

1 **Short communication for Parasitology Research**

2 **Temperature does not influence functional response of amphipods consuming different**  
3 **trematode prey**

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35 **Keywords:** predator-prey, cercariae, transmission interference, *Gammarus lacustris*

36 **Abstract**

37 Direct consumption on free-living cercariae stages of trematodes by non-host organisms  
38 interferes with trematode transmission and leads to reduced infections in the next suitable hosts.  
39 Consumer functional responses provide a useful tool to examine relationships between

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40 consumption rates and ecologically relevant prey densities, whilst also accounting for abiotic  
41 factors that likely influence consumption rates. We investigated how temperature influences the  
42 consumer functional response of the amphipod *Gammarus lacustris* towards the cercariae of  
43 three freshwater trematodes (*Diplostomum*, *Apatemon* and *Trichobilharzia*). Amphipods  
44 displayed different functional responses towards the parasites, with Type II responses for  
45 *Diplostomum* and Type I responses for *Apatemon* prey. Temperature did not alter the  
46 consumption rate of the amphipod predator. *Trichobilharzia* was likely consumed at similar  
47 proportions as *Diplostomum*, however this could not be fully evaluated due to low replication.  
48 Whilst Type II responses of invertebrate predators are common to various invertebrate prey  
49 types, this is the first time a non-filter feeding predator has been shown to exhibit Type I  
50 response towards cercarial prey. The prey-specific consumption patterns of amphipods were  
51 related to cercarial distribution in the water column rather than to the size of cercariae or  
52 temperature influence. The substantial energy flow into food webs by non-host consumer  
53 organisms highlights the importance of understanding the mechanisms that modulate functional  
54 responses and direct predation in the context of parasitic organisms.

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

68 Data will be archived in the Dryad Digital Repository upon acceptance.

69 **Competing interests** The authors declare that they have no competing interests.

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## 86 **1. Introduction**

87 Trematodes are highly abundant parasitic organisms in aquatic ecosystems, with the  
88 biomass of their free-living life stages often equivalent to the most abundant insect orders  
89 (Preston et al. 2013). The immense mass of trematodes is largely attributed to the asexual  
90 production and daily release of thousands of free-living infective cercariae (larvae) from their  
91 first intermediate molluscan hosts (Preston et al. 2013; Soldánová et al. 2016). Whilst successful  
92 transmission of cercariae is essential to complete the trematode's life cycle, direct consumption  
93 of cercariae by non-host organisms can lead to reduced transmission to their next suitable host  
94 (Johnson et al. 2010). The loss of cercariae by consumption from different organisms has been  
95 reported (e.g. rotifers, molluscs, insect larvae, fishes; Orlofske et al. 2015; Welsh et al. 2017),  
96 but its importance appears to be largely overlooked despite the significant contribution parasites  
97 may have to energy flow through food webs when being consumed by non-host organisms  
98 (Thieltges et al. 2008; Johnson et al. 2010). Cercarial consumption may also be context  
99 dependent, influenced by environmental conditions such as temperature or habitat complexity,  
100 in addition to the impact of the size and behaviour of the parasite and the predator (e.g. Orlofske  
101 et al. 2015; Selbach et al. 2019).

102 Temperature, in particular, is recognised as a major driver of both predator-prey and  
103 parasite transmission dynamics as it simultaneously influences both consumption rates (e.g.

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104 Goedknecht et al. 2015; Lavery et al. 2017) and the production of free-living parasite stages  
105 (Poulin 2006). In order to understand how temperature may affect parasite transmission through  
106 its influence on parasite consumption by non-hosts, it is essential to examine potential predator-  
107 prey interactions. Consumer functional responses (hereafter FR) provide a useful tool to  
108 examine relationships between consumption rates and ecologically relevant prey densities (e.g.  
109 Holling 1959; Jeschke et al. 2004), and thus increasing our understanding of the impact  
110 predators have on prey populations (e.g. Bovy et al. 2015; Paterson et al. 2015). Here, we  
111 address the potential consumption of trematode cercariae by the omnivorous amphipod  
112 *Gammarus lacustris* G.O. Sars, 1863 (Malacostraca, Gammaridae), one of the most common  
113 macroinvertebrates in European subarctic lakes. This amphipod co-occurs in the nearshore  
114 benthic habitat with the lymnaeid snail *Radix balthica* L. (Gastropoda, Pulmonata), which  
115 release dense aggregations of larval trematodes, including *Diplostomum* Poirier, 1886  
116 (Diplostomidae), *Apatemon* Szidat, 1928 (Strigeidae) and *Trichobilharzia* Skrjabin &  
117 Zakharow, 1920 (Schistosomatidae) (Soldánová et al. 2017). We utilised a FR approach to  
118 investigate whether the consumption of these three trematode prey taxa by *G. lacustris* was  
119 altered by temperature variation.

120

## 121 **2. Materials and methods**

### 122 **Study organisms and maintenance**

123 All study organisms were obtained from Takvatn, a subarctic lake in northern Norway  
124 (69°07'N, 19°05'E), during August and October 2017 and 2018. *Gammarus lacustris*  
125 amphipods (n = 340 males; length [pereon + pleosome] mean ± SD: 11.9 ± 1.7 mm) were  
126 collected from the lake substrate (4 – 10 m depth) by dredge net with a rectangular metal frame  
127 (20 × 35 cm opening, 1 mm mesh), whereas *R. balthica* snails were hand-picked from the littoral

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128 zone (<1 m depth). Amphipod sex was confirmed under the microscope. Study organisms were  
129 acclimatised for 3 – 10 days in a climatic chamber, with light:dark (20:4, 10:14 h) and  
130 temperature (13, 6 °C) conditions reflecting measures taken in August and October, respectively  
131 (data loggers Onset HOBO UA-002-64 Pendant 64K, MA, US). Amphipods were maintained  
132 in 6-L aquaria with aerated lake water (replaced every 72 h) and provided *ad libitum* with  
133 natural prey (e.g. copepods, cladocerans), whereas snails were individually maintained in 40-  
134 mL beakers with lake water (replaced daily) and provided with lettuce (*Lactuca sativa* L.).

135 Snails were screened for trematode infections by placing each beaker under a light  
136 source for 24 h to stimulate cercarial emergence, with taxa identified *in vivo* (Soldánová et al.  
137 2017). Three trematode genera that do not infect amphipods were selected for the experimental  
138 assays, i.e. *Trichobilharzia*, *Apatemon* and *Diplostomum*. *Trichobilharzia franki* haplotype  
139 “peregra” (hereafter *Trichobilharzia*) has a two-host life cycle with birds as definitive hosts,  
140 whereas *Apatemon* and *Diplostomum* have a three-host life cycle, with fish and piscivorous  
141 birds as second intermediate and definitive hosts, respectively (Soldánová et al. 2017). The  
142 three trematodes display intermittent swimming periods, with *Diplostomum* and  
143 *Trichobilharzia* tending to accumulate close to water surface in relation to the light intensity  
144 and *Apatemon* continuously swimming with only short pauses. However, *Apatemon* exhibits  
145 nocturnal emergence, whereas *Diplostomum* and *Trichobilharzia* exhibit diurnal emergence  
146 (Bell et al. 1995; Karvonen et al. 2004; Haas et al. 2008; Soldánová et al. 2016). Cercarial size  
147 also differed between these trematodes (*Apatemon* ~ 530 µm, *Diplostomum* ~ 690 µm,  
148 *Trichobilharzia* ~ 940 µm; total length, n = 33, 31 and 23 live cercariae specimens,  
149 respectively).

150 To obtain cercarial prey for the experiment, snails were individually incubated in 40-  
151 mL of filtered lake water under a light source (*Trichobilharzia*, *Diplostomum*) or covered with

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152 aluminium foil to mimic night-time (*Apatemon*). Host-induced variability in cercariae (e.g.  
153 maturity or size) was accounted for by preparing a pool of freshly emitted cercariae from  
154 multiple infected snails for each trematode genera. Thereafter, cercariae were individually  
155 pipetted into a plastic beaker with a small volume of filtered lake water (10 mL) in densities  
156 required for the FR experiment. All cercariae were the same age (< 4 h), alive and active when  
157 offered to the predator.

## 158 **Experimental design**

159 A randomised, fully factorial design was used to test the effect of temperatures relevant  
160 to the studied system (13, 6 °C, average water temperatures in August and October,  
161 respectively) on cercarial prey consumption. Each amphipod was supplied with one of eight  
162 prey densities (2, 4, 6, 10, 20, 50, 70 or 100 cercariae) from one trematode genera. Each  
163 treatment combination was replicated six times with the exception of *Trichobilharzia*, which  
164 was replicated twice due to the low availability of *Trichobilharzia*-infected snails.

165 Prior to each assay, amphipods were held without food for 48 h to standardise satiation  
166 levels preceding exposure to cercarial prey. Each amphipod was randomly assigned to an  
167 individual experimental arena (diameter 55 mm) containing 60-mL filtered lake water, which  
168 was placed on a black surface to mimic the amphipod's natural habitat. Amphipods were  
169 provided with a given density of cercariae prey from the prepared beakers, which were rinsed  
170 twice with filtered lake water. Consumed cercariae were not replaced during the assay (non-  
171 replacement design), with amphipods removed after 30 min. Unconsumed cercariae were  
172 counted by filtering the water from each arena through a 12 µm-pore filter membrane  
173 (Whatman, Nuclepore Track-Etch Membrane, Sigma-Aldrich), which was then fixed and  
174 stained in a 70% ethanol and carmine solution prior to counting cercariae under a  
175 stereomicroscope. Control replicates (n = 6 *Apatemon* and *Diplostomum*, n = 2 *Trichobilharzia*)

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176 in the absence of amphipods were run for each prey density to assess the potential loss of  
177 cercariae due to the filtration procedure (0 – 13 *Apatemon*, 0 – 21 *Diplostomum*, 0 – 22  
178 *Trichobilharzia* cercariae lost, Supplementary Table S1), with the number of prey consumed  
179 by each amphipod adjusted by the mean loss of prey specific to each trematode genera and  
180 cercariae density. Prey survival (in terms of vital/mobile cercariae) in the absence of predators  
181 was 100%.

182 All amphipods were maintained for 48 h post-assay in individual arenas under the same  
183 conditions. Amphipods which died or moulted prior to, during or post assay were replaced with  
184 another randomly selected amphipod, as moulting may influence the feeding behaviour of  
185 *Gammarus* (Bovy et al. 2015). Amphipods were then euthanized by immersion in carbonated  
186 water.

## 187 **Statistical analysis**

188 All statistical analyses were completed in R (R Core Team 2017, version 3.6.1). The  
189 presence of outliers and/or differences in prey consumption rates resulting from the  
190 experimental timeframe were determined by visual inspection of raw data plots, with one outlier  
191 detected in the *Diplostomum* dataset at the 10-prey density (Supplementary Fig. S1a).

192 To determine which FR model type (I, II or III) best described the relationship between  
193 cercariae density and the number of cercariae consumed, logistic regressions were fitted to each  
194 amphipod-trematode combination using `frair::frair_test` (version 0.5.100; Pritchard et al. 2017).  
195 Type I FRs assume a linear relationship between cercariae density and the number of cercariae  
196 consumed. Type II FRs (decreasing proportional consumption with increasing prey density) are  
197 indicated by a significant negative first-order term, whereas Type III FRs are indicated by  
198 significant positive first-order term, then a significant negative second-order term (initial  
199 increase then decrease in proportional consumption; Juliano 2001; Pritchard et al. 2017). While

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200 parameters  $a$  (capture/attack rate),  $h$  (handling time, i.e. time spent subjugating, ingesting and  
201 digesting each prey item) and  $T$  (experimental time) describe a Type II response, Type I response  
202 show negligible  $h$ , being thus characterised by  $a$  and  $T$  only. The fixed parameter  $T$  was set to  
203 0.5/24 (30 mins), and after starting estimates had been provided, FR models were fitted using  
204 maximum likelihood estimation (MLE) by using `frair::frair_fit` (Pritchard et al. 2017). Due to the  
205 low number of replicates in *Trichobilharzia* prey, FR analyses could not be run for this prey  
206 and results are presented as a plot in Suppl. Fig. S1c. A single model was fitted for each  
207 trematode-amphipod dataset, including data at 6 and 13 °C, to test for differences in cercariae  
208 consumption between temperatures. Amphipods displayed different FR types towards each  
209 prey genera, and therefore model parameters between these types could not be compared. A  
210 Type II FR was detected with *Diplostomum*, whereas in the analyses involving *Apatemon*  
211 `frair_test` did not show any evidence of Type II or III FRs. Raw data comparing consumed prey  
212 or proportion of consumed prey across densities were plotted to check the fitting of the  
213 *Apatemon* data to a Type I FR. To visualise the uncertainty around the fitted FRs, bootstrapping  
214 ( $n = 1,500$ ) was used to construct 95% confidence intervals.

215

### 216 3. Results and discussion

217 Amphipods showed different consumption rates when preying on *Diplostomum* and  
218 *Apatemon*, with higher proportion of cercariae consumed at low prey densities (<10-prey  
219 densities) and a slight decrease in consumption at 70- and 100-*Diplostomum* prey density.  
220 However, the proportion of consumed cercariae is similar between temperatures within the  
221 same prey genera. Raw data suggests that amphipods consumed *Trichobilharzia* cercariae at  
222 similar proportions to *Diplostomum*; however, this could not be fully evaluated for

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223 *Trichobilharzia* due to low replication. Raw data and percentage of consumed cercariae at each  
224 amphipod-prey combination are summarized in Supplementary Table S2.

225 In the current study, amphipods, namely *G. lacustris*, for the first time have been shown  
226 to efficiently consume trematode cercariae, potentially constituting an additional foraging  
227 resource for this abundant and widespread freshwater crustacean. The amphipods displayed  
228 Type II FRs towards *Diplostomum* at both temperatures (logistic regressions; Fig. 1a,  
229 Supplementary Table S3), which are commonly observed in gammarids and other invertebrates  
230 consuming various invertebrate prey types (e.g. Jeschke et al. 2004; Bovy et al. 2015;  
231 Wasserman et al. 2016; Iltis et al. 2018). In contrast, the consumption of *Apatemon* prey at both  
232 temperatures could best be described by a Type I FR, as the proportion of prey consumed was  
233 not modified by increasing prey density (Fig. 1b, Supplementary Fig. S2). Type I FRs assume  
234 a linear relationship between prey density and consumption, and are usually restricted to filter-  
235 feeding species (Jeschke et al. 2004). Predators showing Type II and Type III FRs typically  
236 exhibit long handling time  $h$  of their prey, while those displaying a Type I FR show negligible  
237 handling time  $h$  and fulfil the satiation condition. This means unless their gut is completely  
238 filled, consumers should search for food at a maximal rate with maximal effort, thus likely  
239 spending longer time foraging (Jeschke et al. 2004). Even if non-filter feeders would neither  
240 fulfil the handling nor the satiation condition described for Type I FRs, our data show for the  
241 first time that a non-filter feeding predator may also exhibit a Type I FR towards specific  
242 cercarial prey. Future experiments should include higher *Apatemon* prey densities to determine  
243 when the saturation point is reached.

244 Differences in FR type depending on the cercarial prey genera consumed by *G. lacustris*  
245 suggest that prey-related traits could be affecting their predation risk (e.g. size or behaviour,  
246 Orlofske et al. 2015; Selbach et al. 2019). Jeschke et al. (2004) showed that in addition to the

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247 low handling time and satiation conditions typically associated with Type I FRs, invertebrate  
248 consumers must also be relatively large compared to their food. The size difference between  
249 *Diplostomum* and *Apatemon* is roughly 160  $\mu\text{m}$  and should not have a major effect on the  
250 handling time displayed by amphipods. However, even if the relative predator/prey size of *G.*  
251 *lacustris* - *Apatemon* would set the limit to justify a Type I response according to the size  
252 condition shown by Jeschke et al. (2004), other factors linked to *Apatemon*'s cercarial  
253 behaviour or distribution in the water column might enable *G. lacustris* to continuously feed on  
254 them. *Gammarus lacustris* remains on or close to the bottom substrate during the day,  
255 swimming throughout the water column at night, mostly feeding on zooplankton (Wilhelm and  
256 Schindler 1999). While both *Apatemon* and *Diplostomum* perform similar swimming  
257 behaviour, *Diplostomum* tends to occupy a position in the upper water column (Haas et al.  
258 2008), and *Apatemon* likely swims faster (Santos et al. 2007; Selbach and Poulin 2018) and  
259 rests for shorter periods (Bell et al. 1995) closer to intermediate positions of the water column  
260 (pers. obs.). The continuous movement and shorter distance to *Apatemon* prey may likely  
261 facilitate a constant consumption by benthic dwelling *G. lacustris* visual predators.

262 The overlapping 95% confidence intervals indicated that temperature did not alter FRs  
263 of amphipods towards any of the trematode prey (Fig. 1), even though FR attack rate *a* differed  
264 significantly between temperatures for amphipods preying on *Diplostomum* (Supplementary  
265 Table S4). Our results thus indicate a low impact of temperature on the consumption of cercarial  
266 prey, similar as previously reported for other invertebrate predator-prey systems (e.g. Paterson  
267 et al. 2015; Wasserman et al. 2016), but contrasting what was observed in gammarids by  
268 Lavery et al. (2017). Lavery et al. (2017) showed an increased prey consumption at increased  
269 temperatures, but their higher temperature range (11 and 20 °C vs current study 6 and 13 °C)

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270 may have triggered this change in consumption rate. Furthermore, the larger prey sizes used in  
271 their experiment (0.4 – 0.8 cm) may have altered the temperature impact on the predator's FR.

272 Cercarial emergence patterns, as well as activity and survival rates can be species-  
273 specific and/or temperature-dependent (Poulin 2006). Therefore, the absence of the ambient  
274 temperature influences on cercariae consumption may demonstrate the ability for amphipods to  
275 exploit the different emergence and active swimming periods intrinsic to *Diplostomum* and  
276 *Apatemon* throughout the year (Bell et al. 1995; Karvonen et al. 2004). Nevertheless, the most  
277 profound impact could be expected on the *Diplostomum* population, as Type II FRs are  
278 considered to have larger implications for prey populations, potentially resulting in local prey  
279 extinction because high proportions of prey are consumed at low densities (Hassel 1978).

280 For the first time, a Type I FR has been revealed for a non-filter feeder species. The  
281 likely constant consumption of *Apatemon* by amphipods represents a continuous energetic  
282 source for this non-host organism, even when different prey densities are available. Therefore,  
283 the increased consumption rate and thus energy profit per unit time that amphipods gain when  
284 preying on intermediate and high abundances of *Apatemon*, in contrast to the lower predation  
285 on *Diplostomum*, might likely impact the energy transfer through the food web, making  
286 *Apatemon* a more profitable prey for amphipods. Further FR experiments on large-bodied  
287 cercariae such as *Trichobilharzia* might help to understand the role of cercarial size for FR  
288 types. Investigating the mechanisms that mediate changes in FRs and direct predation has  
289 important implications for trematode population and disease dynamics, as well as for ecosystem  
290 energetics.

291

## 292 **Figure legend**

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293 **Fig. 1** The effects of temperature (6 and 13 °C) on the consumer functional response of  
294 *Gammarus lacustris* amphipods towards (a) *Diplostomum* (Type II) and (b) *Apatemon* (Type I)  
295 cercariae prey. Lines indicate mean functional response, and coloured bars are 95% confidence  
296 intervals (blue, 6 °C; pink, 13 °C). Attack rate  $a$  and handling time  $h$  are shown by cercarial  
297 prey and temperature (T)

298

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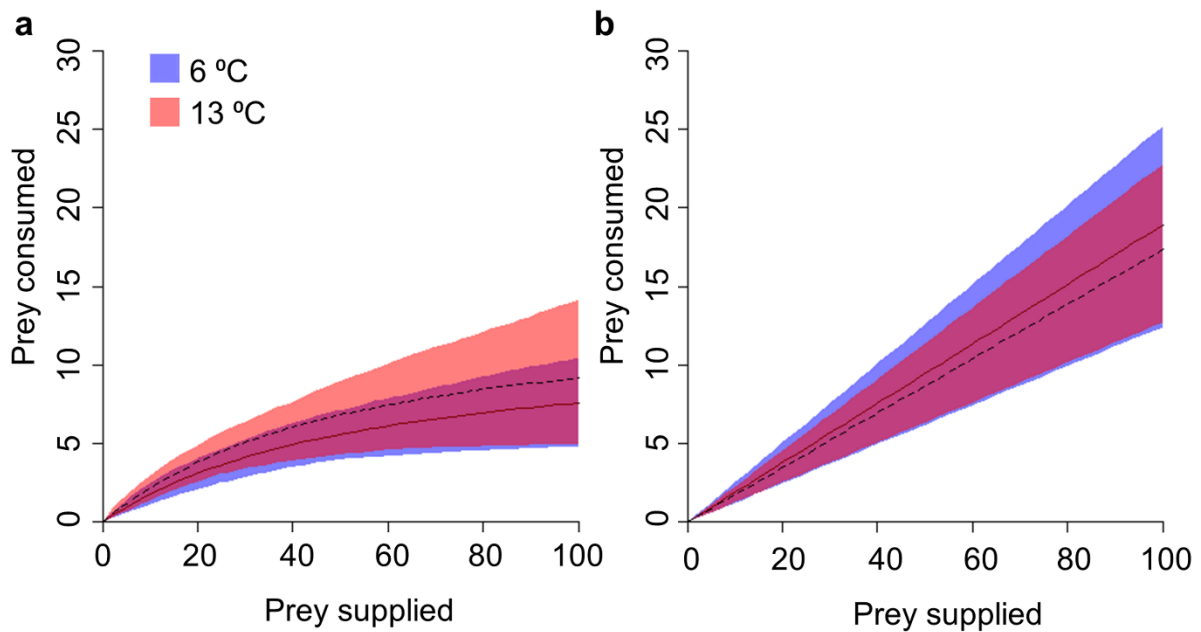
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375 **Figures**

376 **Fig. 1**

377



| Prey               | FR Type | T (°C) | <i>a</i> | <i>h</i> |
|--------------------|---------|--------|----------|----------|
| <i>Diplostomum</i> | II      | 6      | 11.08    | 0.0018   |
|                    |         | 13     | 14.14    | 0.0015   |
| <i>Apatemon</i>    | I       | 6      | 9.07     | -        |
|                    |         | 13     | 8.33     | -        |

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