

Negative phototactic response to UVR in three cosmopolitan rotifers: a video analysis approach

Pierluigi Colangeli  · Ulrike E. Schlägel · Ulrike Obertegger · Jana S. Petermann ·
Ralph Tiedemann · Guntram Weithoff

Received: 14 July 2018 / Revised: 25 September 2018 / Accepted: 12 October 2018
© Springer Nature Switzerland AG 2018

Abstract Ultraviolet radiation (UVR) is an environmental stressor in several ecosystems and can affect organisms' survival and reproduction, and community structure. Rotifers cope with UVR stress adopting preventive behavioral and metabolic mechanisms. However, the demonstration of an immediate behavioral response in rotifers is missing. We investigated the short-term response of rotifers to UVR, by combining video analysis and movement ecology methods, in three common species: *Brachionus calyciflorus*, *Keratella cochlearis*, and *Keratella quadrata*. We recorded the behavior of *B. calyciflorus* (both sexes), *K. quadrata*, and *K. cochlearis* (females) exposed to white light, and to intermittent cycles of

UVR (30:30 s). Individual trajectories were extracted from videos with open-source software. We found that *B. calyciflorus* females exposed to UVR exhibited strong negative phototaxis with increased swimming speed, and a weak positive phototaxis in males. *Keratella cochlearis* and *K. quadrata* showed a weaker response. Our study reveals a species-specific behavioral response to UVR in rotifers. Furthermore, we highlight how sexual dimorphism in *B. calyciflorus* does not only occur in morphology and movement, but also in behavioral traits. Our results help to understand zooplankton community dynamics by providing a mechanistic explanation of UVR response in one major zooplankton taxonomic group.

Guest editors: Steven A. J. Declerck, Diego Fontaneto, Rick Hochberg & Terry W. Snell / Crossing Disciplinary Borders in Rotifer Research

Electronic supplementary material The online version of this article (<https://doi.org/10.1007/s10750-018-3801-y>) contains supplementary material, which is available to authorized users.

P. Colangeli (✉) · G. Weithoff
Department of Ecology and Ecosystem Modelling,
Institute for Biochemistry and Biology, University of
Potsdam, Maulbeerallee 2, 14469 Potsdam, Germany
e-mail: pierluigi.colangeli@gmail.com

U. E. Schlägel
Plant Ecology and Conservation Biology, Institute of
Biochemistry and Biology, University of Potsdam, Am
Mühlenberg 3, 14476 Potsdam-Golm, Germany

Keywords Risk avoidance behavior · *Brachionus calyciflorus* · *Keratella cochlearis* · BEMOVI · Movement ecology

U. Obertegger
Department of Sustainable Agro-ecosystems and
Bioresources, Research and Innovation Centre,
Fondazione Edmund Mach, Via E. Mach 1,
38010 San Michele all'adige, TN, Italy

J. S. Petermann
Department of Biosciences, University of Salzburg,
Hellbrunner Str. 34, 5020 Salzburg, Austria

Introduction

Solar radiation is a major driver of physiological processes across all latitudes and has prominent effects on the structure of both terrestrial and aquatic communities (Field, 1998). In aquatic ecosystems, light determines the productivity and the vertical distribution of organisms, such as phytoplankton and primary consumers (Dodson, 1990; Kaartvedt et al., 1996; Obertegger et al., 2008). Organisms inhabiting the lake epilimnion are exposed to different stressors, such as mechanical disturbance (e.g., wave motion, turbulence) (Verschuren et al., 2002), fluctuations in temperature and water chemistry due to climate (Patalas, 1984; Robertson & Ragotzkie, 1990), and ultraviolet radiation (UVR) (De Mora et al., 2000; Sommaruga, 2001). UVR (280–400 nm) has multiple adverse effects on cellular material and is associated with oxidative stress and aging (De Mora et al., 2000; Snell et al., 2014). In response to UVR stress, organisms may respond with three non-mutually exclusive mechanisms: by avoiding the risk, by shielding themselves with protective compounds, or by DNA-related photoenzymatic repair (Häder et al., 2007; Hansson & Hylander, 2009). Because of the impact that UVR has on aquatic communities' structure and productivity (Leech & Williamson, 2000), it is crucial to understand the mechanisms that regulate UVR photoprotection.

In zooplankton, two major *preventive* mechanisms exist to cope with UVR: photoprotection and risk avoidance (Hansson & Hylander, 2009). Photoprotection is a metabolic response and involves protective compounds (e.g., melanin, mycosporine-like aminoacids [MAAs]) that are either synthesized by the organisms themselves or accumulated through the diet (Sommaruga, 2001; Hansson et al., 2007; Obertegger et al., 2008). Metabolic photoprotection requires multiple steps, and for instance in *Daphnia* spp., it involves stress recognition, melanin synthesis, and carapace impregnation and maintenance after molting (Herbert & Emery, 1990). Risk avoidance

behavior, instead, happens on a considerably shorter timescale (see video 4, *Daphnia* response to UVR, Colangeli et al., 2016). Both mechanisms have direct and derivative costs (Harvell, 1990) associated with metabolites production or acquisition (Hansson & Hylander, 2009), movement across colder and probably less productive layers (Dawidowicz & Loose, 1992), or enhanced predation risk (Hansson et al., 2007). For example, visual predators like fish detect pigmented individuals more easily and thus preferentially prey on the UV-protected populations in comparison to unprotected ones (Hansson et al., 2007).

Some biochemical photoprotection dynamics in freshwater crustaceans and rotifers have been described (Sommaruga, 2001; Hansson et al., 2007; Persaud et al., 2007; Obertegger et al., 2008), and the detrimental effects of UVR have been shown by observing alterations in growth rate, lifespan reduction and vertical community structure in lakes and mesocosms (Cabrera et al., 1997; Leech & Williamson, 2000; Leech et al., 2005; Obertegger et al., 2008). However, a direct, short-term negative phototactic response to UVR at the individual or population level has never been reported in rotifers. Such a response can be expected, because several rotifer species possess a cerebral eye, which is sensitive to white light (Clement et al., 1983; Mimouni et al., 1993); thus, some photoreceptors might be sensitive to the UVR spectrum. Furthermore, phototactic responses towards various light wavelengths and intensities have been described (Jennings, 1901; Viaud, 1950; Cornil-lac et al., 1983).

Species-specific UVR sensitivity has strong influences on the vertical distribution patterns of communities, because it differently affects the water layer preference across different taxa (Cabrera et al., 1997). Leech et al. (2005) reported no preference for deeper layers in lake Giles, Pennsylvania, for *Keratella taurocephala* Myers, 1938 in contrast to other common zooplankton species that clearly preferred deeper lake strata during months of high UVR exposure. This indicates that *K. taurocephala* is adapted to stressful conditions occurring in the epilimnion; however, the underlying protective mechanism remains undisclosed. In another study (Obertegger et al., 2008), the congeneric *K. cochlearis* (Gosse, 1851) responded behaviorally to UVR by moving to the lower layer during the day and returning to the surface layer during the night. In contrast, *Polyarthra dolichoptera*

R. Tiedemann
Unit of Evolutionary Biology/Systematic Zoology,
Institute of Biochemistry and Biology, University of
Potsdam, Karl-Liebknecht-Str. 24-25,
14476 Potsdam-Golm, Germany

Idelson, 1925 persisted in the surface layer during both night and day, probably due to its high content and variety of photoprotective compounds (Obertegger et al., 2008). In a copepod study, either positive or negative phototactic response to UVR was found both within and between species, indicating that this risk avoidance behavior is subject to variation (Overholt et al., 2016).

At a small spatial scale, behavioral responses in microscopic organisms are difficult to quantify because of the associated size constraints. To explore and test behavior, many studies applied choice experiments. In such experiments, a limited number of individuals is given a binary choice (e.g., a corridor with light–dark sides or stress–no stress), and the number of individuals found in each side of the corridor at a given time is used as a proxy for preference, tolerance, or avoidance for a certain environmental cue (Pennak, 1973; Cornillac et al., 1983; Hansson et al., 2007; Overholt et al., 2016). These tests have proven successful and relatively easy to perform; however, only a modest number of individuals can normally be simultaneously monitored, and only limited information related to the relocation of the organisms can be extracted. Thus, with choice experiments, the complex kinematics of the behavior is mostly overlooked. Nowadays, digital imaging is within reach of both citizens and researchers, and the opportunity for sharing direct observations has exponentially increased in the last decade. Digital imaging and image analysis techniques pave the way for easy automatic tracking, allowing the researcher to work with larger sample sizes (tens to thousands of individuals). Moreover, the output of video analysis consists of both morphological and movement-related parameters, which are essential to quantify the organisms' behavior (Coulon et al., 1983; Mimouni et al., 1993; Yúfera et al., 2005; Pennekamp et al., 2015; Soleymani et al., 2015; Obertegger et al., 2018).

In this study, we tested the immediate, small-scale, risk avoidance response to UVR in rotifers, using automatic video tracking in a fully open-source software environment. Two different changes in locomotory patterns were distinguished: oriented movement towards or against a given direction (i.e., phototaxis), and non-directed movement associated with a change in speed (i.e., photokinesis—orthokinesis) (Mimouni et al., 1993).

Materials and methods

Experimental organisms

The experimental organisms were isolated from shallow ponds of the urban area of Potsdam, Germany, fed with a 50:50 mix of *Monoraphidium* sp. and *Cryptomonas* sp. and kept in a UVA-transparent polystyrene 12-well microtiter plate (Greiner Bio-One, catalog number 665 180). The cultures were kept in a climatic chamber having constant temperature of 20°C and with 18:6 light–dark cycle. The *Brachionus calyciflorus* Pallas, 1776 culture consisted of a mixture of genotypes isolated from a natural population of one pond, and reared for four days before filming. At the day of the experiment, it consisted of both males and females (males: ~ 50, females: ~ 200, ~ 60 inds. ml⁻¹). The *Keratella* spp. cultures were established in 2012 and kept as monoclonal cultures since then. At the day of the experiment, the population size of *Keratella cochlearis* was circa 600 females (~ 150 inds. ml⁻¹) and of *Keratella quadrata* (Müller, 1786) was circa 500 females (~ 125 inds. ml⁻¹). Prior to the experiments, cultures were removed from the climatic chamber and brought into a dim-light zone of the laboratory to avoid interference with incoming light. The room temperature was stable at 20°C. The experiments were conducted under a turned-off fume hood.

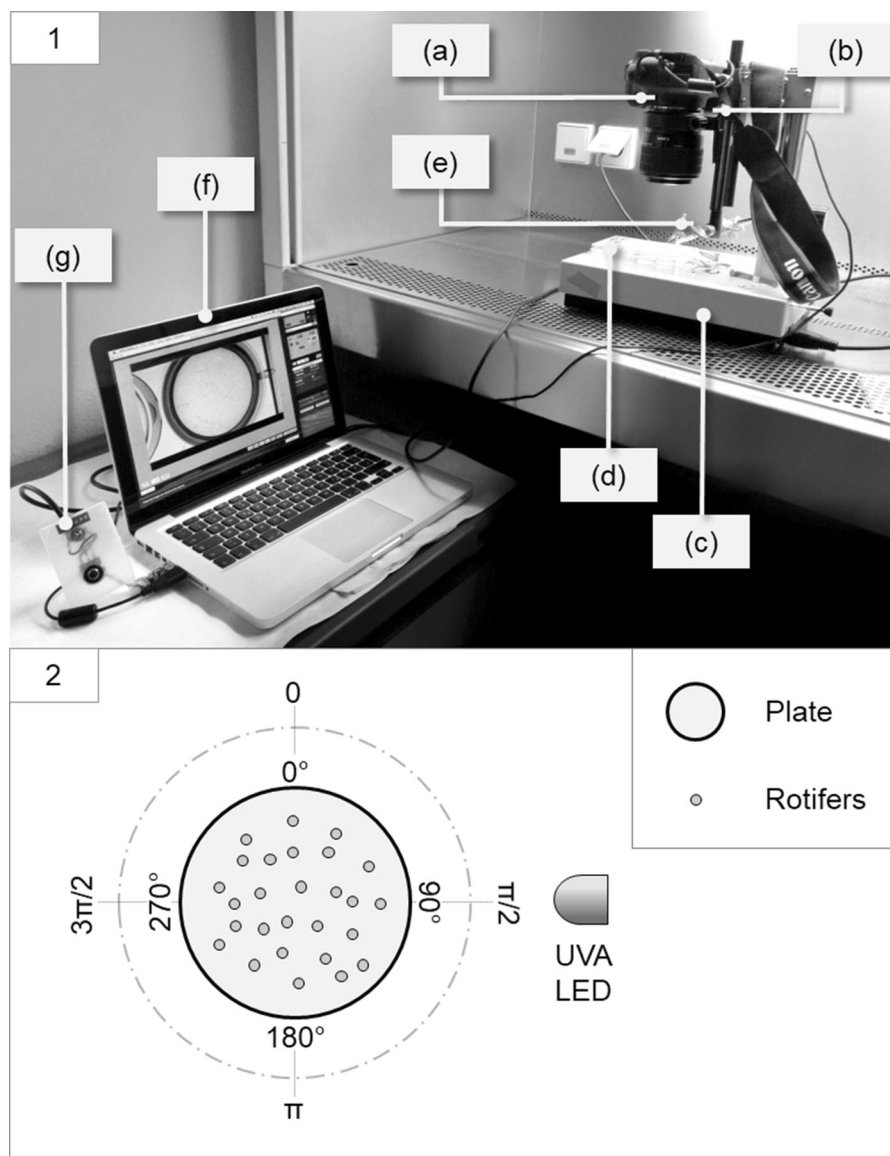
The setup

The filming setup consisted of a Canon 6D camera and Canon MP-65 lens (Fig. 1a), a focusing micrometric slide (Fig. 1b), a stereomicroscope base (Fig. 1c), the microtiter plate with the experimental organisms (Fig. 1d), a generic UVA light-emitting diode (LED: 3 V, 20 mA, UVA_{385 nm}: 0.146 ± 0.045 W cm⁻²), chemistry lab holder (Fig. 1e), a laptop for remote control of the camera (Fig. 1f), and a switch for UVA light (Fig. 1g). It is important to avoid any vibration of the setup during the recordings.

Shooting settings

The cultures were recorded with a full-frame digital camera at 25 frames per second (fps) with × 1 magnification, f/9 aperture, ISO 200, and 1/30 s of exposure time. The only source of light was provided

Fig. 1 Panel 1: The experimental setup consisted of the camera and lens (a), focusing micrometric slide (b), stereomicroscope base (c), microtiter plate with experimental organisms (d), UVA LED and chemistry lab holder (e), laptop for remote control of the camera (f), and a switch for UVA light (g). Panel 2: top view of the plate: UVA was applied from the right side of the plate (90° , corresponding to $\pi/2$ radians)



by the stereomicroscope's white light (WL) and the UVA LED placed 1 cm from the right edge of the plate at 90° (Fig. 1, panel 2). Videos of 62 s were recorded to ensure that at least 1500 frames were captured. The exceeding frames were trimmed prior to the analysis. After filming for 30 s in WL condition, the UVA led was switched on for additional 30 s. Two seconds after the first recording, the cycle was repeated two more times, for three cycles per each culture. The links to original videos can be found in Online Appendix S.

Working hypotheses

Phototaxis

We tested two different hypotheses: (i) no phototaxis in either of the treatments (i.e., WL and WL + UVR; (ii) no phototaxis in WL, but negative phototaxis under UVR stress. These two hypotheses can be statistically tested by observing the distribution of swimming direction in time and fitting an appropriate distribution. In the case of no response to UVR, swimming directions would show a uniform

distribution. Conversely, any preference in swimming direction would better be described by a wrapped Cauchy distribution.

Photokinesis

We tested two different hypotheses when modeling swimming speed: (i) no change in swimming speed under UVR stress; (ii) a change in speed under UVR stress. Swimming speed was modeled as a Weibull distribution. Differences in swimming speed between species were assessed by one-way ANOVA.

Video analysis

In order to extract the trajectories from the recorded videos, we used the BEMOVI package (Pennekamp et al., 2015) of the R environment (R core team, 2017). BEMOVI seamlessly integrates two popular open-source software, namely R (statistics) and ImageJ (image analysis, Eliceiri et al., 2012).

The first step of the analysis was to convert the raw.MOV video files into.avi format, required by BEMOVI and ImageJ. In the conversion step, the videos were trimmed to the exact number of frames (i.e., 1500), colors were desaturated, and the contrast was eventually adjusted to increase the signal-to-noise ratio. All the steps were performed with an R script (Online Appendix S) that controls in headless mode an open-source video editing software (FFmpeg Team, <http://ffmpeg.org>) with scripting capabilities. The corrections were applied automatically to every.MOV file, allowing an efficient, reproducible, and standardized automatic routine. Further information about the functions and the variables can be found in Pennekamp et al. (2015) and Obertegger et al. (2018), and the complete script of the analyses is given in Online Appendix S. The analyses were performed on an Intel® Core™ i7-4790 CPU @ 3.60 GHz, 32 GB RAM, × 64-based processor machine.

Statistical methods

To assess phototaxis and photokinesis in response to UVA exposure, swimming directions and speeds were recorded. Swimming directions were modeled by a uniform distribution (U; non-oriented movement) and a wrapped Cauchy distribution (C; oriented movement in response to UVR). The wrapped Cauchy

distribution has two parameters, the mean direction μ (ranging between 0 and 2π) and the concentration of data ρ (ranging between 0 and 1). The wrapped implementation of the Cauchy distribution adequately represents circular data, in which the minimum and maximum values are actually connected (0° – 360°). For the purpose of our study, we used μ to describe the direction of phototaxis, and ρ to describe the intensity of such behavior (i.e., proxy for the magnitude of phototaxis). For instance, a strong negative phototactic response would be reflected by a high value of ρ (close to 1) and μ located around 270° (corresponding to $3\pi/2$ radians), which is the opposite side of the UVA source located at 90° ($\pi/2$ radians, see Fig. 1, panel 2). For very small values of ρ (close to 0), the wrapped Cauchy distribution resembles a uniform distribution (i.e., lack of phototaxis). To model swimming speeds, we used a Weibull distribution (W), which has two parameters, scale λ and shape k , from which the mean swimming speed can be computed as

$$\text{mean speed} = \frac{\lambda \Gamma(1 + 1/k)}{\text{fps}},$$

where Γ is the Gamma function and fps is the frame rate of the recordings.

With respect to phototaxis, we built two models corresponding to our two working hypotheses: (a) directions were assumed to be uniformly distributed under both WL and UVA exposure (U); (b) directions were assumed to be uniformly distributed under WL exposure and according to a wrapped Cauchy distribution under UVA exposure (C). With respect to photokinesis, we built two models corresponding to our two working hypotheses: (c) speeds were assumed to be distributed according to a Weibull distribution under both WL and UVA exposure (W_1); (d) speeds were assumed to be distributed according to a Weibull distribution under both WL and UVA exposure where the Weibull distributions could differ in their parameters in the two treatments (i.e., W_1 under WL and W_2 under UVR). We fit the models to observed swimming directions and speeds and obtained maximum likelihood estimates for the parameters.

Results

Phototaxis

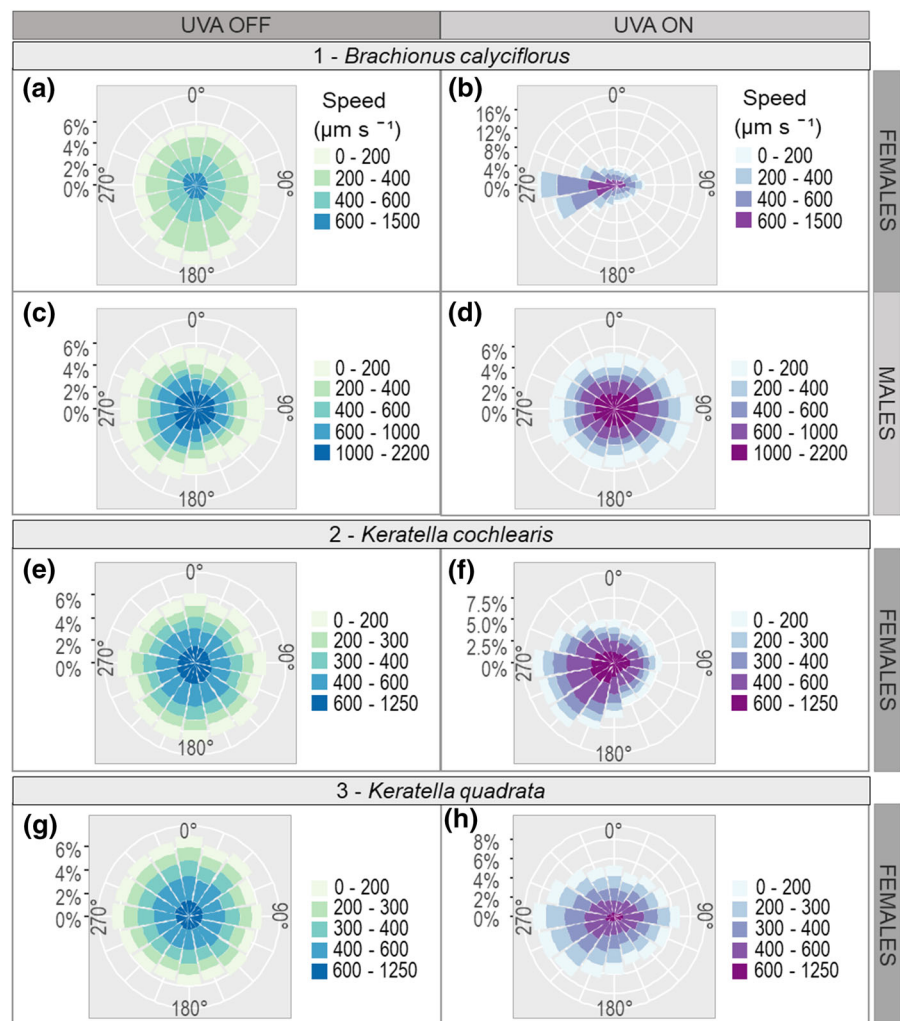
After UVA exposure, applied from the right side of the plate (90° , corresponding to 1.57 radians), *B. calyciflorus* females swam towards the opposite side (wrapped Cauchy μ : 4.56, p : 0.34) (Figs. 2, 3; Table 1), whereas males swam towards the UVA source (105°), but with very weak response (wrapped Cauchy μ : 1.85, p : 0.04) (Figs. 2, 3; Table 1). *Keratella cochlearis* also swam towards the opposite side of the UVA source, (wrapped Cauchy μ : 4.29, p : 0.18) (Figs. 2, 3; Table 1), as also *K. quadrata* did, but with a weak response (wrapped Cauchy μ : 4.36, p : 0.09) (Figs. 2, 3; Table 1). In all three replicates, the

species returned to normal swimming behavior after UVA exposure (Fig. 3).

Photokinesis

In all three replicates, the species returned to pre-UVA exposure swimming speed (Fig. 4). The mean swimming speed of *Brachionus calyciflorus* females under WL was $419 \mu\text{m s}^{-1}$ (± 233 SD), whereas under UVA was $484 \mu\text{m s}^{-1}$ (± 223 SD), showing an increase of 15% ($F = 2,619$, $P < 0.001$) (Fig. 4, Table 1). *Brachionus calyciflorus* males' mean swimming speed under WL was $683 \mu\text{m s}^{-1}$ (± 503 , ± 2.94 SE), whereas under UVA was $721 \mu\text{m s}^{-1}$ (± 522 SD), corresponding to an increase of 1.7% ($F = 77$, $P < 0.001$) (Fig. 4, Table 1). *Keratella cochlearis*

Fig. 2 Radiograms representing the frequency of rotifers moving towards any given direction in space with different swimming speeds ($\mu\text{m s}^{-1}$). UVA incidence was from the right corner (90°). Left side: UVA off. Right side: UVA on. Panel 1: *B. calyciflorus* females (a) and (b); *B. calyciflorus* males (c) and (d). In (b), females show a negative phototactic response to UVA by swimming with higher speed than with no UVA to the left side of the plate, in the opposite direction of the UVA source. No evident response in males was present. Panel 2: *K. cochlearis* showed a medium response to UVA (e, f). Panel 3: *K. quadrata* showed a minor response to UVA (g, h)



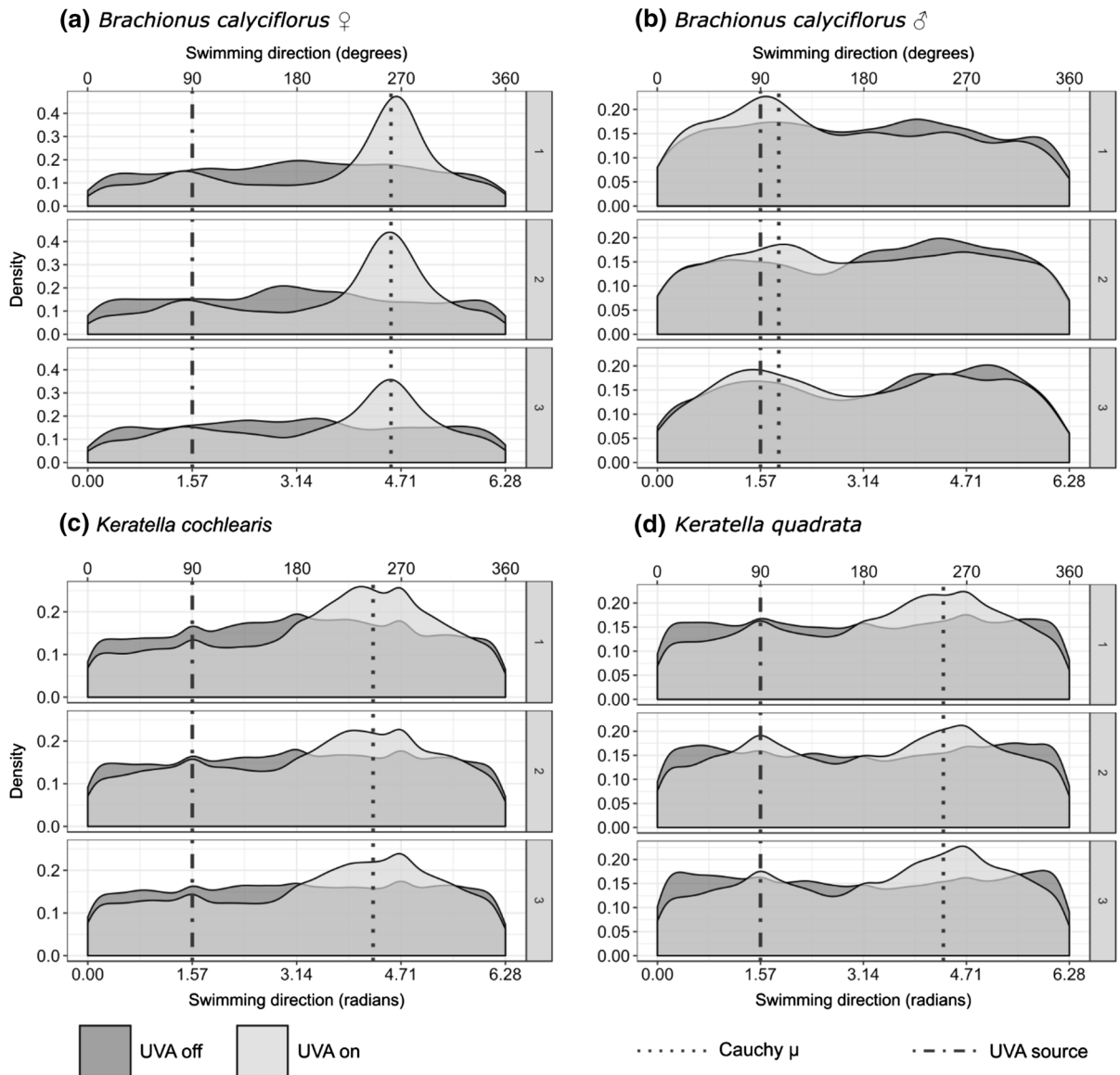


Fig. 3 Density plots showing the swimming direction for each recording cycle (1, 2, and 3), under WL (dark gray), and UVA exposure (light gray). The cycles were recorded in sequence and were separated by a lag of 2 s. The LED emitted UVA from 90° (dash-dotted line). The mean direction of the phototaxis (Cauchy μ) was calculated on cumulative data (dotted line).

Females of *B. calyciflorus* exhibited the strongest negative response (a) (μ : 4.56, p : 0.34) to UVA. *Brachionus calyciflorus* males showed weak positive response to UVA (b) (μ : 1.85, p : 0.04). *K. cochlearis* showed a medium negative response (c) (μ : 4.29, p : 0.18) and *K. quadrata* showed the weakest negative response to UVA (d) (wrapped Cauchy μ : 4.36, p : 0.09).

females' mean swimming speed under WL was $440 \mu\text{m s}^{-1}$ (± 236 SD), whereas under UVA was $471 \mu\text{m s}^{-1}$ (± 237 SD), an increase of 7% ($F = 3,918$, $P < 0.001$) (Fig. 4, Table 1). *Keratella quadrata* females' mean swimming speed under WL was $436 \mu\text{m s}^{-1}$ (± 224 SD), whereas under UVA

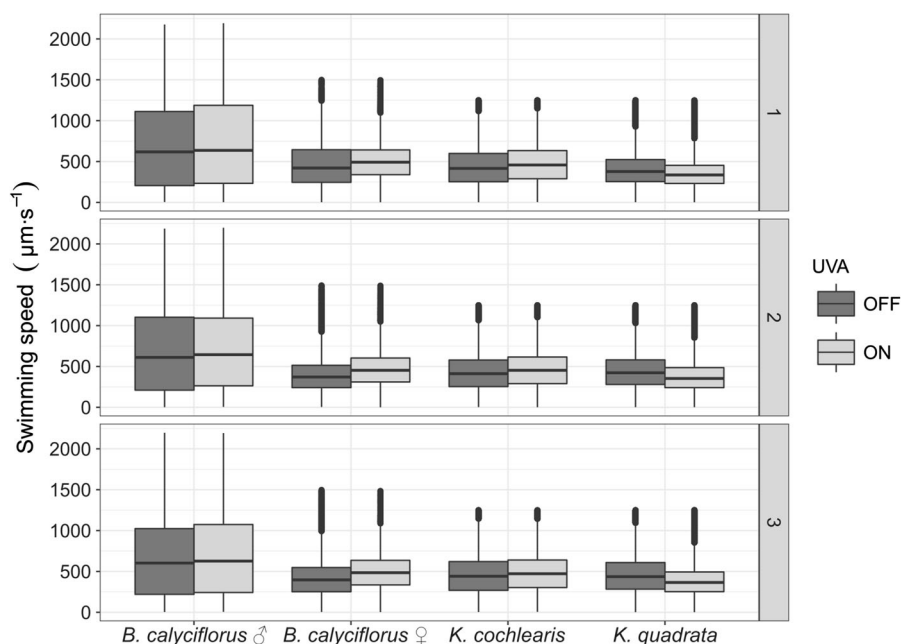
was $371 \mu\text{m s}^{-1}$ (± 182 SD), a decrease of 11% ($F = 21083$, $P < 0.001$) (Fig. 4, Table 1).

Table 1 Parameter estimates of the fitted models for the phototaxis and photokinesis analyses

Behavior	Phototaxis				Photokinesis					
Species	Model	μ	ρ	Angle	Model	λ_1	k_1	λ_2	k_2	Speed
<i>B. calyciflorus</i> ♀	U	–	–		W_1	2.1	20.6	–	–	
	UC	4.56	0.34	261°	W_1W_2	1.9	18.9	2.3	21.8	+ 15%
<i>B. calyciflorus</i> ♂	U	–	–		W_1	1.3	30.3	–	–	
	UC	1.85	0.04	105°	W_1W_2	1.3	29.4	1.3	30	+ 1.7%
<i>K. cochlearis</i>	U	–	–		W_1	2	20.5	–	–	
	UC	4.29	0.18	246°	W_1W_2	1.9	19.9	2.1	21.3	+ 7%
<i>K. quadrata</i>	U	–	–		W_1	2.06	17.8	–	–	
	UC	4.36	0.09	250°	W_1W_2	2.1	19.1	2.1	16.5	– 11%

Phototaxis. Model: U uniform distribution (no parameters), UC: uniform-Cauchy (2 parameters, μ and ρ). The μ values are measured in radians. Angle: direction of phototaxis in degrees. Photokinesis. Model: W_1 Weibull distribution with one set of parameters (2 parameters, λ_1 and k_1), W_1W_2 Weibull distribution with two set of parameters (4 parameters, λ_1 and k_1 , and λ_2 and k_2). Speed: (%) increase calculated on mean speeds before and after UVA exposure

Fig. 4 Boxplots showing the change in rotifers' swimming speed with UVA exposure during every cycles (1, 2, and 3). *Brachionus calyciflorus* females showed the strongest response, increasing their speed by 15%, and *B. calyciflorus* males showed a minor increase of 1.7%. *Keratella cochlearis* swam faster by 7%, whereas *K. quadrata* was the only species swimming slower (– 11%) under UVA exposure



Discussion

We tested the UVR risk avoidance behavior in three eurytherm, euryhaline, widespread species, and observed a gradient of species-specific, negative phototactic responses. All the species that we tested possess a median red eyespot likely containing rhodopsin (Clément, 1980; Kim et al., 2014), the pigment supposed to be involved in phototaxis. To the best of our knowledge, this is the first study that has

observed and quantified the short-term negative phototactic response to UVR in rotifers, revealing a mechanism that may underlie the vertical distribution patterns of some natural communities. Despite some differences, all the tested species responded *immediately* to UVR exposure, and just as rapidly, they returned to normal swimming behavior as the stress ceased. This indicates that, within the experimental timeframe, the UVR exposure did not impair the

species' ability to sense and react to the stress, confirming the high plasticity of protective behavioral mechanisms. The strongest response was observed in *B. calyciflorus* females, but not in conspecific males. A less pronounced response was observed in *K. cochlearis*, whereas *K. quadrata* only showed a weak response to UVR.

UVR risk avoidance behavior

In our experiment, *Brachionus calyciflorus* females had the strongest negative response to UVR. This finding is in contrast to what Cornillac et al. (1983) reported in a study employing choice experiments with *Brachionus calyciflorus*. Specifically, no negative phototaxis could be demonstrated at any tested wavelength (350–700 nm), instead positive phototaxis for females in the boundary region of near UVA and visible spectrum (350–420 nm) and in the 500–640 nm region (Cornillac et al., 1983). We speculate that some diversity might exist at both inter- and intra-specific levels. In the first case, we argue that species with a transparent lorica might be more sensitive in comparison to others with a thicker lorica (e.g., *Keratella* spp.). This is in accordance to studies reporting that *Lepadella ovalis* (O.F. Muller, 1896) and *Asplanchna priodonta* (Gosse, 1950), two rather transparent species, are very sensitive to UVR (Cabrera et al., 1997; Williamson et al., 2001). *Brachionus calyciflorus* males responded to UVR with very weak positive phototaxis and photokinesis. Rotifer males exhibit dwarfism and their anatomy is substantially different in comparison with females (Ricci & Melone, 1998). Accordingly, morphological reductions in males might have affected their ability to sense UVR. Despite males possessing a cerebral eye (Pontin, 1978; Fontaneto & De Smet, 2015; Colangeli et al., 2016—video 3—1'20"), they might lack the specific UVR receptors. However, the observed feeble, unexpected, but detectable response needs further integrative research to be fully discussed and understood from both a behavioral, anatomical, and physiological standpoint.

The response of *K. cochlearis* was less pronounced than the one of *B. calyciflorus* females. This finding is in accordance to Obertegger et al. (2008) finding that *K. cochlearis* avoided the upper layer during the day and moved to the surface during night in Lake Tovel (Italy). Further, *K. cochlearis* had much lower

diversity and concentration of photoprotective compounds in comparison to *P. dolichoptera*, for which no difference in vertical distribution was observed (Obertegger et al., 2008). Weak phototaxis was observed in *K. quadrata*. As reported by Leech & Williamson (2000), *K. taurocephala* persisted in the epilimnion of Lake Giles, Pennsylvania, even during the months of highest UVR exposure, possibly because of a metabolic response (i.e., photoprotection and photorepair). We found a negative photokinetic response as indicated by the reduction of swimming speed. *Keratella quadrata* was the only species that swam slower during UVR exposure. In comparison to *Brachionus calyciflorus*, the long-term culturing (since 2012) of *Keratella* spp. might have influenced their response to some extent. However, even if the magnitude of the response was weaker in *Keratella* spp., the detected negative phototactic behavior indicates that the ability to sense and react to UVR was conserved throughout the culturing period. Additionally, both *Keratella* spp. possess a faceted and thickened lorica, so we speculate that it protects the organisms and screens the photoreceptors from UVR, thus triggering a less marked negative phototaxis. Further research is required to elucidate why some *Keratella* species are more tolerant than others with respect to UVR stress, and we argue that protection is a mixture of physiological adaptation and morphological traits of the lorica.

Methodological considerations

For the purpose of our experiments, we did not discriminate between different life stages of rotifers. The sensitivity to UVR seems to co-vary with age or reproductive status (Luciani et al., 1983; Yúfera et al., 2005). Although the effect of age on swimming speed of rotifers is known (Luciani et al., 1983), we wanted to observe the response to UVR of an average natural population that typically consist of individuals of different ages.

Video analysis is a powerful tool, but like other methodologies, it has limitations. For instance, trajectories might be distorted by identity shifts due to particle collision or temporary loss of detection. Understanding the limits of the software (and hardware) is crucial for planning successful experiments. Each of our recordings lasted 60 s, a timeframe that might seem short, but beholds all the information to

trigger, quantify, and test the short-term behavioral response (e.g., Hansson et al., 2007) we aimed to reveal. A culture of a few hundred individuals can easily generate 10^6 (elaborated) observations; therefore, the recording time is constrained by computational power used to extract the trajectories. Trajectory extraction lasted on average 72 h per species tested on our computer, it is possible to reduce computation time either by increasing the computational resources or by decreasing the number of experimental individuals. Further, metadata notation and code sharing are essential to ensure that experiments are fully reproducible.

We highlight that our setup is highly flexible and can be easily used to characterize movement parameters (e.g., speed, direction, gross distance) of many zooplankton taxa or to test interactions between and within species or trophic levels. A modified setup can be used to test several types of physical and chemical stressors. Future experiments focusing on the response of rotifers to UVR might investigate the photoprotective effect of different diets, for example, by feeding the rotifers with algae synthesizing different photoprotective compounds. Moreover, microplastics might be involved in UVR sensing in rotifers, as the ingested particles might interfere with photoreception, specifically by scattering UVR and disorienting the rotifer. The role of rhodopsin in UVR sensing and phototaxis might be further investigated in sessile rotifers having a swimming larval stage. For example, among the anatomical changes that *Acycclus inquietus* Leidy (1882) undergoes during metamorphosis, the depigmentation of the eyespot was reported (Hochberg et al., 2010). For such organisms, the ability to sense UVR might be necessary (and confined) to the free-swimming larval stage, but might be lost when the settlement occurs in a UVR-shaded location (e.g., inferior margin of macrophytes leaves).

In more general terms, video analysis proves to be an invaluable tool to the study of zooplankton movement, neurobiology, ecotoxicology, and behavioral ecology (Wallace, 2002).

Conclusion

UVR can be a major driver of community structure, and certain rotifer species respond to this stressor by performing vertical migration. Our results showed that

behavioral response mechanisms to UVR in rotifers are species specific under laboratory conditions. Furthermore, we observed that sexual dimorphism in *B. calyciflorus* is not only related to morphology and movement, but also to complex behavioral traits, thus fueling the discussion about the evolutionary significance of separation of sexes in monogonont rotifers. Lastly, we highlight how methods rooted in movement ecology can be applied to plankton research, to obtain novel insight, otherwise not achievable with classical limnological approaches.

Acknowledgements We thank Frank Pennekamp for helping in many occasions with the code, the BioMove group for helpful discussions, and two anonymous reviewers and the Editors of the Special Issue Rotifera XV for their comments. We also thank Christina Schirmer and Christina Luchs for providing rotifers colonies and for helping with the experiments, and Axel Heuer for measuring the LED emission peak. Lastly, we acknowledge the ideas from the master students' practical workshops. This study is supported by the Deutsche Forschungsgemeinschaft (DFG), BioMove research training group (www.biomove.org/), Grant No. DFG-GRK 2118/1.

Data availability Data available on request from the authors.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflicts of interest.

References

- Cabrera, S., M. Lopez & B. Tartarotti, 1997. Phytoplankton and zooplankton response to ultraviolet-radiation in a high-altitude andean lake - short-term versus long-term effects. *Journal of Plankton Research* 19: 1565–1582.
- Clément, P., 1980. Phylogenetic relationships of rotifers, as derived from photoreceptor morphology and other ultrastructural analyses. *Hydrobiologia* 73: 93–117.
- Clement, P., E. Wurdak & J. Amsellem, 1983. Behavior and ultrastructure of sensory organs in rotifers. *Hydrobiologia* 104: 89–129.
- Colangeli, P., A. Cieplinski & U. Obertegger, 2016. Filming of zooplankton: a case study of rotifer males and *Daphnia magna*. *Journal of Limnology* 75: 204–209.
- Cornillac, A., E. Wurdak & P. Clément, 1983. Phototaxis in monochromatic light and microspectrophotometry of the cerebral eye of the rotifer *Brachionus calyciflorus*. *Hydrobiologia*. <https://doi.org/10.1007/BF00045967>.
- Coulon, P. Y., J. P. Charras, J. L. Chassé, P. Clément, A. Cornillac, A. Luciani & E. Wurdak, 1983. An experimental system for the automatic tracking and analysis of rotifer swimming behaviour. *Hydrobiologia* 104: 197–202.

- Dawidowicz, P. & C. J. Loose, 1992. Metabolic costs during predator-induced diel vertical migration of *Daphnia*. *Limnology and Oceanography* 37: 1589–1595.
- De Mora, S., S. Demers, & S. Vernet, 2000. The Effects of UV Radiation in the Marine Environment. Cambridge Environmental Chemistry Series. Cambridge University Press, Cambridge. <https://doi.org/10.1017/cbo9780511535444>.
- Dodson, S., 1990. Predicting diel vertical migration of zooplankton. *Limnology and Oceanography* 35: 1195–1200.
- Eliceiri, K., C. A. Schneider, W. S. Rasband & K. W. Eliceiri, 2012. NIH Image to ImageJ: 25 years of image analysis. *Nature Methods* 9: 671–675.
- Field, C. B., 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science American Association for the Advancement of Science* 281: 237–240.
- Fontaneto, D., & W. De Smet, 2015. Rotifera. In: Schmidt-Rhaesa (eds), *Handbook of Zoology, Gastrotricha and Gnathifera*, 3. De Gruyter, Berlin: 217–300.
- Häder, D.-P., H. D. Kumar, R. C. Smith & R. C. Worrest, 2007. Effects of solar UV radiation on aquatic ecosystems and interactions with climate change. *Photochemical & Photobiological Sciences Royal Society of Chemistry* 6: 267–285.
- Hansson, L. A. & S. Hylander, 2009. Effects of ultraviolet radiation on pigmentation, photoenzymatic repair, behavior, and community ecology of zooplankton. *Photochemical and Photobiological Sciences Royal Society of Chemistry* 8: 1266–1275.
- Hansson, L. A., S. Hylander & R. Sommaruga, 2007. Escape from UV threats in zooplankton: a cocktail of behavior and protective pigmentation. *Ecology* 88: 1932–1939.
- Harvell, C. D., 1990. The Ecology and Evolution of Inducible Defenses. *The Quarterly Review of Biology*. Princeton University Press, Princeton.
- Herbert, P. D. N. & C. J. Emery, 1990. The adaptive significance of cuticular pigmentation in *Daphnia*. *Functional Ecology JSTOR* 4: 703–710.
- Hochberg, R., S. O'Brien & A. Puleo, 2010. Behavior, metamorphosis, and muscular organization of the predatory rotifer *Acyclus inquietus* (Rotifera, Monogononta). *Invertebrate Biology* 129: 210–219.
- Jennings, H. S., 1901. On the significance of the spiral swimming of organisms. *The American Naturalist* 35: 369–378.
- Kaartvedt, S., W. Melle, T. Knutsen & H. R. Skjoldal, 1996. Vertical distribution of fish and krill beneath water of varying optical properties. *Marine Ecology Progress Series JSTOR* 136: 51–58.
- Kim, H.-J., C. Sawada, J.-S. Rhee, J.-S. Lee, K. Suga & A. Hagiwara, 2014. Nutritional effects on the visual system of the rotifer *Brachionus plicatilis* sensu stricto (Rotifera: Monogononta). *Journal of Experimental Marine Biology and Ecology* 460: 177–183.
- Leech, D. M. & C. E. Williamson, 2000. Is tolerance to UV radiation in zooplankton related to body size, taxon, or lake transparency? *Ecological Applications* 10: 1530–1540.
- Leech, D. M., A. Padeletti & C. E. Williamson, 2005. Zooplankton behavioral responses to solar UV radiation vary within and among lakes. *Journal of Plankton Research* 27: 461–471.
- Luciani, A., J. L. Chassé & P. Clément, 1983. Aging in *Brachionus plicatilis*: the evolution of swimming as a function of age at two different calcium concentrations. *Hydrobiologia*. <https://doi.org/10.1007/BF00045960>.
- Mimouni, P., A. Luciani & P. Clément, 1993. How females of the rotifer *Asplanchna brightwelli* swim in darkness and light: an automated tracking study. *Hydrobiologia*. <https://doi.org/10.1007/BF00025827>.
- Obertegger, U., G. Flaim & R. Sommaruga, 2008. Multifactorial nature of rotifer water layer preferences in an oligotrophic lake. *Journal of Plankton Research* 30: 633–643.
- Obertegger, U., A. Cieplinski, M. Raatz & P. Colangeli, 2018. Switching between swimming states in rotifers—case study *Keratella cochlearis*. *Marine and Freshwater Behaviour and Physiology*. <https://doi.org/10.1080/10236244.2018.1503541>.
- Overholt, E. P., K. C. Rose, C. E. Williamson, J. M. Fischer & N. A. Cabrol, 2016. Behavioral responses of freshwater calanoid copepods to the presence of ultraviolet radiation: avoidance and attraction. *Journal of Plankton Research* 38: 16–26.
- Patalas, K., 1984. Mid-summer mixing depths of lakes of different latitudes. *Verhandlungen der Internationalen Vereinigung für Theoretische und Angewandte Limnologie Taylor & Francis* 22: 97–102.
- Pennak, R. W., 1973. Some evidence for aquatic macrophytes as repellents for a limnetic species of *Daphnia*. *Internationale Revue der gesamten Hydrobiologie und Hydrographie Wiley Online Library* 58: 569–576.
- Pennekamp, F., N. Schtickzelle & O. L. Petchey, 2015. BEMOVI, software for extracting behavior and morphology from videos, illustrated with analyses of microbes. *Ecology and Evolution* 5: 2584–2595.
- Persaud, A. D., R. E. Moeller, C. E. Williamson & C. W. Burns, 2007. Photoprotective compounds in weakly and strongly pigmented copepods and co-occurring cladocerans. *Freshwater Biology* 52: 2121–2133.
- Pontin, R. M., 1978. A key to the freshwater planktonic and semi-planktonic Rotifera of the British Isles. *Freshwater Biological Association*.
- R core team, 2017. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.
- Ricci, C. & G. Melone, 1998. Dwarf males in monogonont rotifers. *Aquatic Ecology* 32: 361–365.
- Robertson, D. M. & R. A. Ragotzkie, 1990. Changes in the thermal structure of moderate to large sized lakes in response to changes in air temperature. *Aquatic Sciences* 52: 360–380.
- Snell, T. W., R. K. Johnston, B. Rabeneck, C. Zipperer & S. Teat, 2014. Joint inhibition of TOR and JNK pathways interacts to extend the lifespan of *Brachionus manjavacas* (Rotifera). *Experimental Gerontology* 52: 55–69.
- Soleymani, A., F. Pennekamp, O. L. Petchey & R. Weibel, 2015. Developing and integrating advanced movement features improves automated classification of ciliate species. *PLoS ONE* 10: e0145345.
- Sommaruga, R., 2001. The role of solar UV radiation in the ecology of alpine lakes. *Journal of Photochemistry and Photobiology B* 62: 35–42.

- Verschuren, D., T. C. Johnson, H. J. Kling, D. N. Edgington, P. R. Leavitt, E. T. Brown, M. R. Talbot & R. E. Hecky, 2002. History and timing of human impact on Lake Victoria, East Africa. *Proceedings of the Royal Society B: Biological Sciences* The Royal Society 269: 289–294.
- Viaud, G., 1950. Recherches experimentales sur le phototropisme des planaires. *Behaviour* 2: 163–215.
- Wallace, R. L., 2002. Rotifers: exquisite metazoans. *Integrative and Comparative Biology* 42: 660–667.
- Williamson, C. E., O. G. Olson, S. E. Lott, N. D. Walker, D. R. Engstrom & B. R. Hargreaves, 2001. Ultraviolet radiation and zooplankton community structure following deglaciation in Glacier Bay, Alaska. *Ecology* 82: 1748–1760.
- Yúfera, M., E. Pascual & J. M. Olivares, 2005. Factors affecting swimming speed in the rotifer *Brachionus plicatilis* Rotifera X. Springer, Berlin: 375–380.