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ANIMAL REPRODUCTION

Ions levels in the gastrointestinal tract content of silver catfish *Rhamdia quelen* (Siluriformes: Heptapteridae) are related to the reproduction period

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ABSTRACT: The current study tested the hypothesis that an increase in the ionic levels (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and K^+) in the gastrointestinal tract (GIT), and hepatosomatic index (HSI), gonadosomatic index (GSI), and Fulton's condition factor (FCF) are associated with the reproduction period of silver catfish (*Rhamdia quelen*). So, we verified ionic levels of the fluid phase of the different portions of the GIT content, HSI, GSI, and FCF of silver catfish in different seasons in a dam in southern Brazil. The highest Na^+ and Cl^- levels in GIT were found in spring. The stomach's Na^+ and Cl^- values were lower than the other segments. The K^+ and Ca^{2+} levels in GIT were higher in winter, except in the stomach, where the values increased in summer. The highest K^+ levels were reported in the anterior intestine. The highest Ca^{2+} levels in winter and spring were reported in the anterior and mid-intestines. In summer, the Mg^{2+} levels in the intestine were lower. The values of GSI and FCF were higher in winter and spring, and those of HSI were higher in winter. In conclusion, ions in the GIT and HSI, GSI, and FCF were higher in the reproduction period (winter for spring).
Key words: Fulton's condition factor, gonadosomatic index, Na^+ ions, Cl^- ions.

Níveis de íons no conteúdo do trato gastrointestinal de jundiá *Rhamdia quelen* (Siluriformes: Heptapteridae) são relacionados ao período reprodutivo

RESUMO: O presente estudo testou a hipótese de que um aumento nos níveis iônicos (Na^+, Cl^-, Ca^{2+}, Mg^{2+}, and K^+) no conteúdo do trato gastrointestinal (CTG), bem como no índice hepatosomático (IHS), no índice gonadosomático (IGS) e no fator de condição de Fulton (FCF) estão associados com o período de reprodução de jundiá (*Rhamdia quelen*). Assim, nós objetivamos verificar os níveis iônicos da fase fluida das diferentes porções do CTG, bem como IHS, IGS e FCF de jundiá em diferentes estações do ano em uma barragem no sul do Brasil. Os níveis mais altos de Na^+ e Cl^- do CTG foram encontrados na primavera. Os valores de Na^+ e Cl^- do estômago foram inferiores aos outros segmentos. Os níveis de K^+ e Ca^{2+} no CTG foram maiores no inverno, exceto no estômago, onde os valores aumentaram no verão. Os níveis mais altos de K^+ foram encontrados no intestino anterior. Os níveis mais altos de Ca^{2+} foram encontrados no intestino anterior e médio no inverno e na primavera. No verão, os níveis de Mg^{2+} no intestino foram mais baixos. Os valores de IGS e FCF foram maiores no inverno e na primavera, e do IHS foram maiores no inverno. Em conclusão, íons no CTG e HSI, GSI e FCF foram maiores no período de reprodução (inverno e primavera).
Palavras-chave: fator de condição de Fulton, índice gonadossomático, íons Na^+, íons Cl^-.

INTRODUCTION

Fish living in freshwater demonstrated passive loss of ions by diffusion, water gain by osmosis, and active, ATP-dependent uptake of ions (mainly Na^+ and Cl^-) across the gill epithelium (BARANY et al., 2020). This osmoregulatory capacity is a notable result of the hyperosmotic state of their surrounding environment. The excess water and ammonia are removed by producing large amounts of diluted urine (COPATTI et al., 2015), and the gills absorb the ions (COPATTI & BALDISSEROTTO, 2021). Therefore, drinking is counterproductive to osmotic balance in freshwater, and fish are not thought to drink in this environment. However, FLIK et al. (1995) reported that water is inevitably ingested along with food, and, therefore, the gastrointestinal tract (GIT) is an integral part of osmoregulation in freshwater fish (BECKER et al., 2012; BUCKING & WOOD 2006a, b).

The GIT plays a fundamental role in ion and osmotic homeostasis. The GIT is a complex organ system of fish, and absorption of monovalent ions occurs by several transporter mechanisms: Na^+/K^+-ATPase (NKA) in the basolateral membrane and $Na^+/Cl^-/K^+$ cotransporter (NKCC) and $Na^+/2Cl^-/K^+$ cotransporter (NKCC2) in the apical membrane (BECKER et al., 2006; COPATTI & BALDISSEROTTO, 2021). The intestine can also reabsorb ions discharged by gastric secretion (e.g., Cl^-) and bile when fat enters the intestine (BECKER et al., 2012).

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Besides ionic variables, other parameters can indicate changes in fish's nutritional and welfare conditions, such as Fulton's Condition Factor (FCF) and Hepatosomatic Index (HSI). The FCF indicates the fish welfare, correlated with a weight-length ratio, while the HSI is an analysis that can quantify the energy supply in the liver (CHELLAPPA et al., 1995). In addition, the HSI and the Gonadosomatic Index (GSI) can also show the fish's gonadal maturation because of the vitellogenesis process synthesizing vitellogenin.

Silver catfish (*Rhamdia quelen*, Siluriformes: Heptapteridae) is a freshwater species of omnivorous habit with a Neotropical distribution. There is a lack of data on the GIT for ion regulation of this species, with only one study reporting its importance but without evaluating the differences in the ionic balance between different seasons (BECKER et al., 2006). The current study tested the hypothesis that an increase in the ionic levels in the GIT and indices related to reproduction (HIS, GSI, and FCF) are associated with the reproduction period of silver catfish. Therefore, we evaluated the Na^+, Cl^-, K^+, Ca^{2+}, and Mg^{2+} ions of the fluid phase of the different portions of the GIT content and HSI, GSI, and FCF of this fish species collected throughout the different seasons in one year in a dam in southern Brazil.

MATERIALS AND METHODS

Specimens of silver catfish (647.54 ± 8.52g; 38.90 ± 2.51cm) were collected once a month for one year (between September 2005 and August 2006) with nets (20 m in length and 1 m high, with 2.0, 3.0, 4.0, 5.0, 6.0, and 7.0 cm between nodes) in the Rodolfo Costa & Silva dam in Itaara, RS, southern Brazil (29°29'01" – 29°30'56" S; 53°43'32" – 53°45'29" W).

The fish were grouped in the following seasons: winter, spring, summer, and autumn (n= 4 per month; n= 16 by season). They were weighed, deeply anesthetized with benzocaine (100 mg L^{-1}), and euthanized by a spinal cord section in the capture site. They were kept in Styrofoam with ice until transport to the laboratory, where they were processed. Liver and gonads were used to determine the Hepatosomatic Index (HSI), Gonadosomatic Index (GSI), and Fulton's Condition Factor (FCF) according to the following equations:

HSI (%) = 100 × (liver weight (g) / fish weight (g));

GSI (%) = 100 × gonad weight (g) / fish weight (g));

FCF (g cm^{-3} *100) = 100 × (body weight (g)/body length (cm))^3.

The GIT was removed, according to BECKER et al. (2006), into the stomach and anterior, mid, and posterior intestines. The contents of these segments were collected separately and centrifuged at 5,000 *g* for 5 min at 4 ºC to separate the fluid and solid phases. The fluid phase (supernatant) was stored in microtubes at -20 ºC for later analysis.

The Cl^- concentration was measured according to ZALL et al. (1956). The Mg^{2+} concentration was measured using a GBC 932AA atomic absorption spectrophotometer (GBC Scientific Equipment, Victoria, Australia). The Na^+, K^+, and Ca^{2+} concentrations were measured with a B262 flame spectrophotometer (Micronal, São Paulo, Brazil). Standard solutions were made with analytical grade reagents (Merck, Barueri, Brazil) dissolved in deionized water, and standard curves for five different concentrations were made for each ion (BECKER et al., 2006). The data were expressed in mmol L^{-1}.

All data were expressed as the mean ± SEM. The homogeneity of variances was tested with the Levene test. The data presented homogeneous variances were compared using a one-way (HSI, GSI, and FCF) or two-way (ions levels, season versus GIT portions) ANOVA and Tukey's test. The minimum significance level was set at $P < 0.05$. All analyses were performed using the Statistica 5.1 software.

RESULTS

The GSI was significantly lower in autumn than in other seasons and in summer than in winter. The HSI was significantly higher in winter than in the other seasons and in autumn than in spring. The FCF was lower in summer than in winter and spring (Table 1).

The highest Na^+ and Cl^- levels in all segments of the GIT were reported in spring, except in the anterior intestine, where the values were similar to those found in autumn. The Na^+ and Cl^- values were significantly lower in the stomach in all seasons compared to the other segments. The highest Na^+ and Cl^- levels were found in the winter in the anterior and mid-intestines, in the spring and autumn in the mid-intestine, and in the summer in the posterior intestine (Figure 1A and 1B).

The K^+ levels in all segments of the GIT were significantly higher in winter (except in the stomach, whose higher values were found in summer). The K^+ values in the stomach were significantly lower than in other GIT in all seasons (except in the summer, where K^+ levels were similar between the stomach and the posterior intestine). The highest K^+ levels

Table 1 - Biometric parameters (mean ± SEM) of silver catfish (*Rhamdia quelen*) collected on Rodolfo Costa e Silva dam, Itaara, RS, Brazil, in different seasons.

	---Season---			
Parameter	Winter	Spring	Summer	Autumn
GSI	5.32±0.29[a]	4.34±0.33[ab]	2.36±0.46[b]	0.78±0.08[c]
HSI	1.44±0.08[a]	0.74±0.06[c]	1.01±0.08[bc]	1.14±0.07[b]
FCF	1.14±0.04[a]	1.12±0.08[a]	0.90±0.05[b]	0.99±0.03[ab]

Gonadosomatic index (GSI) and Hepatosomatic index (HSI) are expressed as %. Fulton's Condition Factor (FCF) is expressed as g cm^{-3} *100. Different letters indicate a significant difference between seasons in the same parameter ($P < 0.05$).

in all seasons were reported in the anterior intestine (except in the winter, where the K^+ levels were similar between the anterior and mid-intestines) (Figure 1C).

The highest Ca^{2+} levels in all segments of the GIT were reported in spring (except in the stomach, where the values were similar to those found in summer). The Ca^{2+} values in the stomach were significantly lower than in other GIT portions in winter (except for the posterior intestine) and spring and significantly higher than in other GIT portions in summer. The highest Ca^{2+} levels in winter and spring were found in the anterior and mid-intestines concerning the other segments of the GIT (except in the spring for the posterior intestine) (Figure 1D).

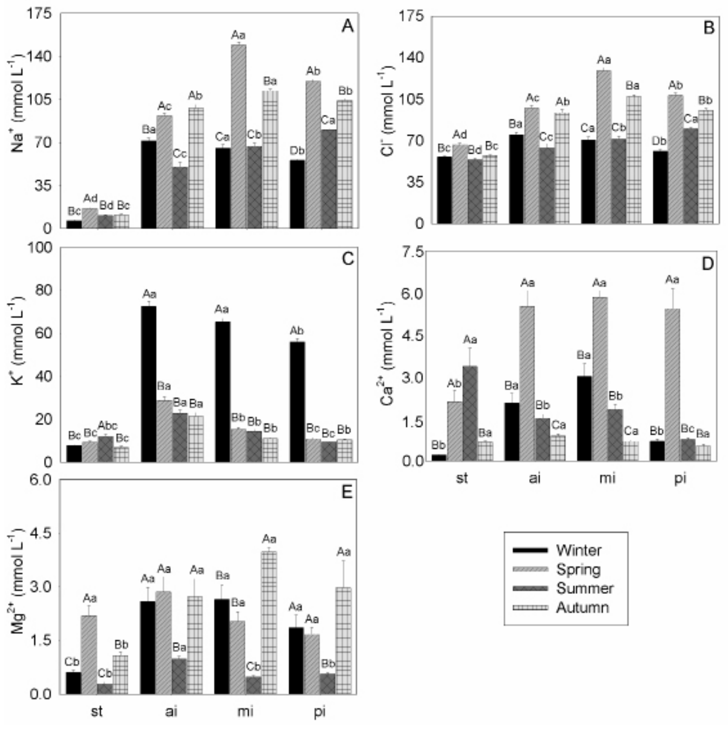

Figure 1 - Na^+ (A), Cl^- (B), K^+ (C), Ca^{2+} (D), and Mg^{2+} (E) levels (means ± SEM) in the fluid phase of the different segments of the gastrointestinal tract of silver catfish (*Rhamdia quelen*) collected on Rodolfo Costa e Silva dam, Itaara, RS, Brazil in different seasons. st = stomach, ai = anterior intestine, mi = mid-intestine, pi = posterior intestine (n = 16 per season). Different capital letters indicate significant differences between the seasons in the same segment of the gastrointestinal tract ($P < 0.05$). Different lowercase letters indicate significant differences between the gastrointestinal tract segments in the same season ($P < 0.05$).

The lowest Mg^{2+} levels in the anterior, mid, and posterior intestines were reported in the summer. In the stomach, the Mg^{2+} values were significantly higher in spring than in other seasons. The Mg^{2+} levels in the stomach were significantly lower in winter and autumn than in the other GIT segments (Figure 1E).

DISCUSSION

The present study characterized the magnitude of ions in the GIT in silver catfish and examined the relationship between ionic content and HSI, GSI, and FCF indices and the differences at ionic levels in different seasons over a year. Initially, an interesting finding of our study was related to the nutritional condition of fish. The HSI, GSI, and FCF values were higher in winter and GSI and FCF also in spring. The HSI can quantify the total energy supply, such as liver glycogen, indicating the energetic metabolic demand (CHELLAPPA et al., 1995). So, the liver hypertrophy found in winter in silver catfish of this study could result from energy mobilization to be used during the breeding period (spring). The GSI data confirmed this hypothesis because the highest values were reported during the winter-spring transition. Therefore, the winter-spring change had the best requirements for an increased weight-length ratio, i.e., an increase in body weight, without variation in length. The higher FCF is probably related to the development of the gonads, where the HSI was decreased by using the energy stored in the liver for gonadal development.

Also, in the spring, the Na^+, Cl^-, and Ca^{2+} levels in all GIT segments of the studied species were increased. The GIT is the first contact site with feeds. Therefore, ion levels in the GIT are affected by fish feeding habits (WOOD & BUCKING et al., 2011; CIAVONI et al., 2023), where an osmotic gradient appeared to be coupled to net Na^+ and Cl^- flux (NADELLA et al., 2014). This pattern resembles unidirectional Ca^{2+} uptake kinetics in GIT (KLINCK et al., 2012). The silver catfish of the current study were collected from the wild, and even though it was impossible to determine when they fed, there was probably a greater intake of food in the spring when the temperature began to increase in these waters. In this season, fish tend to become more active, seeking more foods to generate a stock of energy that will be used for the energy expenditure required for reproduction. A more abundant diet could stimulate the apical GIT transporters, with Na^+ and Ca^{2+} concentrations being 2-3-fold higher in chyme than in freshwater (WOOD & BUCKING, 2011). As seen, GIT has adequate capacity to fulfill fish uptake needs for Ca^{2+} and Na^+ (KLINCK et al., 2012; NADELLA et al., 2014). However, in our study, the stomach's Ca^{2+} absorption rates were not uniform, with spring and summer peaks. It is supposed that GIT uptake rates of Ca^{2+} are top-regulated when the dietary supply of this ion is increased.

Another explanation should also be considered for the increased Ca^{2+} levels in the intestine in the spring. In addition, the levels of this ion were high in the stomach not only in this season but also in the summer, which could be related to nondigested food in the stomach (BECKER et al., 2012). A study in a stream in the same region as this study found that silver catfish's main food item is a crustacean (*Aegla* sp.), followed by larvae of benthic insects (COPATTI et al., 2012). These animals have a large amount of Ca^{2+} in the form of $CaCO_3$ in their chitin exoskeleton, which explains the increased levels of this ion in spring and summer when fish seek more food.

The stomach's Na^+, Cl^-, and K^+ values were lower than in the other segments. This finding was expected because, in this organ, there is a common secretion of gastric juice, which has a low concentration of these ions and a lower absorption of ions compared to the intestine (BECKER et al., 2012). Despite a small amount of K^+ in bile acid secretions (BUCKING & WOOD, 2006a), dietary K^+ was almost completely absorbed from the stomach to the intestine (CIAVONI et al., 2023). The net absorption of K^+ in the stomach occurred following the cessation of Cl^- secretion, demonstrating the K^+ involvement with HCl acid production (BUCKING & WOOD, 2006a).

Interestingly, there was also a decrease in almost all ion levels evaluated in our study in the posterior intestine compared to the anterior or mid-intestine. The exception was Mg^{2+}. As water is absorbed in the mid-intestine (BUCKING & WOOD, 2006b), most ions (Na^+, Cl^-, K^+, and Ca^{2+}) must have been absorbed to maintain their concentration in intestinal portions, while Mg^{2+} was not absorbed, increasing its levels (except for summer) in the posterior intestine. The supply of Mg^{2+} also depends on the type of feeding (vegetable) (BUCKING & WOOD, 2007), which may have been different in summer, when fish may have consumed animals, reducing the values of Mg^{2+} in the intestine.

The higher ion levels in the anterior (and even mid) intestine could be explained by the discharge of ions into the pyloric ceca/anterior intestine by bile (BECKER et al., 2009). In some fish, the pancreatic duct penetrates the gut wall in the transition between the anterior and mid-intestine (EASTMAN & DEVRIES, 1997), where the secretion of Na^+ by the pancreas increases when the chyme of the food reaches the mid-intestine (BECKER et al., 2012). In addition, when Na^+ is absorbed, the osmotic pressure in the intestine rises, stimulating water absorption (BUCKING & WOOD, 2006a; CIAVONI et al., 2023).

Furthermore, in the posterior intestine, the K^+ levels were similar to those found in the stomach (except in the winter). The lower K^+ levels in the intestine in the winter compared to the other seasons might be related to the type of food in this season that would contribute to higher K^+ absorption in the intestine. Silver catfish is an omnivorous species; therefore, it could have increased the consumption of plants with a high K^+ content during the winter. The increase of the K^+ levels in the anterior intestine is due to the discharge of bile into the anterior intestine because high ion levels (Na^+, K^+, Ca^{2+}, and Mg^{2+}) have been reported in the gallbladder bile (BALDISSEROTTO et al., 2004; BECKER et al., 2012). So, in the present study, the reduction of Na^+, Cl^-, K^+, and Mg^{2+} levels in the posterior intestine indicates that they were absorbed in this portion.

CONCLUSION

We verified the hypothesis of our study. There is a relationship between season and the ionic content in the gastrointestinal tract of silver catfish. The ions had the highest levels in winter and spring, which should be related to periods of preparation for reproduction (winter) and reproduction (spring). It was also confirmed by our hepatosomatic index, gonadosomatic index, and Fulton's condition factor results, whose values were higher in these seasons.

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DECLARATION OF CONFLICT OF INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the study reported in this paper.

AUTHORS' CONTRIBUTION

Sample preparation, analysis, and investigation: Alexssandro G. Becker. Data curation: Alexssandro G. Becker, Carlos E. Copatti, and Bernardo Baldisserotto. Writing–original draft, review, and editing: Carlos E. Copatti and Bernardo Baldisserotto. Supervision: Bernardo Baldisserotto All authors agreed to the submitted version of the manuscript.

BIOETHICS AND BIOSECURITY COMMITTEE APPROVAL

This study was carried out according to the principles of the Brazilian College of Animal Experimentation. It was approved by the Committee on Ethics in the Use of Animals of the Universidade Federal de Santa Maria (UFSM), Santa Maria, RS, Brazil, under protocol number 23081.008434/2007-85.

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