Hematology, biochemical profile and thyroid hormones of four species of freshwater stingrays of the genus Potamotrygon

Hematologia, perfil bioquímico e hormônios da tireoide em quatro espécies de arraias de água doce do gênero Potamotrygon

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Abstract

Potamotrygonidae is a family of freshwater stingray endemic in South America. We determined the hematological and serum biochemistry parameters of *Potamotrygon motoro* and *Potamotrygon falkneri* from Parana Basin and *Potamotrygon orbignyi* and *Potamotrygon scobina* from the Amazon Basin. Blood was collected from 55 specimens of *Potamotrygon* sp., and these parameters were evaluated: red blood cell count, hematocrit percent, hemoglobin concentration, leucocytes count, trombocytes count, total protein concentration, albumin, uric acid, urea, creatinine, triglycerides, cholesterol, high-density lipoprotein HDL, calcium, phosphorus, calcium/phosphorus relationship, creatine kinase CK, gamma-glutamyl transpeptidase GGT, Aspartate transaminase AST, alanine transaminase ALT, globulin, albumin/globulin relationship, alkaline phosphatase AP, sodium, chloride, potassium, triiodothyronine T_3, and thyroxine T_4 hormones. The hematological indices were similar for all species, except for levels of heterophils in *P. orbignyi* and *P. scobina* that were higher. Calcium, phosphorus, calcium/phosphorus relationship, sodium, potassium, chloride, AST, ALT, GGT, CK, albumin/globulin relationship, uric acid, creatinine, urea, triglycerides, HDL, T_3, T_4 levels had no significant difference between the species. These results suggest that there is low influence of habitat and feeding habits. AP, total protein, albumin, globulin, glucose, and total cholesterol had significant differences between the studied species. These results support the theory that stingrays migrated from the Atlantic Ocean, adapting to different conditions over time and placed themselves geographically distant from each other.

Keywords: Elasmobranch. Freshwater stingray. Thyroid hormones. Serum biochemistry.

Resumo

Potamotrygonidae é um grupo de raias endêmicas da América do Sul distribuídas nas principais bacias hidrográficas brasileiras, mas informações sobre suas variáveis hematológicas e bioquímicas são escassas. Este estudo teve como objetivo determinar estas variáveis em *Potamotrygon motoro* e *P. falkneri* na Bacia do Rio Paraná, Estado do Paraná e de *P. orbignyi* e *P. scobina* do Rio Piririm, na Bacia Amazônica, Estado do Amapá. Foi capturado um total de 53 espécimes de *Potamotrygon* spp. para colheita de sangue e avaliações do hemograma e variáveis bioquímicas séricas. Os valores do hemograma foram próximos para as quatro espécies e os resultados com diferenças significativas entre *Potamotrygon falkneri*, *P. motoro, P. orbigni* e *P. scobina* ocorreram quanto ao número de eritrócitos e heterófilos. Apesar da diversidade geográfica os resultados apresentaram pouca interferência dos diferentes hábitats nas variáveis avaliadas. Os níveis de proteínas, globulinas, relação albumina:globulina, ácido úrico, creatinina, uréia, HDL-C, relação cálcio:fosfóro, sódio, cloreto, AST, ALT, creatinoquinase (CK), fosfatase alcalina, hormônios triiodotironina (T3) e tiroxina (T4) não diferiram entre as espécies de Potamotrygon. Porém, os níveis de albumina, glicose, colesterol total, triglicerídeos e gama glutamil transferase (GGT) apresentaram diferenças. Este fato sugere pouca interferência do hábitat nas variáveis avaliadas apesar da diversidade geográfica de origem das raias de vida livre.

Palavras-chave: Elasmobrânquio. Raia de água doce. Hormônios tireoidianos. Bioquímico sérico.

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Introduction

Genus *Potamotrygon* is found in the Amazon and Paraguay-Paraná rivers (VASCONCELOS; OLIVEIRA, 2011). However, there are important differences in the diet regarding different species and geographical distribution. *Potamotrygon motoro* from upstream Paraná River feeds on insects (SILVA; UIEDA, 2007); from the Curiaú River, on fish and crustaceans (VASCONCELOS; OLIVEIRA, 2011). *Potamotrygon falkneri* from Paraná River feeds on fish and crustaceans (BALLANTYNE; ROBINSON, 2010), and *Potamotrygon orbignyi* from Negro River feeds on insects (LONARDONI et al., 2006; SHIBUYA; ARAÚJO; ZUANON, 2009).

Hematological and biochemical varieties provide important health information and may be used as a tool for understanding comparative physiology, nutrition influences, and ecological changes (BALLANTYNE; ROBINSON, 2010). Even though there are stingrays in several aquariums worldwide there is scarce literature of their hematological information and biochemical profile (ARNOLD, 2005). The information provided for this study could be also used in clinical pathology studies about stingrays, mainly *Potamotrygon* genus, to determined species status, environmental problems related to these species and phylogenetic relationships.

Triiodothyronine (T3) and thyroxine (T4) are the main hormones secreted by the thyroid. They have different physiological functions in teleost fish and elasmobranchs (PETER, 2011; ABBAS et al., 2012). However, there is a lack of information about stingray physiology, particularly the *Potomatrygon* genus in tropical weather.

We chose these species because they represent their habitats and because there is controversy between authors about the scant information available on these species. The aim of this work was to evaluate and compare the characteristics of hematological findings as hemogram, serum biochemical profile, and thyroid hormones of four species of freshwater stingrays, *P. falkneri* e *P. motoro, P. orbignyi,* and *P. scobina.*

Materials and Methods

We collected 53 neotropical freshwater stingrays of the genus *Potamotrygon* between October of 2010 and July of 2011. *P. motoro* and *P. falkneri* were sampled from Paraná River at Nupelia Study Base, State University of Londrina, Porto Rico city, Parana, Brazil (22°45'40.5"S and 53°19'46.2"W). *P. orbignyi* and *P. scobina* were sampled at Piririm River, Macapa city, Amapa, Brazil (00°45'33.4" N and 50°32'15.0"W). 21 *P. falkneri*, 11 *P. motoro*, 14 *P. orbignyi,* and 7 *P. scobina* were collected.

Stingrays were captured using various fishing gear with natural baits. This study was approved by the Ethical Committee of Animal Welfare of State University of São Paulo (CEUA) n° 18551/09, and licensed of the Brazilian Institute of Environment and Renewable Natural Resources, IBAMA, n° 19978-1/2009.

Stingrays were anesthetized with benzocaine solution (1:20.000) diluted in ethanol-98° (0.1 g/mL), and blood samples were collected from the caudal vessel in tubes containing EDTA (10%). Total length (TL) and maximum disc width (DW) were determined for all animals. Finally, all fish were released in the location where they were captured.

Four mL of blood was collected from the caudal vessel of each animal; 2 mL was transferred to tubes containing EDTA (10%) to hemogram. The other part was transferred to tubes containing separator gel and clot activator which, after clotting, was centrifuged at 750 g for 7 minutes to obtain the serum. This was stored at - 20°C until the biochemical analysis.

Blood samples were used to obtain erythrocyte count, hematocrit, and hemoglobin concentration. Subsequently, we calculated their mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), and mean corpuscular hemoglobin concentration (MCHC).

Total and differential leukocyte counts were performed on blood smears stained with May-Grünwald-Giemsa-Wright. The identification and

nomenclature were performed according to Tavares-Dias and Moraes (2003). The values for total number of leukocytes and thrombocytes were performed by indirect method counting 2000 red blood cells, according to the equation: Total number of leukocytes or thrombocytes (µL) = [(number of leukocytes or thrombocytes counted in the smear) x (erythrocyte global count per µL)]. 2000 / number of erythrocytes counted in the blood smear.

For biochemical assessments, blood samples were placed in tubes and centrifuged at 750 g / 7 minutes to obtain serum which was stored at -20ºC until analysis. Biochemical serum determinations were made using an automatic multichannel analyzer ChemWell® (Awareness Technology Inc.) following the manufacturer's instructions and using commercial kits of Labtest Diagnostica® (Minas Gerais, Brazil).

Total protein concentration was determined by using the biuret method, albumin by using bromocresol green method, uric acid, triglycerides, and cholesterol by using Trinder enzymatic method, urea by UV enzymatic kinetic method, creatinine by alkaline picrate method, high density lipoprotein by HDL-C colorimetric method, calcium by O-cresolphthalein-CPC method, phosphorus by phosphomolybdate method, calcium/phosphorus relationship and creatine kinase by CK UV cinetic method, gamma-glutamyl transpeptidase by modified kinetic colorimetric method of Szasz, aspartate transaminase and alanine transaminase by IFCC UV-kinetic method, globulin, and albumin/globulin ratio.

The concentration of sodium, chlorine, and potassium were determined using EasyLyte Plus Analyzer (Medica Corporation, Bedford, MA, USA). Triiodothyronine (T3) and thyroxine (T4) hormones measurements were performed by direct-ELISA. Blood glucose was measured immediately after the stingray captured using One Touch Ultra Mini™ (Johnson & Johnson Medical).

The results were subjected to analysis of variance and comparison of means using Tukey's test ($P < 0.05$), at the significance level of 5%.

Results

Freshwater stingrays from Paraná River had less weight, and were smaller than stingrays from Piririm River (Table 1).

P. scobina had the highest red blood cell count, with mean of 1.32 (105.µL-1) (Table 2). Heterofils were higher in *P. orbignyi* and *P. scobina*. No eosinophils were observed in *P. scobina.* There were no statistical differences within the stingrays in all the other parameters (Table 3).

P. motoro and *P. falkneri* presented higher levels of potassium at serum than *P. orbignyi* (Table 4).The highest alkaline phosphatase enzyme level was within *P. motoro* and *P. falknerin* (Table 5). The lowest albumin level was noted on *P. orbignyi. P. falkneri* had higher protein, albumin, globulin, glucose, and cholesterol compared with *P. orbignyi* (Table 6).

Table 1 – Means and standard deviation of weight, total length: TL, and disco size: DS, of *Potamotrygon falkneri*, *P. motoro, P. orbignyi,* and *P. scobina.* Different letters represents statistical difference (P < 0.05) – Jaboticabal – 2011

Species	Weight (kg)				TL (cm)				DS (cm)			
	Mean		SD		Mean		SD		Mean		SD	
P. falkneri	2.3	±	0.3	b	55.0	±	2.5	b	32.0	±	1.7	b
P. motoro	1.5	±	0.2	b	51.4	±	2.1	b	30.3	±	1.2	b
P. orbignyi	8.3	±	0.6	a	84.1	±	3.5	a	50.2	±	1.6	a
P. scobina	8.2	±	1.3	a	79.8	±	10.1	a	50.8	±	3.6	a

Table 2 – Means and standard deviation (SD) of blood parameters of: *P. falkneri, P. motoro, P. orbignyi* and *P. scobina*. RBC: Red blood cell count; MCV: mean corpuscular volume; MCH: mean corpuscular hemoglobin; MCHC: mean corpuscular hemoglobin concentration. Different letters indicates statistical differences (P < 0.05) – Jaboticabal – 2011

	P. falkneri				*P. motoro*				*P. orbignyi*				*P. scobina*				
Parameters	**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Unit**
RBC	0.84	±	0.1	a	0.73	±	0.1	a	0.94	±	0.1	ab	1.38	±	0.4	b	$.10^5.\mu L^{-1}$
Hemoglobin	4.31	±	0.2	a	4.19	±	0.4	a	4.96	±	0.8	a	5.22	±	1.1	a	$g.dL^{-1}$
Hematocrit	21.3	±	0.8	a	19.6	±	1.6	a	23.1	±	3.9	a	22	±	4.6	a	%
MCV	288	±	33	a	325	±	59	a	267	±	49	a	171	±	33	a	fL
MCH	57.1	±	5.9	a	59.4	±	13	a	54.5	±	9.8	a	39.5	±	5.6	a	Pg
MCHC	20.8	±	1.1	a	22.2	±	1.9	a	21.9	±	4.1	a	23.7	±	2.2	a	%

Table 3 – Means and standard deviation (SD) of white blood cells of: *P. falkneri, P. motoro, P. orbignyi,* and *P. scobina*. Different letters indicates statistical differences (P < 0.05) – Jaboticabal – 2011

	P. falkneri				*P. motoro*				*P. orbignyi*				*P. scobina*				
Type of cell	**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Unit**
Leukocytes	3618	±	399	a	2520	±	501	a	2555	±	447	a	2299	±	581	a	$.\mu L^{-1}$
Thrombocytes	1255	±	263	a	1077	±	492	a	1232	±	244	a	1736	±	583	a	$.103.\mu L^{-1}$
Neutrophils	1892	±	195	a	1393	±	357	a	1985	±	265	a	737	±	172	a	$.\mu L^{-1}$
Heterophils	232	±	34	b	147	±	35	b	782	±	154	a	851	±	301	a	$.\mu L^{-1}$
Eosinophils	30	±	13	a	20	±	14	a	8	±	6	a	0	±	0	a	$.\mu L^{-1}$
Basophils	9	±	5	b	8	±	5	b	43	±	15	a	43	±	28	a	$.\mu L^{-1}$
Monocytes	337	±	50	a	234	±	76	a	150	±	43	a	73	±	32	a	$.\mu L^{-1}$
Lymphocytes	1123	±	411	a	718	±	189	a	403	±	113	a	594	±	169	a	$.\mu L^{-1}$

Table 4 – Means and standard deviation (SD) of serum minerals and thyroid hormone levels of: *P. falkneri, P. motoro, P. orbignyi,* and *P. scobina.* T_3: Triiodothyronine, T_4: Thyroxin. Different letters indicates statistical differences (P < 0.05) – Jaboticabal – 2011

	P. falkneri				*P. motoro*				*P. orbignyi*				*P. scobina*				
Parameters	**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Unit**
Calcium	9.54	±	0.67	a	9.51	±	1.03	a	8.15	±	1.23	a	9.71	±	2.27	a	$mg.dL^{-1}$
Phosphorus	8.47	±	0.6	a	9.15	±	0.7	a	8.14	±	1.3	a	8.48	±	2.3	a	$mg.dL^{-1}$
Re: Ca^+:P	0.98	±	0.1	a	0.93	±	0.15	a	1.21	±	0.18	a	1.32	±	0.24	a	-
Sodium	174.7	±	20.75	a	173.5	±	16.45	a	164.7	±	19.6	a	156.1	±	31.65	a	$mmol.L^{-1}$
Potassium	14.9	±	13.1	a	10.1	±	11.6	a	6.04	±	1.13	b	8.02	±	3.7	ab	$mmol.L^{-}$
Chloride	157.7	±	17.93	a	159	±	14.56	a	154.9	±	9.8	a	150.9	±	32.59	a	$mmol.L^{-1}$
T_3	1.76	±	0.49	a	1.77	±	0.52	a	2.04	±	0.35	a	1.57	±	0.63	a	$ng.mL^{-1}$
T_4	2.1	±	1.25	a	2.11	±	1.13	a	2.48	±	1.59	a	2.27	±	0.78	a	$\mu g.mL^{-1}$

Table 5 – Means and standard deviation (SD) of serum enzyme and metabolites levels of: *P. falkneri, P. motoro, P. orbignyi,* and *P. scobina*. AST: Aspartate transaminase, ALT:alanine transaminase, AP: alkaline phosphatase, GGT: gamma-glutamyl transpeptidase, CK: creatine kinase. Different letters indicates statistical differences (P < 0.05) – Jaboticabal – 2011

	P. falkneri				*P. motoro*				*P. orbignyi*				*P. scobina*				
Parameters	**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Unit**
AST	119	±	11.7	a	111	±	36.7	a	68	±	13	a	79	±	20.4	a	$U.L^{-1}$
ALT	18	±	2.29	a	25	±	5.27	a	13	±	2.85	a	19	±	8.65	a	$U.L^{-1}$
AP	116	±	17.5	a	124	±	16.9	a	77	±	11.4	a	32	±	6.67	a	$U.L^{-1}$
GGT	8.23	±	0.96	a	9.19	±	2.52	a	8.01	±	1.74	b	9.9	±	2.05	b	$U.L^{-1}$
CK	3593	±	1132	a	904	±	363	a	7248	±	2755	a	1670	±	778	a	$U.L^{-1}$
Uric acid	0.8	±	0.3	a	0.5	±	0.1	a	1.3	±	0.6	a	1.3	±	0.9	a	$mg.dL^{-1}$
Creatinine	0.4	±	0.03	a	0.4	±	0.04	a	0.3	±	0.03	a	0.3	±	0.06	a	$mg.dL^{-1}$
Urea	29	±	3.1	a	30.1	±	3.1	a	38.3	±	17.6	a	24.1	±	4.6	a	$mg.dL^{-1}$

Table 6 – Means and standard deviation (SD) of serum proteins and metabolites levels of: *P. falkneri, P. motoro, P. orbignyi,* and *P. scobina.* Total chol: total cholesterol. Different letters indicates statistical differences (P < 0.05) – Jaboticabal – 2011

	P. falkneri				*P. motoro*				*P. orbignyi*				*P. scobina*				
Parameters	**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Mean**		**SD**		**Unit**
Total protein	3	±	0.1	a	3	±	0.2	a	2	±	0.2	a	2	±	0.2	a	$g.dL^{-1}$
Albumin	0.4	±	0.03	a	0.4	±	0.07	a	0.2	±	0.04	b	0.1	±	0.06	b	$g.dL^{-1}$
Globulins	2.2	±	0.2	a	2.1	±	0.2	a	1.6	±	0.3	a	1.5	±	0.2	a	$g.dL^{-1}$
Rel A:G	0.2	±	0.03	a	0.2	±	0.04	a	0.3	±	0.2	a	0.1	±	0.06	a	-
Glucose	66.9	±	3.7	a	61.8	±	6.7	ab	44	±	8	b	39	±	6.5	b	$mg.dL^{-1}$
Total chol	88.7	±	6.4	a	74.2	±	8.8	a	34.6	±	6.5	b	21.8	±	6.6	b	$mg.dL^{-1}$
Triglycerides	66.7	±	4.4	a	52.4	±	7.1	ab	38.8	±	17.6	b	37.5	±	4.6	b	$mg.dL^{-1}$
HDL-C	8.39	±	1.3	a	9.1	±	1.8	a	8.1	±	2.4	a	9.7	±	2.7	a	$mg.dL^{-1}$

The *P. falkneri* and *P. motoro* from Paraná River had no significant difference between each other in all the analyzed parameters, also no significant difference within *P. orbignyi* and *P. scobina* from Piririm River (Tables 2, 3, 4, 5 and 6).

The principal components analysis showed that the cholesterol, phosphorus, total protein and globulin were the most distinct parameters to distinguish 40.12% of cumulative variance of *P. falkneri and P. motoro* from *P. orbignyi* and *P. scobina* (Table 4 and 6).

Discussion

Freshwater stingrays from Paraná River had less weight and were smaller than stingrays from Piririm River. This may be related to characteristics of each species and their ecosystems.

The ancestors of freshwater stingrays were marine fish that dispersed into the rivers, adapted to new surroundings, and then dispersed throughout South America, speciating along the way (BALLANTYNE; ROBINSON, 2010).

Anthropogenic actions can change the hematology of stingrays due to chronic stress. Semeniuk et al. (2009) showed *Dasyatis americana* from places visited by tourists have hematological differences from stingrays from non-visited places. We found no hematological differences between stingrays from different places. However, hematocrit was similar to

stressed *Potamotrygon histrix* found by Brinn et al. (2012).

Hemoglobin of *Potamotrygon* genus was higher than marine stingrays; while MCV, MCH, MCHC, and red blood cell count (RBC) were lower (WILHELM FILHO et al., 1992). This genus is usually found in fresh water, though they are able to withstand saline environments. Gerst and Thorson (1977) observed that *Potamotrygon* fish in saltwater presented higher hematological indices than marine stingrays, likely due to the atrophy of the rectal gland in these species which leads to an inability to regulate excess salt, causing environmental stress.

All types of leukocytes cells previously described for stingrays were observed in these four species, except the eosinophils that were not seen in *P. scobina*. The most common cells were lymphocytes, neutrophils, monocytes, and heterophils. It is likely the absence of eosinophils in *P. scobina* is physiological in this species in these conditions.

Elasmobranch neutrophils and heterophils have similar morphological and cytochemical characteristics with mammalian eosinophils. Campbell (2006) found that *Raja clavata* and *Raja microcellata* have neutrophils, heterophils, and scarce basophils, similar to our findings in all the stingrays studied. Otherwise, Aragort et al. (2005) found 43% lymphocytes, 35% eosinophils, 20% neutrophils, and 2% monocytes in Ragydae family, *Raja undulata, R. montagui, R. brachyuran, R. microocellata* which differ considerably from our findings. The absence of eosinophils in P. scobina could be physiological, as well as in teleost fish generally there is great variation in their blood percentage (TAVARES-DIAS; MORAES, 2004).

Wood et al. (2002) showed that Potamotrygonidae family presents characteristics of both freshwater and marine fish, based on the study of sodium and chloride exchange and urea excretion in *Potamotrygon hystrix* and *Potamotrygon thorsoni*. Emery (1986) associated the low metabolic activity of these fish with the low rates of ion exchanges in this family, which was also observed in this work.

Serum biochemistry of the four species studied had higher levels of sodium, chlorine, and calcium than a natural population of *Dasyatis Americana* (CAIN; HARMS; SEGARS, 2004) and lower levels of sodium and urea than fish of the same study. However, sodium levels were similar with *Potamotrygon* spp. (164 ± 5.6 $mmol.L^{-1}$), *P. magdalenae* (141 ± 2.4 $mmol.L^{-1}$) and *Potamotrygon* sp (178,2 ± 4.8 $mmol.L^{-1}$) (WOOD et al., 2002; CAIN; HARMS; SEGARS, 2004; BALLANTYNE; ROBINSON, 2010). Chlorine and potassium was also similar with *P. magdalenae* (147 ± 4.5 and 8,5 ± 0.29 $mmol.L^{-1}$) (OGAWA; HIRANO, 1982), but calcium level was higher (3 ± 0.09 $mg.dL^{-1}$).

Chlorine, sodium, and calcium levels of the *Potamotrygon* species evaluated in our study were lower than shark serum levels (STOSKOPF, 1993), likely due to the saltwater habitat of the sharks. Potamotrygonidae fish have an adaptation to low ionic water similar to freshwater bony fishes, with a low affinity ionic transport system (WOOD et al., 2002; BALLANTYNE; ROBINSON, 2010).

Plasmatic concentration of urea was lower comparing with marine elasmobranchs but higher than *P. hystrix, P. magdalenae,* and *P. motoro* (OGAWA; HIRANO, 1982; TAVARES-DIAS; MORAES, 2003). This result is partially due to interspecific and methodological differences. Urea is an important osmolite of marine and euryhaline elasmobranchs. Otherwise, it is not so important in freshwater stingrays (TAM et al., 2003; BALLANTYNE; ROBINSON, 2010). *Potamotrygon* species are ammonotelics (IP et al., 2009) with an atrophic rectal gland; for this reason, they have low capacity to tolerate high salinity (BALLANTYNE; ROBINSON, 2010).

Glucose levels of *P. falkneri, P. motoro, P. orbignyi* and *P. scobina* were higher than within *P. histrix* (1.65 ± 0.06 $mmol.L^{-1}$) (BRINN et al., 2012), probably due to interspecific differences and different habitat.

Lipids and proteins are the main source of energy in fish, the contribution of carbohydrates is limited and it is mainly stored in the liver as glycogen and lipid (GUIJARRO et al., 2003). For this reason, glucose is not abundant in blood plasma of freshwater stingrays (GRIFFITH et al., 1973). The differences in glucose levels in this study are likely due to the type of food. P. falkneri feeds on crustaceans and fish (ARAGORT et al., 2005), whereas *P. orbignyi* and *P. scobina* feed on insects (SILVA; UIEDA, 2007).

HDL levels were similar in the studied species. However, cholesterol levels were lower in *P. orbignyi* and *P. scobina;* and triglycerides were higher in *P. falkneri*. These may be due to feeding habits. Lipids represent an economic way to store energy in tissues. The major body composition alteration is generally due to fatty variations (GUIJARRO et al., 2003).

P. falkneri, P. motoro, P. orbignyi, and *P. scobina* had similar levels of T_4 and T_3 with Atlantic stingray *Dasyatis Sabina* (VOLKOFF et al., 1999), lampreys Entosphenus tridentatus (MESA et al., 2010), and teleostei Epinephelus aeneus (ABBAS et al., 2012). Nevertheless, T_3 levels where higher than Triaenodon obesus shark (CROW et al., 1999).

Most hematological indices were similar in all *Potamotrygon* species. This fact suggests that there is low influence of habitat and feeding habits. However, other parameters such as weight, alkaline phosphatase, glucose, cholesterol, phosphorus, total protein, and globulin had different results. *P. falkneri* and *P. motoro* have similar indices. In the same manner, *P. orbignyi* and *P. scobina* present similar characteristics. However, these have great differences with the first two species. This can be explained since *P. orbignyi* and *P. scobina* are more ancient species (JABLONSKI, 1997). This fact supports the theory that stingrays migrated from the Atlantic Ocean, adapting to different conditions over time and placed themselves geographically distant from each other.

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