GENDER-SPECIFIC LIFESPAN MODULATION IN *DAPHNIA MAGNA* BY A DISSOLVED HUMIC SUBSTANCES PREPARATION

Sandra Euent, Ralph Menzel, Christian E.W. Steinberg*

Laboratory of Freshwater and Stress Ecology, Institute of Biology, Humboldt-Universität zu Berlin, Germany

Received June 4, 2008; in final form August 13, 2008; Accepted August 21, 2008

ABSTRACT

It is well understood that dissolved humic substances (HSs) are taken up by freshwater organisms and interact on various molecular and biochemical levels, including the development of mild chemical stress combined with lifespan modulation. For instance, with the nematode *Caenorhabditis elegans*, a lifespan extension could be observed with certain HS qualities. Intrigued by this result, we studied the potential of lifespan extension with further animals, for instance the water flea, *Daphnia magna*. Upon exposure to the same HS that caused a lifespan extension in *C. elegans*, the usually parthenogenic females started to produce males. Subsequently, males and females were separately exposed. The lifespans of the females were reduced in a concentration dependent manner, whereby the highest exposure concentration 10.75 mg/L DOC reduced the mean lifespan by more than 25% and the mean numbers of offspring. In contrast, the mean lifespan of males was increased in a hormetic manner: 2.15 mg/L DOC exposure increased it by 20% and 10.75 mg/L DOC by less than 10%. This gender-specific modulation of longevity in *D. magna* synchronizes the lifespans of females and males and allows an increased period of sexual reproduction.

Keywords**:** Natural organic matter, humic substances, lifespan modulation, *Daphnia magna*, gender-specificity

* Corresponding author: Laboratory of Freshwater and Stress Ecology, Institute of Biology, Humboldt-Universität zu Berlin, Germany. Phone: +49 30 6322 4715, fax: +49 30 6369 446, e-mail <christian_ew_steinberg@web.de>

Currently, it is well understood that dissolved humic substances (HSs) are taken up by freshwater organisms [1] and interact on various biochemical and molecular levels [for reviews, see 2, 3]. Since HSs are non-endogenous substrates and the aquatic organisms try to get rid of them after being taken up, it is not surprising that HSs provoke all kinds of anti-stress reactions which are well known in ecotoxicology from organismal response to xenobiotic exposure [4]:

- Induction of stress proteins, such as Hsp70 or crystalline Hsps, as well as induction and modulation of biotransformation enzymes [5, 6];
- Development of oxidative stress symptoms and defense, including lipid peroxidation as a likely antioxidant defense [7, 8].

Although the energy-consuming impacts of HSs on freshwater organisms have traditionally been classified adverse, we have shown that the nematode *Caenorhabditis elegans* (Maupas, 1900) actively looks for such a stressful environment and has been requited by a significant lifespan extension [6], provided the HSs contained high spin concentrations (equals high content of organic radicals, indicative of high reactivity), high C:CH_2 and C:H ratios, and high specific UV absorption [9]. Intrigued by this result, we studied the potential of lifespan extension with further animals, for instance the water flea, *Daphnia magna* (Strauss, 1820).

In order to evaluate whether a lifespan extension of HSs-exposed organisms in fact occurs, *D. magna* was exposed to the same lifespan-expanding HS preparation, HuminFeed®, in two concentrations as *C. elegans* was (2.15 and 10.75 mg/L DOC). This HS preparation[1] is a processed leonardite with the aforementioned features and contains 43 % organic carbon and has already been used in biological studies [6, 9]. Dissolved HuminFeed has a specific UV absorption of 12.5 L mg^{-1} m^{-1} and contains 82.0% humic substances, 18.0% low molecular weight compounds, and 0% polysaccharides [9]. All exposure concentrations are environmentally realistic [7]; also, *D. magna* is common in ponds, including stabilization ponds of simple sewage treatment systems. In these ponds, DOC concentrations may reach 30 mg L^{-1} [10]. Yet information concerning whether or not the DOC affects *D. magna* is completely lacking. *D. magna* was cultured in ADaM artificial *Daphnia* medium [11] for 5 generations prior to exposure. The water fleas were fed with the green alga *Pseudokirchneriella*

[1] For more detailed information on HuminFeed®, the reader is referred to http://www.humintech.com/001/animalfeeds/products/huminfeed.html, accessed June, 2008.

subcapitata, which was pre-cultivated in FW04-medium [12]. The exposures were run in 10 parallels with 10 female individuals each in 100 ml-beakers. The animals were kept in permanent light at 21±1 °C. The exposed animals were counted daily in a white tray and placed into freshly prepared dissolved HuminFeed solution.

Usually, *D. magna* is a cyclically parthenogenic cladoceran. This means that it can combine sexual and asexual reproduction and hence benefits from the advantages of both systems. Currently, it is well understood that the production of parthenogenetic male offspring occurs through environmental cues such as temperature, photoperiod and crowding [13]. Obviously, exposure to HS is an environmental stress, since a few days after the onset of the experiments the females produced parthenogenetic males. Yet, this explanation remains conventional. Recent molecular biological studies demonstrate production of male broods of *D. magna* in response to the sesquiterpenoid hormone methyl farnesoate in concert with ecdysteroids [14, 15]. Interestingly, terpenes are the structural feature of both compounds. Recently it has been emphasized that the major precursors of at least the Suwannee River fulvic acid are tannins and terpenoids rather than lignins [16]; it appears most likely that a moiety of the exposed HSs themselves is internalized and triggers the production of parthenogenetic males.

As soon as males could be identified in the *Daphnia* garden, they were isolated and exposed separately in an identical exposure pattern as the females. The results are presented in Figure 1. Exposed females (Fig. 1A) respond with reduced lifespan at any concentration: un-exposed females have a median lifespan of 105 d, whereas 2.15 mg/L reduces it to 95 d and 10.75 mg/L DOC to only 76 d. This equals a lifespan reduction of almost 28%. The opposite can be seen with males: any exposure applied increased the lifespan. Interestingly, it is a biphasic (hormetic) response with the lower exposure concentration being more effective (expansion by 16% of the control) than the higher one (expansion by only 9% of the control). Under control conditions, median lifespans of males (43 d) are much shorter than those of females (105 d). This equals approximately 40%. Yet this discrepancy is clearly reduced by HSs-mediated stress with 2.15 mg/L DOC increasing the male lifespan to 53% and 10.75 mg/L DOC even to 62% of those of the females. Since even aged *D. magna* females can reproduce well, this gender-specific modulation of longevity synchronizes the lifespans of both females and males and allows an increased period of sexual reproduction.

Gender-specific effects on aging have recently been extensively reviewed for human beings [18] as well as for non-humans such as rodent or invertebrates model species [19]. These studies consider infections and diseases in the case of humans, or physical noxes and triggers, such as heat, cold, hypergravity, or food issues such as caloric restriction in the case of small mammals and invertebrates.

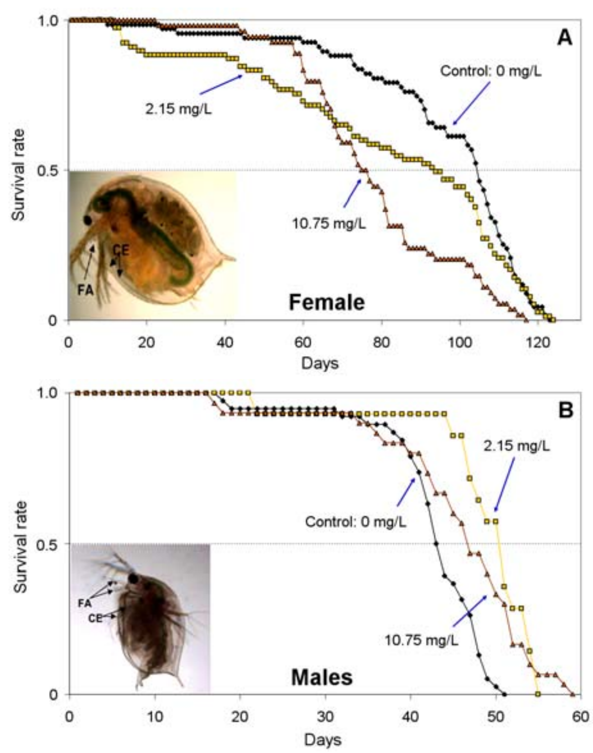

Figure 1 Exposure of *Daphnia magna* to increasing concentrations of HuminFeed® as DOC, a leonardite soil extract rich in phenolics. **A**: Females, lifespan reduction at all concentrations. **B**: Males: lifespan extension at all concentrations. In both graphs, the differences between control and the exposures were significant at the $p<0.01$ level (log-rank test[2], n = 30-77). Note: Time scales (x-axis) in the figures differ! Differentiating sex characteristics include the pair of minute first antennae (FA) of the females, the elongated FA of the males. The bivalved-like carapace of the female has two uniform, symmetrical edges (CE). Both CEs of the male are asymmetrical and are edged by setae. Insert micrographs from [17] with permission.

[2] The log-rank test is available at the Walter & Eliza Hall Institute of Medical Research (http://bioinf.wehi.edu.au/software/russell/logrank/, accessed June, 2008).

Yet to the best of our knowledge, studies on gender-specific longevity modulations in invertebrates by chemical stressors have only occasionally been carried out. For instance, the tropical fruit fly, *Zaprionus paravittiger* (Godbole & Vaidya, 1972), fed on a propyl gallate supplemented diet showed an increased lifespan of females combined with higher activities of antioxidant enzymes [20]. In another study, the cytokinin plant growth hormone kinetin expanded the lifespan of both genders, but this expansion was much more expressed in females than in males [21]. The authors discuss that the anti-aging effects of kinetin are mainly due to a reduction in the age- and gender-specific death rates throughout the adult lifespan.

The adverse effect of HSs on female *Daphnia* must be considered toxic, as not only the lifespan but also the numbers of offspring are affected. With increasing exposure concentrations the numbers decrease to reach only two thirds with the highest concentrations (Fig. 2). With 2.15 mg/L the reduction in offspring numbers was 14%. Toxic effects such as lethality and teratogenicity with freshwater snails or fish embryos have occasionally been observed [22, 23]. Since after the first few generations of offspring the neonates were not allowed any longer to mature, there is no direct information available as to whether or not the HSs stress caused the production of increased numbers of males in order to produce diapausing eggs. Yet the existing papers [13–15] suggest this strategy in *Daphnia*.

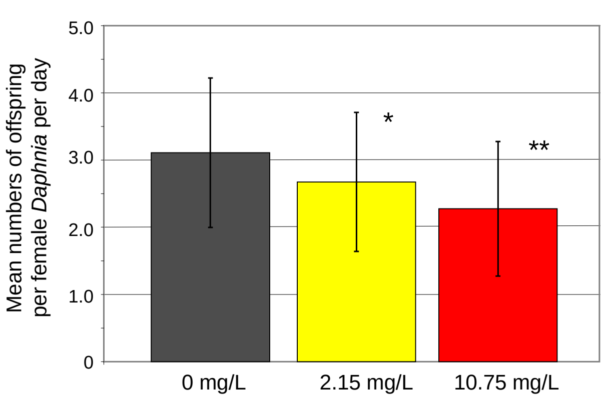

Figure 2 Mean numbers (including standard deviations) of offspring per female *Daphnia magna* per day exposed to increasing concentrations of HuminFeed® as DOC. The differences between control and the exposures were significant at the $p<0.05$ (*) and $p<0.01$ (**) level, respectively (one way ANOVA).

In conclusion, following the observed lifespan modulation in *C. elegans* by dissolved HSs [6], we have presented another example of the fact that these biogeopolymers may serve as a cryptic ecological driving force. Freshwater organisms are exposed to these substances under environmentally realistic conditions and cannot avoid the subsequent chemical stress. There is no need to artificially administer HSs via food or injection - *D. magna* is swimming in these substances and the chemical stress has an ecologically meaningful result. These humic substances modulate the lifespan in the waterflea in a gender-specific way and synchronize the lifespans of females and males. Subsequently, the HS-mediated mild stress allows an increased period of sexual reproduction and hence the formation of diapausing eggs (ephippia) in order to overcome the period of stress.

REFERENCES

[1] Steinberg CEW, Paul A, Pflugmacher S, Meinelt T, Klöcking R, Wiegand C. Pure humic substances have the potential to act as xenobiotic chemicals – A review. *Fresenius Environ. Bull.*, 2003, 12**:** 391–401.

[2] Steinberg CEW, Kamara S, Prokhotskaya VYu, Manusadžianas L, Karasyova T, Timofeyev MA, Zhang J, Paul A, Meinelt T, Farjalla VF, Matsuo AYO, Burnison BK, Menzel R. Dissolved humic substances – ecological driving forces from the individual to the ecosystem level? *Freshwat. Biol.*, 2006, 51: 1189–1210.

[3] Steinberg CEW, Meinelt T, Timofeyev MA, Bittner M, Menzel R. Humic substances (review series). Part 2. Interactions with organisms. *Environ. Sci. Poll. Res.,* 2008, 15: 128–135.

[4] Fent K. *Ökotoxikologie. Umweltchemie, Toxikologie, Ökologie*. 2nd edition. Stuttgart: Thieme, 2003, 232 pp.

[5] Timofeyev MA, Wiegand C, Burnison BK, Shatilina ZM, Pflugmacher S, Steinberg CEW. Direct impact of natural organic matter (NOM) on freshwater amphipods. *Sci. Total Environ.*, 2004, 319: 115–121.

[6] Steinberg CEW, Saul N, Pietsch K, Meinelt T, Rienau S, Menzel R. Dissolved humic substances facilitate fish life in extreme aquatic environments and have the potential to extend lifespan of *Caenorhabditis elegans*. *Ann. Environ. Sci.*, 2007, 1: 81–90.

[7] Timofeyev MA, Shatilina ZM, Kolesnichenko AV, Bedulina DS, Kolesnichenko VV, Pflugmacher S, Steinberg CEW. Natural

organic matter (NOM) induces oxidative stress in freshwater amphipods *Gammarus lacustris* Sars and *G. tigrinus* (Sexton). *Sci. Total Environ.*, 2006, 366: 673–681.

[8] Timofeyev MA, Shatilina ZM, Kolesnichenko AV, Kolesnichenko VV, Steinberg CEW. Specific antioxidant reactions to oxidative stress promoted by natural organic matter (NOM) in two amphipod species from Lake Baikal. *Environ. Toxicol.*, 2006, 21: 104–110.

[9] Meinelt T, Phan TM, Zwirnmann E, Krüger A, Paul A, Wienke A, Steinberg CEW. Reduction in vegetative growth of the water mold *Saprolegnia parasitica* (Coker) by humic substances of different origin. *Aquat. Toxicol.*, 2007, 83: 93–103.

[10] Fadini PS, Jardim WF, Guimarães JR. Evaluation of organic load measurement techniques in a sewage and waste stabilisation pond. *J. Braz. Chem. Soc.*, 2004, 15: 131–135.

[11] Klüttgen B, Dülmer U, Engels M., Ratte H. ADaM, an artificial freshwater for the culture of zooplankton. *Water Res.* 1994, 28:743–746.

[12] Nicklisch A, Shatwell T, Köhler J. Analysis and modelling of the interactive effects of temperature and light on phytoplankton growth and relevance for the spring bloom. *J. Plankton Res.* 2008, 30:75-91.

[13] Hobæk A, Larsson P. Sex determination in *Daphnia magna*. *Ecology*, 1990, 71: 2255–2268.

[14] Eads BD, Andrews J, Colborne JK. Ecological genomics in *Daphnia*: stress responses and environmental sex determination. *Heredity*, 2008, 100: 184–190.

[15] Heckmann LH, Sibly RM, Connon, R, Hooper, H.L., Hutchinson TH, Maund SJ, Hill CJ, Bouetard A, Callaghan A. Systems biology meets stress ecology: linking molecular and organismal stress responses in *Daphnia magna*. *Gen. Biol.*, 2008, 9: R40.

[16] Leenheer JA, Rostad CE. Tannins and terpenoids as major precursors of Suwannee River fulvic acids. *U.S. Geol. Surv. Sci. Invest. Report,* 2004, 1004–5276, 16 p.

[17] Olmstead AW, LeBlanc GA. The environmental-endocrine basis of gynandromorphism (intersex) in a crustacean. *Int. J. Biol. Sci.*, 2007, 3: 77–84.

[18] Candore G, Balistreri CR, Listì F, Grimaldi MP, Vasto S, Colonna-Romano G, Franceschi C, Lio D, Caselli G, Caruso C. Immunogenetics, gender, and longevity. *Ann. New York Acad. Sci.*, 2006, 1089: 516–537.

[19] Minois N. Longevity and aging: beneficial effects of exposure to mild stress. *Biogerontology*, 2000, 1: 15–29.

[20] Bains JS, Kakkar R, Sharma SP. Gender specific alterations in antioxidant status of aging *Zaprionus paravittiger* fed on propyl gallate. *Biochem. Molecul. Biol. Intern.*, 1996, 40: 731–740.

[21] Sharma SP, Kaur P, Rattan SIS. Plant growth hormone kinetin delays ageing, prolongs the lifespan and slows down development of the fruitfly *Zaprionus paravittiger*. *Biochem. Biophys. Res. Comm.*, 1995, 216: 1067–1071.

[22] Steinberg CEW. *Ecology of Humic Substances in Freshwaters. Determinants from Geochemistry to Ecological Niches.* Berlin: Springer, 2003, 440 pp.

[23] Cazenave J, de los Ángeles Bistoni M, Zwirnmann E, Wunderlin DA, Wiegand C. Attenuating effects of natural organic matter on microcystin toxicity in zebra fish (*Danio rerio*) embryos–benefits and costs of microcystin detoxication. *Environ. Toxicol.*, 2006, 21: 22–32.

AES8604