



# Impact of Nuclear Power Plant on Subarctic Habitat and Fish Metabolism

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### Research Article

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## Abstract

The Kola nuclear power plant, which discharges warm water into subarctic Lake Imandra, significantly changes fish habitat and forms a temperature gradient of 8°C compared to the rest of the lake. The stenothermal cold-water native species (whitefish (*Coregonus lavaretus* L.)), living for about half a century under conditions of thermal pollution, has adapted to this stressor. The whitefish population living in the thermal pollution area differs favorably from the population in the natural-temperature environment in terms of its physiological state (blood hemoglobin concentrations, Fulton's condition factor). The adaptive features associated with high respiratory activity (intense Na metabolism) and somatic growth (intense Se metabolism), as well as potentially metabolic vulnerabilities (risks regarding toxic effects and bioaccumulation of Hg, As, and Cu) are shown.

**Keywords:** Lake Imandra; Whitefish; *Coregonus lavaretus* L.; Metabolism

## Introduction

The problem of global climate change became acute in the last century, and related problems arise both in the southern [1] and northern regions [2,3]. Whereas Arctic species are most vulnerable to climate change, and warming and its effects on wildlife and fish are more significant in the Arctic [4]. The global climate models predict a 5–8°C increase in the mean air temperatures by the year 2100 in Europe [5]. Since the middle of the last century, a significant summer warming trend at the lake water temperature has been recorded in Finland [6,7].

Power plants discharging heated water into basins have a significant impact on the ecosystem of the cooling

ponds. The Kola Nuclear Power Plant (Kola NPP), built in 1973, discharges waste water into one of the bays of the subarctic Lake Imandra, located above the Arctic Circle in the Murmansk region, Russia. Kola NPP provides a unique anthropogenic model for studying and predicting the impact of possible climate warming on aquatic organisms.

The stenothermal cold-water native species (whitefish (*Coregonus lavaretus* L.)) is the most widespread species in this region. It is a bottom feeder that leads a relatively settled lifestyle, and can be used as a good local bio-indicator of aquatic environments [8]. Attracted by the large food resources in the thermal pollution area, the whitefish overcame its internal sensitivity to thermal stress. The number of fish with symptoms of general intoxication, kidney

pathologies, and gonadal abnormalities has been decreasing since the 1970s by the 1990s [9].

The aim of the study was:

- To examine the physiological conditions and bioaccumulation of metals in whitefish organs and tissues under thermal pollution and natural-temperature condition in the modern period;
- To identify both positive effects and potentially vulnerable metabolic sites based on the bioaccumulation and the physiological state of fish.

## Materials and Methods

The wastewater from Kola NPP enters the Molochnaya Bay of subarctic Lake Imandra. A thermal pollution area of up to 25 km<sup>2</sup> was formed in the lake, with a temperature gradient from 8°C, near the mouth of the discharge channel [10]. This study was based on data obtained in 2018 on whitefish in the two areas of Lake Imandra. The thermal pollution area is the area of influence of warm water discharges from Kola NPP; and the natural-temperature area is the area not experiencing thermal influence, but the most similar in water chemistry. In total, 12 fish (six from each area of the lake) were examined to determine the metal concentrations in their organs and tissues, as well as their physiological state.

The fish of the same age group (4+–6+) were sampled. The gills, liver, kidney, muscles, and skeleton were sampled to analyze the metal concentrations. The metal concentrations in the biological samples were determined by ICP-AES (ICAP-61 Thermo Jarrell Ash) and ICP-MS (X-7 ICP-MS Thermo Electron) in the Institute of Problems of Microelectronics Technology and Superpure Materials RAS, Chernogolovka, Russia. A certified reference material, DORM-3 and DORM-4 (Fish protein certified reference material for trace metals, National Research Council Canada, Canada), was also

analyzed for quality control, and the observed concentrations were within the certified standard ranges.

The hematology was analyzed using live fish. The blood hemoglobin concentrations (Hb, g/L) were determined using a portable hemometer (MiniGEM523, Russian Federation).

The following data were collected for each fish: body weight (W, g), length (L, cm). Fulton's condition factor (FCF) was calculated as follows [11]:  $FCF = 100 \times W/L^3$

All statistical analyses were performed using SPSS and Statistical. The group differences were tested using the nonparametric Mann–Whitney U test.

## Results and Discussion

The fish physiological state was evaluated by the blood hemoglobin concentration, weight, length, FCF (Table 1). In the past, the most sensitive and vulnerable system of the blood formation of fish showed adaptive effects: if in the late 1970s, there was a huge variation in the concentrations of hemoglobin in the blood (44–163 g/L), leukocytosis, and pathological changes in red blood cells, then by the 1990s, the hemoglobin concentrations in the blood varied at an ecological optimum (80–130 g/L), and the morphological pattern of the blood did not differ from the normal pattern [12]. In the modern period, the blood hemoglobin concentrations were in the range of the environmental optimum in whitefish from the thermal pollution area, while the blood hemoglobin concentration in more than half of the studied fish from the natural-temperature area was below the normal value (Table 1). In anemia, replacing aerobic metabolism with anaerobic glycolysis is only a temporary solution due to less efficient energy use and accumulation of toxic waste [13].

Indicators of the physiological state	Thermal pollution area	Natural-temperature area
<b>Blood hemoglobin concentrations (Hb, g/L)</b>	<b>99</b> (82-131)	<b>68</b> (48-89)
<b>Weight (W, g)</b>	<b>642</b> (530-759)	<b>404</b> (298-459)
<b>Length (L, cm)</b>	<b>33.5</b> (31.4-36.5)	<b>29.7</b> (27.5-31.1)
<b>Fulton's condition factor (FCF)</b>	<b>1.71</b> (1.56-1.77)	<b>1.53</b> (1.41-1.72)

**Table 1:** Indicators of the physiological state (average value and limits of variation) of whitefish from the thermal pollution area and natural-temperature. The bold type shows a reliable group difference (U test with a significance level  $p < 0.05$ ).

Fish homeostasis is largely determined by the concentration of hemoglobin in the blood. Therefore, to survive at elevated temperatures, individuals need to maintain a higher blood hemoglobin concentration. Whitefish with a blood hemoglobin concentration below normal can have a faster fatal outcome in the thermal

pollution area, compared to fish surviving with anemia in the natural-temperature area (Table 1). The frequency of the respiratory cycle depends on the temperature, and gill ventilation increases with an increasing water temperature. Also, the fact that the oxygen saturation of water decreases with increasing temperature contributes to the acceleration

of the respiratory rate.

The high respiratory activity of whitefish in the thermal pollution area is aimed at maintaining the efficiency of the gas exchange of  $O_2$  and  $CO_2$  with the water. Carbonic anhydrase catalyzes the hydration of  $CO_2$ , with the formation of  $H^+$  and  $HCO_3^-$  ions in the cytosol of epithelial cells. Excess  $H^+$  should be eliminated to avoid metabolic acidosis. The following transport systems are located along the apical membrane: the first is the electroneutral  $Na^+/H^+$ -exchanger, which transports  $H^+$  across the apical membrane in exchange for  $Na^+$ ; the second is  $H^+$ -ATPase, which transports  $H^+$  out across the apical membrane, creating an electrochemical gradient for  $Na^+$  to diffuse across the apical membrane through the  $Na^+$ -channel. Along the basolateral membrane,  $H^+$ -ATPase,  $Na^+/K^+$ -ATPase, and the  $Na^+/HCO_3^-$ -cotransporter help to

maintain a very low Na concentration in the cytosol and also removes  $HCO_3^-$  from cells [14]. In addition to the diffusion of  $Na^+$  through the  $Na^+$ -channel, whitefish have intensive  $Na^+$  pumping in exchange for  $H^+$  owing their greater respiratory activity. The Na concentration was slightly increased in the gills of the whitefish from the thermal pollution area, compared to those from the natural-temperature area (Table 2). Whitefish were induced to eliminate excess Na under the thermal impact (Table 2), and the decrease in the Na concentration was enhanced in the kidney with an increasing blood hemoglobin concentration (Figure 1). A similar pattern (increased Na concentrations in the gills and decreased in the liver and kidney) was observed in whitefish exposed to toxic pollution [15]. Also it should be noted that  $Na^+/K^+$ -ATPase activity consumes 20–25% of all ATP production at the whole organism level [16].

Metal	Gills	Liver	Kidney	Muscles	Skeleton
	µg/g dry weight				
Na	6418 (5141-8372)	<b>3558</b> (2522-4998)	<b>6536</b> (4977-8538)	815 (602-1261)	<b>7055</b> (6431-7664)
	5745 (4523-6988)	<b>5316</b> (3874-6618)	<b>8979</b> (7419-10831)	834 (601-970)	<b>6577</b> (6324-6983)
Mg	1083 (824-1326)	<b>713</b> (516-980)	<b>883</b> (590-1260)	1367 (1041-1572)	2033 (1954-2167)
	1163 (1060-1295)	<b>878</b> (772-1010)	<b>1307</b> (1085-1899)	1233 (984-1397)	2120 (1978-2236)
Cu	3.0 (2.0-4.6)	<b>68.1</b> (33.0-117)	27.6 (7.8-109)	1.2 (0.4-1.7)	0.7 (<0.4-1.2)
	2.2 (2.0-2.7)	<b>32.9</b> (19.2-59.1)	10.2 (8.0-13.3)	1.3 (0.9-1.6)	0.6 (<0.4-1.1)
Se	<b>4.8</b> (3.8-6.3)	<b>8.7</b> (7.6-9.7)	<b>9.4</b> (7.1-11.8)	4.9 (3.4-7.6)	<b>1.6</b> (1.0-2.7)
	<b>6.0</b> (4.9-7.2)	<b>14.2</b> (9.9-21.7)	<b>11.6</b> (9.6-14.2)	6.1 (4.1-7.5)	<b>2.5</b> (2.1-3.0)
Mo	<b>0.11</b> (0.06-0.14)	0.67 (0.48-0.93)	<b>0.59</b> (0.41-0.95)	0.02 (0.01-0.02)	<b>0.18</b> (0.08-0.30)
	<b>0.21</b> (0.09-0.31)	0.73 (0.45-0.92)	<b>0.94</b> (0.54-1.45)	0.02 (0.01-0.04)	<b>0.34</b> (0.15-0.54)
Si	<b>54.8</b> (23.1-155)	<b>18.5</b> (15.3-22.9)	<b>40.7</b> (20.4-72.3)	18.4 (13.7-21.8)	12.5 (8.3-17.2)
	<b>272</b> (106-406)	<b>13.1</b> (11.5-16.6)	<b>68.9</b> (39.7-94.4)	15.0 (7.5-29.1)	13.1 (5.2-32.7)
Hg	0.064 (0.042-0.088)	<b>0.162</b> (0.106-0.238)	0.200 (0.154-0.271)	0.098 (0.062-0.200)	<b>0.018</b> (0.004-0.027)
	0.069 (0.048-0.112)	<b>0.120</b> (0.099-0.149)	0.193 (0.146-0.314)	0.099 (0.054-0.218)	<b>0.033</b> (0.024-0.045)
As	0.15 (<0.04-0.49)	0.31 (<0.04-1.59)	<b>0.21</b> (<0.04-0.68)	<0.04	<b>0.21</b> (<0.04-0.73)
	<0.04	<0.04	<b>&lt;0.04</b>	<0.04	<b>&lt;0.04</b>

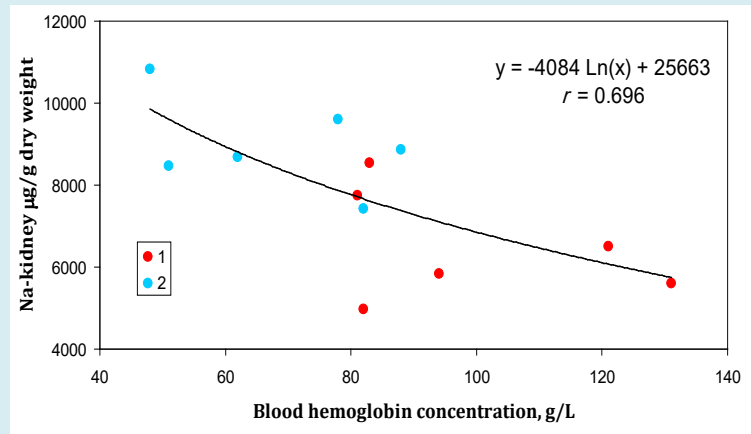
**Table 2:** Average values and limits of variation of some metal concentrations in the organs and tissues of whitefish caught from the thermal pollution area (top line) and natural-temperature area (bottom line). The bold type shows a reliable group difference (U test with a significance level  $p < 0.05$ ).

The basal metabolic rate (the amount of energy that fish requires just to stay alive) does not take into account the costs of growth and reproduction, while only locomotion can increase the basal metabolic rate up to three to five-fold in most fish species [13]. An analytical review of the biological effects of wildlife and fish in the Arctic [4] offers the following examples: the long-term warming of freshwaters is likely to alter fish growth rates, and cold-water native species grow less efficiently in warmer waters. A study of Arctic char revealed

that fish were under greater metabolic stress and had severe glycogen depletion near the end of an abnormally warm summer, compared to colder years [17]. These examples show an acclimatization response to climatic variations, whereas the whitefish from the thermal pollution area adapted to temperature stress, spending energy reserves not only to maintain basic biological functions, but also to induce somatic growth. Fulton's condition factor of the whitefish from the thermal pollution area is significantly higher than

that of the whitefish from the natural-temperature area (Table 1). The approximate average growth rate was 128 g/year for the whitefish from the thermal pollution area versus

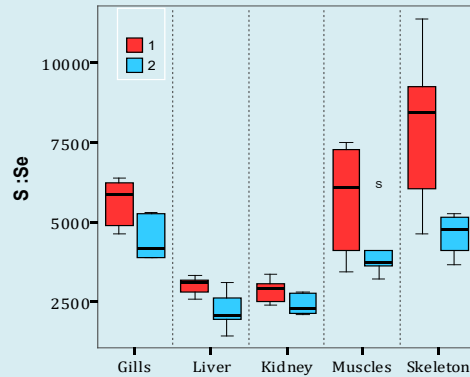
81 g/year for the whitefish from the natural-temperature area, whereas the main metabolic need is Se (Table 2).



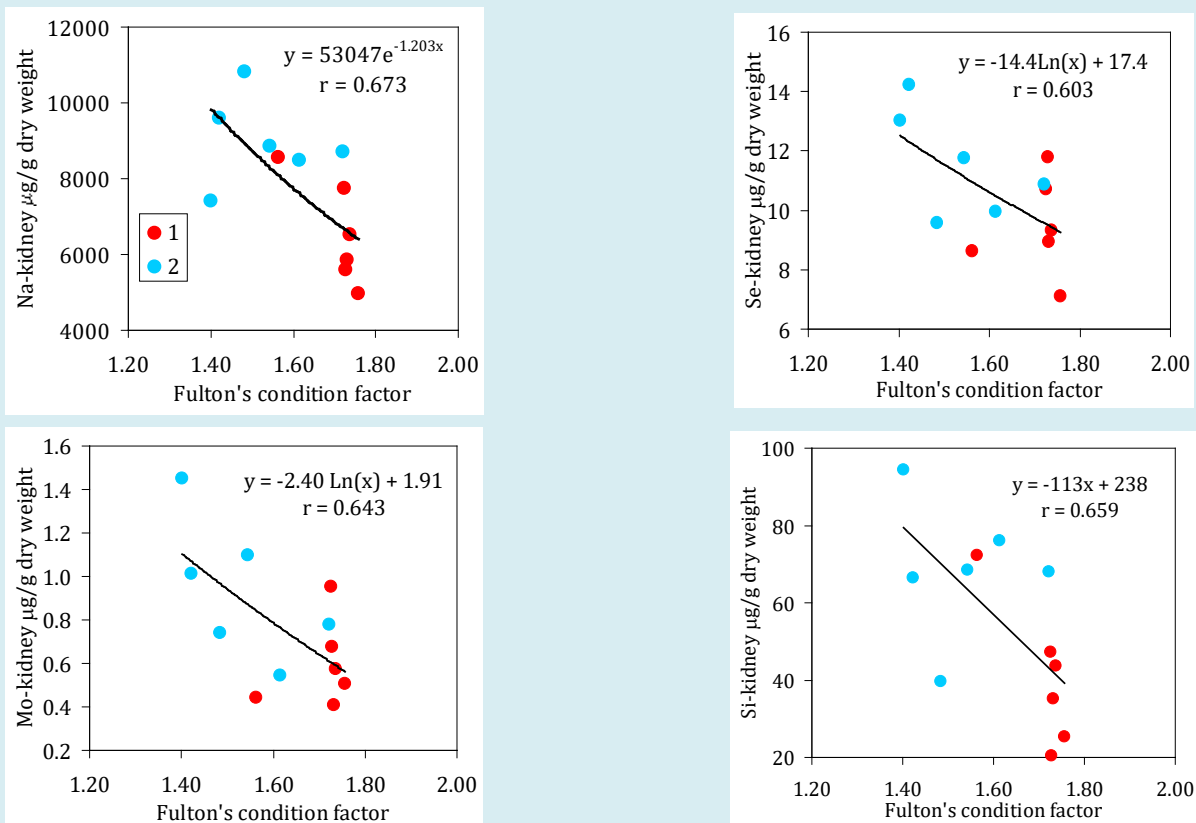
**Figure 1:** Dependence of the Na concentration in the kidney on the hemoglobin concentration in the blood of whitefish from the thermal pollution area (1) and natural-temperature area (2).

As opposed to terrestrial animals, the evolutionary increased reliance on Se is evident in the larger selenoproteomes of aquatic animals, including fish [28]. Se is covalently incorporated as a selenocysteine (SeCys) residue; if Se is specifically incorporated into essential selenoproteins as SeCys, then in all other proteins, selenomethionine (SeMet) replaces methionine (Met) in an unregulated and dose-dependent manner, causing Se toxicity, although SeMet can be considered an unregulated pool of Se for eventual SeCys synthesis [19]. The dose-dependent substitution of S for Se in Met, as a consequence of dietary Se exposure, was illustrated in an experiment with a cage study using wild chub (*Couesius plumbeus*), collected from a reference lake and lake receiving waters of a uranium-processing mill in Northern Saskatchewan, Canada: a decreasing whole-body S isotope signature and an increasing proportion of SeMet-like compounds (determined by X-ray absorption spectroscopy) were observed for the Se-spiked diet treatment groups after 21 days [20]. Having examined the molar ratios of sulfur to Se, these ratios are significantly higher in all organs and tissues of the whitefish from the thermal pollution area, compared to the whitefish from the natural-temperature area (Figure 2). It can be assumed that Se is largely included in the essential SeCys in the whitefish from the thermal pollution area, while SeMet pool is formed in the whitefish from the natural-temperature area. In many cases, S is replaced by Se in Met, and Met is replaced by SeMet. The largest decrease in Se content (more than 1.5 times) is observed in the liver (the dominant site of selenoprotein synthesis and catabolism) of the whitefish from the thermal pollution area (Table 2), and they are the most dependent on its deficit.

The decrease in Mo bioaccumulation in the gills, kidney, muscles and skeleton of the whitefish from the thermal pollution area (Table 2) is apparently associated with an intensive metabolism and, in particular, sulfur-containing amino acids. Molybdenum is a cofactor of at least seven enzymes, the principal of which are xanthine oxidase/dehydrogenase and sulfite oxidase (that is found in the mitochondrial intermembrane space, oxidizes sulfite to sulfate, and is the terminal step in the metabolism of sulfur-containing amino acids) [21]. Molybdate, like selenate [19,21] uses a sulfate transport system. A  $3\text{Na}^+/\text{SO}_4^{2-}$ -co-transporter moves both  $\text{Na}^+$  and  $\text{SO}_4^{2-}$  across the apical membrane, driven by the  $\text{Na}^+$  gradient produced by  $\text{Na}^+/\text{K}^+$ -ATPase on the basolateral membrane; then, the  $\text{SO}_4^{2-}$  is moved across the basolateral membrane by an  $\text{SO}_4^{2-}/2\text{HCO}_3^-$ -exchanger, which is energized by an  $\text{SO}_4^{2-}$  gradient across the basolateral membrane [14]. Evidently, Si also uses the transport system of sulfates. An enhanced excretion of Na by kidneys of the whitefish from the thermal pollution area probably contributes to the Na-dependent transport of Se, Mo, and Si (Table 2). Se, Mo, Si, excretion increases with an increasing FCF (Figure 3). It should be noted that a reduced accumulation of Si was found in the gills and kidneys, but the increased accumulation in the liver of whitefish from the thermal pollution area (Table 2) may indicate support for liver function. In humans, the highest Si concentrations occur in connective and elastic tissues and especially the aorta, where it appears to function as a crosslinking agent that stabilizes collagen and presumably strengthens the vasculature [22].



**Figure 2:** Molar relations of sulfur to selenium (S:Se) in whitefish organs and tissues from the thermal pollution area (1) and natural-temperature area (2).



**Figure 3:** Dependence of the Na, Se, Mo, and Si concentrations in the kidney on Fulton's condition factor of whitefish from the thermal pollution area (1) and natural-temperature area (2).

Se interacts with a variety of other trace elements, primarily in an antagonistic fashion [19], and one of the unfavorable interactions of Se is associated with an increase in Hg bioaccumulation in the liver of fish (Table 2). A vivid antagonistic example (a sufficient accumulation of Se

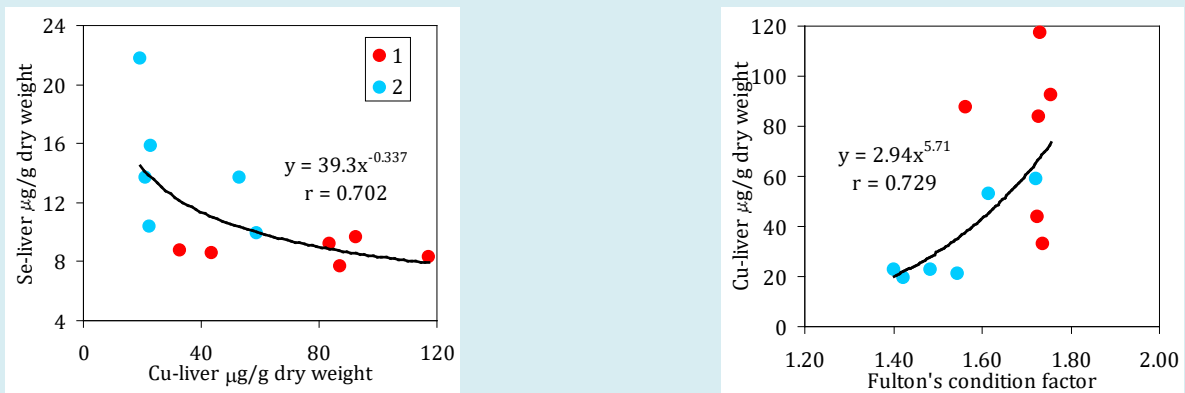
significantly reduced Hg bioaccumulation in the organs and tissues of bream (*Abramis brama* L.) was observed under conditions of strong pollution from a copper smelter [23]. An increase in Hg bioaccumulation in the liver and muscles of fish was observed along a strong temperature gradient [24].

Hg biomagnifies to higher trophic levels in food webs and methylmercury (MeHg), accounting for 91% of the mercury found in the whitefish (*Coregonus clupeaformis*) [25]. In Fennoscandian, an increase in the rate of Hg bioaccumulation in whitefish (*Coregonus lavaretus*) was observed, according to the temperature gradient from the cold oligotrophic lakes in the north to the warmer and increasingly human-altered mesotrophic and eutrophic systems in the south, where, apparently, mercury methylation is more intensive [26]. MeHg is absorbed effectively (>90%) from the gut and enters tissues as a cysteine-bound conjugate that mimics Met, moving freely into cells via amino acid transport proteins [27]. MeHg is by definition a highly specific irreversible selenoenzyme inhibitor, and intracellular MeHg tends to diminish the amount of Se that is biologically available for normal selenoenzyme synthesis [28].

Another unfavorable interaction of Se is associated with the As accumulation, which is significant in all organs and tissues in the whitefish from the thermal pollution area, whereas As does not accumulate under natural conditions (Table 2). Both arsenite and arsenate (interact antagonistically with Se, and the interaction involves complexation with endogenous thiols [19]. The inactivation of the selenoenzymes may be increased in the liver of the

whitefish from the thermal pollution area.

Interactions between Cu and Se in fish decrease the tissue-specific Se accumulation, and the elevated dietary Cu reduced Se concentration in the liver [19]. Cu reduces the Se accumulation in the liver, moreover, the Cu accumulation increases with an increasing FCF (Figure 4). The upper threshold (with higher values representing a risk of toxicity) for the Cu concentration is specified for the yellow perch (*Perca flavescens*) liver at 38.8 µg/g dry weight [29]. Whereas the liver concentrations of Cu exceeded this threshold in most whitefish from the thermal pollution area (Table 2). The toxic effect of Cu can affect the energy metabolism and, in particular, the energy capacity of the liver, which has been demonstrated in cases of toxic pollution [30,31]. Mg is a cofactor for enzymes that transfer phosphate groups, such as the ATPases, involved in energizing the pumps for H<sup>+</sup> and Ca<sup>2+</sup> and the exchanger for Na<sup>+</sup> and K<sup>+</sup>, and most cellular Mg is associated with ATP [14]. A decrease in Mg concentration was observed in the liver of whitefish from the thermal pollution area (Table 2). A reduction in glycolytic capacity may be dependent on a direct competition between Cu<sup>2+</sup> and Mg<sup>2+</sup> for protein-binding sites that will induce enzyme conformational changes and alter activity [32].



**Figure 4:** Dependence of the Se concentrations on Cu concentration in the lever, as well as dependence of the Cu concentration in the lever on Fulton's condition factor of whitefish from the thermal pollution area (1) and natural-temperature area (2).

## Conclusion

The whitefish population living in the thermal pollution area of subarctic Lake Imandra has adapted to heat stress: fish have better indicators of the physiological state compared to the whitefish population in their natural habitat.

One of the main adaptive mechanisms of ion regulation is the intense Na metabolism owing the high respiratory activity of whitefish in warmer water, while Na-dependent transport of sulfates stimulates the excretion of Se, Mo, and Si.

Under conditions of elevated water temperature, the main metabolic requirement may be due to Se deficiency, with intensive metabolism of which the risks of toxic effects and bioaccumulation of Hg, As, and Cu are likely to arise in the case of an increased current stressor or the appearance of new ones.

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