Zooplankton responses to multiple threats within and across generations

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Behavioural responses to co-occurring threats of predation and ultraviolet radiation in *Daphnia*

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Abstract

1. Organisms in the wild are faced with multiple threats and a common response is a change in behaviour. To disentangle responses to several threats, we exposed two differently sized species of the freshwater invertebrate *Daphnia* to solar ultraviolet radiation (UVR) and predation from either moving pelagic or benthic ambush predators.
2. Using an advanced nanotechnology-based method, we tracked the three-dimensional movements of those mm-sized animals at the individual level. Each behavioural trial was performed both under conditions resembling night (no UVR) and day (UVR) and we examined patterns of the depth distribution and swimming speed by *Daphnia* across three treatments: no predator (control); bottom-dwelling damselfly (*Calopteryx* sp.); and fish (stickleback, *Pungitius pungitius*) predators. We also quantified the actual predation rate by the two predators on the two *Daphnia* species, *Daphnia manga* and *Daphnia pulex*.
3. We show that individual *Daphnia* are able to identify predators with different feeding habitats, rank multiple and simultaneously occurring risks and respond in accordance with the actual threat; complex responses that are generally associated with larger animals.
4. In a broader context, our results highlight and quantify how a cocktail of everyday threats is perceived and handled by invertebrates, which advances our understanding of species distribution in space and time, and thereby of population dynamics and ecosystem function in natural ecosystems.

KEYWORDS
Daphnia magna, *Daphnia pulex*, multiple threats, risk assessment, zooplankton

1 | INTRODUCTION

Animal migration is an influential phenomenon universally recognised for at least 2 millennia (e.g. by Aristotle; Nussbaum, 1978) and is common across taxa and scales as a response to biotic and abiotic threats (Hansson & Åkesson, 2014). To maximise the likelihood of survival, an individual has to perceive present threat levels and respond instantly and appropriately. Although all organisms

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on Earth are faced with multiple, simultaneously occurring threats, varying in strength both spatially and temporally, most research on migratory behaviour has focused on a single threat affecting a single taxon. Moreover, different taxa and individuals respond differently in "the landscape of fear" (Ripple & Beschta, 2004), which we define as the combined set of stressors in a natural environment. This suggests that the distribution of animals in the wild may, to a considerable extent, be dependent on individual decisions to move or not to move.

Although seasonal migrations by many bird species or by large ungulates on the African savanna are the most well-known migrations, with respect to biomass the largest migrations are likely to be the diel vertical migrations (DVM) of mm-sized crustacean zooplankton in lakes and oceans (Hays, 2003). Many environmental factors, including light, temperature, food availability, predators, and solar radiation, have been shown to drive the presence and amplitude of DVM (Williamson, Fischer, Bollens, Overholt, & Breckenridge, 2011). Originally, predator avoidance was assigned as the ultimate reason for this behaviour, where organisms migrate from the well-lit surface waters during day into the deeper and darker depths in order to reduce the risk of encountering visually hunting predators, such as fishes (Hays, 2003; Stich & Lampert, 1981). In addition to predation, ultraviolet radiation (UVR) has also been proposed as a proximate cue driving zooplankton movements and migration (Hansson & Hylander, 2009a; Williamson et al., 2011). As UVR attenuates with depth (Scully & Lean, 1994), downward migration also provides a refuge from this dangerous radiation. To reduce predation risk and exposure to damaging radiation, migrating zooplankton normally have to leave warm and food-rich surface waters for cooler depths, which may entail a cost in reduced growth and reproduction rates (Loose & Dawidowicz, 1994). However, the magnitude of such behavioural responses may vary among taxa. Those with protective pigmentation, such as *Daphnia* with melanin, may migrate less distance under UVR exposure than un-pigmented individuals (Hansson & Hylander, 2009a). Moreover, individuals may also exhibit variable behaviours, such as varying their speed when exposed to a predation threat (Schoeppner & Relyea, 2009). Individual behavioural differences allow for novel traits to become established and help species to adapt to new and more challenging conditions (Dall, Houston, & McNamara, 2004; Sih, Bell, Johnson, & Ziemba, 2004). However, in contrast to bird and ungulate migrations, our knowledge of movements and migrations by individual invertebrates within and among species in response to multiple threats is still elusive. Therefore, our study tracked the behavioural threat responses of individually marked aquatic zooplankton.

The freshwater zooplankter *Daphnia* responds behaviourally both to UVR and predation (Dodson, 1988). With respect to predation threat, free swimming, pelagic predators (e.g. fish) have been shown to induce downward migration in zooplankton. Whereas bottom-living, benthic, invertebrate predators may be hypothesised to induce reversed migration, where prey instead favour surface waters during daytime and migrate downwards during night (Ohman, Frost, & Cohen, 1983). At the population level, trade-offs between behavioural responses to different threats have previously been observed in *Daphnia* (Boeing, Leech, Williamson, Cooke, & Torres, 2004; Decaestecker, De Meester, & Ebert, 2002; Van de Meutter, Stoks, & De Meester, 2004). However, here we further advance the understanding of how individual animals respond when exposed to simultaneously occurring threats of UVR and predation by taking advantage of a unique experimental set-up using nanoparticle (quantum dot [Q-dot]) tracking of individuals.

To disentangle and quantify the different responses imposed by predators with different feeding habitats, we exposed two differently sized prey taxa, *Daphnia magna* and *Daphnia pulex*, to UVR in combination with either a pelagic fish or a benthic invertebrate predator, thereby exposing the prey to threats requiring different responses. In addition, we quantified the actual predation rate by the two predators and asked the question: to what extent can invertebrate prey organisms rank everyday threats and make a risk assessment that corresponds to the actual risk? Specifically, we hypothesised that the zooplankters are able to distinguish between the feeding habitats of different predators and adjust their position in the water column accordingly. In which case, the depth distribution will differ depending on which predator is present. Each behavioural trial was recorded in both the presence and absence of UVR to investigate possible day/night modifications in the behavioural strategy. Since solar UVR is a main driver of the vertical migration in *Daphnia* (Hansson & Hylander, 2009a; Rhode, Pawlowski, & Tollrian, 2001; Storz & Paul, 1998), we hypothesised that the depth distribution of the animals should differ between presence and absence of UVR (i.e. between day and night conditions). Finally, in accordance with the size efficiency hypothesis (Brooks & Dodson, 1965) where size-dependent predation can eliminate larger-bodied cladocerans and lead to a zooplankton community dominated by smaller species, we hypothesised that the smaller-sized *D. pulex* would show less response to predators than the larger, more vulnerable, *D. magna*. To test this hypothesis, we exposed the prey to either a bottom-dwelling, ambush predator (damselfly larvae) or a rapidly moving, open water predator (fish), to measure actual predation rates. The study was performed under both presence and absence of UVR, resembling day and night conditions, respectively, allowing assessment of prey behaviour both when they were visible to the predator and when protected by darkness.

2 | METHODS

Daphnia magna, originating from Lake Bysjön (55.67 N, 13.54 E), and *D. pulex* originating from the nearby Lake Dalby quarry (55.67 N, 13.35 E) were used in the experiment. The mean size of *D. magna* was >50% greater than *D. pulex* (2.48 ± 0.18 and 1.55 ± 0.18 mm [mean ± *SD*], respectively). The quarry has a sparse population of brown trout (*Salmo trutta*) and no zooplankton were found in fish guts in a previous study (Ekvall, Hylander, Walles, Yang, & Hansson, 2015), suggesting low predation pressure by fish. The fish community in Lake Bysjön is dominated by crucian carp (*Carassius carassius*) and rainbow trout (*Salmo gairdneri*) (Andersson, Berggren,

Cronberg, & Gelin, 1978). After isolation, each *Daphnia* population was kept in a separate 30-L aquarium with a light:dark cycle of 14:10 hr and fed *ad libitum* three times per week with an algal suspension dominated by *Scenedesmus* sp. Individuals of each species were randomly collected from the source population to perform the behavioural assay.

In many lakes, *Daphnia* are the preferred food item for planktivorous fish and invertebrate predators. Previous studies have shown that all kinds of fishes, including benthic, non-planktivorous and planktivorous species could evoke a behavioural reaction in *Daphnia* (Loose, Von Elert, & Dawidowicz, 1993; Von Elert & Loose, 1996). To quantify the potential trade-off in *Daphnia* threat response, we exposed them to UVR in the absence and presence of either a benthic, ambush invertebrate predator or a fish predator. As benthic predator we used the final instar of damselfly larvae (*Calopteryx* sp., mean size, 30 mm), which were kept in a 20-L aquarium and fed a mixture of *D. magna* and *D. pulex*. In the fish predator treatment, we used adult nine spine sticklebacks (*Pungitius pungitius*, mean size, 50 mm) which were kept in a 20-L aquarium and fed a mixture of *D. magna* and *D. pulex*. The damselflies and the fish were caught in waters close to where the *Daphnia* species originate, and all four taxa naturally co-occur.

Although individual tracking of larger animals, such as birds, mammals, and fish, allows the use of satellites (Hansson & Åkesson, 2014) or PIT tags (Brönmark, Skov, Brodersen, Nilsson, & Hansson, 2008), such devices are too heavy for tracking of mm-sized animals. Therefore, we used an advanced and unique tracking method based on nanotechnology where we labelled individual *D. magna* and *D. pulex* with fluorescent nanoparticles, so-called Q-dots (Ekvall et al., 2013). The Q-dot marking makes it possible to track the movements of even tiny zooplankton through the fluorescence emitted from the nanoparticles upon excitation. The Q-dots do not affect the behaviour, reproduction, or survival of the animals (Ekvall et al., 2013; Lard, Bäckman, Yakovleva, Danielsson, & Hansson, 2010). To separate the two species, we used nanoparticles fluorescent at 585 nm (yellow) and 655 nm (red) wavelength. To ensure that colour had no influence on the results, labelling colour was switched between trials and species using a randomised block design. Experiments and three-dimensional (3D) tracking of the animals were performed in an aquarium with the dimensions 0.2 × 0.2 × 0.85 m (L × W × H), filled with 30 L of water, resulting in a water column 0.75 m deep (Figures S1 and S2). Although the experimental arena was >300 body lengths of the zooplankters, *Daphnia* often perform DVMs of >10 m, suggesting that the experimental conditions may not capture all aspects of DVM under natural conditions. However, to our knowledge, the experimental set-up constitutes the largest volume, and thereby the most natural conditions, ever used to track individual behaviour of zooplankton in 3D. The nanoparticles were excited by integrating eight light-emitting diode (LED) arrays with peak emission at 465 nm (VANQ Technology; Shenzhen, China) and captured by four hardware-synchronised digital cameras (Pike F-210C, Allied Vision Technologies GmbH) arranged as two stereo-pairs along one side of the aquarium (Figure S1). An excitation wavelength of 465 nm was chosen to minimise any effects of the excitation light on the *Daphnia*, and although *Daphnia* can probably see it, this wavelength is outside the four distinctive wavelength classes to which the *Daphnia* compound eye is most sensitive (i.e. 348 ± 4 nm, 434 ± 5 nm, 525 ± 4 nm and 608 ± 8nm), (see Lard et al. (2010) and references therein). Moreover, the animals showed no directional movements in response to the excitation light (465 nm), which was indeed the case when exposed to UVR. Hence, the presence of UVR was here defined as day conditions, whereas time periods with only excitation light were defined as night conditions. Video recordings were tracked to obtain the 3D positions of the organisms at 6 frames/s using the methods described in Palmér, Bianco, Ekvall, Hansson, and Åström (2016), resulting in a 3D track for each individual (for examples of a 3D track see Figure S2). The swimming speed was then calculated using the Euclidean distance travelled between the previous and the successive 3D coordinates, divided by the time lag between two consecutive positions.

We pre-treated the water by leaving either a damselfly larva or a stickleback in the arena–aquarium for 24 hr prior to the start of the experiment to ensure the presence of predator cues in the water. Predators were removed from the arena–aquarium and transferred to a separate aquarium (1.5 L) before the introduction of daphniids. We circulated the water between the predator aquarium and the arena using a peristaltic pump at a flow rate of 5 ml/min (ISMATEC®, Reglo ICC) throughout the trials. After labelling, two *Daphnia*, one individual of each species, were introduced to the arena–aquarium using a 3-ml plastic Pasteur pipette. Daphniids were allowed to acclimatise in the aquarium with the excitation light turned on during 15 min prior to the start of video recording. Each experiment lasted for a total of 6 min and was built up by two phases each lasting 3 min. During the first phase only the top mounted excitation light was switched on, followed by 3 min where a UVR threat was added by switching on an UVR LED array mounted at the top of the aquarium. The UVR LED array provided radiation in the wavelength range of 315–400 nm with a peak emission at 380 nm (VANQ Technology). Therefore, the only radiation sources present in the completely darkened experimental laboratory were the excitation light and the UVA. Therefore, we used the absence/presence of UVR to simulate a DVM situation, that is, a period resembling night conditions (minutes 1–3) when UVR was absent and one resembling day conditions when UVR was present (minutes 4–6). The UVR intensity at the water surface was adjusted to 2.5 W/m^2, corresponding to the solar UVA intensity at noon on a partially cloud covered day (Hansson, 2004). This is within the range of UVR that zooplankton experience in their natural environment, and similar levels of UVR have been used in previous studies (Leech, Padeletti, & Williamson, 2005). For more detailed descriptions of the marking and tracking techniques, see Ekvall et al. (2013) and Palmér et al. (2016). The UVA intensity at the bottom of the aquarium (0.75 m) was about 15% of the surface value, constituting a considerable UVA gradient, as well as a depth refuge with very low UVA intensity at the bottom of the arena (Figure S3). As a control, we used the same design as

described above but without predator cues. Each treatment was replicated 20 times with new, inexperienced *Daphnia* individuals.

To assess whether size-selective predation occurs on the two *Daphnia* species, one fish (stickleback, *P. pungitius*) was placed in each of four 10-L aquaria. After 1 hr of acclimatisation, 10 *D. magna* and 10 *D. pulex* were added to each aquarium and the fish were allowed to feed selectively during 10 min. The predation rate by damselfly larvae (*Calopteryx* sp.) was assessed in an identical set-up ($n = 4$), although the predator–prey interaction was allowed to proceed for 24 hr. At the end of each run the predator was removed and the prey filtered out (100 µm net) and counted. The size of the stickleback and damselfly used in the predation experiments was the same as those used to obtain kairomones during the behavioural trials: 50 and 30 mm for sticklebacks and damselflies, respectively. The fish predation experiments were conducted during daylight conditions with light originating from warm white fluorescent tubes. The same light set-up was used for the damselfly predation experiments with the exception that we used a light:dark cycle of 14:10 hr to mimic both night and day during the 24 hr of the experiment. Predation rate (i.e. the risk level for the prey) was calculated as number of prey eaten per hour in order to compare the size-selectivity between the two predators given the difference in the feeding time provided.

2.1 | Data analysis

The individual mean values of position in the water column (*refuge depth*) and swimming speed were summarised for each UVR phase. Differences in mean depth and speed of the animals when exposed to bottom-dwelling (damselfly), pelagic (fish), or to no predators (control) were compared at day and night conditions, respectively, using one-way ANOVA with multiple comparisons using Tukey's test. All data were log-transformed before analysis in order to meet requirements of the test. One track of a *D. magna* individual was discarded due to technical failure during tracking. The strength of the diel vertical migration (DVM strength) was estimated as a ratio of the mean position of the animals during day and night, and tests were performed using the same procedure as described above. Wilcoxon signed-rank test was used to compare the speed of the same animal during day and night, since these data are not independent of each other. We also analysed the coefficient of variation (CV; the ratio between the standard deviation and the mean value) of depth and speed during night and day for each treatment. CV was used to estimate how similar the response of individual animals was to predator and UVR threats. Student *t*-test was used to compare the differences in predation rate by fish and damselfly.

3 | RESULTS

Individual prey may adjust both depth distribution and speed in response to threats, and *D. magna* and *D. pulex* showed considerable differences in both behavioural dimensions in response to UVR (day versus night conditions) and, for *D. magna*, also in response to different predator feeding habitats. Hence, a graphical overview combining speed and depth dimensions when prey were exposed to different UVR (day and night conditions, respectively) and different predators (cruising fish and ambush bottom-feeding damselfly, respectively), shows that variation in both speed and depth was larger for *D. magna* than for the smaller *D. pulex* (Figure 1). Moreover, while *D. pulex* showed little response to any predator, *D. magna* responded differently to different predator regimes both during day and night conditions (Figure 1).

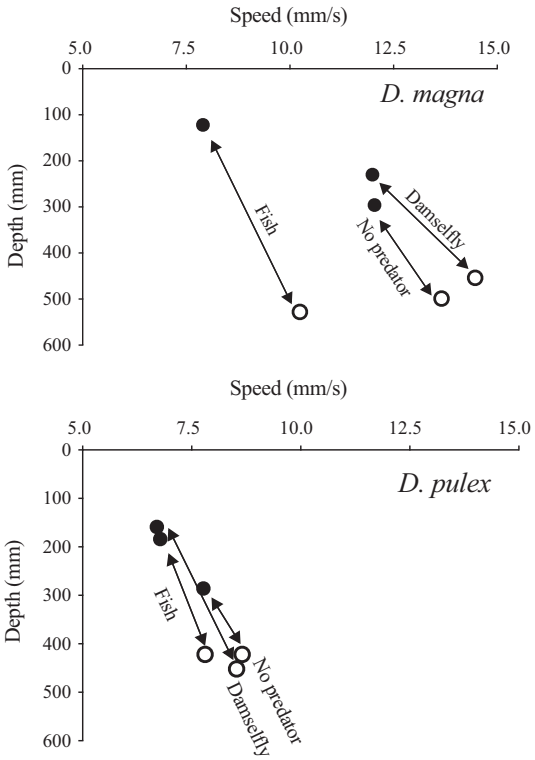

FIGURE 1 Combined response in position and speed. A two-dimensional overview of the behavioural response variables depth (mean; mm) and speed (mean; mm/s) of *Daphnia magna* (upper panel) and *Daphnia pulex* (lower panel) during night (black symbols) and day (open symbols). Arrows connect mean values in depth and speed responses for day (with UVR) and night (no UVR) conditions when exposed to: no predator; bottom-dwelling damselfly (*Calopteryx* sp.); and fish (stickleback, *Pungitius pungitius*) predators. Note: for clarity variance measures are omitted

Irrespective of predator regime, differences in depth distribution between night and day conditions were always significant and both species dove downwards during day conditions (Figure 2; *D. magna*: $F_{1,112} = 21.170$; $p < 0.001$; *D. pulex*: $F_{1,113} = 11.440$; $p < 0.001$). Specifically, *D. magna* swam downwards to a mean refuge depth of 493 ± 17 mm, whereas the mean refuge depth (432 ± 21 mm) for *D. pulex* was significantly shallower ($t_{116} = 2.282$; $p < 0.024$). The mean depth during night conditions (i.e. without UVR) ranged between 100 and 300 mm (Figure 2 and Figure S4). *D. magna* stayed closer to the surface during night when exposed to fish than to

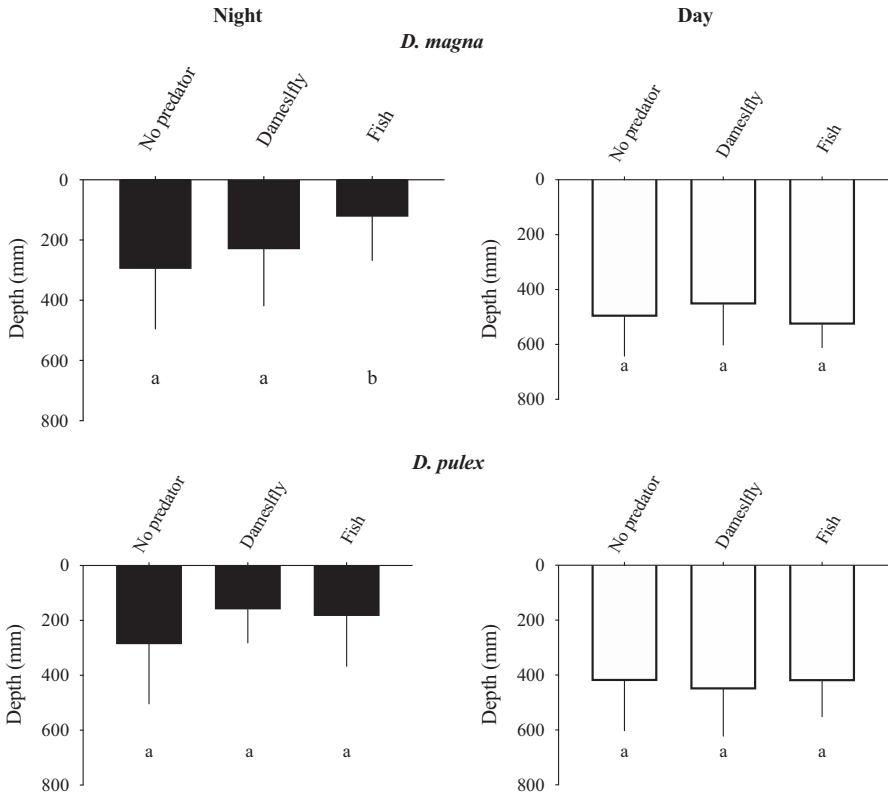

FIGURE 2 Depth responses. Mean depth (± 1SD) distribution (mm) of *Daphnia magna* and *Daphnia pulex* during night (left panel, black bars) and day conditions (right panel, open bars). Treatments (n = 20) are: no predator (control), bottom-dwelling damselfly (*Calopteryx* sp.) and fish (stickleback, *Pungitius pungitius*) predators. Different letters below bars indicate significant differences. *Daphnia magna* at night: $F_{2,56}$ = 6.487; p < 0.003; multiple comparisons: no predator–fish p < 0.004; damselfly–fish p < 0.024

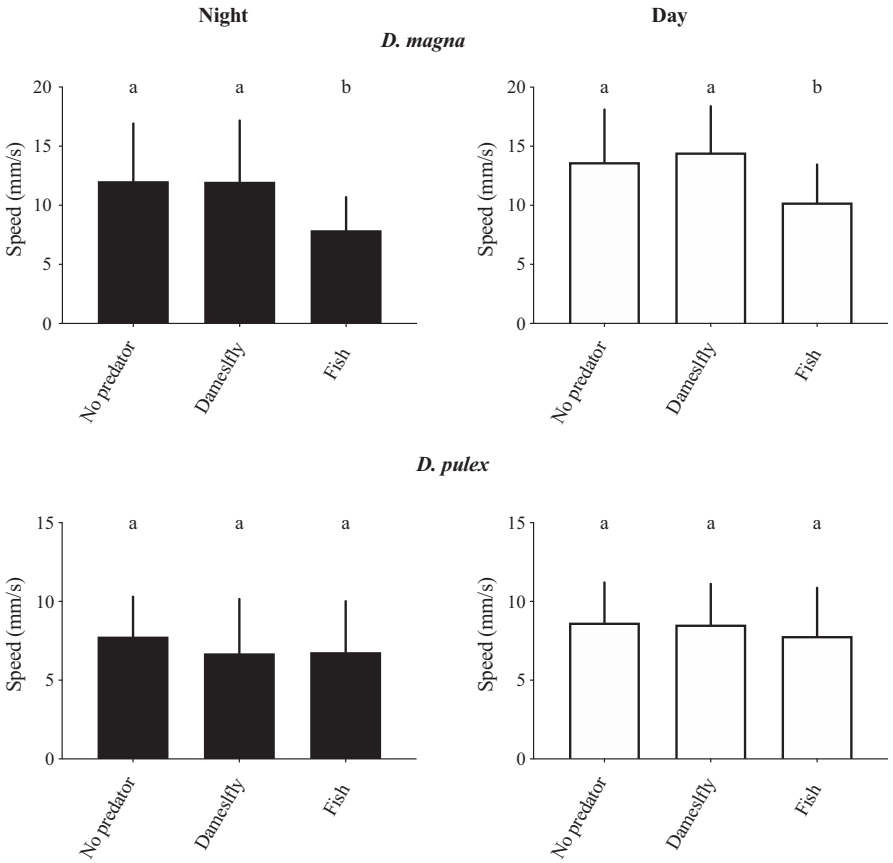

FIGURE 3 Speed responses. Mean speed (± 1SD; mm/s) of *Daphnia magna* and *Daphnia pulex* during night (left panel, black bars) and day conditions (right panel, open bars). Treatments are as follows: no predator (control), bottom-dwelling damselfly (*Calopteryx* sp.) and fish (stickleback, *Pungitius pungitius*) predators. Different letters above bars indicate significant differences. *D. magna* at night: $F_{2,57}$ = 4.950; p < 0.011; multiple comparisons: no predator–fish p < 0.020, damselfly–fish p < 0.026; day: $F_{2,57}$ = 6.664; p < 0.003; multiple comparisons: no predator–fish p < 0.020; damselfly–fish p < 0.004

damselfly or the no predator treatment (Figure 2; $F_{2,56}$ = 6.487; p < 0.003), whereas *D. pulex* showed no response to predators (e.g. $F_{2,57}$ = 2.386; p < 0.067).

The strength of the vertical migration (DVM; i.e. the mean ratio between the depth during day and night conditions), was >five times stronger for *D. magna* exposed to fish than exposed

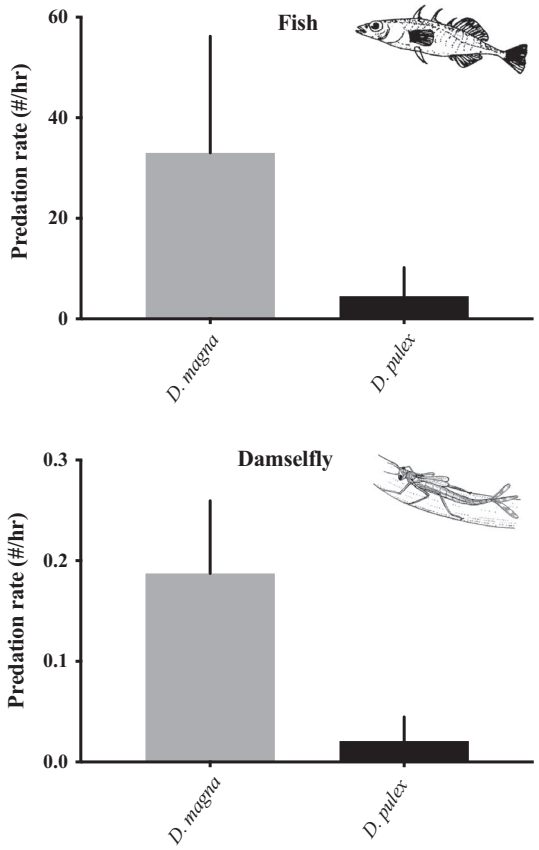

FIGURE 4 Predation rate by different predators. Mean (±1SD) predation rate on *Daphnia magna* and *Daphnia pulex* (numbers eaten/h) by each fish individual (upper panel; stickleback; *Pungitius pungitius*) and each damselfly individual (lower panel; *Calopteryx* sp.). Note different scales on the y-axes

to damselfly or to a predator-free environment (Figure S5; $F_{2,57}$ = 8.586; p < 0.001). In contrast, *D. pulex* showed no differences in migratory strength in response to predation cues (Figure S5). At both night and day conditions, *D. magna* swam slower when exposed to fish than when exposed to damselfly cues or when no predator was present (Figure 3; $F_{2,57}$ = 4.950; p < 0.011 and $F_{2,57}$ = 6.664; p < 0.003, respectively). In contrast, *D. pulex* showed no differences in speed induced by predators for either day or night (Figure 3). The median speed was >30% higher during day than during night conditions for both *D. magna* (12.76 and 9.41 mm/s; p < 0.001, Wilcoxon signed-rank test) and for *D. pulex* (8.23 and 6.29 mm/s, respectively; p < 0.001, Wilcoxon signed-rank test). Specifically, *Daphnia* individuals increased their speed dramatically when UVR was switched on with a peak during minute 4 (Figure S4), but the speed decreased in the following 2 min when they arrived at a relatively safe depth (Figure S4).

Both *D. magna* and *D. pulex* showed larger variation among individuals (i.e. higher CV) in depth distribution during night than during day (Figure S6, i.e. individual animals were more spread out in depth during night). Both species showed the highest CV during night when exposed to fish (Figure S6). However, during day, *D. magna* displayed a very low variance in depth distribution, especially in the presence of fish cues (Figure S6). In contrast to depth distribution, the CVs for speed were more equal with respect to both UVR and different predators (Figure S6).

The actual predation rate imposed by fish was >100 times stronger than that imposed by damselfly on both *D. magna* and *D. pulex*. Mean predation rate per hour was 33.0 *D. magna* and 4.5 *D. pulex* individuals eaten by each fish (t_6 = 2.381; p < 0.027) and 0.19 and 0.021 eaten by each damselfly (t_6 = 4.380; p < 0.003), respectively (Figure 4). Moreover, *D. magna* was almost 10 times more vulnerable to both fish and damselfly predation than the smaller *D. pulex* (Figure 4). Hence, fish imposed a far stronger predation risk than damselfly for both species and the large *D. magna* was the most vulnerable to both fish and damselfly predation.

4 | DISCUSSION

In aquatic ecosystems, zooplankton prey are unlikely to be distributed randomly, but instead they constantly navigate in a *waterscape of fear* from multiple threats, such as predators and damaging UVR radiation. For many species, the primary response to a threat is to migrate or move away from it. For example, *Daphnia* always show strong negative phototactic behaviour when exposed to solar UVR even at very low intensities (Storz & Paul, 1998). Previous studies have also shown that *Daphnia* are able to perceive predators (Dodson, 1988; Weiss, Kruppert, Laforsch, & Tollrian, 2012) and respond behaviourally (Hansson & Hylander, 2009b; Hays, 2003). Here we showed that both *D. magna* and *D. pulex* respond strongly to UVR, whereas only the large *D. magna* adjusted its behaviour in accordance with the level of predation risk. Moreover, the smaller *D. pulex* swam slower than the larger *D. magna* and did not adjust their speed to the presence of predators. In contrast, *D. magna* responded strongly to fish cues and may therefore be expected to appear in deeper waters during day than *D. pulex*. This notion is strengthened by studies in both natural and semi-natural systems where smaller individuals tend to appear closer to the surface than larger individuals (Ekvall et al., 2015; Hansson & Hylander, 2009b). Escaping from well-lit surface waters when exposed to fish predation and UVR may be more adaptive for the larger prey, *D. magna*, than for the smaller *D. pulex*, since fish predation is strongly size selective (Brooks & Dodson, 1965).

In accordance with previous studies (Ekvall et al., 2015; Hansson & Hylander, 2009b), a downward migration was found for both *Daphnia* species during UVR exposure. Although the level of predator cues is difficult to adjust to levels found in natural systems, fish densities that stimulate zooplankton DVM have been shown to range between 1 and 200 fish/m^3 with a median of 16 fish/m^3 (Williamson et al., 2011). Hence, our fish density of about 33 fish/m^3 was well within the suggested range and therefore probably sufficient to elicit a DVM response. Therefore, we expected an additional effect due to predator presence, with a further deeper distribution when exposed to both UVR and fish (Rose et al., 2012) and a relatively shallower distribution when a benthic predator was present

(Boeing et al., 2004). However, the two *Daphnia* species avoided the surface waters in a similar way, irrespective of predator feeding habitats. Hence, our results are consistent with previous studies demonstrating that UVR is the major force driving the vertical migration of *Daphnia* in clearwater systems (Hansson & Hylander, 2009b; Williamson et al., 2011).

Although our experiment was performed on a vertical scale of >300 body lengths of the animals, natural DVM behaviour extends over larger scales and our results may therefore be more applicable for ranking the strength of stressors than predicting natural habitat use. Moreover, our experimental set-up is providing conditions resembling night, with only low intensity blue light to which the animals are not responding with any directional movements, and day with UVR radiation, which is the part of the solar spectrum that the animals experience as a threat. Hence, our experimental design is based on presence (day) and absence of UVR to mirror the natural presence and absence of a major threat to the animals investigated. Although experiments rarely, or never, can copy natural conditions, our design provides a rare opportunity to test the mechanistic responses of both UVR and predation threats. However, it should be noted that, as for all experimental studies, conclusions may not be directly transferred to natural environments, although we argue that experimental studies are crucial for understanding behavioural responses and for identifying mechanisms.

Although *D. magna* and *D. pulex* displayed a significant behavioural response to UVR, the response intensity varied between the two species. When exposed to the UVR threat, each individual was free to dive to 750 mm (the depth of the arena), but they stayed at 400–500 mm, suggesting that this was the depth in our study where UVR was no longer experienced as dangerous. This shows that individual animals are able to sense and adjust their position in accordance with the prevailing UVR threat. Moreover, the mean of this refuge depth was about 15% deeper for *D. magna* than for *D. pulex*, suggesting different sensitivity to UVR (Hansson & Hylander, 2009b) and also that the two species are likely to be separated in space in natural ecosystems.

In addition to differences in depth distribution, both *D. magna* and *D. pulex* showed more variable (higher CV) depth distribution at night when exposed to fish cues, suggesting that some individuals respond to the threat, whereas others identify the night conditions as a predation refuge and therefore use the whole water column. Such individual, or clone, variation in threat response has been noted within several species of *Daphnia* (Langer et al., 2019) and is the likely reason behind the considerable variance detected here. However, it is notable that when exposed to fish during day conditions, both species showed very low variance (CV) in depth, suggesting that when dwelling in a dangerous environment with multiple threats of fish predation and UVR, the majority of the individuals adjust their behaviour accordingly, thereby reducing the behavioural variance in the population. Hence, when exposed to a strong threat, such as fish in well-lit conditions, individual decisions converged, whereas individual decisions were more variable when the threat was weaker (i.e. in darkness).

According to predator–prey theory, a prey organism will have an increased probability of encountering its predator once it, or its predator, increases its speed (Gerritsen & Strickler, 1977). Moreover, when fish were presented to *Daphnia* clones of similar size, but with different swimming speed, the predator selected clones with higher swimming speed (O'Keefe, Brewer, & Dodson, 1998). Accordingly, the large prey species in our study (*D. magna*) responded by reduced speed in the presence of fish predation. As a consequence of both reduced speed and a more restricted water volume explored (lower mean depth distribution), the presence of a fish predator not only imposes a mortal threat, but probably also reduces feeding opportunities, and thereby fitness. Therefore, during conditions resembling night when the pressure from visually hunting predators diminishes, ascending to the surface waters allows *Daphnia* to minimise the costs of residing in a cold, food-depleted deep-water refuge. In addition, we also saw changes in swimming speed, as both *Daphnia* species increased their speed dramatically at the transition from night to day conditions. Generally, the first response to altered conditions is a change in behaviour, that is, to move away from the threat. This was also the case in our study where *Daphnia* individuals swam at higher speed during their escape to deeper waters, suggesting that fast downward swimming allows daphniids to rapidly escape the threat from the damaging UVR.

Previous studies have shown that the original habitat from which the *Daphnia* species originates may influence their anti-predator defence (Dodson, 1988), suggesting that *Daphnia* may respond more strongly to the presence of fish kairomones if they have co-occurred with fish in their natural environment. In our study, *D. pulex* was derived from a habitat with low or absent fish predation and so this may explain why we observed no behavioural response to fish cues in this species. The actual predation rate on *D. pulex* was 10 times lower than on *D. magna* and, accordingly, *D. pulex* did not respond to predation cues. Hence, the actual predation rates on differently sized prey taxa corresponded with the individual ability to rank threats and respond appropriately in a situation where multiple threats have to be handled simultaneously. *D. magna* were able to identify predators with different feeding habitats and efficiency and were more vulnerable to predation. They responded more strongly to the more dangerous, visual predator in a manner dependent on the presence of light. Finally, both species behaved in accordance with the actual threat levels and could be viewed as performing an adaptive risk assessment, although such advanced risk assessments are generally associated with organisms with more complex neurology. Potentially, mm-sized invertebrates are able to respond successfully to several simultaneous risks. It is also possible that differently sized prey taxa make different risk assessments, probably based on different threat levels associated with size-selective predation.

We conclude that in the *waterscape of fear* differently sized zooplankton taxa may alter their response to multiple threats when exposed to UVR and predation risk. Although UVR induced the most pronounced response, the larger sized *D. magna* showed the strongest predator avoidance behaviour, whereas the smaller,

less vulnerable, *D. pulex* did not respond to predator cues. Size may constitute a fitness advantage for zooplankton in the presence of predators. Hence, it is not only higher, more neurologically complex organisms, that are able to rank and respond adaptively to simultaneously occurring, multiple threats. In a broader context, our results highlight how the complexity of a cocktail of everyday, natural threats in nature is perceived and handled by different species. Identifying such complexity may advance our understanding of the spatial and temporal distribution of different sizes and taxa of organisms in the wild.

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CONFLICT OF INTEREST

None of the authors declare any conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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