1   Taxa-specific activity loss and mortality patterns in freshwater trematode cercariae

2   under subarctic conditions

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Abstract

20 Cercarial activity and survival are crucial traits for the transmission of trematodes. Temperature is

21 particularly important, as faster depletion of limited cercarial energy reserves occurs at high

22 temperatures. Seasonal climate conditions in high latitude regions may be challenging to complete

23 trematode life cycle during the six-month ice-free period, but temperature effects on the activity

24 and survival of freshwater cercariae have not been previously identified. After experimentally

25 simulating natural subarctic conditions during warmer and colder months (13 and 6 ℃), a

26 statistical approach identifying changes in the tendency of cercarial activity loss and mortality data

27 was used to detect differences in three trematode genera, represented by four taxa (*Diplostomum*

28 spp., *Apatemon* spp., small- and large-sized *Plagiorchis* spp.). A strong temperature-dependent

29 response was identified in both activity loss and mortality in all taxa, with *Diplostomum* spp.

30 cercariae showing the most gradual changes compared to other taxa. Furthermore, whilst activity

31 loss and mortality dynamics could not be divided into "fish- *vs* invertebrate-infecting cercariae"

32 groups, the detected taxa-specific responses in relation to life-history traits likely indicate the

33 swimming behaviour of cercariae and energy allocation among larvae individuals as the main

34 drivers. Cercariae exploit the short transmission window that allows a stable continuance of

35 trematodes' life cycles in high-latitude freshwater ecosystems.

36

37 **Key words**: larval stages, parasite, transmission strategies, life cycle, high latitude regions

Key Findings

- Cercarial activity loss and mortality show temperature-dependent responses under high latitude conditions

- No division of activity loss and mortality dynamics into "fish- *vs* invertebrate-infecting cercariae" groups

- Taxa-specific responses are related to life-history traits, i.e. swimming behaviour and energy allocation

- Detection of time sections in activity and survival analyses helps suggesting infective periods

- Trematodes exploit short transmission windows under highly seasonal and cold subarctic conditions

Introduction

Many parasite taxa such as trematodes have complex life cycles that involve several developmental stages and subsequent hosts (Galaktionov and Dobrovolskij, 2003). The transmission of trematodes from their first intermediate molluscan hosts to the next invertebrate or vertebrate hosts is typically undertaken by free-living larval stages (cercariae). These cercariae are non-feeding motile stages that need to effectively disperse, localize and infect the next suitable host during their short lifespan (typically 24–72 h; Combes *et al.*, 1994; Morley, 2012). Depending on host type and environmental conditions, transmission strategies involving a great variety of morphological and behavioural features (body size and structure, emergence patterns, swimming activity or vertical

59   distribution in the water column) have evolved making trematodes successful in completing their

60   life cycle (Combes *et al.*, 1994; Morley, 2012).

61   Cercarial lifespan is generally species-specific, with cercarial activity and survival

62   continuously decreasing over time. The active period of cercariae usually represents the functional

63   lifespan, when infectivity is maximal within a few hours post-emergence from the molluscan host

64   (Ginetsinskaya, 1988; Karvonen *et al*., 2003). When activity starts to decrease with cercarial aging,

65   survival starts to decrease as well so that a reduction of longevity is usually linked to a reduction

66   of the active infective period (Lowenberger and Rau, 1994; Pechenik and Fried, 1995). Both

67   cercarial activity and survival are crucial traits for the transmission success, being influenced by

68   several mutually interacting factors under changing environmental conditions (Evans 1985; Rea

69   and Irwin, 1992; McCarthy, 1999a; Pietrock and Marcogliese, 2003; Thieltges *et al.,* 2008;

70   Koprivnikar *et al.*, 2010; Studer *et al.*, 2010). Temperature is a particularly important factor (e.g.

71   Lawson and Wilson, 1980; Evans, 1985; McCarthy, 1999b; Rea and Irwin, 1992; Mouritsen,

72   2002), as the depletion of the limited cercarial glycogen energy reserves (Anderson and Whitfield,

73   1975; Ginetsinskaya, 1988) occurs at higher rates at elevated temperatures, usually resulting in a

74   decrease of cercarial activity and survival (Pechenik and Fried, 1995; McCarthy, 1999b; Karvonen

75   *et al.* 2003; Thieltges and Rick, 2006; Studer *et al.,* 2010; Studer and Poulin, 2013). However,

76   constant cercarial activity and survival over a larger range of temperatures, as well as increased

77   activity at warmer conditions, have also previously been reported (e.g. Koprivnikar *et al.*, 2010;

78   Morley 2011; Selbach and Poulin, 2020).

79   While extensive literature exists on the effects of temperature on the activity and survival of

80   cercariae in temperate regions (e.g. Fingerut *et al.*, 2003; Thieltges and Rick, 2006; Studer and

81   Poulin, 2013; Selbach and Poulin, 2020), research from high latitudes (> 60 ºN) is so far limited

82   to marine snail-trematode systems (Prokofiev, 1999; 2001). At subarctic and arctic latitudes, the

83   seasonal climate may be challenging for lacustrine trematodes to complete their life cycle during

84   the short ice-free period (< 6 months). It has been proposed that trematode taxa can have different

85   survival adaptations to the thermal range experienced in their aquatic habitats, depending on

86   particular geographical environmental conditions (Morley, 2011; Studer and Poulin, 2014). There

87   may also be temperature adaptations in trematode transmission strategies such as cercarial activity

88   and survival to the highly seasonal and cold environments at high latitudes. However, whilst

89   cercarial emergence patterns at low temperatures have been investigated in both temperate and

90   high latitude regions (e.g., 4–20 °C, Lyholt and Buchmann, 1996; 4–18 °C, Brassard *et al.*, 1982;

91   6–27 °C, Prokofiev *et al.*, 2016; 8 °C, Nikolaev *et al.*, 2020), temperature effects on the activity

92   and survival of freshwater cercariae under these conditions still remain to be identified.

93   Investigating general patterns of these important aspects of cercarial biology in relation to seasonal

94   conditions is essential for a better understanding of trematode population and transmission

95   dynamics at high latitudes. Furthermore, this knowledge will be pivotal evaluating the implications

96   of global warming on parasite transmission in colder northern latitudes that are considered most

97   vulnerable to climate change (Kutz *et al.*, 2009; Mas-Coma *et al.*, 2009).

98   In this study, a series of laboratory experiments simulating natural subarctic conditions in

99   warmer and colder months (two temperature scenarios) were conducted to characterize cercarial

100   activity and survival of different trematode taxa parasitizing a freshwater snail species, the

101   lymnaeid *Radix balthica* (Soldánová *et al.,* 2017). We used a statistical approach standardly

102   applied in other research areas different to parasitology such as ichthyology and clinical fields (e.g.

103   Ganna *et al.*, 2013; Halttunen *et al.*, 2017, but see van Beest *et al.*, 2019), where activity loss and

104   mortality of cercariae are analyzed as a proxy for activity and survival. We further detected

105   different time sections that represent a change in the tendency of data, expecting the first time

106   section to represent the most infective period of the cercariae. We investigated four trematode taxa

107   that differ in terms of morphology, life history and transmission strategy with the aim 1) to quantify

108   the effect of the two temperature scenarios on cercarial activity loss and mortality in the different

109   trematode taxa, and 2) to compare cercarial activity loss and mortality among the four trematode

110   taxa and to relate them to specific morphology and behaviour of the cercariae. We hypothesized

111   that cercarial activity loss and mortality are strongly temperature-dependent, likely showing

112   reduced activity loss and higher mortality at higher temperature. Furthermore, we expect that taxa-

113   specific responses will be related to different life-history traits and transmission strategies to

114   localize and infect their next target hosts, with fish-infecting cercariae (as opposed to invertebrate-

115   infecting) showing a faster increase in activity loss and mortality due to the costly swimming

116   activity.

117

118   **Materials and methods**

119   *Parasite and host material*

120   *Radix balthica* snails were collected by hand from the littoral zone of the subarctic lake Takvatn,

121   northern Norway (69°07'N, 19°05'E) during August and October 2018. Snails were individually

122   incubated in beakers containing 30 mL of lake water under a light source for 24 h, and emerging

123   cercariae were identified *in vivo* according to previous research (Soldánová *et al.*, 2017). Three

124   genera, represented by four trematode taxa, exhibiting different cercarial morphological features,

125   emergence and swimming behaviours, were selected for experimental trials of cercarial activity

126   loss and mortality, including *Diplostomum* spp. (Diplostomidae; total length: ~ 690 µm),

127   *Apatemon* spp. (Strigeidae; ~ 530 µm) and *Plagiorchis* spp. (Plagiorchiidae), in which two taxa

128   were distinguished as small-sized *Plagiorchis* spp. (~ 380 µm) and large-sized *Plagiorchis* spp. (~

129   630 µm) due to overlapping morphological features within this genus, encompassing seven

130   species/lineages in subarctic areas (Soldánová *et al.*, 2017; Kudlai *et al.*, 2021). These genera

131   utilise a three-host life cycle involving bird definitive hosts, but two types of second intermediate

132   hosts, with cercariae either infecting fish (*Diplostomum* spp. and *Apatemon* spp.) or benthic

133   amphipods or aquatic insects (*Plagiorchis* spp.) (Fig. 1). *Diplostomum* spp. and *Apatemon* spp.

134   have furcocercous cercariae (i.e., bifurcated tail) that display vertical distribution within the water

135   column and intermittent swimming periods, with *Apatemon* resting for much shorter periods.

136   Furthermore, while *Diplostomum* spp. shows a positive photo-orientation, *Apatemon* shows

137   negative phototaxy (Bell 1996; Haas *et al.,* 2008). Benthic monocercous xiphidiocercariae (i.e.,

138   body with stylet and simple tail) exhibit negative phototaxy and geotaxy, swimming close to the

139   bottom with a continuous and rather slow movement (Lowenberger and Rau, 1994). Cercariae of

140   *Diplostomum* spp. show diurnal emergence, whereas *Apatemon* and *Plagiorchis* show nocturnal

141   emergence (Bell 1996; McCarthy, 1999a; Karvonen *et al.*, 2004; Haas *et al.*, 2008, but see diurnal-

142   emerging *Plagiorchis* species in Gorman, 1980). All snails infected with one of the model

143   trematodes were maintained at experimental temperatures, replacing daily the filtered lake water

144   and regularly fed with lettuce (*Lactuca sativa*).

145

146   *Experimental design*

147   To record cercarial activity loss and mortality of the four trematode taxa, laboratory-controlled

148   experiments simulating field water temperature and photoperiod conditions in lake Takvatn were

149   conducted. These scenarios represented subarctic warmer (August; 13 ºC and 20:4 h light:dark

150   photoperiod) and colder (October; 6 ºC and 10:14 h) months from the ice-free period, based on

151 data recorded in the field by data loggers (Onset HOBO UA-002-64 Pendant 64K). A pool of live

152 and active cercariae of the same age (< 2 h) was collected from different snail individuals to

153 account for potential variability, using the same group of snails throughout the experiments for

154 each temperature setting/field collection. Cercariae were pipetted into 6-well plates, placing

155 approximately five cercariae into 6 mL of filtered lake water (i.e., ~ 30 cercariae per plate). Plates

156 were then placed in climatic rooms (13 and 6 °C) adjusted to reflect natural conditions including

157 sunrise and sunset. Different number of plates with incubated cercariae for each trematode taxon

158 and temperature were used, depending on the availability of infected snails, with a minimum of

159 200 monitored cercariae for most of the trials (Suppl. Table S1). Once the experiment started,

160 cercarial activity loss and mortality was visually checked and recorded under the stereo microscope

161 after four hours, and thereafter every two hours until cercarial death. Live cercariae were classified

162 as active (swimming cercariae) or not active (cercariae barely swimming or crawling, showing

163 erratic movements and spontaneous spasms). Furthermore, cercariae were considered dead when

164 not showing any spasmodic movements and not responding to mechanical stimuli with a fine

165 needle (Koprivnikar *et al.*, 2010; van Beest *et al.*, 2019).

166 To minimise snail mortality in captivity and ensure a sufficient amount of cercariae for

167 experiments, two climatic rooms were used indistinctly for each temperature scenario (13 and 6

168 °C). To be able to do this, a possible confounding effect of climatic rooms on activity loss and

169 mortality of small-sized *Plagiorchis* spp. was tested prior to experiments with other trematodes by

170 running trials before and after inverting the temperature in both rooms. Results of a regression

171 Weibull model (package *survival*, using *survreg*; Therneau, 2020) revealed differences in both

172 activity loss and mortality of cercariae between some individual plates within the same room

173 (RWM, $P < 0.05$) and no significant differences between some individual plates from different

174 rooms (RWM, P > 0.05). Furthermore, activity and survival curves followed the same dynamics

175 in both rooms, suggesting that the observed differences are related to the individual plates and

176 therefore not affected by the climatic rooms themselves.

177

178 *Statistical analysis*

179 To assess the variation in activity loss and mortality rates of cercariae as well as to investigate

180 differences between temperatures and cercariae of different trematode taxa, data were first

181 transformed into individual observations for each cercaria. Data were tested by a Cox proportional

182 hazards regression model (survival::CoxPH, Therneau, 2020). This type of analysis aims to model

183 time to event data, considering death as the event. In this study, we also considered the death of a

184 cercaria as the event to monitor the end of its survival, whereas for activity analyses, we considered

185 the loss of activity of a cercaria as the event to monitor the end of its activity. Therefore, the

186 statistical analyses investigate differences in cercarial activity loss and mortality, as proxies for

187 cercarial activity and survival (i.e. increased activity loss implies lower activity, whereas increased

188 mortality implies lower survival). Similarly, activity loss or mortality represent the probability of

189 cercariae being not active or dead at a given time point, i.e. increased activity loss at higher

190 temperature implies an increased probability of cercariae not being active at that temperature.

191 When analysing the data, if they did not show a constant proportional hazard between levels (using

192 survival::cox.zph, validated at P > 0.05), step functions were used, creating time sections based on

193 the model´s residual plots (using survival::survSplit). Different time sections imply a change in

194 the tendency of the data, i.e. when the activity loss or mortality pattern of a cercaria changes over

195 time, this is detected and data are split into different sections. Thus, the number of time sections

196 depends on the used data set, i.e. selected trematode taxa and temperature. This newly applied

197 analytical approach including time sections helps to uncover changes in the activity loss and

198 mortality patterns of cercariae that otherwise would remain overlooked if considering only the

199 total duration of the active periods (e.g. potential periods when the majority of cercariae are active

200 and thus potentially infective).

201 First, the effect of temperature on cercarial activity loss and mortality was evaluated for each

202 trematode separately (*Diplostomum* spp., *Apatemon* spp., and small- and large-sized *Plagiorchis*

203 spp.), using CoxPH analyses with temperature as a factor (6 *vs* 13 ℃, using 6 ℃ as the baseline to

204 which 13 ℃ is compared), and plates as random effect. When experiments could not be run

205 simultaneously due to insufficient amount of cercariae of the same age, the trial was also included

206 as a random effect (i.e., *Diplostomum* spp., small-sized *Plagiorchis* spp.). Second, the activity loss

207 and mortality among cercariae taxa at the same temperature (either 6 or 13 ℃) were compared.

208 CoxPH analyses were used for each temperature separately with trematode as a factor

209 (*Diplostomum* spp. *vs Apatemon* spp. *vs* small-sized *Plagiorchis* spp. *vs* large-sized *Plagiorchis*

210 spp., each used in different analyses as the baseline to which one of the other taxa is compared),

211 and plates and trial as random effects. Survival function curves were created using the Kaplan-

212 Meier estimator (survival::survfit). Censored data (i.e. when a cercaria survives or remains active

213 unexpectedly longer than the experimental period) were included in statistical analyses according

214 to Crawley (2013) for *Diplostomum* spp. and *Apatemon* spp. All analyses were performed in R (R

215 Development Core Team, version 3.0.1).

216

217 **Results**

218 Our analyses revealed significant differences in cercarial activity loss and mortality of the four

219 trematode taxa belonging to three genera in relation to temperature (6 °C and 13 °C). Depending

220   on the trematode identity and/or temperature combination, our results suggested different time

221   sections according to the proportionality of the Hazard Ratio, implying a change in the activity

222   loss (section "i" in all Tables) or mortality (section "ii" in all Tables) of cercariae (see below). A

223   temperature-dependent response was detected for most of the taxa and, when comparing different

224   trematodes, differences were larger between *Diplostomum* spp. and the other three taxa.

225

226   *Effect of temperature on cercarial activity loss and mortality within trematode taxa*

227   Differences in activity loss rates between temperatures were similar in all tremadode taxa, showing

228   *Diplostomum* spp. the most pronounced difference in the last time section (Table 1i). As for

229   mortality rates, cercariae of *Diplostomum* spp. and large-sized *Plagiorchis* spp. showed the

230   smallest differences between temperatures, whereas differences in mortality of *Apatemon* spp., and

231   especially small-sized *Plagiorchis* spp., were substantial (Table 1ii).

232   The activity loss rate of *Diplostomum* spp. cercariae was divided into three time sections,

233   showing a 2-fold lower activity loss at 13 ⁰C compared to 6 °C during the first 9 h of cercarial age

234   (i.e. higher activity, Table 1i). Thereafter cercariae showed higher activity loss at 13 ⁰C during

235   next two time sections. In contrast, mortality was higher at 13 ⁰C compared to 6 °C during the two

236   detected time sections (1- and 2-fold increased mortality, respectively; Table 1ii). Cercariae of

237   *Apatemon* spp. showed a decreased activity loss and an 8-fold higher mortality at 13 °C, both

238   within the single measured time section (Table 1). Cercarial activity loss patterns of both

239   morphotypes of *Plagiorchis* spp. were similar to some degree, each being split in three time

240   sections. Small-sized *Plagiorchis* spp. showed a 4-fold higher activity loss at 13 °C compared to

241   6 °C during the first 15 h, which later changed, showing a 2-fold decreased activity loss within the

242   following two sections (Table 1i). The activity loss of large-sized *Plagiorchis* spp. cercariae

243 remained comparable between temperatures during the first two time sections and differed

244 significantly only in the last section with a 3-fold decreased activity loss after 44 h at 13 °C

245 compared to 6 °C. Higher mortality of small-sized *Plagiorchis* spp. was detected at 13 ⁰C along

246 their whole lifespan, thus detecting a single time section (12-fold increased mortality, Table 1ii).

247 Cercarial mortality of large-sized *Plagiorchis* spp. split into two time sections, first with 2-fold

248 higher, and second with 3-fold lower mortality at 13 °C. This indicates that although large-sized

249 *Plagiorchis* spp. cercariae suffer from higher mortality at 13 °C at the beginning of their lifespan

250 within the first 44 h, they die at a slower rate during the remaining life period.

251

252 *Differences among trematode taxa in cercarial activity loss and mortality during warm subarctic*

253 *conditions*

254 Activity loss rates of all trematode pairwise combinations were divided in three time sections and

255 mortality rates in two time sections (Table 2). Differences in both activity loss and mortality at 13

256 ⁰C were stronger when comparing the cercariae of *Diplostomum* spp. with the three other trematode

257 taxa, following similar patterns in corresponding time sections with *Apatemon* spp., and small-

258 and large-sized *Plagiorchis* spp. That is, a significantly decreased activity loss was recorded during

259 the first 16 h (2 to 19-fold across compared trematode, Table 2i), followed by increased activity

260 loss in the second and third time sections when comparing small-sized *Plagiorchis* spp. and

261 especially *Apatemon* spp. to *Diplostomum* spp. (8- to 72-fold increased activity loss). The activity

262 loss of large-sized *Plagiorchis* spp. and *Diplostomum* spp. did not differ in the second time section,

263 but showed a significant 6-fold increased activity loss of large-sized *Plagiorchis* spp. in the third

264 section (Table 2i). Mortality of *Diplostomum* spp. at 13 ⁰C was significantly higher during the first

265 27 h compared to the three other trematode taxa (9 to 20-fold lower mortality of compared taxa),

266 thereafter decreasing *Diplostomum* spp.' mortality in the second time sections (14 to 39-fold higher

267 mortality of compared taxa, Table 2ii).

268   Activity loss and mortality of *Apatemon* spp. cercariae was different when compared to small-

269 and large-sized *Plagiorchis* spp. While in the first 16 h small-sized *Plagiorchis* spp. showed a 7-

270 fold activity loss increase compared to *Apatemon* spp., the second time section was comparable

271 between both taxa, whereas in the third section both *Plagiorchis* morphotypes showed an

272 decreased activity loss compared to *Apatemon* spp. (12 to 16-fold across *Plagiorchis* spp.).

273 Comparable mortality rates were found between *Apatemon* spp. and both *Plagiorchis* morphotypes

274 during the first 27 h, whereas small-sized *Plagiorchis* spp. showed a 3-fold higher mortality than

275 *Apatemon* spp. in the second time section (Table 2ii).

276   The activity loss patterns of both *Plagiorchis* morphotypes were similar except for the first 16

277 h, when large-sized *Plagiorchis* spp. showed an 8-fold lower activity loss than small-sized

278 *Plagiorchis* spp. (Table 2i). Mortality of both small- and large sized *Plagiorchis* at 13 °C was

279 similar at both time sections.

280

281 *Differences among trematode taxa in cercarial activity loss and mortality during cold subarctic*

282 *conditions*

283 Both activity loss and mortality rates of all trematode pairwise combinations were divided into

284 three time sections (Table 3). Statistically significant differences in activity loss and mortality at 6

285 ºC were most pronounced when comparing *Diplostomum* spp. with the three other taxa, especially

286 in comparison with large-sized *Plagiorchis* spp. (Table 3, Fig. 2). Cercarial activity loss of

287 *Diplostomum* spp. followed a similar pattern when compared to the other trematode taxa, all the

288 latter showing lower activity loss during the first 13 h (4- to 43-fold across trematode taxa),

289   followed by increased activity loss compared to *Diplostomum* spp. in the second time section, and

290   especially pronounced in the third time section (41- to 57-fold across trematode taxa). The only

291   exception was the comparable activity loss rates between *Diplostomum* spp. and large-sized

292   *Plagiorchis* spp. during the second time section (Table 3i). As for mortality, *Apatemon* spp., small-

293   and large-sized *Plagiorchis* spp. showed lower mortality than *Diplostomum* spp. cercariae during

294   the first time section within 26 h (i.e. 10 to 38-fold), and thereafter showed higher mortality with

295   different change rates depending on the compared trematode taxa and time section (Table 3), the

296   differences being especially strong when compared to large-sized *Plagiorchis* spp.

297   The activity loss of *Apatemon* spp. cercariae was significantly higher in the first time section

298   when compared to both small- and large-sized *Plagiorchis* spp., the latter showing a 5- to 12-fold

299   decreased activity loss compared to *Apatemon* spp. during the first 13 h (Table 3i). Thereafter, a

300   significant change in terms of *Apatemon* spp. decreased activity loss was detected only in the

301   second time section compared to small-sized *Plagiorchis* spp. Similar mortality rates of *Apatemon*

302   spp. cercariae and both morphotypes of *Plagiorchis* were found in the first time sections, but

303   differed significantly after 26 h, showing that both *Plagiorchis* spp. increased their mortality

304   during next two time sections (2- to 11-fold, Table 3ii). These differences were more pronounced

305   in comparison to large-sized *Plagiorchis* spp.

306   The two *Plagiorchis* morphotypes were the most similar in both activity loss and mortality,

307   showing significant differences only in their activity loss rates in the second section, as a 12-fold

308   lower activity loss of large-sized *Plagiorchis* spp. was found compared to small-sized

309   *Plagiorchis* spp. (Table 3i).

310

311   **Discussion**

312   Our results demonstrate the first hypothesis that cercarial activity loss and mortality of the four

313   studied trematode taxa from a subarctic freshwater lake are overall strongly temperature-

314   dependent by showing lower activity loss during the first hours of cercarial lifespan and higher

315   mortality at higher temperature for most of the taxa. The lack of a common pattern in terms of

316   similar changes in activity loss and mortality in relation to temperature variation makes it clear

317   that taxa-specific responses exist at each temperature, suggesting an adaptation of trematodes to

318   the short transmission window under the cold and highly seasonal subarctic conditions by having

319   behaviours that allow active periods during the cercarial lifespan to maximize the transmission

320   rates to their next intermediate hosts. Differences were larger between *Diplostomum* spp. and the

321   other three trematode taxa, not supporting our second hypothesis about the division of activity

322   loss and mortality dynamics into "fish- *vs* invertebrate-infecting cercariae" groups, as among the

323   fish-infecting cercariae of *Diplostomum* spp., as opposed to *Apatemon* spp., exhibited a faster

324   increase in activity loss and mortality during the first hours of their lifespan. Besides, our

325   statistical approach allowed to identify changes in the dynamics of cercarial activity loss and

326   mortality data, thereby recognizing specific time sections. The first time section likely represents

327   the functional lifespan/infective period that cercariae have to infect the next suitable hosts, as

328   shown for numerous species for which the infectivity represents 20–50 % of the survival time

329   (Evans and Gordon, 1983; Evans, 1985; Lowenberger and Rau, 1994; Pechenik and Fried, 1995;

330   McCarthy, 1999b; Karvonen *et al.*, 2003; Thieltges and Rick, 2006). Overall, this study

331   highlights that new knowledge on parasite transmission strategies related to the activity loss and

332   mortality of cercariae is highly valuable to understand the ecological dynamics of lacustrine

333   trematodes in high latitude areas.

334   The strongly temperature-dependent cercarial activity loss and mortality of the studied

335   trematodes, showing taxa-specific responses, is a phenomenon commonly reported in both

336   freshwater and marine trematodes (Poulin, 2006; Thieltges *et al.*, 2008; Morley, 2011). Whilst the

337   life-expectancy of subarctic cercariae was comparable to that reported from other trematodes at a

338   similar temperature range (e.g. 3 to 15 ⁰C; Lo and Lee, 1996; Lyholt and Buchmann, 1996;

339   McCarthy, 1999b; Prokofiev, 1999, 2001; Thieltges and Rick, 2006), it was longer than under

340   warmer conditions (e.g. > 15 ⁰C; Bell, 1996; Pechenik and Fried, 1995; Mouritsen, 2002; Muñoz-

341   Antoli *et al.*, 2002; Karvonen *et al.*, 2003; Koprivnikar *et al.*, 2010; Studer and Poulin, 2013).

342   Nevertheless, both cercarial activity loss and mortality seem to follow similar trends as taxa

343   inhabiting warmer areas in terms of a generally increased mortality at higher temperatures (with

344   exception of the large-sized *Plagiorchis* spp. at an advanced age).

345   *Diplostomum* spp. cercariae remained active during their early life period at 13 ⁰C, thus

346   showing a vigorous swimming behaviour during their most infective period. Thereafter they

347   underwent a loss of activity that drove them to a more passive period until their death. Furthermore,

348   whilst a higher mortality rate of *Diplostomum* spp. was detected at 13 ⁰C, the difference in

349   mortality between the two temperatures was less pronounced than observed for the other taxa,

350   pointing to a higher stability in survival of this trematode across temperatures as suggested for

351   other species (e.g. Koprivnikar *et al.*, 2010; Morley 2011; Selbach and Poulin, 2020). *Apatemon*

352   spp. exhibited lower activity loss during its whole lifespan and higher mortality at 13 °C, which

353   suggests that despite a lower mortality at 6 °C, the activity loss hinders the infection success of

354   cercariae in the subsequent hosts during colder months. Cercariae of both trematodes thus seem to

355   take advantage of the summer months to infect their next intermediate fish hosts. This has been

356   previously described to occur in eye fluke cercariae, which, even though successfully infecting

357   their fish hosts at 7 to 15 ℃, were 4 to 5 times more infective at the warmest temperatures (Lyholt

358   and Buchmann, 1996). Additionally, the cercarial output and transmission of eye flukes to fish in

359   high latitude regions occurs from June to September, peaking in August (e.g. Brassard *et al.*, 1982;

360   Hakalahti *et al.*, 2006). Furthermore, an increase in cercarial productivity to compensate increased

361   mortality at high temperatures has been observed in warmer latitude areas, possibly affecting

362   transmission from the molluscan host (16–20 ℃, Selbach and Poulin, 2020). Once active infective

363   stages are released from molluscan host, the naturally spatio-temporal heterogeneity in cercarial

364   and host densities might lead to variable encounter rates (Combes *et al.*, 1994; Thieltges and Reise,

365   2007). To maximize the number of infections, the total time that a group of cercariae remains

366   active (and thus the energy allocation among them) might be different depending on their infection

367   strategy. A random glycogen allocation would allow variable survival periods among cercariae

368   when next hosts availability is unpredictable, resulting in a certain proportion of stages to remain

369   active seeking for potential hosts while others remain latent/quiescent (Fenton and Hudson, 2002).

370   The gradual increase in the activity loss and mortality of *Diplostomum* spp. in our study, similar

371   to that of *Diplostomum spathaceum* at 20 ℃ (Karvonen *et al.,* 2003), implies a homogeneous

372   glycogen allocation between cercariae. Karvonen *et al.* (2003) suggested that since contact

373   between infective stages and fish hosts is likely to be highly aggregated in both time and space due

374   to fish shoaling behaviour, other aspects different to glycogen allocation, such as cercarial seasonal

375   emergence, might be playing a role in cercarial active periods and longevity. Data on emergence

376   of cercariae would help to explore the existence of seasonal transmission windows in high-latitude

377   areas, if increased emergence, activity and survival of cercariae happen to co-occur under warm

378   or cold subarctic conditions.

379   Small-sized *Plagiorchis* spp. showed higher mortality at 13 ⁰C, however a large percentage of

380   cercariae losing their activity during the first hours. This indicates that these individuals enter a

381   passive stage very soon. The remaining active cercariae are likely those that will later successfully

382   infect their next intermediate invertebrate hosts, as described for other trematodes (Lawson and

383   Wilson, 1980), suggesting an unequal allocation of the energy reserves exploited during the

384   summer months. Nevertheless, their activity loss was lower at the lower temperature, suggesting

385   that this *Plagiorchis* morphotype might be overall more active at colder temperatures or in

386   microhabitats with a more stable temperature, such as the bottom of the water column close to the

387   benthic surface, where *Plagiorchis* spp. are usually distributed (Lowenberger and Rau, 1994).

388   Cercariae of large-sized *Plagiorchis* spp. exhibited similar activity loss and mortality patterns

389   at both temperatures. The higher mortality during the first half of their lifespan at 13 ⁰C, followed

390   by a later decreased activity loss and mortality, suggests that a higher number of cercariae remain

391   alive and active for longer periods during warmer months. This is in accordance with findings of

392   Lo and Lee (1996), who showed that some species of cercariae do not follow the general trend of

393   decreased survival at increased temperatures, thus pointing towards mortality as a consequence of

394   the aging process rather than the temperature itself. The steep changes in cercarial activity and

395   survival curves of large-sized *Plagiorchis* spp., especially at 6 ⁰C, likely suggest an unequal

396   allocation of the energy reserves among the group of cercariae. This may allow some to remain

397   actively host seeking for a longer time, possibly as an adaptation to an unpredictable availability

398   of their next invertebrate hosts (Fenton and Hudson, 2002).

399   The trematode taxa studied here overall showed a temperature-dependent response well

400   adapted to subarctic conditions, allowing them to complete the life cycle even at low temperatures

401   and thus taking advantage of the transmission window during the 6-month ice-free period. The

402   limited energy reserves, mainly concentrated in the main organ of propulsion (i.e. the tail; Lawson

403   and Wilson, 1980), are likely depleted faster at increased temperatures, and thus is the activity.

404   Hence, this supports the activity/survival trade-off (i.e. the longer active periods, the lower

405   survival) suggested for other trematode species as well (Lawson and Wilson, 1980; McCarthy,

406   1999b; Rea and Irwin, 1992; Mouritsen, 2002) with the exception of small-sized *Plagiorchis* spp.

407   during their most infective period.

408   Regarding the differences in cercarial activity loss and mortality among trematode taxa at

409   warm and cold subarctic conditions, the differences between *Diplostomum* spp. and the other three

410   trematode taxa remained similar at both temperatures. The changes in *Diplostomum* spp. activity

411   and survival curves were overall more gradually occurring over time compared to other taxa (see

412   Fig. 2).

413   The swimming behaviour of the furcocercariae of *Diplostomum* spp. was very different to that

414   of bottom-dwelling monocercous cercariae of *Plagiorchis* spp. Therefore, the differences in

415   activity loss and mortality rates may seem obvious, as both cercarial types follow different

416   transmission strategies to locate and invade their next intermediate hosts (i.e. fish *vs* invertebrates,

417   respectively; Lowenberger and Rau, 1994; Bell *et al.,* 1996; Haas *et al.,* 2008; Soldánová *et al.*,

418   2017; see Fig. 1). However, *Diplostomum* spp. and *Apatemon* spp. furcocercariae share similar

419   behaviour, alternating between resting and swimming periods that boost the cercariae into the

420   water column, with *Diplostomum* spp. mostly occupying upper water column positions (Bell 1996;

421   Haas *et al.*, 2008). The unforeseen results showing differences in activity loss and mortality of

422   both fish-infecting furcocercariae with much more gradual changes observed for *Diplostomum*

423   spp. cercariae, leads to the assumption that variations in the relative duration of the active

424   swimming and resting periods might be responsible for the inter-taxa differences among these two

taxa. A reduction in the duration of active periods with cercarial age (while not active periods increase) has been previously reported, but exceptions have been also described (Whitfield *et al.*, 1977; Rea and Irwin, 1995; Chapman, 1974). The longer resting periods of *Diplostomum* spp. may allow a more constant utilization of energy reserves per time unit, thus maximizing their active stage until their death. This would allow a gradual successful infection of fish over time, which might contribute to the higher infection intensity of *Diplostomum* spp. in sticklebacks from Takvatn compared to that of *Apatemon* spp. (mean intensity 30.4 *vs* 3.0, respectively; Born-Torrijos *et al.*, 2021). Additionally, the more gradual changes in activity loss and mortality increase of *Diplostomum* spp. compared to remaining taxa may reflect a dispersal advantage helping to disseminate cercariae and avoiding subsequent heavy parasite-related mortality in next intermediate hosts, as described for various trematode species (e.g. Evans and Gordon, 1983; Lowenberger and Rau, 1994).

Apatemon spp. and *Plagiorchis* spp. showed more similar activity loss and mortality rates among them, being the minor differences in their patterns likely due to their different swimming behaviour (i.e. intermittent swimming with brief resting periods of *Apatemon* spp. *vs* continuous and slow bottom-dwelling of *Plagiorchis* spp.), given that (i) furcocercariae swim faster than mono-tailed cercariae, and (ii) fish-infecting cercariae swim faster than those infecting invertebrates (Selbach and Poulin 2018; Morley, 2020). The amount of glycogen reserves is also different between actively swimming and immobile cercariae (Ginetsinskaya, 1988). Whilst *Plagiorchis* spp. cercariae are not immobile, their displacement speed is much lower (Dixon 1984; Lowenberger and Rau, 1994; Morley, 2020). This, together with their ability to modulate their swimming speed in close vicinity of their next intermediate hosts to facilitate infection (Morley, 2020), could result in lower energetic requirements.

448   The lack of the effect of cercarial body size on the response of individuals to temperature

449   changes (Morley, 2011) could explain our observation that the two *Plagiorchis* spp. morphotypes

450   showed similar activity loss and mortality patterns, despite the only negligible differences in

451   activity loss rates in certain time sections. This could suggest that both morphotypes deplete their

452   energy reserves in a similar way, independently of their size. The overall longer cercarial lifespan

453   and active period of large-sized *Plagiorchis* spp., already reported for *Plagiorchis* species (10 days

454   at 4 ℃ and 90 h at 16 ℃, being infective during 38 h and 18 h respectively, Blankespoor, 1977),

455   could thus simply reflect its larger size and likely larger energy resources, rather than the way it

456   handles the energy. Nevertheless, it cannot be ruled out that the lack of large differences in activity

457   loss and mortality patterns between *Plargiochis* morphotypes could be related to the existence of

458   several lineages within each morphotype in high latitude areas (Soldánová *et al*., 2017; Kudlai *et*

459   *al*., 2021), as different lineages could utilize divergent life cycles and transmission strategies, and

460   thus activity loss and mortality patterns.

461   It is speculative whether the time of emergence might also play a role in cercarial mortality,

462   as some cercariae emerge and actively search for their next intermediate hosts during specific times

463   of the day to enhance the contact (Combes *et al*., 1994). However, although cercariae of the studied

464   genera have been frequently reported as diurnal- (*Diplostomum* spp., Haas *et al.,* 2008) or

465   nocturnal-emerging (*Apatemon* spp., Bell *et al.,* 1996; *Plagiorchis* spp., Lowenberger and Rau,

466   1994; but see diurnal-emerging *Plagiorchis* species in Gorman, 1980), trematodes in high latitude

467   conditions could be highly adapted, similarly to marine species (Prokofiev *et al.,* 2016), by

468   showing different emergence patterns to optimize their transmission to next suitable hosts. The

469   interpretation of cercarial activity loss and mortality in combination with emergence data will

470   enable to better understand the transmission dynamics of lacustrine trematodes in subarctic areas.

Conclusion

Our study shows highly dynamic temperature-dependent activity loss and mortality patterns of trematode transmission stages in subarctic lakes that are likely playing a crucial role in shaping the parasite communities in these systems. Suggestively, these temperature-dependent responses represent adaptations to the short transmission window that allows a stable continuance of trematodes' life cycles during ice-free periods in high-latitude ecosystems. Whilst activity loss and mortality dynamics could not be divided into "fish- *vs* invertebrate-infecting cercariae" groups, there is a taxa-specific response to temperature in relation to life-history traits, with swimming behaviour and energy allocation among cercariae likely being the main drivers of activity loss and mortality dynamics also in subarctic conditions. Since trematode population dynamics are influenced by both cercarial production, emergence and survival (Combes and Theron, 1981), further studies on cercarial emergence are needed to allow a more general understanding of the transmission ecology of trematode populations in high-latitude ecosystems, which are considered most vulnerable to climate change. Although further experimental infection studies should be performed to confirm the first sections as the most infective, the suggestion of those periods through the establishment of different time sections is an advantage of the presented activity and survival analyses.

Supplementary material

Suppl. Table S1. Parameters of each cercarial activity loss and mortality experiment at 6 ℃ and 13 ℃. T (℃), temperature; SD, standard deviation. Number of cercariae reflects the total number of cercariae used in statistical analyses, while number of cercariae per trial represents the different experimental trials performed.

Trematode taxa	T (ºC)	T (ºC) mean ± SD	No. chamber	No. cercariae	No. cercariae per trial	No. plates
Diplostomum spp.	6	5.7 ± 0.3	2	415	208	7
		5.7 ± 0.2	2		207	7
	13	11.9 ± 0.2	1	298	88	3
		11.9 ± 0.2	1		210	7
Apatemon spp.	6	6.1 ± 0.3	2	215		7
	13	11.9 ± 0.2	1	214		7
Small-sized *Plagiorchis* spp.	6	5.7 ± 0.3	2	426	209	7
		4.5 ± 0.4	1		217	7
	13	12.2 ± 0.1	1	423	212	7
		12.9 ± 0.6	2		211	7
Large-sized *Plagiorchis* spp.	6	4.5 ± 0.4	1	62		2
	13	12.5 ± 1.2	2	65		2

Data

Data will be archived in the Mendeley Data database (permanet link: DOI: xxxxx) upon acceptance.

512   **Author Contribution**

513   AB-T and MS conceived the ideas and designed methodology. AB-T, GSB, TV, RKn, RKr, P-AA

514   and MS participated in samplings. AB-T, GSB, TV and MS performed the experimental work.

515   AB-T and GSB statistically analysed the data. AB-T wrote the first draft of the manuscript. All

516   authors contributed critically to the drafts and gave final approval for the publication.

517

523   **Conflicts of Interest**

524   The authors declare there are no conflict of interest.

525

526   **Ethical Standards**

527   Not applicable.

528

References

Anderson, RM and Whitfield, PJ (1975) Survival characteristics of the free-living cercarial population of the ectoparasitic digenean *Transversotrema patialense* (Soparker, 1924). *Parasitology* **70**, 295–310. doi: 10.1017/S0031182000052082.

Bell, AS (1996) *Studies on the biosystematics and biology of strigeids (Digenea) parasitic in freshwater fish*. PhD thesis, University of Stirling, Scotland. **Blankespoor, HD** (1977) Notes on the biology of *Plagiorchis nobfei* Park, 1936 (Trematoda: Plachiorchiidae). *Proceedings of the Helminihological Society of Washington* **44,** 44–50.

Born-Torrijos, A, Paterson R, van Beest, GS, Vyhlídalová, T, Henriksen, EH, Knudsen, R, Kristoffersen, R, Amundsen, P-A and Soldánová, M (2021) Cercarial behaviour alters the consumer functional response of three-spined sticklebacks. *Journal of Animal Ecology* **90**, 978–988. doi: 10.1111/1365-2656.13427.

Blankespoor, HD (1977) Notes on the biology of *Plagiorchis noblei* Park, 1936 (Trematoda: Plagiorchiidae). *Proceedings of the Helminthological Society* **44**, 44–50.

Brassard, P, Curtis, MA and Rau, ME (1982) Seasonality of *Diplostomum spathaceum* (Trematoda: Strigeidae) transmission to brook trout (*Salvelinus fontinalis*) in northern Quebec, Canada. *Canadian Journal of Zoology* **60**, 2258–2263. doi: 10.1139/z82-291.

Crawley, MJ (2013) Survival Analysis. In John Wiley & Sons Ltd (eds). *The R Book*. Chichester, UK: John Wiley & Sons Ltd, pp. 883–893.

Chapman, HD (1974) The behaviour of the cercariae of *Cryptocotyle lingua*. *Zeitschrift f»ür Parasitenkunde* **44**, 211–26. doi: 10.1007/BF00328763.

Combes, C and Theron, A (1981) Les densites cercariennes. *Memoires du Museum National d'Histoire Naturelle, Serie A, Zoologie* **119**, 186–196.

Combes, C, Fournier, A, Mone H and Theron, A (1994) Behaviours in trematode cercariae that enhance parasite transmission: patterns and processes. *Parasitology* **109**, S3–S13. doi: 10.1017/S0031182000085048.

Dixon, MD (1984) *Strategies of host location employed by larval trematodes*. PhD thesis, University of York, UK.

Evans, NA and Gordon, DM (1983) Experimental studies on the transmission dynamics of cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology* **87**, 167–74. doi: 10.1017/S0031182000052513.

Evans, NA (1985) The influence of environmental temperature upon transmission of the cercariae of *Echinostoma liei* (Digenea: Echinostomatidae). *Parasitology* **90**, 269–275. doi: 10.1017/S0031182000050976.

Fenton, A and Hudson, PJ (2002) Optimal infection strategies: should macroparasites hedge their bets? *Oikos* **96**, 92–101.

Fingerut, JT, Zimmer, CA and Zimmer, RK (2003) Patterns and processes of larval emergence in an estuarine parasite system. *Biology Bulletin* **205**, 110–120. doi: 10.2307/1543232.

Galaktionov, KV and Dobrovolskij, AA (2003) *The biology and evolution of trematodes. An essay on the biology, morphology, life cycles, transmissions, and evolution of digenetic trematodes*. Boston, Dordrecht & London, Kluwer Academic.

Ganna, A, Rivadeneira, F, Hofman, A, Uitterlinden, AG, Magnusson, PKE, Pedersen, NL, Ingelsson, E and Tiemeier, H (2013) Genetic determinants of mortality. Can findings from genome-wide association studies explain variation in human mortality? *Human Genetics* **132**, 553–561. doi: 10.1007/s00439-013-1267-6.

574 **Ginetsinskaya, TA** (1988) *Trematodes, their life cycles, biology and evolution*. New Delhi,
575   Amerind.

576 **Gorman, AM (1980)** *Studies on the biology of* Pladiorchis elegans *(Rudolphi, 1802), (Trematoda:*
577   *Digenea) in its mammalian and molluscan-hosts.* PhD thesis, University of Leeds, UK.

578 **Haas, W, Beran, B and Loy, C** (2008) Selection of the hosts' habitat by cercariae: from laboratory
579   to the field. *Journal of Parasitology* **94**, 1233–1238. doi: 10.1645/GE-1192.1.

580 **Hakalahti, T, Karvonen, A and Valtonen, ET** (2006) Climate warming and disease risks in
581   temperate regions – *Argulus coregoni* and *Diplostomum spathaceum* as case studies. *Journal*
582   *of Helminthology* **80**, 93–98. doi: 10.1079/JOH2006351.

583 **Halttunen, E, Gjelland, K-Ø, Hamel, S, Serra-Llinares, RM, Nilsen, R, Arechavala-Lopez,**
584   **P, Skarðhamar, J, Johnsen, IA, Asplin, L, Karlsen, Ø, Bjørn, P-A and Finstad, B** (2017)
585   Sea trout adapt their migratory behaviour in response to high salmon lice concentrations.
586   *Journal of Fish Diseases* **41**, 953–967. doi:10.1111/jfd.12749

587 **Karvonen, A, Paukku, S, Valtonen, ET and Hudson, PJ** (2003) Transmission, infectivity and
588   survival of *Diplostomum spathaceum* cercariae. *Parasitology* **127**, 217–224. doi:
589   10.1017/S0031182003003561.

590 **Karvonen, A, Kirsi S, Hudson, PJ and Valtonen, ET** (2004) Patterns of cercarial production
591   from *Diplostomum spathaceum*: terminal investment or bet hedging? *Parasitology* **129**, 87–92.
592   doi: 10.1017/S0031182004005281.

593 **Koprivnikar, J, Lim, D, Fu, C and Brack, SHM** (2010) Effects of temperature, salinity, and pH
594   on the survival and activity of marine cercariae. *Parasitology Research* **106**, 1167–1177. doi:
595   10.1007/s00436-010-1779-0.

596 **Kudlai, O, Pantoja, C, O'Dwyer, K, Jouet, D, Skírnisson, K and Faltýnková, A** (2021)

597 Diversity of *Plagiorchis* (Trematoda: Digenea) in high latitudes: Species composition and snail

598 host spectrum revealed by integrative taxonomy. *Journal of Zoological Systematics and*

599 *Evolutionary Research*, **59**, 937–962. doi: 10.1111/jzs.12469.

600 **Kutz, SJ, Jenkins, EJ, Veitch, AM, Ducrocq, J, Polley, L, Elkin, B and Lair, S** (2009) The

601 Arctic as a model for anticipating, preventing, and mitigating climate change impacts on host-

602 parasite interactions. *Veterinary Parasitology* **163**, 217–228. doi: 10.1016/j.vetpar.2009.06.008

603 **Lawson, JR and Wilson, RA** (1980) The survival of the cercariae of *Schistosoma mansoni* in

604 relation to water temperature and glycogen utilization. *Parasitology* **81**, 337–348. doi:

605 10.1017/s0031182000056079.

606 **Lo, CT and Lee, KM** (1996) Pattern of emergence and the eEffects of temperature and light on

607 the emergence and survival of heterophyid cercariae (*Centrocestus formosanus* and *Haplorchis*

608 *pumilio*). *Journal of Parasitology* **82**, 347–350. doi: 10.2307/3284178.

609 **Lowenberger, CA and Rau, ME** (1994) *Plagiorchis elegans*: emergence, longevity and

610 infectivity of cercariae, and host behavioural modifications during cercarial emergence.

611 *Parasitology* **109**, 65–72. doi: 10.1017/s0031182000077775.

612 **Lyholt, HCK and Buchmann, K** (1996) *Diplostomum spathaceum*: effects of temperature and

613 light on cercarial shedding and infection of rainbow trout. *Diseases of Aquatic Organisms*, **25**,

614 169–173. doi: 10.3354/dao025169.

615 **Mas-Coma, S, Valero, MA and Bargues, MD** (2009) Climate change effects on trematodiases,

616 with emphasis on zoonotic fascioliasis and schistosomiasis. *Veterinary Parasitology*, **163**, 264–

617 280. doi: 10.1016/j.vetpar.2009.03.024.

618 **McCarthy, AM** (1999a) Photoperiodic cercarial emergence patterns of the digeneans

619 *Echinoparyphium recurvatum* and *Plagiorchis* sp. from a mixed infection in *Lymnaea peregra*.

620 *Journal of Helminthology* **73**, 59–62. doi: 10.1017/S0022149X99000074.

621 **McCarthy, AM** (1999b) The influence of temperature on the survival and infectivity of the

622 cercariae of *Echinoparyphium recurvatum* (Digenea: Echinostomatidae). *Parasitology* **118**,

623 383–388. doi: 10.1017/s003118209900400x.

624 **Morley, NJ** (2011) Thermodynamics of cercarial survival and metabolism in a changing climate.

625 *Parasitology* **138**, 1442–1452. doi: 10.1017/S0031182011001272.

626 **Morley, NJ** (2012) Cercariae (Platyhelminthes: Trematoda) as neglected components of

627 zooplankton communities in freshwater habitats. *Hydrobiologia* **691**, 7–19. doi:

628 10.1007/s10750-012-1029-9.

629 **Morley, NJ** (2020) Cercarial swimming performance and its potential role as a key variable of

630 trematode transmission. *Parasitology* 147, 1369–1374. doi: 10.1017/S0031182020001171.

631 **Mouritsen, KN** (2002) The *Hydrobia ulvae–Maritrema subdolum* association: influence of

632 temperature, salinity, light, water-pressure and secondary host exudates on cercarial emergence

633 and longevity. Journal of Helminthology **76**, 341–347. doi: 10.1079/JOH2002136.

634 **Muñoz-Antolí, C, Trelis, M, Espert, A, Toledo, R, and Esteban, JG** (2002) Survival and

635 infectivity of *Echinostoma friedi* (Trematoda: Echinostomatidae) miracidia and cercariae under

636 experimental conditions. *Helminthologia* **39**, 149–154. doi: 10.1079/JOH2006340.

637 **Nikolaev, KE, Levakin, IA, Galaktionov, KV** (2020) Seasonal dynamics of trematode infection

638 in the first and the second intermediate hosts: A long-term study at the subarctic marine

639 intertidal. *Journal of Sea Research* **164**: 101931. doi: 10.1016/j.seares.2020.101931.

640 **Pechenik, JA and Fried, B** (1995) Effect of temperature on survival and infectivity of

641 *Echinostoma trivolvis* cercariae: a test of the energy limitation hypothesis. *Parasitology* **111**,

642 373–378. doi: 10.1017/S0031182000081920.

643 **Pietrock, M and Marcogliese, DJ** (2003) Free-living endohelminth stages: at the mercy of

644 environmental conditions. *Trends in Parasitology* **19**, 293–299. doi: 10.1016/S1471-

645 4922(03)00117-X.

646 **Poulin, R** (2006) Global warming and temperature-mediated increases in cercarial emergence in

647 trematode parasites. *Parasitology* **132**, 143–151. doi: 10.1017/S0031182005008693.

648 **Prokofiev, VV** (1999) Influence of temperature and salinity on a life span of cercariae of marine

649 littoral trematodes *Cryptocotyle* sp. (Heterophyidae), *Levinseniella brachysoma* and *Maritrema*

650 *subdolum* (Microphallidae). *Parazitologiya* **33**, 520–526 (in Russian).

651 **Prokofiev, VV** (2001) Influence of temperature and salinity on a life span of cercariae of marine

652 littoral trematodes *Podocotyle atomon* (Opecoealidae) and *Renicola thaidus* (Renicolidae).

653 *Parazitologiya* **35**, 69–76 (in Russian).

654 **Prokofiev, VV, Galaktionov, KV and Levakin, IA** (2016) Patterns of parasite transmission in

655 polar seas: Daily rhythms of cercarial emergence from intertidal snails. *Journal of Sea Research*

656 **113**, 85–98. doi: 10.1016/j.seares.2015.07.007.

657 **R Core Team** (2017) R: A language and environment for statistical computing. R Foundation for

658 Statistical Computing, Vienna, Austria. https://www.r-project.org/

659 **Rea, JG and Irwin, SWB** (1992) The effects of age, temperature, light quantity and wavelength

660 on the swimming behaviour of the cercariae of *Cryptocotyle lingua* (Digenea: Heterophyidae).

661 *Parasitology* **105**, 131–137. doi: 10.1017/S0031182000073789.

662 **Selbach, C and Poulin, R** (2018) Parasites in space and time: a novel method to assess and

663   illustrate host-searching behaviour of trematode cercariae. *Parasitology* **145**, 1469–1474.

664   doi:10.1017/S0031182018000288.

665 **Selbach, C and Poulin, R** (2020) Some like it hotter: trematode transmission under changing

666   temperature conditions. *Oecologia* **194**, 745–755. doi: 10.1007/s00442-020-04800-y.

667 **Soldánová, M, Georgieva, S, Roháčová, J, Knudsen, R, Kuhn, JA, Henriksen, EH,**

668   **Siwertsson, A, Shaw, JC, Kuris, AM, Amundsen, P-A, Scholz, T, Laffety, KD and**

669   **Kostadinova, A** (2017) Molecular analyses reveal high species diversity of trematodes in a

670   sub-Arctic lake. *International Journal for Parasitology* **47**, 327–345. doi:

671   10.1016/j.ijpara.2016.12.008.

672 **Studer, A, Thieltges, DW and Poulin, R** (2010) Parasites and global warming: net effects of

673   temperature on an intertidal host–parasite system. *Marine Ecology Progress Series* **415**, 11–22.

674   doi: 10.3354/meps08742.

675 **Studer, A and Poulin, R** (2013) Cercarial survival in an intertidal trematode: a multifactorial

676   experiment with temperature, salinity and ultraviolet radiation. *Parasitology Research* **112**,

677   243–249. doi: 10.1007/s00436-012-3131-3.

678 **Studer, A and Poulin, R** (2014) Analysis of trait mean and variability versus temperature in

679   trematode cercariae: is there scope for adaptation to global warming? *International Journal for*

680   *Parasitology* **44,** 403–413. doi: 10.1016/j.ijpara.2014.02.006.

681 **Therneau, T** (2020) A Package for Survival Analysis in R. R package version 3.1-11,

682   https://CRAN.R-project.org/package=survival.

683   **Thieltges, DW and Rick, J** (2006) Effect of temperature on emergence, survival and infectivity

684   of cercariae of the marine trematode *Renicola roscovita* (Digenea: Renicolidae). *Diseases Of*

685   *Aquatic Organisms* **73**, 63–68. doi: 10.3354/dao073063.

686   **Thieltges, DW and Reise, K** (2007) Spatial heterogeneity in parasite infections at different spatial

687   scales in an intertidal bivalve. *Oecologia* **150**, 569–581. doi: 10.1007/s0044 2-006-0557-2.

688   **Thieltges, DW, Jensen KT and Poulin, R** (2008) The role of biotic factors in the transmission of

689   free-living   endohelminth   stages.   *Parasitology*   **135**,   407–426.   doi:

690   10.1017/S0031182007000248.

691   **van Beest, GS, Villar-Torres, M, Raga, JA, Montero, FE and Born-Torrijos, A** (2019). In vivo

692   fluorescent cercariae reveal the entry portals of *Cardiocephaloides longicollis* (Rudolphi, 1819)

693   Dubois, 1982 (Strigeidae) into the gilthead seabream *Sparus aurata* L. *Parasites & Vectors* 12,

694   92. doi: 10.1186/s13071-019-3351-9.

695   **Whitfield, PJ, Anderson, RM and Bundy, DAP** (1977) Experimental investigations on the

696   cercariae of an ectoparasitic digenean *Transversotrema patialense*: general activity patterns.

697   *Parasitology* **75**, 7–30. doi: 10.1017/S0031182000048307.

Tables & Figures

699   **Table 1**. Evaluation of the differences in (i) activity loss and (ii) mortality of cercariae of four trematode taxa between temperatures (6

700   °C *vs* 13 °C). Results evaluating (i) Cox Proportional Hazards regression (CoxPH) (time active cercariae ~ temperature + plate (random)

701   + trial (random)), and (ii) Cox Proportional Hazards regression (CoxPH) (time live cercariae ~ temperature + plate (random) + trial

702   (random)). If step function was required to accomplish the model assumptions, selected time sections are indicated in the table. The

703   interpretation of results, comparing 6 °C to 13 °C, uses arrows to indicate a higher (▲) or lower (▼) activity loss or mortality of cercariae

704   at 13 ºC. Statistically significant results (at $\alpha = 0.05$) are indicated in bold.

Trematode taxa (N, number cercariae)	(i) Activity loss						(ii) Mortality					
	Time section	$\exp^{(\beta)a}$	$se(\beta)^b$	*z-value*[c]	P-value	Interpretation of activity loss	Time section	$\exp^{(\beta)}$	$se(\beta)$	*z-value*	P-value	Interpretation of mortality
Diplostomum spp. 6 ºC, N=415 13 ºC, N=298	0–9h	0.581	0.137	-3.973	**<0.001**	1.72 times ▼	0–22h	1.354	0.1015	2.985	**0.003**	1.35 times ▲
	9–22h	2.764	0.207	4.916	**<0.001**	2.76 times ▲	22–76h	1.673	0.2398	2.145	**0.032**	1.67 times ▲
	22–76h	5.3506	0.307	5.459	**<0.001**	5.35 times ▲						
Apatemon spp. 6 ºC, N=215 13 ºC, N=214	0–38h	0.669	0.113	-3.558	**<0.001**	1.49 times ▼	0–68h	7.888	0.1462	14.120	**<0.001**	7.89 times ▲
Small-sized *Plagiorchis* spp. 6 ºC, N=426 13 ºC, N=423	0–15h	3.649	0.119	10.923	**<0.001**	3.65 times ▲	0–72h	11.668	0.120	20.470	**<0.001**	11.67 times ▲
	15–20h	0.505	0.177	-3.851	**<0.001**	1.98 times ▼						
	20–48h	0.594	0.262	-1.990	**0.047**	1.68 times ▼						
Large-sized *Plagiorchis* spp. 6 ºC, N=62 13 ºC, N=65	0–33h	1.510	0.373	1.105	0.269		0–44h	1.641	0.2492	1.988	**0.047**	1.64 times ▲
	33–44h	0.762	0.468	-0.582	0.561		44–86h	0.347	0.3986	-2.653	**0.008**	2.88 times ▼
	44–70h	0.299	0.564	-2.142	**0.032**	3.35 times ▼						

705   [a]Hazard ratio (exponentiated coefficients). The hazard rate of cercariae at 6 ℃ in both models (i, ii) is 1, to which the other levels are

706   compared. If $\exp^{(\beta)} < 1$, activity loss or mortality risk is reduced for the trematode taxa at 13 ℃.

707   [b]Standard error of coefficients.

708   [c]Test criterion value.

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712 **Table 2**. Evaluation of the differences in (i) activity loss and (ii) mortality among cercariae of different trematode taxa at 13 ºC.

713 Results evaluating (i) Cox Proportional Hazards regression (CoxPH) (time active cercariae at 13 ºC ~ trematode taxa + plate (random)

714 + trial (random)), and (ii) Cox Proportional Hazards regression (CoxPH) (time live cercariae at 13ºC ~ trematode taxa + plate (random)

715 + trial (random)). If step function is required to accomplish the model assumptions, time sections are indicated in the table. The

716 interpretation of results, comparing the different taxa of cercariae at 13 ºC, use arrows to indicate a higher (▲) or lower (▼) activity

717 loss or mortality of the compared trematode taxa (right place in first column). Statistically significant results (at α = 0.05) are indicated

718 in bold.

Trematode taxa (N, number cercariae)	(i) Activity loss						(ii) Mortality					
Reference *vs* compared	Time section	exp$^{(\beta)a}$	se(β)^b	*z-value*^{c}	P-value	Interpretation of activity loss	Time section	exp$^{(\beta)}$	se(β)	*z-value*	P-value	Interpretation of mortality
Diplostomum spp. – *Apatemon* spp.	0–16h	0.067	0.190	-14.297	**< 0.001**	15.04 times ▼	0–27h	0.10	0.175	-13.448	**< 0.001**	10.47 times ▼
N=298 – N=215	16–27h	13.333	0.343	7.552	**< 0.001**	13.33 times ▲	27–54h	14.714	0.316	8.509	**< 0.001**	14.71 times ▲
	27–52h	71.751	0.667	6.406	**< 0.001**	71.75 times ▲						
Diplostomum spp. – Small-sized *Plagiorchis* spp.	0–16h	0.432	0.112	-7.500	**< 0.001**	2.31 times ▼	0–27h	0.111	0.134	-16.375	**< 0.001**	9.02 times ▼
N=298 – N=423	16–27h	7.756	0.212	9.665	**< 0.001**	7.75 times ▲	27–54h	38.964	0.264	13.897	**< 0.001**	38.96 times ▲
	27–52h	4.420	0.413	3.599	**< 0.001**	4.42 times ▲						
Diplostomum spp. – Large-sized *Plagiorchis* spp.	0–16h	0.053	0.596	-4.934	**< 0.001**	18.96 times ▼	0–27h	0.049	0.473	-6.371	**< 0.001**	20.31 times ▼
N=298 – N=65	16–27h	2.683	0.774	1.274	0.203		27–86h	17.487	0.564	5.072	**< 0.001**	17.49 times ▲
	27–70h	5.768	0.696	2.519	**0.012**	5.77 times ▲						

Apatemon spp. – Small-sized *Plagiorchis* spp. N=215 – N=423	0–16h	6.499	0.168	11.130	**< 0.001**	6.51 times ▲	0–27h	1.161	0.140	1.064	0.288	
	16–27h	0.582	0.334	-1.620	0.105		27–48h	2.648	0.231	4.213	**< 0.001**	2.65 times ▲
	27–38h	0.0616	0.772	-3.612	**< 0.001**	16.23 times ▼						
Apatemon spp. – Large-sized *Plagiorchis* spp. N=215 – N=65	0–16h	0.793	0.619	-0.374	0.708		0–27h	0.515	0.497	-1.334	0.182	
	16–27h	0.201	0.829	-1.935	0.053		27–86h	1.188	0.573	0.301	0.763	
	27–70h	0.080	0.960	-2.627	**0.009**	12.44 times ▼						
Small-sized *Plagiorchis* spp.– Large-sized *Plagiorchis* spp. N=423 – N=65	0–16h	0.122	0.598	- 3.516	**< 0.001**	8.19 times ▼	0–27h	0.444	0.483	-1.682	0.093	
	16–27h	0.346	0.766	-1.386	0.166		27–86h	0.449	0.531	-1.510	0.131	
	27–70h	1.305	0.720	0.370	0.712							

719 [a]Hazard ratio (exponentiated coefficients). The hazard rate ($\exp^{(\beta)}$) of cercariae of each taxon (located in left side of first column) in both

720 models (i, ii) is 1, to which the other taxa are compared. If $\exp^{(\beta)} < 1$, activity loss or mortality is reduced for the compared trematode

721 taxa (right place in first column).

722 [b]Standard error of coefficients.

723 [c]Test criterion value.

724 **Table 3**. Evaluation of the differences in (i) activity loss and (ii) mortality among cercariae of different trematode taxa at 6 ºC.

725 Results evaluating (i) Cox Proportional Hazards regression (CoxPH) (time active cercariae at 6 ºC ~ trematode taxa + plate (random) +

726 trial (random)), and (ii) Cox Proportional Hazards regression (CoxPH) (time live cercariae at 6 ºC ~ trematode taxa + plate (random) +

727 trial (random)). If step function is required to accomplish the model assumptions, time sections are indicated in the table. The

728 interpretation of results, comparing the different taxa of cercariae at 6 ºC, use arrows to indicate a higher (▲) or lower (▼) activity loss

729 or mortality of the compared trematode taxa (right place in first column). Statistically significant results (at α = 0.05) are indicated in

730 bold.

Trematode taxa (N, number cercariae)	(i) Activity loss						(ii) Mortality					
Reference *vs* compared	Time section	exp$^{(\beta)a}$	se$(\beta)^b$	*z-value*^{c}	P-value	Interpretation of activity loss	Time section	exp$^{(\beta)}$	se(β)	*z-value*	P-value	Interpretation of mortality
Diplostomum spp. – *Apatemon* spp.	0–13h	0.281	0.209	-6.078	**<0.001**	3.56 times ▼	0–26h	0.098	0.242	-9.594	**<0.001**	10.23 times ▼
N=415 – N=215	13–22h	10.729	0.273	8.708	**<0.001**	10.73 times ▲	26–44h	5.927	0.299	5.955	**<0.001**	5.92 times ▲
	22–76h	57.114	0.307	13.196	**<0.001**	57.11 times ▲	44–76h	21.123	0.316	9.643	**<0.001**	21.12 times ▲
Diplostomum spp. – Small-sized *Plagiorchis* spp.	0–13h	0.060	0.227	-12.395	**<0.001**	16.69 times ▼	0–26h	0.069	0.187	- 14.307	**<0.001**	14.56 times ▼
N=415 – N=426	13–22h	29.135	0.269	12.560	**<0.001**	29.13 times ▲	26–44h	26.774	0.239	13.739	**<0.001**	26.77 times ▲
	22–76h	46.31	0.276	13.875	**<0.001**	46.31 times ▲	44–76h	52.312	0.326	12.138	**<0.001**	52.31 times ▲
Diplostomum spp. – Large-sized *Plagiorchis* spp.	0–13h	0.023	1.016	-3.711	**<0.001**	43.37 times ▼	0–26h	0.026	0.723	-5.024	**<0.001**	37.76 times ▼
N=415 – N=62	13–22h	2.353	1.250	0.684	0.494		26–44h	67.352	0.776	5.427	**<0.001**	67.35 times ▲
	22–76h	40.694	1.045	3.547	**<0.001**	40.69 times ▲	44–78h	211.540	0.800	6.691	**<0.001**	211.54 times ▲
Apatemon spp. – Small-sized *Plagiorchis* spp.	0–13h	0.213	0.296	-5.225	**<0.001**	4.69 times ▼	0–26h	0.703	0.293	-1.204	0.229	

N=215 – N=426	13–22h	2.716	0.333	3.001	**0.003**	2.72 times ▲	26–44h	4.5174	0.328	4.600	**<0.001**	4.52 times ▲
	22–48h	0.811	0.353	-0.594	0.552		44–72h	2.4766	0.405	2.240	**0.025**	2.48 times ▲
Apatemon spp. – Large-sized *Plagiorchis* spp. N=215 – N=62	0–13h	0.082	1.037	-2.412	**0.016**	12.20 times ▼	0–26h	0.271	0.762	-1.714	0.087	
	13–22h	0.219	1.272	-1.193	0.233		26–44h	11.364	0.815	2.983	**0.003**	11.36 times ▲
	22–64h	0.713	1.079	-0.314	0.753		44–78h	10.015	0.843	2.733	**0.006**	10.01 times ▲
Small-sized *Plagiorchis* spp. – Large-sized *Plagiorchis* spp. N=426 – N=62	0–13h	0.385	1.037	-0.921	0.357		0–26h	0.386	0.741	-1.285	0.199	
	13–22h	0.081	1.264	-1.991	**0.047**	12.38 times ▼	26–44h	2.516	0.783	1.177	0.239	
	22–64h	0.879	1.064	-0.121	0.903		44–78h	4.048	0.788	1.774	0.076	

731 [a]Hazard ratio (exponentiated coefficients). The hazard rate ($\exp^{(\beta)}$) of cercariae of each taxon (located in left side of first column) in both

732 models (i, ii) is 1, to which the other taxa are compared. If $\exp^{(\beta)} < 1$, activity loss or mortality is reduced for the compared trematode

733 taxa (right place in first column).

734 [b]Standard error of coefficients.

735 [c]Test criterion value.
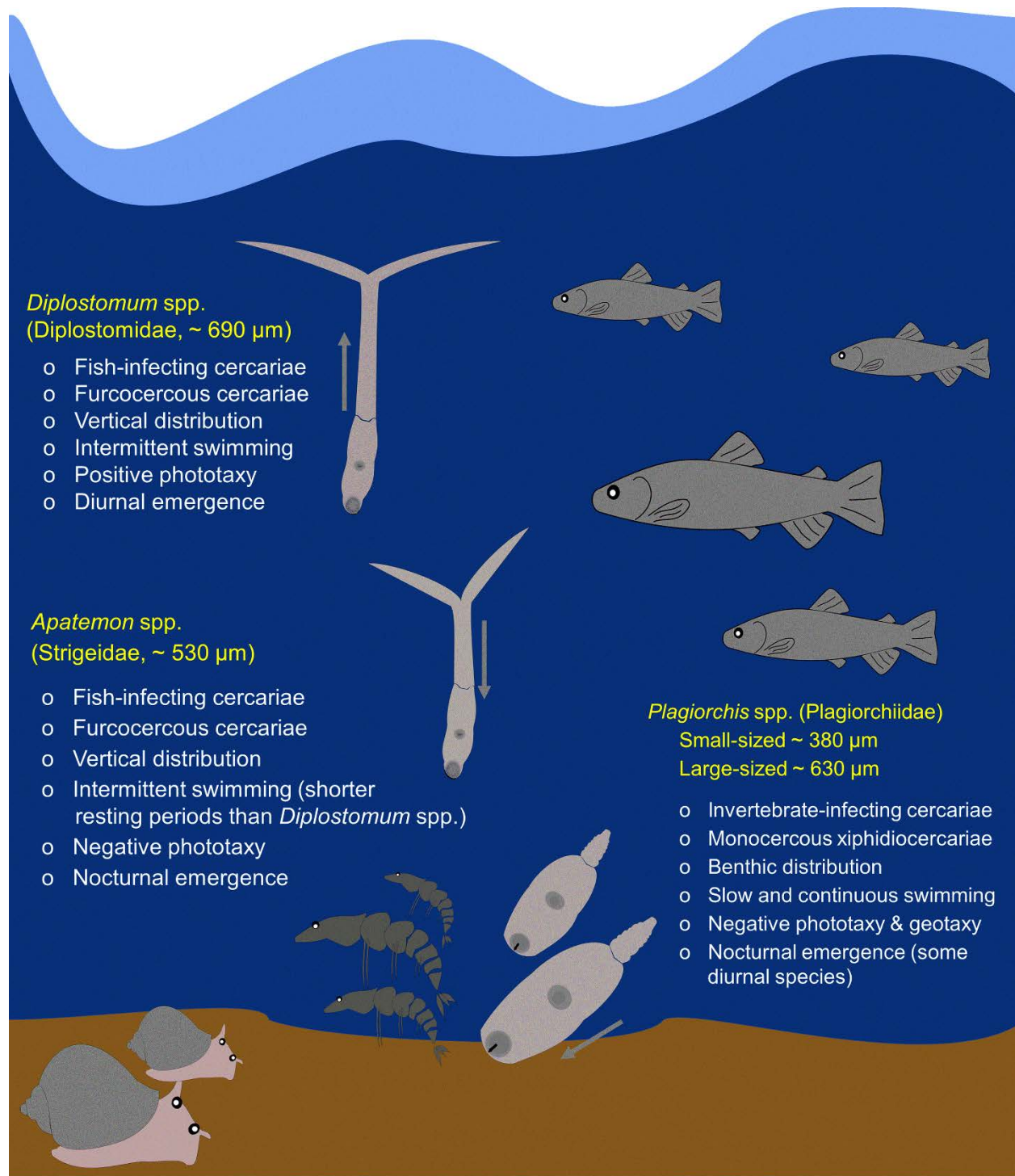
736 **<u>Figures</u>**

737

738 **Fig. 1.** Summary of the life-history traits and transmission strategies (i.e. next target hosts,

739 morphological features, distribution in the water column, swimming behaviours and

740 emergence patterns) of cercariae of the three genera selected for the present study,

741 represented by four trematode taxa: *Diplostomum* spp., *Apatemon* spp., small- and large-

742 sized *Plagiorchis* spp. (see Materials and methods for references). Arrows indicate the

743 direction of phototaxy. The size of cercariae is in proportion to their original size.

744

745 **Fig. 2.** Kaplan-Meier activity (top) and survival (bottom) curves for cercariae of four

746 trematode taxa (*Diplostomum* spp., *Apatemon* spp., small- and large-sized *Plagiorchis* spp.,

747 differentiated by colours) incubated in 6 ℃ and 13 ℃. Censored data included in the

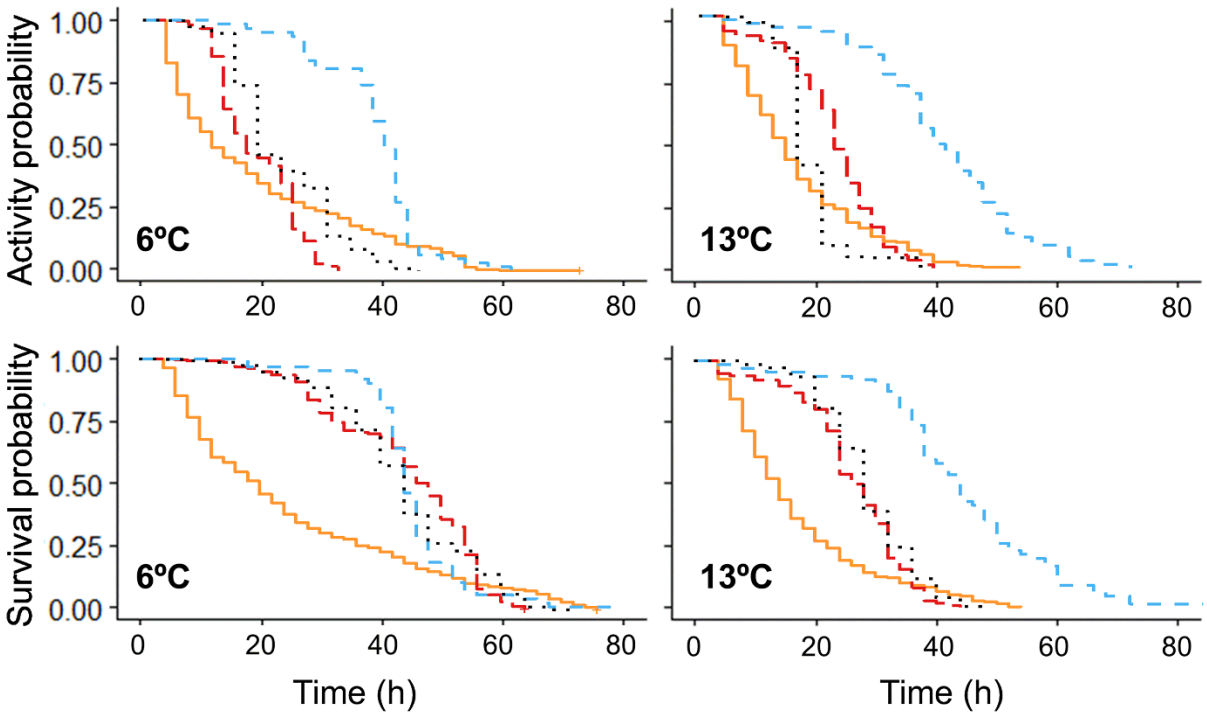748 statistical analyses are indicated by a cross at the end of the curve.

749

Diplostomum spp.
(Diplostomidae, ~ 690 μm)

- o Fish-infecting cercariae
- o Furcocercous cercariae
- o Vertical distribution
- o Intermittent swimming
- o Positive phototaxy
- o Diurnal emergence

Apatemon spp.
(Strigeidae, ~ 530 μm)

- o Fish-infecting cercariae
- o Furcocercous cercariae
- o Vertical distribution
- o Intermittent swimming (shorter resting periods than Diplostomum spp.)
- o Negative phototaxy
- o Nocturnal emergence

Plagiorchis spp. (Plagiorchiidae)
Small-sized ~ 380 μm
Large-sized ~ 630 μm

- o Invertebrate-infecting cercariae
- o Monocercous xiphidiocercariae
- o Benthic distribution
- o Slow and continuous swimming
- o Negative phototaxy & geotaxy
- o Nocturnal emergence (some diurnal species)

750

751

752   Fig. 1

753

Diplostomum spp. - *Apatemon* spp. - Small-sized *Plagiorchis* spp. - Large-sized *Plagiorchis* spp.

754

755

756   Fig. 2