Toxic effects of iron on green and brown hydra

Iron (Fe) is an essential metal for almost all living organisms as it is involved in a wide variety of important metabolic processes. Various human activities can pollute aquatic ecosystems by disposing large quantities of iron compounds[1], which may cause damage to cell structure known as oxidative stress[2]. Hydra (Cnidaria) is found mostly in unpolluted freshwater. Due to its extraordinary regenerative abilities[3], hydra is considered a classical model organism for understanding the fundamental developmental processes[4]. In this experiment, green hydra (*Hydra viridissima* Pallas, 1766), which lives in symbiotic relationship with unicellular green alga[5], and non-symbiotic brown hydra (*Hydra oligactis* Pallas, 1766) were used. The aim of this study was to determine the sublethal effects of iron for two closely related hydra species.

Hydras were collected from Maksimir and Jarun Lakes (Zagreb, Croatia) and kept in laboratory conditions in aerated aquarium water. Five individuals of green and brown hydra were treated with iron dextran. Six concentrations of iron were used for the treatment of green hydra and fourteen concentrations of iron (range from 1 to 4000 mg/l) for the treatment of brown hydra. The experiment lasted for 72 h and the results were compared to the control groups. The experiment was repeated up to three times for each particular concentration.

Various morphological changes such as migration in the experimental dish, changes in body shape, reaction to mechanical stimuli, asexual reproduction and mortality were monitored using a stereomicroscope. Standard histological preparation was performed, using Pearls' reaction that gives blue stained deposits at the site of accumulated iron[6]. Histological slides were analysed by a Nikon Eclipse E600 light binocular microscope and photographed with a Nikon DXM 1200 camera. Lucia G 4.80 software for morphometric measurements was applied after qualitative analysis of histological slides to measure mesoglea thickness by randomly choosing 150 different locations on the sections. The data are shown as mean ± standard deviation (SD). The values of mesoglea thickness between the control and exposed hydra groups were compared with *t*-test (Statistica, $P \leq 0.05$).

We report here the effect of iron on *Hydra viridissima* and *Hydra oligactis* in laboratory conditions. In the control groups hydras were floating in the centre of a water column, but when treated, both green and brown hydras showed permanent migration. When treated with other metals like aluminium, whose chemistry in water is quite similar to iron, hydras reacted alike[7], implying that migration of hydras may be an attempt to search for more favourable micro-environmental conditions (Table 1). In the control group, both species reacted to mechanical stimuli. Exposing hydra to higher levels of iron concentrations slowed down the response, particularly in brown hydra, suggesting that iron

Table 1. Migration of green and brown hydra

Green hydra (%)				Brown hydra (%)			
	Days				Days		
Fe (mg/l)	1	2	3	Fe (mg/l)	1	2	3
C	0	0	0	C	0	0	0
–	–	–	–	0.5	0	0	0
1	0	0	0	1	0	0	0
–	–	–	–	4	0	100	100
–	–	–	–	10	100	100	100
–	–	–	–	20	100	100	100
–	–	–	–	100	0	100	100
–	–	–	–	200	0	100	100
400	100	100	100	400	100	100	100
–	–	–	–	700	100	100	100
–	–	–	–	1000	100	100	100
1400	100	100	100	1400	100	100	40*
1500	100	100	40*	1500	100	100	40*
1600	100	100	40*	1600	100	40*	20*
4000	100	40*	0*	4000	100	20*	0*

*The remaining individuals were dead; –, the experiment was not performed; C, control.

Table 2. Deformations of green and brown hydra

Green hydra (%)				Brown hydra (%)			
	Days				Days		
Fe (mg/l)	1	2	3	Fe (mg/l)	1	2	3
C	0	0	0	C	0	0	0
–	–	–	–	0.5	0	0	0
1	0	0	0	1	0	0	0
–	–	–	–	4	0	0	0
–	–	–	–	10	0	0	0
–	–	–	–	20	0	0	0
–	–	–	–	100	0	0	60
–	–	–	–	200	0	60	40
400	0	20	20	400	100	100	100
–	–	–	–	700	100	100	100
–	–	–	–	1000	100	100	100
1400	100	100	100	1400	100	100	40*
1500	100	100	40*	1500	100	100	40*
1600	100	100	40*	1600	100	40*	20*
4000	100	40*	0*	4000	100	20*	0*

*The remaining individuals were dead; –, the experiment was not performed; C, control.

affected hydra's nervous net and diminished its functionality. As the cross-talk between the nervous and immune system evolved with cnidarians, it is assumed that neurons influence hydra's innate immune response[8].

The body shape of green and brown hydra in the controls was relaxed. At 400 mg Fe/l, green hydra began to relax considerably, while at that level brown hydra contracted intensively, reshaping its body to a sphere-like form, which reduced its body surface, thus preventing further diffusion of toxic substance from the micro-environment (Table 2). Contraction and relaxation are well-known defence mechanisms against unfavourable living conditions[9]. At lower iron concentrations, green hydra individuals reproduced asexually, pointing towards a hormestic effect[10]. Budding in brown hydra was not present, showing a reduced micro-environment adaptation capability. For green hydra LC_{50} was established in the 1400–1500 mg/l, and for brown hydra it was established below 1400 mg Fe/l (Table 3). Acute toxic concentrations of iron used in the laboratory exposures for other studied invertebrates vary between 3 and 400 mg (refs 11, 12). Our results showed that among studied invertebrates, hydras were less sensitive to iron toxicity as they could survive exposure to higher levels of iron concentration.

In addition to morphological changes, histological changes were also observed. Accumulation of iron in the cellular layers of hydra was seen as blue stained deposition (Table 4). The depositions were first found in ectoderm, then in gastroderm (Figure 1). Iron depositions in ectoderm were detected in the apical part of the cells only, pointing towards the possibility of removing the toxicant from

Table 3. Mortality of green and brown hydra

Green hydra (%)				Brown hydra (%)			
	Days				Days		
Fe (mg/l)	1	2	3	Fe (mg/l)	1	2	3
C	0	0	0	C	0	0	0
–	–	–	–	0.5	0	0	0
1	0	0	0	1	0	0	0
–	–	–		4	0	0	0
–	–	–	–	10	0	0	0
–	–	–	–	20	0	0	0
–	–	–	–	100	0	0	0
–	–	–	–	200	0	0	0
400	0	0	0	400	0	0	0
–	–	–	–	700	0	0	0
–	–	–	–	1000	0	0	0
1400	0	0	0	1400	0	0	60
1500	0	0	60	1500	0	0	60
1600	0	0	60	1600	0	60	80
4000	0	60	100	4000	0	80	100

–, Experiment was not performed; C, control.

Table 4. Distribution of iron depositions in the body of hydra

	Green hydra		Brown hydra	
Fe (mg/l)	Ectoderm	Gastroderm	Ectoderm	Gastroderm
C	–	–	–	–
1	–	–	–	–
400	+	–	+	–
1400	+++	++	+++	++
1500	+++	+++	+++	+++

–, No detectable depositions; +, Sporadic dot-like depositions; ++, Dot-like clusters of depositions; +++, Depositions appeared as blue-stained areas.

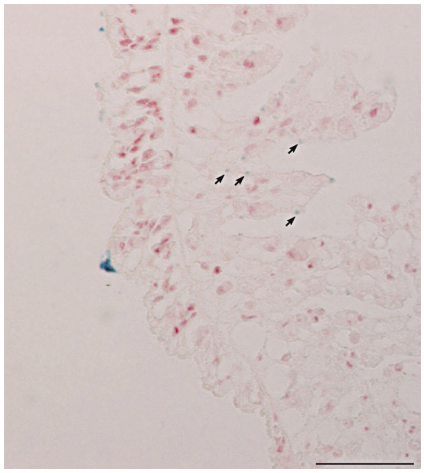

Figure 1. Tissue of brown hydra on the third day of exposure to 1400 mg/l Fe. Arrows point to blue stained iron deposits. Pearls' reaction and 'nuclear fast red'. Bar = 20 µm.

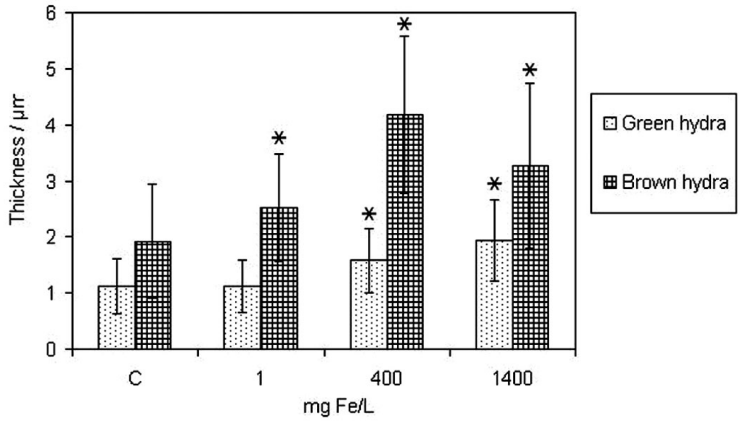

Figure 2. Morphometric measuring of mesoglea, verified various thickness values in green and brown hydra on the third day of exposure to different iron concentrations. Data are presented as mean ± SD. *Indicates significant differences between the mentioned marked concentration for both green and brown hydra and particular control (C); *t*-test by the use of Statistica; $P < 0.05$.

the body of hydra[7]. Long-term exposure to waterborne iron led to a significant accumulation of metal in liver that caused tissue damage in fish[6]. Although iron depositions were not detected in mesoglea, morphometric measurement of mesoglea verified various thickness values (Figure 2), presumably due to its largely non-cellular structure. It is assumed that mesoglea represents a buffer of some sort[13]. Exposure to iron can cause the retention of water in mesoglea, due to its inability to eliminate excess water from the body by contracting[14] and can enhance synthesis of its constituents, which can thicken mesoglea.

In conclusion, non-symbiotic brown hydra exhibited greater susceptibility to iron. Symbiotic green hydra survived better in the given micro-environmental conditions. The present study may point towards the advantages of symbiosis in the living world.

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GORAN KOVAČEVIĆ[1,*,†]
GORDANA GREGOROVIĆ[2,†]
ANA MATIJEVIĆ[3]
MIRJANA KALAFATIĆ[1]

[1]*Faculty of Science, University of Zagreb, Department of Biology, Zoology, Rooseveltov trg 6, HR-10000 Zagreb, Croatia*
[2]*Ministry of Health, Ksaver 200a, Department of Pharmaceutical Inspection, HR-10000 Zagreb, Croatia*
[3]*University Hospital Centre Zagreb, Department of Laboratory Diagnostics, Kišpatićeva 12, HR-10000 Zagreb, Croatia*
**For correspondence.*
e-mail: goran.kovacevic@biol.pmf.hr
[†]Equally contributed to this paper.

Aquilaria malaccensis fruit borer in Peninsular Malaysia

Aquilaria malaccensis Lam. (Thymelaeaceae) has a natural distribution in lowland tropical forests in Peninsular Malaysia, India, Myanmar, Sumatra, Singapore, Borneo and the Philippines. The tree is highly valued for its resin, known as agarwood or popularly known as 'gaharu' in the region, which is utilized in various products such as perfumery, incense, decorative carvings and pharmaceutical products. Agarwood is produced when an agarwood-producing tree is wounded or infected with fungi, microorganisms or insect borers, whereby the borers could also act as a vector of diseases[1]. Only 10% of trees in the wild can become infected by the fungi[2] and produce the much-sought-after resin. Indiscriminate felling of agarwood-producing trees, especially *A. malaccensis*, in the forests has gone beyond control in certain countries. The harvested quantity of agarwood is, however, very low, with less than 0.2 kg per tree for a high-grade resin[3].

A. malaccensis is currently listed as vulnerable according to the IUCN Red List[4] due to overexploitation. Conservation of *A. malaccensis* is important to ensure the sustainability of resources, and this requires an understanding of its reproductive biology[5], which is lacking. Therefore, a series of phenological studies were conducted on wild *A. malaccensis* trees in the forested areas at Penang Island and Perak, Malaysia beginning 2011. The fruits and seeds were also collected from each study site by placing 10–20 square-framed nettings measuring 1 m × 1 m each under the tree prior to the fruiting season for abortion and germination studies. Damaged fruits were scrutinized for the presence of insect pests.

In Penang, one of the aborted and damaged fruits from a total of 1144 had a mature larva living inside and was seen feeding on the fleshy capsule (Figure 1), whereas in Perak a larva was found inside an aborted fruit randomly picked from the ground. A hole measuring about 3 mm in diameter was seen penetrating through the capsule into the fleshy part (Figure 2). The larvae were extracted and

Figure 1. Larva partially concealed within its feeding tunnel (arrow).