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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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ABSTRACT

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

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

Introduction

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

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

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

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

Materials and methods

Experimental animals

Asellus aquaticus (Crustacea: Isopoda) is common in slow flowing waters across Europe (Verovnik et al. 2005). As other asellids, this isopod feeds on detritus and periphyton (Smock & Harlowe 1983; Arakelova 2001). *A. aquaticus* is itself a food source for fish, e.g. perch, *Perca fluviatilis* (Rask & Hiisivuori 1985). In several Swedish lakes, *A. aquaticus* has diverged into two distinct ecotypes, utilising different habitats (Hargeby et al. 2004; Hargeby et al. 2005). In at least two lakes, this differentiation has occurred in parallel with similar morphological and behavioural changes in the novel ecotype (Eroukhmanoff et al. 2009; Eroukhmanoff & Svensson 2009; Karlsson et al. 2010a). In both lakes, divergence has occurred rapidly during the last 20 years after major ecological shifts reviewed in Hargeby et al. 2007 following the emergence of new habitat that mainly consists of stonewort, *Chara tomentosa* (Hargeby et al. 1994; Hargeby et al. 2004). Isopods dispersed into the novel stonewort habitat located in the lake centre, from source populations in the reed stands (*Phragmites australis*) along the shoreline (Hargeby et al. 2004). The major selective agent behind the ecotype divergence is suggested to be predation (Hargeby et al. 2004; Eroukhmanoff & Svensson 2009) because predator regimes differ between the reed and the stonewort habitats (Wagner & Hansson 1998; Eroukhmanoff & Svensson 2009). The reed is mainly inhabited by invertebrate predators, such as dragonfly larvae, while the stonewort is mainly inhabited by fish predators (Eroukhmanoff & Svensson 2009), with small perch (< 15 cm) being the most common (Hargeby et al. 1994).

In June 2009, we collected isopods from both ecotypes (reed and stonewort) in Lake Krankesjön (55°42´N, 13°28´E), in southern Sweden. The reed habitat mainly consists of detritus while the stonewort habitat is a three-dimensional matrix of *Chara* in the lake centre. We sampled individuals from a depth of approximately 0-0.4 m in the reed and 0.5-1.0 m in the stonewort. In total, we collected 50 couples (25 males and 25 females in copula) from each habitat. The couples were carefully separated and we placed each individual in a single container. The isopods were fed decaying leaves, and kept in the laboratory where temperature and light regimes were controlled to mimic natural conditions (17°C, 12L:12D). Wild-caught isopods were allowed to adjust to laboratory conditions for at least 24 h prior to testing, and they were used in the behavioural trials within 4 days of collection in the field. Lab-raised individuals from both ecotypes were kept family-wise in containers in a common-garden setup with the same temperature, equal amount of food and a joint circulating water system. These individuals were the F1-generation from a previous breeding study and they were reared until sexual maturity before being used in the experiment. Animals were sexed by presence (female) or absence (male) of oostegites (Unwin 1920). In total 55 lab-raised individuals were used (28 reed; 27 stonewort).

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

Predator cues

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

height]) for two weeks prior to cue collection, and were fed isopods ad lib three times per week during the acclimatization. The size of the perch is consistent with the species' benthivorous stage in which it mainly feeds on macroinvertebrates (Brönmark and Pettersson 1994). After the acclimatization period, perch were rinsed with dechlorinated tap water and transferred to a stimulus collection aquaria (45 × 25 × 15 cm [length × width × height]) containing 8 l dechlorinated tap water), which was well aerated but contained no filtering device. Cue collection followed standard methodology (e.g. Pettersson et al. 2000; Ferrari et al. 2007), and three days later we removed the cue donor and the stimulus water was immediately frozen in plastic containers (0.8-1.2 l) at -80°C. Perch cues were randomized between trials and pooled in the analysis as cue strength differences between donors offered the same diet are negligible (cf. Brönmark and Pettersson 1994, Pettersson et al. 2000). Frozen stimulus water has been shown to retain its activity for at least two months (Pettersson et al. 2000). Dechlorinated tap water was frozen at the same time, in the same type of containers to be used as control. The cue collection procedure was repeated until there was enough stimulus water. The dragonfly larvae were placed individually in containers (10.5 × 8 × 7 cm [length × width × height]) with dechlorinated tap water, and were fed isopods for a few days during acclimatization. After the acclimatization, we fed each larva with six isopods, and if all isopods were consumed the next day the larva was moved to a circular plastic container (7 cm diameter, 4 cm deep) with 0.2 l dechlorinated tap water, a volume selected to match the ratio of cue donor mass (mean late instar *Aeshna* larval mass = 0.25 g, L.B. Pettersson, unpublished data) to water volume used in the fish cue collection (approximately 1.2 g per l). This matching implicitly assumes stimulus release to be proportional to body mass and is a commonly used compromise solution between alternative standardisation approaches (e.g. Pettersson et al. 2000). The larva stayed in the stimulus collection jar for three days before stimulus water was collected and frozen. We used each larva several times as a cue donor, and the stimulus water was mixed from all 16 larvae. Cue and control water were thawed to room temperature (20°C) the night before the experimental trial started.

Choice experiments

To examine the response to waterborne predator cues from fish or dragonfly larvae, we conducted experimental trials in a two-compartment choice arena (20 × 8.5 × 2.5 cm [length × width × height]) made of transparent PVC plastic (Fig. S1) (cf. Baker & Montgomery 2001; Hale et al. 2009; Wisenden & Dye 2009). The experimental arena consisted of two choice zones, one providing cue and one providing dechlorinated tap water, as well as one no choice zone downstream from the choice zones (Fig. S1). Two arenas were placed side by side on an elevated board in a tank (45 × 25 × 15 cm [length × width × height]), thus we could run two trials at the same time. The elevation facilitated the run off of wastewater. The tank was covered with black plastic on all sides to avoid disturbance from the surrounding environment. The experimental set-up was illuminated by overhead fluorescent tubes, and the experiment was conducted at 20°C. Stimulus and control water was added to the upstream part of the choice zones in separate tubing hoses from two containers using a peristaltic pump (40 ml/min). Test runs with colour dye showed that a stable cue gradient was established within 30 s. A ramp perforated with small holes, at the rear of the no choice zone, counteracted back flow and mixing of the gradients, and the holes also facilitated the outflow of waste stimulus water. We ran two separate trials simultaneously, one in each arena. Hence, in each run we had one arena with fish cue and control water, and one arena with dragonfly cue and control water. One choice arena was always used for fish cue and the other one for dragonfly cue, but within each arena the cue side was randomly determined every 4th trial as well as the position of each choice arena within the main tank. This stratified, randomized design was used to avoid bias for one side in the choice arena.

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

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

Statistical analysis

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

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

To test if the mean number of transitions between the control and cue treatment differed among individuals, we used a General Linear Model (GLM) with number of transitions being the dependent variable, sex and cue (dragonfly or fish) and an interaction term between sex and cue type were included as fixed factors. Differences in final choice were estimated with a Generalized Linear Model (GLZ) with the probability of choosing the cue or control side as the final choice (binomial variable: 0 = cue; 1 = control) as the dependent variable, and sex, cue (dragonfly or fish) and their interaction term as independent factors, using a binomial error structure with a logit link function. The number of transitions and final choice were analysed in wild-caught individuals, and only for those individuals that made a transition (n = 49). Too few lab-raised individuals performed any transitions between the two treatments to be included in the analysis. Mixed model analyses were performed in SAS 9.2 for Windows (Littell et al. 2006), and the additional analyses in SPSS 15.0 for Windows. Model assumptions for all analyses were confirmed using graphical methods.

Results

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

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

Effects of origin, sex and cue type on treatment preference

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

Transitions and final choice in wild-caught individuals

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

Discussion

Waterborne predator cues can have profound effects on antipredator behaviour in aquatic invertebrates, leading to reduced activity, increased refuge use and general changes in habitat preferences (Holomuzki & Short 1988; Wudkevich et al. 1997; Dahl et al. 1998; Åbjörnsson et al. 2004), as well as influencing mating behaviour and foraging (Short & Holomuzki 1992; Mathis & Hoback 1997; Dunn et al. 2008). In the present study, the ongoing differentiation into two distinct ecotypes experiencing contrasting predator communities (Wagner & Hansson 1998; Eroukhmanoff & Svensson 2009), allowed us to quantify prey responses to past as well as present predator communities. In addition, we could address the balance between innate, permanent responses to predator cues, and acquired induced responses to such cues. Our results confirm that waterborne cues for predator recognition are important in *Asellus* from both habitats. Responses were considerably stronger in wild-caught individuals, indicating that prior experience of predators or diet-related predator cues plays an important role in isopod predator recognition compared to more innate, permanent antipredator responses (cf. Pettersson et al. 2000; Brown et al. 2013). However, there was no indication that *Asellus* from the new, stonewort habitat were less responsive to dragonfly larvae typical of their ancestral reed habitat, nor were individuals from the reed habitat less responsive to the fish predator. Interestingly, while antipredator behaviour based on waterborne cues thus appeared general across habitats, there were significant, threat-sensitive differences between the sexes in their responses towards such cues. Furthermore, there were also threat-sensitive differences in response strength to the two standardised predator cues, with fish cues eliciting stronger antipredator behaviour, a finding which is in line with the relative effect of invertebrate and predators on *Asellus* densities in the wild (Wagner & Hansson 1998).

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

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

Interestingly, we demonstrate threat-sensitive predator avoidance between the sexes, where males to a larger extent avoided areas containing predator cues compared to females. In wild-caught individuals males more actively sampled the environment compared to females (increased number of transitions between the control and cue treatment zones), particularly in the fish treatment. In addition, males almost entirely chose the control treatment as the final choice. In the presence of fish, higher activity levels were found in mature males compared to juveniles and females in a stream-dwelling isopod (*Lirceus fontainalis*) (Holomuzki & Short 1990), and recent work has shown sex-specific differences in activity in *A. aquaticus* (Harris et al. 2011). A potential explanation is that males are more active in mate searching than females, and sample the environment more frequently. Thereby, males may more often encounter predator cues, and may therefore show stronger avoidance responses. There was a tendency that males from the ancestral reed habitat showed stronger predator avoidance. Between the two habitats in Lake Krankesjön there is a large difference in population density, which is almost 20 times higher in the novel stonewort habitat compared to the ancestral reed habitat (Karlsson et al. 2010b). Due to the low population size in the reed, males have much lower chance to encounter females, which may favour males that are more active and more responsive to predator cue than the stonewort males.

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

Behavioural responses to predator cues from fish and larval dragonflies differed significantly, with chemical cues from fish eliciting the strongest avoidance behaviour when the ratio of predator body mass to water volume was standardised. There are several plausible explanations for this threat-sensitive pattern. One possibility is that isopods are able to discriminate between different predator types, and thereby responding in different ways. Chemical stimuli from fish tended to induce a behavioural shift in the amphipod *Gammarus lacustris* from spending time in the open water column to spend more time near the bottom, whereas cues from dragonfly larvae did not induce those changes (Wudkevich et al. 1997). Williams & Moore (1985) showed that amphipods exhibited antipredator responses to chemical cues from several different fish species, whereas the response to different invertebrate predators were more variable, and some invertebrate species did not induce any avoidance responses in the amphipod. Thus, there is clearly some evidence that fish and dragonfly predators are able to induce various responses. Threat-sensitive responses can also be caused by different concentrations of chemical cues from perch and larval dragonflies. Different concentration gradients have been used as an indicator of predator presence, and stronger concentrations of, for example, conspecific alarm cues or predator odours have elicited stronger antipredator responses in prey (e.g. Ferrari et al. 2008; Brown et al. 2009). Concentration differences can be caused by different gut retention times, and this can induce variation in emission rates of chemical cues released in the predator diet. Other possibilities are that predator odour is composed of different types of molecules (Ferrari et al. 2007) or that the relative density of predators affects the cues (Ferrari et al. 2006). Threat-sensitive responses in relation to cues from high and low-risk predators were recently shown in guppies (Harris 2010). Predator experienced as well as predator naive individuals exhibited the strongest antipredator behaviour in response towards the most dangerous predator, which indicates that guppies are able to discriminate between different predator types (Harris 2010).

To summarize, in this study we show that cues from past and present predators induce strong predator avoidance in individuals with prior experience of predators irrespective of habitat origin, but weak responses in lab-raised predator naive individuals. Our result suggests that isopods learn to recognise predators, rather than having innate antipredator responses. We also found threat-sensitive responses between the sexes as well as towards different predator cues. Taken together, this study indicates that both ecotypes evaluate and respond to waterborne predator cues in a similar way, but that threat-sensitive considerations play a major role when encountering cues from different predator species or when predator cues are viewed in a sex-specific context.

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

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

Fig. 1 Cue effect (mean + 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