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Predator faunas past and present: quantifying the influence of waterborne cues in divergent ecotypes of the isopod *Asellus aquaticus*

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2 **Predator faunas past and present: quantifying the**
3 **influence of waterborne cues in divergent ecotypes of**
4 **the isopod *Asellus aquaticus***

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22 **AUTHOR CONTRIBUTIONS:** SH, KKG & LP conceived and designed the study,
23 SH & KKG performed the study, SH & LP analysed the data, SH wrote the paper and
24 all authors participated in the editorial process.

25 **ABSTRACT**

26 Waterborne chemical cues are an important source of information for many aquatic
27 organisms, in particular when assessing the current risk of predation. The ability to
28 use chemical cues to detect and respond to potential predators before an actual
29 encounter can improve prey chances of survival. We investigated predator recognition
30 and the impact of chemical cues on predator avoidance in the freshwater isopod
31 *Asellus aquaticus*. This isopod has recently colonised a novel habitat and diverged
32 into two distinct ecotypes, which encounter different predator communities. Using
33 laboratory-based choice experiments, we have quantified behavioural responses to
34 chemical cues from predators typical of the two predator communities (larval
35 dragonflies in the ancestral habitat, perch in the newly colonised habitat) in wild-
36 caught and lab-reared *Asellus* of the two ecotypes. Individuals with prior experience
37 of predators showed strong predator avoidance to cues from both predator types. Both
38 ecotypes showed similar antipredator responses, but sexes differed in terms of threat-
39 sensitive responses with males avoiding areas containing predator cues to a larger
40 extent than females. Overall, chemical cues from fish elicited stronger predator
41 avoidance than cues from larval dragonflies. Our results indicate that in these isopods,
42 prior exposure to predators is needed to develop antipredator behaviour based on
43 waterborne cues. Furthermore, the results emphasise the need to analyse predator
44 avoidance in relation to waterborne cues in a sex-specific context, because of
45 potential differences between males and females in terms of vulnerability and life-
46 history strategies.

47

48 **Keywords:** *Aeshna* spp., antipredator behaviour, chemical communication, learning,
49 *Perca fluviatilis*

50

51 **Introduction**

52

53 In many aquatic habitats, chemical cues are important for predator detection and
54 recognition by prey (Kats & Dill 1998; Pettersson et al. 2000; Schoeppner & Relyea
55 2005; Wisenden & Constantz 2006). The smell of a predator can reveal important
56 information such as the type of prey recently consumed, the hunger state of the
57 predator, and can also provide prey with more general information about predator
58 densities and types of predators present (Åbjörnsson et al. 1997; Dahl et al. 1998;
59 Pettersson et al. 2000; Brown 2003; Schoeppner & Relyea 2005; Ferrari et al. 2006).
60 Waterborne predator cues can thus have profound influences on prey and have been
61 shown capable of inducing morphological defences (Brönmark & Pettersson 1994;
62 Lass & Spaak 2003; Schoeppner & Relyea 2009), antipredator behaviours (Brown &
63 Godin 1999; Pettersson et al. 2000; Åbjörnsson et al. 2004; Gonzalo et al. 2007) and
64 modifying life-history strategies (Benard 2004; Dunn et al. 2008). In general, a prey's
65 ability to assess the local predation threat by the use of reliable environmental cues is
66 of great importance for optimising trade-offs between antipredator behaviours and
67 other fitness-related activities such as mating, foraging, and territorial defence (Lima
68 & Dill 1990; Sih et al. 2000). Predator avoidance behaviours can be energetically
69 demanding depending on the intensity and duration of the response (Lima & Dill
70 1990). To maximise fitness, prey animals that are able to respond in a threat-sensitive
71 manner (Helfman 1989), i.e. adjusting antipredator responses according to the
72 perceived level of risk posed by the predator, will have a selective advantage (Lima &
73 Bednekoff 1999; Lima & Steury 2005). For example, threat-sensitive responses have
74 been shown in relation to different concentrations of conspecific alarm cues or
75 predator odours (McIntosh et al. 1999; Pettersson et al. 2000; Dupuch et al. 2004;

76 Ferrari et al. 2006; Brown et al. 2009), as well as to predator cues from high and low-
77 risk predators (Hawkins et al. 2007). Furthermore, size-specific and sex-specific
78 effects have also been demonstrated to influence antipredator responses by prey. For
79 instance, in mayflies and green frog tadpoles, larger individuals exhibited stronger
80 antipredator behaviours than smaller ones to predator chemical cues (McIntosh et al.
81 1999; Smith et al. 2008) and in Trinidadian guppies, males are significantly bolder
82 than females (Harris et al. 2010).

83 In the present study, we investigate predator recognition and avoidance
84 behaviour in predator naive and predator experienced individuals of the freshwater
85 isopod *Asellus aquaticus*. This isopod has recently colonised a novel habitat and
86 diverged in parallel into two distinct ecotypes (the ancestral *Phragmites australis* and
87 the novel *Chara tomentosum*) in south Swedish lakes (Hargeby et al. 2004; Hargeby
88 et al. 2005; Eroukhmanoff & Svensson 2009). It has been suggested that the rapid
89 ecotype divergence has been heavily influenced by differences in predation pressure
90 (Hargeby et al. 2004; Hargeby et al. 2005; Eroukhmanoff & Svensson 2009). This
91 allows us to quantify how predator recognition and antipredator behaviour develops in
92 a system that moves from one type of predator community to a novel one. That is,
93 when isopods colonised the novel stonewort habitat, this also involved moving from
94 the reed predator community, which is dominated by invertebrate predators (e.g.
95 dragonfly larvae), to a new predator community, which is dominated by fish predators
96 (Hargeby et al. 2004; Hargeby et al. 2005; Eroukhmanoff & Svensson 2009). Both
97 invertebrate and fish predators emit waterborne cues that can be used by prey to
98 assess e.g. the current predation risk (Williams & Moore 1985; Wudkevich et al.
99 1997; Kats & Dill 1998). Hence, it is highly likely that ecotype divergence is

100 paralleled by divergence in predator recognition and threat-sensitive antipredator
101 behaviour.

102 Using a series of laboratory-based choice experiments, we investigated if
103 predator avoidance to olfactory predator cues was affected by sex, predator species
104 (fish or dragonfly larvae), and habitat (reed or stonewort) in wild and lab-raised
105 individuals, respectively. We addressed four main questions: 1) do prior experiences
106 with predators affect avoidance responses; 2) are there threat-sensitive responses to
107 different predator cues, or do all individuals exhibit a general response irrespective of
108 predator type; 3) do males and females differ in predator avoidance; and 4) do
109 ecotypes differ in terms of responses to chemical cues from the two predator types?

110

111 **Materials and methods**

112

113 Experimental animals

114

115 *Asellus aquaticus* (Crustacea: Isopoda) is common in slow flowing waters across
116 Europe (Verovnik et al. 2005). As other asellids, this isopod feeds on detritus and
117 periphyton (Smock & Harlowe 1983; Arakelova 2001). *A. aquaticus* is itself a food
118 source for fish, e.g. perch, *Perca fluviatilis* (Rask & Hiisivuori 1985). In several
119 Swedish lakes, *A. aquaticus* has diverged into two distinct ecotypes, utilising different
120 habitats (Hargeby et al. 2004; Hargeby et al. 2005). In at least two lakes, this
121 differentiation has occurred in parallel with similar morphological and behavioural
122 changes in the novel ecotype (Eroukhmanoff et al. 2009; Eroukhmanoff & Svensson
123 2009; Karlsson et al. 2010a). In both lakes, divergence has occurred rapidly during
124 the last 20 years after major ecological shifts reviewed in Hargeby et al. 2007

125 following the emergence of new habitat that mainly consists of stonewort, *Chara*
126 *tomentosa* (Hargeby et al. 1994; Hargeby et al. 2004). Isopods dispersed into the
127 novel stonewort habitat located in the lake centre, from source populations in the reed
128 stands (*Phragmites australis*) along the shoreline (Hargeby et al. 2004). The major
129 selective agent behind the ecotype divergence is suggested to be predation (Hargeby
130 et al. 2004; Eroukhmanoff & Svensson 2009) because predator regimes differ
131 between the reed and the stonewort habitats (Wagner & Hansson 1998; Eroukhmanoff
132 & Svensson 2009). The reed is mainly inhabited by invertebrate predators, such as
133 dragonfly larvae, while the stonewort is mainly inhabited by fish predators
134 (Eroukhmanoff & Svensson 2009), with small perch (< 15 cm) being the most
135 common (Hargeby et al. 1994).

136 In June 2009, we collected isopods from both ecotypes (reed and stonewort) in
137 Lake Krankesjön (55°42'N, 13°28'E), in southern Sweden. The reed habitat mainly
138 consists of detritus while the stonewort habitat is a three-dimensional matrix of *Chara*
139 in the lake centre. We sampled individuals from a depth of approximately 0-0.4 m in
140 the reed and 0.5-1.0 m in the stonewort. In total, we collected 50 couples (25 males
141 and 25 females in copula) from each habitat. The couples were carefully separated
142 and we placed each individual in a single container. The isopods were fed decaying
143 leaves, and kept in the laboratory where temperature and light regimes were
144 controlled to mimic natural conditions (17°C, 12L:12D). Wild-caught isopods were
145 allowed to adjust to laboratory conditions for at least 24 h prior to testing, and they
146 were used in the behavioural trials within 4 days of collection in the field. Lab-raised
147 individuals from both ecotypes were kept family-wise in containers in a common-
148 garden setup with the same temperature, equal amount of food and a joint circulating
149 water system. These individuals were the F1-generation from a previous breeding

150 study and they were reared until sexual maturity before being used in the experiment.
151 Animals were sexed by presence (female) or absence (male) of oostegites (Unwin
152 1920). In total 55 lab-raised individuals were used (28 reed; 27 stonewort).

153 Due to logistic reasons it was not possible to measure the size of the isopods
154 used in the present experiment. Prior to the experiment, handling of isopods was kept
155 to a minimum to avoid influencing trials. Following each trial, isopods were returned
156 to other experiments with no opportunity to measure them individually. However,
157 using data from our previous field and lab studies we have good evidence that wild-
158 caught females are smaller than males in Lake Krankesjön (mean total length \pm SD:
159 females 7.5 ± 0.9 mm, $n = 200$; males 10.2 ± 1.3 mm, $n = 175$, data from collections
160 in 2008). Wild-caught reed isopods are on average larger than wild-caught stonewort
161 isopods, although the reed isopods are in general more variable and the average length
162 of stonewort isopods fell within the range of reed isopods lengths (Eroukhmanoff et
163 al. 2009). In contrast, lab-raised *Asellus* from Lake Krankesjön show no size
164 differences between sex or habitat (mean total length \pm SD: females 6.1 ± 0.6 mm, $n =$
165 106 ; males 6.2 ± 0.8 mm, $n = 219$; reed 6.1 ± 0.7 , $n = 190$; stonewort 6.2 ± 0.8 , $n =$
166 135 , data from collections in 2009).

167

168 Predator cues

169

170 Two different predator species were used as cue donors in the experiments: Eurasian
171 perch (*Perca fluviatilis*, $n = 2$; 94mm, 8.6g and 108 mm, 12.0 g, respectively)
172 collected in Lake Krankesjön and late-instar dragonfly larvae (*Aeshna* spp, $n = 16$)
173 collected in Vinkeldammen Pond ($55^{\circ}33'N$, $13^{\circ}38'E$) as well as in Lake Krankesjön.
174 The perch were kept individually in 40 l tanks ($50 \times 27 \times 27$ cm [length \times width \times

175 height]) for two weeks prior to cue collection, and were fed isopods ad lib three times
176 per week during the acclimatization. The size of the perch is consistent with the
177 species' benthivorous stage in which it mainly feeds on macroinvertebrates
178 (Brönmark and Pettersson 1994). After the acclimatization period, perch were rinsed
179 with dechlorinated tap water and transferred to a stimulus collection aquaria (45×25
180 $\times 15$ cm [length \times width \times height]) containing 8 l dechlorinated tap water), which was
181 well aerated but contained no filtering device. Cue collection followed standard
182 methodology (e.g. Pettersson et al. 2000; Ferrari et al. 2007), and three days later we
183 removed the cue donor and the stimulus water was immediately frozen in plastic
184 containers (0.8-1.2 l) at -80°C . Perch cues were randomized between trials and pooled
185 in the analysis as cue strength differences between donors offered the same diet are
186 negligible (cf. Brönmark and Pettersson 1994, Pettersson et al. 2000). Frozen stimulus
187 water has been shown to retain its activity for at least two months (Pettersson et al.
188 2000). Dechlorinated tap water was frozen at the same time, in the same type of
189 containers to be used as control. The cue collection procedure was repeated until there
190 was enough stimulus water. The dragonfly larvae were placed individually in
191 containers ($10.5 \times 8 \times 7$ cm [length \times width \times height]) with dechlorinated tap water,
192 and were fed isopods for a few days during acclimatization. After the acclimatization,
193 we fed each larva with six isopods, and if all isopods were consumed the next day the
194 larva was moved to a circular plastic container (7 cm diameter, 4 cm deep) with 0.2 l
195 dechlorinated tap water, a volume selected to match the ratio of cue donor mass
196 (mean late instar *Aeshna* larval mass = 0.25 g, L.B. Pettersson, unpublished data) to
197 water volume used in the fish cue collection (approximately 1.2 g per l). This
198 matching implicitly assumes stimulus release to be proportional to body mass and is a
199 commonly used compromise solution between alternative standardisation approaches

200 (e.g. Pettersson et al. 2000). The larva stayed in the stimulus collection jar for three
201 days before stimulus water was collected and frozen. We used each larva several
202 times as a cue donor, and the stimulus water was mixed from all 16 larvae. Cue and
203 control water were thawed to room temperature (20°C) the night before the
204 experimental trial started.

205

206 Choice experiments

207

208 To examine the response to waterborne predator cues from fish or dragonfly larvae,
209 we conducted experimental trials in a two-compartment choice arena (20 × 8.5 × 2.5
210 cm [length × width × height]) made of transparent PVC plastic (Fig. S1) (cf. Baker &
211 Montgomery 2001; Hale et al. 2009; Wisenden & Dye 2009). The experimental arena
212 consisted of two choice zones, one providing cue and one providing dechlorinated tap
213 water, as well as one no choice zone downstream from the choice zones (Fig. S1).

214 Two arenas were placed side by side on an elevated board in a tank (45 × 25 × 15 cm
215 [length × width × height]), thus we could run two trials at the same time. The
216 elevation facilitated the run off of wastewater. The tank was covered with black
217 plastic on all sides to avoid disturbance from the surrounding environment. The
218 experimental set-up was illuminated by overhead fluorescent tubes, and the
219 experiment was conducted at 20°C. Stimulus and control water was added to the
220 upstream part of the choice zones in separate tubing hoses from two containers using
221 a peristaltic pump (40 ml/min). Test runs with colour dye showed that a stable cue
222 gradient was established within 30 s. A ramp perforated with small holes, at the rear
223 of the no choice zone, counteracted back flow and mixing of the gradients, and the
224 holes also facilitated the outflow of waste stimulus water. We ran two separate trials

225 simultaneously, one in each arena. Hence, in each run we had one arena with fish cue
226 and control water, and one arena with dragonfly cue and control water. One choice
227 arena was always used for fish cue and the other one for dragonfly cue, but within
228 each arena the cue side was randomly determined every 4th trial as well as the
229 position of each choice arena within the main tank. This stratified, randomized design
230 was used to avoid bias for one side in the choice arena.

231 At the start of each trial, arenas were filled with dechlorinated tap water and the
232 pump was turned on to establish the gradients. After approximately 30 s we
233 introduced the isopod in the middle of the no choice section (A) (Fig. S1; position of
234 isopod release indicated by x). For 5 min we then continuously recorded 1) time in the
235 zone with predator cue, 2) time in the zone with control water, and 3) time spent in
236 the no choice zone. All trials were filmed using a centrally placed overhead video
237 camera (Panasonic NV-GS230). We carefully rinsed the experimental arenas with
238 dechlorinated tap water between each set of trials.

239 Finally, we used the video recordings to quantify how *Asellus* individuals
240 sampled the cue environment (cf. Dahl et al. 1998). This was done by counting the
241 number of times each isopod switched between the zones representing the cue and
242 control treatments (“number of transitions”) and to identify which zone the isopod
243 used when the trial ended (“final choice”). An animal was given one transition when
244 it moved from zone B1→A→B2 or from B2→A →B1 (see Fig. S1). In total we
245 recorded 141 individuals, of which 86 were wild-caught and 55 lab-raised (43 wild-
246 reed, 43 wild stonewort, 28 lab-reed, 27 lab-stonewort).

247

248 Statistical analysis

249

250 To be included in the analyses, an individual was required to have visited at least one
251 of the two zones representing the cue and control treatments. To assess whether any
252 groups differed in their propensity to do this, we performed a Generalized Linear
253 Model (GLZ) where the probability of making a choice was the dependent variable
254 (binomial variable: 0 = no choice; 1 = choice), and sex, habitat (reed or stonewort)
255 and origin (wild-caught and lab-raised) were independent factors, using a binomial
256 error structure with a logit link function.

257 Responses to predatory cues were analysed using General Linear Mixed
258 Models (GLMM, SAS Proc MIXED) with a normal error distribution. Time spent in
259 the cue and control treatment zones were the two dependent, associated variables, and
260 sex, habitat (reed or stonewort) and origin (wild-caught and lab-raised), cue type
261 (dragonfly or fish), side (cue or control) and a full set of interaction terms were used
262 as fixed factors. As the time spent in the cue and control zones was dependent within
263 trials, this was explicitly modelled by including individual (isopod) identity as a
264 random factor. The significance of the random effect was evaluated with a Likelihood
265 ratio test, and the Satterthwaite method was used to approximate denominator degrees
266 of freedom.

267 To test if the mean number of transitions between the control and cue treatment
268 differed among individuals, we used a General Linear Model (GLM) with number of
269 transitions being the dependent variable, sex and cue (dragonfly or fish) and an
270 interaction term between sex and cue type were included as fixed factors. Differences
271 in final choice were estimated with a Generalized Linear Model (GLZ) with the
272 probability of choosing the cue or control side as the final choice (binomial variable:
273 0 = cue; 1 = control) as the dependent variable, and sex, cue (dragonfly or fish) and
274 their interaction term as independent factors, using a binomial error structure with a

275 logit link function. The number of transitions and final choice were analysed in wild-
276 caught individuals, and only for those individuals that made a transition ($n = 49$). Too
277 few lab-raised individuals performed any transitions between the two treatments to be
278 included in the analysis. Mixed model analyses were performed in SAS 9.2 for
279 Windows (Littell et al. 2006), and the additional analyses in SPSS 15.0 for Windows.
280 Model assumptions for all analyses were confirmed using graphical methods.

281

282 **Results**

283 Lab-raised individuals were less likely than wild-caught individuals to visit at least
284 one of the two zones representing the cue and control treatments, (GLZ: $\chi^2 = 11.65$, df
285 $= 1$, $P = 0.001$). There was no effect of habitat ($\chi^2 = 0.05$, $df = 1$, $P = 0.83$) or sex (χ^2
286 $= 0.19$, $df = 1$, $P = 0.66$). As a visit to at least one of the zones was required to be
287 certain that an individual did make a choice, the individuals that did not visit neither
288 zone had to be excluded from the analyses (lab-raised $n = 15$, wild-caught $n = 4$). It
289 should be noted that the significant difference between lab-raised and wild-caught
290 individuals indicates that lab-raised individuals had a lower propensity to explore the
291 arena.

292 Overall, isopod individuals differed substantially in their use of the arena, as
293 seen by the highly significant random factor modelling individual identity (Likelihood
294 Ratio test, $\chi^2 = 64.4$, $df = 1$, $P < 0.0001$). Significant differences in total arena use
295 were also detected for other factors (sex, cue, habitat \times sex, habitat \times cue, habitat \times
296 origin \times cue: $F_{1,106} = 6.33 - 4.23$, $P = 0.013-0.042$). However, only significant
297 interactions involving the factor “side” demonstrate active differentiation between
298 predator cues and the control. Hence, only interactions that include the factor “side”
299 are discussed in further detail below.

300

301 Effects of origin, sex and cue type on treatment preference

302

303 The origin, i.e. if the isopods were wild-caught or raised in the laboratory, had
304 significant effects on the time spent in the cue versus control treatment (origin \times side:
305 $F_{1,106} = 5.81$, $P = 0.018$; Fig. 1). Wild-caught individuals spent less time in the cue
306 treatment zone than lab-raised ones (Table 1). The amount of time spent in the control
307 and in the cue treatment zone was similar for lab-raised individuals, whereas wild-
308 caught isopods spent twice as much time in the control as in the cue zone (Table 1).
309 Female and male isopods from both origins reacted in a similar way to predator cues
310 (origin \times sex \times side; $F_{1,106} = 1.87$, $P = 0.174$; Fig. 1). Time in the control and cue
311 treatment zones differed significantly between the sexes (sex \times side: $F_{1,106} = 3.97$, $P =$
312 0.049 ; Fig. 1). Males spent twice as much time in the control compared to the cue
313 zone, but for females there was no such difference (Table 1). There was also a
314 tendency that habitat and sex influenced the time in either treatment (habitat \times sex \times
315 side: $F_{1,106} = 3.71$, $P = 0.057$), where the time difference between cue and control was
316 largest for reed males (not shown). The type of cue significantly affected the amount
317 of time the isopods spent in the control versus cue treatment (cue \times side: $F_{1,106} = 4.22$,
318 $P = 0.043$; Fig. 1). Fish cue elicited the strongest avoidance response, and on average
319 isopods spent twice as much time in the control compared to the cue zone for this
320 treatment. For the dragonfly cue the time spent in either treatment was similar (Table
321 1). No other interaction with the factor 'side' was significant ($P > 0.1$, results not
322 shown).

323

324 Transitions and final choice in wild-caught individuals

325

326 There were significant effects of sex and cue type on the number of transitions
327 between the control and cue zones (sex \times cue: $F_{1,45} = 4.68$, $P = 0.036$), but no
328 significant effect of sex ($F_{1,45} = 3.01$, $P = 0.090$) or cue type ($F_{1,45} = 3.56$, $P = 0.066$).
329 Males increased the number of transitions between the control and cue zones when
330 exposed to fish cue (mean \pm SE: fish = 3.0 ± 0.65 , dragonfly = 1.6 ± 0.26). For
331 females, the average number of transitions was similar between fish and dragonfly
332 cues (mean \pm SE: fish = 1.6 ± 0.20 , dragonfly = 1.7 ± 0.18). Final choice significantly
333 differed between the sexes ($\chi^2 = 5.54$, $df = 1$, $P = 0.019$), but there was no effect of
334 cue ($\chi^2 = 0.24$, $df = 1$, $P = 0.63$) or sex \times cue ($\chi^2 = 0.18$, $df = 1$, $P = 0.67$). Overall, in
335 20 out of 23 observations (87%), males chose the control treatment as the final
336 choice, while the female final choice was almost equally distributed between the cue
337 and control treatment (14 out of 26 females chose the control (54%)).

338

339 **Discussion**

340

341 Waterborne predator cues can have profound effects on antipredator behaviour in
342 aquatic invertebrates, leading to reduced activity, increased refuge use and general
343 changes in habitat preferences (Holomuzki & Short 1988; Wudkevich et al. 1997;
344 Dahl et al. 1998; Åbjörnsson et al. 2004), as well as influencing mating behaviour and
345 foraging (Short & Holomuzki 1992; Mathis & Hoback 1997; Dunn et al. 2008). In the
346 present study, the ongoing differentiation into two distinct ecotypes experiencing
347 contrasting predator communities (Wagner & Hansson 1998; Eroukhmanoff &
348 Svensson 2009), allowed us to quantify prey responses to past as well as present
349 predator communities. In addition, we could address the balance between innate,

350 permanent responses to predator cues, and acquired induced responses to such cues.
351 Our results confirm that waterborne cues for predator recognition are important in
352 *Asellus* from both habitats. Responses were considerably stronger in wild-caught
353 individuals, indicating that prior experience of predators or diet-related predator cues
354 plays an important role in isopod predator recognition compared to more innate,
355 permanent antipredator responses (cf. Pettersson et al. 2000; Brown et al. 2013).
356 However, there was no indication that *Asellus* from the new, stonewort habitat were
357 less responsive to dragonfly larvae typical of their ancestral reed habitat, nor were
358 individuals from the reed habitat less responsive to the fish predator. Interestingly,
359 while antipredator behaviour based on waterborne cues thus appeared general across
360 habitats, there were significant, threat-sensitive differences between the sexes in their
361 responses towards such cues. Furthermore, there were also threat-sensitive differences
362 in response strength to the two standardised predator cues, with fish cues eliciting
363 stronger antipredator behaviour, a finding which is in line with the relative effect of
364 invertebrate and predators on *Asellus* densities in the wild (Wagner & Hansson 1998).

365 In general, predator naive individuals did not avoid areas where predator odours
366 were emitted. On the contrary, during the exposure to predatory cues, naive isopods
367 spent more time in the cue treatment zone compared to the control zone and did not
368 seem to recognise cues from larval dragonflies or fish as something potentially
369 dangerous (Fig. 1). A similar response was shown in predator naive fish (*Gobiusculus*
370 *flavescens*), which showed no avoidance behaviour when exposed to chemical stimuli
371 from predatory cod (Utne-Palm 2001). However, when gobies had been exposed to a
372 live cod on three consecutive occasions they exhibited avoidance behaviours to cod
373 odour alone, which shows that experience (learning) plays a major role in predator
374 recognition based on chemical cues (Utne-Palm 2001; reviewed in Kelley &

375 Magurran 2003). In our study, wild-caught individuals with prior experience of
376 predator exposure showed strong avoidance responses to areas containing predator
377 cues. Thus predator recognition by learning, via the association of visual cues with the
378 smell of a predator which had been fed isopod conspecifics, may apply for this system
379 as well. This learning response can then be further modulated by predation levels
380 experienced in the wild, with high-risk environments selecting for phenotypically
381 plastic, cautious responses in risky situations (Brown et al. 2013). Additionally,
382 studies on isopod behaviour in the presence of a dragonfly larva indicate a role for
383 learning and prior experience of the predator to induce predator avoidance
384 (Eroukhmanoff et al. unpubl. data).

385 Interestingly, we demonstrate threat-sensitive predator avoidance between the
386 sexes, where males to a larger extent avoided areas containing predator cues
387 compared to females. In wild-caught individuals males more actively sampled the
388 environment compared to females (increased number of transitions between the
389 control and cue treatment zones), particularly in the fish treatment. In addition, males
390 almost entirely chose the control treatment as the final choice. In the presence of fish,
391 higher activity levels were found in mature males compared to juveniles and females
392 in a stream-dwelling isopod (*Lirceus fontainalis*) (Holomuzki & Short 1990), and
393 recent work has shown sex-specific differences in activity in *A. aquaticus* (Harris et
394 al. 2011). A potential explanation is that males are more active in mate searching than
395 females, and sample the environment more frequently. Thereby, males may more
396 often encounter predator cues, and may therefore show stronger avoidance responses.
397 There was a tendency that males from the ancestral reed habitat showed stronger
398 predator avoidance. Between the two habitats in Lake Krankesjön there is a large
399 difference in population density, which is almost 20 times higher in the novel

400 stonewort habitat compared to the ancestral reed habitat (Karlsson et al. 2010b). Due
401 to the low population size in the reed, males have much lower chance to encounter
402 females, which may favour males that are more active and more responsive to
403 predator cue than the stonewort males.

404 Alternatively, size-related responses between the sexes may explain the
405 differences in predator avoidance. In *A. aquaticus*, as well as in other isopod species,
406 males guard a female by carrying her in a pre-copula (amplexus) until she moults into
407 mating state (Unwin 1920; Hargeby et al. 2004). Mate guarding may select for larger
408 male size and sexual size dimorphism is common (*Idotea baltica*: Jormalainen et al.
409 2000; *Asellus aquaticus*: inferred from Hargeby & Erlandsson 2006). In mayflies,
410 large individuals responded to trout odour by reducing their nocturnal drift, whereas
411 the night drift density of small nymphs significantly increased (McIntosh et al 1999).
412 The authors suggest that large individuals are more vulnerable to predation during the
413 night because they are more easily detected, and also that the trout preferentially
414 selects larger prey (McIntosh et al. 1999). It should be noted that the present study
415 showed a more pronounced sexual size dimorphism in wild-caught than in lab-raised
416 individuals, something which could potentially influence the relative strength of anti-
417 predator responses. However, males and females from the two origins did not differ in
418 their response to predator cues depending on their origin.

419 Behavioural responses to predator cues from fish and larval dragonflies
420 differed significantly, with chemical cues from fish eliciting the strongest avoidance
421 behaviour when the ratio of predator body mass to water volume was standardised.
422 There are several plausible explanations for this threat-sensitive pattern. One
423 possibility is that isopods are able to discriminate between different predator types,
424 and thereby responding in different ways. Chemical stimuli from fish tended to induce

425 a behavioural shift in the amphipod *Gammarus lacustris* from spending time in the
426 open water column to spend more time near the bottom, whereas cues from dragonfly
427 larvae did not induce those changes (Wudkevich et al. 1997). Williams & Moore
428 (1985) showed that amphipods exhibited antipredator responses to chemical cues
429 from several different fish species, whereas the response to different invertebrate
430 predators were more variable, and some invertebrate species did not induce any
431 avoidance responses in the amphipod. Thus, there is clearly some evidence that fish
432 and dragonfly predators are able to induce various responses. Threat-sensitive
433 responses can also be caused by different concentrations of chemical cues from perch
434 and larval dragonflies. Different concentration gradients have been used as an
435 indicator of predator presence, and stronger concentrations of, for example,
436 conspecific alarm cues or predator odours have elicited stronger antipredator
437 responses in prey (e.g. Ferrari et al. 2008; Brown et al. 2009). Concentration
438 differences can be caused by different gut retention times, and this can induce
439 variation in emission rates of chemical cues released in the predator diet. Other
440 possibilities are that predator odour is composed of different types of molecules
441 (Ferrari et al. 2007) or that the relative density of predators affects the cues (Ferrari et
442 al. 2006). Threat-sensitive responses in relation to cues from high and low-risk
443 predators were recently shown in guppies (Harris 2010). Predator experienced as well
444 as predator naive individuals exhibited the strongest antipredator behaviour in
445 response towards the most dangerous predator, which indicates that guppies are able
446 to discriminate between different predator types (Harris 2010).

447 To summarize, in this study we show that cues from past and present predators
448 induce strong predator avoidance in individuals with prior experience of predators
449 irrespective of habitat origin, but weak responses in lab-raised predator naive

450 individuals. Our result suggests that isopods learn to recognise predators, rather than
451 having innate antipredator responses. We also found threat-sensitive responses
452 between the sexes as well as towards different predator cues. Taken together, this
453 study indicates that both ecotypes evaluate and respond to waterborne predator cues
454 in a similar way, but that threat-sensitive considerations play a major role when
455 encountering cues from different predator species or when predator cues are viewed
456 in a sex-specific context.

457

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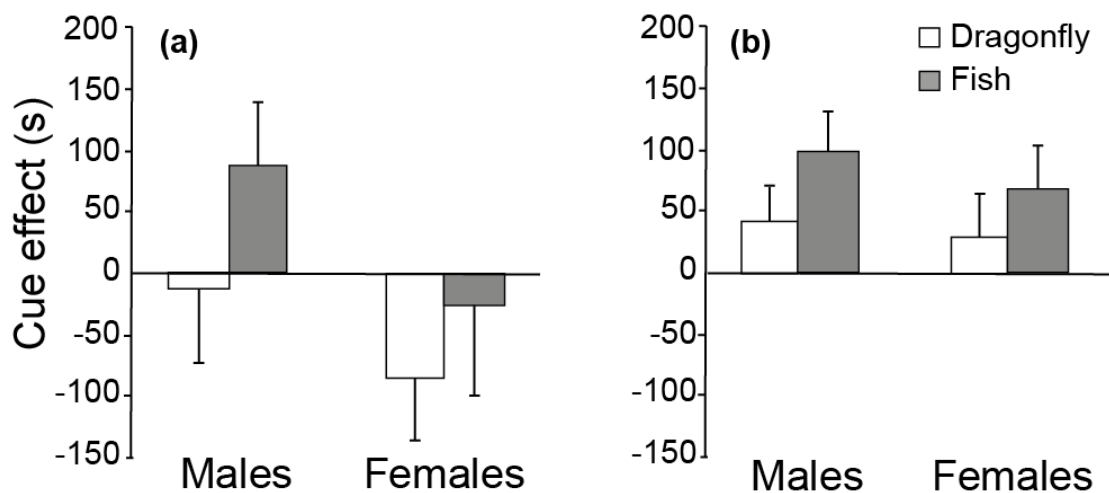
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628 **Table 1** Comparisons of mean time (s) \pm SE spent in the cue versus control treatment
 629 for lab-raised and wild-caught individuals, for males and females, and for all
 630 individuals in relation to cues from larval dragonflies and fish
 631

| | Cue side | Control side | N |
|---------------|-----------------|------------------|----|
| Origin | | | |
| Lab | 96.5 \pm 16.9 | 98.0 \pm 16.7 | 40 |
| Wild | 61.0 \pm 7.9 | 121.0 \pm 10.3 | 82 |
| Sex | | | |
| Males | 56.1 \pm 9.4 | 119.0 \pm 12.9 | 62 |
| Females | 89.5 \pm 12.3 | 108.0 \pm 12.2 | 60 |
| Cue | | | |
| Dragonfly | 80.0 \pm 11.3 | 91.0 \pm 12.3 | 57 |
| Fish | 65.9 \pm 10.8 | 133.0 \pm 12.8 | 65 |

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Fig. 1 Cue effect (mean \pm SE) of chemical stimuli from fish and dragonfly larvae in
 (a) lab-raised and (b) wild-caught male and female isopods. Cue effect is calculated as
 the difference (in seconds) between time spent in the control versus time in the
 predator cue treatment. Positive values indicate that isopods avoid the waterborne
 predator cues



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