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Acclimatization of fish to the higher calcium levels in the water environment

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ABSTRACT
It is established that calcium concentration changes (variations) in the water environment significantly influence its intake and distribution in tissues and organs of hydrobionts. The decrease in calcium concentration in water from 100 to 60 mg.L^{-1} significantly reduces its content in fish liver. In the gills glandular apparatus of fish acclimated to the environment with lower calcium level (in comparison with control one), its concentration on the first day of the acclimation period slightly exceeded the initial level, thus testifying to its possible excretion of endogenous calcium by gills. The increase of calcium excretion through the renal and digestive systems in fish acclimates to the higher water level, and specific changes in phosphates excretion dynamics accompany oral intake. Long keeping fish in water with 100 mg.L^{-1} calcium is accompanied by the increase of total phosphorus in urine (by 2 – 2.5 times), and its day excretion increases by 1.9 – 2.4 times. During fish acclimation to higher calcium levels in the water environment, the excretion of total phosphorus with faecal matter increases. The increase of calcium in the water environment to 100 mg.L^{-1} leads to a temporary increase in total phosphorus excretion with faecal issues. The rise in cation concentration to 200 mg.L^{-1} increases significantly during long-time fish stay in such an environment.

Keywords: *Cyprinus carpio L.*, Ca^{2+} concentration, water environment, regulation, diuresis, excretion.

INTRODUCTION
Calcium and phosphorus are the most plastic elements in bone and other tissue structures, both terrestrial and water animals. Due to its chemical properties, calcium is one element that makes strong connections with proteins, phospholipids, organic acids, and other substances. These properties play an important role and influence many physiological and biochemical processes ongoing in animal organisms **[9]**, **[10]**. It plays an important role in cell membrane permeability since calcium has a considerable stabilizing effect on their structural and functional properties **[1]**. In freshwater fishes, the direct dependency between gill epithelium permeability for Na^+ and Ca^+ and Calcium concentration in the environment is established **[5]**, **[39]**. Under chronic heavy metals' lethal concentration influence, nutrition depression occurs, causing substantial growth retardation in water animals **[40]**, **[41]**. There are literature data on calcium concentration growth with other ions in fishes during their temperature acclimation **[2]**, **[6]**, **[15]**. In fish, the calcium content grows with a temperature rise from 24 to 36 °C **[30]**, thus testifying to metabolism intensification in fish organisms when environmental temperature increases.

According to Grynevych et al. **[7]**, Lall, and others **[16],** this property makes us consider the increase of calcium level in juvenile Atlantic salmon before migration into seawater and in adults during migration to freshwater to spawning grounds as the phenomenon helping to increase moving ability during these periods in salmon's life. Calcium ions decrease the ability of tissue proteins to link water. Under its concentration increase in water environment, the increase in water content in the tissues of brown trout and decrease in marked water turnover and water absorption rate during drinking and its excretion with urine **[23]**, **[36]**. At the same time, in fish in seawater without only calcium, no changes in water outlet through the gills are registered, but the simultaneous absence of both Ca^{2e} and Mg^{2e} increases water outlet through gills by 33% **[35]**, **[42]**.

The introduction of manganese sulfate salts, zinc, and magnesium into artificial intelligence granular feed increases fish growth and makes significant changes in phosphorus-calcium exchange **[21]**, **[26]**.

Changes in water environment calcium concentration can influence acid-base balance in the organism. Researchers **[3]**, **[38]** proved that the increase of calcium level in water from 2.5 to 5 $mg.L^{-1}$ has a short time influence on acid-base balance in the carp's blood, thus decreasing buffer base concentration to 1/3. The animal organism normalizes the ionized calcium concentration with blood pH shift **[31]**.

The role of calcium as a mating factor in muscle fibres contraction and stimulation can be explained by its influence on acetylcholine intracellular distribution **[14]**, **[20]**, **[33]**. As a result, the fish mobility (guppies) quickly changes depending on calcium salts concentration in the environment **[8]**. And the decrease of cation level from 10 to 1 mg makes short, less than a second, stop in cilia beating of mussel's gills **[17]**, **[32]**.

Calcium decreases the nervous system's excitability, and the decrease in its content in the blood leads to its overexcitation. It is considered **[4]**, **[11]** that calcium's role in brain cells metabolism is determined by its activating effect on mitochondrial phospholipids hydrolysis. After removing calcium from the environment, the restoration of neurons' ionic composition in invertebrates doesn't occur **[22]**, **[29]**. In solutions without Ca^{2+}, nerve cells of gigantic neurons in mollusks lose their ability to generate action potentials as the inactivation of Na^{+} transfer takes place, and sometimes, the whole system providing delayed straightening becomes broken. In nerve cells in such conditions, the transport of labeled proteins along axons decreases by 40 – 60% **[25]**, **[37]**.

Scientific Hypothesis

The introduction of hydrobionts into water bodies with high calcium content in the aquatic environment leads to an increase in excretion with metabolic products and total phosphorus.

The results of our studies showed that when fish acclimate to an increased level of calcium in the aquatic environment, along with changes in its content in glandular tissues, the intensity of excretion with urine and faeces, the excretion of total phosphorus with faecal masses also increases.

MATERIAL AND METHODOLOGY

The research of phosphorus calcium metabolism in fishes required developing a unique systematic approach allowing to study tissue, cell, and organ mechanisms in metabolic regulation, taking into account peculiarities of aquatic habitat. We should consider the possibility of calcium and phosphorus intake into fish organisms through the digestive system and directly from the water and the influence of temperature factor in these elements absorption significantly changing the intensity of metabolic process in poikilothermic animals such as fishes.

Samples

The object of investigation is one year and two-year carps *(Cyprinus carpio L.)* with an average mass of 21.0 ±1.6-34.2 ±2.1 and 255 ±9.7 g. Before the experiment, the fish taken from Kyiv region fisheries in the autumn period were kept in stationary capacities of 4 m^3 each. Then 5 specimens of 2-year-old fish and 50 specimens of 1-year-old fish were put in 100 – 130 L aquariums equipped with programmed systems regulating temperature, photoperiods, water gas contents. After the preliminary week acclimation period, the experiments were carried out. The aquariums were filled with aged tap water with the following base mineral components concentration: Na^{+}: 11.7; K^{+}: 6.4; Ca^{2+}: 50 – 100; Mg^{2+}:120.0.

Depending on targets and goals, different experiments on the influence of calcium in different concentrations in the water environment on phosphorus-calcium metabolism in fish and during this cation oral intake were carried out.

Chemicals

The influence of calcium content in a water environment equal to 60, 100, 200, 400 $mg.L^{-1}$ on fish phosphorus-calcium metabolic indices was researched. The preset calcium concentration was reached by adding the calculated amount of calcium chloride to water. The water temperature during the experiment was maintained at 18 – 20 °C; O_2 content fluctuated within 7.59 – 9.76 $mg.L^{-1}$, CO_2: 2.32 – 3 32 $mg.L^{-1}$, HCO_{3}-: 3.6 – 5.3 $mg.L^{-1}$, pH level – from 7.64 to 7.85.

Animals and Biological Material

One year and two-year carps *(Cyprinus carpio L.),* gills, and kidney glandular tissues

Instruments

The study of calcium ions in higher environmental concentration influences phosphorus-calcium metabolism was carried out on gills and kidney glandular tissues after 1, 3, and 7 days of their exposure. In some cases, the investigations were carried out on the experiment's 11 – 14 and 24th days. The tissue homogenate was prepared on 0.2 sucrose (dilution 1:10). The researched tissues were quickly extracted after fish decapitation, weighed, and homogenated in a homogenizer (HG15A, Daihan, South Korea).

Statistical Analysis

The received digital data are processed using standard methods of variation statistics and special computer programs MS Excel and Statsoft Statistica 6.0.

RESULTS AND DISCUSSION

It is common knowledge there is a constant exchange between environment ions and hydrobionts organisms. As for some mineral substances, freshwater hydrobionts have clearly expressed the ability to concentrate them, and the level of these elements (calcium included) greatly exceeds their level in the environment **[34]**. The high calcium content in the extracellular fluid determines its specific role in key organism reactions, including phosphate metabolism. The last one is the base for many structural and functional units of living organisms. Also, they are the most labile component of the main energetic cell-substrate – adenosine triphosphoric acid. In warm-blooded animals, there is a relation between calcium transport and the chemical breakdown of organophosphorus compounds rich in energy **[12]**, **[19]**.

It is important to establish close connections between phosphorus and calcium metabolism to research the effect of calcium in different concentrations on its accumulation in fish functionally different tissues. Also, it is important to research the peculiarities of its excretion from the organism taking into account this cation's high biological activity and its possible influence on tissue phosphatase activity and phosphorus metabolism in glandular and other fish tissue structures.

After investigating calcium tissue metabolism during fish acclimation to its higher content in water and peroral intake, it is established that changes in calcium concentration in the water environment significantly influence its intake and distribution in the tissues and organs of hydrobionts. Thus, the decrease of calcium concentration in water from 100 to 60 mg.L^{-1} results in a significant decrease in the fish liver content (Table 1). The level of general calcium in carp liver decreased more than by three times and didn't return to the initial one even after a 7-day stay of fish in such an environment.

Table 1 The influence of different calcium concentrations in water on its content in fish tissues (mg% of dry tissue) and blood (mg%), M±m.

Days	Calcium concentration in water, mg.L^{-1}	Calcium content in fish tissues and liquids		
		Gills	Hepatopancreas	Blood
1	100	2246.6 ±38.1	116.21 ±3.85	-
	60	2439.55 ±145.2	104.16 ±15.08	-
3	100	1994.00 ±109.1	118.21 ±5.99	14.00 ±0.22
	60	2121.40 ±226.5	32.16 ±2.10*	13.00 ±0.14
7	100	2713.44 ±81.08	103.12 ±11.3	18.90 ±0.20
	60	2547.40±176.34	90.39 ±9.03	12.00 ±0.15*
1	100	650.00 ±14.52	129.00 ±10.00	-
	200	1042.00 ±31.00*	131.00 ±9.00	-
3	100	664.50 ±15.10	146.00 ±0.12	14.00 ±0.32
	200	900.00 ±54.00*	225.40 ±15.10*	16.50 ±0.15
7	100	815.96 ±33.13	124.32 ±11.60	18.90 ±0.20
	200	991.18 ±62.12	98.21 ±14.20	12.10 ±0.13*
1	100	650.00 ±14.52	129.00 ±10.00	-
	400	980.70 ±21.00*	143.00 ±15.00	-
3	100	707.00 ±16.00	166.00 ±2.00	14.00 ±0.22
	400	1117.86 ±90.80*	163.60 ±17.00	16.00 ±0.12
7	100	654.50 ±15.10	146.00 ±12.00	18.90 ±0.13
	400	852.00 ±40.00*	114.00 ±10.00*	13.60 ±0.16*

Note:* the reliable result.

This tendency in general calcium quantity is observed in the fish blood. It is worth noting that in glandular gills apparatus of fish acclimated to the environment with lower, in comparison with control one, calcium level, its concentration in first days of acclimation period slightly exceeded the initial one, thus possibly testifying to the excretion of calcium with gills. But during the 7-day acclimation period in such conditions of the environment, the calcium content in fish gills becomes lower than in control ones. The described changes in general calcium content in gills, glandular tissues, and liver and also in fish blood can be explained by the constant loss of cation in the process of vital fish activity in conditions of reduced intake from water with lower concentration.

The data received by Smart **[28]** testify to possible calcium loss in hydrobionts while keeping them in water with a low level. The author showed that ion excretion into the environment makes up to 10% of the total calcium content in the organism. When the calcium level increases in water to 200 mg.L^{-1}, its increase in gills and liver is registered in the fish organisms. Thus, in fish kept in water with 200 mg.L^{-1} calcium level, its quantity in gills increased by 50 – 60% on the first day and stayed at this level during the whole acclimation period. The calcium content in the liver reached its maximum after 3 days in the water with a 200 mg.L^{-1} cation concentration.

If in fish tissues calcium stayed at a higher level during the whole 7-day period of acclimation to its higher content in water, in the blood, it exceeded its level only on the third day. Then its concentration stayed lower than in the control group level. Registered changes in calcium levels in fish can testify that this organ plays a significant role in calcium metabolism regulation in animals and fish.

Another regularity in calcium accumulation in carp's tissue was registered after its increase to 400 mg.L^{-1} in the environment. Suppose gill tissue accumulates a significant quantity of calcium during the 7-day acclimation period to these conditions, then in the fish liver and blood. In that case, its level increases only at the beginning of the experiment (1 – 3 days), and then it even decreases. Data on calcium content in glandular organs and blood in high cation concentration (400 mg.L^{-1}) suggest that this quantity may cause changes in gill cells membranes permeability, thus, reducing its intake in fish blood and hepatopancreas.

It is known **[24]**, **[27]** that calcium has a significant stabilizing effect on biological membranes' structural and functional properties.

Based on the data provided, we can conclude that calcium level in glandular tissue of gills and liver and biological liquids of carp depends on its concentration in the environment. Thus, after a decrease of calcium concentration from 100 mg.L^{-1} to 60 mg.L^{-1}, hepatopancreas glandular tissue, and the blood loss it. In the gill tissue of the experimental fish, the calcium content increases at the beginning of the experiment, which can testify to endogenic calcium excretion with the gill glandular apparatus. After increasing calcium concentration in water to 200 mg.L^{-1}, its accumulation in gills and hepatopancreas is observed. 7-day acclimation period normalizes processes in fish organisms, and the calcium level in blood decreases. Calcium content in glandular organs and blood of fish from the environment with relatively a high level (400 mg.L^{-1}) let us suppose that this cation concentration can change the permeability of gill cells membranes, thus reducing its intake in blood and hepatopancreas.

Along with non-organic admission into fish organisms directly from water, a significant part of mineral substances, calcium included, enter orally with feed. Depending on ways of calcium intake into fish organisms, peculiarities of its tissue distribution are registered.

The analysis of obtained data showed that in one day after oral calcium intake in 75 mg.kg^{-1} of fish kept in water with 40 mg.L^{-1} cation concentration, the hypercalcemic reaction was observed and characterized with its content increased blood serum from 11.73 ±0.56 to 20.62 ±0.37 mg%. More long (7 days) calcium intake was accompanied by increased its content in blood to 30.39 ±1.03 mg%, almost by 3 times compared with control fishes. The observed rapid growth of calcium content in blood serum testifies its significant intake into the organism orally (Figure 1).

The oral calcium intake is accompanied by its significant accumulation in fish glandular organs (Figure 1). Thus, its amount in the liver during the 7 days exceeded control indices by 3.4 – 3.9 times, in kidneys by 1.5 – 2.1 times. At the same time, the calcium content in gills was lower than in control fishes by 24.8 – 200%. In the researched period, significant accumulation (by 2.1 – 2.2 times) of calcium was registered in fish muscle tissue after oral intake.

Analyzing calcium tissue distribution in fish glandular organs and muscle tissues after its oral intake, it is worth noting the observed effects depended on salt load impact duration. At the same time, the high calcium level in the liver, kidneys, and muscles tissue can testify to their significant depositing possibilities. Its content decrease in gills can result from calcium excretion increase in this way **[13]**.

We also researched renal and extra renal mechanisms of calcium metabolism regulation in fishes. Considering the interrelation in calcium content in water and its intake in general blood flow after absorption with glandular gill cells, it is important to establish the role of renal and digestive systems in its metabolism regulation.

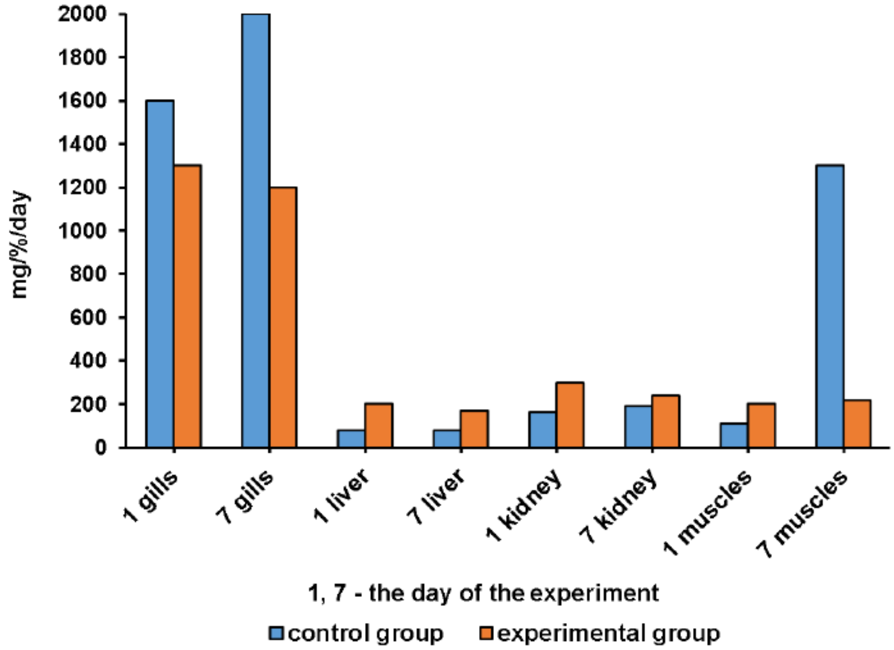

Figure 1 The influence of oral calcium intake on its distribution in carp tissues.

During experiments, it was established (Table 2) that the daily amount of diuresis depends on calcium concentration in water. Thus, in fish kept in water with 40 mg/l calcium concentration, diuresis was 34.12 – 29.2 mg.L^{-1} mass/day with the increase of calcium concentration in water to 100 mg and 200 mg.L^{-1}, the day diuresis in carps decreases.

The higher the calcium concentration in water, the more pronounced its antidiuretic effect is. Thus, with 100 mg.L^{-1} calcium content in water, the diuretic function of carp kidneys decreased by 25.7% and stayed at this level during a short time (1 day) period of fish keeping in such conditions. With the increase of calcium content in water to 200 mg.L^{-1}, the day diuresis in fish decreased by 25.68 – 51.59% and reached the initial level only in seven days. From the literature data **[18]**, we know that calcium significantly influences cell membrane permeability for water, and salts cause the excretion of urine in a lower amount but in higher concentrations.

With the decrease of kidney diuretic function in carps kept in an environment with higher calcium concentration, its amount is registered in urine (Table 2). Thus, in fish acclimated in water with low calcium concentration (40 mg.L^{-1}), its content in the urine was 63.16 – 68.66 mg.L^{-1}, whereas, under 100 mg.L^{-1} concentration, this index increased by 18.3 – 25.8%. Under higher (200 mg.L^{-1}) calcium level in the water, its excretion in the fish urine in one day increased by 40.4% and in 3-7 days by 1.6 – 1.7 times compared to the initial level.

It is known that day calcium excretion with urine is determined both by its concentration and the diuresis level. As the result of the conducted investigation, no direct correlation in calcium concentration increase in water and its day excretion with urine was registered. Thus, on the last day of fish keeping in an environment with higher (100 mg.L^{-1}) calcium concentration, its excretion with urine didn't increase, caused by a rapid decrease in kidney diuretic function. After 3 days, both diuresis and day calcium excretion with urine significantly increased, staying at a higher level in the following 7 days of the experiment. Herewith more direct correlation in day calcium excretion with urine and its level in the water environment was registered during carps acclimation to 200 mg.L^{-1} cation concentration. The obtained results correspond to the results obtained by Buda and others **[2]** which showed the increase of calcium excretion after injection with calcium-chloride during fish acclimation to its higher level in the water, proving the dependence on environmental ionic content and water animals organisms.

The faeces' excretion changes during fish acclimation to higher calcium concentration in the water environment. It should also be noted that calcium faeces excretion in fish acclimation to 100 mg.L^{-1} cation concentration decreased, whereas it increased under 200 mg.L^{-1} concentration (Table 2). It should also be noticed that the most explicit changes in faeces excretion of carps from 2 environmental groups occurred during their long keeping in water with a higher calcium content.

Changes in calcium faeces excretion are closely connected with liver biliary function and thus with metabolic processes levels in fish being acclimated to higher calcium levels in the water. Data on calcium excretion with bile prove it. Therefore, in fish being kept in an environment with 40 mg.L^{-1} calcium level, its concentration in bile fluctuated from 34.04 ±0.73 to 40.67 ±2.60 mg.L^{-1}. In contrast, in 100 mg.L^{-1} Ca^{2+} concentration, it increased in 1 day of acclimation by 28.4% and in 7 days – by 43.3%. The same changes in calcium content in fish bile were registered in fish being acclimated to 200 mg.L^{-1} calcium level in the water environment. It should be noted that fish were kept in an environment with a 200 mg.L^{-1} calcium concentration for a long time. Its excretion with bile increased by 2.1 times compared to the initial level. The minimal calcium loss with faeces in fish was observed on the third day of the experiment under both concentrations in water. As we showed earlier, higher tissue calcium accumulation in fish glandular organs can be caused by higher tissue calcium accumulation.

Table 2 The influence of calcium concentration in the water environment on kidney diuretic function and its excretion with urine and feces in carps, M ±m.

Ca^{2+} concentration in water mg.L^{-1}	Days of the experiment					
		% to control		% to control		% to control
Diuresis mL/kg/day						
40 (control level)	34.12 ±1.68		29.60 ±2.12		29.20 ±2.56	
100	25.32 ±2.92*	-25.79	28.00 ±2.36	-5.41	29.08 ±2.92	-0.41
200	16.52 ±1.28*	-51.58	22.00 ±1.16*	-25.68	32.40 ±1.64	+10.96
Calcium concentration in urine mg.L^{-1}						
40 (control level)	63.13 ±4.18		80.67 ±3.78		68.66 ±3.76	
100	74.67 ±4.08	+18.28	80.67 ±3.55*	+25.83	78.86 ±8.01	+14.86
200	88.66 ±3.87*	+40.44	106.00 ±10.58*	+65.34	117.33 ±10.39*	70.89
Calcium day excretion with urine mL/kg/day						
40 (control level)	2.15 ±0.072		1.90 ±0.060		2.00 ±0.096	
100	1.89 ±0.056*	-12.09	2.26 ±0.144*	+18.95	2.29 ±0.088	+14.50
200	1.95 ±0.048*	-37.21	2.34 ±0.124*	+23.16	3.80 ±0.253*	+90.0
Calcium day excretion with feces						
40 (control level)	4.32 ±0.072		4.92 ±0.272		6.60 ±0.128	
100	2.96 ±0.068*	-31.48	1.64 ±0.104*	-66.67	2.76 ±0.100*	-54.0
200	5.40 ±0.70*	+25.0	1.04 ±0.170*	-78.86	9.00 ±0.150*	+50.0

Note: * the reliable result

It should be noted that during fish acclimation to extremely high (400 mg.L^{-1}) calcium concentration in water, its excretion with bile doesn't increase, but vice versa decreases from 184.00 ±2.4 to 135.00 ±2.1 mg%. It can be explained by the rapid decrease in calcium absorption from water by the gill glandular apparatus and its intake into the organism.

Oral calcium injections caused, together with its tissue distribution, changes in excretion of organs activity. In this case, the urine output in fish after oral calcium injection in the 75 mg/kg/day dose decreased during a short time (1 – 2 days) period by 23.7 – 30.9% (Figure 2.), reaching control indices after 7 days of the exposition. During oral calcium injection, its excretion with urine increased in the first day from 2.15 ±0.072 to 4.16 ±0.16 mg/kg/day or by 1,9 times, on the third day – from 1.90 ±0.060 to 5.26 mg/kg/day (by 2.7 times), on the seventh day – from 2.00 ±0.006 mg/kg/day to 3.92 ±0.224 (by 2 times) in comparison with its excretion in fish without calcium oral injections.

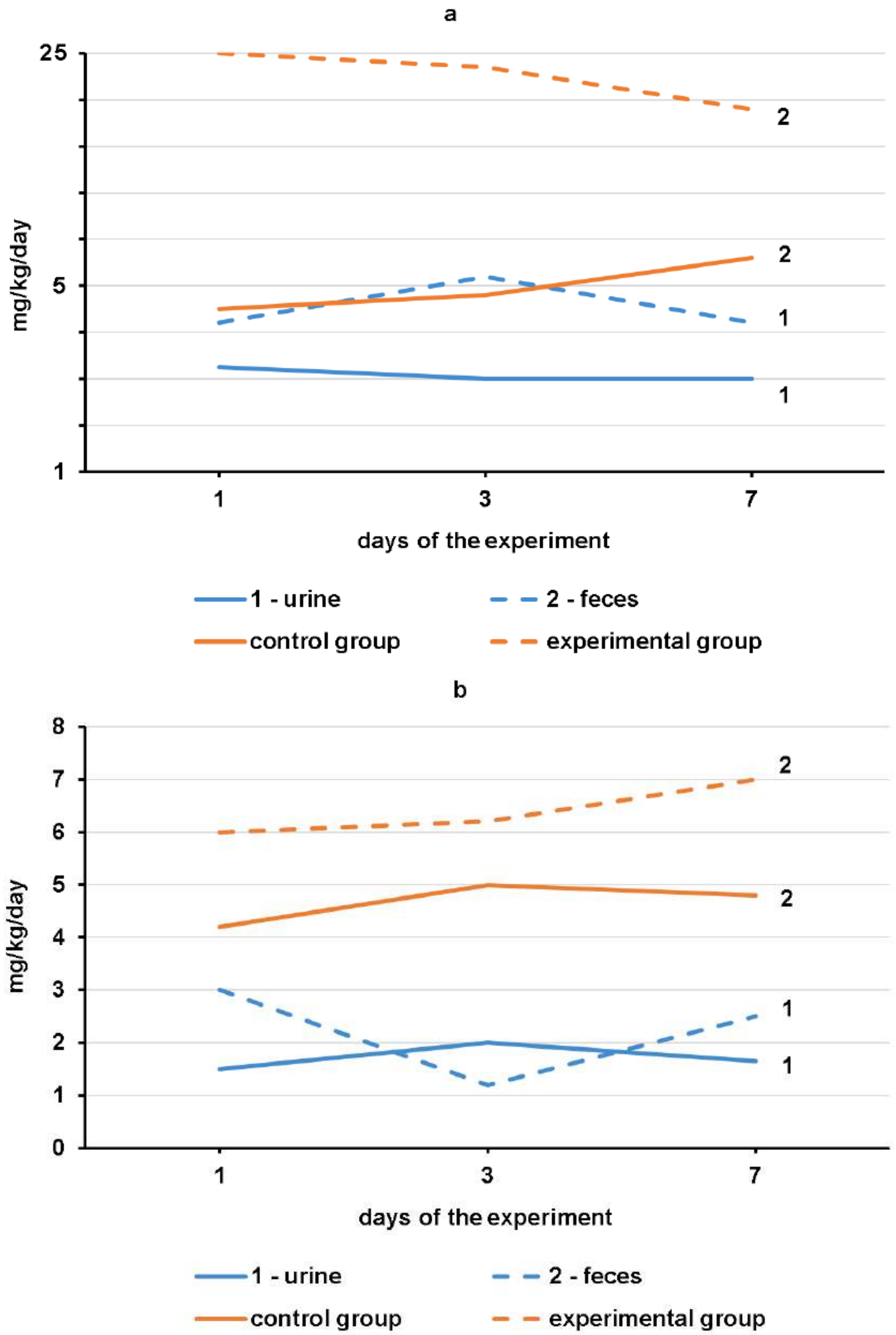

Figure 2 The influence of oral calcium injections on calcium excretion (a) and phosphorus (b) excretion with the fish urine and faeces.

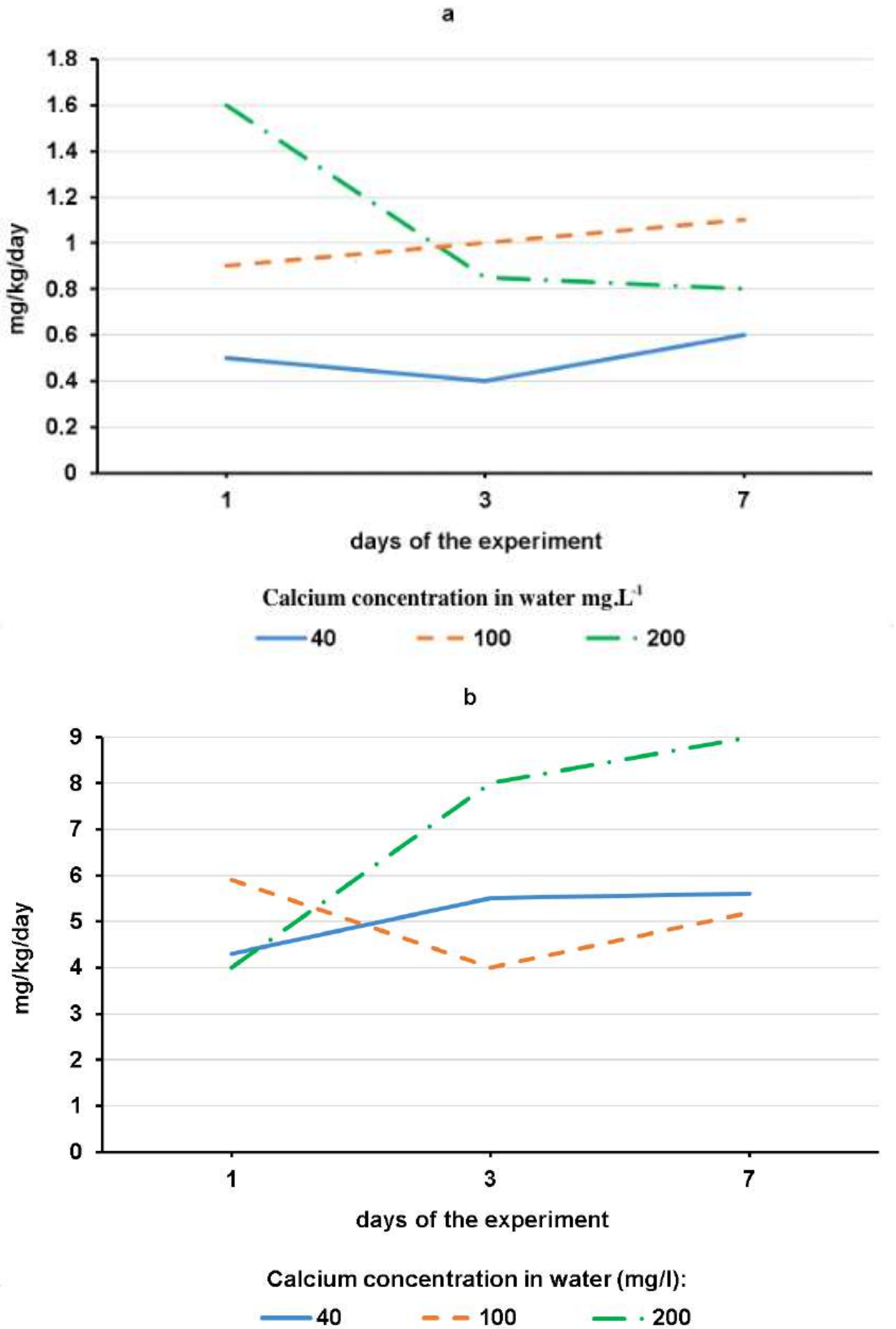

Figure 3 The influence of calcium higher level in water environment on phosphorus day excretion (mg/kg/day) with urine (a) and faeces (b) by carp.

At the same time, its feces excretion sharply increased. It increased after 1, 3 and 7 days of calcium injection from 4.32 ±0.072 to 26.4 ±0.33 mg/kg/day; from 4.92 ±0.272 to 23.5 ±1.60 mg/kg/day; from 6.00 ±0.13 to 16.92 ±2.90 mg/kg/day accordingly.

The increase of calcium excretion through the renal and digestive system of fish being acclimated to its higher level in the water and oral intake is accompanied by certain changes in phosphate excretion dynamics. Thus, in fish being kept in water with 40 $mg.L^{-1}$ calcium level, the phosphate concentration in urine fluctuated from 14.54 ±0.63 to 20.0 ±0.45 $mg.L^{-1}$ and day excretion from 0.496 to 0.584 mg/kg/day (Figure 3). The long-time fish exposure in water with 100 $mg.L^{-1}$ calcium level was accompanied by the increase of total phosphorus level in urine (by 2 – 2.5 times), and its day excretion increased by 1.9 – 2.4 times.

The total phosphorus faeces excretion increases during fish acclimation to high calcium levels in a water environment (Figure 3).

The increase of calcium level in the water environment to 100 $mg.L^{-1}$ causes only a short increase in total phosphorus excretion with faeces. Under the cation, concentration increases to 200 $mg.L^{-1}$ significantly increase during the fish's long stay in such an environment.

It should be noted that during oral calcium intake to fish organism phosphorus day excretion with urine during 7-day exposition exceeded control level by 44.5%. In contrast, it sharply increased during the short time (1 day) (Figure 2).

Phosphate excretion with faeces in fish getting calcium orally during 1, 2, 7 days of the experiment exceeded by 35.4; 15.3 and 26.7% its excretion in fish without cation intake but kept in the same experimental conditions (Figure 2).

So, calcium and phosphorus excretion in fish is determined by the calcium level in the environment and its intake through the digestive tract. The importance of the digestive system in calcium and phosphorus excretion grows with the increase of Ca^{2+} intake into fish organisms. This proves the important role of this system in their metabolism.

CONCLUSION

The investigation results showed that during fish acclimation to increased calcium level in the water environment and the changes of its content in glandular tissues, the intensity of excretion with urine and faeces the excretion of total phosphorus with faeces also increases. The increase of calcium content in the water environment to 100 $mg.L^{-1}$ caused only a short time increase of total phosphorus excretion with faeces. In contrast, cation concentration increases to 200 $mg.L^{-1}$ significantly increases during the long stay of fish in such an environment.

It should be noted that during oral calcium intake into the fish organism, the day phosphorus excretion with urine under 7-day exposition exceeded the level in control fish by 44.5%. In contrast, it was sharply increased in a short time (1 day). The phosphorus excretion with faeces in fish getting calcium orally during 1, 2, and 7 days of the experiment exceeded by 35.4; 15.3 and 26.7% its excretion in fish without cation intake but kept in similar experimental conditions. So, fish's calcium and phosphorus excretion is determined both by their content in the environment and their intake through the digestive tract. The meaning of the digestive system in calcium and phosphorus excretion grows with calcium intake increase in fish organisms, thus indicating the important role of this system in their metabolism.

REFERENCES

1. Ahmad, S. M., Shah, F. A., Bhat, F. A., Bhat, J. I. A., & Balkhi, M. H. (2011). Thermal adaptability and disease association in common carp (*Cyprinus carpio communis)* acclimated to different (four) temperatures. In Journal of Thermal Biology (Vol. 36, Issue 8, pp. 492–497). Elsevier BV. https://doi.org/10.1016/j.jtherbio.2011.08.007
2. Buda, Cs., Dey, I., & Farkas, T. (1996). Role of membrane lipids in temperature acclimatization of carp. In Archiv für Tierernaehrung (Vol. 49, Issue 1, pp. 61–62). Informa UK Limited. https://doi.org/10.1080/17450399609381864
3. Dahlhoff, E. P. (2004). Biochemical Indicators of Stress and Metabolism: Applications for Marine Ecological Studies. In Annual Review of Physiology (Vol. 66, Issue 1, pp. 183–207). Annual Reviews. https://doi.org/10.1146/annurev.physiol.66.032102.114509
4. David, M., Mushigeri, S. B., Shivakumar, R., & Philip, G. H. (2004). Response of Cyprinus carpio (Linn) to sublethal concentration of cypermethrin: alterations in protein metabolic profiles. In Chemosphere (Vol. 56, Issue 4, pp. 347–352). Elsevier BV. https://doi.org/10.1016/j.chemosphere.2004.02.024
5. Fiedler, B., & Scheiner-Bobis, G. (1996). Transmembrane Topology of α- and β-Subunits of Na+,K+-ATPase Derived from β-Galactosidase Fusion Proteins Expressed in Yeast. In Journal of Biological Chemistry (Vol. 271, Issue 46, pp. 29312–29320). Elsevier BV. https://doi.org/10.1074/jbc.271.46.29312
6. Ghosh, T. K., Chauhan, Y. H., & Mandal, R. N. (2019). Growth performance of *Labeo bata* (Hamilton, 1822) in freshwater and its acclimatization in brackish water with betaine as feed additive. In Aquaculture (Vol. 501, pp. 128–134). Elsevier BV. https://doi.org/10.1016/j.aquaculture.2018.11.020
7. Grynevych, N., Sliusarenko, A., Dyman, T., Sliusarenko, S., Gutyj, B., Kukhtyn, M., Hunchak, V., & Kushnir, V. (2018). Etiology and histopathological alterations in some body organs of juvenile rainbow trout

Oncorhynchus mykiss (Walbaum, 1792) at nitrite poisoning. In Ukrainian Journal of Ecology (Vol. 8, Issue 1, pp. 402–408). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/2018_228

8. Hanna, S. M., Thompson, M. J., Dahab, M. F., Williams, R. E., & Dvorak, B. I. (2018). Benchmarking the Energy Intensity of Small Water Resource Recovery Facilities. In Water Environment Research (Vol. 90, Issue 8, pp. 738–747). Wiley. https://doi.org/10.2175/106143017x15131012153176

9. Hudiyash, Yu., Prychepa, M., Potrokhov, A., Zin'kovskiy, O., Gorbatyuk, L., Kovalenko, Ju., & Medovnyk, D. (2020). The effect of environmental conditions of some lakes of the city of Kiev on ichthyofauna condition. In Ribogospodars'ka nauka Ukraini. (Issue 1(51), pp. 28–43). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka). https://doi.org/10.15407/fsu2020.01.028

10. Jesus, T. F., Rosa, I. C., Repolho, T., Lopes, A. R., Pimentel, M. S., Almeida-Val, V. M. F., Coelho, M. M., & Rosa, R. (2018). Different ecophysiological responses of freshwater fish to warming and acidification. In Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology (Vol. 216, pp. 34–41). Elsevier BV. https://doi.org/10.1016/j.cbpa.2017.11.007

11. Ježek, P., & Hlavatá, L. (2005). Mitochondria in homeostasis of reactive oxygen species in cell, tissues, and organism. In The International Journal of Biochemistry & Cell Biology (Vol. 37, Issue 12, pp. 2478–2503). Elsevier BV. https://doi.org/10.1016/j.biocel.2005.05.013

12. Kaloyianni, M., Feidantsis, K., Nteli, I., Stergiou, P., Tsoulia, T., Dimitriadi, A., Antonopoulou, E., & Bobori, D. (2019). Biochemical and molecular responses of cyprinids in two Mediterranean lacustrine ecosystems: Opportunities for ecological assessment and biomonitoring. In Aquatic Toxicology (Vol. 211, pp. 105–115). Elsevier BV. https://doi.org/10.1016/j.aquatox.2019.03.021

13. Kausel, G., Vera, T., Valenzuela, G., Lopez, M., Romero, A., Muller, M., & Figueroa, J. (2010). At least two expressed genes for transcription factors Pitx2 and Rpx are present in common carp and are upregulated during winter acclimatization. In General and Comparative Endocrinology (Vol. 169, Issue 3, pp. 250–257). Elsevier BV. https://doi.org/10.1016/j.ygcen.2010.09.006

14. Kofonov, A., Potrokhov, O., & Hrynevych, N. (2020). Changes in the biochemical status of common carp juveniles *(Cyprinus carpio L.)* exposed to ammonium chloride and potassium phosphate. In Ukrainian Journal of Ecology (Vol. 10, Issue 4, pp. 137–147). Oles Honchar Dnipropetrovsk National University. https://doi.org/10.15421/2020_181

15. Kovalenko, Ju., Shlapak, O., Potrokhov, A., & Zin'kovskiy, O. (2019). Effect of antropogenic pollution on physiological and biochemical parameters of fish and composition of their parasitocenoses. In Ribogospodars'ka nauka Ukraïni. (Issue 3(49), pp. 72–88). National Academy of Sciences of Ukraine (Co. LTD Ukrinformnauka). https://doi.org/10.15407/fsu2019.03.072

16. Lall, S. P., & Kaushik, S. J. (2021). Nutrition and Metabolism of Minerals in Fish. In Animals (Vol. 11, Issue 9, p. 2711). MDPI AG. https://doi.org/10.3390/ani11092711

17. Liebel, S., Tomotake, M. E. M., & Oliveira-ribeiro, C. A. (2013). Fish histopathology as biomarker to evaluate water quality. In Ecotoxicology and Environmental Contamination (Vol. 8, Issue 2, pp. 9–15). Ecototoxicology and Environmental Contamination. https://doi.org/10.5132/eec.2013.02.002

18. Martseniuk, V. M., Potrokhov, O. S., & Zinkovskiy, O. G. (2017). Physiological-Biochemical Peculiarities of Adaptation of Perch and Common Carp to Elevated Water Temperature. In Hydrobiological Journal (Vol. 53, Issue 6, pp. 60–67). Begell House. https://doi.org/10.1615/hydrobj.v53.i6.60

19. Martseniuk, V. M., Potrokhov, O. S., & Zinkovskiy, O. G. (2018). Energy Metabolism in Organs and Tissues of Perch *Perca fluviatilis* under Changes of Water Temperature. In Hydrobiological Journal (Vol. 54, Issue 4, pp. 85–94). Begell House. https://doi.org/10.1615/hydrobj.v54.i4.90

20. Nardocci, G., Simonet, N. G., Navarro, C., Längst, G., & Alvarez, M. (2016). Differential enrichment of TTF-I and Tip5 in the T-like promoter structures of the rDNA contribute to the epigenetic response of Cyprinus carpio during environmental adaptation. In Biochemistry and Cell Biology (Vol. 94, Issue 4, pp. 315–321). Canadian Science Publishing. https://doi.org/10.1139/bcb-2016-0015

21. Pinkina, T., Zymaroieva, A., Matkovska, S., Svitelskyi, M., Ishchuk, O., & Fediuchka, M. (2019). Trophic characteristics of Lymnaea stagnalis (Mollusca: Gastropoda: Lymnaeidae) in toxic environment. In Ekológia (Bratislava) (Vol. 38, Issue 3, pp. 292–300). Walter de Gruyter GmbH. https://doi.org/10.2478/eko-2019-0022

22. Poulsen, H., Khandelia, H., Morth, J. P., Bublitz, M., Mouritsen, O. G., Egebjerg, J., & Nissen, P. (2010). Neurological disease mutations compromise a C-terminal ion pathway in the Na+/K+-ATPase. In Nature (Vol. 467, Issue 7311, pp. 99–102). Springer Science and Business Media LLC. https://doi.org/10.1038/nature09309

23. Prichepa, M., Potrokhov, A., & Zinkovskyi, O. (2017). The features of biochemical parameters change in different ecological groups of fish under conditions of anthropogenic pressure. In Biolohichni systemy (Vol.

9, Issue 1, pp. 39–43). Yuriy Fedkovych Chernivtsi National University. https://doi.org/10.31861/biosystems2017.01.039

24. Prychepa, M. V., Potrokhov, O. S., & Zin'kovskiy, O. G. (2019). Peculiarities of Biochemical Response of Fish to Anthropogenic Load under Conditions of Urbanization. In Hydrobiological Journal (Vol. 55, Issue 3, pp. 44–52). Begell House. https://doi.org/10.1615/hydrobj.v55.i3.50

25. Prychepa, M., Hrynevych, N., Kovalenko, Yu., Vodianitskyi, O., Svitelskyi, M., Khomiak, O., Prysiazhniuk, N., Ishchuk, O., Sliusarenko, A., Kunovskii, J., Mihalskiy, O., Heiko, L., Trofymchuk, A., Gutyj, B., & Levkivska, N. (2021). "Diversity of aquatic animals in water bodies Opechen' (Dnipro floodplain, Ukraine)". In Ukrainian Journal of Ecology (Vol. 11, Issue 3, pp. 285–291). Oles Honchar Dnipropetrovsk National University. https://doi:10.15421/2021_173

26. Prychepa, M., Hrynevych, N., Martseniuk, V., Potrokhov, O., Vodianitskyi, O., Khomiak, O., Rud, O., Kytsokon, L., Sliusarenko, A., Dunaievska, O., Gutyj, B., Pukalo, P., Honcharenko, V., Yevtukh, L., Bozhyk, L., Prus, V., Makhorin, H. (2021). "Rudd (*Scardinius Erythrophthalmus l.,* 1758) as a bioindicator of anthropogenic pollution in freshwater bodies". In Ukrainian Journal of Ecology (Vol. 11, Issue 2, pp. 253–260). Oles Honchar Dnipropetrovsk National University. https://doi:10.15421/2021_108

27. Sinha, A. K., Liew, H. J., Diricx, M., Kumar, V., Darras, V. M., Blust, R., & De Boeck, G. (2012). Combined effects of high environmental ammonia, starvation and exercise on hormonal and ion-regulatory response in goldfish *(Carassius auratus L.)*. In Aquatic Toxicology (Vols. 114–115, pp. 153–164). Elsevier BV. https://doi.org/10.1016/j.aquatox.2012.02.027

28. Smart, G. (1976). The effect of ammonia exposure on gill structure of the rainbow trout (Salmo gairdneri). In Journal of Fish Biology (Vol. 8, Issue 6, pp. 471–475). Wiley. https://doi.org/10.1111/j.1095-8649.1976.tb03990.x

29. Solé, M., Rodríguez, S., Papiol, V., Maynou, F., & Cartes, J. E. (2009). Xenobiotic metabolism markers in marine fish with different trophic strategies and their relationship to ecological variables. In Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology (Vol. 149, Issue 1, pp. 83–89). Elsevier BV. https://doi.org/10.1016/j.cbpc.2008.07.008

30. Solomatina, V. D., Pinkina, T. V., Svitelskyi, M. M., & Feduchka, M. I. (2019). Changes in calcium and phosphorus metabolism in fish with their warm-water growing. In Water bioresources and aquaculture (Vol. 1, pp. 51–62). Kherson State Agricultural University. https://doi.org/10.32851/wba.2019.1.5

31. Souza, M. M., Gross, S., Boyle, R. T., & Lieberman, M. (2000). Na+/K+-ATPase inhibition during cardiac myocyte swelling: involvement of intracellular pH and Ca^{2+}. In Molecular and Cellular Biochemistry (Vol. 210, Issue 1/2, pp. 173–183). Springer Science and Business Media LLC. https://doi.org/10.1023/a:1007154412805

32. Strauch, S., Bahr, J., Baßmann, B., Bischoff, A., Oster, M., Wasenitz, B., & Palm, H. (2019). Effects of Ortho-Phosphate on Growth Performance, Welfare and Product Quality of Juvenile African Catfish *(Clarias gariepinus)*. In Fishes (Vol. 4, Issue 1, p. 3). MDPI AG. https://doi.org/10.3390/fishes4010003

33. Therien, A. G., & Blostein, R. (2000). Mechanisms of sodium pump regulation. In American Journal of Physiology-Cell Physiology (Vol. 279, Issue 3, pp. 541–566). American Physiological Society. https://doi.org/10.1152/ajpcell.2000.279.3.c541

34. Van Bussel, C. G. J., Mahlmann, L., Kroeckel, S., Schroeder, J. P., & Schulz, C. (2013). The effect of high ortho-phosphate water levels on growth, feed intake, nutrient utilization and health status of juvenile turbot *(Psetta maxima)* reared in intensive recirculating aquaculture systems (RAS). In Aquacultural Engineering (Vol. 57, pp. 63–70). Elsevier BV. https://doi.org/10.1016/j.aquaeng.2013.08.003

35. Van der Meer, D. L. M., van den Thillart, G. E. E. J. M., Witte, F., de Bakker, M. A. G., Besser, J., Richardson, M. K., Spaink, H. P., Leito, J. T. D., & Bagowski, C. P. (2005). Gene expression profiling of the long-term adaptive response to hypoxia in the gills of adult zebrafish. In American Journal of Physiology-Regulatory, Integrative and Comparative Physiology (Vol. 289, Issue 5, pp. 1512–1519). American Physiological Society. https://doi.org/10.1152/ajpregu.00089.2005

36. Viarengo, A., Lowe, D., Bolognesi, C., Fabbri, E., & Koehler, A. (2007). The use of biomarkers in biomonitoring: A 2-tier approach assessing the level of pollutant-induced stress syndrome in sentinel organisms. In Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology (Vol. 146, Issue 3, pp. 281–300). Elsevier BV. https://doi.org/10.1016/j.cbpc.2007.04.011

37. Vodianitskiy, O. M., Potrokhov, O. S., & Zinkovskiy, O. G. (2017). Embryonic and Early Postembryonic Development of Carp and Activity of Enzymes of the Energy and Plastic Metabolism under Impact of Water Temperature Fluctuations. In Hydrobiological Journal (Vol. 53, Issue 1, pp. 78–86). Begell House. https://doi.org/10.1615/hydrobj.v53.i1.80

38. Vodianitskyi, O., Potrokhov, O., Hrynevych, N., Khomiak, O., Khudiyash, Y., Prysiazhniuk, N., Rud, O., Sliusarenko, A., Zagoruy, L., Gutyj, B., Dushka, V., Maxym, V., Dadak, O., & Liublin, V. (2020). "Effect of reserviour temperature and oxygen conditions on the activity of Na-K pump in embrios and larvae of perch, roach, and ruffe". In Ukrainian Journal of Ecology (Vol. 10, Issue 2, pp. 184–189). Oles Honchar Dnipropetrovsk National University. https://doi:10.15421/2020_83

39. Vodyanitskyi, A., Potrohov, A., Zinkovskyi, O., & Prychepa, M. (2017). Changes in activity of Na+/K+–ATP-ase in embryos carp fish under different oxygen and temperature of reservoir regime. In Visnyk of Lviv University. Biological series (Issue 75, pp. 14–22). Ivan Franko National University of Lviv. https://doi.org/10.30970/vlubs.2017.75.02

40. Whitehead, P. G., Wilby, R. L., Battarbee, R. W., Kernan, M., & Wade, A. J. (2009). A review of the potential impacts of climate change on surface water quality. In Hydrological Sciences Journal (Vol. 54, Issue 1, pp. 101–123). Informa UK Limited. https://doi.org/10.1623/hysj.54.1.101

41. Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: impacts across multiple levels of organization. In Philosophical Transactions of the Royal Society B: Biological Sciences (Vol. 365, Issue 1549, pp. 2093–2106). The Royal Society. https://doi.org/10.1098/rstb.2010.0055

42. Yang, Y., Cao, Z.-D., & Fu, S.-J. (2015). Variations in temperature acclimation effects on glycogen storage, hypoxia tolerance and swimming performance with seasonal acclimatization in juvenile Chinese crucian carp. In Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology (Vol. 185, pp. 16–23). Elsevier BV. https://doi.org/10.1016/j.cbpa.2015.03.009

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