

FOOD DEPRIVATION ON *GYMNOTUS CARAPO* (LINNAEUS, 1758) - MORPHOMETRIC AND MICROBIOLOGIC STUDY.

Luciano Tavares de Souza (Biologia – Licenciatura, Universidade Federal do Tocantins); *Evilanna Lima Arruda* (Biologia – Licenciatura, Universidade Federal do Tocantins); *Rejanne Lima Arruda* (Biologia – Licenciatura, Universidade Federal do Tocantins); *Vivian Webster* (Biologia - Universidade Católica Dom Bosco); *Eliane Tie Oba Yoshioka* (Bióloga – EMBRAPA – AMAPÁ); *Wagner dos Santos Mariano* (Biologia – Licenciatura, Universidade Federal do Tocantins).

wagnermariano@uft.edu.br

RESUMO

Os peixes estão constantemente sujeitos a modificações no ambiente aquático. O estudo dos aspectos adaptativos dos organismos aquáticos frente as oscilações ambientais passa, necessariamente, pelo conhecimento das estruturas morfológicas e dos mecanismos fisiológicos e bioquímicos. A adaptação é um dos mais importantes conceitos da biologia moderna, unindo muitas áreas de estudo. Em termos gerais, a adaptação permite ao organismo a manutenção da atividade fisiológica e sua sobrevivência quando o ambiente é alterado em um ou mais fatores. Algumas espécies de organismos aquáticos desenvolveram estratégias, para lidar com o pouco oxigênio e alimentos disponíveis. A tuvira, *Gymnotus carapo*, possui alta plasticidade tendo a respiração aérea acessória relativa importância neste contexto, o que possibilita a sua sobrevivência nesses ambientes. O presente trabalho avaliou respostas morfométricas e microbiológicas do sistema digestório da tuvira, *G. carapo*, frente à privação alimentar. Os exemplares foram aclimatados em laboratório e foram divididos em 3 grupos: Grupo controle, 7 e 14 dias de privação alimentar (T0, T1 e T2 respectivamente), onde os indivíduos foram medidos e pesados, obedecendo o protocolo experimental de morfometria e microbiologia. Nenhum peixe morreu durante os experimentos e, durante a primeira semana de privação alimentar os peixes apresentaram maior movimentação e aparente desconforto. A partir do início da segunda semana foi possível verificar uma possível adaptação a falta de alimento, pois os animais voltaram a se movimentar normalmente. A privação alimentar demonstrou que esta espécie lança mão de células do TGI para possivelmente minimizar a demanda energética durante este período e que a microbiota do sistema digestório sofre com a privação do alimento em frequências individuais da população. A população bacteriana que sofre com a privação alimentar são as bactérias transitórias, possivelmente compostas por bacilos gram positivos. A população de bactérias gram negativas sofre uma redução até os sete primeiros dias após a privação e consegue naturalmente recompor a microbiota, como observado no décimo quarto dia.

SUMMARY

The fish are constantly subjected to changes in the aquatic environment. The study of adaptive aspects of aquatic organisms against environmental fluctuations must encompass the knowledge of morphological structures and physiological and biochemical mechanisms. Adaptation is one of the most important concepts of modern biology, bringing together many areas of study. In general, adaptation allows the body to maintain physiological activity and survival when the environment is changed in one or more factors. Some species of aquatic organisms have developed strategies to cope with low oxygen and food available. The tuvira, *Gymnotus carapo*, has high plasticity with

accessory air breathing having a relative importance in this context, enabling their survival in these environments. This study evaluated the microbiological morphometric response and digestive system of the tuvira, *G. carapo*, compared to food deprivation. The specimens were acclimated in the laboratory and were divided into 3 groups: control group, 7 and 14 days of food deprivation (T0, T1 and T2, respectively), where individuals were measured and weighed, following the experimental morphology and microbiology protocols. No fish died during the experiments and, during the first week of starvation, the fishes showed more movement and apparent discomfort. From the beginning of the second week it was possible to verify a possible adaptation to the lack of food, because the animals began to move back normally. A food deprivation demonstrated that this species makes use of cells of the TGI to possibly minimize the energy demand during this period and that the digestorymicrobiota system suffers with the ongoing deprivation of food. A bacterial population of individuals suffering from food deprivation are transient bacteria, possibly composed of gram-positive bacilli. The population of gram-negative bacteria is reduced in the first seven days of deprivation and can naturally replenish the microbiota, as observed on the fourteenth day.

INTRODUCTION / OBJECTIVES

The annual cycle of flood and drought is the most important ecological phenomenon of a river. The area covered by flood and the staying time of water in the fields dictate the availability of habitats and food for fish, crustaceans, birds, reptiles, many mammals, aquatic and semi-aquatic plants. With this dynamic, the South Pantanal of MatoGrosso (Brazil), which has unique topographical features, features dry periods lasting an average of four months per year (VALVERDE, 1972; CADAVID GARCIA, 1984) leading to reduced water levels on the rivers, creating environments popularly known as bays, and streams of ebb and flow (AMARAL FILHO, 1986).

The "Dequada" is characterized by the decomposition of large organic mass that occurs early in the process of flooding of the Pantanal, that depending of its magnitude can cause massive fish kills. The thermal inversion and the decrease in the volume of water, occurred during this phenomenon, eventually generates: a reduced concentration of oxygen available for breathing aquatic animals, momentary loss of homeostasis and tightening environmental biodiversity of ecotones, which can lead to a decrease in food availability.

Animals that live in these conditions maintain different strategies to deal with the limited availability of oxygen and nourishment. When oxygen and food are declining because of some factor, the fish will often respond by leaving to other regions. Different strategies such as hyperventilation, bradycardia, cardio-respiratory timing, peripheral vascular constriction and constriction of the digestive tract are some of the physiological and morphological responses used by fish living in these conditions (MARIANO, 2006, MORAES, *et al.*, 2002).

According to Rotta (2003) the general processes of digestion in fish have been little studied compared to homeotherms (warm-blooded and constant body temperature animals). However, available information suggests that the fishes are, in general, similar to other vertebrates in regard to the digestive processes. Fishes present multiple variations of the basic structure of the gastrointestinal (GI) tract of vertebrates, which are generally correlated to the type of food consumed and the environment, and may influence the presence, position, shape and size of a particular organ.

The gastrointestinal tract and respiratory system of vertebrates are generally the systems that adjust to different environments. The intestine (structurally and functionally) of carnivorous fishes adjust various structures to meet the demands of the organic system, by increasing the area of epithelial surface (microvilli) of the intestine,

increasing selectively the permeable barrier, helping water and electrolyte homeostasis, immunity, microbiota and regulation of digestion and metabolism, and responding quickly to the change of diet of the fishes (RIOS, 2004).

The tuvira, *Gymnotus carapo*, is a fish of the class of freshwater [Actinopterygii](#) and belongs to the order Gymnotiformes, family Gymnotidae. This species has an accessory air breathing, the swim bladder, very vascularized, as an air-breathing organ which enables the maintenance of life in an environment with low oxygen availability (ROTTER, 2004, MORAES, *et al.*, 2002).

Biological and ecological studies of Tuvira in the Pantanal showed that fishes live in lentic environments with dense rooting aquatic plants, which holds a lot of organic matter originating from the process of flooding, where they find their food and shelter. They have nocturnal habits and its occurrence is prevalent in marginal areas of bays, streams, and wetlands of the receding lower reaches of rivers that drain into the Paraguay River (ROTTER, 2004).

Every year during the dry season in the Pantanal, the fish are confined to ponds and streams that have stressful conditions such as hypoxia, exposure to air and food shortages. The shortage in food possibly leads this species to long periods of food restriction. The morphometric and microbiological adjustments during this period are unknown. Anatomical changes, mainly in the digestive tract, are described in several species when subjected to dietary restriction, but were not described for this species. In this context, this paper will demonstrate morphometrics and microbiological parameters of the digestive system of tuvira, *G. carapo*, compared to starvation.

MATERIALS AND METHODS

For the experiment we used 30 adult subjects of *Gymnotus carapo*, both sexes [body mass index (Mc) = 110.8 ± 11 (average \pm SEM)] collected from live bait companies of the city of Campo Grande (MS). The fishes were kept for 21 days in polyethylene tanks of 500 L with aeration and continuous water flow, temperature 25.5 ± 1 ° C and 12/12 photoperiod.

EXPERIMENTAL DESIGN

Acclimatization period: During the acclimatization process tenviras were fed with small fishes every 48 hours. After acclimatization the fish received the same type of food, however with controlled levels (2% of the biomass a day) for 15 consecutive days. The control group was separated immediately after the feeding period. 3 (three) groups with 10 (ten) individuals / each, as the terms G 0 (control group - without food restriction), G1 (1 week food deprivation) and G2 (2 weeks of food deprivation).

MORPHOMETRY OF THE DIGESTIVE TRACT: The fish were anesthetized with benzocaine 0.01% (1 g/10 liter water tank) for 3 to 5 minutes and sacrificed by spinal section, weighed and then measured. The fish were dissected dorsiventral, reverse craniocaudal for further selection process of the organs of interest (digestive tract). The digestive tract was divided into two parts: 1) BEGINNING: oral cavity - foregut, 2) FINAL: bowel itself and the anus. The length of the organs was expressed relative to standard length and not the total length, according to the methodology (RIOS, *et al.*, 2004).

MICROBIOLOGY: The collect was made according to the protocol: 1) We used "swab" to collect the microbiota of the digestive tract, as described above, 2) smears were made and plated on nutrient agar plates, Agar Conckey Mac, Agar mannitol and Brilliant Green, the plates were placed in an oven at 37 ° C for 24 h, for further analysis.

Statistical Analysis: Results are presented as average \pm SEM (Standard error of mean). The Bartlett test was used to determine the homogeneity of the data and define the application of parametric or the nonparametric tests on the variables. The analysis of variance ANOVA followed by post-test Dunnett'S (parametric) was used in all

tests. It was considered for this study 5% ($P < 0.05$) as the level of significance in the statistical analysis using the statistical program GraphPadInstat version 2.01 (GraphPad Software, San Diego, CA, USA).

RESULTS / DISCUSSION

No fish died during the experiments and during the first week of starvation the fish showed more movement and apparent discomfort. From the beginning of the second week was possible to verify a possible adaptation to food shortage, because the animals began to move normally again.

The morphometric parameters of the digestive tract (DT) of *G. carapo* subjected to food deprivation were divided into three parts: full DT, DT initial portion; DT final portion. The complete DT decreased significantly ($p > 0.05$) in both groups of deprivation. The initial portion of the digestive tract did not differ significantly in both groups of food deprivation. The final portion of the digestive tract decreased significantly ($p > 0.05$) in both groups of food deprivation (T1 and T2).

Before depriving them of food, the population of microorganisms consisted of cocci and bacilli. After 7 days of food deprivation there was a sharp drop of microorganisms like bacilli. The population of cocci has not suffered from food deprivation. After 14 days of food deprivation the bacilli population began to re-compose the microbiota of the digestive tract of tuviras.

In analyzing the bacteria on the Gram stain was observed Gram positive and Gram negative bacilli and cocci in the period prior to food deprivation. It was noticed after deprivation of food that the gram-positive bacilli did not return to inhabit the digestive tract of Tuvira as evidenced by the growth profile in different culture plates: nutrient agar (NA), Mac-Conckey agar (CMA), agar mannitol (AMN) and Brilliant green agar.

MORPHOLOGY OF THE DT: The gastrointestinal (GI) tract or digestive tract is the tube that runs from mouth to anus and through which the food passes. Can be subdivided into the oral cavity or bucofaringean, foregut (esophagus and stomach), midgut (intestine itself - IPD) and hindgut (rectum) (RANDALI, *et al.*, 2002). This study separated the GIT in two regions: The initial, which includes the oral cavity, and the foregut and final, where are located the IPD and the anus. Therefore would be better to separate into two parts, because the final portion of the GIT proposed by Rotta (2003) consists of the rectum that does not contribute effectively in the processes of digestion and absorption, but only to the elimination of faeces. The digestive tract (DT) in the first week of food deprivation (T1) modulates the amount of cells that will be available for digestion, reducing the length of the GI tract during the two weeks of deprivation. The energy savings would be the main objective for adopting such organic strategy. The separation of TD in two parts - initial and final - made possible to see where the plasticity was. The initial portion showed no difference compared with control group in both experimental groups (T1 and T2), and the end portion has decreased significantly in both groups. This result shows that the end portion, consisting of the intestine, is the region of the digestive tract that reduces in the first two weeks of food deprivation.

According to Rotta (2003) the gut is a relatively simple tube, starting at the pyloric valve and ending in the rectum, not being separated into thin and thick, like in mammals. This region has the digestive glands and an abundant supply of blood vessels and lymph, where digestion, started in the stomach, is completed. In the intestine is where most of the absorption of nutrients, ions and water from the diet occurs, being the products of digestion kept in solution, which facilitates the absorption. In fish, the intestine can perform other functions as an aid in osmoregulation or respiration in addition digestion and absorption. This all requires a significant energy demand that in tuviras is solved, during food deprivation, by reducing the area of IPD.

MICROBIOLOGY, TD: The oscillation in the presence of microorganisms may be associated with food shortage. The metabolism of enteric bacteria is dependent on the digested food in the intestinal tract for nourishment. With dietary restriction there was a nutritional imbalance that caused a change in the number of individuals of the

population of bacilli. However, these bacilli again populated the digestive tract showing that these microorganisms were not transients, but residents, because the transients are not able to repopulate a surface when removed. Gram's technique was used to classify bacteria. In analyzing the bacteria on the Gram stain we noticed that, after food deprivation in tuviras, gram positive bacilli did not return to populate the digestive tract. This result suggests that among the bacilli that are part of the microbiota of the intestinal tract only gram negative are part of the natural microflora of tuviras (residents) while gram-positive bacilli may be transient and come from the food, not from the water, since the only environmental change for the Tuviras was of food deprivation.

CONCLUSION

The food deprivation demonstrated that this species makes use of cells of the GI tract to possibly minimize the energy demand during this period. The bacterial population that are hit with food deprivation are the transients only, possibly composed of gram-positive bacilli. The population of gram-negative bacteria is reduced in the first seven days of deprivation and can naturally replenish itself, as observed on the fourteenth day. The population of cocci did not suffer from food deprivation.

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