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LETTER

Mesocosm experiments validate induction of *Daphnia* vertical migration by the fish-derived kairomone 5α-cyprinol sulfate

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Scientific Significance Statement

Diel vertical migration (DVM) is, based on biomass, the largest synchronized movement of animals worldwide and has crucial effects in the marine and in standing freshwater systems: In marine environments, DVM significantly contributes to the biological carbon pump, and in freshwater systems, DVM influences predator–prey dynamics and the vertical transport of nutrients. Recently, a chemical cue (kairomone) from freshwater fish that induces DVM in *Daphnia* has been identified using small-scale indoor bioassays. This is the first study about the effects of this kairomone on DVM of a lake-derived *Daphnia* community using mesocosms. We demonstrate that this kairomone induces behavioral changes during daytime that agree with DVM. In general, this is the first field evidence for the activity of an identified kairomone used by metazooplankton in freshwater systems. Furthermore, we provide a methodological approach to determine in situ concentrations of the kairomone by solid phase extraction of water samples and HPLC-MS. With this new method, we demonstrate that in situ concentrations of the kairomone are in accordance with its role as an inducer of DVM in nature.

Abstract

The fish-derived bile salt 5α-cyprinol sulfate (CPS) has been identified as a kairomone inducing the predator avoidance behavior "diel vertical migration" (DVM) in *Daphnia magna* in response to fish. However, conclusions about the ecological significance of CPS have been derived from laboratory experiments only. Using a mesocosm approach, we investigate whether the role of CPS as a kairomone can be confirmed in the field. We demonstrate that CPS induces downward migration during daytime in a field-derived *Daphnia* community mainly consisting of *Daphnia longispina* and *Daphnia cucullata* within the experiment. In the study lake, the actual population of *D. longispina* shows a similar pattern of DVM, while concomitant quantification of CPS by HPLC-MS

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Johanna Ahlers and Meike Hahn contributed equally to this work.

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Additional Supporting Information may be found in the online version of this article.

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confirms that CPS in situ concentrations are sufficiently high for induction of daytime downward migration of *D. longispina* in this oligo-mesotrophic lake. Together, these observations infer that CPS is a significant kairomone-inducing DVM-like behavior in *Daphnia*.

Infochemicals that mediate the perception of predators by prey and which impart a benefit to the receiving organism while not being beneficial for the producer are termed kairomones (Pohnert et al. 2007). Such chemical signaling has been well-established for inducible defenses in freshwater systems, in particular within predator–prey systems by planktivorous fish and prey organisms of the genus *Daphnia*. In response to kairomones from fish, *Daphnia* spp. deploy several inducible defenses against zooplanktivores including life history changes (e.g., earlier reproduction, smaller size at first reproduction; von Elert and Stibor 2006; Hahn and von Elert 2020), morphological defensive structures (e.g., helmets; Laforsch and Tollrian 2004; Hahn and von Elert 2022), and diel vertical migration (DVM; Loose 1993a).

DVM, in response to fish, describes the daytime migration of zooplankton into the dark hypolimnion of a waterbody, which can serve as a refuge from visually oriented predators (Ringelberg et al. 1991). During nighttime, zooplankton migrate back into the epilimnion. The metabolic costs associated with DVM (Loose and Dawidowicz 1994) are compensated by reduced predation losses (Lampert 1993). DVM contributes substantially to vertical nutrient transport (Haupt et al. 2009, 2010) and to the biological carbon pump in marine systems (Brierley 2014), while also affecting the foraging of planktivorous fish and distribution of phytoplankton biomass (Reichwaldt and Stibor 2005; Haupt et al. 2009).

Recently, the first fish-derived kairomone that induces DVM in *Daphnia magna* has been identified (Hahn et al. 2019). The only other identified kairomone perceived by *Daphnia* reported are fatty acids conjugated to the amino group of glutamine that are released by larvae of the insect *Chaoborus* (Weiss et al. 2018). In both cases, kairomone identification was based on small-scale laboratory bioassays. For identification of the fish kairomone, the DVM of a *D. magna* clone was investigated in tubes of 1-m length in which the temperature stratification of a lake was simulated while the length of the water column and the temperature-, light-, and food regime diverged from natural conditions (von Elert and Loose 1996). Using a clone of *D. magna*, the DVM-inducing kairomone was found to be the bile salt 5α-cyprinol sulfate (CPS), a compound exuded by various fish species (Hahn et al. 2019). *D. magna* mainly occurs in ponds where it may coexist with fish; however, DVM traditionally is described in lakes with smaller-sized *Daphnia* species (Larsson and Dodson 1993). Hence, it remains to be demonstrated that CPS also induces DVM in these smaller-sized *Daphnia* species. In addition, unfortunately, little is known about the concentration and effect of CPS in nature.

Here, we conducted a field mesocosm experiment in which we tracked the effect of purified CPS on the vertical distribution of a field-derived *Daphnia* community. We tested two different CPS concentrations to estimate CPS threshold concentrations for DVM induction and quantified CPS concentrations within the mesocosms over time to estimate their chemical stability. Subsequently, we measured vertical daytime and nighttime distributions of *Daphnia longispina* in the experimental lake and quantified concomitant CPS concentrations.

Methods

To test the effects of CPS addition on DVM, we performed a factorial mesocosm experiment during the summer of 2019 in the dimictic oligo-mesotrophic Lake Klostersee. This lake contains *Daphnia cucullata* and *D. longispina* as the major *Daphnia* species (Seitz 1984; Ilić et al. 2021) and has zooplanktivorous cyprinid fish (for details, *see* Supporting Information S1.1). In summer 2022, we determined vertical profiles of light, temperature, chlorophyll *a* (Chl *a*), as well as the daytime and nighttime vertical distribution of *D. longispina* and CPS. To ensure that the chlorophyll profile from 2022 is representative, such data was also collected during 2023. *See* Ahlers et al. (2023) for all raw data used in this study.

Mesocosm experiment

In May 2019, lake plankton (< 250 μm) was enclosed in nine 12-m long bottom-sealed mesocosms (Supporting Information Fig. S1). Thereafter, zooplankton was collected in Lake Klostersee with a plankton net (100-μm mesh) from 12 to 0 m and inserted into the mesocosms. The experiment was started 33 d after the establishment of the fish-free mesocosms to ensure degradation of any initially present fish kairomone. Then, on 11 June 2019, the mesocosms were amended with either CPS (1x or 10x concentrated) or control extract to initiate the experiment (Supporting Information Fig. S1; for details, *see* Supporting Information S1.2). Samples for quantification of CPS in the mesocosms were taken at 0–2 and 9–11 m 48, 72, and 120 h after CPS addition (Supporting Information Fig. S1, for details, *see* Supporting Information S1.2).

Four vertical layers of the mesocosms (0–3, 3–6, 6–9, and 9–12 m) were sampled with 20-cm diameter closing plankton net (type Apstein, Pokorny-Netze GmbH; 140 μm mesh size). To avoid disturbance of vertical gradients, sampling was initiated at the shallowest layer. Each of the nine mesocosms was sampled on 13 June 2019 during daytime (48 h after CPS) and nighttime (60 h after CPS) and on 14 June 2019 during daytime (72 h after CPS, *see* Supporting Information Fig. S1).

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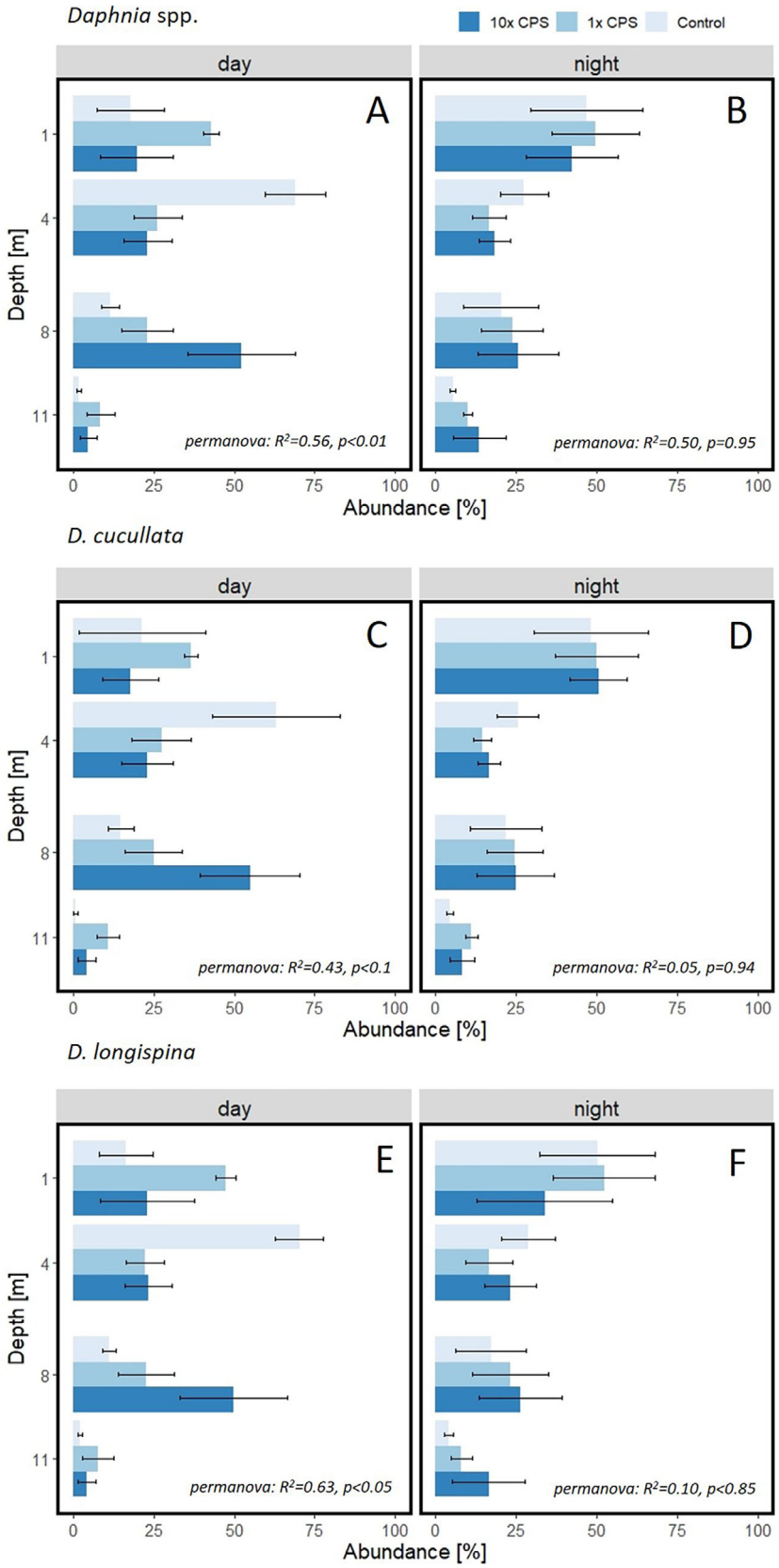

Fig. 1. Legend on next page.

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Zooplankton samples were fixed in 4% (v : v) of formaldehyde and 4% (w : v) saccharose; a dissecting microscope was used for taxa identification and counting. Treatment effects on vertical distributions were tested by PERMANOVAs, and effects on the share of the *Daphnia* community in different depth layers were tested by one-way ANOVA and posthoc tests (for details, *see* Supporting Information S1.5). For analysis of nutritional and physical parameters, *see* Supporting Information S1.6, S1.7.

Vertical profiles in Lake Klostersee

On midday and midnight of 17 August 2022, 18 August 2022, and 19 August 2022, the layers 0–2, 2–4, 4–6, 6–8, 8–10, and 10–12 m were sampled in duplicate in the center of the lake, zooplankton was fixed, and *D. longispina* was counted as indicated above. Deeper strata were not sampled because of muddy sediments. The entire sample was counted for each duplicate before pooling data. A Cochran–Mantel–Haenszel test (software R, version 3.6.2) was performed to test for differences in the vertical distribution of *D. longispina* between daytime and nighttime. On the same dates samples, for CPS were taken (for details, *see* Supporting Information S1.8).

Vertical profiles of temperature were measured on 13 August 2015, 31 August 2016, 04 September 2019, 16 August 2022, and 12 August 2023. A vertical profile of light was measured on 17 August 2022 (*see* Supporting Information S1.7). R-package ggplot2 was used to fit an exponential model to the vertical profiles of light intensity and to apply geomline to connect data points of the vertical temperature profile.

On 17 August 2022, a 2-m long tube sampler was used to collect a vertical profile of Chl *a*. Samples of 2 L were stored at −20°C. After thawing, the samples were filtered onto GFF filters and Chl *a* concentrations were determined spectrophotometrically (Tilzer 1983). On 12 August 2023, a vertical profile of Chl *a* was obtained using a Hydrolab HL4 OTT hydroMet multiprobe.

Results

Mesocosm study in 2019

In an earlier mesocosm study, the *Daphnia* community had been given several days to adapt to changes in fish abundance before vertical profiles of *Daphnia* were sampled (Loose 1993*a*,*b*). Accordingly, on 13 June 2019, we investigated daytime depth distributions at 48 and 72 h after kairomone addition, while the nighttime profile was measured after 60 h (Supporting Information Fig. S1). We found an effect of CPS on the daytime depth distribution of *Daphnia* spp. 48 h after CPS addition (PERMANOVA results, $R^2 = 0.56$, $p < 0.01$; Fig. 1A; Supporting Information Table S5). In the control treatment, most *Daphnia* resided in the layer between 3 and 6 m of depth (~ 68% of the community; Fig. 1A). In the 1x CPS mesocosms, *Daphnia* was located mainly (42%) in the layer between 0 and 3 m depth (Fig. 1A), while after addition of 10x CPS the most favored layer was the one between 6 and 9 m (~ 52% of the community). Accordingly, a significantly larger proportion of the *Daphnia* community resided in the bottom part of the mesocosms (6–12 m depth) during daytime (Supporting Information Fig. S5A; Supporting Information Table S6). The same treatment-dependent trends of vertical daytime distribution were observed for individual species, *D. cucullata* and *D. longispina* (Supporting Information Fig. S5C,E). The effect of CPS on the vertical daytime distribution was significant for *D. longispina* (PERMANOVA: $R^2 = 0.63$, $p < 0.05$; Fig. 1E; Supporting Information Table S5) but not for *D. cucullata* (PERMANOVA: $R^2 = 0.43$, $p < 0.1$; Fig. 1C; Supporting Information Table S5). As expected for animals that perform DVM, CPS did not affect the vertical distribution of *Daphnia* at night (60 h; Fig. 1B,D,F; Supporting Information Fig. S5B,D,F; Supporting Information Table S7). As anticipated, in situ concentrations of CPS in the mesocosms were highest in the 10x CPS treatment and lowest in the control 48 h after its addition. Concentrations were higher in the epilimnion than in the hypolimnion and decreased over time (Table 1; Supporting Information Fig. S6). Seventy-two hours after CPS addition, there was no effect of CPS on the daytime distribution of *Daphnia* (Supporting Information Fig. S7): neither in terms of the vertical distribution of individuals nor the share of the population that resided in the lower half of the mesocosms (Supporting Information Tables S8, S9).

Initial nutrient concentrations in the mesocosms (Supporting Information Table S10) did not differ among treatments

Table 1. Concentrations of 5α-cyprinol sulfate (CPS) in the experimental mesocosms 48 h after CPS addition during 2019. Concentrations represent the means of two technical replicates.

	Control (nmol L^{-1})	1x CPS (nmol L^{-1})	10x CPS (nmol L^{-1})
Epilimnion (0–2 m)	0.67	2.03	32.53
Hypolimnion (9–11 m)	0.22	0.44	6.66

Fig. 1. Vertical distribution of *Daphnia* spp. 48 h (day) and 60 h (night) after the addition of 5α-cyprinol sulfate (CPS) to mesocosms during 2019. Depicted are the relative distributions of *Daphnia* populations in percent (mean ± SE, $n = 3$) over the depth ranges "0–3 m," "3–6 m," "6–9 m," and "9–12 m." Data are depicted for the total *Daphnia* community (**A**, **B**), for *Daphnia cucullata* (**C**, **D**), and for *Daphnia longispina* (**E**, **F**) in response to different CPS concentrations and a control. PERMANOVA results are obtained after 100,000 permutations, testing for the effect of the treatment on distribution patterns.

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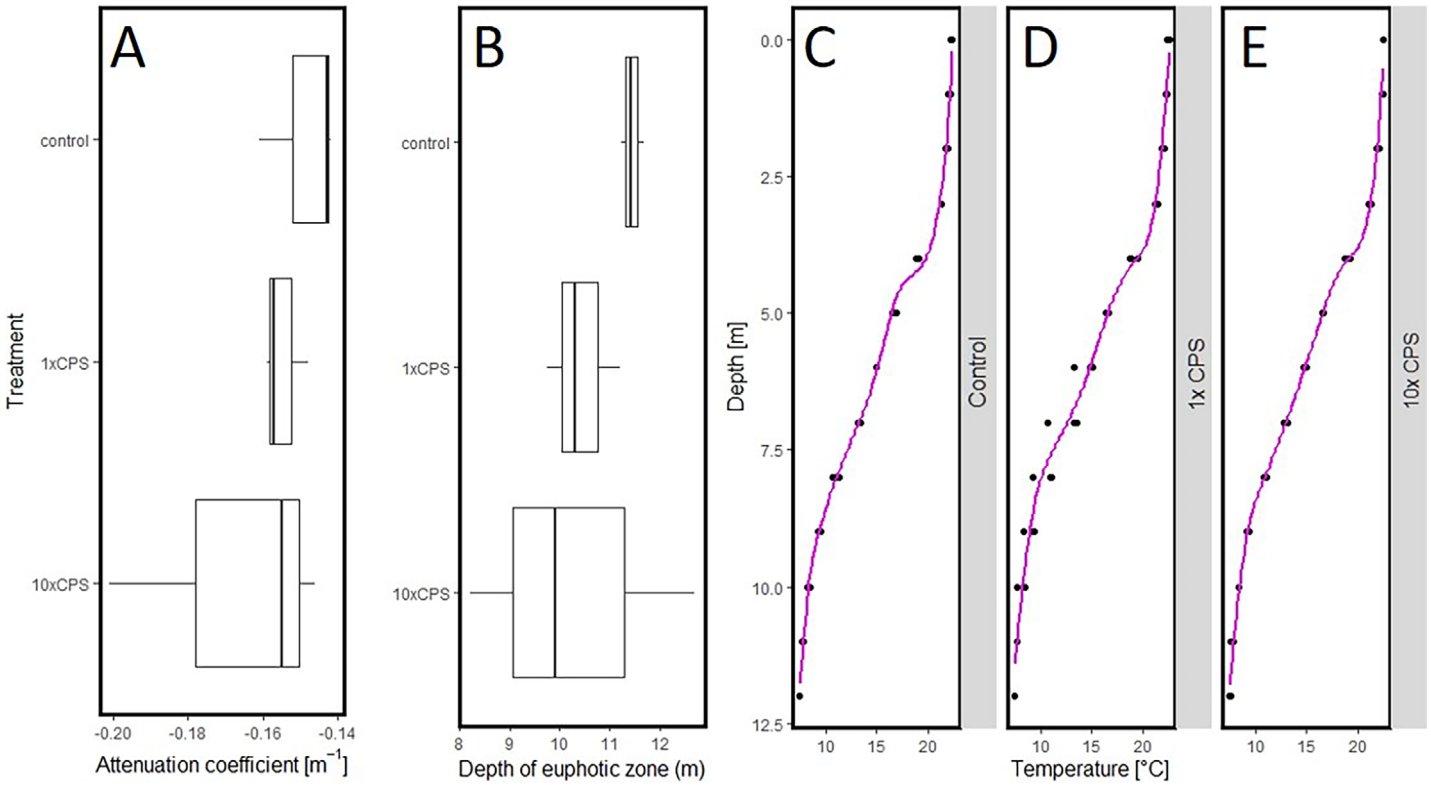

Fig. 2. Depth profiles of temperature and attenuation coefficients and depths of euphotic zone in experimental mesocosms 48 h after addition of 5α-cyprinol sulfate (CPS) during 2019. Boxplots of attenuation coefficients of light (**A**) and depths of euphotic zones (= 1% I_0), (**B**) represent three biological replicates per treatment. Depth-dependent values of temperature are depicted for three biological replicates of control (**C**), 1x CPS-treated (**D**), and 10x CPS-treated (E) mesocosms; the magenta line represents smoothed conditional means of the vertical temperature profiles across all replicates of the respective treatment.

(Supporting Information Table S11); likewise, temperature profiles (Fig. 2C–E) were not distinguishable 48 h after CPS addition (permanova, 1000 permutations, $F_{\text{model},2} = 0.743$, $p = 0.782$). Although light intensities seemed to diverge between treatments mainly at the water surface (Supporting Information Fig. S8), mean coefficients for light attenuation ranged from −0.149 to −0.167 m^{-1} across treatments and were not significantly different among treatments (Fig. 2A; one-way ANOVA, $F_2 = 0.802$, $p = 0.49$). Accordingly, the mean depth of the euphotic zone (1% I_0) ranged from 10.3 to 11.4 m across treatments and was not significantly different (Fig. 2B; Kruskal–Wallis one-way ANOVA on ranks, $H_2 = 2.489$, $p = 0.339$).

Vertical profiles in Lake Klostersee

During daytime on 17 August 2022, 18 August 2022, and 19 August 2022, maximum mean relative abundances of *D. longispina* were observed in the 10–12 m layer of Lake Klostersee, whereas during nighttime the maximum of mean relative abundances occurred at 8–10 m (Fig. 3). The overall number of *D. longispina* did not differ between daytime and nighttime samples (daytime: 1052 ± 147; nighttime 1180 ± 247 mean ± SE, $n = 3$; *t*-test, $t = -0.0477$; $p = 0.678$). In accordance with a pattern of normal DVM, an abundance of *D. longispina* differed significantly between daytime and nighttime (Cochran–Mantel–Haenszel test, $M^2(5) = 15.405$, $p < 0.05$). Accordingly, the proportion of *D. longispina* in the upper part (0–8 m) increased significantly from daytime to nighttime (Supporting Information Fig. S9A; *t*-test, $F = -2.3594$, $p < 0.05$). For the lower part of the water column (8–12 m), there was a trend (though almost significant) that relative abundances decreased from daytime to nighttime (Supporting Information Fig. S9B; *t*-test, $F = 2.1701$, $p = 0.0697$).

On 17 August 2022, the coefficient for light attenuation (−0.234 m^{-1}) was within the 95% confidence interval (CI) of the respective coefficients in the mesocosms; accordingly, the depth of the euphotic zone (1% I_0, 9.1 m) fell within the 95% CI of that in the mesocosms. The vertical profile of Chl *a* revealed a deep chlorophyll maximum (DCM) of 20.4 μg L^{-1} at 12 m during August 2022 (Fig. 3D) and at 11 m during 2023 (Supporting Information Fig. S10), suggesting that DCMs are typical for this lake.

During 2022 and concomitant with DVM profiling in the lake (Fig. 3; Supporting Information Fig. S9), mean CPS concentrations in the lake ranged from 19.6 to 31.5 nmol L^{-1} during daytime and from 5.5 to 10.7 nmol L^{-1} during nighttime (Fig. 4). A two-way ANOVA revealed that concentrations

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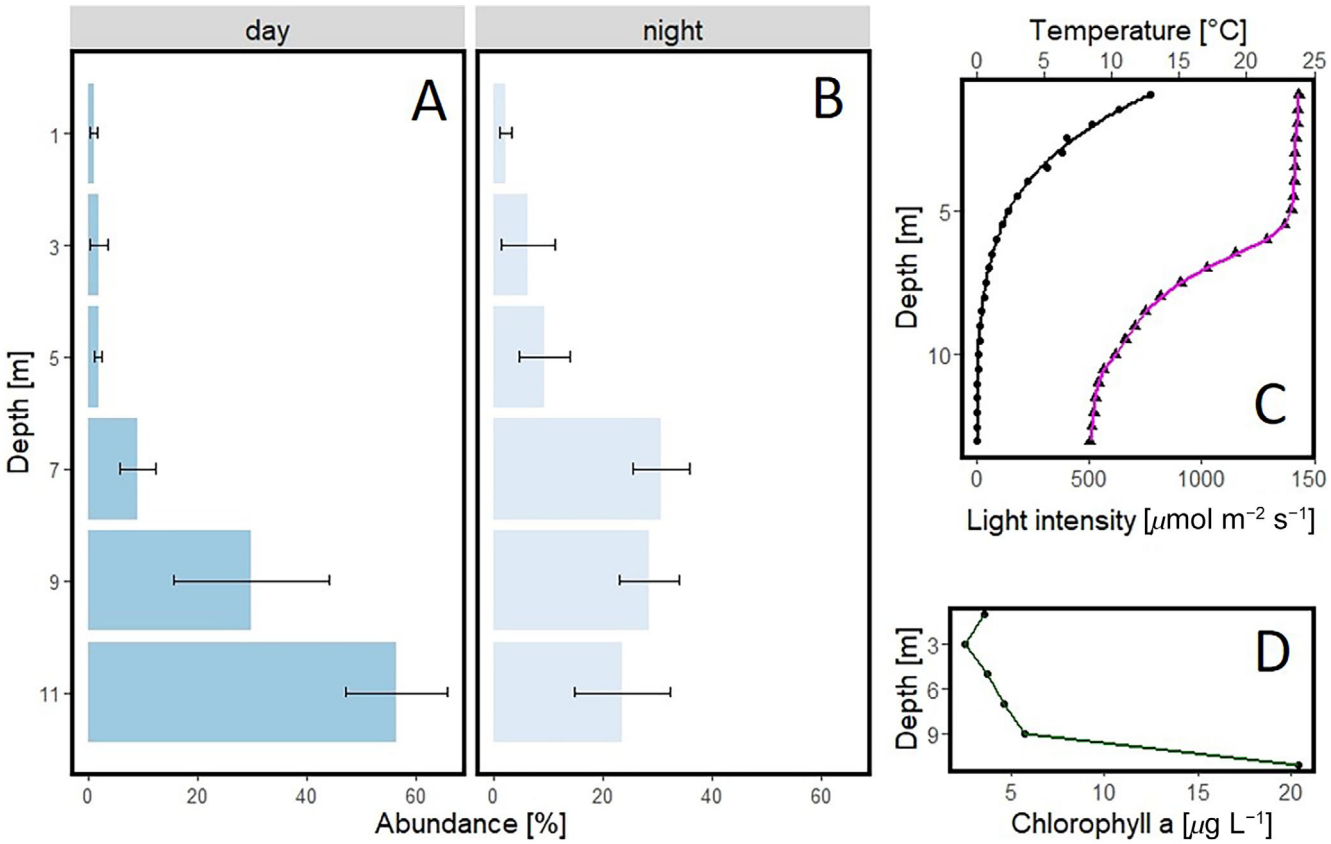

Fig. 3. Vertical profiles of Lake Klostersee during 2022. Distribution of *Daphnia longispina* during daytime (**A**) and nighttime (**B**). Depicted are relative distributions (mean ± SE, $n = 3$) over the depth ranges "0–2 m," "2–4 m," "4–6 m," "6–8 m," and "8–10 m." Data represent samplings from three consecutive dates. (**C**) Vertical profile of light intensity and temperature on 1st day of sampling. The green line represents the fit of an exponential model to the vertical profile of light intensity; the magenta line connects the temperature data points. (**D**) Vertical profile of chlorophyll *a* on 1st day of sampling; the black line connects the chlorophyll data points.

did not differ between the 0–4, 4–8, and 8–12 m layers ($F_2 = 0.23$, $p = 0.793$) but were significantly lower during night ($F_1 = 7.14$, $p < 0.05$); there was no interaction among depth and day/night sampling ($F_2 = 0.44$, $p = 0.648$).

During 2022, at the same time as the DVM profiling, a temperature profile was collected. At each depth, the 2022 temperatures fell within the 95% CI of water temperatures from in total five different years spanning from 2015 to 2022 (Supporting Information Fig. S11).

Discussion

In a small-scale bioassay setup, CPS has been shown to induce "normal" DVM in *D. magna* (Hahn et al. 2019). Hence, we expected similar effects in the field mesocosm experiment in 2019. Under these near-natural conditions, CPS induced a deeper daytime residence in the *Daphnia* community and in *D. longispina*. Such pronounced DVM behavior in response to fish has been demonstrated previously for *D. longispina* (Stich and Lampert 1981; King and Miracle 1995; Makino et al. 1996). Similarly, the limited response of *D. cucullata* is also consistent with previous research that fish prefer larger prey over *D. cucullata* (Laforsch and Tollrian 2004), and that the smaller daphnid does not undergo DVM.

In accordance with the normal DVM pattern, the effect of CPS on vertical distribution in the mesocosms during 2019 was absent during nighttime, although there was a trend that animals preferred the upper layer during nighttime. A similar pattern with a more pronounced absence of upward migration at night was also observed for *D. longispina* in Lake Klostersee 3 years later in 2022. This absence of a strong upward migration at night can be explained by several factors. First, food quantity and quality may not have been optimal in the epilimnion of Lake Klostersee, while upward migration of zooplankton during the night can increase development due to higher epilimnetic temperatures (Williamson et al. 2011). This factor may not override the effects of optimum food conditions. A DCM is commonly present in oligo-mesotrophic lakes in summer (Williamson et al. 2011), like in Lake Klostersee, possibly resulting in elevated food quality of DCMs relative to epilimnetic seston, as seen elsewhere (Rothhaupt 1991; Williamson et al. 1996; DeMott et al. 2004). We hypothesize

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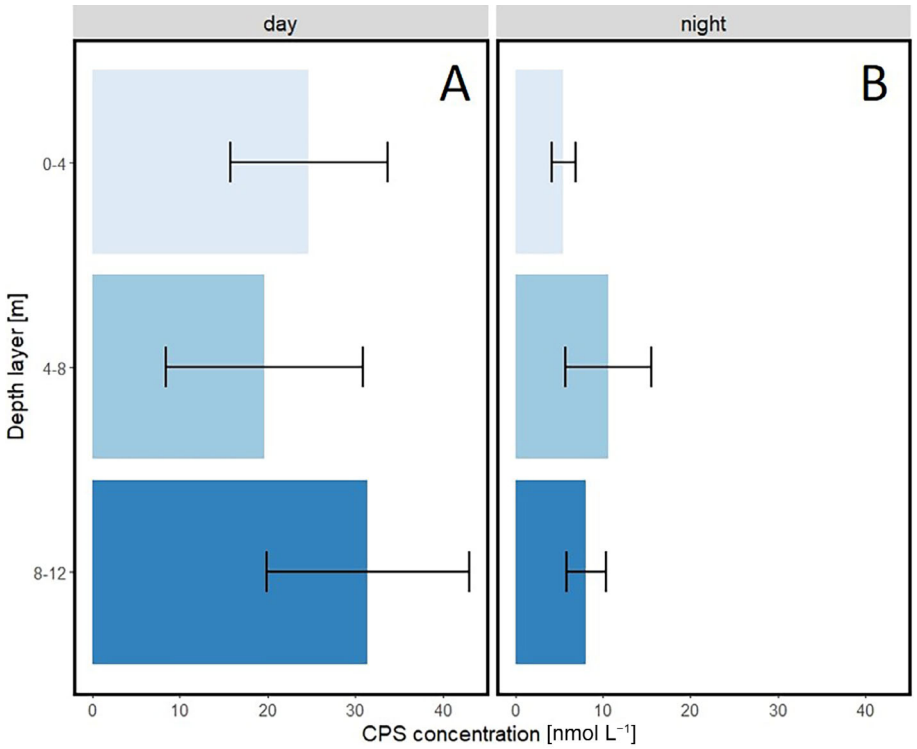

Fig. 4. Concentration of 5α-cyprinol sulfate (CPS) in Lake Klostersee during 2022. Depicted are mean values (± SE, $n = 3$) for the 0–4 m, 4–8 m, and hypolimnion 8–12 m layers that result from three consecutive days.

that food-mediated constraints might have contributed to the absence of a clear upward migration at night in the lake. Pronounced DCMs may result from long stable stratification of mesocosms water columns similar to the situation in the lake, as suggested by common light and temperature profiles in mesocosms and lake. Second, meteorological conditions may have limited DVM at night: light not only serves as a proximate cue for DVM (Ringelberg 1999), but it also reduces the amplitude of upward migration under full moon conditions (Gliwicz 1986; Dodson 1990). Here, the nighttime sampling of zooplankton took place under a clear night sky 4 days before (mesocosm) and 5 days after (lake sampling) full moon. We infer that moonlight may have dampened the upward migration of *Daphnia* at night both in mesocosms during 2019 and Lake Klostersee during 2022.

Comparison among treatments of the mesocosm experiment during 2019 helped identify a threshold for CPS effects on *Daphnia* DVM. Forty-eight hours after CPS addition to mesocosms, there was no effect of CPS on vertical *Daphnia* distribution in the low concentration trials (0.4–2 nmol CPS L^{-1}), whereas *Daphnia* migrated in the high CPS treatment (6.7–33 nmol CPS L^{-1}). This pattern indicates that more than 2 nmol L^{-1} are required for DVM induction in this lake and that the CPS threshold concentration for induction of DVM is reached or even exceeded in the presence of 33 nmol CPS L^{-1} in the respective mesocosms. Furthermore, due to high rates of CPS loss from the mesocosms, it is possible that migration may have been triggered by higher CPS concentrations that had been present earlier in the experiment. Interestingly, in Lake Klostersee during 2022, the pattern for normal DVM coincided with ≥ 20 nmol CPS L^{-1} in the water column, values which lay within the range of CPS concentrations that altered *Daphnia* distribution in mesocosms. In conclusion, CPS concentrations in the lake are most likely sufficiently high to cause deeper daytime residence of *D. longispina*.

These evidence that the DVM of *D. longispina* in the lake can be explained by in situ CPS concentrations was obtained in 2022. The fact that stratification regimes are consistent from 2015 to 2023 shows that the stratification in 2022 was typical for Lake Klostersee and suggests that the finding that DVM in the lake coincides with sufficiently high CPS concentrations is a representative result.

DVM has been induced in a *D. magna* clone by 100 pmol CPS L^{-1} under laboratory conditions (Hahn et al. 2019), whereas 2 nmol CPS L^{-1} did not affect the daytime position of *Daphnia* spp. in mesocosms. The higher threshold in the mesocosms may result from (i) insufficient CPS degradation in control mesocosms so that higher CPS concentrations may have been necessary to affect daytime distributions in comparison to controls; (ii) species-specific CPS thresholds with the smaller-sized *D. longispina* in mesocosms and in Lake

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Klostersee having higher CPS thresholds than large-bodied *D. magna*.

Interestingly, CPS concentrations in Lake Klostersee were significantly lower in nighttime than in daytime samples in 2022. These diurnal fluctuations of CPS concentrations may result from diurnal activity patterns and habitat changes of fish (Helfman 1986). Furthermore, the pronounced decline of CPS concentrations over time in the mesocosms suggests that CPS degradation is rapid. Conceptually, kairomones should show rapid turn-over so that their in situ concentration can reflect predation risk (von Elert 2012). Although bacterial degradation of the DVM-inducing kairomone has been shown in principle (Loose et al. 1993), and for bile salts in particular (Philipp 2011), it remains to be demonstrated to which degree in situ bacterial degradation contributes to a turn-over of CPS.

In summary, CPS is able to induce daytime downward migration according to a normal pattern of DVM in a field-derived experimental *Daphnia* population. Sampling also shows that in situ concentrations of CPS in the oligo-mesotrophic study lake ranged from 6 to 32 nmol L^{-1} and are high enough for induction of daytime downward migration *D. longispina* in a natural lake.

Furthermore, this study also resulted in the establishment of a new method for the in situ quantification of CPS that allows for analysis of the spatial and temporal dynamics of the kairomone in natural lakes and ponds and can be used to describe the "landscapes of fear" (Gaynor et al. 2019) in natural aquatic environments which can be linked to zooplankton DVM.

References

Ahlers, J., M. A. Hahn, M. Stockenreiter, H. Stibor, and E. von Elert. 2023. Mesocosm experiments validate induction of *Daphnia* vertical migration by the fish-derived kairomone 5α-cyprinol sulfate. Dryad. doi:10.5061/dryad.rn8pk0pj3

Brierley, A. S. 2014. Diel vertical migration. Curr. Biol. **24**: R1074–R1076. doi:10.1016/j.cub.2014.08.054

DeMott, W. R., J. R. Edington, and A. J. Tessier. 2004. Testing zooplankton food limitation across gradients of depth and productivity in small stratified lakes. Limnol. Oceanogr. **49**: 1408–1416. doi:10.4319/lo.2004.49.4_part_2.1408

Dodson, S. 1990. Predicting diel vertical migration of zooplankton. Limnol. Oceanogr. **35**: 1195–1200. doi:10.4319/lo.1990.35.5.1195

Gaynor, K. M., J. S. Brown, A. D. Middleton, M. E. Power, and J. S. Brashares. 2019. Landscapes of fear: Spatial patterns of risk perception and response. Trends Ecol. Evol. **34**: 355–368. doi:10.1016/j.tree.2019.01.004

Gliwicz, Z. M. 1986. A lunar cycle in zooplankton. Ecology **67**: 883–897. doi:10.2307/1939811

Hahn, M. A., and E. von Elert. 2020. The impact of diel vertical migration on fatty acid patterns and allocation in *Daphnia magna*. PeerJ **8**: e8809. doi:10.7717/peerj.8809

Hahn, M., and E. von Elert. 2022. One kairomone and multiple effects in *Daphnia* species—5α-Cyprinol sulfate induces morphological defenses in the invasive species *Daphnia lumholtzi*. Front. Ecol. Evol. **10**: 804521. doi:10.3389/fevo.2022.804521

Hahn, M. A., C. Effertz, L. Bigler, and E. von Elert. 2019. 5α-cyprinol sulfate, a bile salt from fish, induces diel vertical migration in *Daphnia*. eLife **8**: e44791. doi:10.7554/eLife.44791

Haupt, F., M. Stockenreiter, M. Baumgartner, M. Boersma, and H. Stibor. 2009. *Daphnia* diel vertical migration: Implications beyond zooplankton. J. Plankton Res. **31**: 515–524. doi:10.1093/plankt/fbp003

Haupt, F., M. Stockenreiter, E. S. Reichwaldt, M. Baumgartner, W. Lampert, M. Boersma, and H. Stibor. 2010. Upward phosphorus transport by *Daphnia* diel vertical migration. Limnol. Oceanogr. **55**: 529–534. doi:10.4319/lo.2010.55.2.0529

Helfman, G. S. 1986. Fish behaviour by day, night and twilight, p. 366–387. *In* T. J. Pitcher [ed.], *The behaviour of teleost fishes*. Springer. doi:10.1007/978-1-4684-8261-4_14

Ilić, M., M. Cordellier, and P. Fink. 2021. Intrapopulation variability in a functional trait: Susceptibility of *Daphnia* to limitation by dietary fatty acids. Freshw. Biol. **66**: 130–141. doi:10.1111/fwb.13623

King, C. E., and M. R. Miracle. 1995. Diel vertical migration by *Daphnia longispina* in a Spanish lake: Genetic sources of distributional variation. Limnol. Oceanogr. **40**: 226–231. doi:10.4319/lo.1995.40.2.0226

Laforsch, C., and R. Tollrian. 2004. Inducible defenses in multipredator environments: Cyclomorphosis in *Daphnia cucullata*. Ecology **1985**: 2302–2311. doi:10.1890/03-0286

Lampert, W. 1993. Ultimate causes of diel vertical migration of zooplankton: New evidence for the predator-avoidance hypothesis. Arch. Hydrobiol. **39**: 79–88.

Larsson, P., and S. I. Dodson. 1993. Invited review: Chemical communication in planktonic animals. Arch. Hydrobiol. **129**: 129–155. doi:10.1127/archiv-hydrobiol/129/1993/129

Loose, C. J. 1993*a*. *Daphnia* diel vertical migration behavior: Response to vertebrate predator abundance. Arch. Hydrobiol. **39**: 29–36.

Loose, C. J. 1993*b*. Lack of endogenous rhythmicity in *Daphnia* diel vertical migration. Limnol. Oceanogr. **38**: 1837–1841. doi:10.4319/lo.1993.38.8.1837

Loose, C. J., and P. Dawidowicz. 1994. Trade-offs in diel vertical migration by zooplankton: The costs of predator avoidance. Ecology **75**: 2255–2263. doi:10.2307/1940881

Loose, C. J., E. von Elert, and P. Dawidowicz. 1993. Chemically-induced diel vertical migration in *Daphnia*: A new bioassay for kairomones exuded by fish. Arch. Hydrobiol. **126**: 329–337. doi:10.1127/archiv-hydrobiol/126/1993/329

Makino, W., H. Haruna, and S. Ban. 1996. Diel vertical migration and feeding rhythm of *Daphnia longispina* and *Bosmina corexoni* in Lake Toya, Hokkaido, Japan. Hydrobiologia **337**: 133–143. doi:10.1007/BF00028514

23782242, 2024, 3, Downloaded from https://aslopubs.onlinelibrary.wiley.com/doi/10.1002/lo2.10375 by Bibl. der Universitat zu Koln, Wiley Online Library on [10/11/2026]. See the Terms and Conditions (https://onlinelibrary.wiley.com/terms-and-conditions) on Wiley Online Library for rules of use; OA articles are governed by the applicable Creative Commons License

Philipp, B. 2011. Bacterial degradation of bile salts. Appl. Microbiol. Biotechnol. **89**: 903–915. doi:10.1007/s00253-010-2998-0

Pohnert, G., M. Steinke, and R. Tollrian. 2007. Chemical cues, defence metabolites and the shaping of pelagic interspecific interactions. Trends Ecol. Evol. **22**: 198–204. doi:10.1016/j.tree.2007.01.005

Reichwaldt, E. S., and H. Stibor. 2005. The impact of diel vertical migration of *Daphnia* on phytoplankton dynamics. Oecologia **146**: 50–56. doi:10.1007/s00442-005-0176-3

Ringelberg, J. 1999. The photobehaviour of *Daphnia* spp. as a model to explain diel vertical migration in zooplankton. Biol. Rev. Camb. Philos. Soc. **74**: 397–423. doi:10.1017/S006323199005381

Ringelberg, J., B. J. G. Flik, D. Lindenaar, and K. Royackers. 1991. Diel vertical migration of *Daphnia hyalina* (sensu latiori) in Lake Maarsseveen: Part 2. Aspects of population dynamics. Arch. Hydrobiol. **122**: 385–401. doi:10.1127/archiv-hydrobiol/122/1991/385

Rothhaupt, K. O. 1991. Variations on the zooplankton menu: A reply to the comment by Smith. Limnol. Oceanogr. **36**: 824–827. doi:10.4319/lo.1991.36.4.0824

Seitz, A. 1984. Are there allelopathic interactions in zooplankton? Laboratory experiments with *Daphnia*. Oecologia **62**: 94–96. doi:10.1007/BF00377380

Stich, H. B., and W. Lampert. 1981. Predator evasion as an explanation of diurnal vertical migration by zooplankton. Nature **293**: 396–398. doi:10.1038/293396a0

Tilzer, M. M. 1983. The importance of fractional light absorption by photosynthetic pigments for phytoplankton productivity in Lake Constance. Limnol. Oceanogr. **28**: 833–846. doi:10.4319/lo.1983.28.5.0833

von Elert, E. 2012. Information conveyed by chemical cues, p. 19–38. *In* C. Brönmark and L.-A. Hansson [eds.], *Chemical ecology in aquatic systems*. Oxford Univ. Press.

von Elert, E., and C. J. Loose. 1996. Predator-induced diel vertical migration in *Daphnia*: Enrichment and preliminary chemical characterization of a kairomone exuded by fish. J. Chem. Ecol. **22**: 885–895. doi:10.1007/BF02029942

von Elert, E., and H. Stibor. 2006. Predator-mediated life history shifts in *Daphnia*: Enrichment and preliminary chemical characterisation of a kairomone exuded by fish. Arch. Hydrobiol. **167**: 21–35. doi:10.1127/0003-9136/2006/0167-0021

Weiss, L. C., and others. 2018. Identification of *Chaoborus* kairomone chemicals that induce defences in *Daphnia*. Nat. Chem. Biol. **14**: 1133–1139. doi:10.1038/s41589-018-0164-7

Williamson, C. E., R. W. Sanders, R. E. Moeller, and P. L. Stutzman. 1996. Utilization of subsurface food resources for zooplankton reproduction: Implications for diel vertical migration theory. Limnol. Oceanogr. **41**: 224–233. doi:10.4319/lo.1996.41.2.0224

Williamson, C. E., J. M. Fischer, S. M. Bollens, E. P. Overholt, and J. K. Breckenridge. 2011. Toward a more comprehensive theory of zooplankton diel vertical migration: Integrating ultraviolet radiation and water transparency into the biotic paradigm. Limnol. Oceanogr. **56**: 1603–1623. doi:10.4319/lo.2011.56.5.1603

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Conflict of interest

None declared.

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