Research on Interrelationship between some Species of Freshwater Fish and Helmintic Larvae within Aquatic Ecosystems Polluted with Heavy Metals

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Abstract
The objective of this study was to investigate the ability of some larvae of cestodes and nematodes which live in freshwater fish (intermediate hosts), to exhibit an uptake of heavy metals. According to some scientifical papers treating this subject, only adult worms were able to absorb successfully heavy metals within their hosts. Furthermore, it is believed that only the adults would act as biofilters and consequently as trustworthy indicators of environmental pollution. This study, carried out on the Danube Delta area, comes to prove the ability of the larvae to absorb heavy metals within their hosts, even when the pollution level with respect to heavy metals is very low. Following the biochemical analyses of water, sediment, aquatic plants, larvae and fish tissues (liver and muscle) samples, it resulted that the larvae were able to absorb important quantities within their hosts, so that only scarce amounts to be found in the muscle and liver. Both parasites were able to accumulate some heavy metals within their hosts, although only one of them did it successfully.

Keywords: Parasitic larvae, fish, biofilter for aquatic pollution, heavy metals

1. Introduction
It is known that heavy metals have only several natural sources - mineral rocks, vegetation, salt, sand, volcanic eruptions - and a lot more anthropogenic sources such as car and truck exhaust, engines, rust and industrial and domestic waste disposal. As well as pesticides, heavy metals adhere to sediments, being transported with it by raining water or melting snow and ice into aquatic systems. Once heavy metals have established into the water, they would remain there for a long time, threatening aquatic life. There are two main characteristics following heavy metals exposure of the animals: bioaccumulation and biomagnification. Heavy metals and some of their compounds have a long retention time and bioaccumulate into the tissues since the excretion rate is very low. Biomagnification occurs as a consequence of bioaccumulation of the toxicant at each trophic level within a food chain. Lately, there has been paid a large interest to the link between heavy metals and certain endoparasites like acanthocephals, cestods, nematodes and trematodes [1]. On this issue, there are some statements on which our study based:
- certain heavy metals, such as Cd, Cu, Pb and Zn were found of six to 280 times higher in cestodes and acanthocephals than in the tissues of their definitive hosts [2-4];
- parasitic larvae might not be able to absorb successfully high quantities of heavy metals within their hosts [1];
- some authors [5, 6] inferred that nematodes would be totally unsuitable to indicate environmental pollution if metals were present in low concentrations.

In order to provide data regarding the interrelationship between some species of freshwater fish and helminthic larvae within aquatic ecosystems polluted with heavy metals, we studied larval eustrongilidosis (phylum Nematoda) and ligulosis (Cestoidea - phylum Platyhelminthes) in freshwater fish, from pathological, biochemical and ecological respects.

2. Materials and methods

The study was carried out during September 2008 - September 2009. Seven species of freshwater fish: silver carp (*Hypophthalmichthys molitrix*), roach (*Rutilus rutilus*), rud (*Scardinius erythrophtalmus*), common bream (*Abramis brama*), rapacious carp (*Aspius aspius*), perch (*Perca fluviatilis*) and pike perch (*Stizosteidon lucioperca*) respectively, were collected from the Lagoonar Complex Razelm-Sinoe, Jurilovca province, Tulcea district. Of them, pike perch, perch and rapacious carp were found infected with the nematode *Eustrogylides* sp. larvae, whereas common bream harboured an infection with the cestod *Ligula intestinalis*.

Water, plant and sediment samples were collected from the site. Fish were dissected and the parasites were examined. Afterwards, a number of samples were taken from the muscle and liver. The larvae were also collected so that the biochemical analyses were carried out.

For assessing the biochemical parameters following the natural exposure of fish to Pb, Hg, Cd, Cu and Zn, liver and muscle samples from both infected and non-infected fish, together with the larvae, water, plant and sediment samples, were analysed using Atomic Absorbtion Sectrophotometry and humid digestion methods.

3. Results and discussion

The pathogenicity of the larvae was obvious in both infections. In eustrongylidosis it consisted of congestion, haemorrhages, chronic inflammation, granulomas, ascites and, especially in rapacious carp and perch, severe visceral and mesenteric adhesions (fig. 1). In most cases, the larvae were found free into the body, whilst in rapacious carp only encysted larvae were found.
Table 1. Comparative heavy metal values found in different sort of samples

<table>
<thead>
<tr>
<th>Sample type</th>
<th>Fish species</th>
<th>Pb (μg/kg)</th>
<th>Hg (μg/kg)</th>
<th>Cd (μg/kg)</th>
<th>Cu (μg/kg)</th>
<th>Zn (μg/kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FISH</td>
<td>Silver carp¹</td>
<td>22.75</td>
<td>Abs.</td>
<td>6.72</td>
<td>330</td>
<td>3810</td>
</tr>
<tr>
<td></td>
<td>Pike perch¹</td>
<td>35.83</td>
<td>31.56</td>
<td>10.82</td>
<td>160</td>
<td>4700</td>
</tr>
<tr>
<td></td>
<td>Roach²</td>
<td>14.21</td>
<td>48.92</td>
<td>7.28</td>
<td>270</td>
<td>4740</td>
</tr>
<tr>
<td></td>
<td>Rudd²</td>
<td>96.03</td>
<td>23.93</td>
<td>4.25</td>
<td>660</td>
<td>7800</td>
</tr>
<tr>
<td></td>
<td>Carp bream³</td>
<td>15.67</td>
<td>Abs.</td>
<td>1.20</td>
<td>450</td>
<td>5330</td>
</tr>
<tr>
<td></td>
<td>Perch¹</td>
<td>24.00</td>
<td>35.81</td>
<td>0.98</td>
<td>380</td>
<td>4070</td>
</tr>
<tr>
<td></td>
<td>Rapacious carp¹</td>
<td>24.88</td>
<td>101.03</td>
<td>1.39</td>
<td>540</td>
<td>4080</td>
</tr>
<tr>
<td></td>
<td>Rapacious carp*²</td>
<td>340</td>
<td>46</td>
<td>36</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Common bream³</td>
<td>684</td>
<td>14</td>
<td>9.5</td>
<td>1530</td>
<td>9370</td>
</tr>
<tr>
<td>EUSTRONGYLIDES SP. LARVAE</td>
<td></td>
<td>420</td>
<td>0.9</td>
<td>0.5</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>L. INTESTINALIS LARVAE</td>
<td></td>
<td>397</td>
<td>8.85</td>
<td>14</td>
<td>2420</td>
<td>27720</td>
</tr>
<tr>
<td>WATER</td>
<td></td>
<td>0.600</td>
<td>Abs.</td>
<td>Abs.</td>
<td>3</td>
<td>39.780</td>
</tr>
<tr>
<td>Common reed leaves (Phragmites communis)</td>
<td></td>
<td>132</td>
<td>-</td>
<td>7</td>
<td>730</td>
<td>21.740</td>
</tr>
<tr>
<td>SEDIMENTS</td>
<td></td>
<td>1.492</td>
<td>15.03</td>
<td>Abs.</td>
<td>5360</td>
<td>3</td>
</tr>
</tbody>
</table>

*Liver samples
¹Infected with Eustrongylides sp. larvae
²Infected with L. intestinalis larvae
³Infection-free

The common bream found infected with the plerocercoid L. intestinalis showed abnormal swimming, a slight abdominal dilatation, ascites and visceral adhesion (fig. 2).

From the data available in the table above (table 1) it can be noticed that the infection-free rudd had in the muscle the highest quantities of Pb (96.03 μg/kg), Cu (660 μg/kg) and Zn (7800 μg/kg). Rapacious carp, which was found heavily infected, had in the muscle the highest quantity of Hg (101.03 μg/kg) and Cu (540 μg/kg). In rapacious carp, making a comparison between the level of lead found in the muscle (24.88 μg/kg) and the one found in the liver (340 μg/kg), it follows that the organ was the main structure for lead storage in this fish. Cd levels didn’t exceed in muscle 10.82 μg/kg (value found in pike perch), neither in infected, nor in the infection-free fishes.

In rapacious carp, there can be noticed the difference between the Cd values in the muscle (1.39 μg/kg) and in the liver (36.00 μg/kg). Perch, which was found heavily infected, showed the lowest levels of heavy metals in the muscle, overall: Pb 24.00 μg/kg; Cd 0.98 μg/kg; Zn 4070 μg/kg (versus rudd: 7880 μg/kg Zn). The amount of Pb found in the Eustrongylides sp. larvae was of over 17 times higher than in the fish (24 μg/kg in perch, versus 420 μg/kg in the larvae).

The plerocercoid L. intestinalis did not manage to accumulate heavy metals within its host, although the parasite showed a great affinity for Zn (table 1, 27720 μg/kg), this being also the highest value obtained, with respect to heavy metal containing in the fish and larvae. The cestod only managed to take in of 0.06-3.00 times more than its host.

At the date, a very low level of Pb detection in the water, plant and sediment samples was registered.
4. Conclusions

As a result of the study, it follows that the third and fourth larval stages of the nematode *Eustrogylides* sp. were able to take in important quantities of Pb within their hosts. Counter to some opinions, the third and fourth larval stages of the studied nematode and cestod managed to store more or less Pb, Cd, Cu, Hg, Cd and Zn, even when the metals were almost undetectable in the water.

By far the most successful at bioaccumulating Pb within its host proved to be the young nematode, which follows that it could be a good indicator of a low chronic environmental pollution.

References


2. Tekin-Özan, S., Kir, I., Accumulation of some heavy metals in *Raphidascaris acus* (Bloch 1779) and its host (E. L., 1758), Turkiye parazitoloji dergisi (Acta Parasitologica Turcica), 2007, 31, p. 4


