Master's thesis

Mercury accumulation in the northern pike (*Esox lucius)* and its intestinal parasite, *Triaenophorus nodulosus* (Cestoda)

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ABSTRACT

Mercury may have neurotoxic, immunotoxic and mutagenic effects. A significant source of mercury is the consumption of piscivorous fish where methylmercury accumulates via aquatic food webs. Parasites are essential components of any aquatic ecosystem. Recent studies have shown that heavy metal concentrations, for example, can be hundreds or thousands of times higher in parasite tissues than in the fish host. However, accumulation of mercury in the host vs. parasite has not been examined. We measured mercury content of the muscles of the predatory fish *Esox lucius*, and its intestinal cestode parasite, *Triaenophorus nodulosus*. Pike were collected from Lake Leppävesi in 1996 and 2009. Results of MANCOVA, with pike length as a covariate indicated higher mercury content in males than in females in pike muscle. The result also suggested that mercury concentration was significantly lower in the parasite than in the host. This could be explained by the life cycle of the parasite; the parasites live for just one year in the host, and so have less time to accumulate mercury than the fishes which were between 7 and 12 of age. The mercury levels did not differ in parasites collected from male and female fish. This indicates that there is no difference between the sexes in exposure to mercury. Thus, we explored if the lower mercury in female pike was due to loosing mercury via eggs. This explanation was not supported as it was mercury content of male gonads that were higher, not the female gonads. So, an alternative hypothesis of different growth rate of male and female pike was investigated. We found evidence for this explanation; males grew more slowly and were, therefore, older at a given size than the females. Thus, the males had more time to accumulate mercury and showed accordingly higher mercury concentrations than the female pike.

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Iwu Nnaemeka:	Elohopeapitoisuudet hauessa (*Esox lucius*) ja sen suolistoloisessa, haukimadossa (*Triaenophorus nodulosus*, Cestoda)
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TIIVISTELMÄ

Elohopealla on neurotoksisia, immunotoksisia ja mutageenisiä vaikutuksia. Petokalojen syönti on merkittävä elohopean lähde, koska metyylielohopea akkumuloituu korkealla ravintoketjussa oleviin eliöihin. Loiset muodostavat olennaisen osan kaikkia ekosysteemejä. Hiljattain on osoitettu, että loiset voivat kerätä raskasmetalleja jopa satoja tai tuhansia kertoja enemmän kuin isäntänsä. Elohopean kertymistä isäntään vs. loisiin ei kuitenkaan ole aikaisemmin tutkittu. Tässä työssä selvitettiin elohopeapitoisuuksia petokalan, hauen (*Esox lucius*) lihaksessa ja haukimadossa (*Triaenophorus nodulosus*), joka on hauen suolessa elävä heisimato. Hauet pyydettiin Leppävedestä huhtikuussa 1996 ja 2009. Tulokset osoittivat pituuskorjatun lihaksen elohopeapitoisuuden olevan korkeamman koiraissa kuin naaraissa. Elohopeapitoisuus oli selkeästi alhaisempi haukimadossa kuin hauessa, mikä johtunee siitä, että loinen on yksivuotinen, jolloin siihen ei ehdi kerääntyä yhtä korkeita elohopeapitoisuuksia kuin haukeen itseensä. Mielenkiintoista oli, että haukimadon elohopeapitoisuuksissa ei ollut eroa koiras- ja naarashaukien välillä, mikä viittaa siihen, että sekä koiraat että naaraat altistuvat yhtälailla elohopealle. Korkeammat elohopeatasot koiraissa eivät siis todennäköisesti johdu koiraiden korkeammasta altistumisasteesta. Tästä syytä työssä tutkittiin kahta selittävää hypoteesia: (1) naaraat menettävät enemmän elohopeaa mätijyvien mukana kuin koiraat maidin mukana tai (2) koiraat kasvavat naaraita hitaammin. Gonadien elohopeapitoisuuksissa koiraiden arvot olivat selkeästi korkeammat kuin naaraiden, mikä on ristiriidassa ensimmäisen hypoteesin kanssa. Sen sijaan kävi imi, että koiraat olivat kokoon suhteutettuna vanhempia kuin naaraat. Tämä tukee hypoteesia kaksi ja viittaa siihen, että korkeammat elohopeapitoisuudet koirashauissa johtuvat niiden hitaammasta kasvusta, jolloin tietyn kokoluokan koiraat ovat ehtineet akkumuloida elohopeaa pitemmän ajan kuin vastaavan kokoiset naarat.

Content

1. INTRODUCTION

1.1. Mercury as a global pollutant

Mercury, Hg, is a pervasive pollutant that accumulates in organisms through the food chains (Downs et al. 1998, Morel et al. 1998). Inorganic mercury is less toxic, but organic mercury, mainly methyl mercury (MeHg), is highly toxic to humans and other fish eating animals, such as seals, bears (Clarkson 1997, 1998, Boening 2000). As a result, mercury is probably the most studied of all trace elements in the environment. Mercury rapidly spreads all over the earth from its natural and anthropogenic sources. Mercury is found naturally in the environment in the metallic form and in different inorganic and organic forms.

Mercury being a pervasive pollutant can be dispersed and deposited thousands of kilometers from its original source by atmospheric circulation. As a result elevated Hg concentrations have not only been observed in fish inhabiting aquatic ecosystems receiving effluents contaminated with Hg, but also in areas that are remote from direct sources of Hg pollution (Håkanson *et al.* 1988; Wren *et al* 1991; Cabana *et al*.1994)

Mercury is emitted to the atmosphere by natural degassing of the earth's surface and by re-evaporation of mercury vapour previously deposited on the earth's surface. It is pertinent to note that mercury is emitted in elemental vapour form (Hg°). Annual natural emissions are estimated to be between 2700 and 6000 tonnes (Lindberg *et al.* 1987), some of which originate from previous anthropogenic activity.

Mercury is produced by the mining and smelting of cinnabar ore. It is used in chloralkali plants (producing chlorine and sodium hydroxide), in paints as preservatives or pigments, in electrical switching equipment and batteries, in measuring and control equipment (thermometers, medical equipment), in mercury vacuum apparatus, as a catalyst in chemical processes, in mercury quartz and luminescent lamps, in the production and use of high explosives using mercury fulminate, in copper and silver amalgams in tooth-filling materials, and as fungicides in agriculture (especially as seed dressings). In total, human activities have been estimated to add 2000–3000 tonnes to the total annual release of mercury to the global environment (Lindberg *et al.* 1987). The global cycle of mercury involves the emission of Hg° from land and water surfaces to the atmosphere, transport of Hg° in the atmosphere on a global scale, possible conversion to unidentified soluble species, and return to land and water by various depositional processes, but the total amount of mercury release to the environment is unknown.

The toxicity of mercury depends on its chemical form, and thus symptoms and signs are rather different in exposure to elemental mercury, inorganic mercury compounds, or organic mercury compounds, this is due to the different source exposure of mercury forms (Kjellstrom et al. 1989). For organic mercury compounds, among which methyl mercury is by far the most important, the major source of human exposure is diet, especially fish and other seafood. For elemental mercury vapour, the most important source for the general human population is dental amalgam, but exposure at work may in some situations exceed this by many times. For inorganic mercury compounds, diet is the most important source for the majority of people.

Over the years, there have been epidemics of mercury poisoning to humans, the famous one was the case of Minamata and Niigata Japan, in 1950s and 1960s, in which thousands of people died and many suffered from fatal neurological diseases as a result of consumption of sea food exposed to methyl mercury. The primary targets for toxicity of mercury and its compounds are the nervous system, the kidney and the developing fetus.

1.2. Mercury in aquatic environment

The production of methylmercury via the microbial methylation of inorganic Hg(II) in the environment is a key process affecting mercury concentration in fish (Rosenberg *et al.* 1997*).* Some of the variation in mercury concentration in fish among northern Ontario lakes, Canada, for example is caused by the effects of temperature and lake size via their influence on the microbial net production of methylmercury in the epilimnia (Bodaly *et al* 1993). Specific rates of mercury methylation in those lakes were positively correlated with water temperature, whereas specific rate of methylmercury demethylation were negatively correlated with temperature. Pathways in the lakes, fish activity levels, and bioavailable fractions of MeHg concentrations for uptake at the base of the food chain are factors which affect in-situ methylation or demethylation (Ramlal *et al.* 1993). Cabana *et al* (1994) stated that the dietary uptake of methylmercury in fish is influenced by size, diet and trophic position.

Concerns over mercury toxicity and the ability of methyl-mercury (MeHg) to biomagnify into potentially harmful concentrations in aquatic food chains have led to a need to understanding the cycling of mercury in aquatic environments. Mercury cycle in aquatic ecosystem is complicated because of the myriad of species and pathways, although the understanding of the biogeochemistry of mercury has increased over the past 10 years with the development of clean protocols for the sampling and analysis of mercury (Gill and Fitzgerald 1987, Gill and Fitzgerald 1989).

In the ocean, inorganic mercury is transformed by micro-organisms (in sediments and in the deep ocean) to the methyl mercury form, which is accumulated by aquatic organisms over their lifetime which is termed *bioaccumulation* and then passed up the aquatic food chain. Mercury builds up progressively in the food chain which is termed *biomagnification*. Hence, predatory fish and mammals that are high up the food chain (high tropic level) typically have the greatest levels of mercury. Such species include whales, dolphins, swordfish, marlin, and sharks and these may bio-accumulate mercury to approximately 1 to 10 million times greater than dissolved mercury concentrations found in surrounding waters (US EPA. 2001. *Mercury Update: Impact on Fish Advisories*. EPA-823-F-01-011. Office of Water, Washington, DC.).

In the aquatic ecosystems, mercury concentration in fish is strongly related to mercury concentration in the lake as well as other biological and ecological process in the system. Piscivorous large fish at the top of the aquatic food chains have the ability to ingest large prey (Werner and Gilliam 1984; Mittelbach and Persson 1998), and often have higher mercury concentration than herbivorous fish species that feed on smaller organisms at the base of a trophic chain (Campbell *et al*. 2003c; Gorski *et al*. 2003; Kidd *et al*. 2004). Such trophic transfer and biomagnification of mercury at high trophic levels led to the understanding that predatory fish are important concentrators of mercury, main source of mercury to humans and serve as an indicator of mercury contamination in aquatic system (Dorea *et al*. 2004).Biomagnification of Hg is the main accumulation pathway and led to the highest total Hg concentration in species highest in the aquatic food chain, as well as an increasing Hg concentration with increasing length, weight, and age for most fishes in freshwater lakes (Petri 2003). .These features of mercury in aquatic ecosystem make it essential to incorporate analysis of food web structure when studying mercury concentration in fish, so that trophic transfer and biomagnification property of the contamination could be better understood.

1.3 Heavy metal and fish parasites

Parasites area an essential component of the aquatic ecosystems. It has been shown that parasites have a remarkable effect on the function of some aquatic communities (Kohler and Wiley 1997; Thomas *et al* 1998). However, their role in the cycle of contaminants has been largely ignored. Recent studies have emphasized the significance of parasites in bioaccumulation of contaminants in their hosts (Sures *et al* 1994), and Sures and Taraschewski (1995) have found concentrations of lead and cadmium as much as 400 and 2,700 times higher in intestinal acanthocephalan parasites than in host fish tissues, respectively. In addition, mollusks infected by trematode parasites contain higher concentrations of organic halogen compounds (Pellinen *et al* 1994) and heavy metals (Kraak and Davids 1991) than uninfected individuals.

Parasites are also attracting increasing interest as environmental indicators due to the variety of ways in which they respond to anthropogenic pollution. Sures and Trachewski (1995) in their experiment showed how acanthophelans act as cadmium sink in fish host. In their experiment, cadmium concentration in parasites were compared with those found in different tissues (liver, muscle and intestine) of their final host, chub and perch. They found out that the parasites showed several times more cadmium than did the tissues of the fish hosts, indicating that some parasites like acanthophelans act sinks to heavy metals, thereby protecting fishes from heavy metal toxicity. The majority of recent investigations have examined the effects of various forms of pollution on the abundance and distribution of parasites (Lafferty 1997, Blanar *et al* 2009), but nowadays there is also an increasing number of papers dealing with the accumulation of toxins within parasites. Changes in the diversity and structure of parasite communities of different fish hosts have therefore received increasing attention due to the possible application of parasites as indicators of ecosystem integrity and health (see review by MacKenzie *et al* 1995). So far, the interaction between mercury accumulation in fish and parasites has not been studied. Thus, it is important to get information about the accumulation of mercury in the host and the parasite tissues, and to figure out the possible role of parasites in the accumulation of mercury in the host organisms used by the human population, like the northern pike, *Esox lucius.*

1.4. Life cycle of *Triaenophorus nodulosus*

Pasternak *et al* (1999) explained that copepods often serve as first intermediate hosts for tapeworms of fishes. Copepods acquire the free-living coracidia larvae of cestodes through feeding. Transmission of the parasite procercoid larva to the next host in the life cycle, fish (second intermediate host), is also achieved via trophic interaction: fish ingest an infected copepod. Finally, the infected second intermediate host fish, such as burbot, together with the plerocercoid larva of *T. nodulosus,* is consumed by the definitive host. Parasites may accumulate to pike throughout the year. Adult parasites live in the intestine of pike at the maximum of one year—from spring to the next spring—and develop eggs which are released to the water with the worms annually during the spawning period of pike.

1.5 Objectives of the study

Purpose of this study was to analyze the mercury concentration of the predatory fish host *Esox lucius* and its intestinal parasite *Triaenophorus nodulosus* (Cestoda) and their associations with the size and sex of the host. Following questions were addressed:

1. Are there differences between male and female pike in mercury concentration of fish muscle?

2. Are there differences in mercury concentration of the parasites between male and female pike?

3. Are there differences in mercury concentration of the gonads of fish between male and female pike?

4, How the mercury concentration in fish muscle and in parasite is related to length and age of fish?

The materials consist of year 2009 samples from Lake Leppävesi, Central Finland, and samples of a similar work done at the same lake in 1996. Thus, the study will also indicate if any long term changes in mercury concentration of pike have taken place in Lake Leppävesi during the 13 years from 1996 to 2009.

2. MATERIALS AND METHODS

2.1. Study site

Lake Leppävesi (Fig. 1), located in Central Finland, is an eutrophic lake with surface area of 63.59 km^2, mean depth of 11m and water retention time of 32 days. The lake is ice covered from late November through mid may, is stratified during the summer and reaches the highest temperature of 18-20^{o}C in late July or August. According to the Finnish Environment Institute, the water quality is graded as good in the lake. Southern Lake Leppävesi discharges into Northern Leppävesi but remains uncontaminated by the effluents. Northern Leppävesi, the actual location of the present fish samples, flows into Lake Päijänne through Vaajakoski, where the mean flow (1961–79) is 155 m^3/sec. The annual average oxygen concentration is usually more than 9 mg/l in the hypolimnion of the Northern Leppävesi during periods of stagnation in most summers. In Southern Leppävesi the oxygen concentration is 8 mg/l (http://www.ymparisto.fi/default.). Lake Leppävesi was chosen for the research because it was established by our pilot study that *E. lucius* in this lake have an intermediate level of mercury and are frequently infected by the cestode, *Triaenophorus nodulosus*.

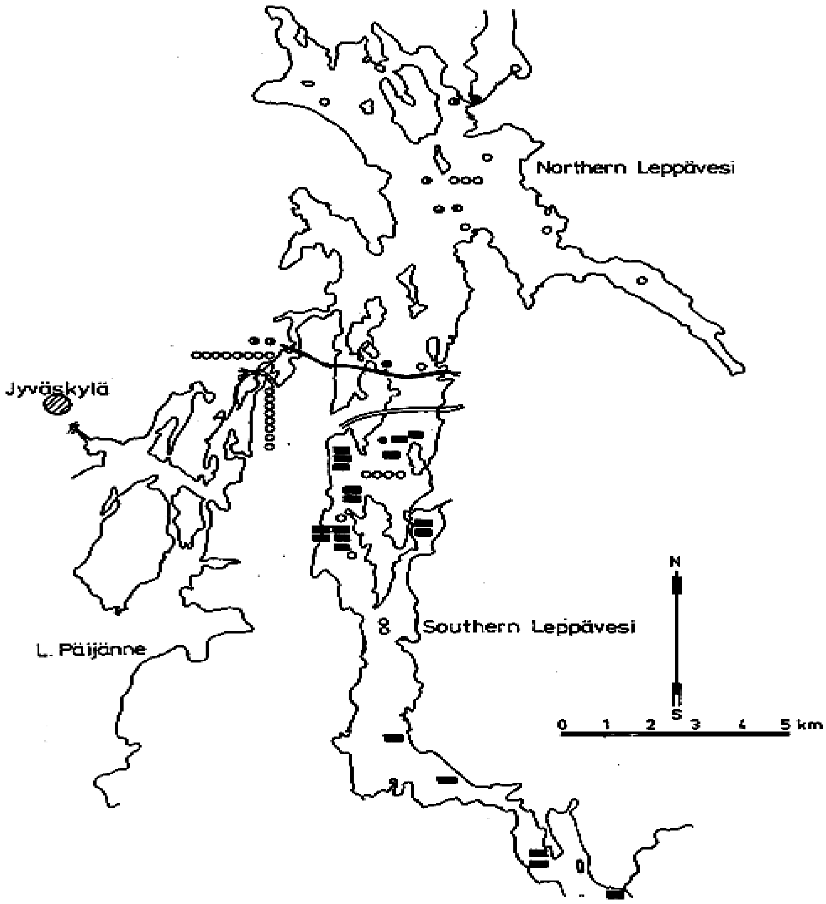

Figure 1. Map of the study site, Lake Leppävesi.

2.2. Sample preparation

Eighteen (n=18) *Exos lucius* were caught randomly from the northern part of the lake with gill net in the last week of April 2009, at the time the lake was covered with ice. In the laboratory, the fish were first weighed and measured for length. Then the fish were carefully dissected and a piece of the dorsal muscle and gonad tissues were removed and sealed, and stored frozen. After that the gut was removed and carefully dissected for removal of the *T. nodulosus* parasites. Parasites were identified using a dissection microscope and transmitted light by pressing them slightly between two large glass plates. In addition to *T. nodulusus*, the nematode parasite *Raphidascaris acus* occurred frequently in the studied fish. *Triaenophorus nodulosus* parasites, which occurred in all the fish examined, were counted, weighed and stored frozen. Fish scales and cleithrum were also removed for age determination of the fish.

After that, all the samples containing the gonads, *T. nodulosus* parasite and muscle tissue, were sent to the laboratory of Institute of Environmental Research of the University of Jyväskylä for mercury content analysis, and the fish scale and cleithrum were used to determine the age of the fishes. Using similar methods, 18 *E. lucius* were caught and studied from the lake in the last week of April 1996. However, in 1996 only the fish muscle and *T. nodulosus* parasite tissues were studied for mercury content, not the gonads. In addition, age of fish was not determined in 1996.

2.3. Mercury analysis

Total mercury was determined in the laboratory of Institute of Environmental Research of the University of Jyväskylä by cold vapor atomic absorption (Perkin Elemr FIMS-400 instrument) with SnCl2 reduction after boiling the samples 30 min with a mixture of conc. HNO3 and H2SO4. Reagent blanks, duplicate samples and a representative reference material were used for analytical quality control purposes. Measurement uncertainty (2U = 95%) was 12 %.

2.4. Data analysis

Multivariate covariate analysis of variance (MANCOVA) was used to study the dependence of mercury content on fish sex and study year in different sample tissues. In MANCOVA, the mercury content in different tissues (fish muscle, parasite) was used as the response variable, while fish sex (male, female) and study year (1996, 2009) were used as fixed factors, and fish length as the covariate. Differences between years (1996, 2009) were analyzed separately using ANCOVA with mercury content in fish muscle as the response variable, year and sex as fixed factors and length as the covariate. MANCOVA on year 2009 material was performed to analyze mercury content in the three different tissues (fish muscle, fish gonad and parasite) with fish sex as a fixed factor and fish length as a covariate. To study the difference in mercury content between male and female gonads, year 2009 materials and ANCOVA were used with mercury content in the gonads as the response variable, sex as a fixed factor and fish length as a covariate. To study if male and female pikes have different growth rates, year 2009 materials and MANOVA were used with fish age and length as the response variables, and sex as a fixed factor. All the data were analyses using SPSS 17. In the text and figures, means are given with ± one standard error (S.E.) of the mean.

3. RESULTS

3.1. Effect of year, sex and length of fish

Multivariate results of MANCOVA did not indicate significant differences in mercury content of fish with respect to year, sex, year-sex interaction or fish length. However, meanwhile the univariate results suggested a significant effect of fish sex ($F_{(1, 32)} = 5.113$, $p = 0.032$) and marginally significant effect of fish length ($F_{(1, 32)} = 3.742$, $p = 0.064$) on the mercury content in fish muscle. Therefore, another MANCOVA was performed with fish sex, only, as a fixed factor and fish length as a covariate. In this case the multivariate results of MANCOVA indicated significant effect of both fish sex and length on mercury content (Table 1). Univariate tests suggested that the effect of sex and length were due to differences in fish muscle (Table 1) so that the mercury content was higher in males than in females (Fig. 2), and that the mercury content increased with pike length. However, the univariate statistics indicated that the mercury content of the parasites tissue was not affected by host sex or host length (Table 1, Fig.2).

Table 1. Multivariate Wilk's lambda and univariate F statistics of multivariate analysis of mercury content with length of fish as a covariate.

	Wilk's lambda		Fish muscle		Parasite	
Effect	F	p	F	p	F	p
Sex	3.373	0.049	6.261	0.018	1.296	0.264
Length	4.248	0.024	8.078	0.008	1.481	0.233

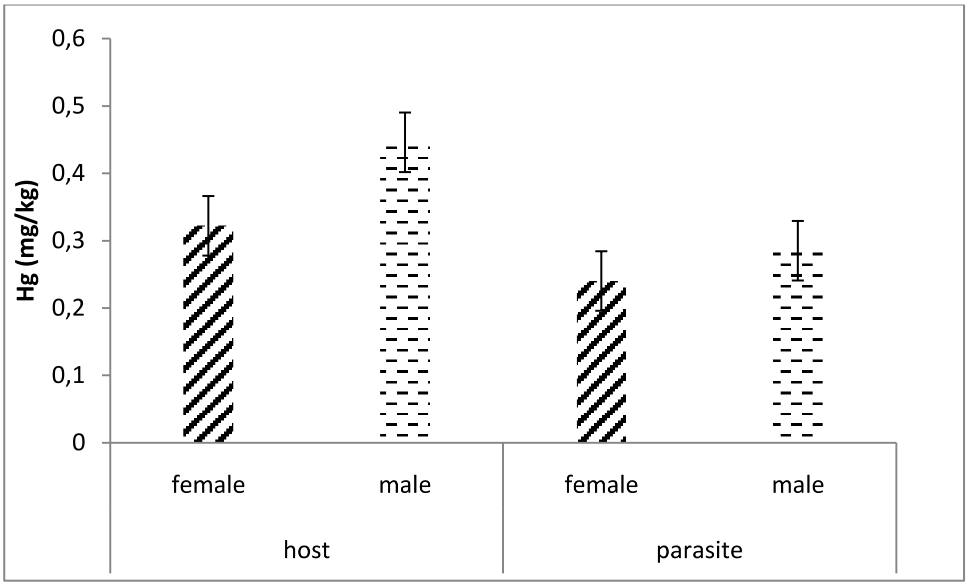

Figure 2. Length adjusted mean (± S.E) mercury content of fish muscle and *Triaenophorus nodulosus* parasite in 10 male and 8 female northern pike, *Esox lucius*, collected from Lake Leppävesi, 2009.

Although a slight increase in the mean mercury content of pike muscle from 1996 to 2009 was detected (Fig. 3), results of a separate ANCOVA on mercury content in pike muscle indicated that the difference between years 1996 and 2009 was not significant ($F_{(1, 32)}$ = 0.324, p = 0.574). However, also the ANCOVA results verified the higher mercury content in males than in females in pike muscle ($F_{(1, 32)}$ = 5.113, p = 0.032).

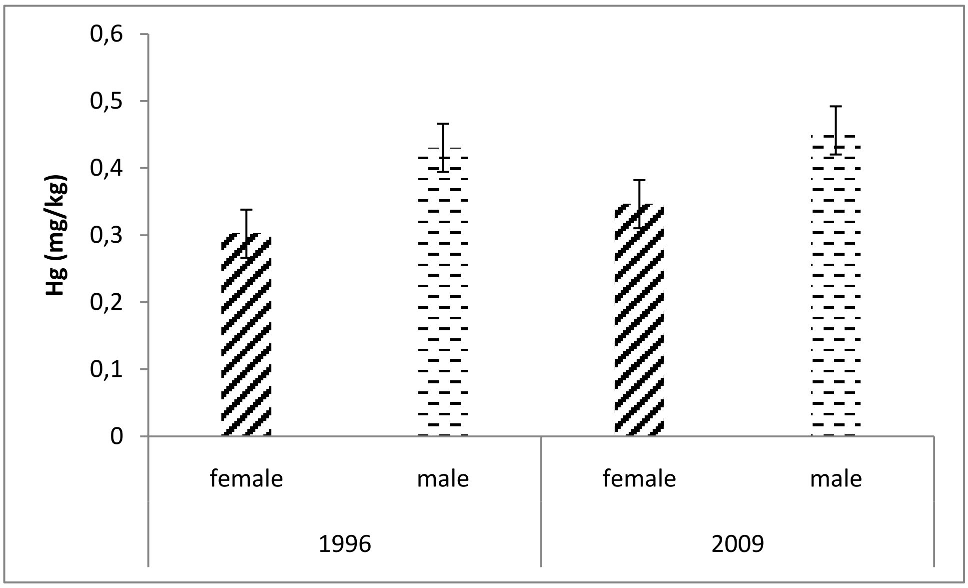

Figure 3. Length adjusted mean (± S.E) mercury content of pike in muscle in male and female fish in Lake Leppävesi in 1996 and 2009.

3.2. Exploration of two hypotheses: Females loose mercury with eggs or male pike grow more slowly?

Two hypotheses were proposed to explain the observed sex difference in fish muscle. First, mercury content may be higher in males if the eggs (roe) contain more mercury than the milt, i.e. females loose mercury with eggs. Second, mercury concentration may be higher in males if the males grow more slowly than the females—males of a given size have had more time to accumulate mercury than females of the same size.

Results of ANCOVA suggested the effect of sex on mercury content of gonads was significant ($F_{(1, 17)} = 9.754$, $p = 0.007$). However, opposite to our first hypothesis, mercury content was higher in the gonads of males than in females (Fig. 3).

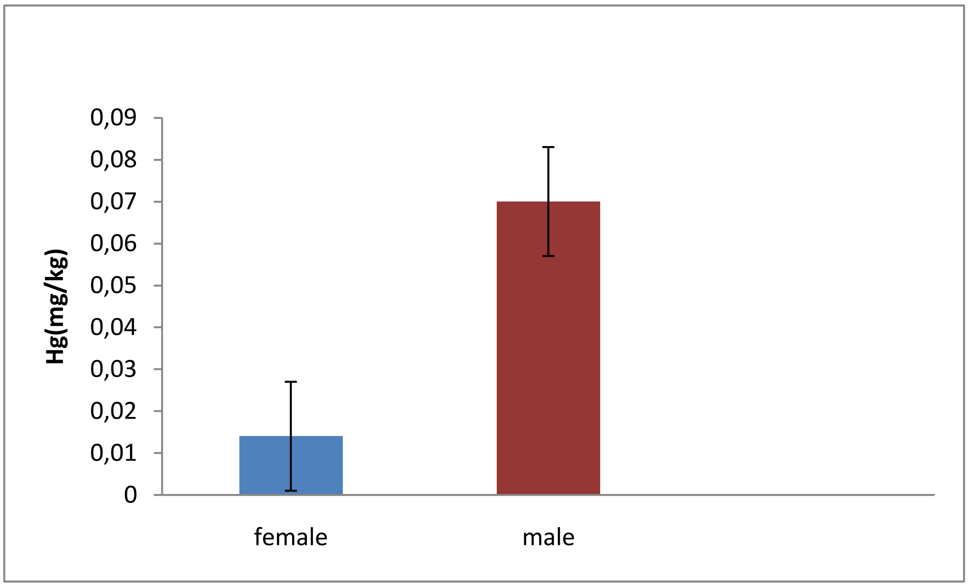

Figure 3. Mean (± S.E) mercury content of gonads of male and female pike in 2009 materials from Lake Leppävesi.

Multivariate results of MANOVA indicated that there was a general sex-difference when the age and length of males and females was analyzed (Wilk's lambda = 0.639, $F_{(2, 14)}$ = 3.951, P = 0.044). Univariate results revealed that there was a significant age difference between males and females ,[$F_{(1, 15)} = 7.165$, $P = 0.017$], the males being older (Fig. 4). However, by length the males and females did not differ ($F_{(1, 15)} = 2.819$, $P = 0.114$, Fig. 5). Thus, in the materials from Lake Leppävesi 2009, males and females were of the same size but males were about 1,5 years older than the females, i.e. they grow more slowly than the females.

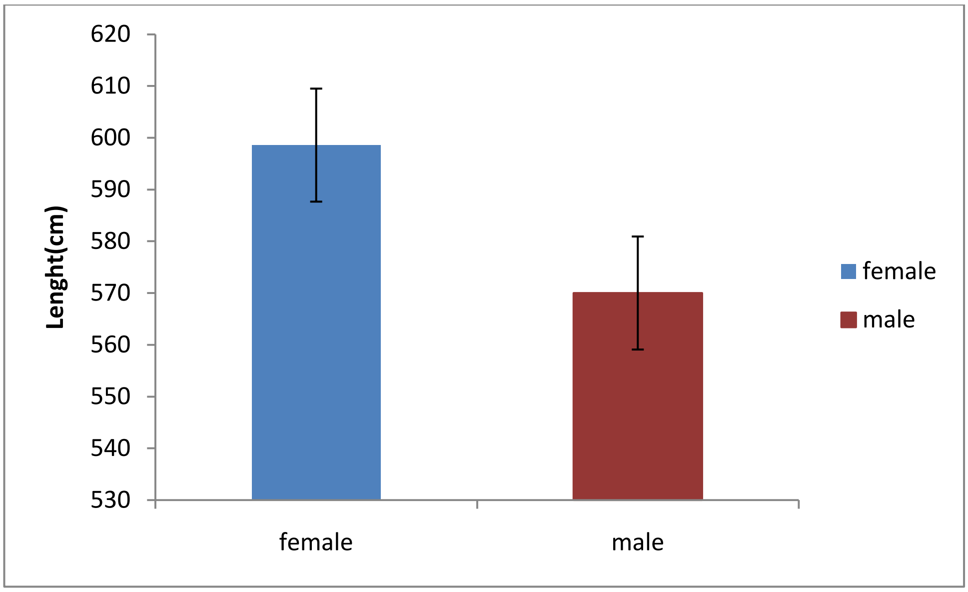

Figure 4. Length adjusted mean (± S.E) length of male and female pike in Lake Leppävesi 2009.

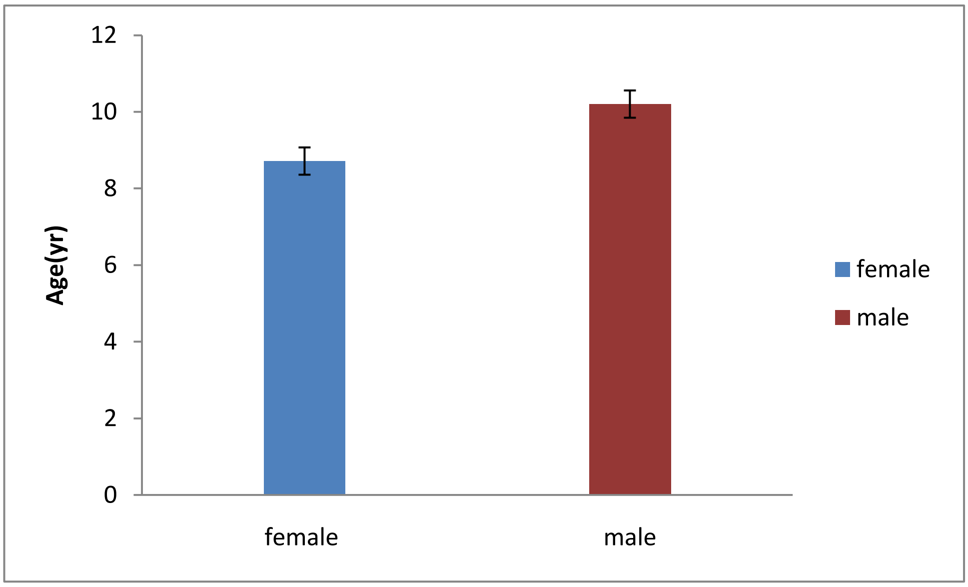

Figure 5. Length adjusted mean (± S.E) age of male and female pike in Lake Leppävesi 2009

3.3. Exploration of the protection hypotheses: Is high intensity of infection associated with low mercury level in fish?

Intensity of *T. nodulosus* infection did not correlate with mercury content of the fish muscle ($n = 32$, Pearson correlation coefficient = 0.221, $P = 0.232$). Thus, mercury level in pike muscle was not related to the number of cestode individuals in the gut.

4. DISCUSSION

Within a given fish population, concentration of methylmercury in muscle tissue or whole fish typically increase with increasing age and size, a pattern that has been observed repeatedly in surveys of mercury in fishes (Wiener and Spry 1996).The increasing concentration with age results from the very slow rate of elimination especially methylmercury by fish relative to its uptake (Huckabee *et al* 1979). Typically, younger individuals show lower mercury levels than adults, because mercury levels increase as individuals get older (Adams and McMichael 1999). This can be explained by bioaccumulation, distinct feeding habits in young and adult individuals or higher growth rates in juveniles resulting in growth dilution and shorter exposure. Increasing mercury content with increasing fish size was also observed in the present study.

During sample preparation and collection, there may negligible sources of error as the fishes were accurately measured, and weighed. The only possible error occurrence may be during fish age determination, as it was not an easy task, but at the end of the project the exercise was done with the help of a professional. Also the proof of result reliability was that the same methods of sample preparation was used in 1996 and 2009, fish caught by the same fisherman, and the mercury analysis was done in the same laboratory and the same method was used in 1996 and 2009.

One of the main results of the present study was the higher mercury concentration in male pikes when compared to females. Two possible hypotheses were proposed to explain this pattern: (1) females loose mercury via eggs during spawning, or (2) male pikes grow more slowly and thereby accumulate more mercury (are older at a given age). Results did not support the first explanation; mercury concentrations were remarkably higher in male gonads compared to females. However, the present results indicated that the male pike grow more slowly than the females, indicating that the observed sex-difference may be due to a longer time of accumulation of mercury in the male fish. The result is in line with findings by Forrester *et al.* (1972) who stated that if growth rate in males is slower, then females of the same length are younger with less time for mercury exposure. Differences in mercury concentration due to different growth rate have also been explained by "growth dilution" associated with high growth rate of fish (Lyle 1984).In a study carried out by Verta *et al* (1986) they found out that the growth rate of northern pike did not correlate significantly with the Hg concentration. The effect of the growth rate on Hg accumulation in the lakes became evident when they further compared lakes with unusual high pike Hg levels compared to its prey were compared to lakes with normal pike Hg concentrations. The results showed that the pike in high-mercury-level lakes grew at a significantly slower rate than those in the other lakes. The negative effect of fish growth rate on mercury accumulation in fish was also reported by Rodgers (1996) and Harris and Watras (1996). In addition, Trudel and Rasmussen (1997), in their critical analysis of experimental data on methylmercury elimination by fish, showed that elimination of methylmercury in fish is negatively correlated to body size.

Until now different helminth species have been investigated in respect of their heavy metal accumulation capacity (Sures *et al* 1999). The most promising parasites are acanthocephalans, a group of intestinal worms, commonly found in fish. Parasite data can be utilized when analyzing the difference in mercury concentration between males and females in the present study. The fact that mercury content in the intestinal parasite, *T. nodulosus*, did not differ between male and female pike can be interpreted so that both sexes are equally exposed to mercury through their diet. Mercury content in the parasite reflects the exposure during the last year because the parasite *T. nodulosus* has an annual life cycle, but there may be considerable variation in the age of the parasites. Each year a new parasite generation infects the pike in the spring/early summer, grow during the year, and die off and release their eggs next spring at the time of pike spawning. Therefore it can be concluded that the higher mercury level in male pike is not due to differential exposure to mercury by male and female fish, but rather due to other factors, such as differential elimination of mercury in males and females, or longer duration of exposure and accumulation in males.

Wiener and Spry (1996) hypothesized that storage of methylmercury in skeletal muscles serves as a protective mechanism in fishes, given that sequestration in muscles reduces the exposure of the central nervous system to methylmercury. Relating this to the present study, it could be hypothesized also that the intestinal cestodes would “protect” pike from exposure to mercury. Sures and Tarachewski (1995) have calculated intestinal acanthocephalans may protect fish host against cadmium exposure due to the accumulation of Cd to acanthocephalans in large quantities. Present results do not support the view that *T. nodulosus* would give any protection against exposure to mercury in pike. The result revealed that the there is less mercury in the parasites than in the pike host. This is probably because *T. nodulosus* parasites live for just one year in the gut of the host, which makes their accumulation level of mercury relatively small.

The result of this research can be compared with result of other related work done in finland, Verta *et al* 1986, demonstrated that the mean Hg concentration in the 1 kg standard pike in 67 lakes situated throughout the country was 0.56 µg g^{-1}. This result is closely related to our findings (0.3-0.45 µg g^{-1}). We can draw conclusion here relating our lake of study that the Hg concentration in Lake Leppävesi has not really increased or decreased much even after a number of years between 1996 and 2009.

There was a slight increase in the mercury content in Lake Leppävesi in respect to year 1996-2009, but it could be said that the difference is not significant. Thus, over the years, mercury in fish has not decreased in the lake even as mercury output from anthropogenic sources has decreased. Thus the changes in aquatic food web and especially in predator fish seem to be slow.

In conclusion, this research has demonstrated that there is more Hg accumulation in muscles of male northern pike than the female fishes, probably due to the fact that the male pike grow more slowly than the female pike, hence they have more time to accumulate more Hg than the female pike. Also, the fact that the mercury content in the parasite *T nodulosus* did not differ between male and female fish showed that both sexes are equally exposed to mercury via their diet—Hg content in the parasite represent only one year of accumulation, because the parasite live for one year in the host. There is an insignificant increase of Hg in the lake over the 13 years, indicating slow changes in mercury levels in natural fish populations.

Lipid and protein content may affect the accumulation and concentration of mercury Therefore, it will be a good idea for future researchers to take into account the lipid and protein content of the examined tissues.

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