01.001 Production optimization and partial characterization of proteases produced by Streptomyces capoamus isolated from Paullinia cupana rhizosphere.

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Resumo

Production optimization and partial characterization of proteases produced by Streptomyces capoamus isolated from Paullinia cupana rhizosphere. Coelho, L.V.A.*, 1Patriota, L.L.S., 1Paiva, P.M.G., 2Silva, L.A.O., 1Napoleão, T.H. 1Departamento de Bioquímica-UFPE, Recife/PE. 2Departamento de Antibióticos-UFPE, Recife/PE. Introduction: Proteases are a group of hydrolytic enzymes with important market value due to their several applications in the pharmaceutical, cosmetological and biomedical industries. They are extensively found in microorganisms. Actinobacteria of the genus Streptomyces have gained prominence in the production of several metabolites with biotechnological potential, such as hydrolytic enzymes. Objective: To evaluate the best conditions for the production of proteases by Streptomyces capoamus and to achieve partial purification of the produced enzyme. Methodology: The isolate used (171X) was collected from the rhizosphere of Paullinia cupana in Maúes, Amazonas. To stimulate the production of proteases, the following carbon sources (1%, w/v) were evaluated: gelatin, wheat flour, soybean meal, milk whey, and Manihot esculenta (macaxeira) leaf powder. The liquid fermentation in protease inducing medium was performed at 37 °C for 7 days. The proteolytic activity assay utilized azocasein as the substrate. After determination of the best carbon source for protease production, a central composite rotational design (CCRD) was adopted, with temperature (30, 34 or 40 °C), source concentration and pH (3.4 to 8.5) as variables for optimization of production conditions. The metabolic fluid obtained using the better conditions was dialyzed against 0.1 M Tris-HCl pH 8.0 and loaded onto a DEAE-Sephadex column equilibrated with the same buffer. The adsorbed proteins were eluted with 1.0 M NaCl. Results: The isolate 171X was able to use all the carbon sources and highest specific proteolytic activity (12.31 U/mg) was detected in the metabolic liquid of the microorganisms incubated with M. esculenta leaf powder. The CCRD assay revealed that the best conditions for protease production were: 2.0% (w/v) of M. esculenta powder, 40 °C, pH 7.5. In the chromatography, a single peak with protease activity was obtained, corresponding to adsorbed proteins that were eluted with 1.0 M NaCl. After dialysis, the peak showed specific activity of 13.43 U/mg, corresponding to a purification fold of 1.1. Conclusion: S. capoamus 171X is capable of producing proteases using low cost carbon sources, being the best results obtained with M. esculenta leaf powder. The best fermentation conditions were determined and the enzyme purification process was started successfully.

Palavras-chaves: Proteases, Streptomyces capoamus, optimization

01.002 HEPATIC AND GILL ISOENZYMES OF LACTATE DEHYDROGENASE AND MALATE DEHYDROGENASE OF Astyanax bimaculatus (LAMBARI) AS BIOMARKERS OF ENVIRONMENTAL IMPACT

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Resumo
Introduction: The waters of the Una river basin are used mainly for public supply of the municipality of Taubaté (SP). Studies have shown that water quality is below the class II river standards in some parts of the basin, suggesting the need for a more rigorous monitoring of this basin. Biochemical markers have been used for the early detection of sublethal metabolic alteration in fishes, and they are useful for assessing environmental pollution. Enzymes of energy metabolism such as lactate dehydrogenase (LDH) and malate dehydrogenase (MDH) of fishes are candidate markers of environmental pollution. Both enzymes are expressed as isoenzymes or isoforms. Changes in the levels of LDH and MDH isoforms in the liver and gills of Astyanax bimaculatus due to deterioration of water quality of the habitat has not been investigated.

Objective: To evaluate the potential of expressed activity of isoforms of LDH and MDH of the hepatic and gill tissues of Astyanax bimaculatus as biomarker of pollution of the Una river basin.

Methodology: Fish were collected from November 2013 to February 2014 at three points, P1, P2 and P3, all located in Una river basin, municipality of Taubaté (SP). P1 was localized at Itaim river, inside the experimental farm of the University of Taubaté, where there was less human activity; P2 was in a ditch located at the edge of the Estrada Municipal de Remédios, adjacent to a rice field; and P3 was located in a stream close to Estrada Municipal Dr. José Luiz Cembranelli, near cattle grazing pasture. The fish capture was authorized by SISBIO (No. 41097-1) and the Animal Ethics Committee (CEUA / UNITAU No. 013/2013). Hepatic and gill tissues of 10 and 12 specimens were used, respectively. The tissues were homogenized in 50 mM Tris-HCl buffer (pH 7.4). The homogenates were sonicated and centrifuged (10,000g, 10min) and the supernatant was used for electrophoresis (7% polyacrylamide gel) under non-denaturing conditions for 3.5 hours (4oC, 100V)<Physiol Zool. 61: 114, 1995>.

Results: The gill LDH of all fish, independent of collection site, presented two electrophoretic bands (two isoforms) clearly distinguishable, one of them with high activity and the other with low activity. However, gill MDH presented three isoforms clearly distinguishable in the fish from P1 and P3, and two isoforms in those from P3. Two isoforms of hepatic MDH were identified in the specimens collected at P2, while only one isoform was detected in those from P1 and P3. Specimens from P3 presented two or three isoforms of hepatic LDH. However, only one isoform of this LDH was clearly detected in specimens from P1 and P2.

Conclusion: The activities of the hepatic isoforms of MDH and LDH and the gill isoforms of MDH were modulated according to the environment, showing that these enzymes of Astyanax bimaculatus have potential as a biochemical marker of environmental change.

Palavras-chaves: Environmental impact, Isoenzymes, Una River, Water pollution, Yellow Tail Lambari

01.003 Foraging behavior of benthic and nektonic tadpoles in diurnal and nocturnal periods

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Resumo

Introduction: Usually differences in tadpole’s morphology are used to infer the resource they explore, such as the depth in water column. In this context, there are several proposals of tadpole’s classification in ecomorphological guilds. However, this relationship is hardly tested experimentally.

Objective: We aimed to test two hypotheses: (i) tadpoles with different morphotypes forage in different depths along water column; (ii) the period of the day influences where tadpoles forage along the water column.
Methodology: We chose species that represent two ecomorphological guilds: benthic (*Physalaemus nattereri*) and nektonic (*Scinax fuscovarius*). Tadpoles were acclimatized for 24-hour in aquaria with different depths. We covered the bottom with clay and placed artificial plants in standardized places. Each aquarium (120x60x40cm) represented a replicate, with 10 tadpoles of the same species and similar size (developmental stages: 29 and 35). In total, we tested 12 replicates for each species. We established three treatments along the water column for each aquarium: bottom of the deepest area, middle of water column and bottom in the shallowest area. We offered a commercial algal-based food in flat glass plates (15x15cm) simultaneously in the three treatments for 45 minutes, at morning and night. The plates were digitized (after drying naturally) and the percentage of food consumed was quantified (software Image J). We analyzed six plates per replicate (three diurnal and three nocturnal), totaling 72 plates per species. A factorial repeated measures ANOVA was used to test each hypothesis separately. All procedures were approved by Comissão de Ética no Uso de Animais (CEUA nº179/2017) of IBILCE.

Results: We found differences in food consumption due to interaction between species and microhabitats during the day (F=4.24, p<0.05) and night (F=6.25, p<0.01). In both periods, tadpoles of *S. fuscovarius* consumed food similarly in the three depths (day: bottom/deep = 2.50±2.06%, middle = 2.12±1.80%, bottom/shallow = 2.12±1.76%; night: bottom/deep = 4.12±3.47%, middle = 4.46±5.10%, bottom/shallow = 3.55±4.22%). Tadpoles of *P. nattereri* consumed eight times less food in the middle of the water column in comparison to the others treatments in the morning and nineteen times less at night. Also, tadpoles of *S. fuscovarius* consumed five times more food than *P. nattereri* in the middle depth during the day, and six times more at night. Both species consumed more food at night (*P. nattereri*: F=4.00, p<0.05; *S. fuscovarius*: F=5.45, p<0.05).

Conclusion: The present study complements with empirical data the inferences about the relationship between tadpole’s morphology and microhabitat choice behavior. We verified that the preference to explore food resources differs between morphotypes. Tadpoles of *S. fuscovarius* are more generalists, while *P. nattereri* forage preferentially on the bottom, indicating that their morphology is more restrictive than those of nektonic tadpoles. In addition, both species were more active and consumed more food at night.

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Bibliography: 1Herpetologica. 16:183,1960

Palavras-chaves: Anura, Ecomorphology, Experimental ecology, Guilds, Resource use
Introduction: Iron oxides adsorb environmental contaminants such as glyphosate. Glyphosate, ingredient of Roundup® is widely used in Brazil, but it has many toxic effects. Objective: This work aim to verify possible metabolic and behavioral changes promoted by ferrihydrite isolated or associated with herbicides and if it acts as a protector against the effects of herbicides.

Methodology: Male adult Wistar rats (270-290g) and females (240-260g) were used. Behavioral evaluation was performed based on Guidelines OPPTS870.6200 (EPA, 1996) and OECD424 (OECD, 2001), analyzing behavioral, autonomic alterations, respiratory frequency, and motor changes. It genre constituted 6 groups (3 rats/group), which received, via gavage, 1000 mg/kg body weight of ferrihydrite (F), glyphosate (G), Roundup® (R), ferrihydrite associated with glyphosate (FG), ferrihydrite associated with Roundup® (FR) or water (control C), and were observed post-administration for 15, 30, 45 min, 1, 2, 4, 24 h, and once a day until 14 days. Another set of rats was carried out for daily evaluation of food intake and body weight (b.w.) receiving the same compounds (5 rats/group) during 4 days (one dose by day). After, all rats were submitted to 18h of fasting, euthanized by decapitation, and the blood was collected to biochemical dosages. Data were treated by two-way ANOVA followed by SNK test (significance level 5%).

Results: All results of isolated ferrihydrite were not different from the control indicating no change in the parameters evaluated. The R and G groups showed several behavioral changes (hyperactivity, spasms, repetitive trunk, head movements) and autonomic disorders (diarrhea, polyuria, palpebral ptosis, exophthalmia), the FR group showed intense polyuria and diarrhea which were not observed in the FG group suggesting protective role of the nanoparticle over glyphosate but not over Roundup®. After 4 days of the daily administration of compounds, FG and C groups gained corporeal mass (FGm: +8.43±5.7 g, Cm: +7.4±1.7 g, 0% mortality) instead Gm group that lost body mass (-3.2±5.4 g, p<0.01, 80% mortality); high body mass loss and significant mortality (80%) were observed in both Rm group (-55.5±2.3 g) and FRm group (-40.9±0.0 g) showing protection of glyphosate but not of Roundup® by ferrihydrite. These changes were similar for females, however, with zero mortality. Food intake was significantly lower (p<0.05) in the Gm (4.9±0.6 g/100g b.w.), Rm (1.3±0.3 g/100g b.w.), Gf (3.4±0.7 g/100g b.w.), and Rf (2.1±0.5 g/100g b.w.) groups in relation to controls (Cm: 7.6±0.4 g/100g b.w.; Cf: 6.0±0.2 g/100g b.w.). Ferrihydrite was effective in preventing ingestive reduction in glyphosate groups (FGm: 7.0±0.4 g/100g b.w.; Gf: 4.9±0.5 g/100g b.w.) but not Roundup® groups (FRm: 0.3±0.1 g/100g b.w.; FRf: 2.9±0.4 g/100g b.w.). The plasma concentration of cholesterol and glucose were similar among all groups except for glycemia in the FRm (109.1±3.2 mg/dL) and FRf (101.4±4.7 mg/dL) groups that decreased significantly (p<0.05) compared to controls (Cm: 115.9±5.3 mg/dL; Cf: 122.0±3.5 mg/dL, respectively). Ferrihydrite did not change behavior, body weight, food intake, blood glucose and cholesterolemia during the time studied and was able to prevent changes caused by glyphosate.

Conclusion: The nanoparticle ferrihydrite showed no toxicity and was effective as a protector against the toxic effects of the glyphosate but not Roundup®.

Funding: PRONEX-CNPq-Fundação Araucária, PIBITI-CNPq.

Palavras-chaves: Behavior, Ferrihydrite, food intake, glycemia, Herbicide
Usually species that explore the same resources are morphologically similar, as they are exposed to similar selective pressures — e.g., tadpoles that inhabit the bottom of the water body typically have depressed bodies and narrow fins. Although many studies describe this relationship between morphology and resource use, the consequences of this interaction for species performance and growth, remains not tested experimentally.

Objective

Test the hypotheses that: (i) benthic tadpoles have higher growth rates at the bottom of the water column, (ii) morphology influences the feeding performance along the water column.

Methodology

We conducted an experiment with species that commonly coexist at the bottom of the water body and share resources: *Physalaemus cuvieri*, *P. nattereri* and *Rhinella schneideri*. We tested tadpoles individually in glass aquaria where food was offered in microscope blades at one of the following treatments: bottom, middle of the water column and close to the surface. Each treatment lasted 4 days with 12 replicates for each species. We measured the total length of each tadpole before and after the experiment to calculate the growth rate, and also the body and tail sizes to calculate a body/tail size proportion index. Every microscope blade with food was measured to determine the amount of food consumption. We excluded tadpoles that lost weight during the experiment from data set because we considered they were not healthy and did not respond well to laboratory conditions. We used an ANOVA two-way to test our first hypotheses and ANCOVA two-way to test our second hypotheses. The experiments were proceeded in accordance with Ethics Committee on the Use of Animals (121/2015).

Results

Tadpole’s growth rate differed among species (F=19.12; p<0.001) but not among treatments (F=2.36; p=0.1) — probably due to the low number of tadpoles of *P. cuvieri* sampled in each treatment (e.g. surface: n=5). Despite this, we observed differences in mean and standard deviation among treatments for each species. For example, for *P. cuvieri* the increase in total size was the least when tadpoles foraged close to the surface (0.04 ± 0.03%) as well as for *R. schneideri* when these tadpoles foraged in the middle of the water column (0.17 ± 0.03%). Growth rate of *P. nattereri* was similar among treatments (bottom: 0.19 ± 0.10%; middle: 0.15 ± 0.04%; surface: 0.15 ± 0.07%) When we analyzed tadpoles’ performance, we found differences in food consumption among species (F=20.90; p<0.001) and treatments (F=9.17; p<0.001). Also, morphology was related to tadpole’s performance — the higher the index (body/tail size proportion next to 1), higher the food consumption (F=12.24; p<0.001). — and influenced the feeding performance along the water column.

Conclusions

Our study indicates that morphological differences, as the body/tail size proportion, are related to variation in performance along the water column, which is, consequently, reflected on the tadpoles’ growth. Differences in feeding efficiency can be an important key for species to explore different niches.
Thus, although all these species are benthic, there are still differences in microhabitat use among them that can be fundamental for their coexistence.

**Funding**

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**Palavras-chaves:** anuran larvae, behavior, ecology

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**01.006 TOTAL MERCURY CONCENTRATIONS (HgT) IN HEMIODUS UNIMACULATUS FISHES FROM TAPAJÓS RIVER IN SANTARÉM, BRASIL**

**Autores**

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**Resumo**

**Introduction:** Tapajós River has for a long time, a history of goldmine activity using mercury (Hg). Thus the Hg in concentrations in Tapajós basin are higher than other areas in Amazon region. The discharged Hg may be methylated to methylmercury (MeHg), the most toxic Hg chemical form for humans. The MeHg is concentrated in fishes, which ones are consumed by riverine populations. This, it’s important monitoring Hg fish concentrations from Tapajós River fishes. This study analyzed HgT concentrations in a very consumed herbivorous fish knowned as charutinho.

**Objective:** To analyze HgT in fish of the species Hemiodus unimaculatus commercialized in the city of Santarém-Pará-Brazil.

**Methodology:** The fishes were bought in open fish market during the months of January and February of 2018. The weight and length were recorded. The HgT concentrations of 50 samples were performed in duplicate using a DMA 80 (Italy). The analyses quality control was performed using a certificates reference material BCR 463 (SIGMA). For statistical analyses mean and standard deviation were used.

**Results:** The weight of analyzed fishes ranged from 30 to 50g and the HgT concentration was 0,03±0,01 ppm.

**Conclusion:** The herbivorous species as Hemiodus unimaculatus, showed Hg concentrations low than carnivorous species, however these Hg levels should be monitored frequently, as besides gold mine activities, some hydroelectric power plant will be built in Tapajós River, increasing the Hg contents in that environment.

**Funding:** CAPES (PVE), PIBIC/CNPQ, UFOPA.

**Palavras-chaves:** concentrations, herbivorous fish, mercury, monitoring, tapajós river

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**01.007 CONCENTRATIONS OF TOTAL MERCURY (HgT) IN FISH SOLD IN SUPERMARKETS IN SANTARÉM, BRAZIL.**

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Resumo

**Introduction:** The Hg is one of most toxic chemical agents for life. It's methylated chemical form, methylmercury (MeHg), crosses biological barriers, being accumulated in all human tissues. For humans, fish diet is the main way for contamination, since 95% of fish HgT is MeHg. Following the Brazilian legislation, fish HgT concentration (ppm) of 0.5 (no carnivorous) and 1.0 (carnivorous) is considered safety for human diet. Thus, it's important to know if fishes sold in supermarkets are in accordance with the law.

**Objective:** To analyze HgT concentrations in fishes sold in supermarkets in Santarém city, Pará state, Brazil.

**Methodology:** We analyzed 51 fish samples. Theses ones were analyzed in duplicated using a DMA 80. The quality control was performed through the certificated reference material BCR 463 (SIGMA). The analyzed species were *Carcharrhinus spp* (n=4), *Theragra chalcogramma* (n=7), *Urophycis brasiliensis* (n=8), *Brevoortea pectinata* (n=18), *Paralichthys brasiliensis* (n=7) e *Micropogonias furnieri* (n=8). Statistical analysis was done using the mean ± standard deviation.

**Results:** The HgT concentrations (ppm) in *Theragra chalcogramma*, *Urophycis brasiliensis*, *Paralichthys brasiliensis*, *Micropogonias furnieri*, *Brevoortea pectinata* were (0,01 ±0,00), (0,06 ±0,02), (0,05 ±0,02), (0,12 ±0,08), (0,04 ±0,01) respectively. The HgT concentrations in *Carcharrhinus spp* was1,4 ±0,10.

**Conclusion:** The HgT concentrations in almost all analyzed fishes were in accordance with legislation. However, *Carcharrhinus spp* showed HgT concentrations 47% above the legislation limit. Thus, this fish should not be sold.

**Funding:** CAPES (PVE), CNPq (PIBIC), UFOPA.

**Palavras-chaves:** concentrations, contamination, fish, mercury, sold

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01.008 THE OXIDATIVE PROFILE OF BRAZILIANS TORTOISE VARIES ACCORDING TO SEASONALITY

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Resumo

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**Resumo**
Title: THE OXIDATIVE PROFILE OF BRAZILIANS TORTOISE VARIES ACCORDING TO SEASONALITY. 1Castro, G.S.*, 1Cornacini, F.H., 1Pereira, L.R., 2Silva, D.H. 1Bonini-Domingos, C.R., 1Department of Biology, 2Department of Chemistry and Environmental Sciences, UNESP/IBILCE, São José do Rio Preto/SP.

Introduction: Reptiles are animals that have dependence on the environment for maintenance of temperature and regulation of their metabolic activities. Thus, its metabolic homeostasis is constantly challenged in response to environmental variations such as temperature difference and rainfall patterns. The concept of oxidative stress refers to the imbalance between the production of reactive oxygen species and the antioxidant defenses in organisms. In Brazil, the species that represent the Class Chelonia, SubOrder Cryptodira are: Chelonoidis carbonarius e Chelonoidis denticulatus.

Objective: Analyze the effect of seasonality in the oxidative response of male and female of Chelonoidis carbonarius e Chelonoidis denticulatus.

Methodology: Eleven individuals, six C. carbonarius (three males and three females) and five C. denticulatus (two males and three females) were assessed from the Municipal Zoo Missina Palmeira Zancaner, from Catanduva/SP. Blood sampling was performed at the zoo ambulatory where the animals were sanitized and the puncture was performed in the occipital vein, twice a year, in the dry season and in the rainy season, after approval of the Committee on Ethics in the Use of Animals (protocol nº164/2017). The levels of oxidized biomolecules were determined spectrophotometrically by plasma dosage of thiobarbituric acid reactive species (TBARS) expressed in ng/mL of plasma. As antioxidant marker, the Trolox equivalent antioxidant capacity (TEAC), expressed in mM, was analyzed in plasma. Statistical analysis was performed in groups with at least three individuals using General Linear Models (GLM). We applied GLM with ANOVA design for comparison between the sexes of C. carbonarius and GLM with repeated measures ANOVA design for comparisons between seasons. Data were expressed as mean ± standard deviation. We considered P<0.05 as statistically significant.

Results: For the C. carbonarius species, in the dry season, TBARS levels were lower in males than in females (696.4±283.4 versus 1295±201.3; P=0.0406); while the antioxidant capacity had the opposite effect (2.12±0.05 vs 1.96±0.08, P=0.0469). In the rainy season, males and females did not present differential expression for both TBARS (724.5±659.6 vs 365.8±96.94, P=0.413) and TEAC (1.996±0.14 vs 1.576±0.34, P=0.12). As for seasonality, no difference was found for TBARS between dry and rainy seasons (995.5±394.6 vs 545.1±473.1; P=0.16); meanwhile, the antioxidant capacity was higher in the dry season than in the rainy season (2.04±0.1 vs 1.78±0.32, P=0.04). For C. denticulatus, it wasn’t possible to perform a comparison between males and females because of the irregularity of pais (male/female). As for seasonality, TBARS showed no difference between seasons (1023±259.3 vs 638±540.2; P=0.17) while TEAC was higher in the dry season (2.10±0.13 vs 1.86±0.1; P=0.06).

Conclusion: The results suggest an adaptive response in the dry season, independent of the species, with the increase of the total antioxidant capacity, which possibly avoided a more pronounced increase in the levels of oxidative lesions, except for females of the C. carbonarius species.

Funding: Capes

Palavras-chaves: antioxidant capacity, C. carbonarius, C. denticulatus, oxidative lesions, seasons