percentage of parasite to fish weight as an estimate of infection severity was on average (mean \pm SD) $34.3 \pm 32.0\%$ (range: 5.1–189.9%) for severe infections and $0.5 \pm 1.0\%$ (range: 0.0–5.0%) for mild infections. Thus, the mild-infected fish group had either very small infections or were uninfected, having none of the recognizable physical or behavioural alterations characteristic in heavily infected individuals. A subsample of fish was dissected for *Diplostomum* (Diplostomidae) and *Apatemon* (Strigeidae) eye infections, with no heavy infections causing visual impairment observed (see Suppl. Information).

Raw data and the percentage of consumed cercariae for each stickleback-prey, temperature and infection severity combination are summarised in supplementary Table S2. Sticklebacks exhibited an overall higher consumption towards *Trichobilharzia*. Differences in the proportion of consumed cercariae was particularly evident at low prey densities (<10 prey), with more *Plagiorchis* consumed than *Trichobilharzia*. Despite considerable variation across *Plagiorchis*-prey densities, the proportion of consumed cercariae at intermediate (20- to 70-) or high prey densities (100- or 200-) was higher for *Trichobilharzia* (Suppl. Table S2).

Functional responses (FR)

Logistic regressions indicated that sticklebacks displayed Type II FRs when consuming *Plagiorchis* prey, and Type III FRs towards *Trichobilharzia* prey across both temperatures and cestode infection levels (Table 1).

In the stickleback-*Plagiorchis* predator-prey combination, FR attack rate a and handling time h differed significantly between all infection severities and temperatures, except for sticklebacks with severe infections (Table S4, Fig. 1). Sticklebacks with mild cestode infections at 6 °C showed the highest attack rate and handling time, whereas the attack rate was lowest for sticklebacks with severe cestode infections at 6 °C. Handling time was lowest for sticklebacks with mild cestode infections at 13 °C. Consumer FRs towards *Plagiorchis* prey showed context-dependencies in regard to temperature and predator infection severity, with severe-infected sticklebacks showing higher attack rates at 13 °C than at 6 °C. The opposite pattern was observed

for mildly infected individuals, who in addition showed a more than 10-fold greater attack rate in comparison to other temperature and predator infection combinations. However, as the high attack rate coincided with a higher handling time, together these acted to limit the FR asymptote, thus showing decreased consumption. Severely infected sticklebacks showed overlapping 95% confidence intervals indicating similar feeding rates at both temperatures (Fig. 1 a, b), while 95% confidence intervals indicated that mild-infected sticklebacks displayed a significantly higher FR to *Plagiorchis* at 13 °C than at 6 °C.

In the stickleback-*Trichobilharzia* predator-prey combination, FR search coefficient b and scaling component q differed significantly between all infection severities and temperatures, except the scaling component for sticklebacks at 6 °C with mild and severe cestode infections (Table S4, Fig. 2). The highest scaling component was shown by sticklebacks with severe infections at 6 °C, and the lowest by those with mild infections at 13 °C (Table S4). Handling time h differed significantly between mild- and severe-infected sticklebacks at 6 °C, as well as in mild-infected sticklebacks between 6 °C and 13 °C, which showed the highest and the lowest handling time, respectively (Table S4). Consumer FRs towards *Trichobilharzia* indicated that mild- and severe-infected sticklebacks had a higher search coefficient b at 13 °C than at 6 °C, while the scaling component q decreased (moving to a more destabilizing type of FR such as Type II). However, overlapping 95% confidence intervals suggested that neither temperature nor cestode infection severity altered the FR of sticklebacks towards *Trichobilharzia* prey (Fig. 2).

Discussion

Three-spined sticklebacks have for the first time been proven to efficiently consume trematode cercariae, thus demonstrating that parasites can constitute an additional foraging resource for this widespread fish species. To our knowledge, this study is also the first to describe the FR types, and the parameters characterizing them, of a non-host fish species consuming larval trematode prey. We demonstrate that sticklebacks display trematode genera-specific FRs towards cercarial prey (*Plagiorchis* – Type II, *Trichobilharzia* – Type III), which are robust to the influences of both temperature and cestode infection status of the predator. Stability of FR types across different temperatures and infection status has been observed in other predator-prey systems (e.g. amphipods-invertebrates, Paterson et al., 2015; notonectid-cladoceran, Wasserman et

al., 2016), thus suggesting that cercarial traits determining their dispersion and transmission strategies are most likely the main drivers shaping the observed FR here.

Cercariae consumption by sticklebacks may have major impacts on trematode populations, since these non-host organisms may severely reduce or even functionally eliminate free-living parasitic stages from the environment. Furthermore, given the vast geographic distribution of three-spined sticklebacks (25–75°N) coupled with their large population density fluctuations (Klemetsen et al., 2002), cercariae consumption by sticklebacks could represent a significant and underestimated driver of trematode infection dynamics across aquatic ecosystems. In many European lakes, shallow vegetated littoral zones represent a preferred habitat for both sticklebacks and trematode-infected snails (Jørgensen & Klemetsen, 1995), thus facilitating access to parasite prey. Although larval trematode availability may vary in time and space, our FR experiments suggest that sticklebacks can successfully exploit parasite prey in low densities during autumn conditions when snail infection is scarce, and at higher densities in summer months when highly infected snails release clouds of cercariae. Further prey choice studies are required to elucidate whether sticklebacks show a preference for particular parasite species, in addition to whether parasites are preferentially consumed over other similar sized prey (e.g. zooplankton). Thus, our data highlight the necessity to include commonly occurring parasites into food web studies as food resources themselves, concurrently reflecting their density-depending character as an energy source for non-host organisms.

High cercarial consumption by sticklebacks, such as observed for *Plagiorchis* at lower densities and *Trichobilharzia* especially at higher densities, could severely reduce the likelihood of subsequent hosts becoming infected. Not only does this suggest that abundant sticklebacks populations have the potential to shape aquatic parasite community composition, but it also emphasizes the value of non-host fish species as biological control agents for parasites of medical and veterinary importance. Previous studies have shown that both copepods and fish to be effective parasite control agents (e.g. Marten, 1990; Griffin & Knight, 2012), including the control of bilharziasis transmission to humans (Siau et al., 1992), however FR parameters are only beginning to be used as evaluation metrics for potential control agents (Cuthbert et al., 2018). Although our study demonstrated that sticklebacks showed Type III responses to *Trichobilharzia* prey, the identification of a fish species associated with prey population destabilizing Type II responses would be highly beneficial in the suppression of this parasite, which is the main

causative agent of human cercarial dermatitis (swimmer's itch) in Europe (Horák et al., 2015; Soldánová et al., 2013).

The efficient use of fish as biocontrol agents should include targeting systems with an evidenced interaction between trematode dispersal strategy and predator feeding behaviour, as this has been shown to alter cercariae predation susceptibility (Selbach et al., 2019). The differential predation rates and FR types towards the two trematodes potentially reflect genera-specific behavioural and size-related interactions linked to their dispersion strategies. For instance, *Trichobilharzia* is twice as large as *Plagiorchis*, so the higher consumption rates towards *Trichobilharzia* may indicate a preference for larger prey as noted elsewhere (Catania et al., 2016; Kaplan et al., 2009; Orlofske et al., 2015). On the other hand, sticklebacks are known to preferentially attack the densest region of prey swarms (Heller & Milinski, 1979), thus aggregated pelagic *Trichobilharzia* cercariae may facilitate consumption. Moreover, predation could also be triggered by the 'rest-swim-rest' behaviour of *Trichobilharzia* cercariae (Horák et al., 2015). In contrast, the continuous and slow swimming behaviour of *Plagiorchis* cercariae may largely be unnoticed by pelagic fish due to their benthic (negative phototaxy) location (Lowenberger & Rau, 1994). This, together with their smaller size, draws our attention to the fact that a higher proportion of *Plagiorchis* are consumed at lower prey densities, which likely determines the Type II response. These cercariae might be easier to prey at lower densities due to their slow swimming behaviour, while *Trichobilharzia* may be more difficult to catch when swimming isolated from a cercariae cloud. Moreover, the different FR types displayed towards each trematode species may also indicate the potential for prey switching (Barrios-O'Neill et al., 2014; Leeuwen et al., 2007) to exploit the species-specific emergence peaks, from *Trichobilharzia* during the day to *Plagiorchis* during the night (McCarthy, 1999; Soldánová et al., 2016). Future studies simultaneously employing different parasite species, especially those that occupy the same habitat (e.g. diurnal pelagic cercariae), would help to further elucidate a potential prey switching effect.

Surprisingly, temperature and infection status had only a limited impact on the consumption of both cercarial prey. Sticklebacks infected with *S. solidus* grow faster than uninfected individuals, suggesting increased consumption rates (Arnott et al., 2000). At 6°C, severe-infected sticklebacks showed higher consumption towards *Plagiorchis* than mild-infected individuals. However, although some parameters of the FRs differed between mild- and severe-infected individuals there were no consistent patterns across the two temperature regimes or parasite prey species. Similarly, only a positive temperature effect for mild-infected sticklebacks

consuming *Plagiorchis* at 13°C was observed, even though a food consumption by sticklebacks increases with temperature (Wootton et al., 1980). This would point to stronger impacts on *Plagiorchis* populations during warmer than cool water periods as this species might then be consumed by both mild- as well as severe- infected fish. In a meta-analysis investigating the relationships between FR parameters and temperature (Englund et al., 2011), temperature had a positive impact on both the attack rate and handling time of ectothermic animals, including fish. Our results show this only partially, probably because the attack rate is influenced by both predator and prey behavioural performance (Englund et al., 2011).

Furthermore, species-specific cercarial activity and survival rates are temperature-dependent (Poulin, 2006). *Trichobilharzia* has a wide temperature tolerance (Horák et al., 2015), thus its activity might not change in the 6 °C to 13 °C range, whereas *Plagiorchis* slows down its activity at decreased temperatures (*pers. obs.*), thus lowering predator-prey encounter rates. Whilst it has been suggested that FRs should tend towards Type II when environmental factors such as temperature reach the predator's optimal value (Barrios-O'Neill et al., 2016; Uszko et al., 2017), *Trichobilharzia* FRs showed a more sigmoidal shape (higher q) at decreased temperatures, where sticklebacks are well-adapted (Franke et al., 2017). This emphasises the need to assess a variety of local temperature regimes that encompass the organisms' range of experienced temperatures (e.g. like sub-Arctic extremes) when exploring displayed FRs, especially in order to uncover global trends in a predator's feeding behaviour.

In conclusion, our consumer FR experiments have provided a platform for continued investigations of infection severity and temperature effects on FRs and trematode consumption. Whilst temperature and cestode infection in sticklebacks do not appear to have strong impacts on FR, traits inherent to the trematode species transmission and dispersion strategies (e.g. morphotype or swimming behaviour; Morley, 2012), are likely the main drivers of cercarial consumption by sticklebacks. Future studies on the role of non-host organisms in the modulation of trematode populations could benefit from the integration of FRs displayed towards species with different dispersion and transmission strategies, as opposed to single-density experiments. Such experiments would enhance our understanding on the key roles non-host predators play in the regulation of trematode population dynamics, which may help in the reduction of disease risks and implementation of effective control measures, especially in the context of climate change when increased spread and transmission of water-borne diseases is expected.

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Authors' contributions. AB-T and MS conceived the ideas and designed methodology, with assistance from RAP. All authors participated in samplings. AB-T and GSB performed the experimental work. AB-T and RAP analysed data and functional responses. AB-T wrote the first draft of the manuscript. All authors contributed critically to the drafts and gave final approval for the publication.

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Figures and table

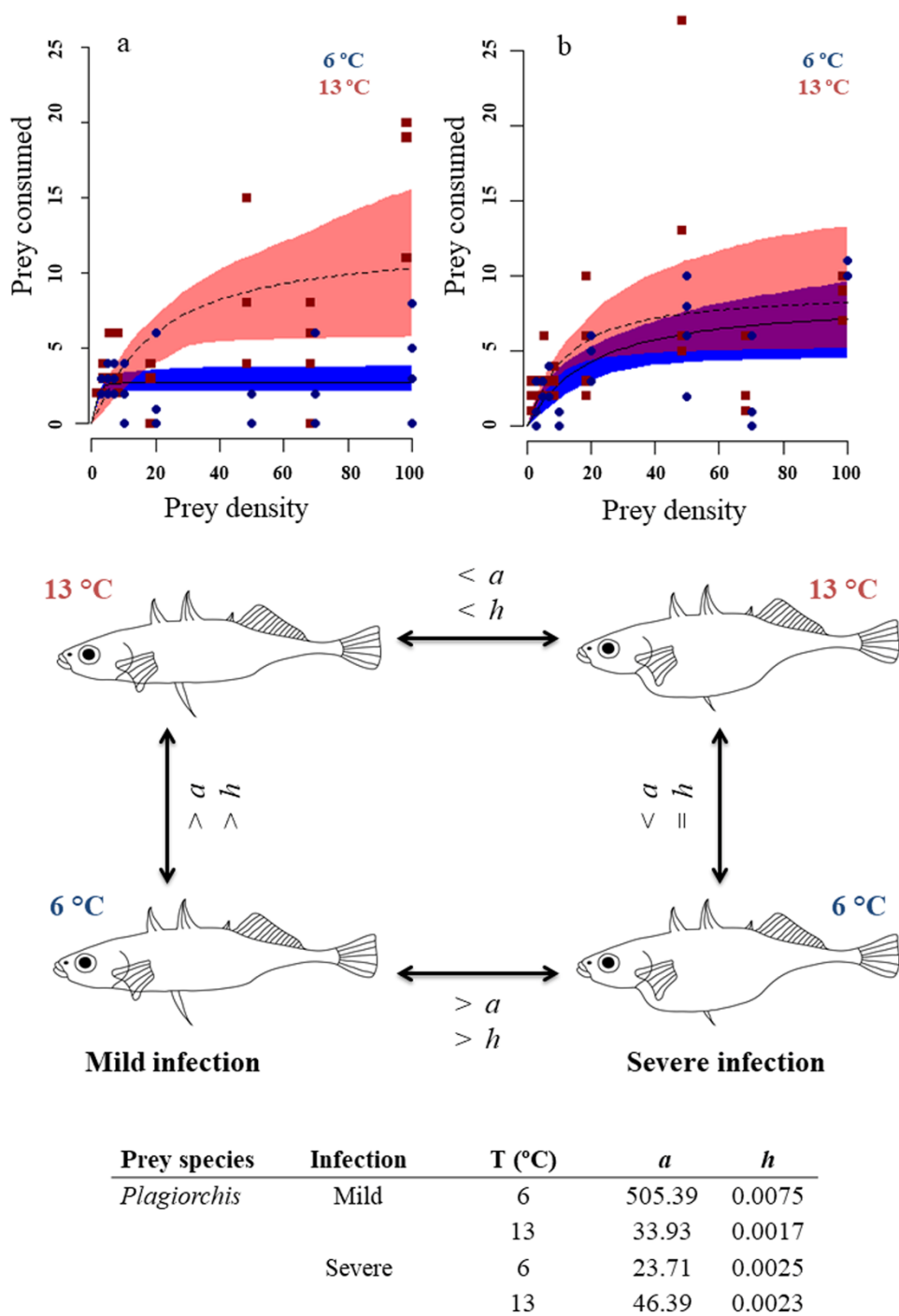


Figure 1. The effects of infection severity (a, mild; b, severe) and temperature on the consumer functional response of three-spined sticklebacks towards cercariae of *Plagiorchis*. Lines (solid, dashed) and coloured bars (blue, pink) represent the mean bootstrapped FR and 95% confidence intervals at 6 °C and 13 °C, respectively. Dots represent the raw data of functional response

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experiments (blue 6 °C, pink 13 °C). Significant differences (shown as < or >, $\alpha = 0.05$) in FR attack rate a and handling time h are shown by infection severity and temperature (T).

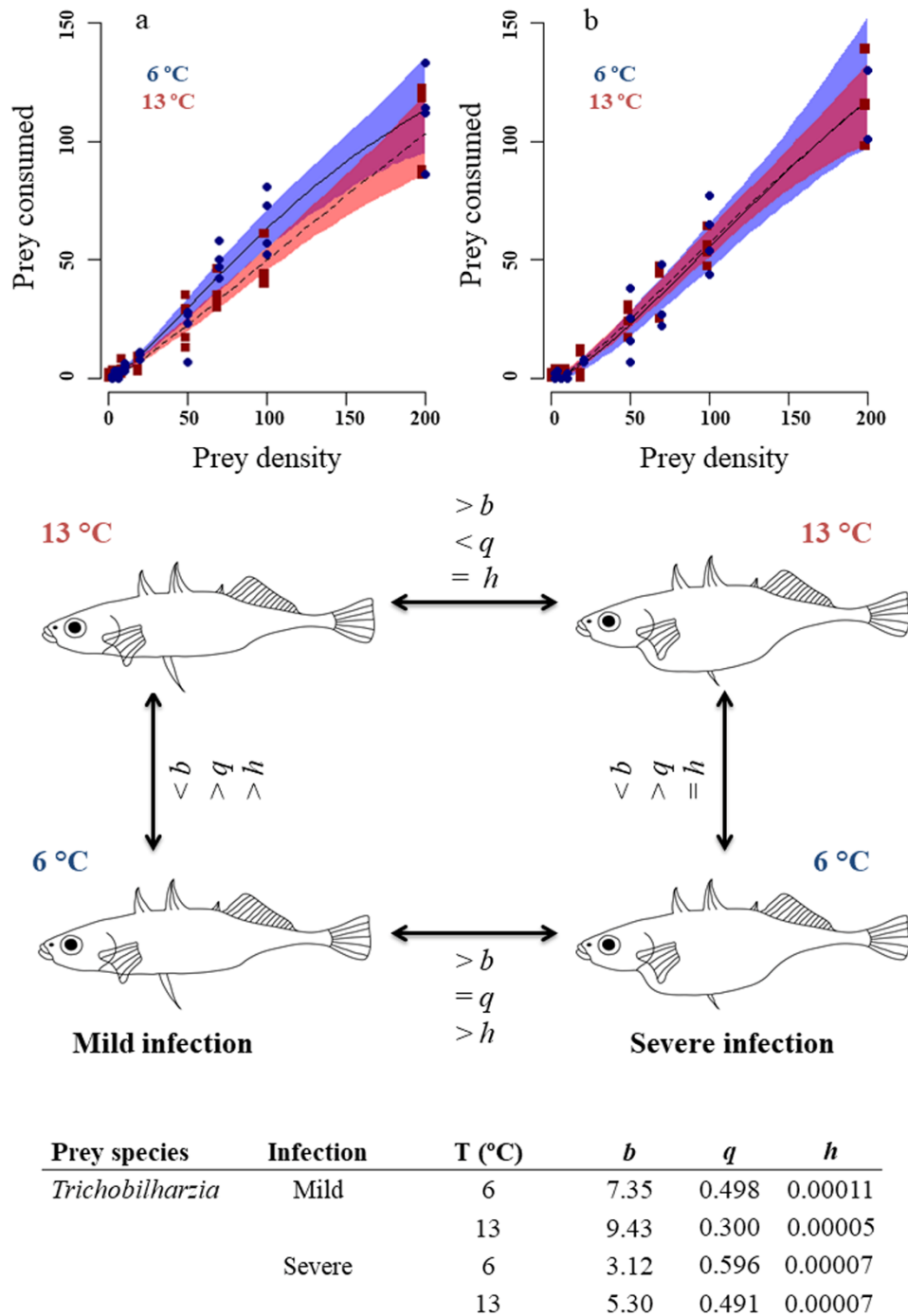


Figure 2. The effects of infection severity (a, mild; b, severe) and temperature on the consumer functional response of three-spined sticklebacks towards cercariae of *Trichobilharzia*. Lines (solid, dashed) and coloured bars (blue, pink) represent the mean bootstrapped FR and 95% confidence intervals at 6 °C and 13 °C, respectively. Dots represent the raw data of functional response

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experiments (blue 6 °C, pink 13 °C). Significant differences (shown as < or >, $\alpha = 0.05$) in FR handling time h scaling component q and search coefficients b are shown by infection severity and temperature (T).

Table 1. Results of logistic regressions for selecting categorical Type II or III functional response models. Statistically significant differences in parameters ($\alpha = 0.05$) are indicated in bold.

Predator	Prey species	Logistic regression Type II		Logistic regression Type III		
		1st term	P	1st term	2nd term	P
Three-spined sticklebacks	<i>Plagiorchis</i>	-0.0198386	<0.001	–	–	–
	<i>Trichobilharzia</i>	–	–	2.0095e ⁻⁰²	-7.1434e ⁻⁰⁵	<0.001