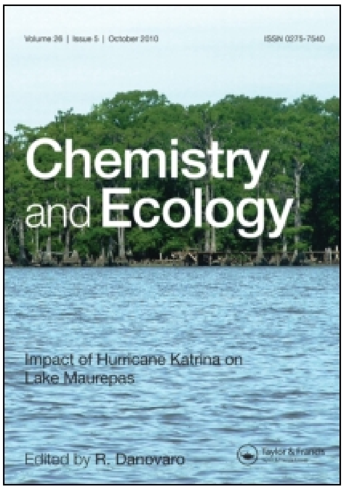

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Exposure to humic material modulates life history traits of the cladocerans *Moina macrocopa* and *Moina micrura*

Christian E. W. Steinberg[a]; Laura Vićentić[ab]; Ramona Rauch[a]; Rihab Bouchnak[ac]; Albert L. Suhett[d]; Ralph Menzel[a]

[a] Department of Biology, Humboldt-Universität zu Berlin, Berlin, Germany [b] Beuth-Hochschule für Technik Berlin, Germany [c] Department of Freshwater Conservation of the Brandenburg University of Technology Cottbus, Bad Saarow, Germany [d] Laboratório de Limnologia, Universidade Federal do Rio de Janeiro (UFRJ) & Post-Graduation Program in Ecology (PPGE/UFRJ), Rio de Janeiro, Brazil

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Exposure to humic material modulates life history traits of the cladocerans *Moina macrocopa* and *Moina micrura*

Christian E.W. Steinberg[a]*, Laura Vićentić[a,b], Ramona Rauch[a], Rihab Bouchnak[a,c], Albert L. Suhett[d] and Ralph Menzel[a]

[a] *Department of Biology, Humboldt-Universität zu Berlin, Berlin, Germany;* [b] *Beuth-Hochschule für Technik Berlin, Germany;* [c] *Department of Freshwater Conservation of the Brandenburg University of Technology Cottbus, Bad Saarow, Germany;* [d] *Laboratório de Limnologia, Universidade Federal do Rio de Janeiro (UFRJ) & Post-Graduation Program in Ecology (PPGE/UFRJ), Rio de Janeiro, Brazil*

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Humic substances (HS) are ubiquitous biogeochemicals and major constituents of all surface waters. HS are internalised by exposed organisms which, in turn, respond with anti-stress reactions. However, the outcome of these reactions is not necessarily negative; rather, it can lead to increases in individual lifespan and lifetime reproductive output. If clones of two closely related species respond differently, HS have the potential to shape the community structure. We obtained clones of *Moina macrocopa* and *Moina micrura* from Brazilian habitats and this potential to affect the community structure was tested with HuminFeed® (HF), a commercial HS preparation exotic to the animals, and with regional HS from a Brazilian coastal lagoon. Upon exposure to HF, the *M. macrocopa* clone responded with only a slight increase in lifespan and reproductive output. However, when exposed to the Brazilian HS, a strong lifespan extension and a reduction in lifetime reproductive output occurred in this clone. Upon exposure to HF, the *M. micrura* clone responded with a reduced lifespan and reproductive output. Upon exposure to HS, none of the *Moina* clones showed a delay in the onset of reproduction. Our results show that HF impacts the clones of two sister *Moina* species differently, and hence has the potential to shape zooplankton communities.

Keywords: *Moina macrocopa*; *Moina micrura*; humic substances; longevity; fecundity; stress ecology

1. Introduction

A classical paradigm states that dissolved humic substances (HS), mainly fulvic acids (FA), are too large to be taken up by aquatic organisms [1–3] and consequently do not interact with their metabolism and DNA. However, recent eco-chemical and ecological studies have shown that FA building blocks have molecular masses of $\sim$0.5 kDa [4]. One may argue that the paradigm cited above might be based on a lack of appropriate experiments in the past. In fact, recent studies with radioactively labelled material have shown that all exposed organisms take up HS and HS-like substances easily [5,6]. After HS are internalised, aquatic organisms try to get rid of them; hence,

*Corresponding author. Email: christian_ew_steinberg@web.de

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it is not surprising that HS provoke all kinds of anti-stress reactions which are well known from exposure to man-made chemicals:

- transcriptionally controlled induction of stress proteins and antioxidant enzymes [7];
- development of oxidative stress symptoms, including lipid peroxidation [8]; and
- induction and modulation of biotransformation enzymes [6,8–12].

We have shown recently that these stress symptoms do not necessarily result in overall adverse effects, such as early death or reduced fecundity, provided that the stress remains mild, i.e. non-lethal; instead, lifespan extension [12–14] and increased fecundity [14,15] have been reported. Longevity is often combined with the acquisition of multiple stress tolerance, as shown in *Moina macrocopa* Straus, which developed an increased osmotic tolerance when pre-exposed to HS (Suhett, unpub.), and in the swordtail *Xiphophorus helleri* Heckel, which also developed an increased tolerance to a physical stressor [16].

This article tries to answer the following questions by evaluating life table data which is common in ageing research:

- Do closely related species show identical, or at least similar, reactions to HS exposure? There are indications that closely related algal species react differently upon identical HS exposures [17]. We therefore tested one clone of each *Moina* species, *Moina macrocopa* and *Moina micrura* Kurz, and exposed them to a HS preparation which has previously been shown to be effective in bioassays with *Caenorhabditis elegans* Maupas and *Daphnia magna* Straus [12–14].
- If the cladocerans respond to HS exposure, is there a difference between exotic and regional (Brazilian) HS? To answer this question, we compared the responses of *M. macrocopa* exposed to the aforementioned HS preparation and to HS from a Brazilian coastal lagoon.

2. Materials and methods

2.1. Moina *species and cultivation*

One clone of each species, *M. macrocopa* Straus and *M. micrura* Kurz, was tested. *M. macrocopa* was isolated from a puddle in Rio de Janeiro, Brazil [18]. The *M. micrura* clone was found in the oligotrophic Lajes Reservoir (Piraí, Rio de Janeiro State) [19] and has been cultivated in the Laboratory of Ecophysiology and Ecotoxicology of Cyanobacteria of the Universidade Federal do Rio de Janeiro since 2002.

Stock cultures of both species were maintained in artificial *Daphnia* medium [20], although with a 10-fold higher bicarbonate concentration to avoid any risk of acidification by the addition of HS. In order to evaluate only the impact of the biogeochemicals on the life performance of *Moina*, further stresses, such as crowding and the subsequent development of males, were avoided. Densities of individuals which allow pure parthenogenetic reproduction were evaluated in pre-tests. If male *Moina* occurred, the density was reduced. All exposure scenarios with the exotic and Brazilian HS were not stressful with respect to crowding.

In the series with the exotic HS (HuminFeed®), animals were pre-cultivated at 0.1 mM dissolved organic carbon (DOC) for three generations, and fifth-generation neonates of both *Moina* clones were used in the experiments. Both cultures and exposures were held at $23 \pm 1\,^{\circ}$C under permanent light. *M. macrocopa* individuals were cultivated in mineral water (Minalba brand, Brazil) when exposed to Brazilian HS. The pH was adjusted to 7.0 with 0.1 M H_2SO_4. The cultures and exposures were held in a climatic chamber at $24.5 \pm 1\,^{\circ}$C, with a 12:12 light/dark cycle.

2.2. *Humic materials and exposure scenarios*

The HS preparation HuminFeed®[1] (HF) was applied to evaluate potential HS-mediated lifespan modulation. HF is processed leonardite, contains 43% organic carbon, and is comprised of 82% humic substances and 18% low-molecular-mass compounds [21]. For the HF series, animals were exposed in 100 mL vessels with five animals in each (10 replicates). The following exposure concentrations were applied: 0, 0.18, 0.54 and 1.08 mM DOC. Vessels were checked daily for dead individuals. The exposed animals were placed into freshly prepared dissolved HF solutions every second day and the offspring were removed and counted.

A small lagoon, Lagoa Atoleiro, in the sandy soil of the Restinga de Jurubatiba National Park (Rio de Janeiro State) was the source for the Brazilian HS. The groundwater in this region is extremely rich in HS, with concentrations of up to 18 mM DOC. HS may account for >90% of DOC; hence, an isolation and enrichment procedure was not necessary. Water was sampled from Atoleiro, filtered in 0.7 μm glass fibre filters (GF 75, Advanctech), and the DOC concentration measured using a Shimadzu TOC-5000 total organic carbon analyser. Samples were diluted to exposure concentrations of 0.83, 1.67, 4.17 and 8.33 mM DOC. The control solution was pure mineral water, as used for the stock culture. The exposures were run in 30 mL vessels with one female neonate in each (10 replicates). The pH of all solutions was adjusted to 7.0 with H_2SO_4 or NaOH to avoid the confounding effects of pH variability.

2.3. *Food*

Clones of both *Moina* species that were exposed to HF were fed the green algal species *Pseudokirchneriella subcapitata* (Koršikov) Hindák, a high-quality food that supports cladoceran

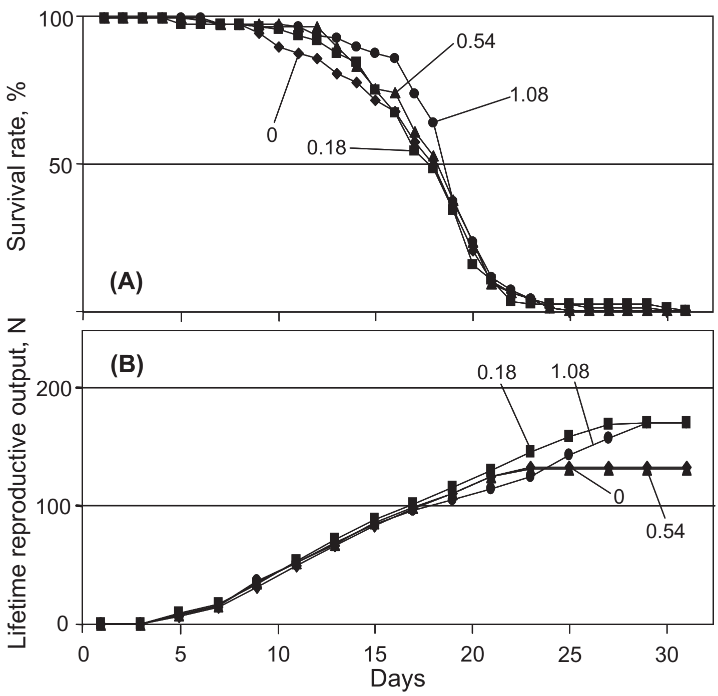

Figure 1. Lifespan modulation (A) and lifetime reproductive output (B) of female *Moina macrocopa* exposed to HuminFeed®, mM DOC, at 23 ± 1°C and in permanent light. Standard deviations are not shown in (B) for the sake of clarity. Diamonds: control; squares: 0.18 mM; triangles: 0.54 mM; circles: 1.08 DOC.

Table 1. Median, mean and maximal lifespan of the tested *Moina* clones exposed to humic substances.

	Median		Mean		Maximal		
HF, mM 24 ± 1 °C permanent light	Days	% of control	Days	% of control	Days	% of control	p (entire lifespan)
Moina macrocopa and HuminFeed®							
0	17.5	100	17.4	100	23.5	100	
0.18	17.5	100.1	17.7	101.7	25.5	108.5	ns
0.54	18.2	103.7	18.1	104.0	22.5	95.7	ns
1.08	18.6	105.9	18.7	107.5	26.0	110.6	ns
	Median		Mean		Maximal		
DOC, mM 24.5 ± 1°C 12:12 light/dark cycle	Days	% of control	Days	% of control	Days	% of control	p (entire lifespan)
Moina macrocopa and Atoleiro DOC							
0	15.0	100	15.6	100	20.0	100	
0.83	24.0	160.0	22.4	143.3	27.0	135.0	<0.0001
1.67	22.5	150.0	21.1	135.0	26.0	130.0	<0.0001
4.17	15.5	103.3	15.4	98.5	22.0	110.0	<0.05
8.33	19.0	126.7	18.7	119.5	25.0	125.0	<0.0001
	Median		Mean		Maximal		
HF, mM 24 ± 1°C permanent light	Days	% of control	Days	% of control	Days	% of control	p (entire lifespan)
Moina micrura and HuminFeed®							
0	14.6	100	15.1	100	22.0	100	
0.18	13.5	92.3	13.8	91.5	19.0	86.4	<0.001
0.54	13.8	94.6	14.3	94.7	22.5	102.3	ns
1.08	13.8	94.8	14.6	96.7	22.0	100.0	ns

Note: ns, not significant.

development well [22]. *P. subcapitata* strain NIVACHL 1 was obtained from the Culture Collection of Algae at the University of Göttingen, Germany. Algae were cultivated in artificial freshwater medium [23] and grown in batch cultures under permanent light at $\sim$40 μmol photons $\cdot$ m^{-2} $\cdot$ s^{-1}. Purified air was pumped through the culture and algae were harvested during logarithmic growth. The animals were fed *ad libitum* with 5 mL of a logarithmically growing culture with $\sim 5 \times 10^6$ cells $\cdot$ mL^{-1}.

M. macrocopa exposed to the Brazilian HS were fed 5 mL of *P. subcapitata* culture with a final density of 10^5 cells $\cdot$ mL^{-1}. The algae were cultivated in MBL medium [24], without vitamin addition, in a climatic room at 20 ± 2 °C, with a 12:12 light/dark cycle, and cells were harvested at the logarithmic phase.

2.4. *Data interpretation and statistical analysis*

Mean, median and maximum lifespan were assessed for each condition. Alterations in the lifespan values of treated versus untreated *Moina* were specified in percentages. The results of the HF series are the means of two independent trials; for the Brazilian HS, we show a representative single trial of a series with almost identical results.

The statistical significance of the entire lifespan was tested by means of the log-rank test, which was specifically developed for lifespan curves (Bioinformatics at the Walter and Eliza

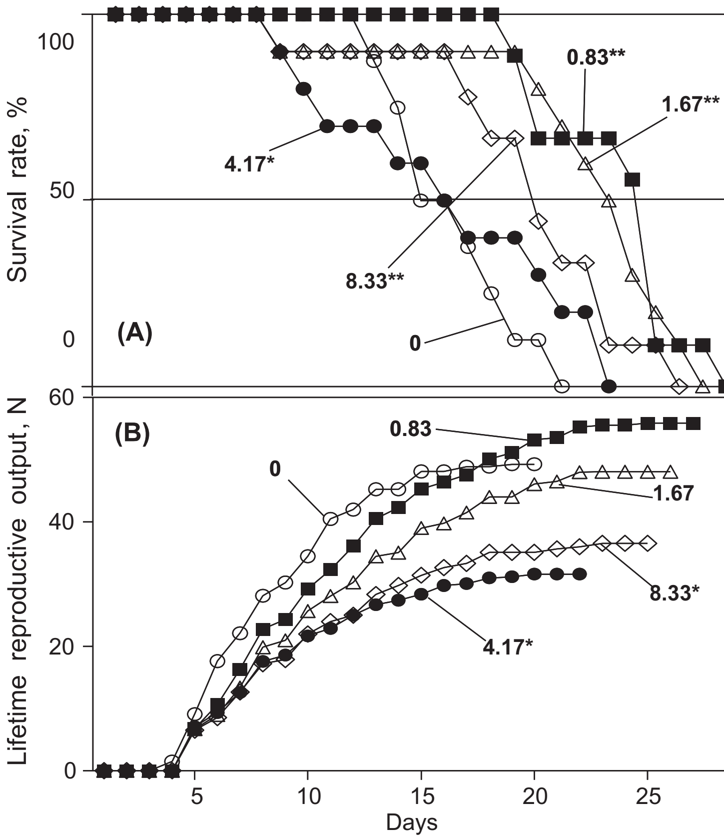

Figure 2. Lifespan modulation (A) and lifetime reproductive output (B) of female *Moina macrocopa* exposed to material from the Lagoa Atoleiro, Restinga de Jurubatiba National Park, Rio de Janeiro State, mM DOC, at 24.5 ± 1 °C with a 12:12 light/dark cycle, $^{*}p < 0.05$, $^{**}p < 0.01$. Standard deviations are not shown in (B) for the sake of clarity. Clear circles: control; filled squares: 0.83 mM; clear triangles: 1.67 mM; filled circles: 4.17 mM; clear diamonds: 8.33 mM DOC.

Hall Institute of Medical Research; http://bioinf.wehi.edu.au/software/russell/logrank/). To gauge reproduction, cumulative offspring numbers are presented to evaluate the potential impact on the timing of the reproduction in the animals. The statistical significance of the reproductive output in the various exposure scenarios was calculated from the means of total offspring per female in each treatment using one-way analysis of variance (ANOVA) on ranks (Sigma Stat 3.5, SPSS Inc., USA). Differences were considered significant at $p < 0.05$ and highly significant at $p < 0.01$. Cumulative curves of the reproductive output are presented in the figures to identify potential phases of reproduction.

3. Results

3.1. *Responses of the* Moina macrocopa *clone*

3.1.1. *HuminFeed®*

When *M. macrocopa* individuals were exposed to HF, there was a tendency for lifespan expansion only at higher HF concentrations (Figure 1(A)), which can be best seen with median and mean values (Table 1). A tendency for increased reproductive output could also be observed at two exposure concentrations, because some individuals reproduced until a few days before death (Figure 1(B)). A delay in the onset of reproduction was not seen (Figure 1(B)).

3.1.2. *Humic substances of the Lagoa Atoleiro*

The aforementioned results for lifespan modulation were obtained with HS material exotic to the animals; however, what happens when they are exposed to natural humic material from habitats

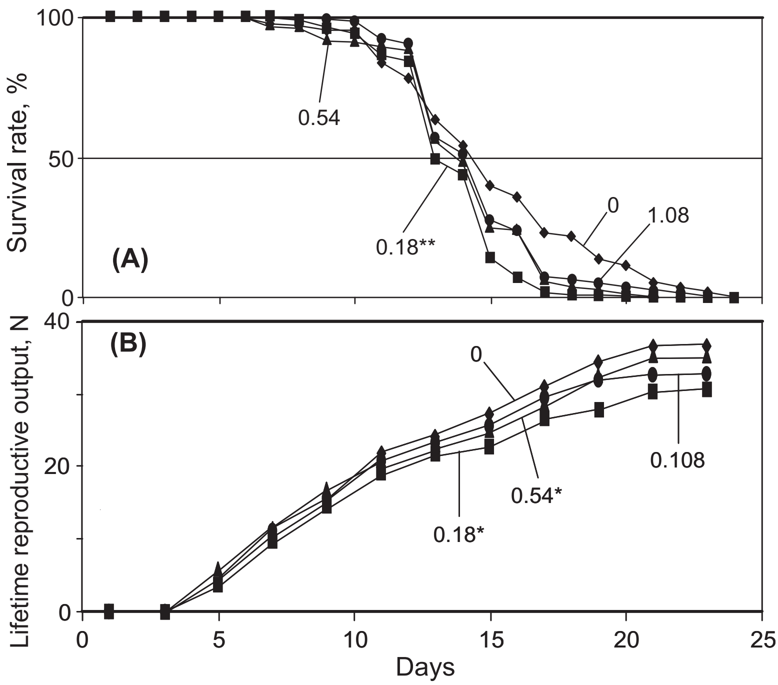

Figure 3. Lifespan modulation (A) and lifetime reproductive output (B) of female *Moina micrura* exposed to HuminFeed®, mM DOC, at $23 \pm 1\,^{\circ}C$ and in permanent light, $^{*}p < 0.05$, $^{**}p < 0.01$.

in the region where these *Moina* clones live? The results for *M. macrocopa* are shown in Figure 2 and given in Table 1: dissolved HS extended *M. macrocopa* survival, with an increase in the median lifespan in 0.83 mM DOC of up to 60% in relation to controls. Exposure to Atoleiro DOC did not significantly affect offspring numbers up to 1.67 mM DOC; higher DOC concentrations reduced the number of offspring significantly. As in previous experiments, the animals reproduced until a few days before death and a HS-mediated delay at the beginning of reproduction was not observed.

3.2. *Responses of the* **Moina micrura** *clone tested*

Exposure of *M. micrura* to HF that was identical to that of *M. macrocopa*. Exposure to 0.18 mM HF significantly reduced the lifespan (Figure 3(A), Table 1), and exposure to 0.18 and 0.54 mM reduced lifetime reproductive output (Figure 3(B)) by ∼20% compared with controls. *M. micrura* females, like *M. macrocopa*, reproduced until a few days before death and a delay in the start of reproduction was not observed. Body growth was also reduced, and optical inspection revealed that growth reduction was combined with an increase in darker pigmentation in the individuals, particularly at the highest HS concentration (data not shown).

4. Discussion

Humic substances are ubiquitous; even in so-called clear-water lakes, they are present at low concentrations (up to 0.1 mM) [25]. However, extreme values of up to 18 mM are reported from Brazilian coastal lagoons [26], many of which still house zooplankters, such as the *Moina* species [27]. The presence of various functional groups enables HS to interact with organisms even at the subcellular, biochemical and molecular biological levels; consequently, HS have the potential to act as natural chemical stressors [6,11,28,29]. Internalised HS lead to the activation of oxygen as one major response pathway [8] and induce anti-stress reactions which are energy-consuming and may eventually be lethal [25,29]. In a recent study, we have shown that the response to HS exposure is transcriptionally controlled [7].

The applied HS exposures did not have lethal effects on the *M. macrocopa* clone; instead they have the potential to expand lifespan and increase fecundity. This potential of HS has recently been shown with *C. elegans* [12] and also *D. magna* [13,14]. There appears to be a difference in the timing of reproduction in *C. elegans* and the cladocerans species: if *C. elegans* is exposed to plant polyphenols, such as catechin or caffeic acid, the onset of reproduction is slightly, and in the case of caffeic acid even significantly, delayed [30,31], whereas the onset of reproduction is unchanged in *D. magna*, *M. macrocopa* and *M. micrura*. Another difference between *C. elegans* and the tested cladoceran species is noteworthy, whereas extension applies only to the post-reproductive phase of the nematode lifespan [12,30,31]; in the cladocerans, even the reproductive phase is extended [13,31]. Because all the tested strains and clones of *C. elegans* and *Moina* spp. are *r*-strategists, the potential advantages of these changes are not yet fully understood, but may affect intra- and interspecific competition [31].

The humic material, HF, used in the first assay is a preparation by alkaline extraction from a leonardite; this means that it is exotic to the exposed animals, which were isolated from water bodies in Brazil. *M. macrocopa* responds to elevated HS exposure with almost unchanged life traits, because only slightly increased lifespan and reproductive output were found. In contrast to the tested *M. macrocopa* clone, *M. micrura* reacted differently upon exposure to the exotic HS material: HF is obviously not lethal, but is slightly toxic to this species and reduces lifetime reproductive output and growth. This indicates that two the related species did not necessarily develop a similar strategy to overcome adverse situations. Comparable results with various HS have been found previously with two sister coccal green algal species [17].

There appears to be an explanation for the lifespan extension on exposure to HS, because it is understood that a large set of genes exists whose activity is linked to longevity; genes and pathways for ageing are also known [32]. We assume that genes or regulatory pathways for ageing, such as the one with the insulin-like growth factor I in its centre, may be blocked by small internalised HS molecules, and the blockage, in turn, may extend the lifespan of exposed individuals.

Lifespan was also extended in *M. macrocopa* exposed to Brazilian HS, but reproductive output was not stimulated. Instead, it seemed unaffected at concentrations up to 1.7 mM, and adversely affected on exposure to higher concentrations. Whether this difference can be attributed to structural differences in the exposed humic materials is open to further study.

5. Conclusion

This article shows that cladocerans do not always respond adversely to mild natural chemical stresses. Exposure to ubiquitous HS at environmentally realistic concentrations clearly impact ecologically relevant parameters in clones of *M. macrocopa* and *M. micrura*, such as lifespan and reproductive output. This should feed back to the population structure, intra- as well as interspecies competition, and succession, because clones of two related *Moina* species responded differently to an identical stress exposure: fecundity did not change in *M. macrocopa*, whereas in *M. micrura* it was reduced.

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Note

1. The use of HuminFeed® is not an advertisement for this product. For more information on this commercial product, the reader is referred to http://www.humintech.com/001/animalfeeds/products/huminfeed.html (accessed June 2009).

References

[1] R.G. Wetzel, *Limnology. Lake and River Ecosystems*, 3rd ed., Academic Press, San Diego, CA, 2001.
[2] R.C. Playle, D. Dixon, and K. Burnison, *Copper and cadmium binding to fish gills: modification by dissolved organic carbon and synthetic ligands*. Can. J. Fish. Aquat. Sci. 50 (1993), pp. 2667–2677.
[3] A.Y.O. Matsuo and A.L. Val, *Acclimation to humic substances prevents whole body sodium loss and stimulates branchial uptake capacity in cardinal tetras* Paracheirodon axelrodi *(Schultz) subjected to extremely low pH*, J. Fish Biol. 20 (2007), pp. 989–1000.
[4] T. Reemtsma and A. These, *On-line coupling of size exclusion chromatography with electrospray ionisation-tandem mass spectrometry for the analysis of aquatic fulvic and humic acids*, Anal. Chem. 75 (2003), pp. 1500–1507.
[5] W.H. Wang, C.M. Bray, and M.N. Jones, *The fate of* ^{14}*C-labelled humic substances in rice cell in cultures*, J. Plant Physiol. 154 (1999), pp. 203–211.
[6] C.E.W. Steinberg, A. Paul, S. Pflugmacher, T. Meinelt, R. Klöcking, and C. Wiegand, *Pure humic substances have the potential to act as xenobiotic chemicals – a review*, Fresen. Environ. Bull. 12 (2003), pp. 391–401.
[7] C.E.W. Steinberg, N. Ouerghemmi, S. Herrmann, R. Bouchnak, M.A. Timofeyev, and R. Menzel, *Stress by poor food quality and exposure to humic substances*: Daphnia magna *responds with oxidative stress and lifespan extension, but reduced offspring numbers*, Hydrobiologia 642 (2010), pp. 224–236.

[8] M.A. Timofeyev, Z.M. Shatilina, A.V. Kolesnichenko, V.V. Kolesnichenko, and C.E.W. Steinberg, *Specific antioxidant reactions to oxidative stress promoted by natural organic matter (NOM) in two amphipod species from Lake Baikal*, Environ. Toxicol. 21 (2006), pp. 104–110.
[9] R. Menzel, S. Stürzenbaum, J. Kulas, A. Bärenwaldt, and C.E.W. Steinberg, *Humic material induces behavioral and global transcriptional responses in the nematode* Caenorhabditis elegans, Environ. Sci. Technol. 39 (2005), pp. 8324–8332.
[10] A.Y.O. Matsuo, B.R. Woodin, C.M. Reddy, A.L. Val, and J.J. Stegeman, *Humic substances and crude oil induce CYP1A expression in the Amazonian fish species* Colossoma macropomum *(tambaqui)*, Environ. Sci. Technol. 40 (2006), pp. 2851–2858.
[11] M. Bittner, J. Janošek, K. Hilscherová, J. Giesy, I. Holoubek, and L. Bláha, *Activation of Ah receptor by pure humic acids*, Environ. Toxicol. 21 (2006), pp. 338–342.
[12] C.E.W. Steinberg, N. Saul, K. Pietsch, T. Meinelt, S. Rienau, and R. Menzel, *Dissolved humic substances facilitate fish life in extreme aquatic environments and have the potential to extend lifespan of* Caenorhabditis elegans, Ann. Environ. Sci. 1 (2007), pp. 81–90.
[13] S. Euent, R. Menzel, and C.E.W. Steinberg, *Gender-specific lifespan modulation in* Daphnia magna *by a dissolved humic substances preparation*, Ann. Environ. Sci. 2 (2008), pp. 7–10.
[14] R. Bouchnak and C.E.W. Steinberg, *Modulation of longevity in* Daphnia magna *by food quality and simultaneous exposure to dissolved humic substances*, Limnologica 40 (2010), pp. 86–89.
[15] S. Höss, M. Bergtold, M. Haitzer, W. Traunspurger, and C.E.W. Steinberg, *Refractory dissolved organic matter can influence the reproduction of* Caenorhabditis elegans *(Nematoda)*, Freshwater Biol. 46 (2001), pp. 1–10.
[16] T. Meinelt, K. Schreckenbach, K. Knopf, A. Wienke, A. Stübe, and C.E.W. Steinberg, *Humic substances affect physiological condition and sex ratio of swordtail* (Xiphophorus helleri *Heckel*), Aquat. Sci. 66 (2004), pp. 239–245.
[17] A.T. Karasyova, O.E. Klose, R. Menzel, and C.E.W. Steinberg, *Natural organic matter differently modulates growth of two closely related coccal green algal species*, Environ. Sci. Pollut. R. 14 (2007), pp. 88–93.
[18] L.M. Elmoor-Loureiro, J.M. Santangelo, P.M. Lopes, and R.L. Bozelli, *A new report of* Moina macrocopa *(Straus, 1820) (Cladocera, Anomopoda) in South America*, Braz. J. Biol. 70 (2010), pp. 225–226.
[19] F.G. Araújo and L.N. Santos, *Distribution of fish assemblages in Lajes Reservoir, Rio de Janeiro, Brazil*, Brazil. J. Biol. 61 (2001), pp. 563–576.
[20] B. Klüttgen, U. Dülmer, M. Engels, and H. Ratte, *ADaM, an artificial freshwater for the culture of zooplankton*, Water Res. 28 (1994), pp. 743–746.
[21] T. Meinelt, T.M. Phan, E. Zwirnmann, A. Krüger, A. Paul, A. Wienke, and C.E.W. Steinberg, *Reduction in vegetative growth of the water mold* Saprolegnia parasitica *(Coker) by humic substances of different origin*, Aquat. Toxicol. 83 (2007), pp. 93–103.
[22] G. Muñoz-Mejía and F Martínez-Jerónimo, *Impact of algae and their concentrations on the reproduction and longevity of cladocerans*, Ann. Limnol. 43 (2007), pp. 167–177.
[23] A. Nicklisch, T. Shatwell, and J. Köhler, *Analysis and modelling of the interactive effects of temperature and light on phytoplankton growth and relevance for the spring bloom*, J. Plankton Res. 30 (2008), pp. 75–91.
[24] H.W. Nichols, *Growth media – freshwater*, in *Handbook of Phycological Methods: Culture Methods and Growth Measurements*, J. Stein, ed., University Press, London, 1973, pp. 7–24.
[25] C.E.W. Steinberg, *Ecology of Humic Substances in Freshwaters. Determinants from Geochemistry to Ecological Niches*, Springer, Berlin, 2003.
[26] A.L. Suhett, F. MacCord, A.M. Amado, V.F. Farjalla, and F.A. Esteves, *Photodegradation of dissolved organic carbon in humic coastal lagoons (RJ, Brazil)*, in *Humic Substances and Soil and Water Environment*, L. Martin-Neto, D.M.B.P. Milori, and W.T.L. Silva, eds., Embrapa, São Carlos, Brazil, 2004, pp. 61–63.
[27] J.M. Santangelo, R.L. Bozelli, A.D.M. Rocha, and F.A. Esteves, *Effects of slight salinity increases on* Moina micrura *(Cladocera) populations: field and laboratory observations*, Mar. Freshwater Res. 59 (2008), pp. 808–816.
[28] C.E.W. Steinberg, S. Kamara, V. Yu. Prokhotskaya, L. Manusadžianas, T. Karasyova, M.A. Timofeyev, J. Zhang, A. Paul, T. Meinelt, V.F. Farjalla, A.Y.O. Matsuo, B.K. Burnison, and R. Menzel, *Dissolved humic substances – ecological driving forces from the individual to the ecosystem level?* Freshwater Biol. 51 (2006), pp. 1189–1210.
[29] D. McMaster and N. Bond, *A field and experimental study on the tolerance of fish to* Eucalyptus camaldulensis *leachate and low dissolved oxygen concentration*, Mar. Freshwater Res. 59 (2008), pp. 177–185.
[30] N. Saul, K. Pietsch, R. Menzel, S.R. Stürzenbaum, and C.E.W. Steinberg, *Catechin induced longevity in* C. elegans: *from key regulator genes to disposable soma*, Mech. Ageing Dev. 130 (2009), pp. 477–486.
[31] K. Pietsch, S. Hofmann, R. Henkel, N. Saul, R. Menzel, and C.E.W. Steinberg, *Caffeic acid affects life traits differently in the nematode* Caenorhabditis elegans *and the cladoceran* Moina macrocopa, Fresen. Environ. Bull. 19 (2010), pp. 1238–1244.
[32] C.T. Murphy, S.A. McCarroll, C.I. Bargmann, A. Fraser, R.S. Kamath, J. Ahringer, H. Li, and C. Kenyon, *Genes that act downstream of DAF-16 to influence the life-span of* Caenorhabditis elegans, Nature 424 (2003), pp. 277–284.