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SYMPOSIUM PROCEEDINGS

Adalberto Val

Don MacKinlay

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PREFACE

Fish are so important in our lives that they have been used in thousands of different laboratories worldwide to understand and protect our environment; to understand and ascertain the foundation of vertebrate evolution; to understand and recount the history of vertebrate colonization of isolated pristine environments; and to understand the adaptive mechanisms to extreme environmental conditions. More importantly, fish are one of the most important sources of protein for the human kind. Efforts at all levels have been made to increase fish production and, undoubtedly, the biology of fish, especially the biology of unknown species, has much to contribute.

As we prepare this brief introduction to the "Advances in Fish Biology Symposium," we are including 48 oral and poster papers on a diverse range of species, covering a number of topics. From the description of new tropical fish species to the hardy nature of fish species of the Amazon, from the chromatic organization of the retina of salmon to the relationship between flood pulse and fish biology in the Pantanal, from fish as environmental biomarkers to pain perception in fish, this symposium is a "voyage" across the world of fish biology.

The contributions are stimulating and we sincerely wish to thank all the investigators who contributed to this symposium. We hope that your participation result in new insights and new approaches of fish biology and so contribute to the advancement of this central theme of our lives. We wish that all contributors and participants have a productive meeting.

Symposium Organizers:

Adalberto Val, INPA, Manaus, Brazil
Don MacKinlay, Fisheries & Oceans Canada

CONGRESS ACKNOWLEDGEMENTS

This volume is part of the Proceedings of the 6th International Congress on the Biology of Fish, held in Manaus, Brazil in August, 2004. Ten years have passed since the first meeting in this series was held in Vancouver, BC, Canada. Subsequent meetings were in San Francisco, California; Baltimore, Maryland; Aberdeen, Scotland; and again in Vancouver, Canada. From those meetings, colleagues from over 30 countries have contributed more than 2,500 papers to the Proceedings of over 80 Congress Symposia, all available for free viewing on the internet.

We would like to extend our sincere thanks to the many people who helped us organize the facilities and program for this 6th Congress.

The local arrangements team worked very hard to make this Congress a success. The leaders of those efforts were Vera Almeida Val, Adriana Chippari-Gomes, Nivia Pires Lopes and Maria de Nazare Paula Silva (Local Arrangements); Marcelo Perlingeiro (Executive Secretary) and Maria Angelica Laredo (Fund Raising). The enormous contribution of time and effort that was required has led to an unforgettable experience for the participants, thanks to the imagination, determination and dedication of this team.

Many sponsors helped ensure the success of the meeting through both monetary and in-kind contributions, including: Fundação Djalma Batista, Honda, Merse, Cometais, Turkys Aquarium, Banco da Amazônia, Banco do Brasil, FUCAPI, SEBRAE/AM, IDAM/SEPROR, FAPEAM, SECT-AM, SUFRAMA, PETROBRÁS, CAPES, FINEP, CNPq, the Physiology Section of the American Fisheries Society, UFAM - Federal University of Amazonas, Fisheries and Oceans Canada and INPA - National Institute for Research in the Amazon.

Travel arrangements were ably handled by Atlantic Corporate Travel (special thanks to Maria Espinosa) and Orcal Planet, and the venue for the meeting was the spectacular Tropical Hotel Conference Center in Manaus.

The Student Travel Award Committee of the Physiology Section of the American Fisheries Society, led by Michael Redding, evaluated 65 applications from 15 countries and awarded 40 Travel Grants, after an ambitious and trying fund-raising effort. Special thanks must go the US Department of Agriculture, the US Geological Survey, US National Science Foundation and the World

Fisheries Congress for providing funds. In addition, the American Fisheries Society contributed books to be used as prizes for the best student papers.

The editorial team compiled the short abstracts into an abstract book and formatted and compiled the papers for the Symposium Proceedings. Thanks to Karin Howard, Christie MacKinlay, Anne Martin, Callan MacKinlay and Marcelo Perlingeiro.

In particular, we would like to extend a sincere 'thank you' to the organizers of the individual scientific Symposia and their many contributors who took the time to prepare a written submission for these proceedings. Their efforts are very much appreciated. We hope that their participation will result in new insights, new collaborations and new lines of research, leading to new papers to be presented at the 2006 Congress in St. John's, Newfoundland.

Congress Chairs:

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Introduction

Ozone depletion is the cause of increased ultraviolet radiation at the ground level, especially at high latitudes. Most of the compounds causing ozone depletion have now been phased-out but we cannot yet be sure whether global ozone reached a minimum. In the Equatorial regions, there are no statistically trends in total ozone what mean that the UV-B (280-315 nm) irradiance will continue to be the highest one in this region (Winckler & Fidhiany, 1996), thought local attenuation caused by clouds, smoke from burning forest, etc., can indeed occur. This is a cause of concern, particularly because (a) plenty of evidence shows that changes in spectral composition exceeding those experienced during the evolution of exposed animals cause stress, and (b) it may affect the existence of the stupendous life diversity in the tropics. Many organisms possess protective and effective cellular repair mechanisms against UV exposure, but excessive exposure to UV radiation may overload their capabilities. In addition, changes of solar radiation on aquatic ecosystems may result in decreased biomass productivity including fish yields.

Three physical processes attenuate solar radiation in the water column: water surface reflection, scattering and absorption. Surface reflectance is a function of solar elevation and incidence angle, while scattering and absorption depends on inherent properties of the water and are independent of the incident radiation. The UV absorbance for natural waters is dependent on the concentration of dissolved organic material (DOM). The absorbance spectra for DOM are low at the red end and rise exponentially with decreasing wavelength in the UV. These processes limit UV penetration to the first centimeters of the top of the water column in natural freshwater bodies. This means that for a given water type, the amount of solar radiation reaching water surface roughly determines the amount of radiation penetrating the water column. Because UV irradiance prevailing at the Equator is high, even compared to ozone-depleted region in Antarctic, any environmental disturbance that exposes the aquatic animal to water surface can be highly stressful.

Hypoxia is an environmental condition that can drive fish to water surface. Early in the evolution, fish had to deal with hypoxia and improved a myriad of adaptive strategies to explore all available sources of oxygen, including air and air-water interface, or to conform themselves to low oxygen availability. Fish managements in aquaculture facilities can also involve situations that enhance exposure to UV. Under these environmental conditions, tambaqui, *Colossoma macropomum*, and pirarucu, *Arapaima gigas*, are emblematic animals to analyze the effect of UV on tropical fish.

Biological aspects of pirarucu and tambaqui

Pirarucu is an air-breather. Except for the first few weeks of its life, up to 7 cm in length, pirarucu surfaces regularly to breathe. In adults of *A. gigas*, near 80% of oxygen is taken up at the well-vascularized swim-bladder and 20% at the gills, while carbon dioxide is preferentially excreted at the gills (~78%) and the rest at the gills (~15%) and kidney (~7%). Therefore, adults of *Arapaima* are dependent of air uptake into the modified swim-bladder to transfer oxygen to tissues. Under normal environmental conditions, pirarucu surfaces every 5 minutes to breathe and may drown within 10 minutes without access to air. Pirarucu is the largest scaled freshwater fish and one of the most important species farmed across South America.

Tambaqui is a favorite culture species in many parts of South America and in several other countries. The young fish filter plankton, but the adults eat mainly

fruits during the flooded season, using powerful jaws that crash fruits and hard seeds. Tambaqui is hypoxia-tolerant; this species can survive oxygen levels as low as 10% air-saturation. Beyond this point, tambaqui starts to breathe at the water surface, i.e., under environmental hypoxia the animal comes to the surface and skims the well-oxygenated surface layer of water (reviewed by Val and Almeida-Val, 1995). To improve this, the animal expands the inferior lips that form a funnel that directs the surface water across the gills. The lips are not involved with gas exchange; they serve strictly to facilitate the skimming of water surface. The animals remain skimming as long as oxygen depletion persists. As the oxygen returns to normal levels, the lips swelling disappear in about the same time required for the expansion, *ca*. two hours, and the animals move to the middle of the water column. Undoubtedly, this mechanism provides tambaqui and other fish species, as *Mylossoma* and *Brycon*, an important alternative to ventilation in hypoxic waters. When access to water surface is denied, the animals exposed to hypoxia expand the lips but a massive drop of blood oxygenation occurs and the animals die. Thus, pirarucu and tambaqui face UV radiation exposure when managed in aquaculture stations, ventilating in hypoxic water, and eating.

Effect of UV exposure

Experimental aquaria were fitted from above with three different sources of radiation (UVA, UVB and UVR (UVA+UVB)) provided by Philips TL40W/05 and TL40W/12 lamps. The radiant intensity at the surface of the water column was previously set (250; 500; and 125+250 $mW.cm^{-2}.h^{-1}$, respectively) and monitored continuously over the experimental period. We aimed to access the effect of irradiance duration and the differential effect of UVA and UVB on tambaqui and pirarucu, kept initially under normoxia and neutral pH, in standard water.

Exposure of pirarucu and tambaqui to UVA, UVB and UVR (UVA+UVB) under controlled conditions in the laboratory revealed several differences between the two species. Even at reduced duration, the animals presented extensive physiological and biochemical disturbances. Large skin damage was observed on the head of pirarucu and this is related to its inability to self protect by reducing the distance to UV source, i.e., to stay away from water surface, as do the tambaqui. Contrasting to tambaqui, pirarucu also experienced high mortality at a given irradiation. To analyze the effects of irradiance duration on pirarucu a new set of experiments are in progress using lower UV doses. This

difference between the two species may be reflecting the amount of time they spend at water surface, what roughly relates to UV exposure dose. Pirarucu spends only few seconds to uptake air, while tambaqui stays there until dissolved oxygen returns to normal levels in their natural habitats. Therefore, one can assume that tambaqui has being in contact with higher doses of UV radiation in nature and thus acquired adaptive mechanisms to reduce the effects of UV exposure.

Exposure to UVB is more dangerous to tambaqui than UVA or UVR exposure. Tambaqui exposed to UVB presented significant changes of hematological indexes, white cells and swimming performance. U_{crit} was reduced to one seventh of the control values in animals exposed to UVB while the animals exposed to UVA presented no changes. In pirarucu, the proportion of nuclear erythrocytic abnormalities (ENA) increased from 0.5% to 2.0% under this condition. The preliminary results obtained here for tambaqui are similar to those described for *Cichlasoma nigrofasciatum* and for *Rutilus rutilus* (Winkler and Fidhiany, 1996; Salo *et al*., 2000). Under natural conditions, tambaqui can avoid UV exposure by escaping to deeper waters or to flooded forest. However, under hypoxia the animals must remain skimming water surface. In our experimental setup, escape response was also useless as we used clear water with no suspend solids or DOM, a situation in many cases similar to that found in aquaculture facilities.

Concluding Remarks

These preliminary observations suggest that these two tropical fish are very sensitive to UV exposure, despite the high incidence of UV light on their pristine environments. Additional analysis are needed before we can have a clear picture on the effect of UV exposure on tropical fish, particularly species from different groups and fish from different water type habitats.

Acknowledgements

National Institute for Research in the Amazon (INPA), The Amazonas State Research Council (FAPEAM), and The National Research Council of Brazil (CNPq) supported this work. ALV and VMFAV are recipients of research fellowship from CNPq/Brasil.

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INVESTIGATION INTO THE DIFFERENCES IN NITROGEN METABOLISM BETWEEN RAINBOW TROUT AND ATLANTIC SALMON OF DIFFERENT AGE CLASSES

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Studies have highlighted significant differences between rainbow trout (*Oncorhynchus mykiss*) and Atlantic salmon (*Salmo salar*) in terms of digestible nitrogen retention efficiency (NRE, N gain / digestible N intake). Azevedo et al. (1998) found that Atlantic salmon retained a greater proportion of digestible protein than rainbow trout of similar size growing at similar rates and fed similar diets. Refstie et al. (2000) reported that despite poorer nitrogen digestibility, Atlantic salmon showed greater nitrogen retention and feed efficiency than rainbow trout of similar size fed same diet. Fish size also appears to affect the efficiency of amino acid utilization. A very significant decrease in efficiency of N retention was observed as rainbow trout grew while Atlantic salmon showed no differences (Azevedo et al. Unpub.).

Most of the nitrogenous waste excreted by fish originates from the catabolism of amino acids. Improvement of the efficiency of utilization of dietary amino acids may reduced the impact of waste products on the environment and reduce production costs. Regulation of amino acids utilization is a poorly understood issue in fish nutrition. There is a need to investigate the regulation of the main metabolic pathways of amino acid utilization. The present project aim is to study the metabolic differences between Atlantic salmon and rainbow trout in order to gain a better understanding of the metabolic mechanisms involved in differences in the efficiency utilization of dietary amino acids between fish

species. This information could then be translated into the development of methodologies for genetic selection or metabolic and nutritional modulation.

Materials and Methods

A 16-week growth trial was conducted using rainbow trout (40 and 382g initial weight) and Atlantic salmon (2.5 and 270g initial weight) reared at 15°C. Age class (0+ & 1+) and specie (AS & RT) were the factors compared. Additionally, two planes of nutrition were compared with the younger fish (near satiety (S) and 50% feed restriction (R)). An extruded open formulae feed (MNR-02HS) was used as the sole diet. Carcass samples were taken at 8-week intervals for carcass composition analyses. Liver samples were also taken at 8-week intervals, snap-frozen in liquid nitrogen, and stored at -80°C until analyzed. Glutamate dehydrogenase (GDH) and aspartate aminotransferase (AST) were determined according to standard methodology. All data were analyzed using a factorial ANOVA.

Results

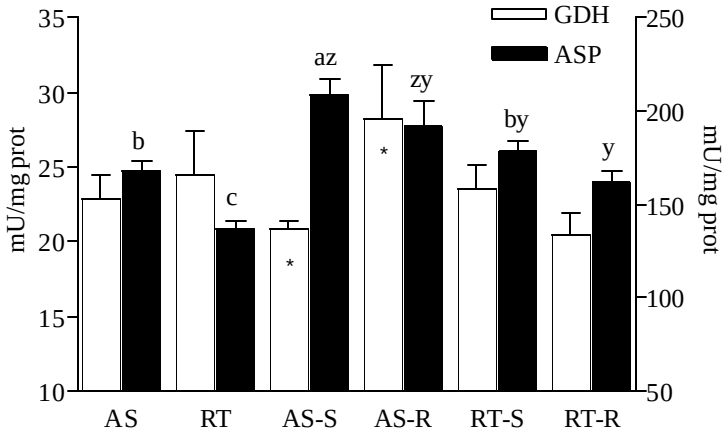
Growth performance is reported in Table 1. Growth rate (expressed as thermal-unit growth coefficient, TGC) and feed efficiency (gain:feed) were significantly different between species, age classes, and planes of nutrition. Nitrogen (N) retention efficiency (N gain/N intake) was affected by species and age class, and energy retention efficiency (energy gain/ energy intake) was affected by species and plane of nutrition. GDH activity was only affected by plane of nutrition in Atlantic salmon. AST was significantly higher in Atlantic salmon compared to rainbow trout at the two age classes. A significant positive correlation was found between AST and N retention efficiency ($p<0.05$).

Table 1 – Growth performance of rainbow trout and Atlantic salmon fed an extruded open formu lae feed for 112 days at 15°C.

		IBW g/fish	Gain g/fish	FE[1]	TGC[2]	NRE[3]	ERE[4]
A. salmon 0+	R	2.4	11.7	1.49	0.065	55.9	47.6
	S	2.6	25.6	1.49	0.100	57.0	51.0
R. trout 0+	R	40.7	143.7	1.42	0.135	54.1	48.2
	S	40.4	249.0	1.28	0.190	53.5	54.9
Specie			***	***	***	*	*
Plane			***	**	***	NS	**
Spe*Plane			***	**	***	NS	NS
A. salmon 1+	S	270.0	520.9	1.24	0.159	50.6	51.8
R. trout 1+	S	382.5	512.4	0.93	0.135	35.5	46.3
Specie			**	**	**	**	NS
Age			***	**	NS	**	NS
Spe*Age			**	NS	***	*	NS

1. Gain/feed; 2. Thermal-unit growth coefficient = 100×[(FBW1/3-IBW1/3)×(sum T×D)-1]; 3. Nitrogen retention efficiency (%, N intake/total N Intake); 4. Energy retention rfficiency (%, gross energy gain/ gross energy intake)

Figure 1 – Enzyme activities for GDH and AST. (Columns with different letters are significantly different) (Means ±SEM).



Discussions

The results from the present study agree well with the results of Azevedo et al. (1998) who observed significantly higher N retention efficiency in Atlantic salmon compared to rainbow trout. The significant difference in nitrogen utilization observed between age classes in rainbow trout is also consistent with results from previous studies (Azevedo et al. Unpub.). The increase in GDH activity with feed restriction is similar to what was observed by Walton and Cowey (1982) who interpreted this as a strategy for compensation in energy metabolism. AST was higher in Atlantic salmon compared to rainbow trout. AST was positively correlated with NRE. Determination of the activity of other amino acid catabolizing enzymes, assessment of gene expression, and nutrient flux studies are needed to better understand differences between species and age classes in terms of amino acid utilization.

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Acknowledgments

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TISSUE SPECIFIC CHANGES IN PROTEIN SYNTHESIS ASSOCIATED WITH SEASONAL METABOLIC DEPRESSION IN CUNNER (*TAUTOGOLABRUS ADSPERSUS*)

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Cunner (*Tautogolabrus adspersus*) are the northern most representative of the Labridae family, which is composed of mainly tropical marine fishes. Cunner are year round residents of inshore waters along the western Atlantic coastline, ranging from Newfoundland to Chesapeake Bay. During the winter, cunner remain in shallow inshore waters. To cope with the stress of low water temperatures, cunner decrease their metabolic rate and spend the winter in a torpid state sheltering beneath rocks and in crevices. Cunner found in Newfoundland waters become dormant in the fall when water temperatures reach 5°C and remain until May or June when water returns to approximately 5°C (Green and Farwell, 1971).

During metabolic depression, energy utilizing processes must be decreased in order for the animal to survive. Protein synthesis is a major contributor to the metabolic rate of a cell (11-42% of basal metabolism) and has been shown to decrease significantly during periods of hypometabolism (Houlihan et al., 1995).

The objective of this study is to compare rates of protein synthesis in various tissues in cunner in an active state, during torpor and during arousal from winter

torpor. In order to achieve this, cunner were injected with a flooding dose of ^{3}H phenylalanine, as described in Garlick et al. (1980). Due to the low metabolic rate of this species, an incubation time of 24 hours was necessary to fulfill the validation criteria for the flooding dose method (ie complete flooding of the free phenylalanine pool, stable specific activity and linear incorporation rates).

Preliminary results of protein synthesis rates in liver, white muscle and brain tissue sampled at 14°C and 8°C demonstrate a downregulation of protein synthesis in cunner when exposed to decreasing water temperatures. A comparison of tissue specific protein synthesis rates at both temperatures demonstrated liver > brain > white muscle (Table 1), a pattern which is supported by much of the literature on protein synthesis in fish. The Q_{10} values calculated from the protein synthesis rates at 14°C versus 8°C demonstrate that protein synthesis is extremely temperature sensitive in cunner (Table 1).

Table 1. Phenylalanine incorporation rates (ηmol phe g protein^{-1} hour^{-1}) and Q_{10} values in cunner liver, white muscle and brain at 14°C and 8°C.

Tissue	14°C	8°C	Q_{10} (14°C to 8°C)
Liver	505.5	185.4	5.3
White Muscle	0.048	0.010	13.6
Brain	0.606	0.091	23.6

At these temperatures cunner are still visually active and feeding. The dramatic decrease in protein synthesis rates before the animals enter winter torpor may indicate that cunner exhibit intrinsic metabolic depression, where metabolic depression occurs in anticipation of a potential environmental stress (Guppy and Withers, 1999), in this case the stressor being decreased temperature.

It is expected that during winter torpor, rates of protein synthesis will decrease even further, if able to be detected at all. However, in the spring, during arousal from dormancy, cunner may exhibit a hyperactivation of protein synthesis in order to compensate for loss of growth and tissue maintenance during the extended period of winter dormancy. The results of this study will expand upon the behavioural observations of winter dormancy in cunner by showing changes in the

physiological and biochemical mechanisms underlying seasonal metabolic depression. As well, it will extend the knowledge base on protein synthesis in fish to include the effects of extreme low temperatures and temperature induced dormancy.

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DEVELOPMENT OF RAINBOW TROUT HEPATOMA CELL LINES:

EFFECTS OF RAINBOW TROUT PRO-IGF-I EA4-PEPTIDE

ON MORPHOLOGICAL CHANGES AND

ANCHORAGE-INDEPENDENT CELL GROWTH

IN RTH CELL LINES

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Our laboratory have shown previously that recombinant rainbow trout (rt) Ea4-peptide of pro-IGF-I exhibited anti-tumor activities against cancer cell lines derived from various human cancer tissues (Chen et al., 2002; Kuo and Chen, 2002). To confirm that rtEa4-peptide can exhibit the same spectrum of anti-tumor activities in fish tumor cells, we had developed permanent single-cell clones (RTH1B1A, RTH1B1D, RTH1B2A and RTH1B2C) from a rainbow trout liver tumor induced by dibenzo(a,l)pyrene treatment. At 135 passages, the doubling time of these single-cell clones in CO2-independent medium at 20oC was 3.9 days, 3.5 days, 3.0 days and 4.5 days, respectively. Reverse transcription (RT)-polymerase chain reaction (PCR) analysis showed that the expression of liver-signature genes (e.g., aldolase B, G-6-Pase, PEPCK, HNF-I, IGF-I, IGF-II and GH receptor-2 genes) and CYP1A1, CYP1A3 genes was detected in these four single-cell clones (Figure 1). To confirm whether these cell lines possess malignant properties, in vitro colony formation assay in a soft-agar medium was conducted and the results showed different degrees of colony formation activities were observed among these single-cell clones. This is the first report of the development of a true hepatoma cell line from rainboiw trout. Treatment of RTH1B1D with recombinant trout Ea4-peptide resulted in the induction of a dose-dependent morphological change (Figure 2) and the

suppression of colony formation in a soft-agar medium (Figure 3). In addition, both morphological change and reduction of colony formation (Figure 4) were also observed in permanent transfectants of RTH1B1D cells carrying a trout Ea4-peptide gene or its human counterpart, hEb-peptide gene. These results confirm our earlier observations that trout pre-IGF-I Ea4-peptide and hEb possess activities counteracting malignant properties of cancer cells in vitro. (This research was supported by grants from NSF (IBN-0078067), USDA (CONTR # 58-1930-0-009) and Connecticut Sea Grant College (R/A 18) to TTC and grants ES03850. ES00210 and CA34732 to GSB.

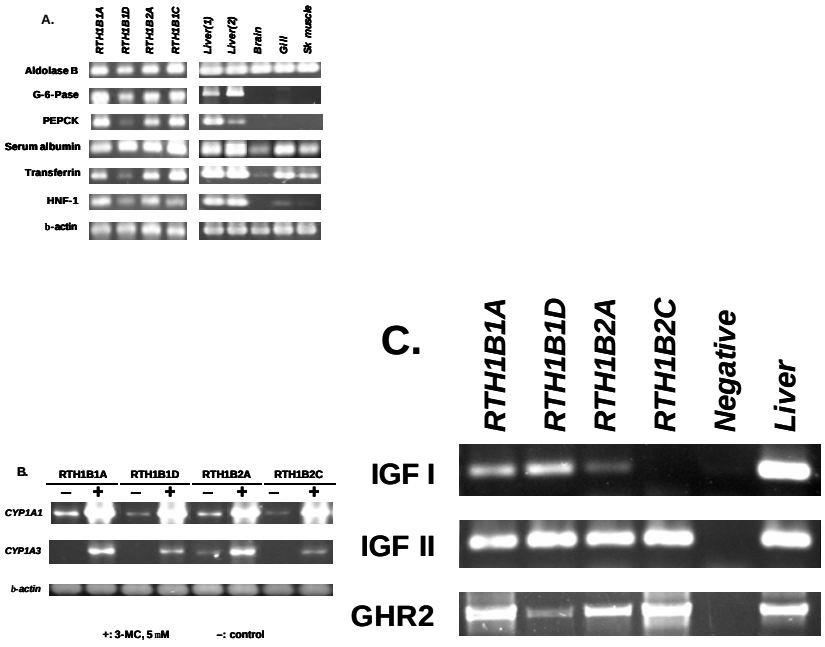

Figure 1: Reverse transcription-polymerase chain reaction (RT-PCR) determination of genes expressed in RTH single-cell clones. RNA was isolated from RTH single-cell clones and used as a template for first strand cDNA synthesis. Double-stranded cDNA of specific mRNA was amplified by PCR, using the first strand cDNA as a template. (A), expression of aldolase B, G-6-Pase, PECK, serum albumin, transferrin, HNF-1 and ?-actin genes; (B), expression of CYP1A1 and 1A3 genes; and (c) expression of IGF-I, -II and GHR-2 genes in RTH single-cell clones. G-6-Pase,

glucose 6-phosphatase; PECK, phosphoenolpyruvate carboxykinase; HNF-1, hepatic transcription activator-1; 3-MC, 3-methylchloranthrene; IGF-I, insulin-like growth factor –I, IGF-II, insulin-like growth factor II; and GHR2, growth factor receptor 2.

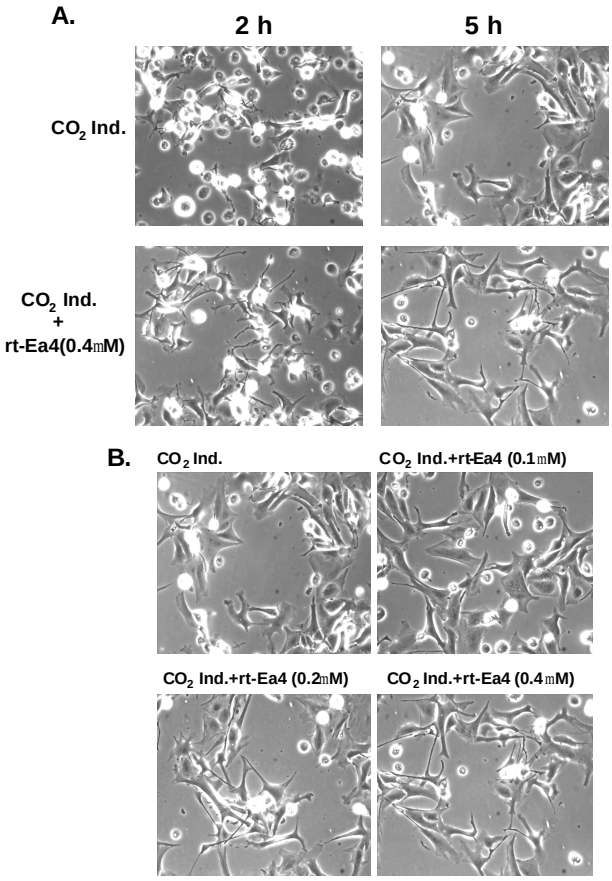

Figure 2: Morphological change in RT H1B1D cells induced by recombinant trout Ea4-peptide. About 1-2 x 105 cells, resuspended in a serum-free CO2-independent medium, were plated in a six-well culture chamber supplemented with various concentrations (0.1, 0.2 and 0.4 ?M) of recombinant trout Ea4-peptide or control proteins (0.4 ?M). Cells were

incubated at 20oC. Two to five hours after addition of Ea4-peptide, cells were observed under an Olympic IX50 microscope equipped with phase-contrast objective lenses (original magnification, 200x). (A) Time course of morphological differentiation; (B) Dose-dependent morphological differentiation at 5 hrs. CO2 ind, CO2-independent medium supplemented with control proteins

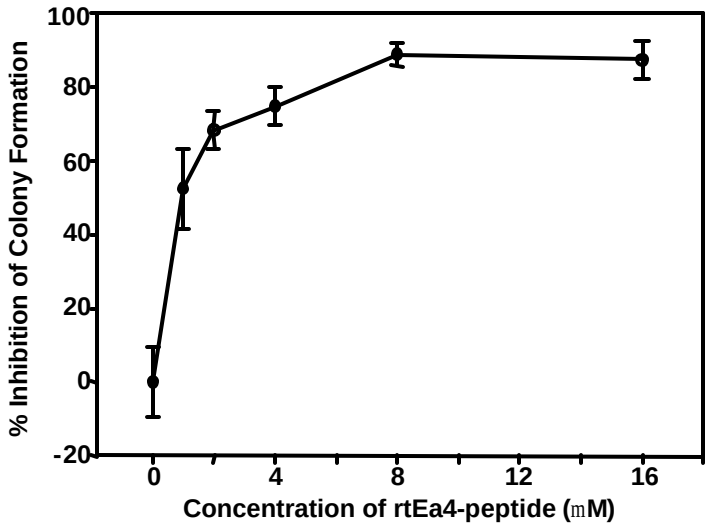

Figure 3: Dose-dependent inhibition of the colony formation activity of RTH1B1D cells in a semi-solid medium by recombinant trout Ea4-peptide. About 2 x 104 RTH1B1D cells were plated in a CO2-independent medium containing 0.5% agar, 1.25% FBS, and various concentrations of recombinant trout Ea4-peptide or control proteins, in 24-well culture chambers. After the medium was solidified, each well was overlaid with 1.5 ml of CO2-independent medium supplemented with the same concentrations of trout Ea4-peptide or control proteins. The plates were incubated at 20oC in a humidified incubator. Colonies were observed under an Olympic inverted microscope (Model IX50) equipped with phase-contrast objectives (40x, original magnification). Each data point is the average of four samples and the assay was conducted twice. Standard deviations of the two independent assays were presented.

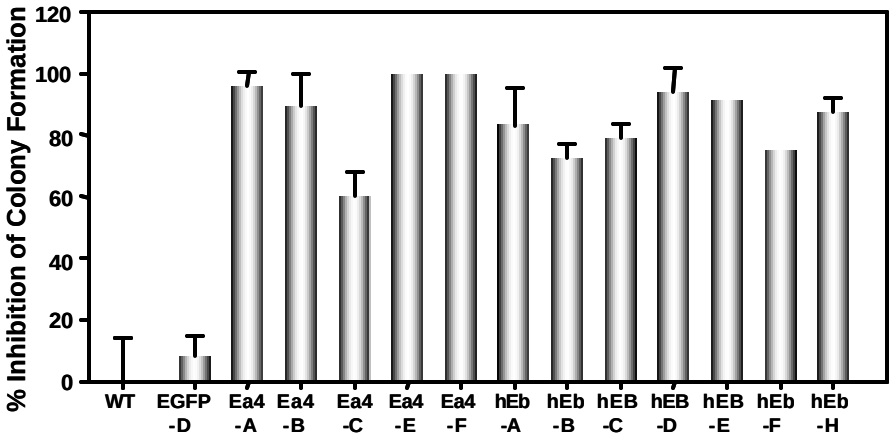

Figure 4: Colony formation activities of RTH1B1D transfectants expressing rtEa4-peptide or hEb-peptide genes in a semi-solid agar medium. Transgene construct containing rtEa-4-peptide or human (h) Eb cDNA/EGFP gene driven by a CMV promoter or EGFP gene driven by a CMV promoter was transfected into RTH1B1D cells and permanent transformants isolated. Transformants expressing rtEa4-peptide or hEb-peptide were subjected in vitro colony formation assay. Each data point is an average of four samples and the assay was conducted twice. Deviations of the two independent assays were presented.

ULTRASTRUCTURAL STUDY

OF SKELETAL MUSCLE FIBER TYPE

IN TWO TROPICAL FISH

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Abstract

The aim of this work was to determine the muscle fiber type that holds the skeletal musculature of two species (*Caquetaia kraussii & Colossoma macropomum*). Samples of caudal and dorsal muscles were studied using TEM techniques. In *Caquetaia kraussii* we found only one type of white twitch fiber. For *Colossoma macropomum* only one fiber type with intermediate characteristics (IIa and IIb) was determined. The Z line thickness was similar to IIb fibers (approximate 50 nm). The sarcotubular system was IIa type fiber.

Lipids droplets of IIa type were not found. This fiber type could be related with the food and predators for those species.

Keywords: *Caquetaia kraussii*, *Colossoma macropomum*, ultra-structure muscle.

Introduction

The musculature of locomotion in a great amount of teleost fish comprises 60% of the total body mass, giving also the power for a quick swimming (Sanger & Stoiber, 2001). The skeletal muscle of vertebrate consists of tonic fibers (specialized for slow steady contraction) and twitch fibers (specialized for rapid movements). The twitch fibers may show three different types: a) slow twitch fibers (type I) contract slowly and fatigue slowly. They are used both for maintaining posture and for moderately fast, repetitive movements, because, they contain a large number of mitochondria and depend on oxidative phosphorylation. Also, they use ATP a relatively slow rate. This type is usually called red muscle because they contain a high concentration of myoglobin. b) The second twitch fiber is the fast twitch oxidative (type IIa) fibers specialized for rapid, repetitive movements as in sustained, strenuous locomotion. They have many mitochondria and can produce ATP quickly by oxidative phosphorylation and thus fatigue slowly. c) The last are the fast twitch glycolytic (type IIb) fibers that contract rapidly and fatigue quickly. These fibers are generally recruited when a very rapid contraction is required. They contain few mitochondria and thus depend on anaerobic glycolysis to generate ATP. These categories are somewhat arbitrary, because some muscle fibers combine properties of different types. Also, the absolute values for many of the parameters may vary among species. Within a given muscle, however, the fiber types can be distinguished by their histological properties. Another useful method is based on the abundance of oxidative enzymes such as succinic dehydrogenase. Until now, there is limited information about ultra-structure muscle of continental tropical fish. The aim of this work was to determine the muscle fiber type that holds the skeletal musculature of two species: *Caquetaia kraussii*, (Pisces, Ciclidae) which is a great food source for its excellent flavor, not very bony axial skeleton, low fat and high protein contents (Segnini & Chung, 2001) and *Colossoma macropomum* (Pisces, Characeae), a species widely found in South America from the Orinoco basin to the Amazon basin river. In Venezuela, it is abundant in the Guanare, Portuguesa, Meta, Apure,

Caroní and Orinoco rivers. Also, they have a high aquaculture potential because they can be cultured and reproduced in captivity (González y Heredia, 1998).

Materials and Methods

The specimens of *Colossoma macropomum* (Characiformes: Characidae) and *Caquetaia kraussii* came from fish cultivated at the Experimental Station Guanapito, an experimental center of the National Fund for Agricultural Investigations (FONAIAP), Guárico State, Venezuela. 8 fish from each species were selected. After that, fishes were decapitated and approximately 2mm diameter of caudal and dorsal muscles were rapidly dissected and fixed with glutaraldehyde (2.5%) in Millonig buffer (pH = 7.8, 320 mOsm) for 45 min, trimmed to give blocks of approximately 1 mm^3 which were washed three times with Millonig buffer (pH = 7.8) for 1 min. The blocks were postfixed in osmium tetroxide (1%) using the same buffer for 1 hour at 4ºC, washed for 15 min in distilled water and dehydrated gradually through an ascending concentration of ethanol at 4ºC for 5 min in each stage. They were submerged twice in propylene oxide (15 min at room temperature) and infiltrated with a 1:1 mixture of propylene oxide-resin for 30 min. and four changes of pure resin (LX-112) in each stage. Finally, they were placed in plastic moulds for 48 hours at 60ºC. Sections of 80 nm were cut on an ultra microtome (200 mesh), stained with uranyl acetate and lead citrate and observed in a Hitachi H-7100 electron microscope at 75 kV.

Results and discussion

The electron microscopy is one analytical method used to characterize and identify muscle fiber types where the properties like Z line thickness, volume density and types of mitochondria, volume density and distribution of sarcoplasmic reticulum and T system can be observed. In these two species the results showed one fiber type of white twitch fiber IIb, (glycolitic) for *C. kraussii* and intermediate for *C. macropomum.*

Colossoma macropomum display a mixture of white and red fiber features (pink fibers) this type of fiber commonly appear relatively late in the development. In some species their appearance coincides with the end of yolk sac absorbed and the switch to exogenous nutrients (Sanger & Stoiber, 2001). *C. macropomum* showed the sarcotubular system developed with triads to Z line level, such as in

fast white type fibers (glycolitic or IIb). The mitochondria were abundant as well in myofibril spaces as in subsarcomeres spaces, and a motor nerve subsarcolemic. Also they have abundant glycogen as IIa or oxidatyve fibers. However, lipids droplets of IIa type were not found, which does not agree with this type of fiber (Figures 1 to 3). These results are similar to those obtained for Coporo, (*Prochilodus mariae* (Pisces: Prochilodontidae)) (Heredia & Finol, 1999), however both species differ because the Coporo showed lipid droplets between the intramiofibrilar spaces and in the subsarcolemic region. Transversal section of *Colossoma macropomum* muscle fiber showed six thin filaments for one thick filament like other vertebrates.

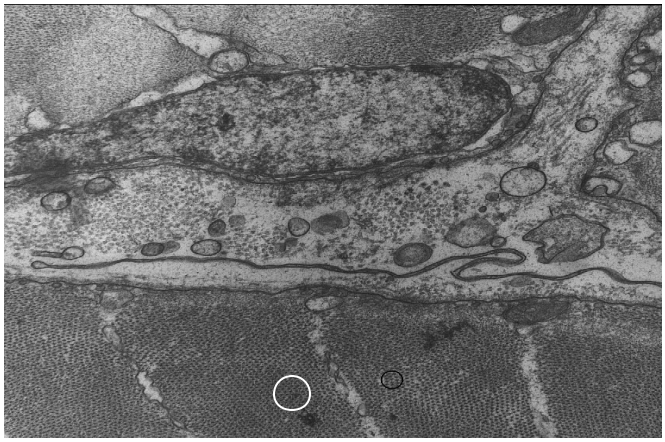

Fig. 1: Transversal section of *Colossoma macropomum muscle* fiber. Notice areas of six thin filaments for one thick filament. 36.000 X

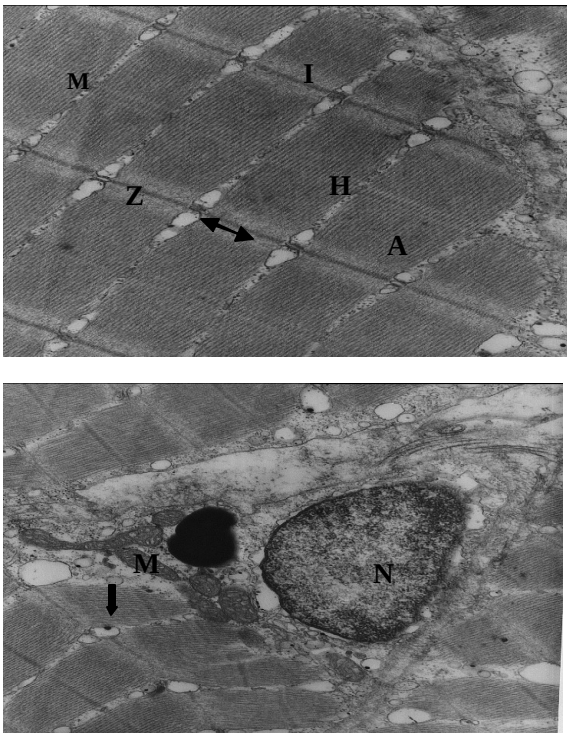

Fig. 2: Micrography of the longitudinal section of *Colossoma macropomum* muscle fiber. Upper: dark A-band with the central H-band, light I-band bisected by Z-line with straight orientation and the triad for sarcomera to Z line level. 45.000 X. Down: a nucleus (N), mitochondria (M) and calcium salts inside terminal tubules (arrow). 36.000 X.

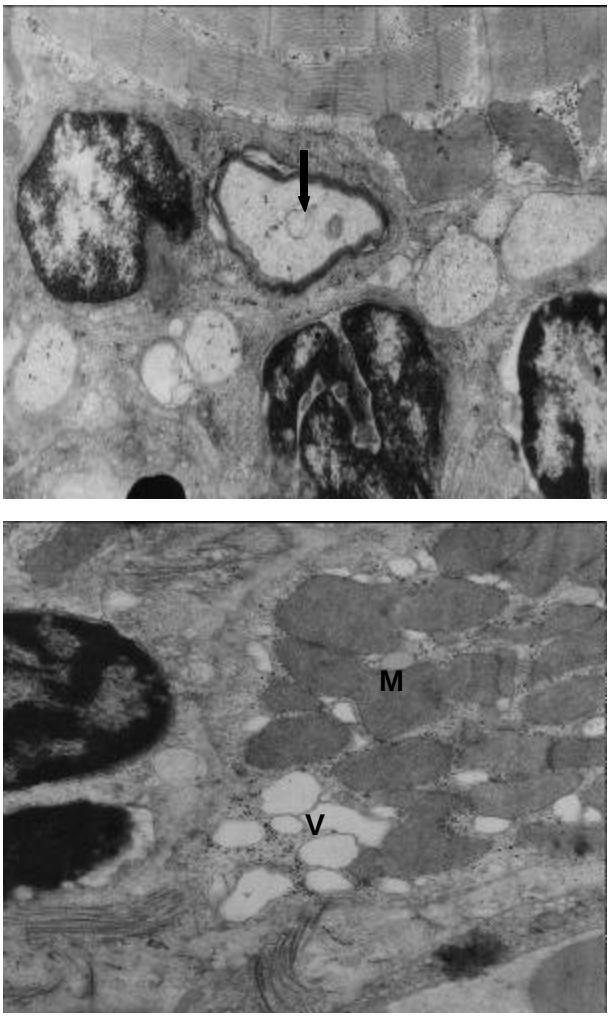

Fig. 3: Micrography of the longitudinal section of *Colossoma macropomum* muscle fiber. Upper: a motor nerve (arrow) next to muscle fiber. 30.000 X. Down: notice abundant mitochondria (M) and vacuoles (V). 24000 X.

White fibers are tightly packed with myofibrils occupying between 75 and 95% of fiber volume, organelles such as mitochondria which interrupt the arrays of myofibrils are a few and lipid droplets and myoglobin are present in only at low levels and the vascularization is poor. Glycogens content is also low with granules mainly located between myofibrils and exhibit a well developed sarcotubular system. The triad is situated at the Z line. This white fiber characteristic is shown in *Caquetaia kraussii*, Z-line thick is 50 ± 22.04 nm (N=72) and triad is situated at Z line. o in this species, the droplets of lipid were not observed in all section (Figures 4 and 5). In conclusion, we can say that the Z gross and trace, mitochondria abundance and lipid droplets observations can be used in the ultrastructural determination of the different types of skeletal fibers

Acknowledgement

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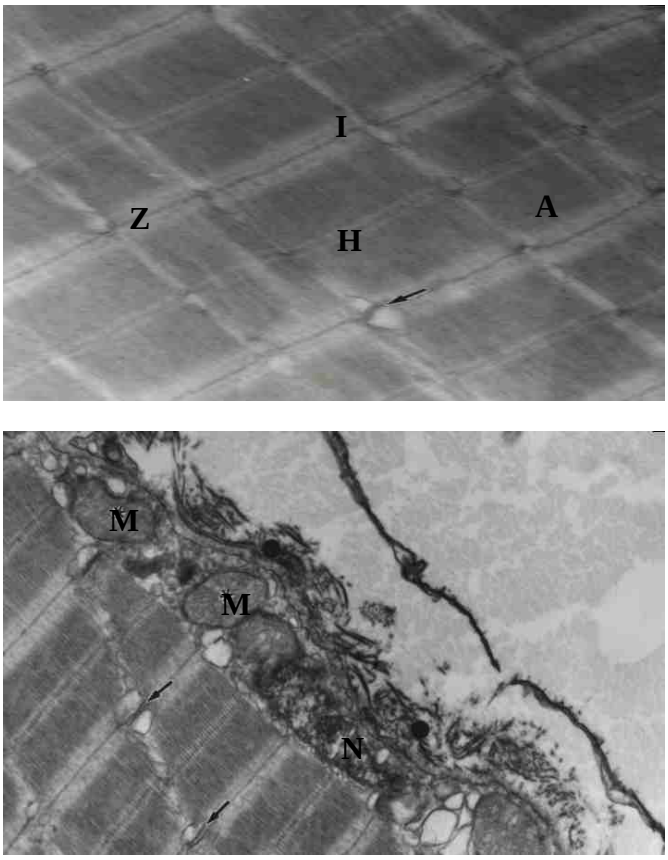

Fig.4: Micrography of the longitudinal section of *Caquetaia kraussii* muscle fiber. Upper: dark A-band with the central H-band, light I-band bisected by Z-line with straight orientation and the triad for sarcomera to Z-line level (arrow). 33.600 X. Down: a nucleus subsarcolemic (N), mitochondria (M). 30.000 X.

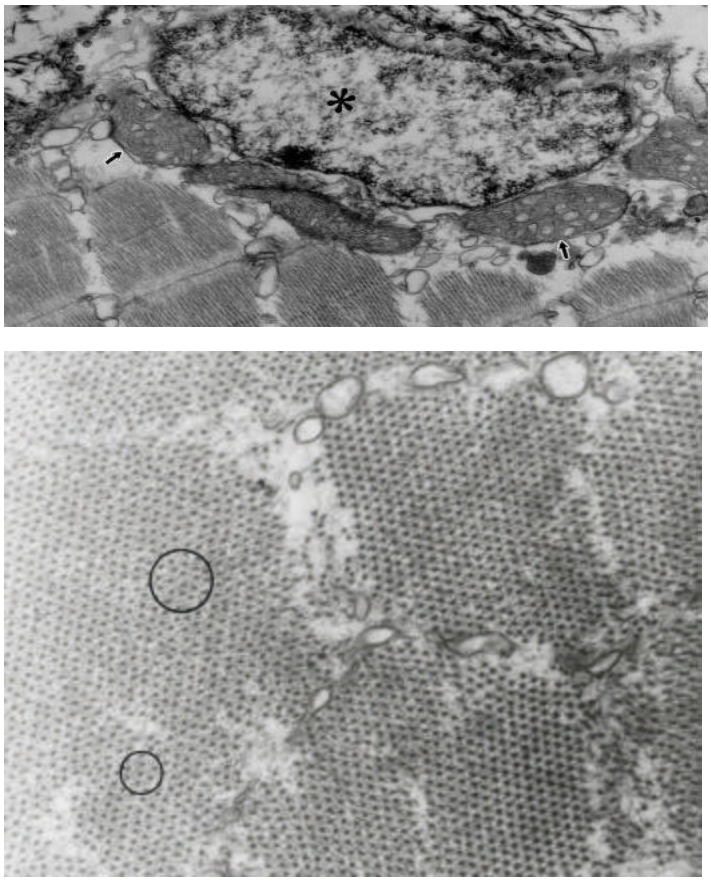

Fig. 5: Micrography of the longitudinal section of *Caquetaia kraussii* muscle fiber. Upper: notice mitochondria next to the nucleus. 30.000 X. Down: transversal section of *Caquetaia kraussii* muscle fiber. Notice areas of six thin filaments for one thick filament. 60.000 X

CARBON STABLE ISOTOPE DYNAMICS IN HERBIVOROUS

LORICARIID CATFISHES

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Fish in the Amazon basin are inaccessible during rainy seasons when most fish disperse into submerged forests. Thus, studies of the ecology of tropical fishes are usually performed on fish caught in dry seasons. Stable isotopes may present a way to investigate trophic ecology of fish during inaccessible seasons.

Plant materials have distinct $\delta^{13}C$ values that are determined by their photosynthetic pathways and metabolism. The relationship between $\delta^{13}C$ in fish diets and otoliths (sagittae) may reveal factors about Amazonian herbivorous loricariid catfish diets and their life history.

This project will determine whether otolith isotope chemistry can be used to gather information about the dietary history of wood-eating and algivorous loricariid catfishes (*Panaque nigrolineatus* and *Hypostomus regani* respectively).

Methods

Otoliths were extracted from *Panaque nigrolineatus* (22 fish from Rio do Peixe, Aruana, Goias, Brazil) and *Hypostomus regani* (14 fish from Rio Mogi-Guaçu, Pirassununga, São Paulo, Brazil) in the field and brought back to laboratory to count the growth rings under the light microscope. Otoliths were then micromilled by a computer-controlled dental drill in the Stable isotope laboratory (Syracuse University). Pre-programmed three-dimentional digitized paths guide the robotic micromill along the annuli of otolith to sample. A subsample was collected after each run then a series of subsamples from one otolith were separated.

Forty-eight juvenile *Hypostomus* sp. were purchased from aquarium wholesalers and randomly assigned to one of four dietary treatments: 1) corn (*Zea mays*), a C_4 plant with relatively high $\delta^{13}C$ values (approximately –10‰ (Vogel, 1993; Hanba et al., 1997; Stable Isotope/Soil Biology Laboratory, Institute of Ecology, University of Georgia, 1997)); 2) commercially obtained freshwater algae, *Spirulina* sp. (approximately –23.7‰, present study); 3) broccoli (*Brassica oleracea*, $\delta^{13}C$= –35.4‰ (Evershed et al., 1999)); and 4) a C_3 wood, red maple (*Acer rubrum* with $\delta^{13}C$ value = –26.1‰ (Brown, 1999)). All tanks were covered with opaque material to exclude naturally occurring algae and maintained minimizing environmental differences among tanks other than diet. After nine months on the experiment, eight, seven, and four fish survived on the corn, algae, and broccoli diets respectively. The surviving fish were sacrificed by an overdose of anesthetic, and then otoliths were removed. Although all *Hypostomus* sp. on the wood-only diet gradually died over the first five months of the experiment, the otoliths of four longest-lived fish were extracted for further analysis.

Milled otolith samples (from the field) or whole otoliths (from laboratory-reared *Hypostomus* sp.) were roasted at 200°C to remove any volatiles and measured $\delta^{13}C$ values by a gas ratio mass spectrometer at Syracuse University. $\delta^{13}C$ values of *Spirulina* sp. were measured in the stable isotope laboratory at the University of Wyoming.

$\delta^{13}C_{(Otolith)}$ values from 10 *P. nigrolineatus* and 7 *H. regani* were not successfully obtained because of deficiencies of the milled sample and subjected to elimination for quality control considerations.

Results

The mean $\delta^{13}C_{(Otolith)}$ values of field-collected *P. nigrolineatus* and *H. regani* differed by 3.67‰ (Table 1.) and were significantly different from each other despite their occurring in a similar geographic area (the Kruskal-Wallis test, $P<0.001$). These values also did not correlate with body length, weight, or growth rings for either species ($P>0.05$).

	Min	Max	Mean	St Dev	n
(a)	–15.15	–13.78	–14.40	±0.35	12
(b)	–12.50	–8.81	–10.74	±1.35	11

Table 1. Summary of $\delta^{13}C_{(Otolith)}$ measurements on field-caught *Panaque nigrolineatus* (a) and *Hypostomus regani* (b) (Units are ‰ except the number of fish at the last column. "St Dev" is standard deviation.)

The distribution pattern of individual $\delta^{13}C_{(Otolith)}$ values in *Panaque nigrolineatus* showed a strong tendency of possessing values around –13.78~–15.15‰ (Table 1.), whereas those of *H. regani* were more dispersed (around –8.81~–12.50‰ (Table 1.)). There are, however, no time effects in $\delta^{13}C_{(Otolith)}$ values for either species ($P>0.05$, general interaction ANCOVA).

The different dietary treatments in *Hypostomus* sp. produced different carbon stable isotope values in their otoliths (Figure 1.). This difference in $\delta^{13}C_{(Otolith)}$ values mirrors the differences in $\delta^{13}C_{(Diet)}$ values (Figure 1.).

The mean $\delta^{13}C_{(Otolith)}$ of the corn diet showed a significant difference from the other diets ($P<0.05$, Scheffe's test). The mean $\delta^{13}C_{(Otolith)}$ values of broccoli and wood diets were also significantly different from each other ($P<0.05$, Scheffe's test), but not from the algae diet.

Discussion

The most likely causes of the difference in the mean $^{13}C_{(Otolith)}$ values of field collected two loricariid species are species differentiation in metabolic status, otolith formation processes, or diet preferences.

A narrow range of $\delta^{13}C_{(Otolith)}$ values in *P. nigrolineatus* could be due to a constant metabolism in the field throughout their life or a constant diet (Table

1.). *Hypostomus regani*, on the other hand, may change activity levels throughout their life or may have varied and/or changeable diets (Table 1.).

According to the dietary controlled experiment, the $\delta^{13}C_{(Diet)}$ values are probably a very important component for controlling the $\delta^{13}C_{(Otolith)}$ values in laboratory-reared *Hypostomus* sp..

Since a majority of research in Neotropical fish biology has been dependent on sampling during the dry seasons, stable isotope measuring techniques could improve the study of temporal trophic dynamics in the Neotropics.

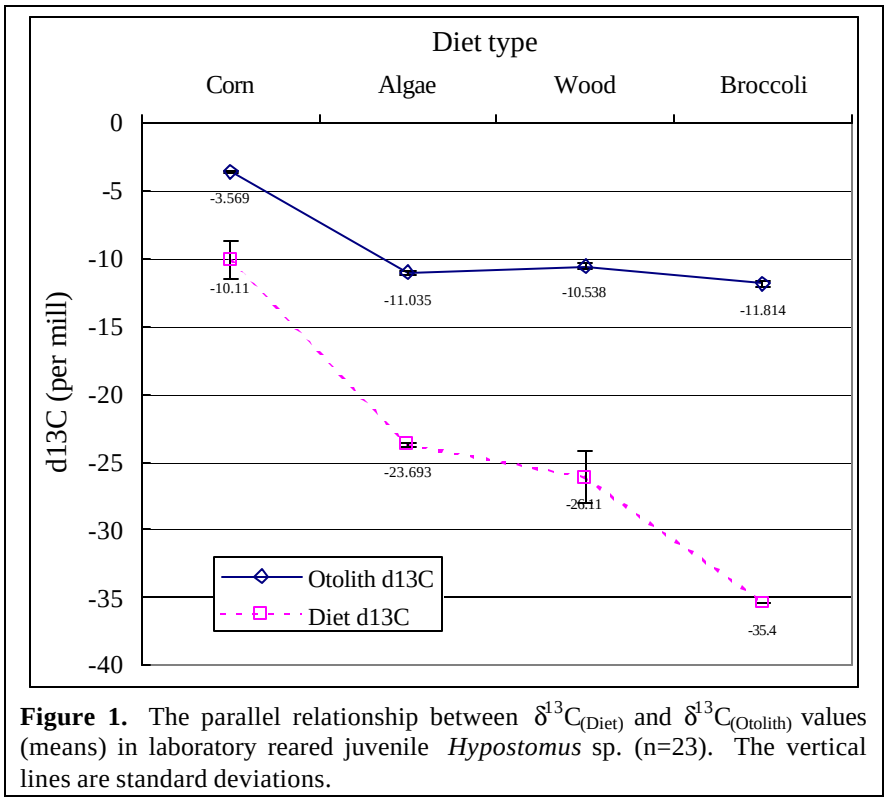

Figure 1. The parallel relationship between $\delta^{13}C_{(Diet)}$ and $\delta^{13}C_{(Otolith)}$ values (means) in laboratory reared juvenile *Hypostomus* sp. (n=23). The vertical lines are standard deviations.

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GROWTH RATES AND MORTALITY OF RED EMPEROR AND FINGERMARK SEA PERCH (LUTJANIDAE) IN QUEENSLAND, AUSTRALIA

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Abstract

Growth and mortality of red emperor *Lutjanus sebae* and fingermark sea perch *L. johnii* were studied in Queensland, Australia using data collected as part of a cooperative angler tagging-program. Growth was modeled using the computer program GROTAG. Annual growth increments for red emperor were estimated as 6.38 mm for individuals 20-cm TL and 6.94 mm for those 40-cm TL. Annual growth increments for fingermark were estimated as 11.01 mm for individuals 20-cm TL and 8.04 mm for those 40-cm TL. Thus, the grow rate of fingermark exceeds that of red emperor. Instantaneous mortality rates (Z) were estimated for each species as the slope of the regression of the natural log of number of recaptures and time at liberty. Our results suggest annual mortality rates of 0.12 for red emperor and 0.18 for fingermark. Slow growth rates and low annual mortality rates suggest both species might be sensitive to overexploitation.

Introduction

Members of the family Lutjanidae are found throughout the world in warm seas. Adults are demersal and feed primarily on fishes and crustaceans, and occur from shallow to inshore areas to depths of 550 m (Anderson, 1987). Red emperor *Lutjanus sebae* and fingermark sea perch *L. johnii* are distributed from eastern Africa to western and south central Pacific, respectively. In Australia,

both species occur most abundantly in tropical waters along the states of Queensland, Northern Territory, and Western Australia. Despite the wide distribution of both species and their importance as food and recreational fishes throughout their geographic range, little is known of their basic biology.

Members of the Australian National Sportfishing Association (ANSA) Queensland have conducted a cooperative tagging program, known as Suntag, in collaboration with personnel of the Queensland Department of Primary Industries (DPI) since the 1980s. Anglers participating in this program, capture fish, tag them with dart or t-bar anchor tags, and record and report the time and place of capture, total length (TL), and the tag number. The tags are uniquely numbered and bear a toll-free telephone number that anglers can call to report information (time and place of capture, total length, and the tag number recaptured fish). Information on recaptured fish also can be reported by mail or email. Capture and recapture records are maintained in an Access database.

Although there are limitations to data collected by cooperative tagging programs (Kearney, 1988; Gillanders et al., 2001), data from the various collaborative tagging projects in which ANSA members have participated have been used to describe movement, migration, stock structure, growth, mortality, and other population characteristics for a number of Australian fishes (e.g., Morton et al., 1993; Begg et al., 1997; Gillanders et al., 2001; Wilde and Sawynok, *in press*). In this paper, we use data collected as part of the ANSA-DPI Suntag collaborative tagging program to estimate growth and mortality rates of red emperor and fingermark.

Materials and Methods

ANSA members have tagged red emperor and fingermark since 1983. Red emperor reported on herein initially were captured between 5 September 1986 and 2 November 2003, and recaptures were made during 7 May 1988 to 4 November 2003. Fingermark were captured between 23 May 1986 and 17 October 2003, and recaptures were made during 15 December 1987 through 10 October 2003.

We assessed growth of red emperor and fingermark using the computer program GROTAG (Francis, 1988), which uses maximum likelihood to estimate growth parameters from tagging data. GROTAG estimates $g_?$ and $g_?$, the mean annual growth rates of fish of lengths ? and ?, respectively, which were chosen to lie within the range of lengths at tagging. Francis (1988) suggested that $g_?$ and $g_?$

give a better description of growth information provided by tagging data than do the usual von Bertalanffy growth parameters L_8 and K. The expected growth or length increment, ? L, for a fish of initial length L_1 at liberty for time ? T is given by:

$$\Delta L = \left[\frac{\left(b g_a - a g_b \right)}{g_a - g_b} - L_1 \right] \left[1 - \left(1 + \frac{g_a - g_b}{a - b} \right)^{\Delta T} \right].$$

GROTAG also estimates the mean (m) and standard deviation (s) of measurement error, the coefficient of growth variability (v), seasonal variation in growth (two parameters, u and w), and the proportion (p) of outliers (Francis, 1988). Estimates of the standard errors of $g_?$ and $g_?$, as well as for other model parameters, can be obtained by simulation.

We fitted an initial GROTAG model that estimated only the growth parameters $g_?$ and $g_?$, and growth variability. We then sequentially added parameters describing seasonal variation in growth, measurement error, and outliers by refitting the model. We included individual parameters in the final model if likelihood ratio tests indicated that their inclusion resulted in a significant ($P \leq 0.05$) increase in model fit (Francis, 1988). We assessed the adequacy of the final model by inspecting scatterplots of standardized residuals versus length at capture, time at liberty, and expected growth increment.

We estimated the instantaneous rate of mortality for red emperor and fingermark as the slope of the relationship between the natural log of number of recaptures and time at liberty (Gillanders et al., 2001). Gillanders et al. regressed the number of recaptures per month against time (months) at liberty and considered only recaptures made within the first 35 months at liberty. The analyses presented herein analyze the number of recaptures per month (30 d) for 19 months for red emperor and 16 months for fingermark.

Results

A total of 3948 red emperor and 3939 fingermark were tagged and released by ANSA members; 795 red emperor and 327 fingermark were recaptured. Recapture rates (based on reported tags) for the two species were 20.1% for red emperor and 8.7% for fingermark. After removing records that either lacked

information necessary to the analysis or could not reliably be matched with capture data, we had final samples of 723 red emperor and 292 fingermark.

Length at tagging for fingermark ranged from 11- to 120-cm TL, most (81%) were 20- to 40-cm TL. Length at tagging for fingermark ranged from 15- to 60-cm TL, most (86%) were 20- to 40-cm TL. GROTAG models were fitted separately for each species. The final models included growth parameters, as well as estimates of growth variability, seasonal variation in growth, measurement error, and the proportion of outliers (Table 1). Plots of standardized residuals versus length at tagging, time at liberty, and expected annual growth increment showed no pattern, suggesting that the models provide adequate descriptions of growth for both species (Francis, 1988).

For red emperor, the estimated annual grow increment was 6.38 mm for fish 20-cm TL and 6.94 mm for fish 40-cm TL (Table 1). For fingermark, the estimated annual grow increment was 11.01 mm for fish 20-cm TL, and 8.04 mm for fish 40-cm TL.

Instantaneous mortality of red emperor and fingermark were estimated by regressing the natural log of the number of recaptures (lnN) against time at liberty (T) (Figure 1). Both regressions were significant ($P < 0.0001$) and imply instantaneous mortality rates of 0.22 ($\ln N = 4.853 - 0.218 \times T$, $r^2 = 0.96$) for red emperor and 0.19 ($\ln N = 3.83 - 0.194 \times T$, $r^2 = 0.85$) for fingermark. Using the relationship $A = 1 - e^{-Z}$, where Z is the instantaneous mortality rate, the annual rate of mortality A can be determined as 0.12 for red emperor and 0.18 for fingermark.

Table 1. Annual growth increments (cm) and parameter estimates for GROTAG (Francis, 1988) growth models fitted to red emperor and fingermark tagging data.

Parameter		Red emperor		Fingermark	
		?	se	?	se
Mean growth increments	g_{20}	6.38	0.046	11.01	0.039
	g_{40}	6.94	0.031	8.04	0.029
Growth variability	v	0.61	0.004	0.33	0.003
Seasonal amplitude	u	0.83	0.005	0.70	0.006
Seasonal phase	w	0.75	0.001	0.43	0.002
Outlier contamination	p	0.03	0.001	0.04	0.002
Measurement bias	m	0.87	0.003	0.91	0.005
Measurement error	s	0.02	0.006	-0.14	0.009

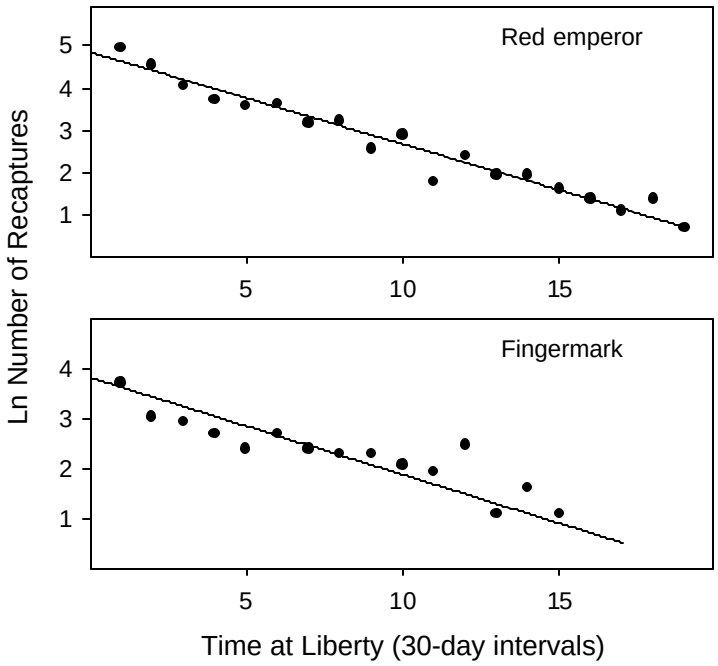

Figure 1. Regression of the natural log of recaptures on time at liberty (in 30-day intervals) for red emperor and fingermark captured from coastal waters of Queensland, Australia.

Discussion

Growth of red emperor and fingermark is slow, as has been observed for other Australian lutjanids (Davis and West, 1992; Milton et al., 1995; Newman et al., 1996; Kritzer 2004), including red emperor (Newman and Dunk, 2002). The annual growth increment (11.10 mm) of fingermark 20-mm TL is approximately 50% greater than that of comparably sized red emperor (6.38 mm). The difference between these species in annual growth increments decreases with increased length.

Our estimates of growth increments for red emperor suggest that the annual growth increment increases with length. Although this is unlikely, our results suggest there is little variation in annual growth increments among red emperor 20 to 40 mm in length, the size best represented in our samples. The apparent increase in annual growth increment with increased length is most likely a methodological artifact and may be attributable to length-related differences in sex ratios among our samples. A number of lutjanids exhibit sexually dimorphic growth (Newman et al., 1996; Newman and Dunk, 2002) with greater growth among males. We have no means of determining the sex of fish in our samples; however, if small females and large males were disproportionately common in our red emperor samples, we might expect to observe an apparent relationship between growth increment and length.

Annual mortality estimates presented in this paper for red emperor (0.12) and fingermark (0.18) are comparable to those reported for other Australian lutjanids (Newman et al., 1996; Newman and Dunk, 2002

This combination of slow growth and low annual mortality rates observed in red emperor and fingermark captured by recreational anglers in Queensland waters are commonly used to infer susceptibility of fish stocks to over exploitation. Ralston (1997) reported that the ratio of fishing mortality to natural mortality in several species of lutjanids ranged from 1.25 to 3.33. We cannot distinguish between mortality attributable to natural causes and that attributable to fishing in our samples. However, the low rates of total mortality (including natural and fishing mortality) observed for red emperor and fingermark suggest that, at this time, fishing mortality is relatively low for both species.

Acknowledgments

We wish to acknowledge the efforts of ANSA members who tagged the fish reported on herein.

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PAIN PERCEPTION IN RAINBOW TROUT

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Nociception is the detection and reflex response to a tissue-damaging, potentially painful stimulus and is distinct from pain perception since pain also comprises adverse behavioural and physiological reactions. To demonstrate nociception in a fish, techniques in neuroanatomy and electrophysiology were adopted to examine the trigeminal nerve, which conveys nociceptive information from orofacial areas in higher vertebrates, for the presence of A-delta and C fibres that may act as nociceptive neurons. Behavioural experiments were also conducted to assess the rainbow trout's (Oncorhynchus mykiss) responses to potentially painful stimulation.

Methods

Rainbow trout were killed by overdose in anaesthetic, perfused with saline and the fixative, formal acetic alcohol (Sneddon 2002). Transverse sections of the trigeminal nerve were examined by staining with toluidine blue. To investigate if there were receptive fields on the fish's head that had a nociceptive function, electrophysiological recordings were made from afferent cell bodies in the trigeminal ganglion (Sneddon 2003a). To assess pain perception, behavioural responses to administration of acutely acting noxious substances, bee venom and 1% acetic acid, were assessed and compared with handled controls and saline administered fish as well as fish administered with a painkiller, morphine (Sneddon 2003b; Sneddon et al. 2003a, b).

Results

The trigeminal of the rainbow trout did possess A-delta and C fibres (25% and 4% total fibre type respectively) that may act as nociceptors. A variety of somatosensory receptor types were located on the head of the trout and 31% of these were polymodal nociceptors preferentially stimulated by noxious mechanical, thermal and chemical stimuli. A further 7% of these receptors were mechanothermal nociceptors that only responded to noxious mechanical and thermal stimuli. The fish administered with venom and acid performed anomalous behaviours, did not feed until the venom and acid effects had subsided and also showed an almost double fold increase in respiration rate. These effects were not seen in the controls or in fish that had been treated with an analgesic, morphine.

Discussion

The rainbow trout does possess nociceptors that are strikingly similar to those found in higher vertebrates. The behavioural results suggest the potential for pain perception in the rainbow trout. Comparative analysis of nociception may provide insights into the evolution of nociceptive systems. By examining results from lower vertebrates, we can compare and contrast properties of nociceptors with higher vertebrates including humans. Hypotheses shall be presented to discuss the possible causes of the different properties between lower and higher vertebrates (Sneddon 2004).

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PARALLEL TRENDS IN THE BIOLOGY

OF ARCTIC ANADROMOUS FISHES

AND THE CONSEQUENCES FOR FISHERIES MANAGEMENT

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Canadian Arctic communities in the Nunavut and the Northwest Territories depend upon a limited selection of anadromous species for subsistence needs and sport and commercial enterprises. In the Arctic portion of the lower Mackenzie River the principal species are the broad whitefish, *Coregonus nasus* and the inconnu, *Stenodus leucichthys*. In Arctic Nunavut Territory only one species, Arctic charr, *Salvelinus alpinus,* is harvested.

These species are targeted because their unusual life cycles give them the characteristics most desirable to primitive harvesters. As implied from the anadromous designation they all migrate via rivers to and from the marine environment. The fish are large and abundant (5 to 10 million broad whitefish according to Thera (1998)). The migrations are seasonal , occurring in the spring and fall. Resultingly, at particular predictable times of the year their numbers are highly concentrated in time in space. Harvesters are able to use gillnets or weirs to capture large numbers of these fishes with little effort.

At the same time there are alternative forms of each species that do not leave freshwater lakes and thus provide a stable source of food throughout the entire year. Thus, each species has both a migratory anadromous form and a

freshwater resident non-migratory form. Harvesters have made considerable use of both types.

Broad Whitefish Life Cycle

In the lower Mackenzie River anadromous broad whitefish begin life under the ice in gravel beds in the large tributaries of the system such as the Peel River, Arctic Red River and, also in the mainstem Mackenzie (Thera 1998). The eggs and larvae remain in the gravel until they are flushed northward from the spawning areas by the spring freshet (Fig. 1). Larvae generally travel eastward along the still ice-covered coasts of Richard's Island and the Tuktoyaktuk Peninsula aided by the Coriolis Effect on the plume of freshwater from the Mackenzie River. Upon reaching the mouth of one of the many small freshwater systems on the coast, the larvae migrate upstream and remain in these systems until they mature at age 6-8 (Tallman and Reist 1997). The mature fish migrate along the coast to the Mackenzie Delta area and then onward during the fall with the rest of the adults to the spawning grounds upstream. Spawning takes palace under the ice during October.

There is also a freshwater form that occurs in larger stable lakes in along the Mackenzie Basin (Tallman et al. 2002) . These lake resident broad whitefish do not migrate to the coast but complete their life cycle within the lake basin.

Inconnu Life Cycle

The Arctic portion of the Inconnu anadromous life cycle is nearly identical to that of the broad whitefish using the same tributaries for spawning and the same lakes for rearing. The adult spawning migration and spawning is earlier in the season taking place in August and late September, respectively (Tallman unpublished data).

Lake resident populations exist but occur much further south in Great Slave Lake (Howland et al. 2000). In both cases, the lake forms are large and form the basis for major fisheries.

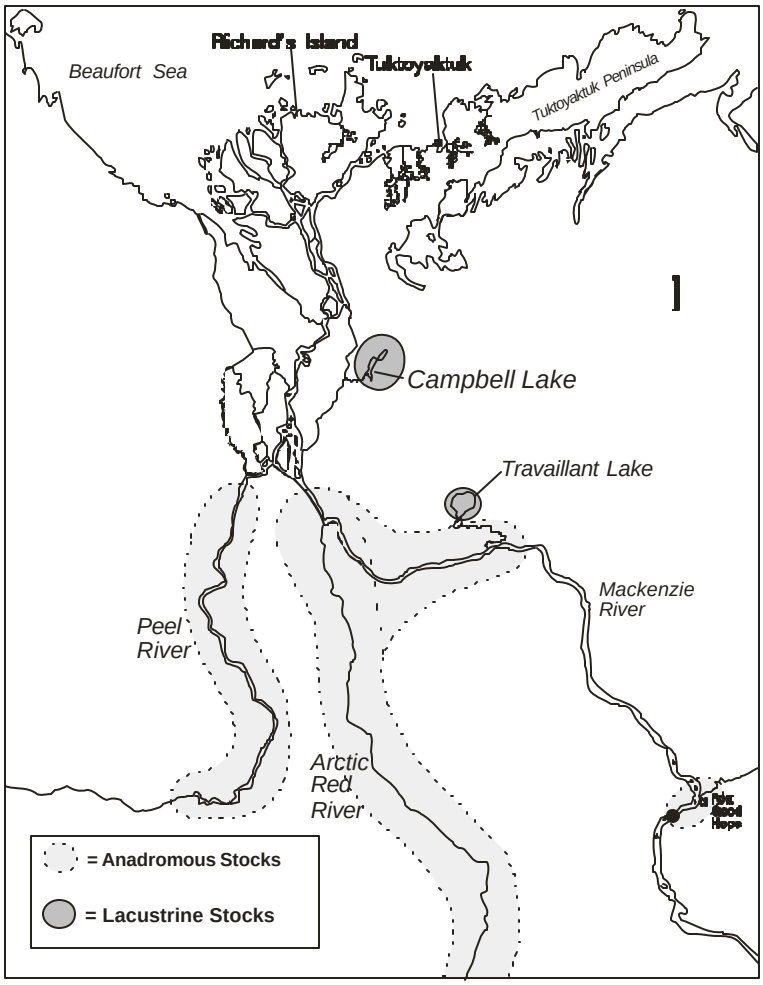

Figure 1. The lower Mackenzie River showing spawning sites of resident (lacustrine) and anadromous stocks of broad whitefish. Rearing sites of the Tuktoyaktuk Peninsula and Richard's Island are shown also.

Arctic Charr Life Cycle

Arctic charr spawn in river systems with attached lakes along the Nunavut coast (Tallman et al. 1996). They rear in the lakes for several years at which point

they smolt and migrate to sea for the summer. Some remain in the lake as freshwater resident charr, completing their life cycle with going to sea. The anadromous form must return to freshwater to over-winter. However, unless they are spawning they do not necessarily return to their natal system. Charr can migrate along the coast up to 250 kms. Depending upon the local they may spawn between every 3 to 10 years in the Canadian Arctic. Thus, large portions of the population may be overwintering elsewhere as resting fish.

Fisheries Management Nightmares

While anadromous Arctic charr, inconnu and broad whitefish are easy to harvest they are very difficult to manage due their complex life cycles. In the case of broad whitefish and inconnu growth curves to determine production are difficult to estimate for individual stocks because the offspring rear in locations geographically remote from the spawning grounds. The stock range is enormous and determining the sampling rate is difficult. Like Arctic charr, adults may spend several seasons resting after spawning and therefore catch-per-unit-effort indices are suspect. Arctic charr present an even greater difficulty for fishery management because a large and variable portion of the adult population may be resting and migrating into other systems. Thus, most abundance estimates suffer from a lack of a closed population. Fishery analyses rely on observations of age structure and comparisons with other situations. Precise levels of exploitation rates are not known. These problems have not been solved as yet and there is no management model that is satisfactory.

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ENVIRONMENTALLY RELATED LIFE HISTORY

OF THE RED-BELLIED PIRANHA, *PYGOCENTRUS NATTERERI*,

IN TWO RIVER BASINS

OF THE BOLIVIAN AMAZON (BENI, BOLIVIA)

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EXTENDED ABSTRACT ONLY: DO NOT CITE.

The influence of white, black or clear water systems on Amazonian fish distribution and abundance is largely discussed in the literature. On the other hand, the influence of the different types of waters on the fish life histories has received much less attention. However, for the fish species which inhabit both white and clear or white and black waters, the associated differences in chemical composition, production and community patterns must probably influence reproductive and growth characteristics.

The red-bellied piranha *Pygocentrus nattereri* is one such species widely distributed in tropical South America independently of the water types. As for many other sharp-teethed piranhas, its reputation of voracious predator has focused attention on its feeding habits. However, many piranhas among which the red-bellied, mainly feed upon sick and injured fishes as well as upon other animal remains, and are believed to play an important ecological function as

"cleaning and health squads" (Pauly 1994, quoting Schulte 1988). Despite its ecologically important role in Neotropical food webs and its impact on human local consumption, *P. nattereri* reproductive and growth characteristics in natural environments in relation with genetic structure have received little attention. In this study, we tested the hypothesis that the contrasted environmental conditions will induce phenotypic differences in breeding and growth patterns between the geographic populations of the two basins.

Material and methods

Reproductive (histological analyses of gonads, breeding season, age and size at first sexual maturity, fecundity and oocyte size) and growth characteristics of *P. nattereri* were compared in a white water (Mamoré) and clear water (Itenez) rivers of the Bolivian Amazon. At the same time, the filogeography of *P. nattereri* in the high Madeira was established by sequencing the control region of the D-Loop (mtDNA).

Fish samplings were carried out between July 2001 and January 2004. A total of 849 fishes were analysed: 568 in the Mamoré basin and 281 in the Itenez basin. For the Mamoré basin, sampling points were located on the Isiboro-Sécure river between 14°49'S and 16°20'S. For the Itenez basin, sampling points were located in the San Martín and San Joaquín between 13°9'S and 14° 8"S.

Fish age and growth were determined using polished sections of the sagitta otoliths (Figure 1) of 75 individuals in the Itenez and 80 in the Mamoré.

Results and conclusions

Histological analyses revealed two modes in oocyte distributions for every studied female in both rivers, indicating that a same female spawns at least twice during the breeding season.

In both river basins, *P. nattereri* reached maturity during their first year, females maturing later than males. Sizes at maturity ranged from 118 to 140 mm. Both age and size at maturity differed significantly between the two river basins.

Breeding periods were highly seasonal, starting with the rising waters and lasting through part of the high water period. Breeding seasons in both basins

were very similar, with a possible delay of about one month in the Itenez, which would correspond to the delay in water rising between the two basins.

In the Mamoré, batch fecundities ranged from 6,056 to 27,071 oocytes for females of 258 g and 471 g, respectively. In the Itenez, it ranged from 3,551 to 21,213 oocytes for females of 126 g and 518 g, respectively. The comparison of linear regression models between fecundity and body weight indicated a significantly higher fecundity for the Mamoré population. As an example, a 500 g female would lay about 19,225 eggs in the Mamoré and 13,562 eggs in the Itenez basin.

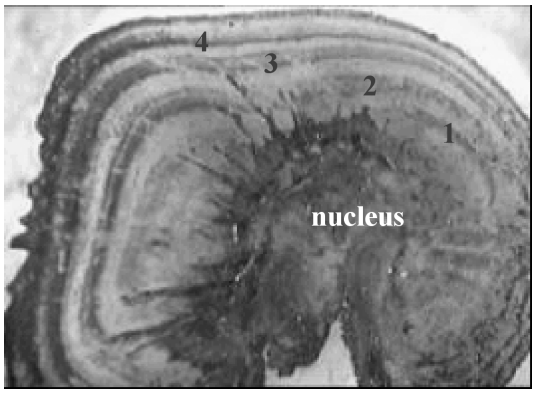

Figure 1. Horizontal polished section of a 4 years old otolith of *Pygocentrus nattereri*.

However, oocyte sizes were very similar in both basins: 1.62 mm ± 0.074 (SD) and 1.651 ± 0.054 for the Itenez and Mamoré respectively.

Seven age classes of one year intervals were observed in the two rivers. Length at age were fitted with non-linear iterative procedure to the von Bertalanffy growth model. Parameter estimates were L_{∞} =237 mm, K= 0.47, t_0= -0.832 for the Mamoré and L_{∞} = 250 mm, K=0.289, t0 = -1.363 for the Itenez. Comparisons indicated a significantly higher growth in the Mamoré than in the Itenez.

Filogeography results indicated that in Bolivia *P. nattereri* constitute a genetic unit clearly distinct from the Peruvian (Ucayali) and central Amazon (Solimoes) (Figure 2). On the other hand, *P. nattereri* in Bolivia represents a monophyletic group with very little diversity. This suggests that there would be a single population in Bolivia which would have recently and rapidly radiated throughout the high Madeira.

Therefore, it is very likely that the significant differences in size and age at maturity, fecundity and growth observed between the populations of the two rivers are environmentally induced phenotypic responses rather than genetically based differences.

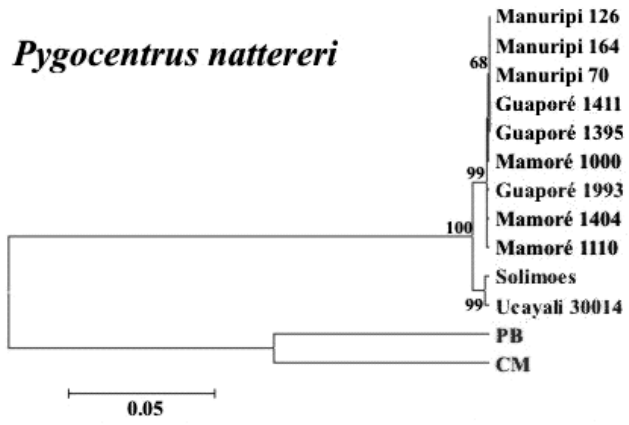

Figure 2. UPGMA tree showing the divergence between *Pygocentrus nattereri* sequences in Bolivia rivers (Mamoré, Manuripi, Itenez/Guaporé), central Amazonia (Solimoes) and Peruvian Amazon (Ucayali). PB and CM refer to sequences of *Piaractus brachypomus* and *Colossoma macropomum*, respectively.

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LIFE HISTORIES AND GENETIC STRUCTURE

OF *COLOSSOMA MACROPOMUM*

IN THE BOLIVIAN AMAZON

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Colossoma macropomum is one of the most exploited species in all the Amazon region and in the Bolivian rivers. In the Mamoré this species is the most exposed to fishing effort. Previous studies in the Mamoré (Loubens & Panfili, 1997) have been undertaken when fishing pressure over this species was quite low compared to the actual situation. On the other hand, in the Itenez basin a recent work of Reinert and Winter (2000) showed a relatively non-threatened

population based on age frequency data. This situation leaded us to conduct a study on life histories and genetic structure of *Colossoma macropomum* in the Bolivian Amazon, which includes the three main rivers basins (Béni, Mamoré and Itenez). One of the main interests of this work is the comparison of life history traits between different environments (clear or dark waters from the Itenez basin and white waters from the other two basins) and to determine a possible correlation of these factors with the genetic structure observed.

Material and methods

Population growth parameters have been characterized using the Von Bertalanffy growth curve model ($Lt = L_{\infty} (1-e^{(-K(t-t0))})$. Basically we determined L_{∞} (length at infinite age) k (growth coefficient) and t_0 (age at length 0), which allowed us to fit log linear curves for each population. The determination of differences between growth curves has been performed using a comparison of regression lines. Age determinations were made with otoliths, which were previously embedded in resin, polished and stained with 1% toluidine blue in EDTA 0,1 M, pH 7.0.

Among all the possible reproductive traits we focused our study mainly in fecundity and age or length at first maturity and reproductive cycle. Fecundities were determined on stage 4 (ripe) females. Oocyte numbers were determined using three ovary samples of approximately 0,25 g. Oocytes have been isolated under a binocular and counted using NIH Image Software. Length at first maturity was determined during reproductive period, using a logistic regression between the percentage of mature fish and consecutive standard length size classes using Statistica 5.0 software package to evaluate the parameters of the following logistic equation, $\%MF=1(1/\exp(-a(L-L_{50})))$. Where **%MF** represents the percentage of mature females in each size class); **a** a fitting coefficient (determined by the model); **L** the standard length class and $\mathbf{L_{50}}$ the length at which 50% of the females are mature (determined by the model).
The age at first maturity (A_{50}) was derived from the L_{50} value using the corresponding growth curves.

Fish were sampled from 2001 to 2003 with a bi-monthly periodicity in the Mamoré and Itenez basins and more punctually (2 samplings in average during reproductive period) in the Béni basin .

Genetic characterization of the fish was performed using nuclear DNA in the intronic regions using the Exon-Primed-Intron-Crossing (EPIC) technique to delimit the genetic populations.

Results and conclusions

We found significantly different growth curves in the three river basins, and different fecundities between Mamoré and Itenez river basins (Table 1). Since fecundity was positively correlated with standard length and weight, we used the regression lines to compare these parameters between river basins.

Size at first maturity (L_{50}) also varied between males and females and between the three river basins. Age at first maturity (A_{50}) was significantly higher in the Itenez basin reaching 6,1 and 5,5 years for females and males respectively, while in Mamoré and Béni basins the A_{50} varied only between 3,3 and 4,4 (Table 1). Breeding season started at the maximum of flooding in the three basins. Each female spawned only once a year between October and December.

River Basin	L8 (mm)	k		L50 (mm)	A50 (Years)	Relative Fecundity (eggs/g B.W.)
MAMORÉ	744	0,246	Male	427	3,4	
			Females	455	3,4	30-160
ITENEZ	881	0,184	Male	582	5,5	
			Females	551	6,1	10-85
BÉNI	748	0,276	Male	495	3,3	
			Females	562	4,4	N/A

Table 1: Comparison of Von Bertalanffy growth curve parameters (L_8 and k) and reproductive traits (L_{50}, A_{50} and Relative Fecundity) of *Colossoma macropomum* in three river basins of the Bolivian Amazon.

EPICS showed a marked genetic structure of *C. macropomum* between the rivers of the Bolivian Amazon (Figure 1). Three different populations have been characterized: Itenez-Béni-Espéranza-Yata, Mamoré-Isiboro and Béni-Salinas-Manuripi-Cardenas. This structure is correlated with geographic distance following river beads indicating that genetic fluxes are probably maintained by fish migrations within the main river course and not through the flooded plains.

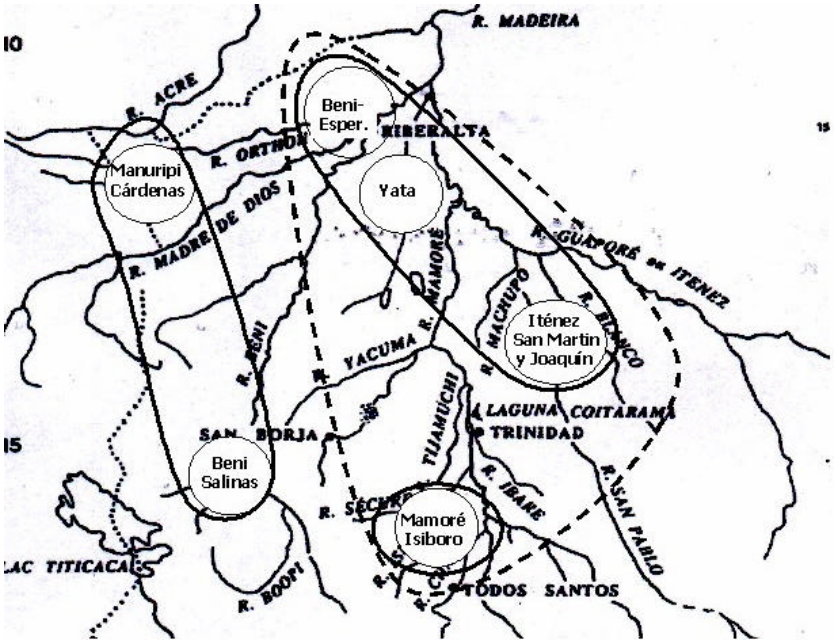

Figure 1. Genetic structure of *Colossoma macropomum* populations in the Bolivian Amazon.

Life histories and genetic studies showed that *C. macropomum* populations were structured in the Bolivian Amazon. Nevertheless the relative influence of genetic characteristics and environmental factors on the variations of life history traits cannot be completely outlined in this study and needs further work in order to understand population structure mechanisms. We also found that Isiboro-Mamoré population had a significantly reduced age and size at first maturity since the last study of Loubens and Panfili (1997). This could be an indicator of the increased level of fishing pressure in this area and should be taken into account to elaborate a sustainable use of this species in the Mamoré basin.

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GENETIC STRUCTURE OF *CICHLA CF. MONOCULUS* IN THE BOLIVIAN AMAZON AS REVEALED BY INTRON LENGTH POLYMORPHISM (EPIC-PCR)

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EXTENDED ABSTRACT ONLY: DO NOT CITE.

The largest Neotropical piscivorous cichlids belong to the genera *Cichla*, known as 'tucunaré' in Brazil, Colombia, Peru and Bolivia, or 'pavones' in Venezuela. Owing to their voracity and tenacity, they are appreciated worldwide as sport-fishes, which has motivated their introduction in several tropical or sub-tropical regions, such as Hawaii, Panama, Puerto Rico, or Florida, frequently at the expense of native species (review in Winemiller 2001). Paradoxically, *Cichla* species have received little attention in their natural environments. Until recently, most of the available information on *Cichla* came from introduced or cultivated populations. From little more than ten years, Winemiller and his collaborators started studying the ecology of *Cichla* species in Venezuela, providing the first detailed researches on wild *Cichla* populations in Latin America (see for example Jepsen *et al*., 1997, 1999, Winemiller *et al*., 1997). However, outside Venezuela, natural populations of *Cichla* remain poorly studied. Even fewer genetic studies have been carried out so far.

Five *Cichla* species are formally described in South America, although Kullander and Nijssen (1989) recently suggested the existence of 11 taxa in the genera. These are *C. ocellaris*, *C. temensis*, *C. orinocensis*, *C. intermedia* and *C. monoculus*, but Andrade *et al*. (2001) using mt rRNA (16S) sequencing, showed the existence of *C. monoculus* and *C. temensis* hybrids wherever they are found in sympatry in Brazil. In Bolivia, the only known species is referred to as *C. monoculus*, although it might be a distinct species endemic from Bolivia, smaller and with different meristic characteristics (Kullander, pers. com.; Duponchelle *et al*., unpublished data). Its taxonomic status and life histories are currently under study. The present work presents the genetic structure of *Cichla cf. monoculus* in the main rivers of the Bolivian Amazon.

Material and Methods

186 fishes were analyzed from 10 localities in 6 rivers of the Bolivian Amazon. The length polymorphism of 6 intronic loci was used with the Exon-Primed Intron-Crossing Polymerase Chain Reaction (EPIC-PCR) technique. Amplified loci were resolved by acrylamide gel electrophoresis and sizes ranged from 250 bp to 740 bp.

Results and Conclusions

Within the ten sampled sites along the 6 main rivers of Bolivia, 4 very distinct populations of *C. cf. monoculus* were found (Figure 1). One regroups the individuals sampled in the Yata, “Alto” and “Medio-Itenez” (San Martin and San Joaquim). The other populations are located in the Ichilo, Manuripi and Sécuré rivers. Figure 2 illustrates the geographic distribution of these populations. The comparison of geographic distances (at bird's flight or following the river's course) between sampled sites and the corresponding genetic distances showed that genetic fluxes between populations are made through the main river courses and not through the flooded plains. The most isolated populations are the more geographically distant (Ichilo and Manuripi). The highest polymorphism was observed in the Yata-Itenez population, which presented two exclusive alleles and possessed all the alleles found in the other populations. This suggested that the Itenez has been a refuge zone or an intermediate step during the colonization of Bolivian rivers by *Cichla* from the Madeira. The other Bolivian rivers were probably colonized from this "source" population.

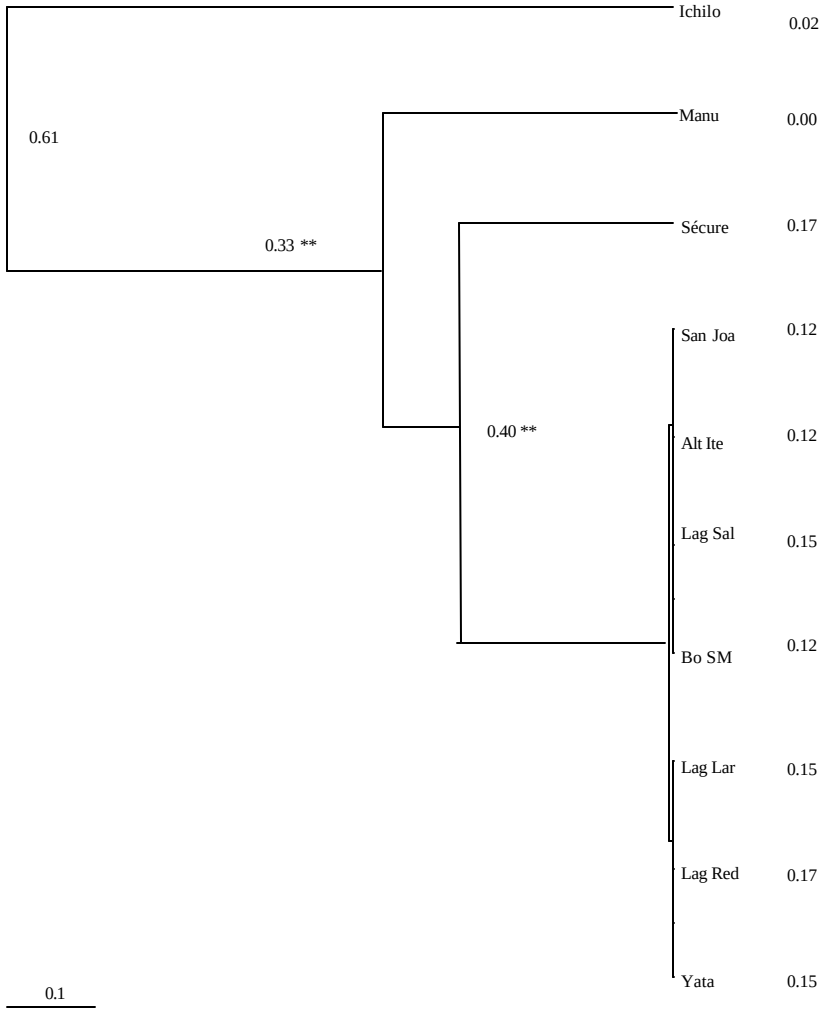

Figure 1: Dendrogram UPGMA based on the genetic distance between the 10 sampled localities in Bolivia. Fst values at the ramifications. Rivers Ichilo, Manuripi (Manu), Sécure, Yata, Alto Itenez, and Medio-Itenez (San Martin [laguna Sala, laguna Larga, laguna Redonda] and San Joaquín).

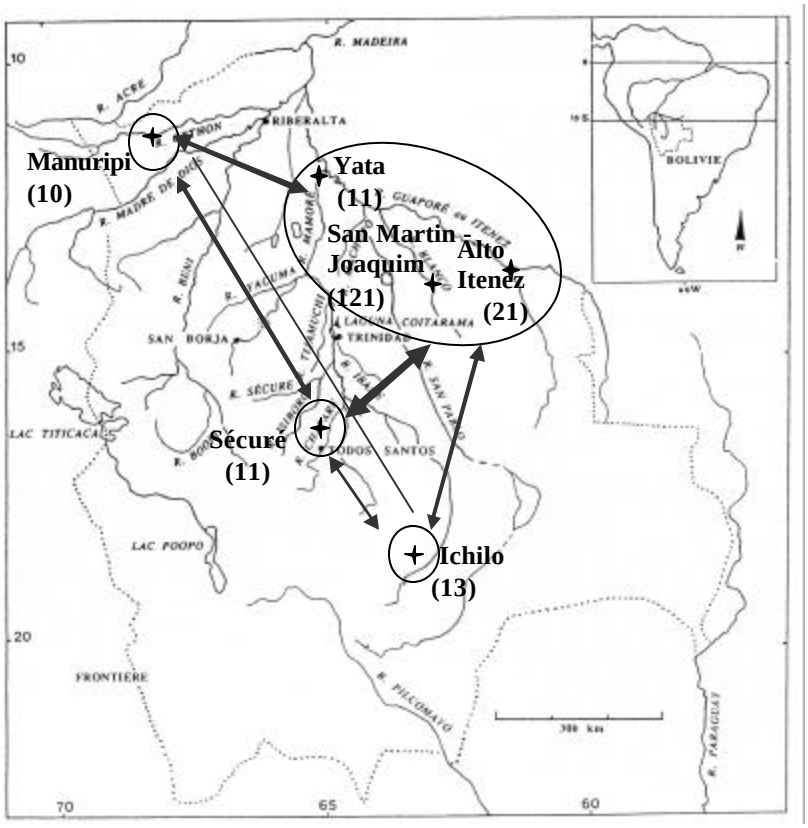

Figure 2. Geographic distribution of the 4 populations of *Cichla cf. monoculus* in the river of the Bolivian Amazon. Each circle is a population. Arrows represent genetic fluxes between populations and their size are proportional to the flux intensity. Number of individuals analyzed per locality between bracket.

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INTRON LENGTH POLYMORPHISM (EPIC-PCR)

AS A MOLECULAR SYSTEMATIC TOOL

FOR THE IDENTIFICATION OF PIRANHAS SPECIES

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EXTENDED ABSTRACT ONLY: DO NOT CITE

Lessa (1992) introduced intron-targeted PCR, in which a non-coding intron was amplified using primers designed from highly conserved exon sequences. This approach, called Exon-Primed Intron-Crossing (EPIC)-PCR, has been shown to yield substantial variability, mainly from intron length polymorphism, and was successfully used in several population genetic surveys (Daguin & Borsa 1999, Hassan *et al.* 2003a). EPIC-PCR has several advantages in populations genetic studies: (i) by using primers from heterologous genes, cloning and sequencing of target can be avoided; (ii) cross-species amplification should be easier than when primers are designed in non-coding sequences because exon sequences are more conserved across species; (iii) for the same reason, within species, PCR artifacts such as null alleles are expected to be less frequent.

Piranhas are a well known problematic group in systematic and a recent cytogenetic study (Nakayama *et al.* 2002) showed that NOR pattern failed to

offer substantial information between closely related species of the genus *Serrasalmus.* For the reasons mentioned above, we applied the EPIC-PCR in the case of the piranha fauna in order to attempt for a molecular characterization of the 9 species of the Bolivian Amazon: *Serrasalmus* (*Serrasalmus*) *rhombeus* (SR), *Serrasalmus* (*Serrasalmus*) *spilopleura* (SS), *Serrasalmus* (*Pristobrycon*) *eigenmanni* (PE), *Serrasalmus* (*Serrasalmus*) *compressus* (SC), *Serrasalmus* (*Serrasalmus*) *elongatus* (SE), *Serrasalmus* (*Serrasalmus*) *hollandi* (Sho), *Serrasalmus* (*Serrasalmus*) *marginatus* (SM), *Pygocentrus nattereri* (PN) and *Catoprion mento* (CM).

Material and Methods

We used 5 pairs of primers (Bierne *et al*., 2000, Hassan *et al*., 2003b) and obtained 14 polymorphic loci as most of them belong to multigene families. All the amplified loci were easily resolved by acrylamide gel electrophoresis and sizes ranged from 325 bp up to 1090 bp. The number of alleles varied greatly between loci and ranged from 2 to 10 alleles.

Results and Conclusions

Results obtained from this pool of loci are shown in table 1.

Table 1. Levels of genetic differentiation between piranha species. Upper matrix represent Fst for pairwise comparisons and lower matrix give the number of diagnostic and semi-diagnostic locus respectively.

	SR	**SS**	**PE**	**SC**	**SE**	**PN**	**Sho**	**SM**	**CM**
SR	-	0.82**	0.75**	0.83**	0.82**	0.86**	0.74**	0.83**	0.88**
SS	**6**-3	-	0.83**	0.82**	0.77**	0.79**	0.84**	0.84**	0.85**
PE	**5**-3	**8**-2	-	0.87**	0.86**	0.87**	0.82**	0.88**	0.89**
SC	**4**-3	**6**-3	**8**-1	-	1**	0.92**	1**	0.83**	1**
SE	**5**-1	**7**-2	**8**-1	**8**-1	-	0.92**	1**	0.93**	1**
PN	**8**-2	**3**-5	**10**-2	**8**-1	**8**-2	-	0.96**	0.92**	0.92**
Sho	**1**-6	**9**-2	**5**-2	**4**-2	**6**-1	**9**-2	-	0.91**	1**
SM	**5**-2	**8**-2	**7**-2	**4**-2	**6**-0	**8**-2	**4**-4	-	0.95**
CM	**8**-2	**7**-1	**10**-1	**8**-2	**8**-0	**10**-1	**6**-1	**8**-1	-

**. Significant at a p=0.01 level

All the F-statistics calculated by pairwise comparisons are highly significant ranging from 0.75 between SR and Sho up to 1 between Sho and SC for example. The number of diagnostic locus, defined as locus with alternative alleles between species, varied greatly with a minimum of 1 diagnostic locus between SR and Sho and a maximum of 10 loci between CM and PE for example. The number of semi-diagnostic locus, defined as locus presenting alternative as well as shared alleles between species, is globally low except between SR and Sho indicating a recent divergence between this 2 species. Although alleles distribution across species is greatly informative, hierarchical analyses at the population level confirm the F-statistics results (Figure 1). For each of the 9 species, all the populations constitute monophyletic units defined in the population genetic sense as a group of populations derived from an ancestral population.

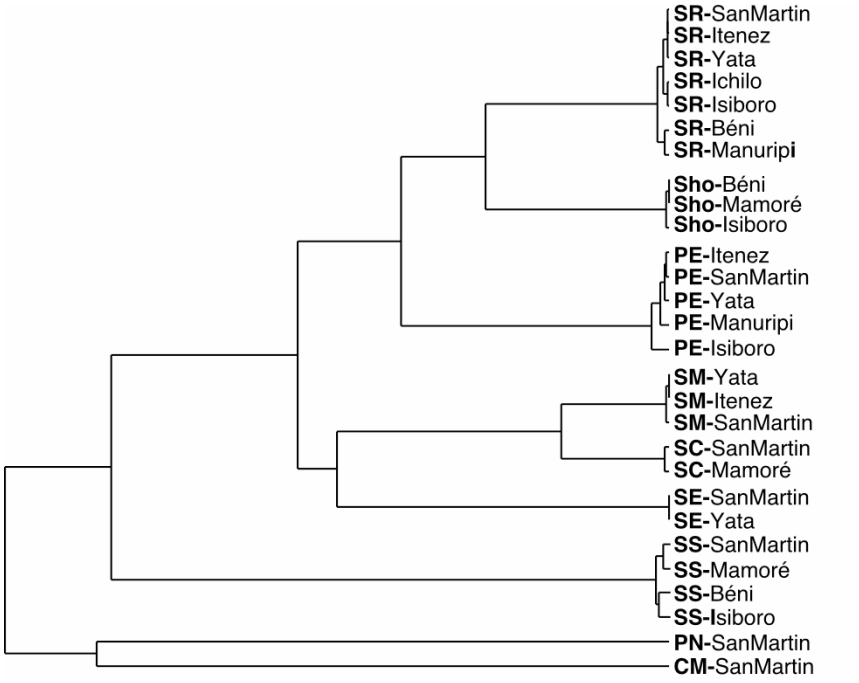

Figure 1: Dendrogram UPGMA based on the genetic distance between 28 populations of the 9 species of the Bolivian Amazon.

We conclude that intron length polymorphism assessed by EPIC-PCR is a powerful tool for the identification of the Bolivian species and intra-specific polymorphismobserved at least for 4 species suggests a potential candidate for population or species complex genetic studies.

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OXYGEN CONSUMPTION DURING ACUTE TEMPERATURE STRESS IN YOUNG OCEAN POUT (*MACROZOARCES AMERICANUS*): A BENTHIC, COLD-WATER MARINE SPECIES

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Most work in the area of fish thermal physiology has been performed using temperate freshwater or pelagic marine species (e.g. salmonids, goldfish, cod, etc.). In a rare study examining the thermal physiology of a benthic cold-water species, Zakhartsev *et al*. (2003) found that warm-acclimated adult eelpout (*Zoarces viviparus*) exhibited the same routine metabolic rate as cold acclimated fish when transferred to colder water. This result differs from the traditionally accepted view that warm acclimated fish have lower metabolic rates compared with cold acclimated individuals after acute transfer to low temperatures. Zakhartsev *et al*. (2003) speculated that the eelpout in their study were "permanently adjusted" to life at colder temperatures, and thus did not show the response expected from previous research with temperate species. However, the thermal physiology of other cold-water marine fishes has received little attention, particularly with regard to early life stages.

The current study examined the metabolic response of young ocean pout (*Macrozoarces americanus*) to acute temperature change. Populations of ocean pout that inhabit cold waters off the coast of Newfoundland show very high levels of antifreeze proteins during the entire year (Fletcher *et al*. 2001),

suggesting that these fish inhabit colder waters year-round. Experiments were conducted to examine how thermoacclimation alters the thermal sensitivity of metabolism, and whether the pattern of response to temperature acclimation differs from that typically reported for temperate species.

Methods

Ocean pout eggs were collected from the wild and placed in laboratory incubators until hatch. After hatching, groups of ocean pout were acclimated to 3°C, 7°C or 11°C. The fish remained at these temperatures for a minimum of three weeks prior to experimentation.

After fasting for at least 24 hours, individual ocean pout (mean wet mass 280±19 mg) were transferred to a small Blazka-type respirometer (60 ml volume) set at their acclimation temperature where they remained undisturbed overnight. The next morning ventilation rate and routine oxygen consumption (MO_2) was measured using a Presens fibre-optic oxygen measurement system. For the 7°C and 11°C treatments, the temperature within the respirometer was then reduced to 3°C over the course of 1.5 h, and MO_2 was measured at 3°C. For all groups, the water temperature was then increased by 2°C every 1.5 h until the temperature reached a maximum of 17°C. MO_2 was measured every 2°C increase.

Results and Discussion

Initial measures of MO_2 at each acclimation temperature revealed a Q_{10} of 7.7 between 3°C and 11°C. This Q_{10} value is much larger than would normally be expected over this temperature range. Furthermore, at all measurement points during the gradual temperature increase from 3°C to 17°C, the warm acclimated pout showed higher MO_2 compared to cold acclimated individuals. For example, the oxygen consumption of the 11°C acclimated fish only decreased by 20% after the initial temperature drop to 3°C (from 481 mg O_2 kg^{-1} h^{-1} at 11°C to 383 mg O_2 kg^{-1} h^{-1} at 3°C), and was 4-fold greater than the 3°C acclimated fish at this temperature. Further, at 17°C, the MO_2 of the 11°C fish was about 40%

higher than that of the 3°C acclimated individuals. This apparent acclimation-induced elevation in metabolism was also reflected in the ventilation data, where ventilation rates were higher at each temperature for the warm acclimated fish.

These results are in direct contrast to the large body of previous work which shows warm acclimated fish are unable to match the metabolic rate of cold acclimated fish when exposed to cool temperatures (e.g. Evans 1990). Although extremely interesting, our results are difficult to resolve. At present, we have three possible explanations for the observed trends. First, the 'shock' associated with the acute temperature drop in this study may have caused a stress-associated increase in oxygen consumption in the warm acclimated fish. Second, ocean pout are adapted to life in cold waters, and it is possible that they possess only a single, cold-water adapted isoform of various metabolic enzymes whose production is increased during acclimation to warm temperatures (to compensate for their decreased efficiency at higher temperatures). A drop to colder temperatures in these warm acclimated fish would then result in a higher than expected metabolic rate because of the high concentrations of enzymes that are optimised for function at cold temperatures. Finally, the MO_2 of ocean pout may become depressed (i.e. torpor) when they are acclimated to colder temperatures.

At present it is unclear why young ocean pout possess a unique thermal physiology. Each of the above hypotheses can be used to explain portions of the data set but are inconsistent when applied to the results has a whole. Future studies are planned that will measure the whole body concentrations of certain aerobic enzymes (e.g. citrate synthase, cytochrome oxidase) in young ocean pout acclimated to a range of temperatures, and also examine the changes in MO_2 that take place in the 24 hour period following a sudden drop in temperature (i.e. from 11°C to 3°C).

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MULTISENSORIAL CONVERGENCE TO THE HYPOTHALAMIC

NUCLEUS ANTERIOR TUBERIS IN *GYMNOTUS CARAPO*

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Fishes have several extra senses (e.g. mechano and electrosensorial lateral line) in addition to the more familiar vision, hearing, touch, taste and smell of most terrestrial animals and thus they provide a rich array of sensory systems for investigating multisensory questions.

Gymnotiform fishes comprise a relatively small group of about 85 species of weakly electric fish that inhabit South and Central America's freshwaters. Although they are potentially able to use visual, tactile and acoustic information for orientation or prey capture, they nonetheless rely mainly on their active orientation system by emitting electric organ discharges and monitoring the feedback from these discharges. Animate or inanimate objects located in the animal's immediate vicinity that differ in electrical impedance from the surrounding water change the transepidermal current flow generated by the fish's own discharges. The electrosensorial system serves for electrolocation (Lissmann e Machin 1958) and electrocommunication (Hagedorn 1986) and the

evolution of such mechanisms allow nocturnal activity in waters of poor visibility; as a consequence they are relatively safe from visual predators and can exploit food sources that remain hidden for other orders of fish.

Electrosensory systems might share a common evolutionary lineage with the mechanosensory (lateral line) system. It has been suggested that electrosensory brain structures could have evolved by duplication from mechanosensory areas, yielding a strong relationship between these systems (McCormick and Braford, 1988). The hypothalamic nucleus anterior tuberis (TA) has been involved in the processing of acoustic and mechanosensorial information in most teleosts (Striedter 1991) and thus was chosen as the focus of the present study.

The aim of the present study was to investigate the afferent connections of the TA of *Gymnotus carapo*, providing a description of its connection patterns, in order to confirm its participation on mechanosensorial processing also in this species and analyze the relationship between this nucleus and nuclei involved in electrosensory/motor tasks.

Methods

The knifefish Gymnotus carapo, used throughout this "in vivo" study, were bought from a local dealer and kept on individual aquaria in the laboratory until the experiments were hold and during survival time (4 days). Animals with sizes ranging from 20 to 26 cm total length were used without sex distinction. Animal care, anesthesia, surgery and euthanasia were carried out in compliance with guidelines set forth by the Brazilian Society for Neuroscience.

Unilateral biotinylated dextran amine (BDA: 3000MW; Molecular Probe, USA) iontophoretic depositions to the TA (six cases; one case exemplified on figure 1) were made in accordance with previous studies (Corrêa et al., 1998). Briefly summarizing, after transport, perfusion and Vibratome sectioning (80 µm thickness), and during tissue processing, BDA labeling was visualized using a standard avidin-biotinylated HRP method (Elite Vectastain ABC Kit, Vector Laboratories Inc. 1997) with a nickel intensified peroxidase 3-3´ diaminobenzidine dihydrochloride (DAB: Sigma, St. Louis, Missouri) reaction.

Results

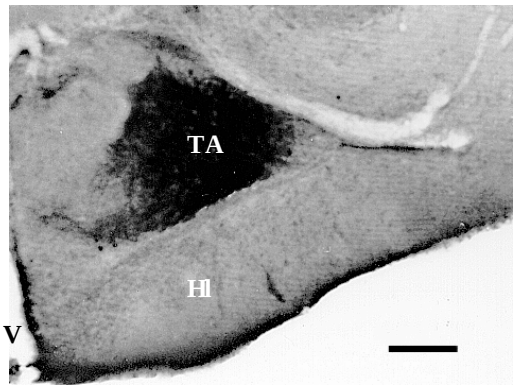

Figure 1. Photomicrograph through the diencephalon of *Gymnotus carapo* showing a typical injection site confined to the nucleus anterior tuberis (TA). Hl: hypothalamus lateralis; V: ventricle. Scale bar: 100 µm.

The torus semicircularis is a mesencephalic hypertrophied structure which receives topographically segregated mechano, electric and octaval information carried by lateral lemniscal fibers; its ventral subdivision receives secondary mechanosensorial input from the rhombencephalic mechanosensorial lobe. We found that TA is massively afferented from the ventral torus semicircularis and in a minor extent from the nucleus praeeminentialis, the latter also being a secondary mechanosensorial station possibly involved in feedback control of this sensorial modality.

Also, afferent connections from the pre-electromotor central posterior/ prepacemaker complex and from the nucleus electrosensorius (beat related and acousticolateral subdivisions), an electro-sensorial/motor interface, were observed. Both nuclei have an important role in the execution of electromotor commands.

Furthermore, the ventral telencephalon, the major target of secondary and tertiary olfactory projections in teleosts, and the preglomerular complex (medial subdivision), target of multisensory input and a relay station to the telencephalon, together with the nucleus glomerulosus, a relay station of visual information to the hypothalamus, project to the TA.

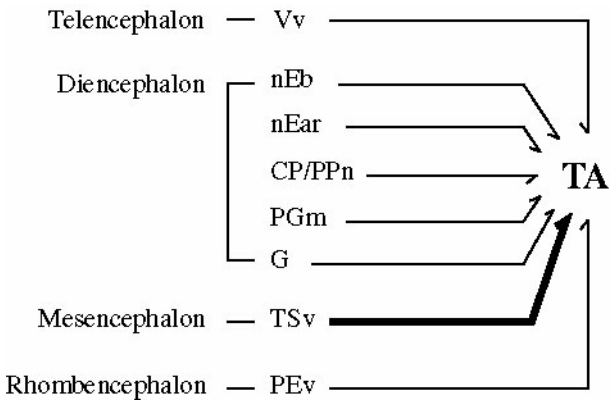

Figure 2. Afferent connections to the nucleus anterior tuberis (TA). Arrow thickness indicate the density of the connections. CP/PPn: central posterior/prepacemaker complex; nEb: nucleus electrosensorius, beat related subdivision; nEb: nucleus electrosensorius, acousticolateral subdivision; G: nucleus glomerulosus; PEv: nucleus praeeminentialis, ventral subdivision; PGm: preglomerular complex, medial subdivision; TSv: ventral semicircular torus; Vv: ventral telencephalon.

Conclusion

The TA is primarily a mechanosensorial processing center as extensively mechanosensorial input from ventral torus semicircularis and additional nucleus praeeminentialis input suggests. The present results also point to the existence of multisensorial convergence (mechano, electro, visual olfactory and multisensory from preglomerular nucleus) at this diencephalic nucleus allowing its possible involvement in processes that depend on multisensorial integration (figure 2). However a study of the efferents of this nucleus should be carried to allow a broader discussion.

Acknowledgements

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ESTIMATION OF SYSTEMATIC ERROR IN STEREOLOGICAL AND NON-STEREOLOGICAL DETERMINATION OF THE SURFACE AREA OF GILLS OF THE RAINBOW TROUT, *ONCORHYNCHUS MYKISS*

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Although stereological methods have been used for the quantitative evaluation of vertebrate respiratory organs for more than 35 years (Weibel 1963), their application to gills has remained rudimentary (Hughes 1972). Instead, the surface area has been determined from vertical projections of isolated secondary lamellae (SL), sampled from prescribed sites and multiplied by the total number of SL (Hughes 1966). The diffusion distance has been estimated from orthogonal measurements obtained from sections that appeared to pass

perpendicularly to the general surface plane of the SL. This method, while intuitively appealing, is contains numerous untestable assumptions and lacks a mathematical foundation. In addition it is applicable only to fish gills that have flat, isolatable SL. Thus, lungfish or other obligatory air-breathers, in which the gills are reduced or otherwise modified, remain unmeasurable. Recently we have developed a morphometric method that employs exclusively mathematically founded stereological techniques and is applicable to gills of any structure (Costa et al., 2000). The stereological methods, however, yield a surface area for trout gills that is approximately double that previously determined using non-stereological methods (Höller S, and Perry, S.F., 2003. Comparison of non-stereological and stereological methods for determination of gill surface area in the rainbow trout, Oncorhynchus mykiss.). Therefore it is neceessary to determine if the present stereological methods or the previously used non-stereological methods are in error.

Using six rainbow juvenile trout rainbow trout (*Oncorhynchus mykiss*; M_B 43.33g, SD 12.90), we tested the hypothesis that the stereological method yields a systematic overestimate of surface area due to significant expansion of methacrylate-embedded tissue after sectioning. On a second group of six trout of similar body mass, we tested the hypothesis that the non-stereological method results in significant underestimation of the surface area due to diversion of the secondary lamellae from the planar projection model. An index of actual area of the secondary lamellae was compared with one for the area of vertical projections of the same lamellae using intersection counting techniques and methacrylate-embedded tissue.

In the first experiment, significant shrinkage occurred during acetone dehydration (28% of area), but was compensated by expansion of the sections (22.5%), such that no significant difference between the native and sectioned tissue was present (Figure 1). The non-stereological method, however, would underestimate the area by 42%. At is, the actual area was 172% of that measured by the non-stereological method (Figure 2). Considering that the base of the secondary lamellae could be masked by the gill filament and thus escape sampling in the non-stereological method, the systematic underestimate could be even greater. We therefore conclude that the observed factor-of two error lies entirely with the non-stereological method.

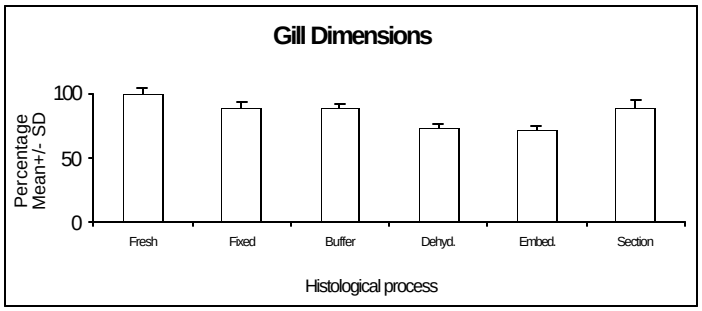

Figure 1. Gill dimensions during the histological process in *O. mykiss*. Data are presented as mean of six fish ± SD. There wasn't significant difference between 'fresh' and 'section'.

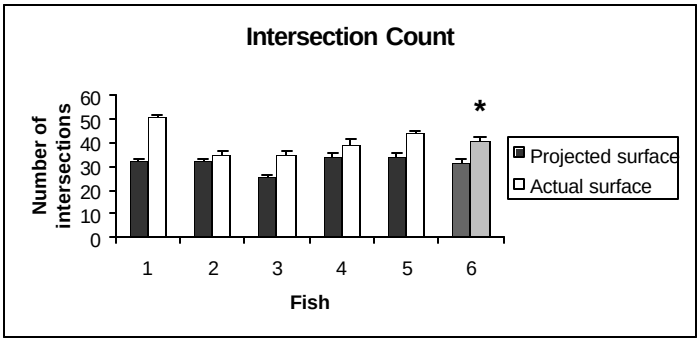

Figure 2. Comparison between non-stereological (projected surface) and stereological method (actual surface). Data are presented as mean of five fish ± SD. 'Fish 6' is value mean of 'Fish 1-5"; (*): significant difference.

Acknowledgments

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THE RETINA OF CHILODUS PUNCTATUS:

TOPOGRAPHIC ORGANISATION OF NEURONAL DENSITY

IN THE GANGLION CELL LAYER

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EXTENDED ABSTRACT ONLY - DO NOT CITE

The topographic distribution of neurons (ganglion cells and displaced amacrine cells) within the retinal ganglion cell layer has been extensively investigated in several species of teleosts (Collin, 1999) and to a lesser extent in elasmobranchs (Bozzano and Collin, 2000). The analysis of the topographic distribution of retinal neurons permits the identification of high visual acuity zones with elevated neuronal density. A variety of topographies have been observed in several species of teleosts both from shallow water and deep sea. These topographies are thought to be associated to the ecological niche or the foraging strategy a given species employs. However, a detailed phylogenetic basis is fundamental to understand how the retinal topographic specialisation could be

involved with a specific ecological task or even represent an ancestral heritage. We aim to analyse the topography of neurons in the ganglion cell layer of the spotted headstander *Chilodus punctatus* Müller & Troschel, 1844, a freshwater fish species which inhabits the Amazon and western Orinoco basins. This fish adopts a very singular posture. The snout is pointed to the bottom of the river as the body rotates its longitudinal axis in an oblique fashion. *Chilodus* feeds basically on zoobenthos and detritus obtained from the bottom (Mills and Vevers, 1989).

The fish were commercially acquired and transported to the laboratory. They were decapted and the heads transferred to a Petri dish containing phosphate buffer saline (PBS) pH 7.2. The eyes were enucleated and opened at the equator under saline bath. Then, the posterior eyecups were fixed in 4% paraformaldehyde in phosphate buffer pH 7.2 for one hour. After the fixation period, the eyes were placed in PBS. The retinas were dissected by removing the sclera and severing the falciform process. The pigment epithelium was bleached in a solution of 10% hydrogen peroxide in PBS for 24 or 48 hours. After this, the retina was rinsed and radial cuts were made to flatten it with the retinal ganglion cell layer uppermost onto a gelatinised slide. The retina was then exposed to formaldehyde vapours at 60^0C for two hours. Then, the retina was rehydrated, satined for Nissl substance with an aqueous solution of 0,1% cresyl violet for approximately 10 minutes, dehydrated, cleared and finally mounted. The retinas were topographically analysed by constructing a matrix on which the density values were plotted. The retina was sampled at 0.5 mm intervals with a sampling window of 0.0064 mm^2. Maps were constructed by using the program Arcview GIS 3.3.

The isodensity contours revealed the presence of a non-prominent horizontal visual streak with a mean peak of 21.823 ± 1.097 cells/mm^2 (N=3) (Figure 1). In the horizontal visual streak, we observed a predominance of neurons with small perikarya (Figure 2A). At dorsal periphery, density values fall to approximately 7×10^3 cells/mm^2; in this region there are many neurons with large perikaria (Figure 2B). At nasal and temporal peripheries, the values of isodensity contours are slightly higher.

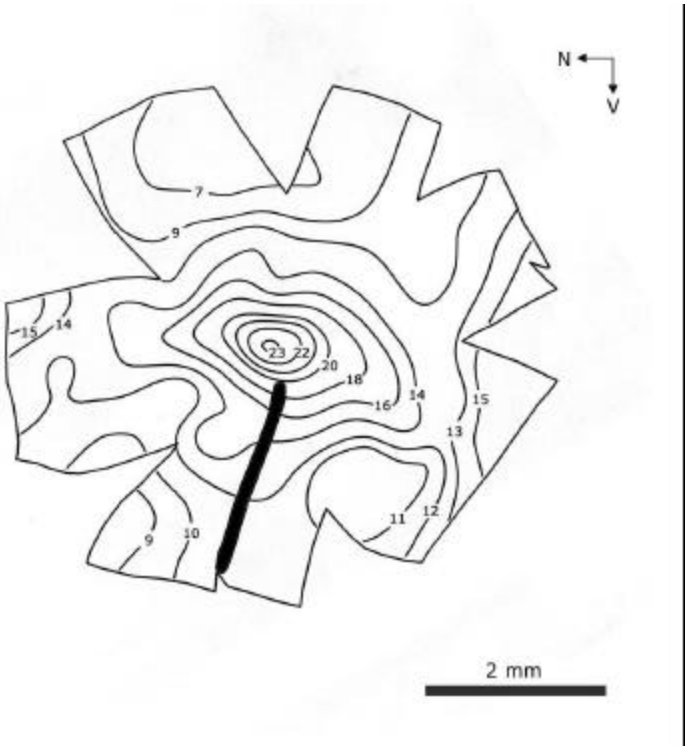

Figure 1. Topographic map of neuronal density within the ganglion cell layer of the spotted headstander *C.punctatus.* Note the presence of a moderate horizontal visual streak. The dark strip represents the position of the falciform process. Densities are x 10^3 cells/mm^2 ; N= nasal; V= ventral.

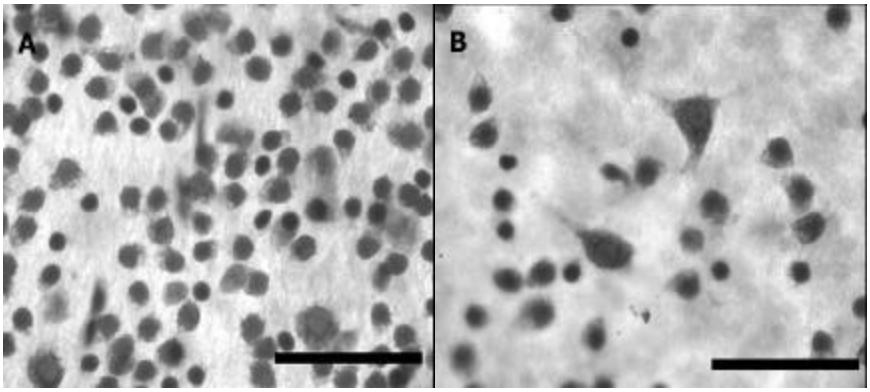

Figure 2 (A). Photomicrograph of neurons in the elevated density zone. Note the small bodied neurons and the elevated packing. (B) Photomicrograph of large perikarya neurons at the dorsal periphery. Scale bar = 30 µm.

According to Hughes (1977), animals that inhabit open areas are supposed to possess a retinal specialisation known as visual streak. The visual streak is a strip of elevated neuronal density along the horizontal meridian. On functional grounds, this retinal specialisation is devoted to provide high visual acuity along the horizon. Hughes (1977) emphasised that the presence of a visual streak would be clearly advantageous for animals that live in open areas without the obstruction of vegetation in the visual fields. The presence of a visual streak, more or less conspicuous, has been observed for an amount of fish species that inhabit open areas (Collin, 1999). The presence of a horizontal visual streak in the retina of *C. punctatus* is in agreement with the habitat this species live in. On the other hand, one would also expect the presence of a temporal area, which would be of great functional value to discriminate objects in the binocular visual field, but this was not the case as this species does not swim in a horizontal aspect. Actually as this fish swims in an oblique fashion, a temporal area would project to the inferior visual field and aid the animal to better discriminate food items located on the bottom. A more complete description of retinal organisation

of other species with different swim postures of the family Chilodontidae is of great importance to assess if species phylogenetically close with different foraging strategies share the same type of retinal specialisation.

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INNATE IMMUNE RESPONSE

OF FRESHWATER FISH *PROCHILODUS LINEATUS*

DETECTED BY ANALYSIS OF LEUCOCYTES REACTIVE OXYGEN

PRODUCTION

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EXTENDED ABSTRACT ONLY - DO NOT CITE

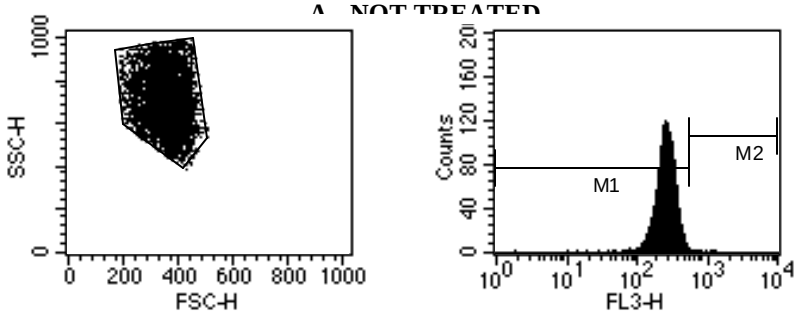
Prochilodus lineatus is a freshwater fish belonging to the Prochilodontidae family being found in a Brazilian river basin called Paraná. This specie has proctactile mouth adapted to suck microorganisms and organic deep river substrate, forming great shoals migrating down the river to feed and along up to reproduce. The process in which leucocytes release reactive oxygen species (ROS) is called respiratory burst and is correlated with an innate immunity response. The tests used to evaluate ROS production are based on fluororescence, luminogenic and chromo metric tests, some recently ones have been made using flow cytometry (Morimoto *et al*., 2003; Rothe & Valet, 1990). This work investigates *P. lineatus* monocytes, neutrophils and large lymphocytes respiratory burst in flow cytometry. Ten *P. lineatus*, 2-3 years old, males and females, weighing 933±84 g and measuring 42.0±3.0 cm were obtained in São Paulo Energetic Company (CESP), Environment Department in Paraíbuna, São Paulo. Before each experiment, the fishes were anaesthetized in

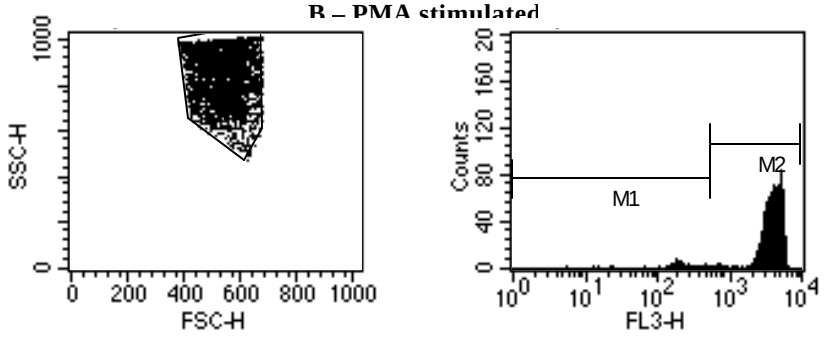
a 40-ppm benzocain water solution (Labsynth products®, Diadema, SP, Brazil). One milliliter of blood was laid in 3 ml of Histopaque®, Sigma®, St. Louis, MO, USA) in a 5-ml plastic tube. The buffy coat was gently collected and washed once in a Phosphate Buffer Saline solution (PBS) (0.1 M, pH 7.2). The cell populations were submitted at two different stimuli: PMA (100 nM/ml) and LPS (50 µM/ml) (all drugs from Sigma®, St. Louis, MO, USA). All the stimuli were performed for 30 min. Cyanide acide (KCN) (1 mM/ml) was used as a control of metabolic superoxide production. All populations were incubated for 30 min with 1 µM of dihydroethidium (Fluka®, St. Louis, MO, USA) and analyzed in a FL-3 fluorescence emission in a histogram graphic in FACS cytometer (Scalibur®) in order to measure reactive oxygen products. The average percentage of M2 (percentage of increase of fluorescence) in monocytes was detected in cells stimulated with PMA (Figure 1, A and B).

The other stimulus did not show any increase. Monocytes were the only cell type responsible for this increasing. The results with monocytes were 4±2% in no treated cells, 89±4% in PMA treated cells. There was found significant differences comparing monocytes PMA treated cells with non-treated cells (ANOVA, $p<0.001$). The significant increasing of ROS production only in monocytes stimulated with PMA is in accordance with literature. Increases ROS production is described for neutrophils, monocytes/macrophages and eosinophils. The lack of neutrophils response to PMA stimulus was not expectable, since neutrophils are described as the first inflammatory cells in fish. This non-response may be due the neutrophils from blood were not activated enough or these cells needs different kind of stimuli to produce ROS. There are very few works that uses flow cytometry analysis for ROS production in fish leukocytes, like the study of ayus, carps and rainbow trout neutrophils from Kidney and blood, using dihydrorhodamine 123 as a green marker fluorescence. The neutrophils presented great positively after PMA treatment (Novoa et al., 1996). It is difficult to compare quantitatively these data with the present work, since the authors investigate only neutrophils, different PMA concentration, different stimulation time and different marker. This present work confirms the macrophage importance in the cellular non specific immune response in *P. lineatus*, bring perspectives to future investigations in order to find correlations between respiratory burst and others immune phenomenon. These comprehensiveness of the *P. lineatus* in a non-specific immune system are important to increase the production in captivity and to develop vaccines and others non specific immune stimulants. Another importance of this work is a

perspective to investigate molecular and cellular mechanisms of respiratory burst in *P. lineatus* to generate data that in the future could be correlate with other vertebrates to find keys in respiratory burst biology.

Figure 1





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EFFECT OF VITAMIN D SUPPLEMENTATION ON HAEMATOLOGICAL PARAMETERS AND WEIGHT GAIN OF TAMBAQUI (*COLOSSOMA MACROPOMUM*)

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Fish of the Amazon exhibit high plasticity in their feeding behavior, using many natural sources of proteins, fibers, carbohydrates and vitamins. Vitamin D is important to animal nutrition, acting on skeleton growth through bone mineralization. The primitive form of vitamin D is obtained through ultraviolet radiation. In fishes, its synthesis depends on the limited amount of sunlight available in the water column. Filter fishes obtain vitamin D through the feed,

from phytoplankton. However, fishes from intensive and super intensive culture are harmed by the density of animals and by the high water renovation, which reduces plankton production. Thus, in this case, a supplementation of vitamin D in the diet is needed (Lovell, 1989). Tambaqui (*Colossoma macropomum*) is the most important fish of the Amazon reared in South America. The aim of this study is to assess the effect of different vitamin D concentrations on growth, weight gain and hematological parameters of tambaqui.

Material and Methods

Tambaqui juveniles were fed twice a day over 60 days, with the same experimental ration, except for the vitamin D amount (0, 250 and 1000 IU vitamin D_3/Kg) administered as Colecalciferol D_3. Twenty-one fishes were distributed in nine tanks of 500L, three tanks per treatment, being two experimental units and one as a spare. On the 30^{th}day, eight fishes were withdrawn from the tanks and taken to the laboratory for the analyses; eight other fishes from the spare tank replaced them to keep the animal density. The animals were anaesthetized and the blood was extracted from the caudal vein. The blood was used to estimate hematocrit (Hct) (%) by microhaematocrit method, hemoglobin concentration [Hb] (g/dL) by cyanmethemoglobin method, and total red blood cell count (RBC) ($x10^6/mm^3$ of blood), using a Neubauer chamber and a 40X magnification microscope. Using these results, the corpuscular constants (MCV – mean corpuscular volume, MCH- mean corpuscular hemoglobin, and MCHC- mean corpuscular hemoglobin concentration) were calculated according to Brow (1976). Plasma levels of Phosphorus (mg/100mL) were analyzed with a Doles commercial kit and calcium levels (mEq/L) were analyzed by a flame photometer (CELM FC 180). The values of weight gain were calculated by equation: Weight gain (%) = (Pf – Pi) x 100/Pi, where Pf is the final weight (g), Pi, the initial weight (g). Food conversion rate was calculated by the equation: Food conversion = food amount (g)/ weight gain (g). Data re expressed as mean±SEM and were subjected to one-way analysis of variance, and a *post hoc* Tukey test (5%), when appropriate.

Results and Discussion

Vitamin D affects animal growth. Studies with this vitamin in tilapia (*Oreochromis niloticus*) showed an increase of 2576% in weight gain and decrease feed conversion (Shiau & Hwang, 1993). Andrews *et al*. (1980), studying with *Ictalurus puntactus*, observed an increase in weight gain when 1,000 IU of vitamin D were supplemented in the diet. In the current study,

tambaqui showed a significant weight gain with vitamin D supplementation presenting the best growth with the ration containing 1000 IU/ Kg (Table 1). There was no change on feed conversion values during the first 30 days trial. On the 60th, however, a significantly better feed conversion was observed in fishes fed with vitamin D-supplemented ration. These results suggest that tambaqui is able to efficiently use colechalciferol, as tilapia and channel catfish (*Ictalurus punctatus*).

Table 1. Hematological parameters, weight gain and food conversion of *Colossoma macropomum* fed on vitamin D-supplemented diet. (*) shows significant differences (P<0.05) with control.

Parameters		Control	0 IU	250 IU	1000 IU
Ht	0 d	28.06±0.83			
(%)	30 d		28.25±0.71	30.31±0.39	28.50±0.90
	60 d		20.88±2.29	21.94±1.62	25.75±1.34
Hb	0 d	7.57±0.26			
(g/dl)	30 d		7.52±0.21	7.66±0.14	7.23±0.27
	60 d		5.24±0.75	5.47±0.71	7.00±0.43
RBC	0 d	2.11±0.09			
(10^6/mm^3)	30 d		2.06±0.10	1.92±0.07	1.64±0.08*
	60 d		1.16±0.18*	1.42±0.19	1.69±0.11
MCV	0 d	134.21±4.9			
(? m3)	30 d		139.20±6.0	159.29±4.9	174.93±6.0
	60 d		188.07±15.4	182.78±33.9	154±7.77
MCH	0 d	36.04±0.83			
(pg)	30 d		37.06±1.58	40.22±1.26*	44.24±1.35*
	60 d		46.13±3.88	38.79±1.88*	41.94±1.89*
MCHC	0 d	27.01±0.73			
(%)	30 d		26.77±1.12	25.28±0.45	25.36±0.50*
	60 d		24.61±0.84*	24.35±2.85	27.31±1.23
Weight	30 d		40.6±1.69	55.8±4.6*	59.7±1.3*

(%)	60 d		58.4±8.5	106.0±9.4*	137.7±3.8*
Food	30 d		1.37±0.23	0.91±0.08	0.81±0.00
Conversion	60 d		2.10±0.04	1.01±0.09*	0.82±0.00*

Blood tissue is responsible for the transportation of nutrients, metabolites, inorganic ions, etc., allowing a coordinated integration of organs and tissue functions. Blood is altered following animal's health (Blaxhall, 1972). These modifications can also occur after changes in diet, including changes in vitamin levels. Hematocrit and hemoglobin did not change in this study. The red blood cell count (RBC) decreased after 30 days in animals fed with 1000 IU and was in the same level after 60 days. A decrease of RBC count was also observed in animals fed on 0 and 250 IU after 60 days. The derived parameters followed this profile, i.e., no changes were observed for MCHC, while MCV and MCH change significantly among the treatments (Table 1).

According to Lovell (1989), vitamin D_3 is a calcium and phosphorus absorption hormone regulator forerunner, regulating the most important vitamin D metabolite: $1,25(OH)_2$-D_3. In the current study, phosphorus did not present a significant difference among the treatments along the first 30 days. After 60 days, phosphorus levels increased in animals fed on the vitamin D-supplemented diets (250 and 1000 IU) (Figure 1). However, calcium levels showed significant variations during the entire experimental period.

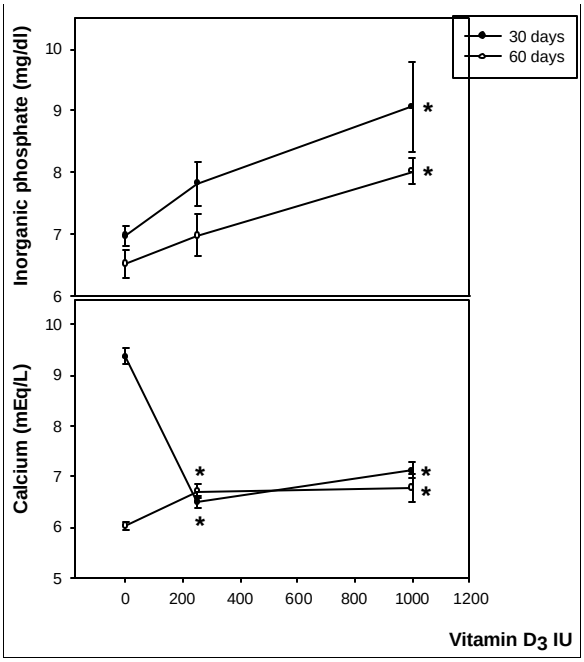

Figure 1**.** Inorganic phosphorus plasmatic levels and total calcium of *Colossoma macropomum* fed on vitamin D-supplemented diets. (*) shows significant differences (P<0.05) from control.

Conclusion

Vitamin D is needed in the diet of tambaqui and we suggest a supplementation of 1000 IU/kg of food. Vitamin D supplementation in the diet of tambaqui does not affect the health of the animals but grant an improvement of growth.

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HOW BIG AND DIFFERENT IS THE GH -INTRON 3 OF AMAZONIAN

FISH SPECIES ?

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EXTENDED ABSTRACT ONLY – DO NOT CITE

The growth hormone - GH is a 22 kDa polypeptide containing 180 to 210 amino acids. In most fish species GH is structured with five exons and four introns. However a fifth intron in cichlids and salmonids have been described. The pattern for GH evolution in fishes seems to be a slow rate with occasional bursts of rapid evolution. Phylogenetic interrelationships of fishes have been examined with GH sequences (amino acids and nucleotides) and the results in general is in agreement with traditional morphological hypotheses. In addition to the GH coding sequences, the intron uncoding regions have also been employed to advance phylogenetic interrelationships of Salmonids or even to detect intraspecific polymorphisms. As part of an effort to develop molecular markers for Amazonian fishes, the aim of the present study was to design primers complementary to the flanking regions of GH-intron 3 for PCR amplification and DNA sequencing in order to assess whether or not this region is a candidate for intra- or interspecific analysis.

Nine characiform fish species belonging to the families Characidae (Serrasalminae), Gasteropelecidae, Lebiasinidade, and one species from *incertae sedis* were sampled (Tab. I). All species are commercially important for fisheries either as food, sport fishing or aquarium hobby. We extracted DNA from tissue samples preserved in 95% ethanol using standard phenol-chloroform methods. We amplified our sequences using new primers designed from on our alignments and analyses of published and unpublished sequence data for GH in

several Cypriniformes and Siluriformes (GenBank). PCR reaction products were purified and then sequenced using the amplification primers and an automated DNA sequencer (MegaBace 1000). Fragments were assembled using Sequence Navigator and sequences were aligned using ClustalW. We reviewed and fine-tuned the alignments visually using Bioedit.

Three new primers designed on the basis of the nucleotide sequences of GH exons 3 and 4 available on the GenBank were developed. The first set amplified the end of exon 3, intron 3, and begining of exon 4 (GHEX3F:5'-CCGCTGTCTTCTTTCTGCAAYTC-3' and GHEX4R (5'-GGG-AAC-TCC-CAG-GAC-TCR-AT-3') and the second set amplified the end of exon 3, intron 3, and the end of exon 4 (GHEX3F:5'-CCGCTGTCTTCTTTCTGCAAYTC-3' and GHE4R2: 5'-GATGCCCATTTTCAGGTCAG-3', as schematically shown in the Figure 1.

We obtained PCR amplification (only a single band) and DNA sequences for the nine fish species. When available, the coding sequences were successfully aligned but the alignment of intron 3 was possible only in related fish species since high sequence divergence across families was detected.

In the Serrasalminae, the size of amplicons varied from 300-600 bp. The two sets of primers amplified well only for *Colossoma macromopum*. Four out of five Serrasalminae species (Pacu - *Mylesinus paraschomburgkii* and *Mylesinus paucisquamatus*, Piranha - *Serrasalmus rhombeus* and *Serrasalmus* sp.) presented similar intron sequences and few diagnostic polymorphic sites. The fifth species (tambaqui - *Colossoma macropomum*) presented the most derived sequence among serrasalmins and in a population genetic analysis we uncovered 5 genotypes that seems not to be geographically related. In this group, the size of intron 3 varied from 303 to 405 bp. Moreover, we observed that distinct from Cypriniformes, but similar to Siluriformes, the *Colossoma macropomum* (Characiformes) lacks 10 amino acids in the middle of exon 4.

In regard to the ornamental fishes, only in the cardinal tetra *Paracheirodon axelrodi* the two set of primers amplified DNA. However, the amplicons were not homologues after DNA sequence analysis. For the first set of primers the amplicon seems to be homologue to the GH-intron of other characiforms. For the second set, apparently a second GH copy or a GH-like sequence was amplified. These sequences are totally distinct one of each other. To the other ornamental fishes, sequence analysis among the hatchet fishes (*Carnegiella*

marthae and *Carnegiella strigata*) shows discrete nucleotide divergence as well as for the pencilfish *Nannostomus eques*.

Because introns are considered useful for tracing polymorphism in nuclear DNA, since its trend to retain more mutations than protein coding genes, usually is recommended using them as a counterpart of mitochondrial DNA. However, despite that the intron can be considered a neutral marker in general, we advised that small intraspecific divergence was detected in the GH – intron 3 of the nine Characiformes species analysed in this study.

Acknowledgements

This work was supported by Pronex-CNPq (Grant:), PNOPG-CNPq (Grant:) and INPA (Grant: PPI 2-33750). CNPq and Fapeam provided fellowships to the junior author. We also thank Asher Benzaquen (Turkys Aquarium) and Chihiro Yamamoto (K-2 Aquarium) for occasionally provided ornamental fishes.

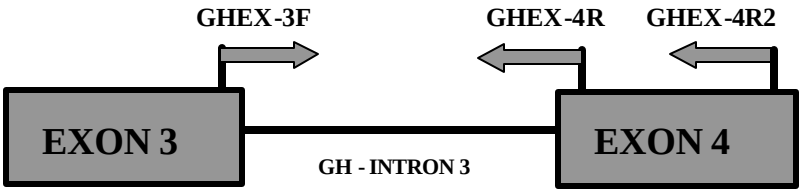

Figure 1. Schematic representation of GH – Intron 3 and positioning of primers.

Table 1. GH sizes of exons 3, intron 3 and exon 4 of Cypriniformes, Siluriformes and Characiformes.

Species / GenBank access	EX 3	I 3	EX 4
Cyprinus carpio / X51969	117	565	162
Hypophthalmichthys molitrix / M94348	117	423	162
Ctenopharyngodon idella / X60419	117	447	162
Ctenopharyngodon idella / X60988	117	446	162
Misgurnus mizolepis / AF133815	117	161	162
Alburnus alburnus / Y09421	-	280	-
Ictalurus punctatus / AF267989	117	717	132
Ictalurus punctatus / S69215	117	717	132
Clarias gariepinus / AF416488	117	261	132
Heteropneustes fossilis / AF416489	117	591	132
Colossoma macropomum	-	405	-
Mylesinus paraschomburgkii	-	303	-
Mylesinus paucisquamatus	-	303	-
Serrasalmus rhombeus	-	303	-
Serrasalmus sp. (group rhombeus)	-	303	-
Carnegiella marthae	-	197	-
Carnegiella strigata	-	197	-
Paracheirodon axelrodi I	-	117	-
Paracheirodon axelrodi II	-	380	-
Nannostomus eques	-	200	-

MYOSIN HEAVY CHAIN (MHC) EXPRESSION AND MYOFIBRILLAR-ATPASE (M-ATPASE) ACTIVITY IN THE MYOTOMAL MUSCLE IN *BRYCON CEPHALUS*

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

In most adult fish, the myotomal musculature is usually composed by a superficial thin layer of red fibers and a main mass of white ones. This pattern differs from that observed in skeletal muscle of other vertebrates, whose fibers type are intermingled. The superficial red fibers are associated to slow cruise swimming whereas the bulk of white fibers are used in fast, burst locomotion. An intermediate region that varies in the fiber type composition and in the physiology is between these two main layers (Sanger and Stoiber, 2001).

Myosin is the most abundant protein in the muscle tissue and it is a molecular motor which produces the force for muscular contraction (Goldspink et al., 2001). Distinct isoforms of the myosin have been described in slow, red and white, fast muscle fibers, with great variability among the species and developmental stages (Mascarello et al., 1995).

In general, red fibers express slow isoform of Myosin heavy chain (MHC) I and in the white fibers can express fast isoform MHCIIa or MHCIId or MHCIIb. However, hybrid fibers can express two or more MHC isoforms (Staron, 1991).

We analyzed the pattern of the mATPase activity and the MHC isoform expression in red, intermediate and white fibers, in *Brycon cephalus* adults.

Material and Methods

Specimens of *B. cephalus* adults were sacrificed by MS-222 (3-Aminobenzoic acid ethyl ester - Sigma) anesthesia. Small fragments of red intermediate and white muscle were frozen in n-Hexane, previously cooled in liquid nitrogen (-196°C). Cryostat transverse sections (7 to 10 µm) were submitted to mATPase after acid (pH 4.6) for myosin ATPase characteristic analysis.

MHC isoform analysis was performed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). Six to ten serial cross sections (12-µm thick) were placed in 250 µl of a solution containing 10% (wt/vol) glycerol, 5% (vol/vol) 2-mercaptoethanol, 2.3% (wt/vol) SDS, and 0.9% (wt/vol) Tris HCl for 10 min at 60°C. Small amounts of the extracts (8 µl) were loaded on a 7-10 % SDS-PAGE separating gel with a 4% stacking gel, run overnight (19-21 h) at 120 V, and stained with Coomassie Blue. MHC isoforms were identified according to their molecular mass.

Results

The histochemical technique for identifying fibers with different myosin ATPase activities, following pre-incubation in acid pH, showed a variable reaction intensity among red, intermediate and white muscle layers. In the red layer, fibers were strongly acid-stable. In white, most fibers were acid-labile, but some acid-stable small fibers were observed distributed among the acid-labile ones. In the intermediate muscle layer, there were three different fiber types distributed in two layers: near to the red layer, the fibers were acid-labile and near to the white layer, there were fibers with weak acid-stable or acid-labile characteristics.

SDS-PAGE revealed similar myosin heavy chain isoform expression between white and intermediate muscle, represented by a thick single band comparable with the migration pattern of mammalian MHC I. In the red muscle, there were two slight bands comparable with the migration pattern of mammalian MHC I and MHC IIb.

Conclusion

There was not a correlation between m-ATPase activity in muscle fibers from the red, white and intermediate muscle layers and the MHC expression pattern.

A co-migration between MHCs may explain the single band in the white and intermediate layers that contains two and three fiber types, respectively. The presence of one fiber type in the red layer shows that these fibers express two MHCs. Our results revealed the existence of chemical composition differences between the MHCs in red, white and intermediate muscle layers.

The expression of the different isoforms is related to the variations in the contractile properties of the muscle fibers during locomotory activities.

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Acknowledgements

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USING MEIOTIC ANALYS IS IN ORDER TO INVESTIGATE THE

EVOLVING MECHANISMS IN THE CHROMOSOMAL VARIABILITY

OF *Symphysodon aequifasciatus* (CICHLIDAE, PERCIFO RMES)

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Among the 196 ornamental fish species approved by IBAMA to be harvested, the discus is one of the most famous being very popular in the international market. The genus *Symphysodon* is endemic to the amazon basin and comprises two species, *Symphysodon discus* Heckel, 1840 and *Symphysodon aequifasciatus* Pellegrin, 1904. However, some authors have proposed five subspecies that differ on the stripe and colouring patterns, and geographic distribution.

Cytogenetically, there are evidences that *Symphysodon* species present the most derived karyotype among cichlids given the pronounced differences observed in the diploid number (2n=60 chromosomes – Figure 1) and nucleolar organizer region (NORs) pattern, when compared to the 135 cichlid species karyotyped so far. (Felberg, 2003; Mesquita, 2002).

In order to understand how chromosomal evolution takes place in the process of speciation of *Symphysodon* genus we analyzed the meiotic behaviour investigating the diplotene cells of 14 male *Symphysodon aequifasciatus*. The meiotic preparations were obtained using the technique reported by Kligerman & Bloom (1977) and adapted to fish by Bertollo *et al*. (1978). Overall, the gonads were removed, fragmented, placed in a 0.075M KCI hypotonic solution for 30 minutes and fixed in Carnoy's fixative (methanol 3:1 acetic acid). The gonads were macerated in 50% acid acetic, in a glass wells plate, the cells were dropped and reaspired immediately on a glass slide over a heating metal plate at a temperature of 40º C. The slides were stained with 5% Giemsa solution for 10 minutes. Light microscope photomicrography was carried out using an Olympus photomicroscopy.

The analysis of diplotene cells of *S. aequifasciatus* showed the presence of a chain like structure with a ring or linear configuration, and a variable number of bivalent and univalent chromosomes (Figure 2a and b). The presence of this chain probably reflects a series of reciprocal translocations occurred among their chromosomes. When this chain assumes a linear configuration could be an indicative that some chromosomes possess early synapsis and/or chiasma termination, during the diplotene. The presence of univalent chromosomes might be related to the early termination of the chiasma among the chromosomes of the chain or among the bivalent chromosomes. In addition to that, it was found that the bivalent chromosomes present one or two terminal chiasmata, however the exact number of uni and bivalent chromosomes still cannot be determined due to the great degree of condensation of the chromosomes and their reduced size. The application of G-banding technique and the obtaining of microspread prophasic chromosomes will help on the identification and determination of the number of chromosomes involved in the chain.

However, these preliminary data lead us to advance two hypotheses: 1) that karyotype 2n=60 originated from an ancestral with a diploid number equal to 48 acrocentric chromosomes, by polyploidisation, followed by the chromosomal number decrease through fusions and deletions; 2) that one series of events, such

as pericentric inversions, followed by centric fissions and new pericentric inversions, might have originated 2n=60 metacentric and submetacentric chromosomes type from the ancestral karyotype 2n=48 acrocentric chromosomes.

Therefore, further studies are necessary regarding this meiotic behaviour for infer whether or not these proposed events were the predominant evolving pathways in the speciation of genus *Symphysodon*.

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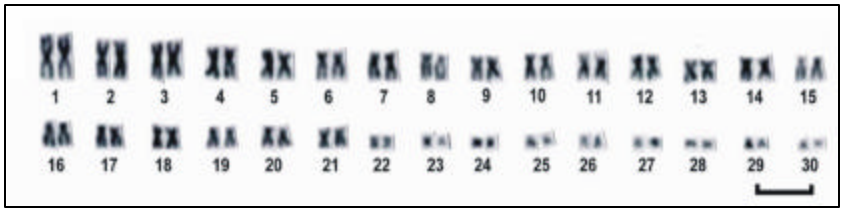

Figure 1- Karyotype of *Symphysodon aequifasciatus* with 2=60 chromosomes, submitted to standard staining (Débora Rabello Mesquita, 2002).

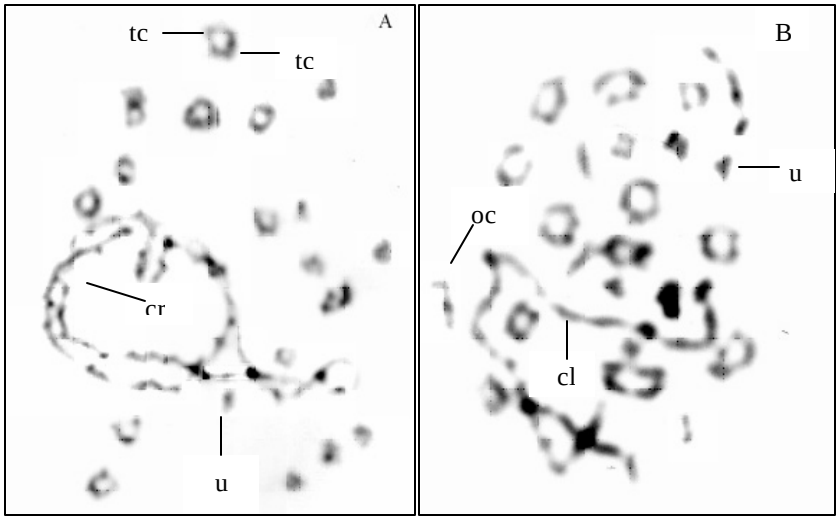

Figure 2. Male diplotene nuclei of *Symphysodon aequifasciatus* submitted to standart staning. A) Cell showing a chromosomal chain (cr) and bivalent

chromosomes with two terminal chiasmata (tc), both in ring configuration and univalent (u). B) Diplotene nuclei showing the chromosomal chain with linear configuration (cl), bivalent chromosomes with one chiasma (oc) and univalent (u).

EFFECTS OF ULTRAVIOLET RADIATION EXPOSURE ON THE SWIMMING PERFORMANCE AND HEMATOLOGICAL PARAMETERS OF TAMBAQUI, *Colossoma macropomum*

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Introduction

Tambaqui is the most reared Amazon fish. It is usually reared in ponds (0.5 – 1.5m) using oligotrophic waters with high transparency (0.4 – 1.2m). Thus, ultraviolet radiation penetrates water causing many detrimental effects. The handling for transportation, for biometry and for partial fishing cause high swim exercise. This study analyses the effect of UVR (UVA+UVB) exposure and compare the effects of UVA, UVB and UVR on the *Ucrit* and hematological (haematocrit, RBC counts, corpuscular constants, haemoglobin, ENA, differential leukocytes counts) and biochemical parameters (glucose, protein, lactate, LDH activity) of tambaqui.

Material and Methods

I) Fish and irradiation

Colossoma macropomum specimens were obtained from local aquaculture suppliers. The fish were kept in 500-l outdoor flow-through tanks and fed daily *ad libitum* with commercial extruded pellets. During the experiment the fish were kept on a 12:12h light/dark regime. The animals were exposed to UVA and UVB from above by Philips TL40W/05 and TL40W/12 lamps, respectively.

Table 1. UV irradiation used on tambaqui

Test 1: Tambaqui (weight 70.6±4.98) exposed to UVR (UVA+UVB)			
Treatments	Irradiations days	UVA ($W/cm^2.day$)	UVB ($W/cm^2.day$)
Control	13	0	0
2h/day	13	0.504	1.08
4h/day	13	1.008	2.16
8h/day	6	2.016	4.32
Test 2: Tambaqui (weight 45.6±8.29) exposed to UVA, UVB and UVR			
Control	4	0	0
UVA	4	1.08	-
UVB	4	-	2.088
UVR	4	0.504	1.08

II) Hematological assays

Haematocrit, RBC, corpuscular constants, and haemoglobin levels were determined according to Houston (1990). Erythrocytic nuclear abnormalities (ENA) and leukocytes were determined after staining with Wrigt-Giemsa. A total of at least 400 leukocytes were used to estimate the relative proportion of neutrophils, basophils, eosinophils, lymphocytes and monocytes. At least 3,000 erythrocytes were evaluated for ENA. An optical microscope, 100x-magnification oil immersion objective, was used. The percentages of the various leukocyte types and ENA were calculated.

III) Biochemical parameters
The glucose concentration was determined using the Glucox 500 kit (DOLES S.A), the protein concentration using the B36 kit (DOLES S.A.), the lactate concentration using the 826 UV kit (Sigma Chemical Co.) and the LDH activity was determined according to Driedzic and Almeida-Val (1996).

IV) Statistic
The statistical significance of the difference between data set was determined by one-way ANOVA with $p \leq 0.05$, followed by a *post hoc* Tukey test. Data is reported as mean ± SEM.

Results and Discussion

Test 1
Exposure of tambaqui to UVR affected the hemoglobin, MEHC, lactate, protein, LDH activity, ENA (%) and the proportions of leukocytes (Table 1).

Table 1. Haematological parameters of tambaqui exposed to UVR in different times.

Parameters	Control	2h/day	4h/day	8h/day
Haematocrit (%)	29.6±0.89	29.6±0.86	28.4±0.98	30.1±1.31
RBC (cel.10^6/mm^3)	1.47±0.13	1.83±0.22	1.71±0.15	1.90±0.09
[Hb] (g/dl)	6.78±0.18 ^{a}	7.96±0.28 ^{b}	7.69±0.19 ^{ab}	7.71±0.43 ^{ab}
MEV (μm^3)	217.5±24.9	215.3±60.9	173.5±11.8	160.2±8.08
MEH (pg/cell)	50.2±6.62	56.8±15.44	46.8±2.70	41.1±2.75
MEHC (%)	22.9±0.34 ^{a}	26.8±0.46 ^{b}	27.3±1.08 ^{b}	25.7±1.11 ^{ab}
Glucose (mg/dL)	59.3±5.84	74.2±3.21	68.6±6.47	73.4±11.53
Lactate (mmoles/L)	6.5±1.42 ^{a}	6.1±1.48 ^{a}	6.4±0.78 ^{a}	3.84±0.33^{b}
Protein (g/dl)	1.99±0.12 ^{a}	2.10±0.10 ^{ab}	2.26±0.13 ^{ab}	2.55±0.11^{b}
LDH activity (UI/ml)	0.03±0.01^{a}	0.06±0.01 ^{ab}	0.14±0.04 ^{b}	0.13±0.04 ^{ab}
ENA (%)	0.42±0.10 ^{a}	1.36±0.09 ^{b}	1.14±0.11 ^{ab}	0.53±0.10 ^{a}
Neutrophils (%)	14.0±1.93 ^{a}	34.1±2.90 ^{b}	26.5±2.87 ^{b}	33.0±2.18 ^{b}
Eosinophils (%)	0.00±0.00	0.31±0.15	0.38±0.16	0.44±0.26
Basophils (%)	11.5±1.62 ^{a}	32.2±3.83 ^{b}	32.9±5.13 ^{b}	50.5±0.85^{c}
Limphocytes (%)	73.7±2.98 ^{a}	30.8±4.19^{b}	36.6±5.40 ^{b}	13.7±0.85^{c}
Monocytes (%)	0.75±0.21 ^{a}	2.48±0.60^{ab}	3.50±0.63 ^{b}	2.25±0.49 ^{ab}

Different superscript letters indicate difference (p=0.05) between treatments.

Tambaqui exposed for 2h/day during 13 days increased haemoglobin, MEHC, ENA (%), neutrophils and basophils, and decreased the proportion of lymphocytes by 40%. When exposed for 4h/day during 13 days, MEHC, LDH activity, ENA (%), neutrophils, basophils and monocytes increased, and lymphocytes decreased by 50%. Tambaqui exposed for 8h/day during 6 days increased lactate, protein, neutrophils and basophils (aprox. 440%), and lymphocytes decreased by 20%.

The results indicate that UVR affects hematological and biochemical parameters of tambaqui. UVR exposure results in increased LDH activity and lactate, suggesting an increase of the anaerobic activity. UVR induced lymphocytopenia and granulocytosis. Lymphocytes are very sensitive to the damaging effects of UV radiation. Fish lymphocytes are functionally equivalent in many aspects to mammalian B and T cells (Salo *et al.,* 2000). Leukocytes have close relationships with primary responses of the immune system. A direct relationship was found between the increase of UVR dose (h/day * number day) and alterations on the hematological and biochemical parameters.

Test 2

Table 2 summarizes post-exercise hematological and biochemical parameters of tambaqui exposed to UVA, UVB and UVR.

Table 2. Ucrit and hametaological parameters post-exercise of tambaqui

Parameters	**Control**	**UVA**	**UVR**	**UVB**
Ucrit (BL/s)	6.86±0.68^{ab}	6.61±0.27^{a}	5.31±0.54^{b}	1.01±0.08^{c}
Hematocrit (%)	31.0±0.76^{a}	35.7±0.71^{b}	31.3± 0.47^{a}	37.1±0.90^{b}
RBC(cel.10^6/mm^3)	2.8±0.08^{a}	2.8±0.18^{a}	1.9±0.6^{b}	3.1±0.06^{a}
[Hb] (g/dl)	5.9±0.27^{a}	6.9±0.26^{b}	5.8±0.17^{a}	7.38±0.19^{b}
MEV (μm^3)	108.6±4.5^{a}	126.3±8.5^{a}	162.5±5.7^{b}	117.6±1.9^{a}
MEH (pg/cel)	20.7±1.03^{a}	24.4±1.47^{a}	30.1±1.42^{b}	23.4±0.61^{a}
MEHC (%)	19.11± 0.48	19.60±0.91	18.4± 0.34	20.0± 0.43
Glucose (mg/d)	188.8±17.6^{ab}	148.8±14.5^{a}	183.0±13.4^{ab}	227.5±26.3^{b}
Protein (g/dl)	2.77±0.10^{a}	2.78±0.09^{a}	3.27±0.16^{ab}	3.59±0.18^{b}
Lactate (mmol/l)	7.32±0.66^{a}	1.92±0.27^{c}	4.91± 0.62^{b}	2.40± 0.37^{c}

Different and superscript letters indicate difference (p=0.05) between treatments.

Ucrit decreased six times for UVB exposed animals. Exposure of tambaqui to UVA increased post-exercise haematocrit and haemoglobin and decreased lactate. When exposed to UVR, tambaqui increased post-exercise RBC, MEV and MEH and decreased lactate. Tambaqui exposed to UVB radiation increased post-exercise haematocrit, haemoglobin and protein and decreased lactate.

Results clearly suggest that UVB strongly affect swimming performance of tambaqui. Winkler and Fidhiany (1996) found a decrease of the metabolic rate in *Cichlasoma nigrofasciatum.* In the present study we observed alterations in haematocrit, corpuscular constants and haemoglobin, an indication that blood gas transport capacity may be altered by UVA, UVR and UVB. Alterations of glucose, protein and lactate indicate that the aerobic activity was affected. This study found UVB as more dangerous compared to UVA and UVR. Similarly to Jokinen *et al*. (2000), these findings suggest that hematological and biochemical parameters are more affected by UVB, while UVA exposure had minor effects.

Under natural conditions fish may try to avoid exposure to solar radiation by escaping to deeper waters or to shade (behavioral adjustments). In clear and shallow waters and in fish farming, however, fish are subject to exposure to solar radiation forcing morphological and physiological adjustments. Ultraviolet radiation has strong effects on the hematological and biochemical parameters and on swimming performance of tambaqui. Reduction in immune functions can result in increased susceptibility to disease. These results indicate that ultraviolet radiation is a potential environmental stressor.

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EFFECTS OF ULTRAVIOLET ON THE INCIDENCE OF ECTOPARASITES IN PIRARUCU, *Arapaima gigas* (CUVIER, 1829), (OSTEICHTHYES: OSTEOGLOSSIFORMES)

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Studies on the harmful effects brought about by the higher ultraviolet (UV) radiation incidence on living organisms have been increasing in the past few years. UV radiation makes up a small portion of the total radiation received from the sun. It is subdivided into ultraviolet A (UVA) 320-400nm, ultraviolet B (UVB) 280-320nm and ultraviolet C (UVC) 100-280nm, being UVB radiation the most dangerous due to its adding effect (Seeling, 2003). UV radiation effects on aquatic ecosystems are in directly related to the suspended particle number and depth. In some water bodies the UV intensity diminishes with depth. However, organisms that use the water surface, such as phytoplankton and obligatory air breathing fish, suffer direct influence from this radiation (Kirchhoff, 2003). Pirarucu, *Arapaima gigas* (Cuvier, 1829), is one of the most valuable fish found in the Amazon region. It can reach a total length of up to 3m, is widely distributed throughout the Amazon and is an obligatory air breathing fish, which needs coming up to the surface at regular intervals of few minutes to breathe oxygen from the atmosphere (Queiroz & Crampton, 1999). Thus, pirarucu ectoparasitas must suffer influence from UV radiation. Pirarucu ectoparasitas are: Monogenoidea, *Dawestrema cycloancistrium* Price & Nowlin, 1967, *D. cycloancistrioides* Kritsky, Boeger & Thatcher, 1985, and *D. punctatum* Kritsky, Boeger & Thatcher, 1985; Copepoda, *Ergasilus* sp.; Branchiura, *Argulus* sp. and *Dolops discoidalis* (Bouvier, 1899). Few studies have been performed to evaluate the UV radiation effect on ectoparasites organisms. The purpose of the present study is to evaluate the UVR (UVA +

UVB) radiation effects on the pirarucu ectoparasite fauna. Fish were acquired from a fish-culture station and acclimated for eight days at the Molecular Evolution and Ecofisiology Laboratory (LEEM) in the National Research Institute of Amazon (INPA). Then, they were transferred to a room that had been adapted for the experiments. Fish on treatment 1 were exposed to UVR radiation for 1h/d and on treatment 2 to 2h/d. Control treatment fish were exposed for 4h/d to fluorescent light of the same voltage as that of ultraviolet. At the end of the experiments, the fish were slaughtered and underwent necropsy at the Fish Parasitology and Pathology Laboratory (LPP) in INPA. Parasite indexes were determined according to Bush *et al.* (1997) and analysed according to the different UV radiation exposure times. Only *D. cycloancistrium* species was found. Mean intensity and standard deviation in the control treatment were 583 monogenoids and 278.56 respectively. In treatment 1 they were 564 and 227.56. And in treatment 2 they were 265 and 86.76. There is little difference between control and treatment 1. However, there is a much bigger difference between control and treatment 2, which points out that the longer the exposure time the lower the parasite number. Studies have yet to be carried out on the effects of UV radiation on fish monogenoids. Nevertheless, studies conducted on shrimp, crab, and other invertebrates larvae found that the longer the UV radiation exposure time the higher the mortality rate of those organisms (Seeling, 2003), thus corroborating the findings in the present study.

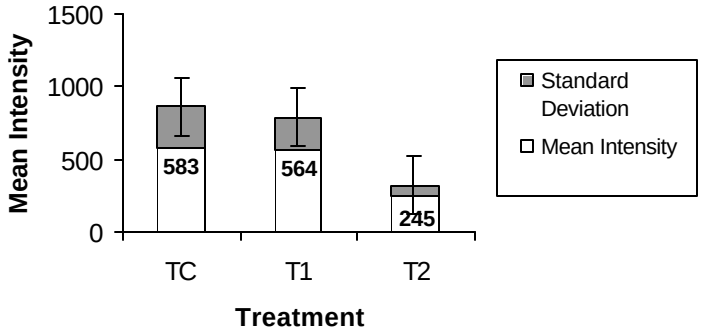

Figure 1 – Mean intensity variation of pirarucu parasite *Dawestrema cycloancistrium*, when exposed to UVR (UVA+UVB) (n = 3).
TC=control treatment; T1=1h/day exposure; T2=2h/day exposure.

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DAILY VARIATION OF THE DIGESTIVE ENZYMES :

AMYLASE, MALTASE, LIPASE, AND TOTAL PROTEASE

IN JUVENILES OF TAMB AQUI, *Colossoma macropomum*

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

Tambaqui is one of the most important food fish of the Amazon and has demonstrated an excellent potential for rearing (Val and Honczaryk, 1995). Fish culture is a recent activity in the Amazon region and the lack of proper technology and scientific information impair its development. In aquaculture, nothing is more important than sound nutrition and adequate feeding. Food not digested generates several problems with consequent economic losses. To achieve an efficient alimentary regime, continuous adjustments in ration, schedules and frequency of the feeding are needed to compensate for changes of requirements, mainly in the juvenile phase. The alimentary chronology, in this sense, is useful to guide the schedules of the meals reducing the losses for not consumed food and lowering the feeding costs. Studies on the response of the digestive enzyme activities to find the physiological basis of the relationship between schedules and the effective use of the food could help establish more economic feeding systems. To provide information for protocols of feeding time that result in better weight gain and feeding conversion rate, we have analyzed daily variations of activities of amylase, maltase, lipase and general proteases in *C. macropomum.*

Materials and Methods

I) Collection of samples

Juveniles of *C. macropomum* (35.5±16.7 g and 9.8±3.2 cm) were obtained from fish culture stations. Fishes were transferred to the National Institute for Research in the Amazon (INPA) and were kept in 3000 l circular tanks with continuous aeration. They were fed to satiety with commercial pellets for two weeks prior to the experiments and exposed to natural photoperiod and temperature. After the acclimation period, the fishes were submitted to four experimental protocols: 1) starved; 2) fed at 7:00 AM; 3) fed at 5:00 PM; and 4) fed randomly. The pellet diet was offered once a day *ad libitum* for ten days, according to the experimental protocol. At the end of the experiments, one hour after the last feeding, six fishes were taken every two hours during 24 hours, killed by cervical ablation and their digestive tracts were immediately excised and kept frozen at –70 °C for posterior analyses of the digestive enzymes.

II) Preparation of enzyme extracts

The preparations of enzyme extracts were carried out at 4ºC. The digestive tracts of each fish were homogenized in 0.02M -phosphate buffer (1:0.5 w/v) at pH 7.0. Homogenates were centrifuged for 15 minutes at 15,000 rpm. The supernatants were taken for enzymatic assay. The protein concentration was estimated according to biuret assay (Doles, Inc).

III) Enzyme assay

Amylase activity was measured according to Caraway (1959), using Doles amylase-test kit. Maltase activity was assayed by glucose-oxidase method (Dahlquist, 1961), lipase was assayed using In Vitro lipase test kit and non-specific protease activity was measured using azocasein as substrate, according García-Carreño (1992). Specific activities, estimated in triplicates for each sample, are expressed in µmol of hydrolyzed substrate per minute and expressed per gram of protein (U/g protein).

IV) Estatistical analysis

The statistical significance of the difference between data set was determined by one-way ANOVA with $p \leq 0.05$, followed by a post hoc Tukey test. Values are reported as arithmetic means ± SEM.

Results and Discussion

Changes in specific activities of all analyzed digestive enzymes in unfed fish were significant showing cyclic and ordered patterns with peaks of maximum activity followed by valleys with minimum activity. Except for maltase, the maximum activities peaked at dawn (4:00-6:00 AM) in unfed animals. The maximum and minimum amylase activities were found at 6:00 AM and 10:00 AM respectively (402.5±34.9 and 80.1±4.3 U/g protein). A different pattern was observed for maltase, that showed maximum and minimum activities between 10:00-12:00 PM (12.9±1.2 and 4.7±0.6 U/g protein). Lipase and general protease activities showed a similar pattern. The maximum activities occurred at 6:00 AM (31.9±2.9 and 308.6±36.9 U/g protein) and the minimum ones at 12:00 PM (14.3±1.5 and 78.8±9.7 U/g protein) (Fig 1).

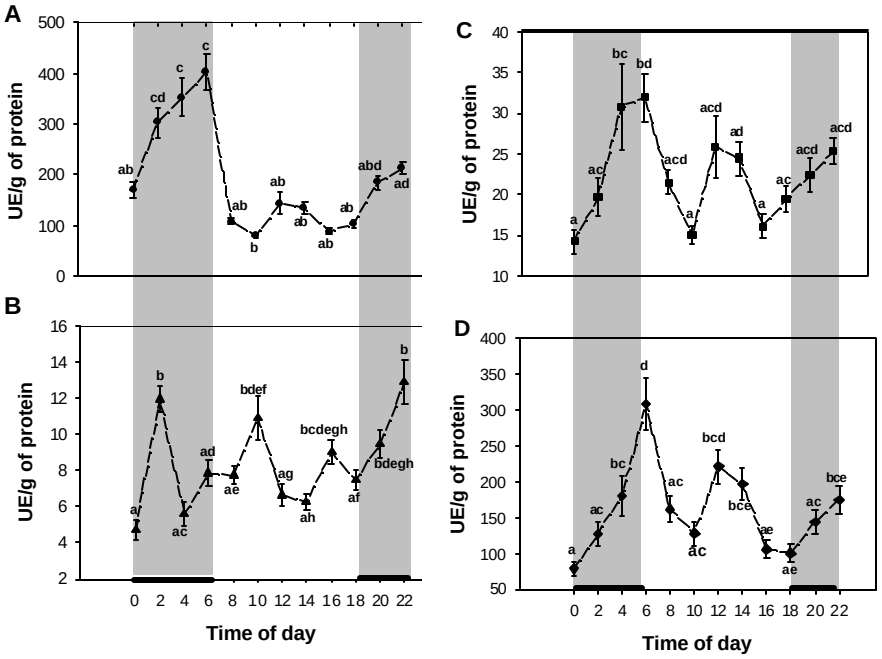

Figure 1. Daily variation of the digestive enzymes: amylase (A), maltase (B), lipase (C) and non-specific protease (D) in starved juveniles of *C. macropomum* (*n=12*). Vertical bars indicate dark periods. Different letters stand for statistical differences (P< 0.05)

Different enzyme activity patterns were found in fed fish when compared to the unfed group. They suggest that digestive enzymes are induced by food intake, which may be due to different alignments or re-alignments of endogenous rhythms. Fishes fed early in the morning (7:00 AM) reach earlier time the maximum activities for all enzymes studied (Table 1). That should be indicating that the fishes has a relatively rapid digestive enzyme production in the morning.

Table 1. Moment of the maximum enzyme activity in *C. macropomum* juveniles, fed at different times of day. All values are shown in hours past feeding.

Feeding time	7:00 AM	5:00 PM	Random
Amylase	17	19	21
Maltase	7	17	11
Lipase	17	19	21
Non-specific Protease	21	23	21

The opportunistic feeding habits of tambaqui and its ability to use a wide range of nutrients efficiently are due to its digestive enzymes. Adjusting feeding times for early in the morning should be a good choice because the enzymatic machine is ready to act. Further investigations are needed, however, to check if feeding at these times really results in better performance.

Acknowledgements

This work was supported by National Institute for Research in the Amazon (INPA) and The National Research Council of Brazil (CNPq), K.L.V. and C.A.C.P. are recipients of fellowships from CNPq/Brazil.

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DIGESTIVE ENZYMES OF SOME TELEOSTS OF THE AMAZON WITH DIFFERENT FEEDING HABITS

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EXTENDED ABSTRACT ONLY - DO NOT CITE

Introduction

The Amazon region presents a wide variety of aquatic environments that shelter an ichthyofauna richer than that of any other river system, as well as a great amount of food sources. Food preferences were established in the process of adaptive radiation and colonization of these different habitats. The ability of fishes to use such nutrient variety depends on the synthesis of appropriate digestive enzymes. Digestive enzyme profile is a clue to understand feeding ecology and digestive process. In the present study, the activities of non-specific protease, lipase, amylase and maltase of the alimentary tract of eight teleost species from four different orders have been reported. An attempt is made to correlate the enzyme activities with their feeding habits.

Material and Methods

I) Collection of samples
Fishes of eight teleost species from four different Orders and with different feeding habits (Table 1) were collected in Anavilhanas archipelago at Negro River at 2º 43'S and 60º 45'W during an expedition aboard of INPA's Research Vessel Amanaí II in December, 1999. The fishes were caught with a small-mesh

gill net and killed by a head blow and punched the spinal cord immediately after capture. The digestive tracts were individually weighed and frozen at -70°C until their use for enzyme analyses.

Table 1. Weight, length, and feeding habits of the studied species.

Species	**Weight (g)**	**Length (cm)**	***Feeding habit***
Osteoglossiformes			
Osteoglossidae			
Osteoglossum bicirrhosum (9)	852.7 ± 263.7	50.3 ± 4.2	Omnivore
Characiformes			
Prochilodontidae			
Semaprochilodus taeniurus (64)	311.2 ± 127.9	22.6 ± 2.9	Detritivore
Semaprochilodus insignis (18)	259.4 ± 65.6	20.5 ± 1.7	Detritivore
Serrasalmidae			
Metynnis hypsauchen (65)	88.2 ± 28.4	12.2 ± 1.3	Omnivore
Siluriformes			
Pimelodidae			
Phractocephalus hemioliopterus (7)	1404.2 ± 337.9	38.9 ± 2.7	Omnivore
Perciformes			
Cichlidae			
Geophagus aff. altifrons (79)	219.5 ± 47.4	19.0 ± 1.9	Omnivore
Cichla temensis (9)	379.4 ± 91.6	27.0 ± 2.8	Piscivore
Cichla monoculus (21)	379.5 ± 81.2	26.1 ± 1.7	Piscivore

II) Preparation of enzyme extracts

The preparation of enzyme extracts was carried out at 4°C. The digestive tracts of each fish were homogenized in 0.02M -phosphate buffer (1:0.5 w/v) at pH 7.0. Homogenates were centrifuged for 15 minutes at 15,000 rpm. The supernatants

were taken for enzyme assay. The protein content was estimated using a commercial protein-test Kit (Doles®).

III) Enzyme assay

Amylase activity was measured according Caraway (1959), using amylase-test kit (Doles®). Maltase activity was assayed by glucose oxidase method (Dahlquist, 1961), lipase was assayed using lipase test kit (In vitro®) and protease activity was measured using azocasein as substrate according García-Carreño (1992). Specific activities are expressed in µmol of hydrolyzed substrate per min and expressed per g of protein (U/g protein).

IV) Statistical Analysis

All data were subjected to one-way analysis of variance. Differences among the group of means were analyzed for significance by Tuckey's multiple range test. $P \leq 0.05$ was considered statistically significant. Results are shown as mean±SEM.

Results and discussion

As general rule both proteolytic and amylolytic activities are related to the feeding habit. Usually protease content of carnivorous fish is higher than omnivorous and carbohydrase activities in omnivorous is higher than piscivorous (Kuz`mina, 1996). In this study, these relationships were not found (Figure 1). The piscivorous fishes showed lower proteolytic activities and their amylolytic enzymes were not different from other studied groups and from the omnivore *O. bicirrhosum* that showed the highest proteolytic activity.

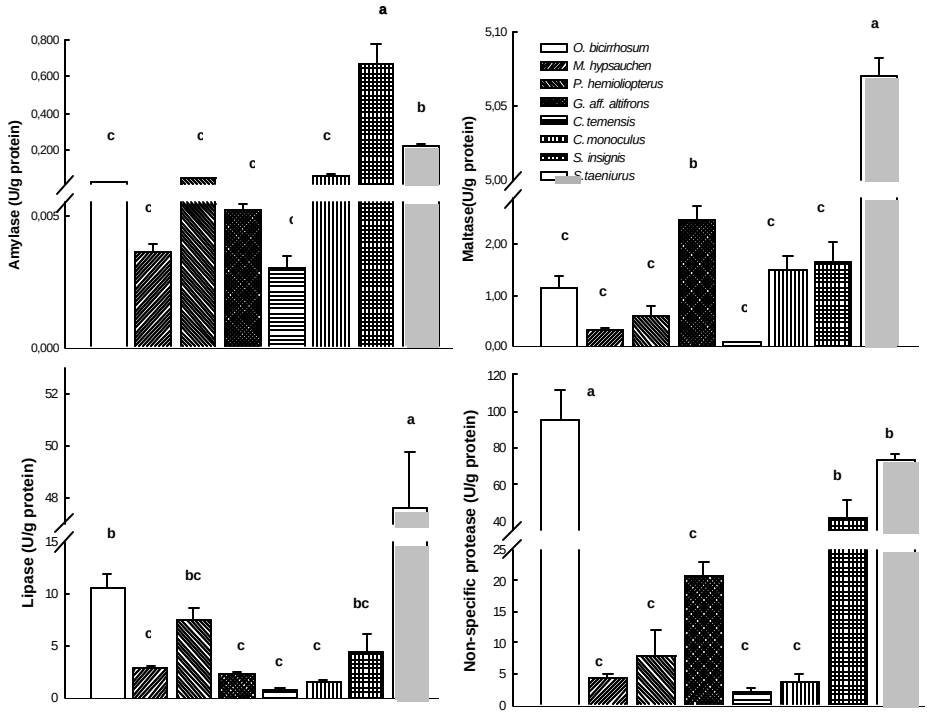

Figure 1. Specific enzymes activities of amylase, maltase, lipase and non-specific protease in some teleosts of the Amazon with different feeding habits. Different letters stand for statistical differences ($p < 0.05$).

Semaprochilodus taeniurus, a detritivorous fish, showed higher maltase and lipase activities than other species, including its congener *S. insignis* that, in its turn, showed the highest amylase activity. We suppose that high carbohydrases and lipase activity in these species are an adaptation to extract high energy levels from detritus, a poor nutrient source. This adaptation may be specie-specific. The occurrence of lipase higher than amylase activity suggest that fat is more important than carbohydrate as energy source for all groups.

Many factors other than feeding habit can influence enzymes activities in fishes. Among these factors are, for example, the degree of gut stuffing, the nutritional condition, the age, the structural complexity of substrates, the temperature and circadian and seasonal physiological rhythms. Also, as state elsewhere (Val and Almeida-Val, 1995) the fishes of the Amazon are opportunistic as regard as their feeding habit, what may strongly influence their digestive enzyme profile. More studies are needed before we can draw a clear picture of this issue.

Acknowledgements

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REPRODUCTION AND GROWTH OF FISH ASSOCIATED TO DIFFERENCES BETWEEN ESTUARINE ENVIRONMENTS

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Abstract

Features related to reproduction and growth are auxiliary indicators in evaluating the receiving capacity of the habitat. Relative condition factor (Kr), population structure and gonadal maturation stages at six species were considered in order to compare two neighboring mangrove estuaries from the south of Brazil: Guaratuba Bay and Barra do Saí Lagoon. The former mangrove-estuary, because of its larger area and depth, receives a greater fish population for reproduction and growth than the latter, where the renewal of the seawater is supposedly smaller. Both estuaries are used in a similar manner by the fish reproduction, but differ in the population structure, Kr and growth parameters. It can be concluded that the physiographic differences among estuaries might not alter their importance for reproductive activity, although they influence the individual biological parameters of coastal fish.

Key-words: fish assemblages, bay, coastal lagoon, relative condition factor.

Introduction

Lately, the trend of fishing management plans has been to give greater emphasis to the quality of the habitat, which includes both abiotic and biotic elements, as

well as to the traditional data showing the characteristics of the stock (capture and estimates of density and biomass). This ecosystematic vision (Stergiou, 2002) extends to other localities than those used directly by fish, since most of the target species are migratory. Lloret et al. (2002) and Lloret and Planes (2003) proposed several criteria based on the evaluation of the condition of the fish in their habitat because the energy needed for growth, reproduction or maintenance can be inferred through the analysis of the population structure and the condition of the fish in the environment (Pope & Kruse, 2001). It is assumed that a better "condition" of the individuals in an assemblage is associated to the better quality of the habitat (Lloret et al., 2002, Écoutin & Albaret, 2003).

The present study analyses the "condition" of the fish in a broad sense - gonadal maturity, size distribution, mass/length relationship and relative condition factor - as the distinguishing element between estuarine environments. The populations of two mangrove estuaries in the south of Brazil were analysed. Guaratuba Bay (25º52'S - 48º39'W) and Barra do Saí Lagoon (25º59'S - 48º36'W) are 15 kilometers apart and situated close to an important fishing zone of the continental shelf. The differences in river discharge, as well as in area and topographical characteristics (45 km^2, up to 6 m deep or 0.12 km^2, up to 2 m deep, respectively), indicate that the two estuaries can contribute in different ways to the growth of the juveniles that are the targets of commercial fishing as well as to the growth of the foraging species.

Materials and Methods

The individuals were collected in 144 launches of a beach trawl net, between 1999 and 2001, in the interior of Guaratuba Bay (continental and marine sectors) and in Barra do Saí Lagoon. After measuring (total length) and weighing the fish, their gonadal maturity was classified macroscopically following the Vazzoler scale (1996) – immature stages, stages in maturation, mature and spawned stages.

The reproductive analysis involved six species, common to the Bay (without any distinction between the continental and marine sectors) and to the Lagoon, the occurrence of which was superior to 100 individuals: *Anchoa parva, Atherinella brasiliensis, Citharichthys arenaceus, Eucinostomus argenteus, Sphoeroides greeleyi* and *S. testudineus.* The individuals whose maturity stage could not be determined macroscopically were disregarded (ND). The sexes were grouped after verifying the absence of significant differences in length between males and females ($t_{49;49} = 0.931$; $p = 0.354$; $t_{63:54} = 1.004$; $p = 0.317$).

The size distribution and the values of mass/length relationship and relative condition factor were studied in *Anchoa parva* and *Atherinella brasiliensis*, with specimens distributed along 5mm classes of total length. The relative condition factor (Kr) represents the relationship between the real mass and the mass calculated by the mass/length equation. The Mann-Whitney test (p=0.05) was used to compare the Kr values between the Bay and the Lagoon since the data had a normal distribution and was made up of different sized groups (Zar, 1996). Because the Kr values are relative, they were not compared between systems, but with their maximum values; values greater than one would represent individuals of higher higidity. Populations of the continental and marine sectors were considered together for the Bay.

Results

Reproduction

All the maturation stages were registered, both in the Bay and in the Lagoon, in individuals from the five studied species: *A. parva, A. brasiliensis, Citharichthys arenaceus, Sphoeroides greeleyi* and *S. testudineus.* In the two estuaries, 74% of the collected individuals were immature, because they were juveniles. In the Bay, no species was solely responsible for more than 45% (in *A. brasiliensis*) of the individuals collected in this stage; on the other hand, in the Lagoon 86% of the immature individuals belonged to *E. argenteus.*

Size classes

The size distribution of *A. parva* was of 34 to 157 mm. The most predominant size was 65-69 mm in the continental sector, 40-44 mm in the marine sector and 35-39 mm in Barra do Saí. The lengths above 80 mm appeared with a frequency of less than 2.6%, both in the Bay and in the Lagoon (Fig. 1). The size distribution of *A. brasiliensis* was of 12 to 151 mm. The class with the highest frequency had greater lengths in the continental (105-109 mm) and marine (85-89 mm) sectors of the Bay than in the Lagoon (40-44 mm) (Fig. 2).

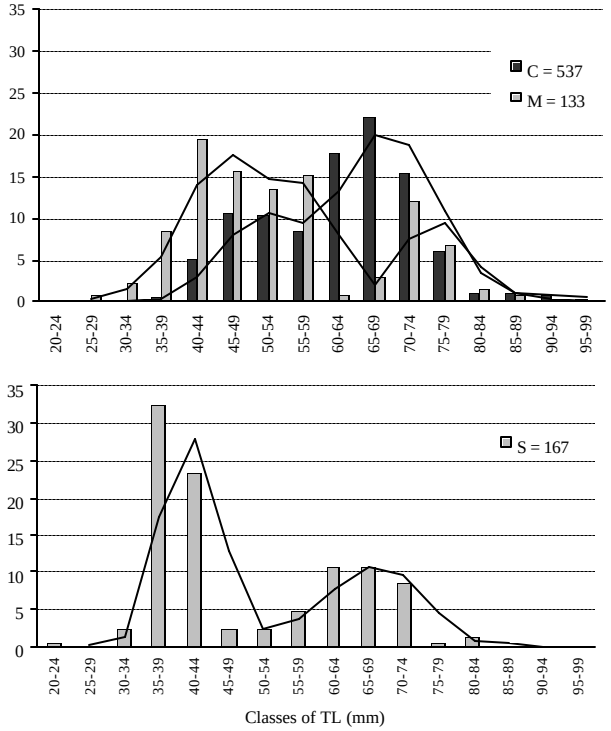

Figure 1 - Distribution of the individuals of *Anchoa parva* in classes of total length (TL) in the continental (C), marine (M) and Barra do Saí (S) sectors.

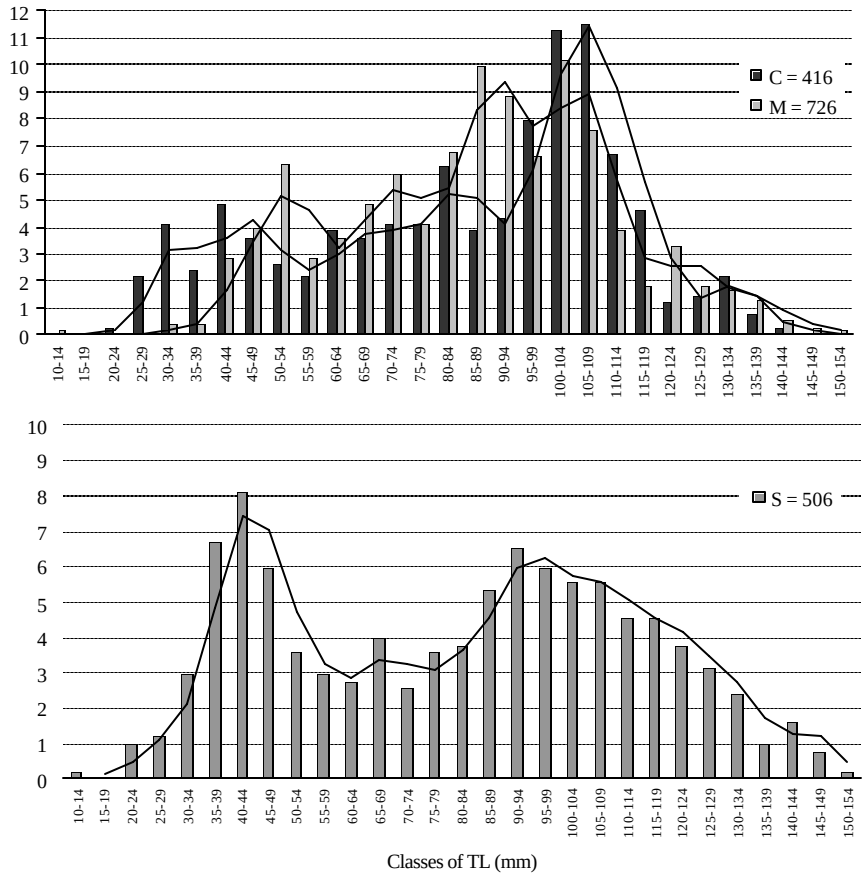

Figure 2 - Distribution of the individuals of *Atherinella brasiliensis* in classes of total length (TL) in the continental (C), marine (M) and Barra do Saí (S) sectors.

Mass/length relationship

The angular coefficient given by the mass/length relationship calculated for the population of *A. parva* was significantly different (Mann-Whitney: $T_{168;670}$ = 30438.0; $p<0.001$) between the Bay and the Lagoon. The same occurred for the

population of *A. brasiliensis* (Mann-Whitney: $T_{506;1143}$ = 351788.0; p<0.001). Positive allometry was shown for the ichthiofauna in the Bay (Tab. I).

Table I - Parameters of the mass/length relationship of *Anchoa parva* and *Atherinella brasiliensis* in Guaratuba Bay and Barra do Saí Lagoon. n: number of individuals; a: linear coefficient; b: angular coefficient; R^2: determination coefficient.

Species	Site	n	a	b	R^2	Relationship
A. parva	Barra do Saí	168	1,04 x 10^{-5}	2,942	0,98	$TM=1,04.10^{-5}.TL^{2,942}$
	Guaratuba	670	0,55 x 10^{-5}	3,056	0,96	$TM=0,55.10^{-5}.TL^{3,056}$
A. brasiliensis	Barra do Saí	506	1,13 x 10^{-5}	2,886	0,98	$TM=1,13.10^{-5}.TL^{2,886}$
	Guaratuba	1143	0,64 x 10^{-5}	3,007	0,98	$TM=0,64.10^{-5}.TL^{3,007}$

Relative condition factor

The average Kr values were smaller than 1 in the Bay, both for *A. parva* (Fig. 3a) and *A. brasiliensis* (Fig. 3b). The Mann-Whitney test showed that the average Kr differed (p<0.05) for both species between the study sites, being significantly greater in the Lagoon than in the Bay.

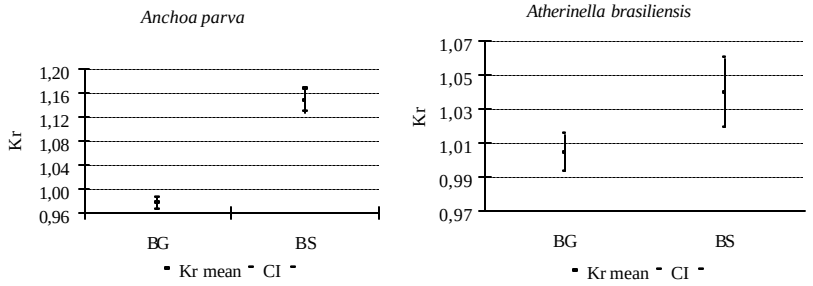

Figure 3 - Mean relative condition factor (Kr) and confidence interval (95%): *Anchoa parva* (BG: Guaratuba Bay, n: 670; BS: Barra do Saí, n: 168); *Atherinella brasiliensis* (BG: Guaratuba Bay, n: 1143; BS: Barra do Saí, n: 506).

Discussion

Due to the occurrence of all the maturation stages, it is probable that *A. parva*, *A. brasiliensis*, *Citharichthys arenaceus*, *Sphoeroides greeleyi* and *S. testudineus* are resident species in the two studied estuaries, and therefore reproduce in both. *Eucinostomus argenteus*, however, is a trophic migrant that is extremely abundant in the Lagoon, although it occurs only as a juvenile. This species is, therefore, answering for the similarities between the high percentages of juveniles in the two systems. This species, like other Gerreidae, does not reproduce in the estuary, but migrates in spring or summer to reproduce at sea (Chaves and Otto, 1999).

Considering the size distribution of *A. parva* and *A. brasiliensis*, the dominance of juveniles in the studied assemblages can be confirmed, mainly in the Lagoon. In fact, Guaratuba Bay was more efficient than the Lagoon at sheltering larger individuals.

Anchoa parva and *A. brasiliensis* are resident species in estuaries with a high reproductive potential, low longevity and ample tolerance to abiotic variations. Factors such as temperature, salinity, food availability, sex, time of year and maturity stage could be causing differences in the relationship parameters. According to Agostinho and Gomes (1997), these factors regulate populational parameters such as growth, nutritional conditions and gonadal development of the individuals.

The populations of *A. parva* and *A. brasiliensis* can be distinguished regarding the place of capture, leading to the perception of a large higidity in the Lagoon. Lloret and Planes (2003) observed that there are differences in the condition between samples of a same species captured in different habitats and that its value is generally higher in individuals that occur in shallow areas compared to those captured in deeper areas. Another example that reflects the condition of the fish was described for *Diplodus sargus*. The individuals captured in rocky habitats showed a better condition than those captured in habitats with sandy beds (Lloret and Planes, 2003). Using the relative condition factor, Morato et al. (2001) also found significant differences between three different islands in the Açores archipelago, Portugal, although only in less than 20% of the investigated

species. The authors believe that this significance may be reflecting the influence of differences in the environment or factors of the habitat, for example, differences in the thermal regime of the water, which is known to influence the growth of the fish. However, comparing the mass/length relationship of 16 species of fish from two estuaries on the west coast of Africa, Écoutin and Albaret (2003) noticed the absence of an effect of the ecosystem over this variable. It is possible, therefore, that the differences registered between Guaratuba Bay and Barra do Saí Lagoon are associated to differences in the size at which the studied species frequent each of these estuaries. As the age structure of these populations is unknown, one cannot disregard the hypothesis that these environments are exerting pressures over the individual growth of the species.

The majority of the species that occupy the two estuaries studied here, especially the six used in this investigation, do not have a direct fishing value, but integrate the food web of migratory, estuarine-dependent fish that are exploited in the adjacent continental shelf. From the results, one can conclude that the reproduction and growth variables are directly related to the "general condition" of the individuals and, therefore, express differences between estuarine areas that must be considered when evaluating priority coastal areas for the management of stock.

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REPRODUCTIVE BIOLOGY OF

SOUTHWESTERN ATLANTIC YELLOWTAIL SNAPPER

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Introduction

Yellowtail snapper *Ocyurus chrysurus* is a tropical Lutjanidae, known by form aggregations in the Western Atlantic, and it's usually seen above the bottom, mostly around coral reefs. The species ranges from Florida to southeastern Brazil, and is most common in the Bahamas, off south Florida and throughout the Caribbean (Allen, 1985). According to Coleman et al. (1999) all managed stocks of reef fish for which the status is known are either overfished or in danger of being so (with few exceptions) and clearly, changes in fisheries management are needed. The economic importance of *O.chrysurus* for eastern Brazilian coast (landings accounting for 4300 t or 43% of all catches) makes protecting the sustainability of the fishery a critical consideration. We present analysis of spawning season, length at first maturity and fecundity for yellowtail snapper that may help defining future management practices.

Materials and Methods

Samples were taken from small-craft commercial fisheries that landed at Porto Seguro between 1997-2000. Fork length (FL), whole and gutted weights (WW e GW)), weight of gonads (gW), sex and maturation condition were recorded for all collected fish (N=990). Females and males were divided into four sexual classes (immature, in maturation process, mature, spent) according to macroscopic observation of the gonads. Spawning was evaluated for both sexes by examining seasonal variations in the gonadossomatic index (GSI = 100 x gW

/ GW). Size at which 50% of females were sexually mature (L_{50}) was determined by using the Loglikelihood ratio applied to the logistic curve. Sub-samples of three different parts of gonads (anterior, medial, posterior) were taken and tested for significant differences. To estimate total fecundity (total number of vitellogenic or advanced yolked oocytes at any time in the ovary) and batch fecundity (total number of advanced yolked oocytes matured per females, uncorrected for atretic losses), the gravimetric method was used and oocytes were measured using an ocular micrometer. An ANOVA was used for comparison of median number of oocytes by gonad region. Sex by size frequency distributions and GSI by maturation condition were compared by using the Kolmogorov-Smirnov and the Kruskal-Wallis nonparametric test, respectively. Significance level was 0.05 in all instances.

Results and Discussion

Females and males ranged, respectively, from 23.0 - 55.4 cm and 22.7 - 51.2 cm FL. The larger size-classes (> 52 cm) were composed solely by females. Sex by size frequency distributions didn't differ significantly (Kolmogorov-Smirnov; N=930). Females captured were mostly immature or spent (58%) and had low individual GSI values (GSI ranged from 0.02 to 0.14%). Females in maturation process first appeared in March and were captured until December, while females were mature between June and December. Beginning in June mean GSIs for females increased (0.65%), reaching a maximum value (3.2%) in October, then declining to a minimum value in May (0.56%). In males, mean GSI began to increase in May (1.1%), reached a peak in January (3.2%), and declined to a minimum value in April (1.0%) (Figure 1). Spawning season between October and May, as suggested by the mean GSIs, was different from spawning seasons suggested for other areas. In the U.S. spawning occurs between May and August (Mueller et al., 2003), while in Cuba spawning extends from March to November (García-Cagide et al., 1994).

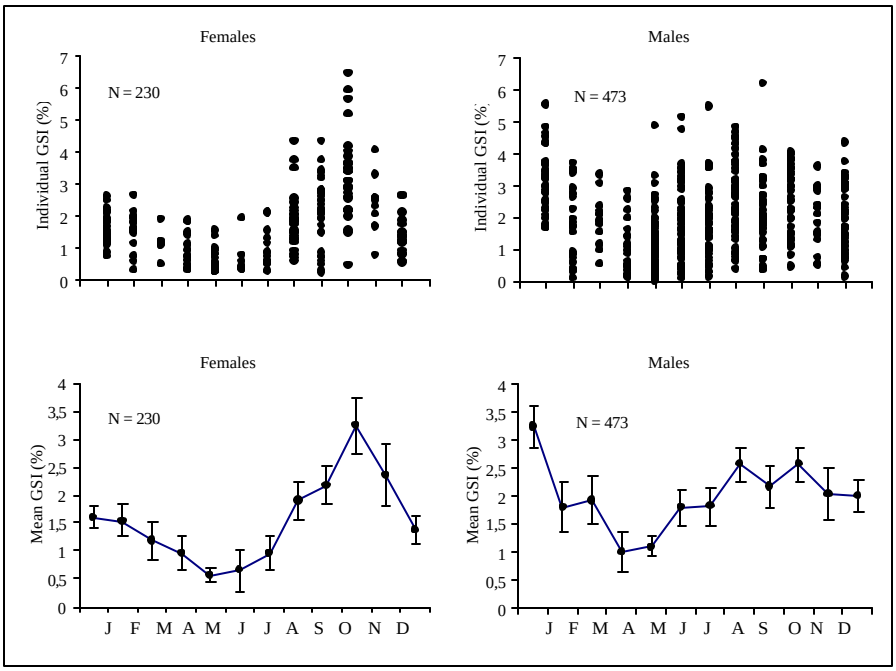
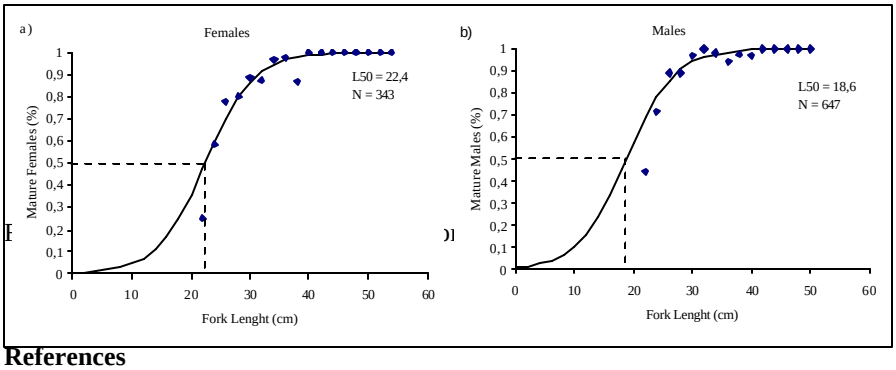


Figure 1. Individual and mean gonadosomatic index (GSI) values for females (N = 230) and males (N = 473) over a single year.

The smallest mature female had FL = 22.0 cm. Fifty percent maturity of females was attained at 22.4 cm, and all females larger than 40.0 cm were mature. Apparently, males reach maturity prior to females. The smallest mature male caught had FL = 22.0 cm, and fifty percent maturity of males was observed at 18.6 cm (Fig. 2). According to Thompson & Munro (1983), length at first maturity in Jamaica was 29.0 cm and 26.0 cm (fork length) for females and males, respectively.

Median oocytes values by gonad region didn't differ significantly (ANOVA; N = 50). Total fecundity had an estimated value between 40 - 870 thousand oocytes, while batch fecundity was estimated between 10 - 470 thousand oocytes.



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ENVIRONMENTAL FACTORS INFLUENCING THE DISTRIBUTION OF FISH GROUPS IN HEADWATER STREAMS, JAÚ NATIONAL PARK, AM

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Introduction

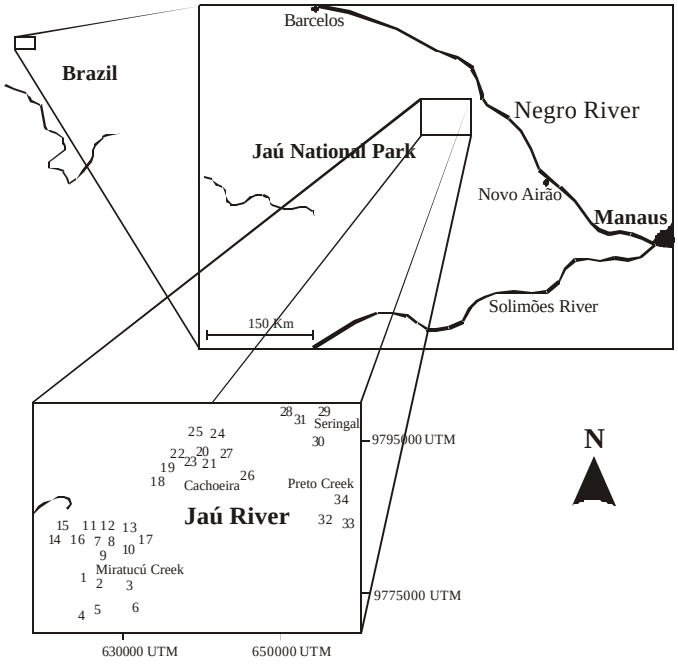
The River Continuum Concept (RCC) indicates how the longitudinal variations of physical characteristics influence the distribution of aquatic invertebrates along a fluvial system (Vannote *et al*., 1980). Although, RCC does not make predictions about the ichthyofauna, it is probable that the systematic variations in the physical-chemical and habitats characteristics along the fluvial continuum also influence the fish communities in these systems. The general objective of the present study is to investigate the influence of the substrate variation and physical characteristics of the canal in fish family groups of headwater streams of a Jaú River, a large Amazon tributary, at the beginning of flood period.

Study Area

The Jaú River is located in the Central Amazon between Moura and Novo Airão cities. This river is 300 Km long, with about 1500 streams in a 10000 Km^2 drainage area, is situated 300 Km Northwest of Manaus, completely enclosed in Jaú National Park. The climate in the studied region is tropical rain with temperatures varying between 22 and 33 ^{o}C. The annual rain varies between 1700 and 2500 mm. Two seasonal periods that extends for November to June, with March as the rainiest, with a average of 350 mm, and November as the driest, with

an average of 140 mm (FVA/IBAMA, 1998). The present study was undertaken in 34 headwater streams of the Jaú River (Figure 1). The streams studied have pH varying between 3 and 5.5, water temperature between 23 and 26 ^{o}C, width between 0.3 and 8.0 meters, and depth varying from 0.3 to 1.7 meters.

Figure 1. Localization of 34 studied streams in Jaú River, Central Amazonian.



Materials And Methods

Samples were taken in November and December of 1998. From each stream samples were taken from a stretch of water continuing two complete meanders to guarantee that most of the habitats would be represented. The physical-chemical parameters were determined at a central point in each stretch. The depth and the width of the canal measured for a distance of ten equidistant lateral transections with the

aid of a tapeline and one meter measuring rod. The substratum was classified in six categories: leaves, sand, trunks, mud, roots and rocks. The result was used to estimate the percentage of substrate coverage in each stretch, the habitat diversity was calculated using the Shannon & Weaver (1949). Two different artifacts, namely, a wicker fish trap and a hand nets were used to collected fish. The parameters physical, chemical and environmental of the streams were grouped together in orthogonal axes by the NMDS (Non-Metric Multidimensional Staggering) method, and then the most significant independent variable in the axes were related directly with the abundance and diversity of fish groups by Linear Regression.

Results

As for the identified fish groups, the family most representative in number of individuals was Characidae with 568 individuals in one stream. However, we captured only one individual of the family Electrophoridae, Nandidae and Ctenoluciidae. We collected 5771 individuals belonging to 67 species and 24 families, with a total biomass of 5146 g. 21 species of the family Caracidae, belonging 12 the (genera) *Hemmigramus* with 2745 record individuals were collected, in other words, 47.56% of all the fishes, amount all Caracidae we get 3445 individuals (59.69% of the total) (table 1).

To reduce the degree of co-variation among independent variables and the degrees of liberty in the final analysis, ordinary orthogonal axes were created by way of 14 original independent parameters. Only two axes explain the greater part of the environmental variations. Axis one was strongly associated to the physical gradient of the canal and dissolved oxygen, as postulated by Vannote *et al*. (1980) in the Continuum River Concept. Axis two, in turn, was highly influenced by two components of the substratum; the sand with interfered positively, and leaves negatively. This axes shows the effect of differences in the abundance of these two-bottom substrate, independent of river size. Thus, the influences of the variables related to the axes on the relative abundance in the fish groups were direct analyzed through Linear Regression.

The depth of channel shows the significant effect in three groups of fishes that were collected in headwater streams. The number of Caracidae increase with width and depth. Although, the number of Erythrinidae and Lesbiasinidae decrease with the depth (figure 2). The individual number of Curimatidae and Lebiasinidae increase with depth and width, also the Caracidae are affecting too

(figure 3). The physical characteristics of the canal affect more the groups than by presence and abundance of bottom substratum. The biomass of Gimnotiformes Group (families: Hypopomidae, Gymnotidae and Sternopygidae) increase in channels with a higher percentage of sandy bottom substrate. The Lebiasinidae number increase with leafy bottom substratum and decrease with sandy (figure 4).

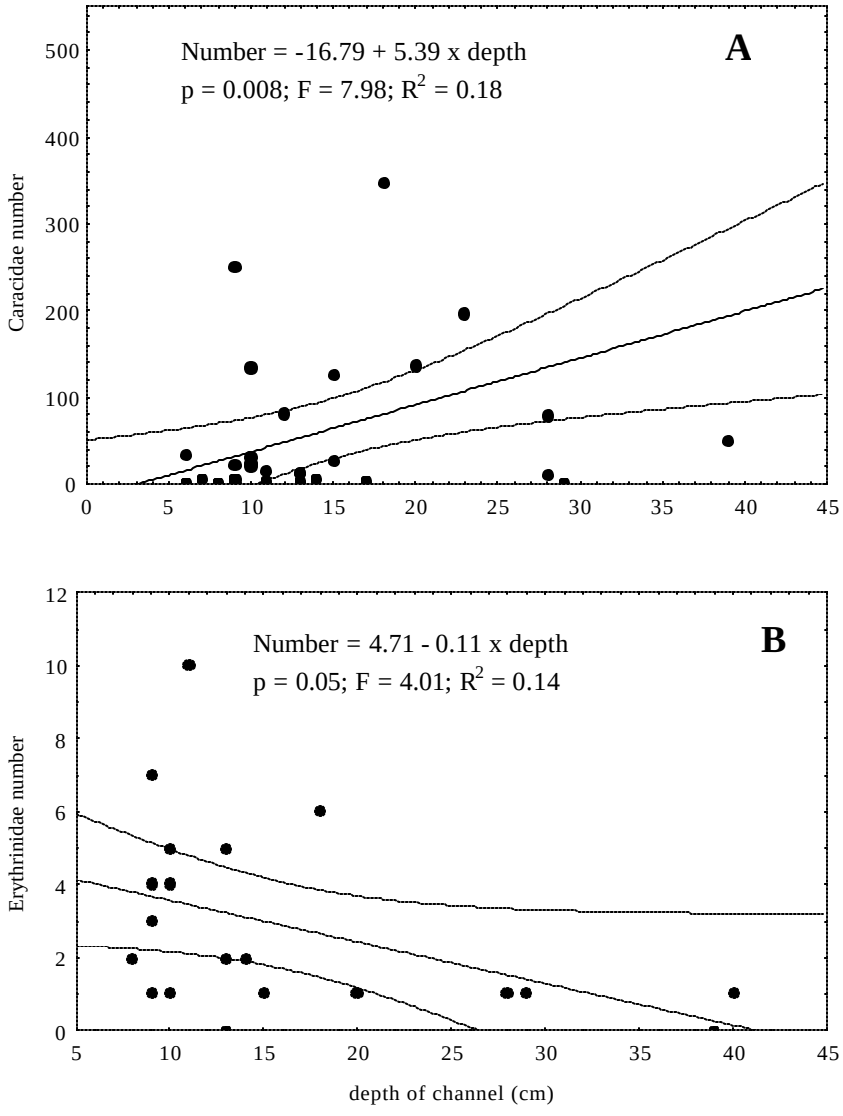
Table 1. Species of fish groups in families, with: the number of indivi duals (N).

Familie	Specie	N
Auchenipteridae	***Trachelyichthys* cf. *decaradiatus***	**2**
Callichthyidae	***Megalechis thoracata***	**4**
Cetopsidae	***Helogenes marmoratus***	**5**
Characidae	***Hemmigramus* cf. *analis* 1**	**322**
	Hemmigramus cf. *bellottii*	413
	Hemmigramus cf. *ocellifer* 1	16
	Hemmigramus cf. *vorderwinkleri* 1	4
	Hemmigramus gr. *analis* 2	212
	Hemmigramus aff. *iota* 1	211
	Hemmigramus aff. *iota* 2	241
	Hemmigramus aff. *analis* 3	348
	Hemmigramus aff. *vorderwinkleri* 2	118
	Hemmigramus aff. *ocellifer* 2	44
	Hemmigramus cf. *schmardae* 1	582
	Hemmigramus aff. *schmardae* 2	234
	Moenkhausia cf. *collettii*	18
	Moenkhausia cf. *cotinho*	136
	Moenkhausia cf. *lepidura*	10
	Iguanodectes cf. *geisleri*	62
	Astyanax cf. *anterior*	5
	Gnathocharax cf. *steindachneri*	43
	Acestrorhynchus aff. *grandoculis*	2
	Acestrorhynchus sp.	1
	Hyphessobrycon aff. *melazonatus*	423
Characidiidae	***Klausewitzia* sp.**	**81**
	Microcharacidium cf. *eleotrioides*	4
Cichlidae	***Apistogramma* sp.**	**419**

	Crenicichla cf. *notophthalma*	24
	Crenicichla sp.1	3
	Crenicichla sp.2	4
	Aequidens sp.	15
	Heros severus	2
	Cichlasoma sp.	3

Crenuchidae	***Crenuchus* sp.1**	**200**
	Poecilocharax weitzmani	82
Ctenoluciidae	***Boulengerella lateristriga***	**1**
Curimatidae	***Curimatopsis evelynae***	**10**
Doradidae	*Physopyxis* cf. *lira*	**3**
	Acanthodoras sp.	1
	Acanthodoras cf. *spinosissimus*	9
Electrophoridae	***Electrophorus electricus***	**1**
Eleotrididae	***Microphilypnus* sp.1**	**66**
	***Microphilypnus* sp.2**	11
Erythrinidae	***Erythrinus erythrinus***	**52**
	Hoplias cf. *malabaricus*	25
	Hoplias cf. *lacerdae*	10
Gasteropelecidae	***Carnegiella strigata***	**6**
Lebiasinidae	***Pyrrhulina* cf. *laeta***	**49**
	Copella cf. *nattereri*	529
	Copella nigrofasciata	474
	Nannostomus marginatus	15
	Nannostomus eques	26
Nandidae	***Monocirrhus polyacanthus***	**1**
Pimelodidae	***Nemuroglanis lanceolatus***	**20**
Rivulidae	***Rivulus* cf. *ornatus***	**23**
	Rivulus cf. *compressus*	1
Scoloplacidae	***Scoloplax cf. dolicholophia***	**1**
Synbranchidae	***Synbranchus* sp.**	**16**
Trichomycteridae	***Tricomycterus* sp.**	**3**
Hypopomidae	***Hypopygus lepturus* 1**	**19**
	Hypopygus lepturus 2	55
	Microsternarchus bilineatus	1
	Stegostenopos criptogenes	1
Gymnotidae	*Gymnotus anguilaris*	10
	Gymnotus cf. *pedanopterus*	2
Sternopygidae	*Sternopygus macrurus*	1

Figure 2. Relationship among the depth of channel and individual numbers of Caracidae, Erythrinidae and Lebiasinidae in headwater streams.



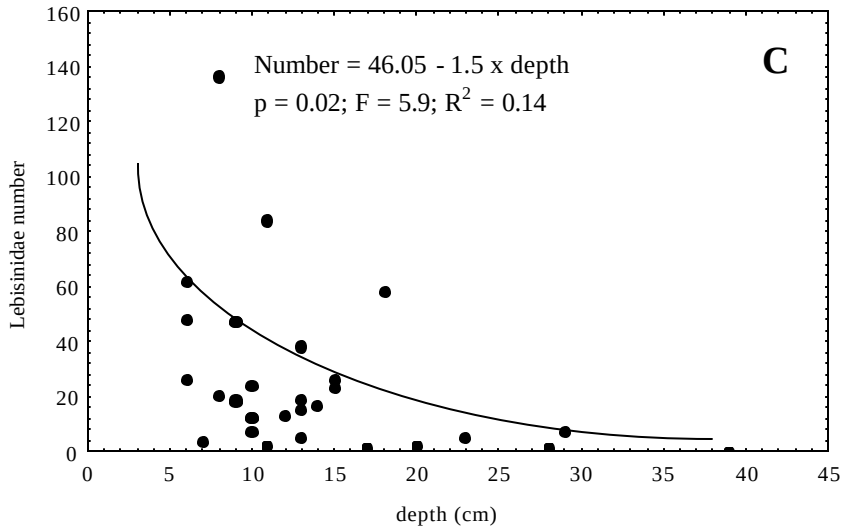
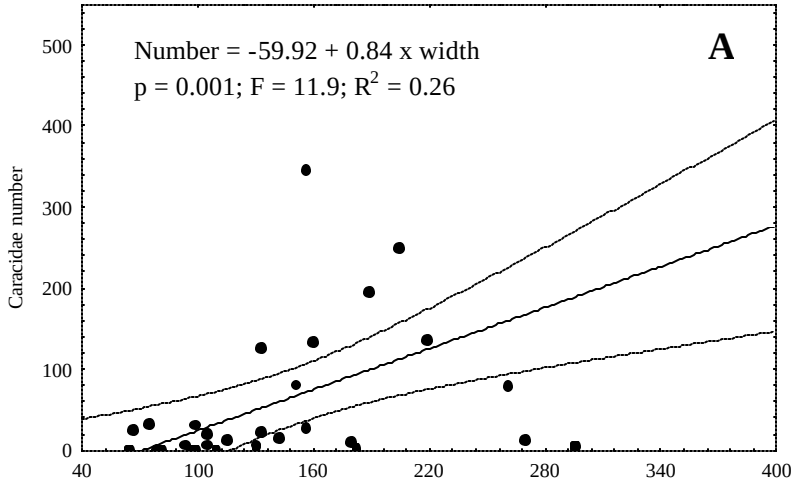
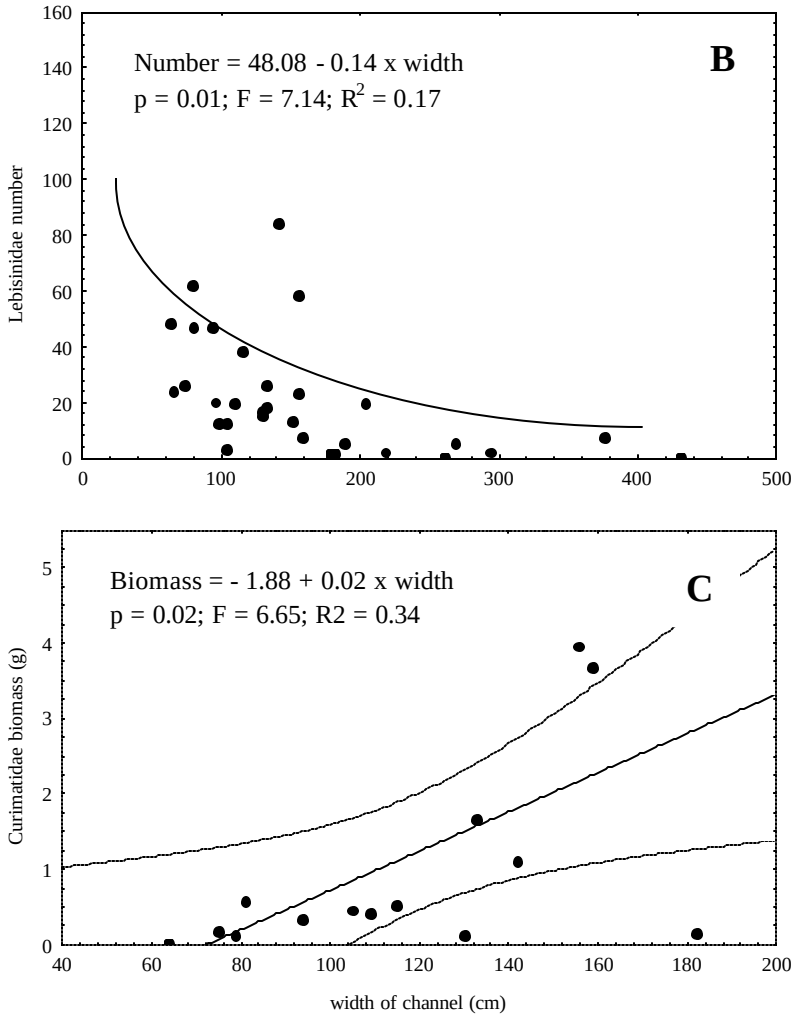


Figure 3. Relationship among the width of channel and abundance of Caracidae, Lebiasinidae and Curimatidae in headwater streams of Jaú River.




Number = 48.08 - 0.14 x width
p = 0.01; F = 7.14; R2 = 0.17
B
Lebisinidae number
0
20
40
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100
120
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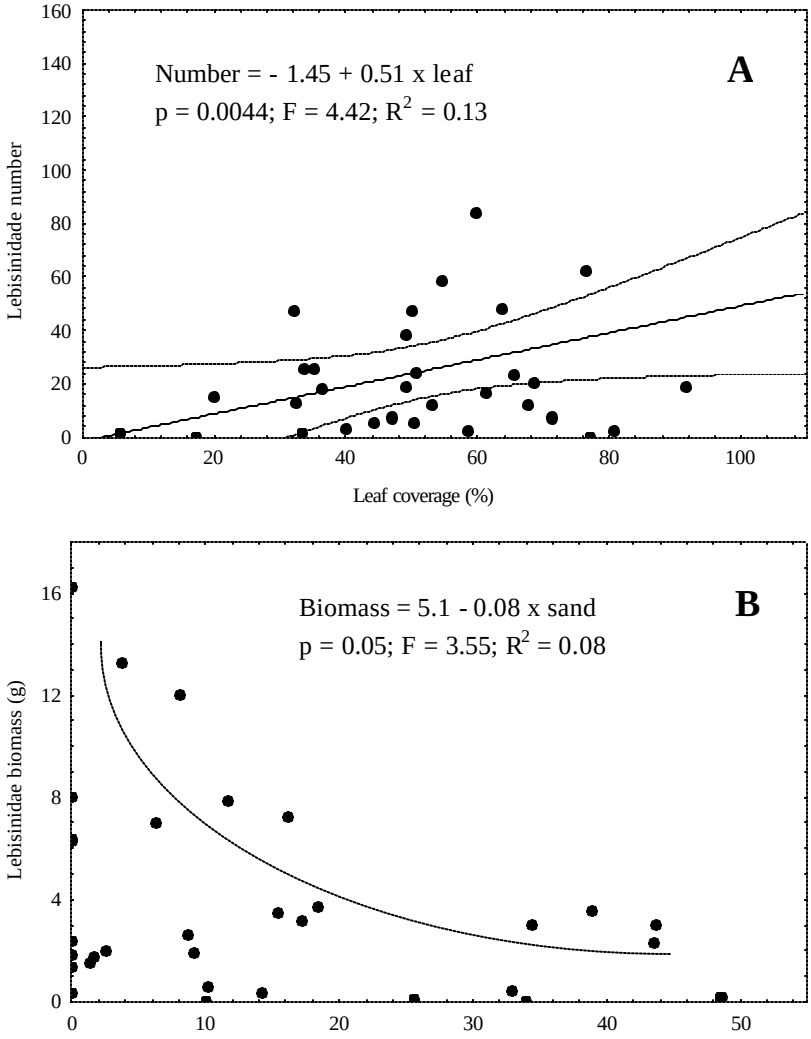
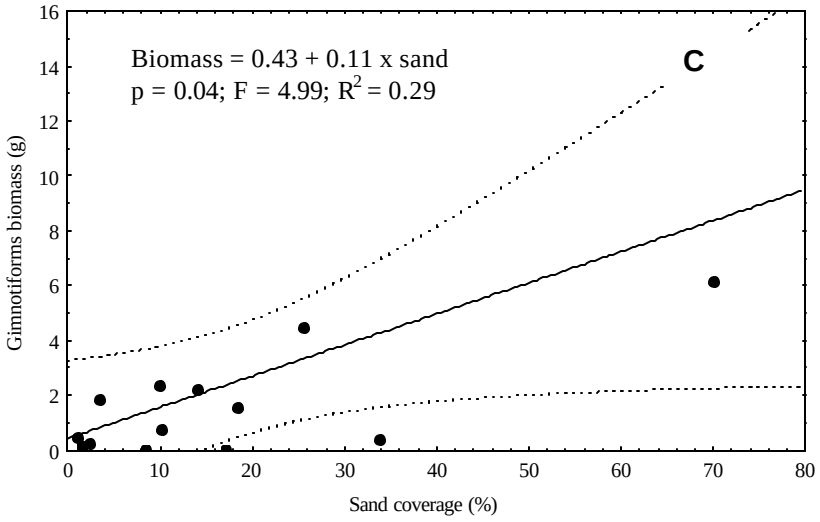
160
0
100
200
300
400
500
Biomass = - 1.88 + 0.02 x width
p = 0.02; F = 6.65; R2 = 0.34
C
Curimatidae biomass (g)
0
1
2
3
4
5
40
60
80
100
120
140
160
180
200
width of channel (cm)

Figure 4. Relationship among the habitats coverage of bottom substratum (leaf and sand) and abundance of Lebisianidae and Gimnotiforms in headwater streams of Jaú River.





Discussion

We identified some biotic characteristics that systematically varied with the canal morphology; in this study the majority of the relationship obeyed the previsions of RCC. (Vannote *et al*., 1980). However, the distributions of families of fishes identified were influenced by local variations, in presence and in abundance of determined bottom substrates, associated to the size of the stream. By and large, the physical characteristics of each stream as current flux, width and depth of the canal elaborated a mosaic complex of habitats. This elaborated environmental combination made up of a variation of different types of substratum in accordance with the locality of each studied was reflected in the trophic structure of aquatic mic ro invertebrates (Cargnin-Ferreira, 1998), and in fish communities in headwater streams of Jaú River.

Some fish families were influenced by a mixture of physical and habitat structure in headwaters of Jaú. The Lebiasinidae group appears to be associated with depth and width, these fishes were found in shallow and narrow stream. However, the leaf and sand bottom coverage also affect the Lebiasinidae, leaf positively and sand negatively. Perhaps, the small size of fishes in this group family aversion to exposed sandy bottoms, connecting with the predation increase with both channel width and depth (figures 2 and 3). This is probably

happen because should has more food and refuges in leafy bottom substratum (figure 4).

Various species or group of species associated themselves to diverse habitat types without clear specific preference. *Crenicichla* sp. was found to be associated with the sand substratum as well as rock or leaves (A. Kemenes, personal observation). Many authors have diffused the theory that fish have specific living habitats. However, the results of this study suggest that this type of association exists only for smaller groups and is not clear from the families' point of view. In these analyses, leaf and sand substratum played significant roles in the fishes of streams, indicating that the presence of these substrates in the streams making the survival of fish species possible. The Gimnotiformes were abundant in streams with sandy bottom substratum. Perhaps, this habitat offer few refuges for their preys and facilitates their visualization at bottom coverage (figure 4).

Conclusions

Although the variability of substrate covering independent of river size may be important for some groups of family, it explains the fish variability in stream. However, the River Continuum Concept had a greater influence on the family groups. The results of this study have important implications for the management and conservation of ichthyofauna in this and other tropical fluvial systems. The preservation of aquatic habitat is essential for the maintenance of the structure of headwater fish communities. The loss of a habitat, in a degraded stream, can generate the disequilibria of a delicate structure with the possible disappearance of fish groups. About 80 % of the fish genera captured in this study were ornamental fish that are exported to Europe and USA (Chao, 1998). Thus, the Jaú National Park can be considered a protected reproduction area of these organisms highly coveted internationally. This will probably be very important for the future development of ecological and economical in the Central Amazon.

Acknowledgements

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MIGRATION OF MANDI (*PIMELODUS MACULATUS*) PASSED UPSTREAM OF THE IGARAPAVA FISH LADDER, GRANDE RIVER, BRAZIL

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

The Grande River, located in the upper Paraná Basin, is segmented by 11 dams. Igarapava Dam is located at rkm 501 (20°00'S and 47°45'W) between the Volta Grande (rkm 425) and Jaguara (rkm 547) dams. Igarapava Dam was built in 1998 and the reservoir fills an area of 36.5 km^2. A 17.5-m high vertical-slot fishway was built at Igarapava Dam to provide passage for upstream migrant fish around the dam.

Mandi is a small (< 2 kg), widely distributed catfish in the Paraná Basin. It is common in natural (e.g. riverine) and non-natural (e.g. reservoir and dam tailrace) habitats. In the Igarapava reservoir, it is one of the most abundant fish and an important commercial fisheries resource. Mandi spawn from October to February (Braga, 2001; Vono *et al*., 2002, Dei Tos *et al*., 2002) and experts disagree whether the species is a long (Va zzoler *et al.*, 1997) or a short (Agostinho & Júlio Jr, 1999) distance migrant.

The objective of the present study was to track movements of migrant radio-tagged mandi to develop a conceptual model of their migration after they exit the Igarapava fish ladder and enter the Igarapava reservoir.

Methods

From November 2002 to March 2004, we radio tracked six female and five male mandi. Reproductive stage of all females was late stage (ripe) and all males were early stage (not running ripe). Fish were 32.0–37.5 cm (standard length) and weighed 790–1,100 g. We captured fish that had entered the fishway by dewatering the fishway and netting the fish. We used electronarcosis to immobilize fish while we internally implanted a 8.9-g Lotek® coded radio tag with the antenna extending outside the body. Tagged fish were released in the Igarapava Reservoir 4 km upstream of the dam.

We used a boat-mounted tracking system to survey eight times for tagged fish in the Igarapava and Volta Grande reservoirs, mostly during the spawning season. Also, fixed-location data-logging radio telemetry receivers tracked movements of tagged fish in the forebay and in the tailrace just below Volta Grande and Igarapava dams, and in the tailrace below Jaguara Dam.

Results and Discussion

Home range (maximum distance between most up- and downstream locations) was 24 $\pm$ 24 km (mean $\pm$ sd) and ranged from 3 to 80 km (Fig. 1). Four males and one female migrated upstream 31–43 km from the release point during November 2002 to January 2003 and in February 2004. One fish reached the Jaguara Dam tailrace and stayed for 3 days. Five fish remained within 5 km of the release point in the Igarapava reservoir. Two of these five fish remained for 10 h to 5 days at the dam's forebay, then moved upstream into the reservoir. One fish remained at the dam's forebay for 5 months until the tag signal disappeared.

Three fish stayed 2 weeks–2 months in the Igarapava reservoir, then migrated downstream passing Igarapava Dam in January 2003. Two of these fish passed the dam during the same hour they arrived at the forebay and one fish passed the dam 3 days after arrival at the forebay. The three fish passed downstream through the bulb turbine in 4–6 min. After passing the dam, the three fish stayed < 1 km downstream from Igarapava Dam and were detected by the data-logging receiver for 90 min–20 days. One fish continued to move downstream and was harvested ~ 40 km downstream in the Volta Grande Reservoir. The fate of the other two downstream migrants is unknown.

The data suggest the following conceptual model for mandi that pass upstream of Igarapava Dam using the fish ladder. After exiting the fishway, migration

distance is highly variable. Most fish (64%) stay within the Igarapava reservoir, few (18%) migrate through the reservoir into the Grande River reach (~ 5 km long) between Igarapava Reservoir and Jaguara Dam, and even fewer fish (9 %) reach the Jaguara Dam tailrace. Mandi stay upstream in the Igarapava reservoir for a few weeks to a few months, then some (27%) migrate downstream passing Igarapava Dam via the turbines. Some mandi delay a few days in the Igarapava Dam forebay before passing through a turbine. The mandi that pass downstream of Igarapava Dam continue moving downstream to the Volta Grande reservoir, the likely place of origin prior to their upstream migration to the Igarapava fish ladder.

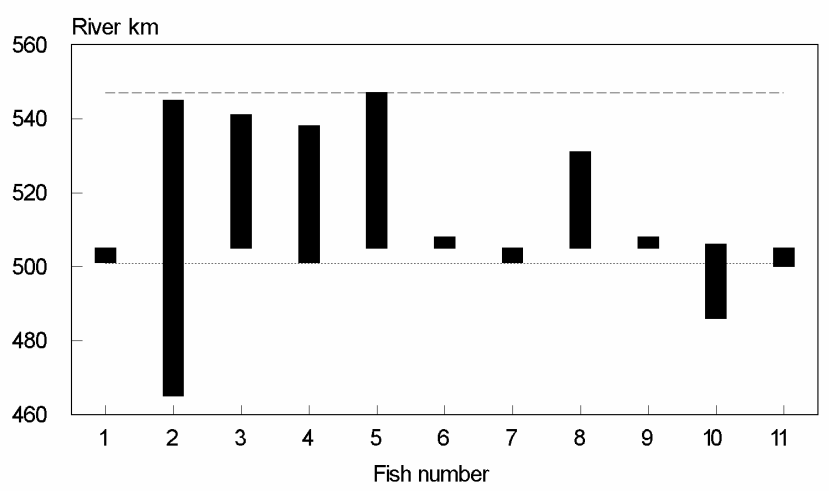

Figure 1. Home range of mandi (males = 1 to 5) in the Grande River. Horizontal lines indicate river km location of Igarapava (dotted line) and Jaguara (dashed line) dams.

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FISH PASSAGE AT THE IGARAPAVA FISH LADDER,

RIVER GRANDE, BRAZIL

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Igarapava Dam was built in 1998 on the Grande River (Upper Paraná Basin, Brazil, 20°00'S and 47°45'W) between the Volta Grande (76 km downstream) and Jaguara (46 km upstream) dams. To allow upstream migrant fishes to pass around Igarapava Dam, a vertical-slot fish ladder, 325.0 m long and 17.5 m high, with a 6% slope was built. A fish counting window near the ladder exit allows visual counts of fish as they exit the ladder. We monitored the fish that passed the counting window to evaluate use of the ladder by various fish species.

Methodology

In this study, data was gathered using on-site visual counts from July 2000 to February 2004 and video-image counts similar to Haro & Kynard (1997) from June to September 2003. The on-site visual counts were done for 15 min of each working daylight hour (8:00–17:00 h). Fish were observed by video system for 24 h/day, 7 days/week. We used an infrared light (Hiebert *et al*. 2000) to illuminate the viewing area and see fish at night. To determine the species available for passage, we sampled for fish near the ladder entrance with gill nets of variable mesh sizes one night each 3 months beginning in August 2000.

Results and Discussion

We identified 35 fish species at Igarapava Dam: 25 in the fish ladder and 32 in the river. Thus, the ladder passed 71% of the species present. Six species were long-distance migratory species in the order Siluriformes and Characiformes. Seven species were exotics, most from another Brazilian river basin. Characiformes, Siluriformes and Perciformes were the dominant orders, a common pattern for migratory species in the Neotropical region (Lowe McConnell 1987). Fish passed in the ladder during all months of the year, but most fish passage (75%) was from October to February, the wet season used by many fishes for migration and spawning. The standard length of fish using the ladder ranged from 4 cm (*Bryconamericus stramineus*) to 80 cm (*Pseudoplatystoma* sp.). Thus, even small fish were able to pass upstream in the ladder. The four species that dominated abundance during video observations (Figure 1) had different patterns of diel passage. *Pimelodus maculatus*, the most abundant species, was nocturnal and passed almost exclusively at night from 18:00 to 07:00 h with a peak between 1:00–5:00 h. *Leporinus octofasciatus*, the second most abundant species, was diurnal passing mainly during the day from 6:00 to 18:00 h, with a peak at 16:00–18:00 h. *Prochilodus lineatus*, the third most abundant species, was nocturnal passing during 18:00–20:00 h, and *Schizodon nasutus*, the fourth in abundance, had a bimodal peak of passage – dawn and dusk.

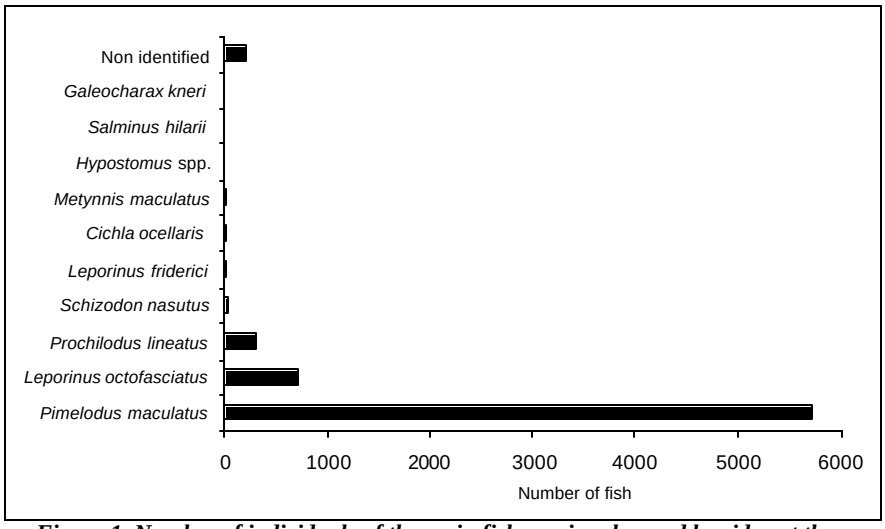

Figure 1. Number of individuals of the main fish species observed by video at the Igarapava fish ladder viewing window during June–September 2003.

Conclusion

The Igarapava fish ladder enabled fish species of diverse lineage that differed greatly for body size and diel movement timing to pass around the dam during upstream migration. Upstream migrations of fishes outside the wet season suggest non-spawning fish on foraging migrations or fish staging for future spawning. A long-term comprehensive monitoring program of fish passage at Igarapava is necessary to determine the importance of the facility to upstream fish passage and restoration of local fisheries.

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PARASITIC ISOPOD ANILOCRA APOGONAE,

A DRAG FOR CARDINAL FISH

CHEILODIPTERUS QUINQUELINEATUS

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EXTENDED ABSTRACT ONLY-DO NOT CITE

Cymothoid isopods, Anilocra apogonae, are regular ectoparasites of the cardinal fish Cheilodipterus quinquelineatus on the Great Barrier Reef. Our aim was to determine whether this large isopod, attached to the head of the fish, affects the physiology and behaviour of its host. The study was conducted at Lizard Island Research Station (14° 40'S, 145° 28'E), Great Barrier Reef, Australia.

We found that the condition of wild-caught C. quinquelineatus, did not differ between fish with and without the parasitic isopod A. apogonae. However, in a laboratory experiment, where we had parasitized and non-parasitized fish given low food or high food regimens, we found that the parasitized fish lost 35% more weight than non-parasitized fish when held on a low-food regimen. By contrast, the fish receiving large rations of food, all gained a similar amount of weight regardless of being parasitized or not. Thus, A. apogonae had a significant effect on fish weight only when food supply was limited. This clearly indicates that the isopods do affect the energy balance of the host, but that this can be compensated for with high levels of food intake under laboratory conditions. This may also be the case in the wild. If parasitized fish have to

increase their foraging efforts, this could have drastic consequences for their survival, as foraging is often associated with the risk of being predated. Previous studies have shown that fish who are under energetic stress due to parasites have a greater need for energy and are therefore more willing to compromise safety from predation for foraging gains (Godin & Sproul 1987, Giles 1987). Alternatively, selection may explain why wild caught parasitized fish did not display reduced condition. Thus, it is possible that fish surviving the parasite association may be high quality individuals that normally would have had a higher condition than the population average.

That the isopod infection increased the demand for energy was collaborated by our measurements of resting oxygen consumption, suggesting that the resting metabolic rate was 25 % higher in parasitized fish. This was most likely attributed to the energy involved in overcoming the extra hydrodynamic resistance encountered by the bloated fish.
Our measurements of pectoral fin-beat frequency in resting fish, showed that parasitized fish were beating their pectoral fins at a 23 % higher rate than non-parasitized individuals. Pectoral fins are used to maintain balance in fish (Jobling 1995). Most likely, the increased pectoral fin-beat activity was related to a need for increased finning to maintain the equilibrium of the body posture when burdened with an asymmetrically placed parasite. In the wild, we found that C. quinquelineatus remained relatively stationary rather than constantly swimming. Thus, much of the energy devoted to fin movements is possibly used for posture control rather than sustained movement in this species.

Swim tunnel experiments showed that the maximum sustainable (aerobic) swimming speed, and the swimming endurance at a very high (anaerobic) speed, were both lower in parasitized C. quinquelineatus. This implies that the impaired swimming performance was related to the increased hydrodynamic drag caused by the parasite. Slower sustained swimming speeds and more rapid times to fatigue may have a number of implications for fish. Decrease in rapid anaerobic swimming bursts may reduce an individual's ability to avoid and escape predation. This may be especially detrimental for parasitized C. quinquelineatus as they may have to spend more time foraging for food due to the energetic stress caused by the association of the isopod, as shown by the feeding experiments. Their ability to swim against water currents may also be compromised by the isopod A. apogonae.

Acknowledgements

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IMPORTANCE OF THE COLLECTIONS TO STUDY PARASITISM: ISOPODS (CYMOTHOIDAE) ON THE ICHTHYOLOGICAL COLLECTION AT THE NATIONAL RESEARCH INSTITUTE OF AMAZON (INPA), MANAUS, AM, BRAZIL

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Introduction

Collections are mainly used for studying the organisms there deposited. However, it is possible to obtain relevant parasitological data from well-conserved fish. Studies regarding parasitism in collections are historically known for their contribution for better understanding the geographical and temporal distribution of these organisms, as well as studying their parisitism indexes. The Cymothoidae are ectoparasites of the marine and freshwatter fishes (Romestand *et al*., 1982). Their length vary from 0,5 to 440 mm. Trilles (1991) listed 334 species of cimothoids in 42 genera parasitizing fishes worldwide. Thatcher (2000) reported that 45 of these occur in South America. These organisms are easily found in collection's fishes because they have well defined fixation locus. So, the species of the genera *Artistone* Shioedte, 1866 and *Riggia* Szidat, 1948 are found perforating the peritoneal cavity; *Asotana* Shioedte & Meinert, 1881 and *Vanamea* Thatcher, 1993, on the mouth cavity; *Anphira* Thatcher, 1993, on the dorsal part of the opercullar cavity; and *Braga* Shioedte

& Meinert, 1881 on the ventral part of the opercullar cavity or on the mouth of their hosts (Araujo, 2002).

This study intends to show the importance of Fish Collections for the knowledgement of the Cymothoidae.

Material and Methods

10,299 fishes collected in the ichthyological collection at INPA were analysed. There were selected fishes considered as appropriated hosts for the cimothoids, such the ones from the Gymnotiform order, subfamily Serrasilminae, genera *Cichla* from the Cichlidae family and *Triportheus* from the Characidae family. The Cymothoidae were removed from the mouth, branchial chambers and external surfaces of their hosts and preserved in 70% alcohol. Mouthparts and other appendages were removed with dissecting needles and cleared in pure phenol for study. Permanent preparations were made of some appendages utilizing the phenol-balsam method describid in Thatcher (1991). Photographs were taken and drawing were made with the aid of a camera lucida. Measurements are in micrometers (µm) unless designated as millimeters (mm).

Results and discussion

105 isopods were found parasitizing the gill cavities, mouth, and perforating the abdominal cavity. Parasites belonging to five genera were recorded and two new ones were added to the Cymothoidae family. It also widened the knowledge regarding the geographical distribution of these parasites in the Amazonian basin, and made it possible to get new records for different hosts as well as to describe new parasite species.

Anphira junki Araujo & Thatcher, 2003 from the gill cavities of the fish *Triporteus. albus*, Cope, 1872 caught in the Amazonian region (Lago Grande, Amazonas river, Monte Alegre, Pará) from 1976 a 1995 and part of the INPA's Collection, was also described. This species is the third one described of the genus and brings to 11 the number of species from Amazonia.

Gen. n.1. et sp. n. is described from the buccal cavity of an Amazonian fish, *Metynnis lippincottianus* caught in the Lake Santa Fé, Guaporé river, near the Costa Marques town, in Rondonia state, in 1983. The new genus differs from all others known in the family principally by having large anterior projections on the merus and carpus of pereopod 7. The 7th dactyl and the rounded projection

on the carpus together form a grasping organ which helps to secure the animal on the tongue of its host (Fig. 1). The new genus constitutes the seventh genus recorded parasitizing fishes in the Amazonian region.

Gen. n.2. et sp. n. is described parasitizing the ventral part of the gill cavities of *Sternachella othos, Sternachella sp* distributed in the Amazonas river near Tapajós river; Madeira river; Purus river; and Solimões river. This new genus presents simetric body; convex pereon, cephalon immersed in pereonit 1, with ventral rotation and mouth vental (Fig. 2). Coxal plates evidents, large and present on all 7 pereonits. The male is similar to the female and not as convex.

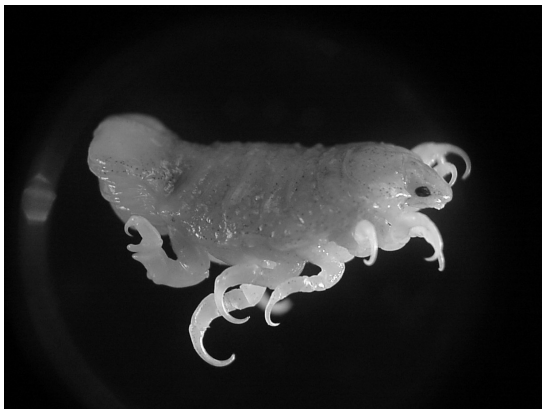

Fig. 1. Gen. nov.1. sp. nov. Length: 16,9 µm.

Fig. 2. Gen. nov.2. sp. nov. Length: 8,2 µm.

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SKIN FISH TANNAGE PROCESS

BY CHROME (STATIC AND MECHANIC)

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Abstrac t

The main objetive of this study was to release skin tannage process of pacu fish cropped to filleting industries of Mato Grosso do Sul. Skins were submited to tannage process and physics and mechanics analisys (BASF/RS Laboratory). Finished leathers (static and mechanic) presents bigger laceration resistence (36,63 N/mm²) than the non-finished leathers (26,55 N/mm²). Pacu fish leather showed appropriated to clothing and shoes fixed assets due high tensile stress resistence with final touch (22,23 N/mm²) and in transversal cut position (25,45 N/mm²).

Key words: Laceration resistence, mechanics analisys, pacu, skin

Introduction

The Mato Grosso do Sul State with its perfect weather conditions and big quantity of sweet water has great potential to psicoculture developing.

The activity has been desenvolved, atracting investment and at the moment the state jail is the biggest alevin productor aknoledged as the biggest native species productor. (PROJETO PACU, 2003)

The above mentioned facts were important to the state psicoculture to be in discussion among state and federal orgains, private companies and local associations with the intention of to increase new groth filleting technologies and consequently product usage .

Joined to the increasement and development of new technologies in the fish growth is the skin usage to tannage what has been turning possible the comercial utilization from this part that generally is disposed by filleting industries.

The usage of this sub product in Mato Grosso do Sul has been happening due to many social programs from ancient and recent govenments that have intensified through programs that intend to use the fish as much as possible, contributing to fishermen families incomes; as the finished product gives material that is transformed in acessories, shoes, wallets and other products.(JORNAL AGRÍCOLA, 1999).

The fish skin has been called the attention of leather industries not only in Brazil as in foreign contries too, as this product, considered to be exotic, when finished offers the same advantages of cattle leather. The fish leather shoes garantee the feet sweting and steam passage through the colagen fibers (FAERTES,1988).

.Besides, the exotic drawing makes a compasation in relation to the cattle leather (colagen fibers interlacing (resitence) and the original drawing of these skin hardly can be imitated by pressing on other leathers, what turns impossible the product fasification.(JORNAL AGRÍCOLA,1999).

According to Adeodato (1995), the mechanic resistence test realized in IPT laboratory (Franca), BASF (RS) proved that fish leather cut in the same thickness as the cattle leather, showed bigger resistence.

Although the great interest of leather industries in this product usage, still today the fish skin tannage has been hapened in handcraft way form and consequently insatifactory, once studies that wanto to evaluate the kind o skin, texture and resistence are rare and give privileges only for some fish species, however it is cientificaly known that depending on the skin structure the tannage process could be not satisfactory giving origin to a low quality product (PEDERZOLLI *et al*.,1995).

In this way, it is important to think in the general usage of this product and its subproducts from the procedure, among them, the fish skin.

Taking in consideration all the points mentioned in this work the purpose is to criate a pattern methodology in order to take advantage of the skin product, generated in the fish production as an alternative to the local fishermen in the state of Mato Grosso do Sul/MS.

Objectives

The present work has an objective evaluate the pacu (*Piaractus mesopotamicus*) epidermis cell structures and analyse the colagen fibers pattern through special methods and also making comparisosns in traction resistence, stretching an tearing power of tannaged skin.

Methods

To this work fish sample were used and obtained in the Mar&Terra psicoculture, located in Itaporã City – MS.

The chosen fish for this study was the pacu (*Piaractus mesopotamicus*), for being the most cultivated species in psicocultures.

Capture Methodology and species preparation for the analsis

The fish in the adult phase were collected by net n.º 12, not considering the sex. Right after were submited to 4^{O}C temperature for 30 min, for turning the fish in a non resposive action and right after were sacrified by spine medule destruction.
The used samples in the experiment were measured in relation to the total length (Lt in centimeters from the nose extemity to the fin caudal extremety), using the ictiometricand the adopted unit in centimeters with aproximation to the imediate inferior unity.

Tannage Process

The used skins in the static and mechanic tannage in chrome were taken out of the fish wtih a narrow point pliers, and immediatelly identified and freezed to conservation until tannage process. For it the skin were naturaly defreezed to ambient temperature and identified by colored little balls sticked to them by line.

The skins were weighted and based on this weight the product and water quantities were set and add in all tannage process. The chrome tannage process both mechanic and static were used in the skins according to the following stages: rewetting, "caleiro", "desencalage", purge, unpolishing, piquel, tannage, neutralization, retannage, polishing, drying, softing, and finishing. (following)

Physical Mechanic analysis

For the physic mechanical measuring procedures 10 samples were taken out from the dorsal line and 10 perpendicular to the fish dorsal line. The samples were analyzed in the physic mechanic laboratory BASF/RS.

The samples were identified and climatized being under 22ºC and relative humidity of 65 ±2%. Before doing the physic-mechanical tests, the samples were measured in the longitudinal and tranversal ways.

For traction and stretching resistence determination a Kratos dynamometer was used, where the result was given by N/cm and in Kgf/cm^2.

Results

The analized species leather tannaged with chrome salts both mechanic (fulão) and static (bucket) and showed in tis visual aspects with a similar structure toa a sand paper, buto to touch showed soft with no smell that requires tannage process.
After skin tannage it was observed that the leather showed an uniformity in the alveolos size (spaces where the escamas were taken out) having, so, a structure regularity, being soft and consistent. Tannage process parts: with chrome salts (static and mechanic)

Re-wetting: The skin were put in re-wetting bath for the initial remotion and existent fat retirada existent in the worked species, besides their hydratation.

Desengraxe: due to a high fat level, it was necessary the desengraxe process due to the difficulty of chemical substances penetration and curtentes.

"Caleiro": during this process it was observed the skin intrumescimento, opening the fibers and consequently scales liberation.

Desencalagem: In this process the cal was eliminated and all the alcaline products in the skin interior were removed, doing in this way the alcaline desentumecimento of the skin.The “desencalagem” control was done trough a cut in the skin, putting some fenolftaleina alcoholic solution drops on the mentioned cut, that in total calcium missing it shows itself with no clolour.

Purge: this is an optative process.

Piquel: This is an salineacid process taht prepares the skin to the tannage.

Tannage: The material turned estable and unrotable once in this process the skin resistance against microorganisms attacks increases, taking its hidrotermic stability.

Basification: strong alcalis were used as sodium carbonate or bicarbonate (changing drastically the enviroment pH). This pH value elevation can be suficient to cause precipitation causing stains in the tannaged leather. The basificants agents usage solubilizes and liberates the alcalinity slowly what takes to a gradual and slight basicity in the bath.

Neutralization: the material was prepared to the retanning chemical products receiving, with the aim of liberate the nocive acids existent in leather through light assist products and with no damage to leather fibers and flower.

OBS: before and after the neutralization it is important to do good washes, as with it before the neutralization part of the water soluble products is extracted and with the posterior wash formed salts are eliminated by the used basis.

“Recurtimento”: in this part the leather elasticity excess from tannage was corrected. This part demands from leather a greater rigidity in the flower, that's why the retannage with other agents that will leave them less elastic.

Polishing: in this part olis are incorporated to hte leather to it promotes the softness where the fibers are involved by materisl shower, that works as lubrificant, avoiding their aglutination during the drying.

Drying: the leather drying was realized stretching them in wood plains leaving them to dry in the shadow. (natural drying).

Finishing: attached

Physic Mechanical Analysis

There was a cut position effect (longitudinal and tranversal) of the test body and in the tannage form and finishing to the analized icognitas. These results are found in the Table 1. However, in the tannage progressive tearing test (estatic and mechanic) didn´t influence as well as the maximum loading in N aplied in the same test.(Table 1).

The leather finishing process influenced in the progressive tearing test. The tannage leather and with finishing showed greater tearing resistence. (36,63 N/mm) comparing to the semi finished leathers (26,55 N/mm). The loading in N and the maximum power used in the test didn´t show significant difference.

The cut position in the leather body test didn´t show significantly diference to the progressive tearing test and the maximum power used in the test.(Table1).

However the aplied load in the test showed difference as for the oblique cut it was necessary a bigger load to tear the leather.

Observed the Table 1 it is noticed that the average values were superior to the ones obtained in the longitudinal cut, although the diference was not significant.

In Table 1 pacu leather stretching and traction tests results are observed. The tannage process didn´t influenced in tearing load, elongation or lenghten and traction lenghthen. The rupture load aplied in the traction test and the elongation value didn´t differ between leathers with finishing and without finishing.

The aplied load to the rupture test in the body test and the leather enlongation differed in relation to the longitudinal and oblique ways. The oblique position (186,70 N e 80,56%) showed a bigger value compared to the longitudinal (98,71 N e 55,72%).

For the traction test there was interaction between the finishing (leather with or without finishing) and body test cut position (longitudinal and transversal). With the interaction development consequences. Cut position X .Leather finishing we can notice that leathers with finishing (29,56 e 14,89 N/mm^2) showed a greater

resistence to traction compared to semi finished leathers (21,89 e 11,68 N/mm^2).So the finishig gave a better quality to the final product increasing its resistence. (Tabela2). The leather tranversal way with or without finishing, showed a better traction resistance 29,56 e 21,34 N/mm^2).

Table 1 – Resistance tests averages in pacu leather (*Piaractus mesopotamicus*) with different tannage process by chrome, with and without finishing and leather position.

Factors	Progressive tearing			Traction and stretching		
	Load N	Tear (N/mm)	Maximum power (N)	Load in rupture N	Traction tension (N/mm^2)	Elongation (%)
Tannage process (FC)						
Estatic	26,79a	30,28a	67,79a	137,26	17,57	70,20
Mechanic	28,10a	32,91a	67,16a	151,15	16,23	66,08
Finishing (AC)						
With	27,71a	36,63a	65,72a	143,23	22,23	69,03
Without	27,17a	26,55b	69,23a	142,18	16,51	67,25
Cut Position (PC)						
Longitudinal	24,79b	30,07a	63,00a	98,71b	13,29	55,72b
0blique	30,10a	33,12a	71,95a	186,70a	25,45	80,56a
Test F						
Tannage process (FC)	0,45ns	1,33ns	0,02ns	2,54ns	8,44**	1,47ns
Cut position	0,076ns	19,57**	0,57ns	0,01ns	21,22**	0,27ns
	7,27**	1,80ns	3,72ns	68,94**	96,07**	53,26**
Interaction FC x AC	2,91ns	0,85ns	0,87ns	0,0	0,24ns	2,85ns
Interaction FC x PC	0,15ns	1,78ns	0,84ns	0,02ns	0,86ns	2,74ns
Interaction AC x PC	0,13ns	1,65ns	0,07ns	0,61ns	4,06*	1,42ns
Interaction FC x PC x AC	1,92ns	1,24ns	0,33ns	0,37ns	0,18ns	0,051ns
C.V. (%)	25,47	26,93	24,10	26,24	23,63	13,50

ns – non significative (P>0,05) * - significative (P<0,05) ** - significative (P<0,01)

Table 2 – Interaction development consequences cut position X Leather finishing for traction tests.

Cut/finishing	With finishing	Without finishing
Transversal	29,56	21,34
Longitudinal	14,89	11,68

Discussion

The manner how colagen fibers are disposed in dermis garantee each other a tied attachment, and by this raise the leather resistence. When the resistence tests on leather were done the weight and fibers orientation (longitudinal and transversal) classes, it was realized that there were dermis structural differences in this species, and the leather showed superior resistence, in most of the test analysis, in transversal allignment than than the longitudinal orientation.
In fish that locomotion is made with ondulatotory motions dermis is composed by crossed arrangement with large thickness of colagen fibers that covering with helicoidal form in the border of the body sustained the strenght tranmission through the spine column (Greven *et al.*, 1995). The characteristic colagen fibers skin arrangement , after the tannage process, presents high resistence, specially in transversal allignment of fish body.

The compact piavuçu (*Leporinus macrocephalus)* dermis is formed with wavy large thickness colagen fibers, and some are found perpendicular to the epidermis allignment (Lorentz,1999).

According to Junqueira et al (1983) it is interesting to do the histological study of colagen fibers architecture in fish compact dermis, because these ones are the main resposible components by skin stabilization during the tannage process.The skin resistence varies with dermis colagen fibers distribution and disposition and the tannage technical process used.

There are few works refering to fish leather resistence in the academic literature (Machado, 2001; Souza et al. 2002a).

There are several facts that increase the leather resistence, so the tannage technical process used in skins increase in a thin leather a reasonable resistence.

According to Craig et al. (1987), in skin of some animals species the colagen fibers distribution is disposed as size and heterogenical degree of fiber size. The parameters that show the traction (loading strenght, traction, tension and elasticity) can be related to each other with the quantity and the colagen fiber orintation. The dermis thickness is determinated, specially, by colagen fibers proportions (Fujikura et al., 1988).

The leather resistence is relationed to the colagen fibers disposition and orientation, that is different in each fish species. According to Machado (2001), in the silver pacu skin analysis, it was observed that dermis colagen fibers were disposed as tiles on the roof, and as a consequence, the leather of these fish species showed the higher progressive tearing tension. The maximum strenght (44,30 N) used at tearing test (36,51 N/mm) was significantly ($P<0,01$) bigger than the maximum strenght observed results in piavuçu leather (17,05 N) and piraputanga leather (6,72 N), that didn´t present that colagen fibers orientation found in silver pacu species.

The utilization of this leather type in clothing and shoes industry shows that the pacu leather is indicated to the clothing usage, as defined in NIVELES de calidad aceptables em la indústria del cuero (1976), written by HOINACKI (1989),with the significant values to the tanned clothing in chome technical process, and the leather must present a minimal traction resistence of 9,80 N/mm^2. Otherwise, the finished leather (22,23N/mm^2) and the transversal leather cut position (25,45N/mm^2) can be aplied in shoes sole, as showed in SENAI tannage school, this leather must have 17,65 N/mm^2 of minimal traction resistance.

The length criterion for this type of leather can be used to clothing manufacture as presented in Vademécum para el técnico em curtición BASF, written by HOINACKI (1989), the maximum value indicated to tanned leather clothing in chrome techinical process is 60% of length until rupture.

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SILAGEM'S FLOUR USIN G

RESIDUES OF CORVINA (MICROPOGONIAS FURNIERI):

OBTAINING AND CHARACTERIZATION

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ABSTRACT

The use of the residues of the fish industrialization in the region is still small being only destined for for the flour preparation, that is of low quality. The fish chemical silage in study at FURG is a liquid product preserved by the action of acids and it can be done starting from the whole fish or its residual material. In order to obtain fish chemical silage, the corvina residues were milled, added with acetic acid P.A. (10% V/P), and stored to the room temperature. Periodic homogenization was accomplished, also control of the pH and temperature of the process, as well as determination of the proximal composition of the raw material and of the fractions of the obtained silagem. The temperature stayed above 20ºC and the pH below 4,5. The text of soluble nitrogen increased during the hydrolysis period reaching 65%. The solid fraction was dry in stove, originating a flour with 59,54% of gross protein.

Keywords: Industrialization; Hydrolysis degree; Chemical Silage; Fish.

Introduction

The municipal district of Rio Grande - BRAZIL captures 99% of the total fish of Rio Grande do Sul. Reaching 38 thousand ton/year (IBAMA, 2002) and generating around 18,24 thousand tons of residues, that corresponds to everything that remains of the filleting stage (head, fishbone, tail and visceras). Nowadays this residue is being used in its totality for production of fish flour, or then, it leaves discarded, contributing to increase the problem of the environmental contamination (Seibel & Souza-Soares, 2003).

According to Espíndola & Oetterer, (1993) silage is a liquid product prepared with entirely fish or in parts, to which acids have been added, and liquefaction of the mass has happened for the action of the enzymes, already presents in the fish.

This work looked for obtaining a chemical silage of corvina residues, and in order to characterize the different fractions obtained during its processing.

Materials and Methods

The residues of the fishing industries were milleed, distributed in plastic pails and added with acetic acid (10% v/p), with daily manual homogenization, pH control and temperature control in the first 5 days. To the 15 days of process, it happened the natural separation of the solid and liquid phases. The different fractions were obtained through the separation of the bones (retained portion) and centrifugal machine (5000 rpm). The dry process of the solid fraction was accomplished in stove with circulation of air for ± 24 h to 55ºC and milled (Figure 1).

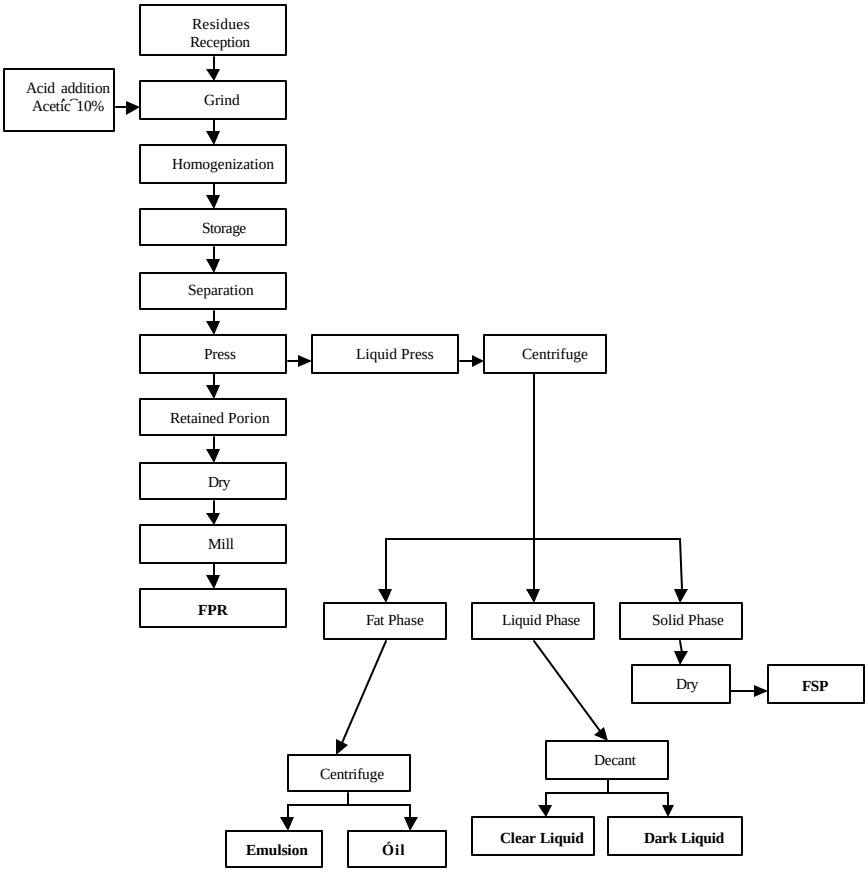

Figure 1: Flux of chemical silage process.

The proximal composition was made, as well as the accompaniment of the degree of hydrolysis of the proteins, through the determination of soluble nitrogen for the macroKjeldahl method (A.O.A.C. 1995). The data were submitted to the variance analysis (ANOVA), in the softwear Statistica for Windows 6.0.

Results and Discussion

During the processing of silage, the room temperature varied between 21 and 28°C, while the intern (of the system) stayed between 20 and 25ºC, what contributed to the fast increase in the text of soluble nitrogen, until the eighth day, reaching in the fifteenth day a text of 62%, in relation to the total nitrogen.

The pH after the addition of acetic acid was around 3,35, reaching a final value of 4,47 that is below the critic (4,5) for growth of bacterias that can cause diseases, even so very close, could have risk of contamination of the silage.

For presenting high text of ashes, the flour of bones can be used as complement of rations. The portion obtained starting from the solid fraction of the silage were drought creating the flour, with 59,54% of gross protein. This percentile can be elevated through a wash of the retained portion. The fish oil obtained presented 97,00% of lipids, 0,73% of humidity and, insignificant proteins and ashes texts (Table 1).

Table 1. Composition (%) proximal of the raw material and the different phases obtained

Sample	Humidity	Gross Protein	Minerals Totals	Extrat Ethereal
Residue	71,77	14,17 cd	5,23 c	6,85 d
FPR	6,80	31,21 b	37,71 a	18,68 c
FSP	11,24	59,54 a	13,27 b	7,41 d
Emulsion	53,99	10,97 d	1,77 d	30,49 b
Oil	0,73	0,4 e	0,03 e	97,00 a
Clear Liquid	84,72	10,55 d	2,13 d	0,14 f
Dark Liquid	83,07	11,57 d	2,10 d	1,10 e

- average of 3 repetitions
- FPR = Flour of the Retained Portion, FSP = Flour of fish silage; Different lower cases in the column indicate significant difference for the Tukeys test ($p < 0,05$).

Conclusions

The acid hydrolysis was effective in the experimental conditions.
The technological conduct of retreat of the bony material was effective, resulting in a silage flour with smaller content of minerals and another with high text of the same ones.

The silage acid flour of corvina residues it presented protein value of 59,54%.

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ALLOZYMIC VARIATION OF CULTIVATED AND NATURAL POPULATIONS OF *Caquetaia kraussii* (Perciformes: Cichlidae)

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Abstract

The genetic structure of a population may be altered by factors such as genetic flux and natural selection. The aim of this work was to know the endogamy coefficient, to determine the variation degree and genetic similarity in cultivated and natural populations of *Caquetaia kraussii* (Petenia), using electrophoresis. 26 enzymatic systems were revealed, a total of 28 presumed loci, 26 monomorphics, only one allele in equilibrium and a higher endogamy level in both populations. The results indicate low genetic variability in the populations, with strong influence of consanguinity in both populations.

Key words: selection, polymorphism, endogamy, electrophoresis, *Caquetaia kraussii*.

Introduction

It has been suggested that changes in genics frequencies, inside isolated populations, are continuous small evolutive events; but they are very slow, which makes difficult direct observation. However, often in individualized populations, it is possible to observe genics frequencies changes, which produce new adaptive characteristics, and some times it is possible to find new varieties and species (Gaggitti & Vetter, 1999).

The difference between individuals from a same population can be attributed to phenotypic plasticity (non genetics) according to Mayr (1963), while the genetics differences or genetics variations can be considered as any alteration that occur in the DNA when compared with the initial state. Depending on the nature of the change it may result in variations in the protein that codifies by substitution of one or more aminoacids, altering the tridimensional protein conformation (Reilly *et al*., 1999).

In the last decades, the electrophoresis has been extensively used as standard analysis in the genetics characterization of cultured fish populations stocks for commercial purposes. The information obtained is used to monitoring genetics variability of these stocks as a way to assure their maintenance.

The aim of this paper was to determine the iso and aloenzymatic patterns in *Caquetaia kraussii* (Petenia), from natural (Guanapito´s water reservoir) and cultured populations (Guanapito, Guárico Experimental Station - Venezuela) to evaluate the possible reproductive selection effect over the genetics variability (H and P) of *Caquetaia kraussii* cultured populations. This species (Fig. 1) was initially described for Steindachner in 1878 at Magdalena River, Colombia. In Venezuela, Pellegrin (1903, cited by Infante, 1979) suggested that this species came from Maracaibo Lake basin, but nowadays it is distributed in most of hydrographical basins of the country, mainly due to involuntary or voluntary insertion. This species has several characteristics that do them suitable for fish culture: resistance to manipulation, resistance to dissolved oxygen sudden changes and adaptation to pH water changes. The disadvantages are their precocious reproduction (they can reproduce when they are 90 days old), which can produce an overpopulation and reduced body growth. If we want to use this species in pisciculture it is necessary to know their genetics identity.

Figure 1. *Caquetaia kraussii*

Materials and methods

Caquetaia kraussii cultured were collected from culturing tanks and ponds at Guanapito´s Experimental Station and the natural species in Guanapito´s water reservoir with the help of different fishing nets ("chinchorros" and "salabardos"). The organisms were taken to Guanapito´s Experimental Station laboratories (INIA – GUÁRICO). Some of them and the necessary tissues (liver and muscle) were preserved in dry ice, carried out to The Ictiology Laboratory (IZT, Facultad de Ciencias, UCV) and kept in an ultra-freezer (-80ºC) for further studies. The electrophoretic analyses were done following Soudsuk, (1993) protocol. Twenty five (25) animals from each population were analyzed with twenty six (26) enzymatic systems: colored bands were produced to the end, indicating the enzymatic activity location.

Results

From twenty eigth (28) presuntive loci obtained for the specie, 26 were monomorphous for the two studied populations* and only two (2) showed certain polymorphism, at least in one of analyzed populations: *EST-1** and *GPI-1**. The *EST-1** loci showed two alleles for natural populations with the presence of two genotypes: homocigous and heterocigous, while the cultured population was found to be monomorphic for the faster allele. For *GPI-1** the obtained loci indicates monomorphism in the cultured population and polymorphism in the natural population, showing only one of the homocigous and the heterocigous genotypes. According with the allele´s frequencies and genetics variability, from the polymorphics loci percentage (P), it is possible to observe that the variability is higher in natural population (7.40) when compared with the cultured population (0.00). The average heterocigosity (H) in Petenia populations is very small. In some cases it is possible to find 0.00 values for cultured Petenia.

Discussion

The comparative genetic studies of cultured and natural fish populations have been made following the criteria suggested by Morales *et al.* (1998), Alarcón & Alvarez (1999) and Kohlmann & Kersten (1999). Such studies allow to monitoring the population genetic variability acting as prevention measures indicators in those cases where the altering factors are working.

The results show a small genetic variability in the species, estimated from the proportion of polymorhics loci and the average frequency of heterocigous loci per organism. The enzymatic patterns found in this work for Petenia natural population allow registering a percentage of 7.40 for polymorphic loci (P) and 0.007 for heterocigosity (H).

It has been shown in several studies done over the different genus constituents of the Tilapia groups that ciclides variability is low. A good example is Feresu-Shonhiwa & Howard (1998) where they indicate a percentage of polymorphics loci of 0.00 and an average heterocigosity of 0.000 in natural populations of *Tilapia rendalli*.

Also, due to the low genetic variability observed in the natural population of Petenia, the results indicate a very clear reduction of these for the fish cultured population.

No only the isolation of a small number of ancestors can reduce the genetic variability but also the parent´s choice in a continuous way from individual family related because the children of the same ancestors lead to the consanguinity from one generation to another.

Conclusions

The alozymic analysis to characterize the cultured and natural population of *Caquetaia kraussii* (Petenia), was possible by applying the proteins electrophoresis technique.

With the genetic study of cultured and natural Petenia it was possible to identify twenty eight (28) loci with twenty five (25) monomorphics, 3 polymorhics *EST*-1*, *G6PDH** and *GPI*-1* in the natural population measuring a decrease of the polymorphous loci in the cultured population, with only G6PDH*.

The research also demonstrates that the variability in the cultured population is less than in the natural populations, and that the consanguinity is working in the species cultured with an heterocigous sharp decreasing in the polymorphic loci *G6PDH** in the cultured population.

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AGE AND GROWTH OF SOUTHWESTERN ATLANTIC

YELLOWTAIL SNAPPER

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EXTENDED ABSTRACT ONLY – DO NOT CITE

Introduction

Yellowtail snapper, *Ocyurus chrysurus*, is an important tropical shallow-water reef fish species that either form large schools or swim in small groups well above the substrate. The species, distributed in the Western Atlantic from North Carolina to southeast Brazil, is mostly abundant in waters off south Florida, the Bahamas and in the Caribbean (Manooch and Drennon, 1987). Brazil was the highest producer of yellowtail snapper during 2000 (4,165 metric tons), accounting for roughly 56 % of total landings, followed by Mexico (18 % of total landings), the U.S. and Cuba. Data from the Brazilian REVIZEE Program (Living Resources of the EEZ) indicate yellowtail snapper as one of the main fishery resources from northeast-southeast Brazil. Nevertheless, information on the populational structure of the species is limited (Diedhiou, 2000; Araújo et al., 2002). We have estimated age and growth of southwestern Atlantic *O.chrysurus* (16^{o} and 20^{o} S) from counts of increments on whole sagittae otoliths.

Materials and Methods

A total of 966 individuals of *O.chrysurus* were sampled from commercial landings at Porto Seguro (eastern Brazilian coast, southwestern Atlantic) between September 1997 and June 2000. For each fish fork length (FL), whole

weight (WW), weight of gonads (Wg) and sex were determined. Sagittal otoliths were removed, cleaned, and stored dry for later age determination. All female undamaged otholits were used, while male otholits were chosen to best represent each length class observed in the catches. A total of 642 (322 males, 320 females) otoliths were measured (length, width), weighted and examined. The whole otolith was placed in mineral oil on a dark-backed dish illuminated from above, with the external concave face upwards. Opaque bands were counted from the focus to the outer edge of the sulcus. Individual blind counts were made independently by two readers. If band counts differed and no agreement could be reached in a second count, the otolith was rejected. The von Bertalanffy (1957) growth equation $Lt = L_8(1 - e^{-K(t-t0)})$ was fitted to observed age-length data using nonlinear regressions.

Results and Discussion

The 996 fishes that were examined ranged in length (FL) from 227 to 535 mm. The relationship between whole weight and FL followed the potential mo del, with 0.99 correlation. The relationship between otolith weight (OW) and FL followed the potential model (r=0.92), while the relationship between OW and observed age was linear (r=0.68). The regression of FL and observed age on otolith length and height revealed linear relationships with correlation coefficients > 0.64. Coincidence among independent opaque band readings of two readers was 30 % in the first reading and 54.6 % in the second reading. Opaque bands are laid down between October-January (spring and early summer), as shown in Figure 1. Opaque bands deposition in southwestern Atlantic yellowtail snapper seems to be related to spawning season, since the increase in frequency of opaque margins coincides with an increase in the mean gonadosomatic index.

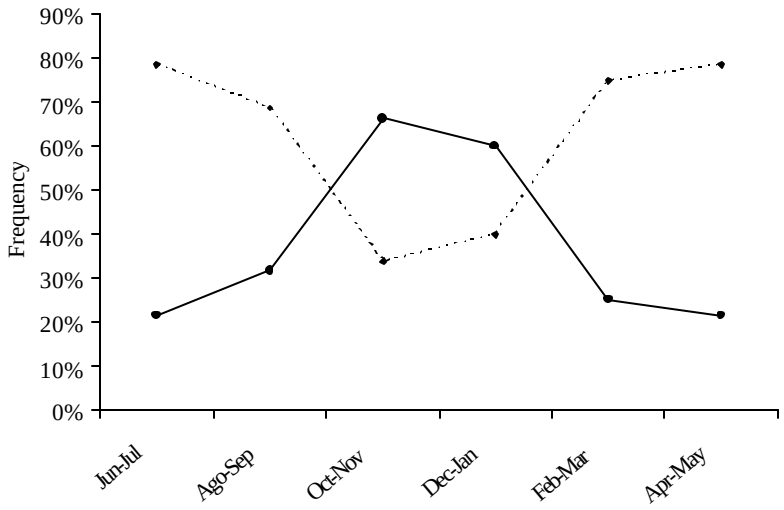

Figure 1. Bimonthly frequency of border type (opaque: solid line; translucent: dotted line) in whole otoliths (n=642) of Southwestern Atlantic yellowtail snapper.

Despite the high variability in sizes-at-age, observed lengths for ages 4-15 fit the von Bertalanffy growth model well. No differences in growth rate were found between sexes. The von Bertalanffy growth function for observed total length at age (pooled sexes) is FL (mm) = 516.2 $(1 - e^{-0.108(t + 2.059)})$, confirming that the species is relatively long-lived and slow-growing. The growth parameters obtained here (L_∞=516.2; K = 0.108) were similar to those obtained in Cuba (L_∞=500.0; K =0.150) and Porto Rico (L_∞=502.5; K =0.139) using, respectively, vertebrae (Piedra, 1969) and sectioned otoliths (Manooch and Drennon, 1987). Sectioned otoliths have been found to be more legible than whole otoliths (Manooch and Drennon, 1987), and have become the preferred method of age determination for yellowtail snapper. For *Lutjanus erythropterus*, *L. malabaricus* and *L. sebae* from the central Great Barrier Reef, Newman et al. (2000) found that age estimates obtained from counts of increments on whole otoliths were consistently much lower and more imprecise, at all ages compared with counts from sectioned otoliths. Further studies using transverse sections of

these same *O.chrysurus* otoliths will allow the comparison of age estimates by both methods for Southwestern Atlantic yellowtail snapper.

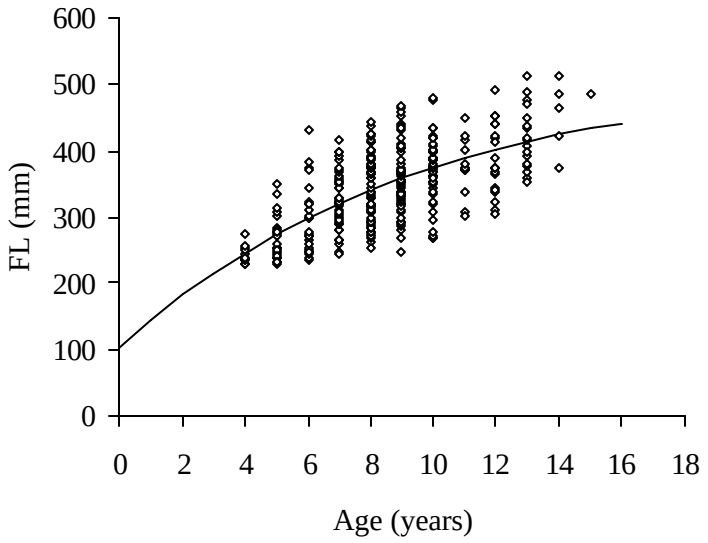

Figure 2. The von Bertalanffy growth curve for Southwestern Atlantic yellowtail snapper.

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SEXUAL AND GEOGRAPHICAL VARIATION OF MORPHOMETRICS IN THE BLUE SHARK (*Prionace glauca*)

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Abstract

Specimens of *Prionace glauca* were collected from November 1996 to April 1998 by pelagic longline on the continental slope of southern Brazil between latitudes 27°S and 35ºS. Forty body measurements were taken from 35 females with total length from 148.5 to 294.5 cm, and 78 males with total length from 135 to 292 cm. The sexes differed significantly in six body proportions. The female had a broader pectoral fin, a greater pectoral-pelvic space and a shorter tail than the male. These differences may be a secondary sexual characteristics related to reproduction and to the hydrodynamics of swimming. Thirty-four body proportions were compared with published data from northeastern Brazil: 8 differed in both sexes, 5 in males only, and 5 in females only. In *P. glauca*, geographical variation of body proportions exists and can be measured. Morphometrics may be useful for identifying unit stocks of this species.

Introduction

Throughout its area of distribution, the blue shark has been caught in increasing numbers since the early 1960`s as bycatch or target species in pelagic longline fisheries, and in recent years the catch per unit effort has declined in several fishing areas. The species is classified as especially vulnerable to overfishing (Castro *et al*., 1999). One of the requirements for fishery management is to define unit stocks and their distribution in space. In the case of *P. glauca*, unit stocks have not been recognized yet.

Morphometry is often applied in the taxonomical study of sharks and can be used to separate species which are otherwise very similar in morphology (Compagno, 1984). Therefore, unit stocks, of a species may differ from each other in their typical body proportions. Morphometric measurements are easily made with simple equipment. If morphometrics could separate unit stocks of *P. glauca*, a useful tool for fisheries management would become available. This possibility can be explored through study of geographic variation of morphometrics of the species. For the Atlantic Ocean, there are published data on morphometrics of *P. glauca* from northeastern Brazil and the Atlantic coast of Canada (Mckenzie and Tibbo, 1964; Hazin, 1991). In the present study, the morphometrics of *P. glauca* from southern Brazil are presented, and are compared with data from Northeastern Brazil.

Material and methods

This study was conducted at the "Laboratório de Elamobrânquios e Aves Marinhas da Universidade Federal do Rio Grande". The study area was the continental slope of Southern Brazil, between latitudes 27ºS and 35ºS, longitudes 46ºW and 51ºW (Figure 1). The samples were taken by R. V. "Atlântico Sul", using tuna longline as follows: mainline monofilament ∅ 3.5 mm; buoy line monofilament ∅ 3.5 mm and length 16 m; gangion monofilament ∅ 1.8 mm and length 7 m, with steel wire tracer ∅ 1.4 mm and length 3 m; tuna hook Mustad 9202 SKR 8/0; baskets of 6 hooks with 60 m between gangions; longline of 50 baskets and 300 hooks. Longline was baited with squid, set at 5 pm, and retrieved at 9 am of the next day.

Longline sets were distributed randomly over depths from 200 to 1000 m, with few samples over greater depths: 7 sets in November and December 1996, 11 sets in July 1997, and 10 sets in March and April 1998.

Fishing depth was monitored with depth recorders mounted in hook position, and varied between 30 and 80 m. Morphometrics were measured immediately after capture. Specimens caught alive were immobilized by cutting the spinal cord behind the head, and passing a steel wire through the neural arches. Complete sets of measurements were obtained from 113 specimens, 35 females and 78 males.

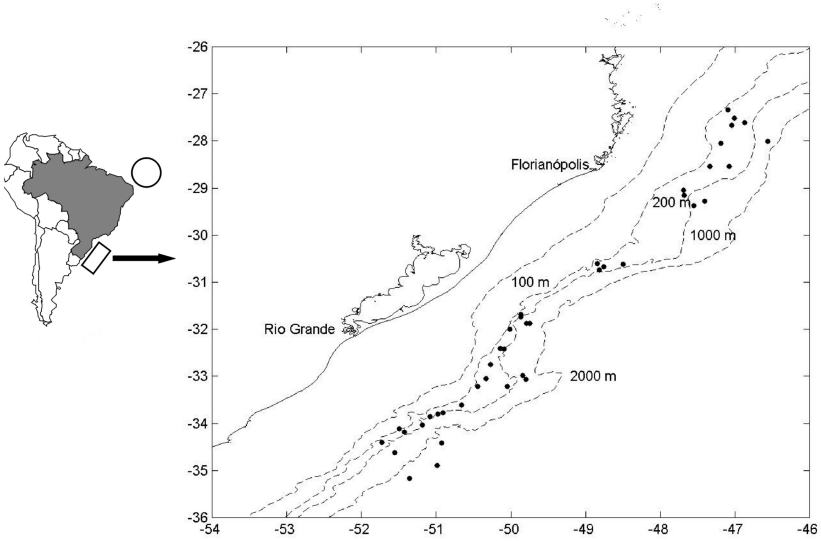

Figure 1. Location of the study area ( ) and positions of the longline sets (•) made by R.V. "Atlantico Sul" from November 1996 to April 1998. The circle (o) represents the study area of Hazin (1991).

For measuring, the specimen was placed outstretched on a measuring board. Thirty-seven measurements were taken as defined by Compagno (1984), whose terms are used here (Tables 1 and 2). Total length and fork length were measured on the measuring board. All other measurements were taken with a builder's measuring tape made of plastic-coated glass fibre. All distances from the snout to body points were taken in a straight line parallel to the body axis. All other distances were measured in a straight line, point to point.

The total length (TL) ranged from 148 to 294 cm in the females and from 135 to 292 cm in the males. In the analysis, the measurements were transformed into percent of TL. Differences between sexes, for each body proportion, were tested by analysis of variance using the Tukey`s significant difference method (Sokal and Rohlf, 1969). The variables which differed significantly between sexes ($p<0.5$), were used to compute the discriminant function for sexes according to Johnson and Wichern (1992).

For northeastern Brazil (latitudes 2ºS to 7ºS, longitudes 32ºW to 38ºW), Hazin (1991) presents means, standard deviations, range and sample sizes, for males and females, of 34 body proportions measured in the same way as in the present study, but given as proportions of fork length (FL), of 20 specimens with FL from 166 to 227 cm in the males and from 162 to 208 cm in the females. For comparison, all 81 specimens within this range of FL were selected from the present data, and their body proportions were expressed as percent of FL. The differences between the means within sexes were tested by t-test according to StatSoft Inc. (1993), with $p<0.05$ being significant.

Results and Discussion

Four variables differed between sexes with probability < 0.01, and two variables differed with probability < 0.05 (Tables 1 and 2). The female had the following body proportions greater than male: prenarial length, posterior margin of pelvic fin, and distance between pectoral and pelvic fins. The following body proportions were greater in the male: upper lobe of caudal fin, and distance between pelvic and anal fins.

The following discriminant function was found, with Z being the discriminant score: (Z) = -5.31189 – 0.54827 (pelvic-anal space) + 0.827511 (pelvic posterior margim) + 0.252561 (pectoral-pelvic space) + 0.889528 (prenarial length) – 0.2642(dorsal caudal margin) + 0.413261 (pectoral base). The frequency distribution of Z consisted of two overlapping curves, the centroids being Z= -0.47 for the males and Z= 1.04 for the females (Figures 2 and 3). The critical value of Z was 0.21. The discriminant function classified 82% of the specimens correctly as to sex.

Table 1. Mean, range, standard deviation(SD), of proportional body dimensions of males and females of *Prionace glauca* from Southern Brazil in % of total body length. Total length from 135.0 to 292.0 cm in the males, n = 78, and from 148.5 to 294.5 cm in the females, n = 35. Variables marked with # differ significantly between sexes with $p<0.01$, variables marked with * differ with $p<0.05$. Morphometric measurements taken in the present study according to Compagno (1984).

	Males			Females		
Body proportion	Mean	Range	SD	Mean	Range	SD
Fork length	81.54	75.34 – 88.59	1.74	81.68	79.10– 89.24	1.82
Precaudal length	74.55	71.14 – 79.17	1.23	74.78	70.99– 81.17	1.67
Pre-first dorsal length	37.16	33.64 – 41.67	1.46	37.30	33.97– 39.61	1.17
Pre-second dorsal length	64.02	59.09 – 68.75	1.51	64.55	61.80– 69.93	1.56
Prepectoral length	22.12	17.94 – 28.60	1.57	21.84	18.68– 26.67	1.73
Prebranchial length	22.45	18.39 – 26.74	1.35	22.39	18.08– 25.15	1.65
Head length	22.95	19.97 – 25.51	1.25	23.29	20.70– 26.46	1.52
Preorbital length	7.75	5.85 – 9.17	0.84	7.79	5.29– 9.18	0.90
Prenarial length *	4.87	3.18 – 6.08	0.44	5.09	4.29– 5.77	0.38
Eye height	1.44	1.15 – 2.11	0.17	1.48	0.99– 1.85	0.18
Eye length	1.43	1.15 – 1.84	0.18	1.43	1.12– 1.73	0.13
First gill slit height	2.66	1.92 – 3.67	0.38	2.58	1.87– 3.20	0.38
Fifth gill slit height	1.99	1.02 – 3.23	0.36	1.92	1.43– 2.42	0.24
Interdorsal space	20.24	17.77 – 22.55	1.00	20.33	19.06– 22.49	0.83
First dorsal anterior margin	10.07	7.35 – 11.72	0.94	9.86	8.19– 12.22	0.91
First dorsal posterior margin	8.19	6.58 – 10.07	0.87	7.94	6.90– 9.29	0.67
First dorsal base	7.08	5.00 – 8.25	0.62	7.11	5.95– 8.56	0.59
First dorsal height	7.36	5.59 – 9.13	0.81	7.36	6.17– 9.36	0.88

Table 2. Mean, range, standard deviation(SD), of proportional body dimensions of males and females of *Prionace glauca* from Southern Brazil in % of total body length. Total length from 135.0 to 292.0 cm in the males, n = 78, and from 148.5 to 294.5 cm in the females, n = 35. Variables marked with # differ significantly between sexes with $p<0.01$, variables marked with * differ with $p<0.05$.

	Males			Females		
Body proportion	Mean	Range	SD	Mean	Range	SD
Second dorsal anterior margin	4.08	3.11 – 8.15	0.72	4.02	2.76– 5.48	0.48
Second dorsal posterior margin	4.53	3.64 – 7.93	0.54	4.36	2.93– 5.05	0.42
Second dorsal base	3.57	1.14 – 7.93	0.77	3.52	2.96– 4.39	0.29
Dorsal-caudal space	7.55	6.25 – 8.82	0.54	7.55	6.49– 8.61	0.55
Dorsal caudal margin *	25.61	23.76 – 35.05	1.34	25.06	22.02– 27.87	1.14
Preventral caudal margin	11.94	8.89 – 14.17	1.03	12.02	10.49– 14.18	0.86
Anal-caudal space	7.34	6.08 – 8.82	0.54	7.32	5.97– 8.52	0.58
Anal anterior margin	5.28	4.35 – 6.49	0.44	5.42	3.88– 6.85	0.56
Anal posterior margin	3.78	2.37 – 5.63	0.63	3.65	2.49– 4.79	0.50
Anal base	3.79	3.01 – 4.72	0.38	3.88	3.17– 6.20	0.54
Pelvic-anal space #	9.49	6.64 – 12.34	1.13	8.53	5.83– 12.35	1.07
Pelvic anterior margin	5.90	3.80 – 9.04	0.68	5.89	4.14– 7.25	0.62
Pelvic posterior margin #	5.01	3.79 – 5.90	0.41	5.26	4.56– 6.36	0.41
Pelvic base	4.78	2.50 – 6.25	0.72	4.71	4.10– 5.38	0.30
Pectoral-pelvic space #	25.02	20.22 – 29.22	1.68	26.05	24.19– 28.52	0.96
Pectoral anterior margin	21.53	17.00 – 33.64	2.15	21.14	18.99– 26.41	1.40
Pectoral posterior margin	18.40	15.13 – 22.17	1.36	18.24	15.43– 23.41	1.76
Pectoral base #	4.89	3.55 – 6.36	0.65	5.31	4.01– 8.15	0.81

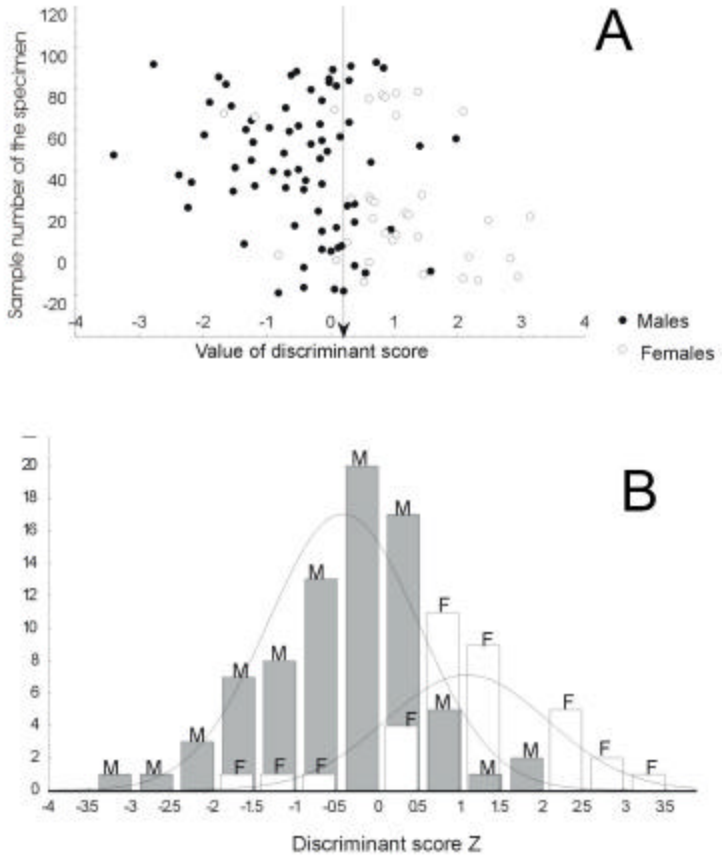

Figure 2. Morphometrics of *Prionace glauca* from southern Brazil A) Distribution of discriminant score values of each specimen. Arrow indicates limit value of separation. B) Frequency distribution of discriminant score Z, with. F = females, M = males

Hazin (1991) found the following morphometrics of *P. glauca* in northeastern Brazil: pectoral-pelvic space, 30.5 in males, and 31.5 in females; pelvic-anal space, 11.5 in males, and 10.7 in females (values in % of FL). The sample was small (only 10 specimens of each sex), and as a result the differences between sexes are not statistically significant. However, they are similar in sign and magnitude to those found in the present study. This is evidence that the

morphometric differences between sexes found in the present study, may be a general feature of *P. glauca*.

In the male, the pelvic fin is situated nearer the pectoral fin, and further away from the anal fin, than in the female. Both sexes have the same value of anal-caudal space, and the male has a greater dorsal caudal margin; the male has a relatively longer tail. Summing the values of pelvic-anal space, anal base, anal-caudal space, and dorsal caudal margin in Table 1, the male's tail is 1.44 % longer, a difference of 2.75 cm at TL of 210 cm. According to the values of pelvic-anal space in Table 1, in sexually mature (Pratt, 1979) specimens with TL of 215 cm, the space between the pelvic and anal fins is 19,5 cm in the male and 18 cm in the female. The functional aspect of this difference may be that the greater spacing of the pelvic and anal fins in the male results in the space necessary for the claspers to develop and move.

For any given value of TL, the distance between the pectoral and pelvic fins was 4% greater in the female than in the male (Table 1). If the distance between these fins is proportional to the size of the abdominal cavity of the fish, and if in fishes, body dimension scale approximately to the third power of volume or mass (Von Bertalanffy, 1977), then for any body size within the range of total length examined, the volume of the abdominal cavity of females approximately 13% larger than for male. *Prionace glauca* is viviparous and the greater abdominal cavity of the females may provide the space necessary for gestation. In conclusion, the morphometric differences between the sexes of *Prionace glauca* include secondary sexual characters, related to the functioning of reproductive organs.

Bass (1973) found that in 13 species of carcharhiniform sharks from South Africa of the genera *Mustelus*, *Sphyrna*, *Carcharhinus*, *Galeorcerdo*, *Cephaloscyllium*, *Halaelurus*, *Holohalaelurus* and *Rhizoprionodon*, the relative distance between the pectoral and pelvic fins was greater in the female than in the male. Thus, it seems that this morphometric feature occurs in carcharhiniform sharks in general.

The base of the pectoral fin was about 9% greater in the female than in the male (Table 1) but the anterior and posterior margin of the pectoral fin were not significantly different between the sexes. The broader pectoral fin and the shorter tail of the female of P. glauca are evidence of sexual differences in the hydrodynamics of swimming in this species. Bass (1973) found that the pectoral fin was greater in the female than in the male in carcharhiniform sharks from

South Africa. Therefore, such differences between the sexes may also be a general feature of these sharks.The morphometrics presented by Mckenzie and Tibbo (1964) for P. glauca from the Atlantic coast of Canada refer to a pooled sample of males and females. Therefore, comparing these data with the present results is difficult.

In the comparison of 34 morphometrics between the present data and those cited by Hazin (1991) for the northeast of Brazil, there are significant differences in 18 variables, 8 differing in both sexes, 5 in the males only, and 5 in the females only (Table 2). If such differences were due to methods, then they would always occur in both sexes, and in all or most of the morphometrics, but no difference occurred in 16 out of 34 variables. The close agreement in almost half of the variables is evidence that the observed differences in the remaining variables are real. The principal differences are that in both sexes, the specimens from southern Brazil have a longer head, smaller eyes, a lower first dorsal fin and a smaller pelvic fin. Between the two areas, the females differ in shape and relative size of the second dorsal fin, the anal fin and the lower caudal fin lobe, and the males differ in the shape of the pectoral fin. On the whole, the southern specimens have 11 body proportions smaller than the northern ones, but have a longer head.

These results are evidence that in *P. glauca* body proportions vary geographically. This could be further examined through discriminant function analysis of morphometrics from different ocean areas. In this way, unit stocks of *P. glauca* could be recognized, and their geographical range could be established. However, little can be done with the headed, gutted and finned carcass. Data for stock identification through morphometrics needs to be collected at sea by research vessels or on board of commercial vessels before the catch be processed.

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FISH SPECIES

AS INDICATORS OF CHEMICAL POLLUTION

IN A TROPICAL ESTUARY

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Abstract

Santa Cruz Channel estuary (Pernambuco, Brazil) has a history of chemical contamination and environmental degradation by sugar-cane plantations, industries and intensive aquaculture. There are 57 families encompassing 145 species of fish registered in the area. Mugil liza, Mugil curema, Cathorops spixii, Achirus lineatus, Centropomus undecimalis and Trichiurus lepturus were chosen for having different feeding habits, relationships with the estuary, size classes and market value. As a group, they can be considered suitable bioindicators of the level of chemical contamination.

Keywords: bioindicators, chemical pollution, tropical estuary, Brazilian Northeast.

Introduction

Estuaries have been the first coastal environment to be occupied by human populations. Historically they have been used for shelter, food supply and discharge of effluents. Some of these uses are clearly incompatible. The chemical contamination of estuaries is a major concern worldwide. However, it is not always well monitored, and the environmental degradation and quality loss of fisheries products may be poorly estimated.

Fish are well recognized bioindicators of environmental changes, including chemical pollution (FAO/SIDA, 1983; Espino, 2000). Since fish span over a wide variety of feeding and living habits, they are exposed to chemical contamination from different food sources and water conditions within the estuary. They are also an important link between the environment and human populations through fisheries and consumption by the local and other markets.

Depending on the feeding and living habits of each fish species, they will represent a different type of exposure of the estuarine biota to chemical pollution. A group of species comprising many of these habits can indicate in a more comprehensive way the level and special distribution of chemical contamination of an estuary than a single species response.

Study area

The Santa Cruz Channel estuary (7o34'-7o55'S and 34o48'-34o52'W) in Pernambuco State, Brazilian Northeast, is an estuarine complex which margins are bordered by mangrove forests. It is a U-shaped tidal creek which separates Itamaracá Island from the continent. The extension of the main channel is about 22km and its width can reach 1.5km at the widest point. Depths varies from 1-17m. It receives contributions from numerous small rivers which drain forested, agricultured, urbanized and industrialized land.

It has a history of chemical contamination (e.g. nutrients, organic matter, oil, Hg, PCBs, Dioxins, Organochlorines) (Silva, 2002a and therein). The anthropogenic influences in the area started with the removal of the native forest

(Atlantic Rain Forest) for sugar-cane plantations since 450 years ago. The development of sugar-cane plantations and milling has lead to the introduction of persistent chemicals and irrigation of the land with the liquid organic rich effluents from the milling process. The land drainage carries the chemicals and the excess of nutrients to the nearby channels which form the estuarine complex.

During the last century an industrial estate was developed around its margins. The industries include chlor-alkali, fertilizers and agricultural defensives plants, paper mills, cement, aluminium, and others. The population in the small drainage basins which form the estuarine complex have also increased significantly. The region of Santa Cruz Channel is the fastest growing in Pernambuco costal zone (Costa and Souza, 2002). More recently it has also been under the threat of the effluents of intensive aquaculture (prawns farming) (which can cause severe cultural eutrophication and water mobilization) and dredging for leisure boats circuits.

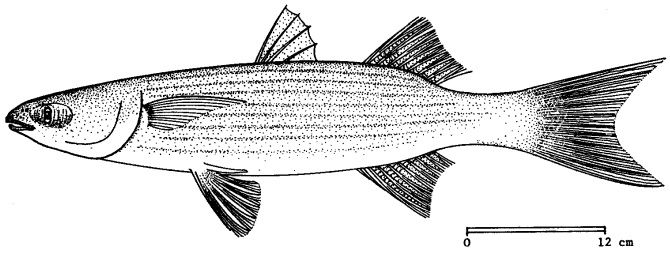
The present work has examined the fish species registered for Santa Cruz Channel in the literature and selected six species to be used as bioindicators of chemical pollution based on their living and feeding habits, socio-economic value and previous use as bioindicators.

Results and Discussion

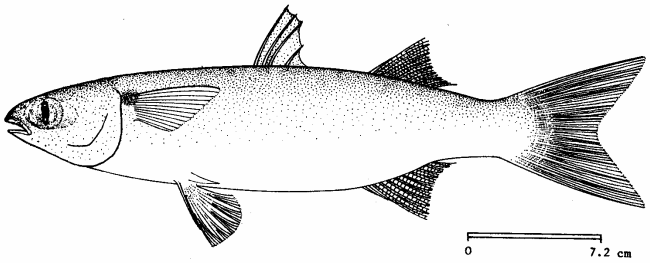
There are 57 families encompassing 145 species of fish registered for Santa Cruz Channel. The Class Chondrichthyes is represented by 2 families and 2 species; the Class Actinopterygii is represented by 55 families and 143 species (Vasconcelos Filho and Oliveira, 2000).

The species Mugil liza, Mugil curema, Cathorops spixii, Achirus lineatus, Centropomus undecimalis and Trichiurus lepturus were chosen because they cover a wide range of feeding habits (three trophic levels), different relationships with the estuary, size classes and market values. Also, these species occur along the Brazilian coast and are frequently captured in estuaries by subsistence and commercial fisheries.

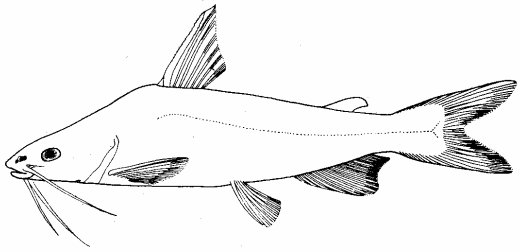
The fish species chosen are reproduced in Figure 1. The most important characteristics of each species which make them suitable bioindicators of chemical pollution are listed and briefly commented.



Mugil liza (Valenciennes, 1836). Blueback mullet. Taken from FAO, 1978.

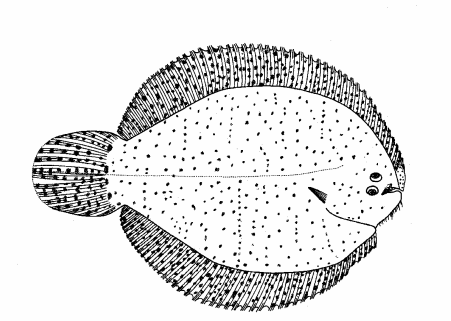


Mugil curema (Valenciennes, 1836). White mullet. Taken from FAO, 1978.

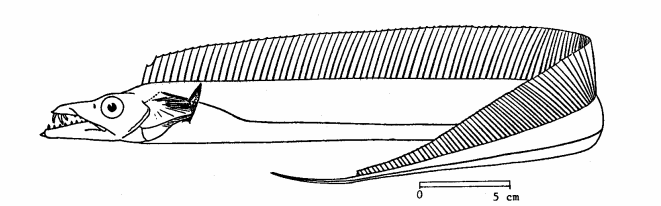


Cathorops spixii (Spix and Agassiz, 1829). Madamango sea catfish. Taken from Figueiredo and Menezes, 1978.

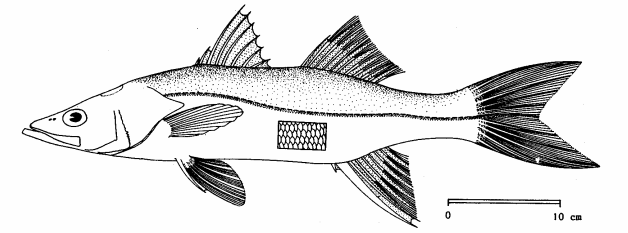
Figure 1: Fish species occurring at Santa Cruz Channel chosen to be used as indicators of chemical contamination of the area in monitoring programs.



Achirus lineatus (Linnaeus, 1758). Lined sole.
Taken from Figueiredo and Menezes, 1978.



Trichiurus lepturus (Linnaeus, 1758). Swordfish, cutlassfish or largehead hairtail. Taken from FAO, 1978.



Centropomus undecimalis (Bloch, 1792). Common snook. Taken from FAO, 1978.

Figure 1 (cont.): Fish species occurring at Santa Cruz Channel chosen to be used as indicators of chemical contamination of the area in mo nitoring programs.

Mugil liza and Mugil curema: species from the Mugilidae Family inhabit shallow waters in estuaries, beaches and reefs. They occur in both marine and brackish waters. Almost all species in the family are omnivorous. The species of Mugilidae usually have a high fat content in their bodies, favouring the bioaccumulation of lipophilic pollutants. Have been used in Brazil as bioindicators of chemical contamination of coastal ecosystems, inclusive at Santa Cruz Channel (see also: Costa and Kehrig in this volume; Sant'Anna, Costa and Akagi in this volume; Silva, 2002a). Have a significant commercial importance along the whole Brazilian coast.

Cathorops spixii: demersal species, lives in tropical estuarine and marine environments. They feed on invertebrates, being exposed through benthic preys in their diet as well as through direct contact with the sediments. Have limited commercial importance, but are consumed by human populations directly linked to the estuarine environments. No previous works using this species as indicators of chemical pollution were found.

Achirus lineatus: demersal species, lives in tropical estuarine and marine environments. Their close relationship with the bottom sediments is important to evaluate the contamination conditions in that environmental compartment. They are exposed through benthic preys in their diet as well as through direct contact with the sediments. Previous works in the region have used this species as indicators of oil pollution.

Centropomus undecimalis: Nektonic demersal species inhabiting shallow waters , bays, estuaries and brackish waters. Carnivorous fish preying on smaller fishes and invertebrates. No previous works using this species as indicators of chemical pollution were found.

Trichiurus lepturus: Cosmopolitans of tropical and temperate warm waters. This species is benthopelagic and frequently penetrates estuaries. Carnivores which prey on small fish and invertebrates. Have been successfully used as bioindicators of mercury pollution (Silva, 2002b; Pinho, 1998).

The main parameter used for choosing these species was their relationship with the estuarine environment. The greater the species dependence on the estuarine environment and the longer it spends in there, the better for its use as bioindicator. These should better reflect the water and sediments chemical parameters prevailing in the estuary (Table 1).

The species trophic level was also taken into consideration. The three levels of consumers were included: primary, secondary and tertiary (Table 1). Analysis of different trophic levels can inform about bioaccumulation and biomagnification processes which may be occurring, even if the species indicated do not prey directly on each other.

Table 1: Relationship with the estuarine environment and trophic level of the six fish species chosen to be used as bioindicators of chemical Pollution at Santa Cruz Channel, Pernambuco, Brazil (Mathieson et al., 2000).

Species	Ecological Guilds	Dietary Preference Guilds
Mugil liza	Diadromous migrants	Omnivorous
Mugil curema	Truly estuarine resident	Omnivorous
Cathorops spixii	Truly estuarine resident	Strictly invertebrate feeders
Achirus lineatus	Truly estuarine resident	Strictly invertebrate feeders
Centropomus undecimalis	Diadromous migrants	Feeding on invertebrates and fishes
Trichiurus lepturus	Marine adventitious visitors	Feeding on invertebrates and fishes

As a group, these species have been considered to be suitable bioindicators of the level and forms of chemical contamination of Santa Cruz Channel. These six species occur along the whole Brazilian coast, allowing for comparisons with other regions which maintain biomonitoring programs of chemical pollution in coastal environments.

Priority should be given to the study of the ecology of these potential bioindicators of chemical pollution. Their temporal and spatial distribution in the estuary and adjacent coastal environments needs to be investigated in order to subsidize a monitoring program in Canal de Santa Cruz.

The authors suggest a biomonitoring program using these species of fish. Sampling can be done either along the contamination gradients, starting at the sources, or at the fishermen colonies along the channel. There are three main

landing points in the area. The program should involve seasonal (rainy and dry seasons) sampling of 10 to 20 individuals of each species (if they occur all year long in the estuary). Muscle tissues can be analysed for their contents of metals and organic compounds which are know to pose chemical contamination threat to the estuarine environment under investigation. The intensive analysis scheme should be carried out for three to four years, until it is clearer which is the best time of the year (more critical) to assess the chemical contamination of the estuarine environment. Only then a consistent spatio-temporal schedule could be designed to monitor the environment on a long term basis.

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FISH SPECIES USED AS BIOINDICATORS

OF MERCURY POLLUTION ALONG THE BRAZILIAN COAST

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Abstract

A literature review shows that fish has been widely used as bioindicators of mercury contamination along the Brazilian coast during the last 25 years. Fish muscle tissue is the most used matrix to evaluate mercury contamination. Other tissues are also used, as well as the whole animal after homogenisation. The group is considered a suitable bioindicator due to its ecological diversity. Thirty four species and ten genera of fish are registered as having been used for mercury biomonitoring of Brazilian coastal waters. The fish species most frequently used as bioindicator of mercury pollution were Micropogonias furnieri, Mugil spp. and Trichiurus lepturus.

Keywords: bioindicators, mercury, total mercury, methylmercury, Brazilian coast.

Introduction

The contamination of aquatic systems by mercury in its various forms is a major concern in conservation ecology and public health. Since the Minamata episode during the 1960s in Japan the subject has been studied worldwide. Fishes are

commonly used as bioindicators of the environmental conditions where ever there is threat of mercury contamination. They cover a wide range of trophic levels and are an important link of the aquatic food chains with human populations. These two main features made fish very interesting for mercury contamination studies and monitoring programs.

Eighteen works, concerning mercury contamination studies of the estuarine, coastal and marine fish species along the Brazilian coast, were selected from a number of references and are chronologically listed and shortly described here. They range from 1981 to 2002. The works reviewed cover a large range of latitudes (tropical – 8oS, to sub-tropical – 32oS) and therefore very different climatic and oceanographic conditions, as well as different stages of industrial and economic development, urban occupation of coastal areas, deforestation and sustainability of agricultural land. All the reference sources available in Brazil were searched. The works were chosen based on their availability and reliability.

The number of fish species reported as being used for mercury environmental contamination assessment was 34, another 10 works report only genera. Table 1 summarizes the species and genera which appeared in the references considered.

Table 1: Summary of the fish genera and species used in mercury contamination assessments along the Brazilian coast. The fish species appear on the table as they are reported in the original reference, followed by the actual valid name when necessary.

Elasmobranchii:	Actinopterygii:
Carcharhinus spp.	Achirus sp.
Isurus sp.	Bagre spp.
Mustelus spp.	Caranx sp.
Odontaspis sp.	Centropomus spp.
Sphyrna sp.	
Squalus spp.	Anchoviella lepidentostoli
	Achovia clupeoides
Galeocerdo cuvier	Arius spixii (Cathorops spixii)
Mustelus higmani	Bagre bagre
Prionace glauca	Centropomus undecimalis
Rhizoprionodon lalandei	Chaetodipterus faber
Rhizoprionodon porosus	Cynoscion virescens
Squatina argentina	Eucinostomus gula
	Jenysia lineata
	Katsuwonus pelamis
	Lile piquitinga
	Lopholatilus villarii
	Lycengraulis grosidens
	Macrodon ancylodon
	Menticirrhus americanus
	Micropoganias furnieri
	Mugil brasiliensis
	Mugil curema
	Mugil liza
	Mugil platanus
	Netuma barba
	Odontesthes bonairensis
	Orthopristis ruber
	Pseudopersis numida
	Sphoeroides testudineus
	Stellifer rastrifer
	Trichiurus lepturus

The intensive work on mercury contamination of fish done by some research groups in the Amazon and Pantanal regions resulted in a change in the Brazilian regulation in 1998. The new Brazilian legislation establishes that only non-predatory species destined to human consumption should still present total mercury content of <0.50 µgHg.g-1 w.w. (Brasil, 1975; Brasil, 1998), while the predatory fish species could have total mercury contents of up to 1.00 µgHg.g-1 w.w, and still be fit for human consumption (Brasil, 1998).

Muscles of fish of various feeding habits was the most used tissue for mercury analysis. Sometimes, the whole fish, homogenized, other tissues or the stomach contents were also used. Micropogonias furnieri, the Atlantic croaker is a carnivore and Mugil spp., mullets, are herbivores. These, together with Trichiurus lepturus, which is also a carnivore, were the most frequently used fish species.

Review

In 1981, CETESB analysed mercury in Lile piquitinga and Anchovia clupeoides from Santa Cruz Channel, Pernambuco State. They reported concentrations of 0.4±0.15 µgHg.g-1 d.w. and 0.39±0.19 µgHg.g-1 d.w..

The bioaccumulation and the toxicity of mercury in Jenynsia lineata from Patos Lagoon, Rio Grande do Sul State, was investigated by La Reza (1983), who concluded that it could be used as indicator of mercury contamination in the lagoon in some situations.

Five fish species from Guanabara Bay were studied for their total mercury content in the muscle tissues. They choose the fish species based on their feeding habits and their consumption by the population. Samples presented total mercury concentration below 0.26 µgHg.g-1 w.w. Only two species presented a direct relationship between total length and total mercury content (Moreira and Pinto, 1989).

Eysink (1990) work in São Paulo State results from a long term study. Mercury was present in the physical compartments within the minimum criteria for the preservation of aquatic life, in spite of eventual high concentrations in the biota (>0.50 µgHg.g-1 w.w.). There were larger concentrations of mercury in the muscles and edible parts than in the digestive tract. There was also a direct relationship between size and mercury content for various species. In general,

concentrations were higher towards the top of the trophic web. Only carnivorous fish were, exceptionally, unfit for human consumption. Slightly high values were detected in the muscle and stomach contents of Sphoeroides testudineus (0.40 and 0.12 µgHg.g-1 respectively) and Centropomus spp. (0.34 and 0.10 µgHg.g-1 respectively). The species of economic interest in the estuarine region of Iguape and Cananéia were represented in this study by Lycengraulis grossidens, Anchoviella lepidentostoli, Mugil curema and Mugil liza. The highest mercury content was detected in the muscle of L. grossidens (0.26 µgHg.g-1).

Also in São Paulo State, Boldrini (1990) studied the evolution of mercury contamination in the different environmental compartments along the Santos coastal plains, which encompasses Cubatão. From 1974 to 1978 an early study indicated that the aquatic systems were contaminated by mercury, above the levels recommended for the protection of aquatic life. In 1975 the first evaluation of the biota found maximum values of 0.80 µgHg.g-1 and, 16.7% of the fish samples were above the recommended 0.50 µgHg.g-1 limit. A monitoring program was undertaken from 1979 to 1980. High levels were observed for Santos and São Vicente estuaries in the stomach contents of Mugil liza (0.98 µgHg.g-1), Achirus sp. (0.56 µgHg.g-1) and muscles of Eucinostomus gula (0.73 µgHg.g-1). In Santos Bay high levels were observed in all sampling campaigns for Cathorops spixii (up to 1.00 µgHg.g-1), Micropoganias furnieri (up to 4.80 µgHg.g-1), Stellifer rastrifer (up to 0.90 µgHg.g-1) and Netuma barba (up to 1.00 µgHg.g-1). These high levels were more frequently observed in the stomach contents of fishes with bottom feeding habits. A significant mercury bioconcentration factors was observed in muscles (444 to 5611 fold) of Caranx sp., Centropomus undecimalis, Eucinostomus gula, Chaetodipterus faber, Bagre bagre, Cathorops spixii, Netuma barba, Trichiurus lepturus, Cynoscion virescens and Micropogonias furnieri; and in the stomach contents (167 to 5944 fold) of Caranx sp., Mugil brasiliensis, Mugil curema, Bagre bagre, Cathorops spixii, Netuna barba, Achirus sp., Micropogonias furnieri and Stellifer rastrifer.

Moreira and Pinto (1991) used Micropoganias furnieri and Orthopristis ruber. In O. ruber mercury concentration in the muscles (N=22) ranged from 0.083-0.32 µgHg.g-1 w.w. and; in M. furnieri (N= 23) from 0.023-0.20 µgHg.g-1 w.w.. M. furnieri showed a relationship between mercury concentration and age (in this case calculated based on total length, cm).

Kehrig (1992) and Kehrig et al. (1998) compared the total mercury levels in fish muscles from four Brazilian estuaries. The main species used for this work was M. furnieri. This fish occurs along the Brazilian coast, and further down to

Argentina, being more abundant south of Cabo Frio (Rio de Janeiro State). A total of 224 individuals were sampled in three estuaries in Rio de Janeiro State (Guanabara Bay (N=61), Sepetiba Bay (N=63), Ilha Grande Bay (N=57)) and also at Conceição Lagoon (N=43), Santa Catarina State, from the summer of 1990 up to the spring of 1991. The concentrations ranged from 0.014–0.43 µgHg.g-1 w.w.. The other fish species studied was Macrodon ancylodon, a herbivore. Total mercury content in the muscle of M. ancylodon sampled in Sepetiba Bay in the autumn 1991 ranged from 0.010-0.13 µgHg.g-1 w.w.. Both species presented direct and significant relationships between the biotic parameters, represented by their total length and weight, and the total mercury content in their muscle tissues. It was possible to observe seasonal variations in the weight normalised mercury concentrations for M. furnieri, showing that the physico-chemical variables of the water have an influence on the assimilation of the metal by this species.

Mauro et al. (1997) determined the methylation potential in Guanabara Bay, which was considered low. Total and methylmercury content of different trophic levels: Micropogonias furnieri (carnivorous fish), Menticirrhus americanus (detritivorous fish) was measured in a seasonal characterisation of the mercury content in the edible parts of each species. The total mercury levels were 0.017–0.27 µgHg.g-1 w.w. with the percentage of methylmercury to total mercury being 93% for M. furnieri (N=61). M. americanus (N=19) showed levels between 0.010–0.13 µgHg.g-1 w.w..

Pinho (1998), measured total mercury concentration in the muscle of 55 samples of four fish species (Katsuwonus pelamis, Lopholatilus villarii, Pseudopersis numida and Trichiurus lepturus) from the eastern part of the Brazilian Exclusive Economic Zone (EEZ) (14-22oS). Mean concentration was 0.27±0.37 µgHg.g-1 w.w., lower than the sharks and rays group (1.01±0.94 µgHg.g-1 w.w.). The muscle tissue of 160 sharks, of different trophic levels, represented by six species were analysed. The two species of Mustelus, which preys mostly on small invertebrates had 0.05-1.54 µgHg.g-1 w.w. (x=0.40±0.34 µgHg.g-1; N=92). Galeocerdo cuvier, less selective feeder, had 0.17-0.25 µgHg.g-1 (0.21±0.04 µgHg.g-1; N=3). Three species of Carcharhinus and two of Squalus, which are mainly fish feeders, had 0.32-2.57 µgHg.g-1 (1.37±0.74 µgHg.g-1; N=9) and 0.34–0.4.06 µgHg.g-1 (x=2.07±0.68 µgHg.g-1; N=55) respectively. Higher trophic levels showed a higher risk for human consumption, based on WHO recommendations, especially for those human groups with a high shark meat intake in their diet.

Morales-Aizpurúa et al. (1999) analysed the total mercury contents in 26 sharks (Squatina argentina, Prionace glauca, Sphyrna sp., Odontaspis sp., Isurus sp.) from São Paulo State coast. Levels ranged 0.04-4.71 µgHg.g-1 being 54 % of them above the new Brazilian legal acceptable limit for human consumption of 1.00 µgHg.g-1 for predatory fish (Brasil, 1998).

Di Beneditto et al. (2000) examined Trichiurus lepturus, which is preferably preyed by Sotalia fluviatilis (porpoise) was also successfully investigated as an attempt to assess the amount of mercury which could be transferred through the food chain.

Kehrig et al. (2000) made a comparison amongst different trophic levels in Guanabara Bay. A total of 291 specimens of Micropogonias furnieri and Mugil liza were collected in differents periods between 1988 and 1998. Total mercury and methylmercury concentration in tissues were determined. All presented low total mercury and methylmercury concentrations. M. furnieri showed higher total and methylmercury concentrations. Also, the ratios of methylmercury to total mercury were lower than organisms with other feeding habits and different trophic levels. There was a significant difference in the methyl:total mercury ratio between the species. Carnivorous fish presented higher methylmercury percentage (98 %) than detritivorous fish (54 %). This indicates that biomagnification of the organic form of mercury is probably occurring in the food chain.

Kehrig et al. (2001), examined 101 M. furnieri and M. liza collected in different periods between 1990 and 2000 at Guanabara Bay. The total mercury and methylmercury contents of muscle varied according to the sampling point and water quality.

Lacerda et al. (2000) analysed three shark species (Rhizoprionodon lalandei, R. porosus and Mustelus higmani) from the Southeastern Brazilian coast. Total mercury values ranged 0.0008-0.28 µgHg.g-1 d.w., ad were considered low, when compared to values reported for other larger shark species from the Southwestern Atlantic.

Sant'Anna et al. (2000a and b) made an exposure survey at Santa Cruz Channel to assess the possible risk of mercury contamination of human populations through fish consumption. Fish muscle presented relatively low values for total and methylmercury concentrations. Mugil sp. presented mean values of total mercury and methylmercury of 0.027±0.026 µgHg.g-1 and 0.020±0.016 µgHg.g-

1 respectively (N=60). The mean methylmercury % in the samples was 70.2±29.9%.

Ustra (2001) studied total mercury concentrations in fish from the Patos Lagoon. The fish species examined were Micropoginias furnieri (0.276±0.087 mgHg.kg-1 w.w.), Bagre sp. (0.0804±0.0054 mgHg.kg-1 w.w.), and Mugil platanus (0.0267±0.0007 mgHg.kg-1 w.w.), all below the Brazilian legislation limit for human consumption. Other species were also investigated during this study, but not identified.

Niencheski et al. (2001) analyzed mercury in fish from the Patos and Mirim Lagoons. Micropogonias furnieri and Odontesthes bonairensis had mercury levels below 200 ngHg.g-1w.w..

Kehrig et al. (2002) compared M. furnieri from Guanabara Bay collected in 1990 (N=61) and 1998 (N=20). In 1990 mercury levels were 108.9±58.6 µgHg.kg-1 and in 1998 199.5±119.3 µgHg.kg-1 of total mercury. Methylmercury in 1998 was 194.7±112.7 µgHg.kg-1 (98%). In the same study M. liza is reported as having 15.4±5.8 µgHg.kg-1 and 9.6±2.6 µgHg.kg-1 of total and methylmercury respectively.

Monitoring of mercury in coastal environments in Brazil has suffered of an intermittent character, it has nevertheless successfully established a baseline for mercury in marine and estuarine biota. Mercury levels, with very few exceptions, remained relatively low in the approximately 20 years comprised in this review.

The situation concerning mercury contamination of coastal environments is comparable to other developing and developed countries such as Argentina, Ghana, Portugal, France, United States and others.

Studies have reduced the number of species. Now, less species, of better know biology, more cosmopolitan and widely consumed provide the basis for comparisons amongst different coastal environments in Brazil and other adjacent countries.

The Northern coast remains the less studied of all coastal regions in Brazil. As a consequence, we still don’t know which may be the effect of the Amazon and other mercury impacted basins on coastal environments.

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MUGIL SP. USED AS BIOINDICATOR

OF MERCURY POLLUTION

IN SANTA CRUZ CHANNEL, PERNAMBUCO, BRAZIL.

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Abstract

Mugil sp. was used to indicate the levels of mercury contamination of Santa Cruz Channel Estuarine complex. Sixty individuals were analysed. The total Mercury concentration ranged from 4.6-167.0 μg.g-1 (26.9±26.1 μg.g-1), and methylmercury concentrations ranged from 1.8-91.6 μg.g-1 (19.6±16.0 μg.g-1) w.w.. Methylmercury percentuals were in the range of 5.7-100% (80±23.8%). This species is widely sold in the region and consumed by the local population. The total mercury and methylmercury concentrations found are bellow the permitted levels for human consumption according to the Brazilian legislation. The results are comparable to other regions in Brazil where the same gender was also used.

Keywords: Mugil, bioindicators, mercury, total mercury, methylmercury, Brazilian coast.

Introduction

Mercury contamination of coastal environments and fisheries products is a global concern. It must receive special attention near historically contaminated sites, as the vicinities of chlor-alkali plants. Even after the switch of the production process (from Hg electrodes to membranes) or the start of more efficient effluent treatments, the sites tend to remain contaminated for a long time. Therefore, monitoring is necessary to guarantee that the mercury remaining in the system is immobilized in the sediments, or, to evaluate at which rate it is being methylated or exported to the adjacent coastal waters.

At Santa Cruz Channel, a tropical estuary in Pernambuco State, Northeast Brazil, a chlor-alkali plant has operated without adequate effluent treatment for over 35 years. It is estimated that the plant released 22 to 35 tons of inorganic mercury in the estuarine system until 1987. Some of the mercury (estimative goes up to 2.5 tons) is still buried in the sediments (Meyer, 1996).

Biological monitors have been used to assess the environmental contamination and the transformations the mercury remaining in the system may be undergoing, (Meyer, 1996; Meyer et al., 1998; Nilson et al., 2001; Sant'Anna, 2001). These studies also help to assess the possibility of transfer of the mercurial forms present in the natural environment to the human populations which live around the channel and consume fish and shellfish from the area (Nilson et al., 2001).

Species from the family Mugilidae, especially Mugil, have been favoured as biomonitors of metals (inclusive mercury) contamination at coastal areas due to their feeding habit (they feed on the organic matter and diatoms overlaying the bottom sediments), relation to the estuarine environment, high fat content in the muscles and market value (e.g. Yilmaz, 2003; Filazi et al., 2003; Canli and Atli, 2003; Kehrig et al., 2001; Alonso et al., 2000).

Also, these species occur along the whole Brazilian coast, allowing for comparisons of the contamination levels of different coastal ecosystems of the country.

Material and Methods

Sixty individuals, of a non identified species of Mugil, probably M. liza or M. curema, were sampled at the fishermen colony at Santa Cruz Channel in the spring of 1999 (August and September). This is the end of the dry season in the region. The fish were caught inside the channel by local fishermen.

The muscle tissue was removed according to standard procedures for metal analysis (FAO/SIDA, 1983). The samples were frozen (-18oC) until analysis. The total and methylmercury analysis were made at the National Institute for Minamata Disease (NIMD), in Minamata, Japan.

Results and Discussion

The standard length (SL) of the sample varied from 27.2 to 36.6 cm (30.6±1.9 cm; n=60). Mercury concentrations in the muscle tissue of the individuals analysed varied from 4.6 to 167.0 ug.kg-1w.w. for total mercury and 1.8 to 91.6 ug.kg-1w.w. for methylmercury. The mean values of the mercury contents in fish muscles were 26.9±26.1 ug.kg-1w.w. of total mercury and 19.6±16.0 ug.kg-1w.w. of methylmercury. The percentage of methylmercury in the samples was between 5.7 and 100%, with mean values of 80±23.8%.

There was no correlation between size (SL in cm) and weight (g) of the fish analysed and their mercury contents. This might be due to the relatively small size interval sampled. The fish sampled came form a single catch.

The total mercury concentrations found in fish from Santa Cruz Channel are within acceptable levels for human consumption according to the Brazilian sanitary legislation (Brasil, 1975 and 1998) and the World Health Organization (WHO, 1989 and 1990). The maximum permitted mercury concentration for human consumption is 0.50 µg.g-1 w.w. for fish and shellfish. Predatory fish may have up to 1 µgHg.g-1 w.w. (Brasil, 1998).

The mercury chemical species initially introduced to the system was inorganic, elemental, mercury Hg(0). The analysis showed that most of the mercury in the fish muscle is in the organic form (mono methylmercury – CH_3Hg^+). The presence of the methylated form indicates that the metal is being transformed in the estuary and transferred to the rest of the estuarine trophic web already in its

most toxic form. The methylation process is probably taking place in the sediments, driven by bacterial activity.

If this species can rapidly respond to environmental changes (mercury contents in superficial sediments), then the present results would be reflecting a period of low continental runoff and decreased river flow. Therefore, at the time of sampling there was less particulate matter discharge and probably the chance of mercury contamination of the fishes which feeding habits are related to the bottom sediments. It is not know how fish from this group reacts to seasonal variations in the mercury contents of the sediments and in their diet. The concentrations reported here could be on the lower end of the range at the end of the dry season in the region.

Mercury availability in the water column could also be explained by the eutrophic conditions of the environment which leads to intense precipitation of the mercury to the sediments, after adsorption onto the particulate matter.

Any process which favours the re-suspension of the contaminated sediments (as dredging for instance) can remobilise mercury to the water column and further expose the biota.

Other authors have also used species of Mugil spp. for the same purposes (Figure 1). The total and methylmercury levels found in the muscles of fish from Santa Cruz Channel are comparable to the levels measured in other regions in the country.

A work done on the coast of São Paulo State, near areas under the influence of severe industrial pollution investigated the mercury concentrations of Mugil curema, M. liza and M. brasiliensis. The mean mercury value found in the stomach contents was 098 ug.kg-1 w.w (Boldrini, 1990). Also in São Paulo State Mugil curema and Mugil liza showed less than 0.5 ug.kg-1w.w. of total mercury in their muscles (Eysink, 1990).

Although the mercury and methylmercury levels found in fish from Santa Cruz Channel are relatively low, periodic sampling and analysis is highly recommended. A monitoring program would help to observe whether the channel is being cleared from the mercury contamination. We suggest to decision makers the establishment of a regular monitoring program which should include:

- Sampling of abiotic environmental compartments and bioindicators of different trophic levels every 3 to 5 years;

- Sampling of fish of as different size classes and feeding habits as possible;

- Sampling at different fishermen colonies along the channel (there are three main landings);

- Sampling of water, particulate matter and surface sediments from fish regular feeding grounds for mercury analysis;

- Sampling of the abiotic environmental compartments along the contamination gradient starting from the releasing point of the effluent;

- Monitoring of dredging activities (before, during and after) for the risk of mercury re-suspension from the sediments, at least at the most contaminated portion of the channel.

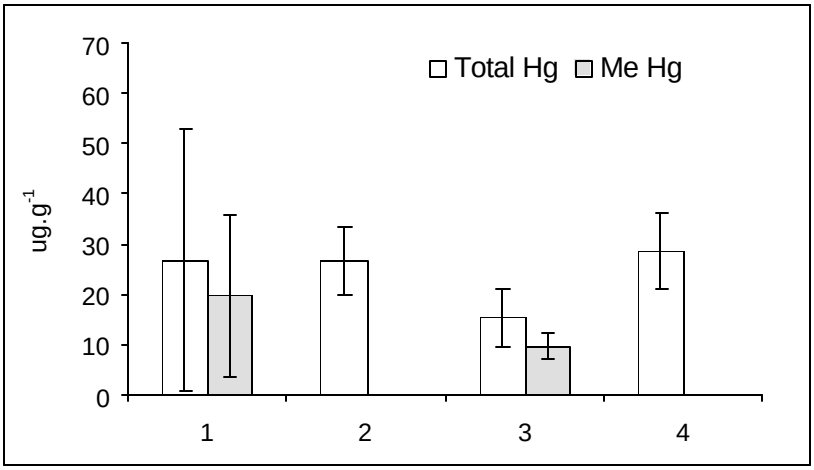

Figure 1: Comparison of works which used species of Mugil spp. as bioindicator of mercury pollution along the Brazilian coast. (1) present work at Canal de Santa Cruz, fish sampled in 2000 (n=60) Mugil sp.; (2) Ustra, 2001 at Lagoa dos Patos, fish sampled in 2000 (n=10) Mugil platanus; (3) Kehrig et

al. (2002) at Guanabara Bay, fish sampled in 1998 (n=20) Mugil liza; (4) Kehrig et al. (1988) at Guanabara Bay, fish sampled in 1988, Mugil sp..

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CHROMATIC ORGANIZATION

OF SINGLE CONES IN THE RETINA OF JUVENILE SALMON

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EXTENDED ABSTRACT ONLY- DO NOT CITE

Salmonid fishes have two morphological types of cone (single and double) arranged in a square mosaic throughout the retina. The unit of a square mosaic consists of four double cones whose long axes align with and lie along the sides of a square surrounding a central single cone and four single corner cones located at the corners of the square (Engström, 1963) (Figure 1). Previous studies have shown that corner cones are partially lost from the ventral retina of some juvenile salmonids around the time of smoltification (the physiological transformation that readies the fish for life in saltwater) (Kunz, 1987; Kunz et al., 1994; Novales Flamarique, 2000 and 2001). During this time, a reduction in ultraviolet (UV) sensitivity has also been noted, and attributed to the partial loss of corner cones (Novales Flamarique and Hawryshyn, 1996). With the exception of the rainbow trout and the Atlantic salmon, the extent of this cone loss in the retina of salmonid fishes has not been documented. As well, the association between corner cones and UV visual pigment has not been fully established. We have performed in-situ hybridization with visual pigment mRNA probes and histological analyses to determine the distribution of UV and blue pigment bearing cones and the density of single cones in the retina of four species of juvenile Pacific salmon [pink (*Oncorhynchus gorbuscha*), chum (*O. keta*), coho (*O. kisutch*), and chinook (*O. tshawytscha*)], and in the Atlantic salmon (*Salmo salar*).

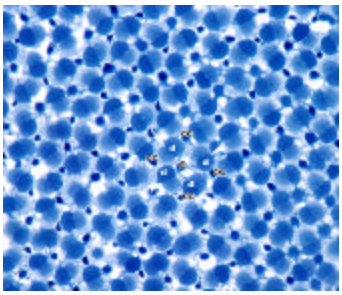

Figure 1. Tangential retinal sections showing the square cone mosaic in chinook parr; double cones (d) make the sides of the unit square, and single cones are located either in the center position (c) or at the corners (cc). Arrows point to the common partition separating double cone members.

All single cones in the retina of pink alevins contained UV visual pigment and loss of corner cones was restricted to the peripheral ventro-nasal retina of the young smolt. Single cones in this area of the smolt failed to label with the UV opsin probe but labeled with the blue opsin probe. Most of the single cones were present in the retina of chum parr; as in pink salmon, the loss of corner cones began in the ventro-nasal periphery, and in this species progressed throughout the retina. The dorso-temporal quadrant was the only area where corner cones remained in the retina of the smolt. Most of the single cones labeled with the UV probe in the retina of chum parr (Figure 2); however, the single cones in the smolt retina labeled with the blue probe. Corner cones and UV opsin label were present throughout the retina of the coho alevin. In the smolt, however, corner cones were restricted to the dorsal retina and UV opsin labeling persisted in a small patch of cones located in the dorsal periphery. In other regions, the majority of single cones labeled with the blue probe. The chinook parr had but ~ 25% of the corner cones remaining in the ventral retina, and UV opsin expression was absent from its lower half. The loss of corner cones continued in the smolt leaving a small patch of cones in the centro-dorsal region where labeling with the UV probe persisted; other regions of the smolt retina had single cones that labeled with the blue probe. Unlike the retina of Pacific salmon, that of Atlantic salmon had already lost most of the corner cones, except in the dorsal periphery, by the parr stage. The single cones in this region were the only cones that labeled with the UV probe. The retina of the

smolt was devoid of corner cones, and the labeling of UV opsin was further confined to a smaller region of the dorsal periphery.

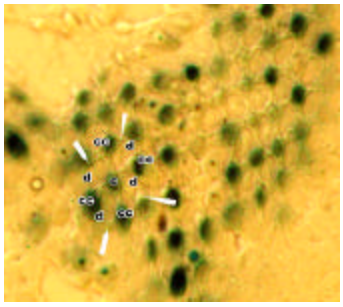

Figure 2. Tangential sections showing labeling (dark stain) with the UV mRNA probe of all single cones inner segments in the square mosaic of recently hatched chum salmon.

Overall, all single cones in the recently-hatched fish (yolk-sac alevin) contained UV visual pigment exclusively, while the single cones in saltwater-ready smolts had predominantly blue visual pigment. Salmon in between these stages had single cones expressing both UV and blue visual pigment, in accordance with a UV-blue transformation event that progressed from the ventral to the dorsal retina. The timing of the transformation preceded the partial loss of single cones and may be a triggering signal for the latter event. The extent and timing of the transformation and the magnitude of single cone loss varied between species, but both events showed the same topographical trend in all species.

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ORGANIZATION OF COLAGEN FIBERS

AND DERMIS MORPHOMETRY

OF PEIXE CACHORRO (Acestrorhunchus pantaneiro).

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Abstract

The main objective of this study was to find out the colagen fibers arrangement in the compact three dermis regions of the fish Acestrorhunchus pantaneiro , collected in Rio Negro region, IPANN/UNIDERP/MS. Skin samples of 06 adult species were removed, processed and stained with eosin-hematoxylin and with Mason trichromic.Three dermis region present medium size colagen fibers and parallels to epidermis, differing in measured thickness. Dermis in this species comparing to others teleostis fish showed differences between compared three studied regions.
Password: fibers arrangement, dermis of fish Acestrorhunchus pantaneiro, skin, morphometry

Introduction

The aquiculture is specially, the fish growth. This culture has been recently introduced in Brazil. This situation can be justified by the geogephic situation from the country, which has the third hidric potential on earth, formed by the

basins: Amazônica, Tocantins-Araguaia, Platina, e do São Francisco, involving an aproximately area of six milions nine hundred thousand square meters in the Brazilian Territory.

The " dog" fish (Acestrorhunchus pantaneiro – Menezes, 1992) from the Characidae family and sub family Acestrorhynchinae is a representant found in all rivers in the Paraguai basin and doesn´t belong to the many economic important fish in the State of Mato Grosso do Sul what justify such a small number of works on this species.

The "dog" fish is a pelagic icitofaga species from open waters. It is a fish with a long body, redish coloration, and fast swimmer, being found specially in calm waters surface, as bays and dead arms from the rivers. It has got different aerodinamic forms, as well as, mouth position, teeth kinds and head shape, turning it possible to this meat eater fish to use different strategies end consumption of many different kinds when it is abundant in the region, feeding itself preferivelmente of small fish and sweet water shrimps.

Their reproduction is in the "pantaneira" plain, in the flood period, and do not go further than 25 cm lenght (Resende et al., 1996).

This fish skin study will contribute with more information to its biology as the skin is the first orgain to be in contact with the enviroment and it also gives very important infomation about the animal.

The fish dermis in general are different from mammals as they are covered by thick epidermis, having or not scale and not presenting sebaceous glands. The dermis is composed by thick colagen sheaf, paralely disposed perpendicular to the surface in many fish species (JUNQUEIRA, 1983)

The teleosteos dermis organization shows that this skin layer is constituded by a conjunctive tissue in which there are colagen fibers predominance. This skin shows in the majority of times two sublayers: the more superficial one, located under the basal membrain, which is made of a narrow conective conjunctive tissue piece (superficial dermis) the internal layer is composed by conjunctive tissue modelated and dense in which colagen fibers are showed oriented in only one direction (compact dermis, deep dermis or reticular dremis)

Junqueira et al. (1982) & Montes (1996) utilized the Picrosirius polinization to identify the colagen types in many fish skin. These researchers showed that this

methodology can be used to study the disposition and orientation of the colagen fibers.

The colagen is the most important protein to the tanning, as it the one that reacts to the curtents agents. This protein is resistent to many proteolitic enzims including tripsin and quimiotripsin, however some anaerobic bacterias excretate proteolitic enzims capable to attack the native collagen.

Collagen absorbs and keeps huge water quantities, due to the presence of certain polar groups reactives in the molecule which permit the interaction with water molecule.

The intumesciment is a result of the association of water molecule with reactive groups of colagen molecule, and during the tanning these respective groups react with the curtents, loosing the capacity of keeping water.

Objectives

With the intention of knowing the three regions of the dermis (dorsal, lateral line and ventral) from the "dog" fish (Acestrorhunchus pantaneiro – Menezes, 1992) a study was realized a study to make possible a better usage fo this species which is greatly found in Paraguai River bacia/MS and in many cases both skin and fish are disposed by local fishers.

Materials and Methodology

In this study 06 (six) adult species were used, males and females of Acestrorhunchus pantaneiro, collected in Correntoso stream, Rio Negro Region, Research Institute – IPAN/UNIDERP/MS, located in Sante Emília Farm, Aquidauana Pantanal ($j = 19^0 30' 22".00$ e $\lambda = 55^0 36' 40".8$).

The fish in adult phase, were collected by with n.º 12 net, not considering sex. Right after were submited to a temperature of 4OC for 30 minutes, this procedure was realized with the objective of turning the animal to a non responsive action, and after, they were sacrified by spine medule destruction.

The used samples in the experiment were measured in what concerns to total lenght (Lt in centimetrs: the distance from the top of the nose until the caudal

padle), using the ictiômetric with centesimal scale; they were weight (Wt: in grams) in weigthing machine with 1 g sensibility.

Histological Analisys

Fragment samples from the skin dorsal region, lateral and ventral line were taken out and put in Formol 10% solution tamponated for 24 hours. After fixation, the fragments were processed according to common methodology and included in parafin. The 5-7µm esperssura cuts were submited to Hematoxilina-eosina (HE; Luna, 1968) coloration, and by Masson (Michalany, 1980) tricomic to describe the general skin histology. The histologis analisys was realized in Histopatology Laboratory at UNIDERP.

Quantitative Methodology

Morphometric analisys were realized in the compact dermis of "dog" fish to measure its thickness. In the corresponding laminas, were analyzed 02 cuts obtained from the dorsal and ventral regions; in each cut were evaluated 3 different regions (median and lateral) totalizing end of 24 measures, with an 10x tambor ocular (with removable filamento) help.

Before the dermis measurements were done, it was realized an ocular calibration with a special lamina (Carl Zeiss) provided by divisions of 0,01mm (10? m), intending to transform the ocular divisions in micrometres. The measured values were multiplied by the micrometric coeficient of each objective and expressed in micrometres (0,01mm (10? m). All the obtained values in the morphometric analisys were realized in the Histopatology Research Laboratory.

Results

The dermis from the dorsal region is narrow with colagen fibers in medium size and paralels to the epidermis. It was observed that among the horizontal colagen fibers vertical fibers that initiate in in the dremis surface can be seen (near to the frouxa dermis) and these fibers go until inferior region of it. The lateral region dermis is thicker and the colagen fibers show themselves in desorganized location, waved and from spaces to spaces vertical fibers were observed.

The ventral dermis region showed itself narrower in relation to the lateral line dermis. These ones are desorganized, waved and there are little vertical fibers.

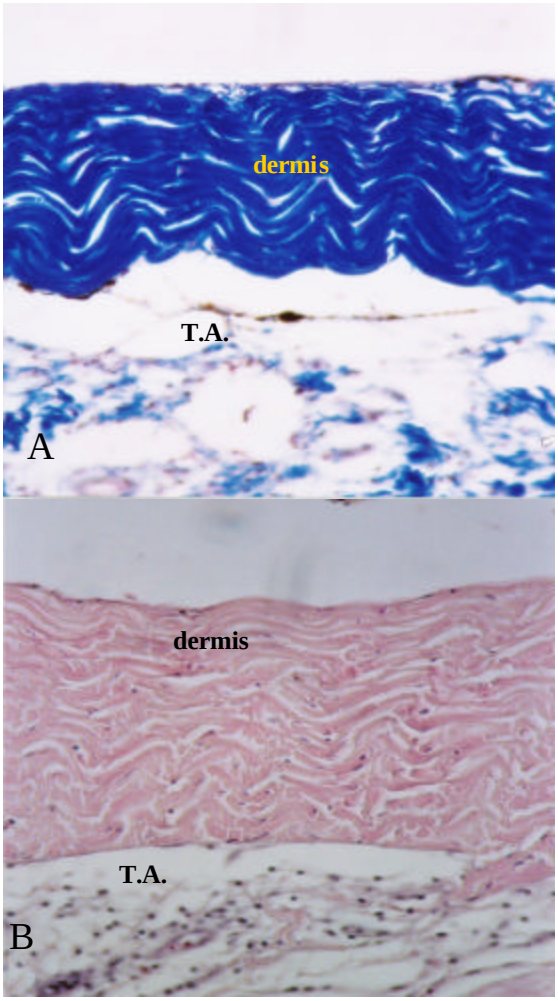
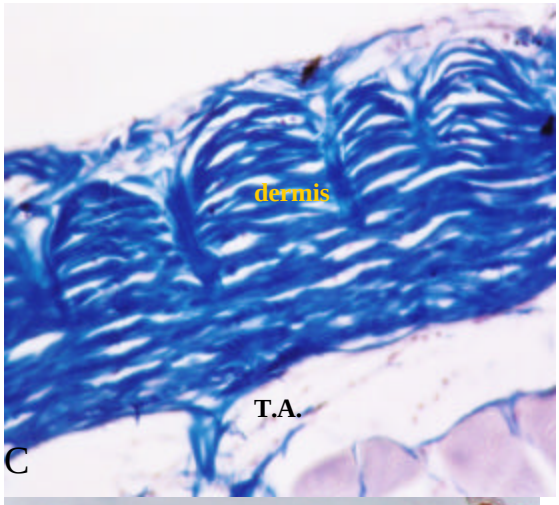


Fig. 1 - Peixe cachorro. Região dorsal. Observar a derme e tecido adiposo (TA). 400XM

Figure 1 – Dog fish. Dorsal Region.Observe the dermis and fat tissue (TA). 400XM

Dog fish dorsal region 400xM

Coloration: (A) TM e (B) HE.



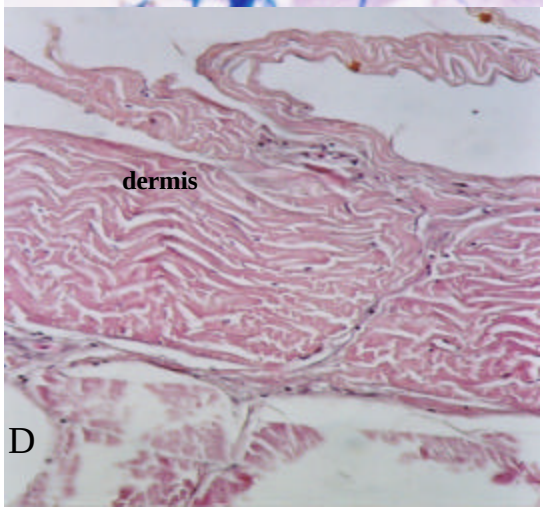


Figure 2 – Dog Fish. Lateral Region. Observe the fat tissue TA). 400X

Coloration: (C) TM e (D) HE.

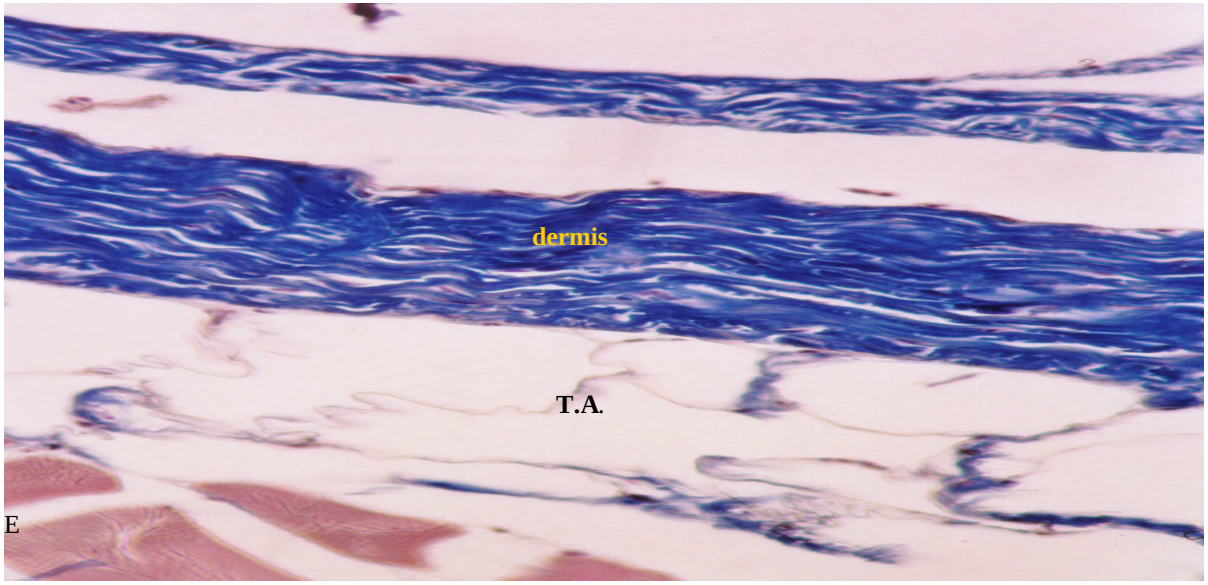


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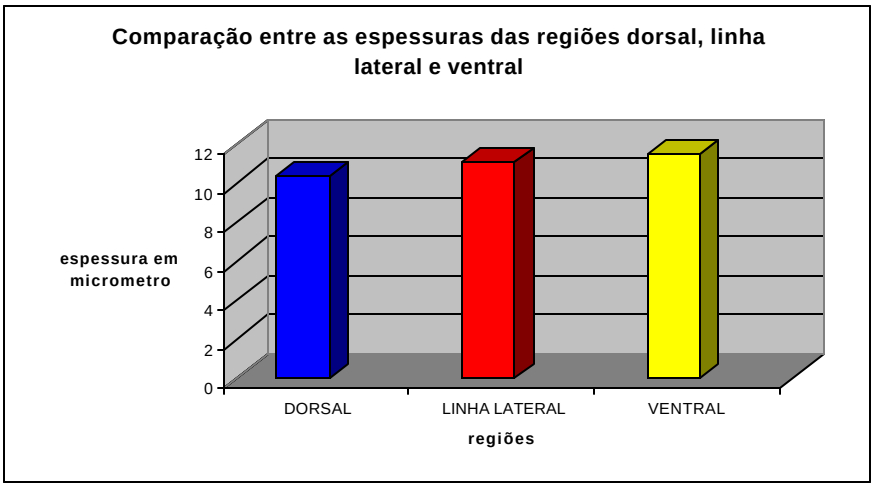
Figure 3 – Dog Fish. Ventral Region. Observe the dermis and fat tissue (TA). 400X

Coloration:(E) T

Morphometric data:

The measurements were compared to the statistics using the Newman-Keuls test for different averages comparisons. Three regions were analyzed: dorsal, ventral and lateral line. Using a significance level of 5% results were found and sre registered in table 1, where averages followed from the same letter do not differ one from each other.

REGIONS	THICKNESS(? m)
VENTRAL	11,5a
DORSAL	10,4a
LATERAL LINE	11,1a



Comparison between the dorsal, ventral and lateral line.

Discussion

The skin fish is divided in two layers: the epidermis and the dermis (papilar and compact). The epidermis functions, according to many authors (Van Oosten, 1957; Ingran, 1980; Mittal & Banerjee, 1980; Whitear, 1986; Mittal et al., 1994) are: act as a protection barrier not allowing the organism invasion by different patogens; regulate the ions fluid permeability, reduce the water and body atrict, making it easier, in this way, the animal movements and protect the corporal surface from mechanic abrasion. These two last functions are related to the mucous production of the epidermis mu cous cells.

The fish histologic skin structure is different among the species and with the same individual, as it can be observed in the Mandi skin (Pimelodus maculatus) and Jurupoca skin (Hemissurubim platyrhinchus).

The escama or skin fish compact dermis, is similar in different species, as these are composed by a dense conjunctive modelated tissue and the predominant colagen fibers generally are oriented and thick. In relation to the "dog" fish skin dermis it was different a little in the three compact dermis (dorsal, lateral and ventral)

In the "dog" fish the compact dermis of three analyzed regions is divided is layers: superficial, medium and inferior. The dorsal dermis is narrow and in the superficial layer the colagen fibers are narrower, waved and paralels. Besides the longitudinal colagen fibers thick vertical fibers were observed, these fibers cross all the layer thickness

In the medium layer until the inferior layer the longitudinal fibers were thicker and with no ondulations. In the lateral line region the observed structure in the colagen fibers was different in relation with the described to the dorsal region,

because the longitudinal fibers were more desorganized very ondulated and the vertical fibers in lees quantity.

And in the ventral region the fibers were thinner desorganized since the superior layer until the inferior one and the vertical fibers in less quantity.

Souza et al. (2002b) analyzed the "Tilápia" from the Nile River skin (Oreochromis niloticus) and related that it presents a dermis formed by a thick colagen fibers layer and disposed in paralel to the surface, making a thick conjunctive tissue. The compact dermis shows a superficial and a deep one. In the superficial dorsal dermis, the colagen fibers are thin, waved and paralels, and from the point that they become distant from the superficial layer the colagen fibers sheat become thicker, closed and interlaced. In the most superficial part, ther are transversal colagen fibers. So, the way the colagen fibers are disposed in the dermis it is possible a good connection between them, consequently the leather has a bigger resistence.

According to Junqueira et al (1983) it is interesting to realize the histologig study of the colagen fibers architecture in the fish compact dermis, as these dermis are the principal responsable component for the rough matetial estabilization during the tanning process, generating the leather. The skin resistance varies according to its distribution and colagen fibers distribution and the composition of dermis colagen fibers dispose, the curtimento technique used, the fish species, as well as the skin thickness.

Through morphometric analysis measurements were realized (lateral and median) in the compact "dog" fish dermis using the ocular 10x with re movable filament. Three regions were analyzed: dorsal, lateral line and ventral and the thickness of the ventral region was realized (11,5µm) and dorsal (11,1µm) are similar but different from the lateral line (10,4µm) that showed to be thicker. (Picture: 4, Chart 01).These results were seen through measurements done in the "dog" fish compact skin and the dermis thickness in the ventral region and line were, however they showed themselves thicker in the dorsal region..

According to Machado (2001), the dermis thickness in the dorsal region in the fish "pacu prata"(Mylossoma sp), "piau"(Leporinus macrocephalus) and "piraputanga"(Brycon hilarii) are very little different, but the "piau" and "pacu" show as much as the same thickness in the ventral dermis and the "piraputanga" a thinner one in relation to the other above mentioned.

As the skin thickness is important for the curtimento process, the "piau" and the "pacu" skin are more apropriate to this process than the "piraputanga" skin.

The aproximatlely skin thickness from the dorsal dermis of escama fish: pacu (20µm), piau (22 µm), e piraputanga (17 µm) and the average of the ventral dermis is around 15 µm for the "piau", 18 µm for the "pacu" e 8 µm for the "piraputanga".

Hertwig et al., (1992), describing the density and thickness from the compact extract of T. Steindachneri related that the great quantity of colagen fibers in the dermis give a tense resistance, providing to the skin a similar consistence to the cattle leather, apropriate to resist to pressions generated when the animal body is distended. The compact dermis from the T. Steindachneri, in this way, is apropriate mechanic protection to the animal and facilitate its movement in the aquatic enviroment.

Conclusion

From the morphologic poin of view the dog fish skin (Acestrorhunchus pantaneiro – Menezes, 1992) studied, it has got an structural organization common to the one found in the teleosteus.

This species dermis in relation to other fish species showed differences between the three regions and besides that the dremis from the ventral region have the same thickness of the lateral line, diferring very little in relation to the dorsal region dermis.

Through the coloration by tricromico de Masson it´s possible to notice differences in relation to colagen fibers disposition in compact dermis, as these ones show themselves more orgaized in the dorsal region in relation to the lateral line.

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EFFECT OF CRUDE OIL

ON RESPIRATORY AND LOCOMOTION BEHAVIOR

OF AMAZON FISH PIRARUCU (*Arapaima gigas*).

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Introduction

Large amount of oil has been extracted near the Urucu River, a tributary of Amazon River, and transported in tanks across the Solimões River to Manaus City to be refined. In such trip, there is always a risk of an oil spill. Although the effects of oil on marine and terrestrial environments have been largely documented, its effect on freshwater habitats are still unknown.

The toxicity of crude oil is related to thousands of organic compounds (water-soluble fraction), short chain hydrocarbons (volatile, short lived in the aquatic environment) and long chain hydrocarbons (persist on the top of the water column, physical barrier at the air-water interface)(Val and Almeida-Val, 1999).

To survive extreme periods of low dissolved oxygen or even anoxia, fish of the Amazon developed several adaptations to uptake oxygen directly from air or from the upper part of the water column. Pirarucu (*Arapaima gigas*) developed the ability to breathe air continuously. In case of a petrol spills, these animals must break through the oil slick to uptake oxygen.

While the role of environmental stressors, including toxic agents, on biochemical and pathological aspects of aquatic animals have been frequently described (Wester *et. al*., 2002), the use of behavioural theories and methods are almost unknown, what can be observed by very little works using behavioural indicators of animal well being (Shumway, 1999). Despite of this, Barton (1997) considered behaviour patterns as more immediately indicators of environmental stress. In the present study we investigated the role of oil exhibition on respiratory and locomotion behaviour.

Methods

Isolated fishes (length mean = 15.30 ± 1.30 cm) kept in 50 litters tanks were submitted to a low amount of crude oil (0.45 ml/L, oil slick = 0.01 cm). The air breathing respiration and locomotion activity were monitored before, 20 min., 2, 6 and 24 hours after oil exposition using a video camera. Each animal was filmed for 15 minutes in each time session. Dates were obtained by registration of the frequency of air breath respiration in each session and the time spent in locomotion in the correspondent periods. Statistical analyses was done by two way ANOVA followed to Tukey test.

Results and Conclusion

The frequency of air breathing respiration decreased significantly 20 minutes after oil exposition and stayed low until the end of the experiment (Fig. 1). The time spent in locomotion also decreased significantly after 6 hours the animals were exposed to crude oil and stayed lower until the end of the experiment (Fig. 2).

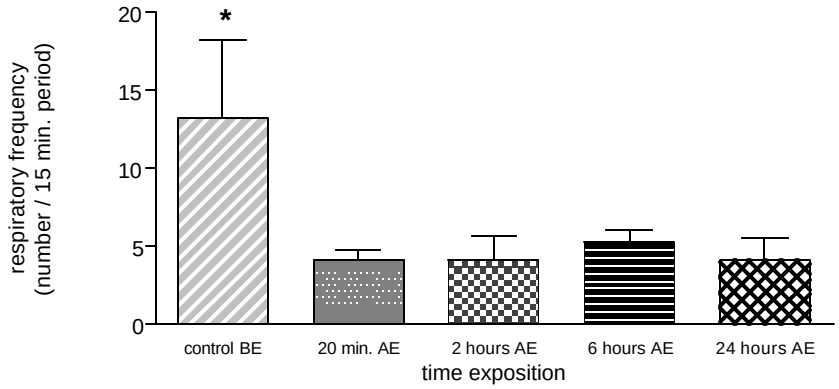

Figure 1. Respiratory activity of isolated pirarucu submited to crude oil. BE = before oil exposition; AE = after oil exposition (P < 0.001).

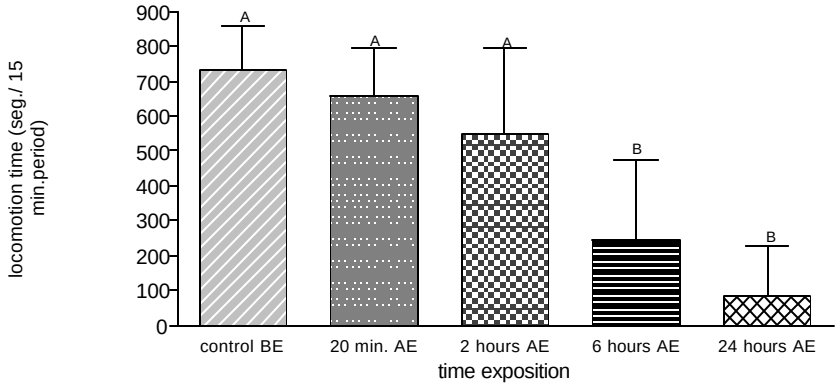

Figure 2. Locomotion activity of pirarucu submited to crude oil. Different letters indicate statistical difference among time exposition (P < 0.01).

The frequency of air breathing respiration is a very sensitive parameter and the decreased found in such behaviour must promote significant physiological and behavioural accommodation on fishes exposed to such stressor, including locomotion activity. However, with the protocol used is not possible to identify if the response obtaind is correlated to physical effects, chemical effects or both.

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THE FLOOD PULSE CONCEPT

AND ITS RELATION TO FISH BIOLOGY IN THE PANTANAL.

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The main challenge for sustainable use of the Pantanal means that we need to understand the main ecological process that makes Pantanal to work. This is the flood pulse concept thought by Junk et al., (1989), "*the principal driving force responsible for the existence, productivity and interactions of the major biota in river-floodplain systems is the flood pulse*"; '*a predictable pulse of long duration engenders organismic adaptations and strategies that efficiently utilize attributes of the aquatic/terrestrial transition zone*".

Comparing available fish production data, from 1995 to 2000 (Catella et al., 1998; Catella & Albuquerque, 2000 a,b; Catella et al., 2001; Catella et al., 2002; Campos et al., 2002) and from 1979 to 1985 (Silva, 1986), a decreasing of fish production can be viewed between this two periods, being something 7 times low now than in the past. What would be the causes for this decrease? An answer to this question needs to understand the flood pulse in rivers with large floodplain as Upper Paraguay river basin, particularly to Paraguay river and its tributary as Taquari river.

Welcomme (1979; 1985), Junk et al., (1989), Junk (1980, 1997, 2001) wrote a lot about the flood pulse concept in rivers with very well developed floodplains in South American tropical environments. The flooplain, due to their characteristics of being periodically flooded, acts as a bioprocessor. Inorganic nutrients carried from the river to the floodplain are used by different communities of primary producers during the terrestrial and aquatic phases to produce organic material that is used by aquatic and terrestrial consumer communities, resulting in high primary and secondary productions. Internal cycles of organic material and correlated nutrients among terrestrial and aquatic

phases results in nutrient accumulation in the floodplain that makes the system to work in a trophic level higher than expected only by the nutrients carried by the water rivers (Junk, 2001). In this way, biological and biogeochemical processes in the system river-floodplain are described by the floop pulse concept, which considers the lateral exchanges between the rivers and their floodplains as well exchanges between terrestrial and aquatic phases in this same plain. The importation of dissolved and particulated organic material from the headwaters have few importance, due to a small amount and low quality in comparison with the organic material produced in the floodplain.

South American rivers with well developed floodplain have a very diverse and abundant fish fauna that feed on organic detritus that is not known in other places. The most known of these species are the curimbatá (*Prochilodus lineatus*) in the Paraguay-Paraná river and jaraquis(*Semaprochilodus spp*), curimatãs (*Prochilodus nigricans*) and branquinhas (Curimatidae family) in the Amazon river basin. These species feed on the organic particulated material produced by the decomposition of terrestrial vegetation during the flooding period. At the same time, during the flooding, the flowers, fruits, seeds and even the stems and leaf of the riparian and flooded vegetation are fed by herbivorous and omnivorous fishes, belonging to Myleinae, Bryconinae, Thiportheinae and Characinae sub-families. These group, together organic detritus feeding fishes are the base of food chain that support large carnivorous fishes as dourado "*Salminus brasiliensis*", pintado, "*Pseudoplatystoma corruscans*" and others and a particularly interesting group, the piranhas (Serrasalminae) and a rich fish-eating species like cormorants, jabirus, wood storks, herons, caimans, otters and giant-otters.

Finally, the flooded vegetation, terrestrial or aquatic, act as a filter retaining in their roots, stems and leaves the detritus and other organic debris, on which develop a rich community of algae and microorganisms, which are used as food by fish larva, alewives and small sized-fishes. During the flooding season, abundant insect community develops on the macrophytes, which is got by insect feeding fishes.

Flood pulses are responsible for the fish richness and for fish production, more high the flood, more fish production and lower the flood, less fish production. In the dry season, terrestrial vegetation grows again, supported by the nutrients that comes trough the flooding water and from the decomposition of aquatic vegetation of the previous inundation. In this way, the system can incorporate and use the organic material in a very efficient way, explaining the richness and

diversity of rivers with floodplains, even the river drains poor soils as happen in the Upper Paraguay basin. The flooding also allows development of the aquatic vegetation that gives shelter and food for fishes. The flood pulse also explain the abundance of animals that depends on fish for their survival, as caimans, fisheater birds (cormorants, jabiru, woodstork, spoonbill, herons, ...), giant otters and neotropical river otters.

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