

SEASONAL PATTERNS OF INFECTION BY *Kudoa* sp. (MYXOZOA) IN THE CATFISHES IN THE BRAZILIAN AMAZON REGION

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ABSTRACT

The myxosporidians of the genus *Kudoa* cause *post mortem* myoliquefaction in fishery products and may potentially transmit zoonoses. The present study describes the infection of the skeletal musculature by *Kudoa* sp. in two sea catfish species, *Cathorops spixii* (Agassiz, 1829) and *Cathorops agassizii* (Eigenmann & Eigenmann, 1888), captured monthly in an estuary of the municipality of Vigia de Nazaré, in Pará, northern Brazil between March, 2015, and August, 2016. The morphological features of the spores are described, and the influence of the seasonal variation on the occurrence of the parasite is discussed. The specimens were taken to the laboratory for analysis using a hand lens and light microscopy. When parasites were identified in the musculature, small fragments of the tissue were removed for histological processing and staining by the Hematoxylin-Eosin, Ziehl-Neelsen, May Grunwald-Giemsa, and Gomori techniques. The pseudocysts were found in the muscle fibers, and the spores were star-shaped with elongated extremities and 4 piriform/rounded polar capsules of equal size. No infections were observed in the fish specimens collected during the rainy season, whereas all (100%) of the *Cathorops* specimens examined during the dry season were infected. This indicates that the ecology and infection patterns of the parasite (identified as a species of the genus *Kudoa*, on the basis of its morphological features) is influenced by salinity levels.

Key words: parasite; multivalvulid; histology; myxosporidian; estuary; *Cathorops*.

VARIAÇÃO SAZONAL DE INFECÇÃO POR *Kudoa* sp. (MYXOZOA) EM BAGRES NA REGIÃO AMAZÔNICA BRASILEIRA

RESUMO

Os mixosporídios do gênero *Kudoa* são causadores de mioliquefação pós-morte em pescado e apresentam potencial zoonótico. O presente trabalho apresenta infecção por *Kudoa* sp. em musculatura esquelética em duas espécies de bagres, *Cathorops spixii* (Agassiz, 1829) and *Cathorops agassizii* (Eigenmann & Eigenmann, 1888) capturados em estuário amazônico, destacando aspectos morfológicos do esporo e a influência da sazonalidade em sua ocorrência. No estuário do Município de Vigia de Nazaré, Pará, Brasil, foram capturados 160 espécimes de *Cathorops* sp. no período de março de 2015 a agosto de 2016 em coletas mensais. Os exemplares foram transportados até o laboratório, utilizando lupa e microscópio de luz. Constatada a presença de parasito na musculatura, pequenos fragmentos do tecido foram retirados para processamento histológico e coloração por Hematoxilina-Eosina, Ziehl-Neelsen, May Grunwald-Giemsa e Gomori. Os pseudocistos encontravam-se dentro das fibras musculares e os esporos apresentavam forma estrelada com extremidades prolongadas, com 4 cápsulas polares de formato piriforme/arredondadas de tamanhos iguais. Durante os meses que apresentaram maiores índices pluviométricos, não foi observada infecção parasitária, entretanto, nos meses com menor índice pluviométrico, a taxa de infecção foi de 100%, dados que sugerem influência da salinidade nos aspectos ecológicos e infecciosos do parasito, identificado pelos dados morfológicos como pertencente ao gênero *Kudoa*.

Palavras-chave: parasito; multivalvulida; histologia; mixosporídeo; estuário; *Cathorops*.

INTRODUCTION

The diversity of myxosporidian species has been the focus of a range of studies in many different geographic regions (Lom and Dyková, 2006). In South America, the Amazon basin, in particular, has enormous potential for the understanding of the diversity of these parasites, given the region's considerable fish diversity.

One myxozoan microparasite that has received considerable attention in recent years is the genus *Kudoa*, which causes *post mortem* myoliquefaction (degradation of the muscle fibers, caused by the action of proteolytic enzymes) in fishery products, rendering the produce unfit for human consumption (Kawai et al., 2012; Sugita-Konishi et al., 2014). More than 100 *Kudoa* species have been described (Eiras et al., 2016), but of these, only *Kudoa orbicularis* is known to occur in a freshwater environment (Azevedo et al., 2016). All other species have been found infecting marine or estuarine fish (Lom and Dyková, 2006; Eiras et al., 2016).

The species of this genus infect an ample diversity of fish in a number of different geographic regions, where they may have a considerable impact on local fisheries and aquaculture operations, causing economic losses through the myoliquefaction of the produce (Moran et al., 1999). These authors also recommend the classification of *Kudoa* species as zoonotic agents, given their capacity to cause enterotoxinfections in humans, which provoke symptoms such as diarrhea, vomiting, and abdominal pain, caused by the rupture of the gastrointestinal mucosa (Ohnishi et al., 2013; Sugita-Konishi et al., 2014; Yahata et al., 2015).

To contribute further to the understanding of the distribution and ecology of *Kudoa* in South America, the present study investigated the presence of these parasites in two sea catfish species (*Cathorops* spp.) captured in an estuary on the Amazon coast of Brazil. The morphology of the parasite spore is described, together with the seasonal pattern in the occurrence of infection.

MATERIAL AND METHODS

Fish sampling

The specimens analyzed in the present study were obtained from the estuary of the Guajará-mirim River, in the municipality of Vigia de Nazaré (00°51'12" S, 48°08'41" W), in Pará, northern Brazil. The sample included 82 specimens of *Cathorops spixii* and 78 specimens of *Cathorops agassizii*, which were collected using cast-nets and hand-lines between March 2015, and August 2016 (SISBIO license number 27,119). The physical-chemical parameters of the water (temperature, pH, and salinity) were registered using a multi-parameter apparatus.

The specimens were preserved on ice in styrofoam coolers for transportation to the Carlos Azevedo Research Laboratory (LPCA) at UFRA in Belém, Brazil. In the laboratory, the fish were measured and weighed. For necropsy, the specimens were anesthetized with 50 mg L⁻¹ tricain methanesulfonate (MS222 SIGMA), following the recommendations of the UFRA ethics committee for the use of animals in experimental research (CEUA: 013/2014). The specimens were then dissected and the gonads were analyzed to determine their sex. Subsequently, the specimens were observed under Zeiss stereomicroscopes to determine the presence of microparasitic cysts. Small fragments of skeletal muscle were placed on microscope slides in a drop of water and sealed with a cover slip for the examination of the fresh material under light microscopy (LM; Zeiss) for the confirmation of the parasitism.

When the presence of parasites was confirmed, micrographs of the fresh spores were prepared using a digital camera attached to the LM, using the AxioVision 5.1 software, to determine the morphometric features of the species, following Burger and Adlard (2010). A total of 30 spores per fish were measured to determine their mean, length, and thickness, and the length and width of the polar capsules. These findings were compared with the morphometric data available on the spores of other *Kudoa* species.

Preparation of the samples and histological analysis

For the histological analysis, fragments of the muscle tissue, approximately 0.5 cm thick, were cut from the muscle tissue of the specimens, and fixed in Davidson solution for 24 hours. The samples were then dehydrated in a progressive series of alcohol, and diaphanized in xylol for impregnation and embedding in paraffin. Sections of 5 µm were then obtained in a microtome, warmed to 60 °C in a water bath, and then dried in an oven at 60 °C for 24 hours to guarantee adhesion to the slide. The sections were then stained with Hematoxylin-Eosin, Ziehl-Neelsen, May Grunwald-Giemsa, and Gomori (Luna, 1968). Stained slides were selected for photographic documentation using a Zeiss microscopic Primo Star AxioCam ERc 5s camera with AxioVision 5.1 software.

RESULTS

In the Guajará-mirim estuary, the mean water temperature during the study period was 28.91 ± 0.55 °C (27.67-29.86), with little variation being found between the first (rainy season) and second (dry season) halves of the year. However, the pH and salinity varied considerably between periods, reflecting the influence of rainfall levels. During the rainy season (January-June), the mean pH was 6.73 ± 0.34 (6.26-7.3), while salinity was 0.01 ± 0.01 (0-0.02), whereas during the dry season (July-December), the mean pH was 7.71 ± 0.35 (7.3-8.41), and salinity was 6.18 ± 0.87 (5.00-7.55).

The *Cathorops* specimens collected during the present study had a mean furcal length of 14.1 cm (10-18 cm) and weight of 48.2 grams (20-102 g). Most (80%) of the individuals were females (128/160 specimens). The prevalence of parasite infection varied considerably between seasons, with none of the 80 *Cathorops* specimens collected during the rainy season (January-June) presenting *Kudoa* sp. (Figure 1) in the musculature. In July, by contrast, half (10/20) of the *Cathorops* specimens collected were infected with *Kudoa* sp., and all 60 specimens collected between August and December were infected. The parasites were found in both the epaxial and hypaxial musculature (Figure 2A).

The pseudocysts were found within the skeletal muscle fibers, with an elongated shape in the longitudinal view, 1.2(± 0.2) mm long and 0.3 (± 0.1) mm in width (Figure 2B, C), containing mature tetra-capsuled spores (Figure 2D). The pseudocysts in the musculature of the host were identified by the different techniques (Hematoxilina-Eosina, May-Grunwald-Giemsa, Ziehl-Neelsen, and Gomori) used to stain the histological sections (Figure 3).

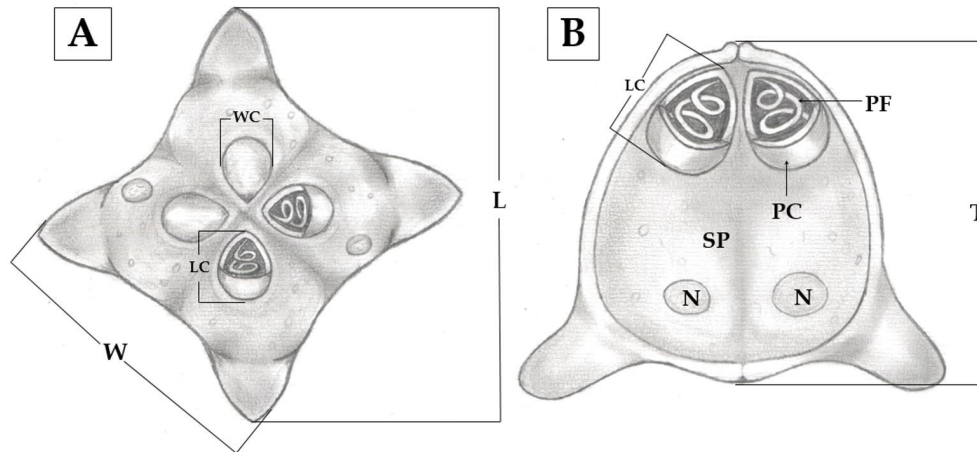


Figure 1. Diagram of the *Kudoa* sp. parasite found in the skeletal musculature of *Cathorops spixii* and *C. agassizii* collected in the Guajar-mirim estuary in Par, Brazil. (A) apical view (a.v.): L = spore length; W = spore width; LC = length of the polar capsule; WC = width of the polar capsule; (B) lateral view (l.v.): T = spore thickness; PC = polar capsule; PF = polar filament; SP = spore plasma; N = nucleus.

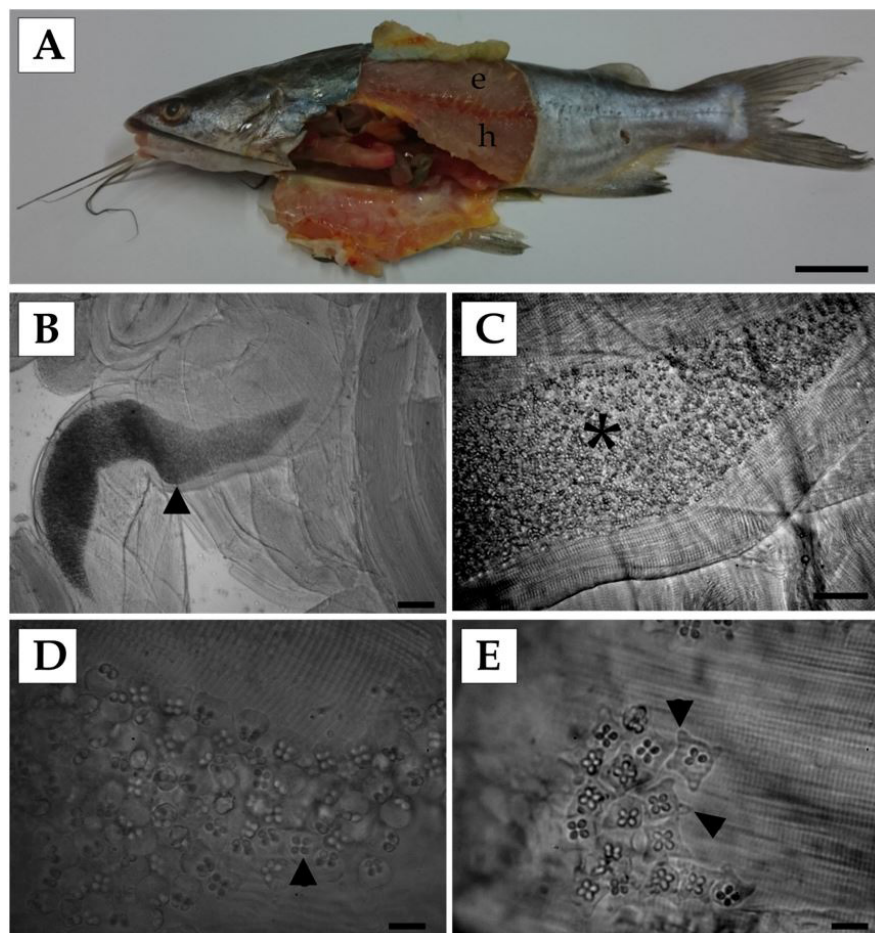


Figure 2. (A) *Cathorops spixii* host collected in the Guajar-mirim estuary in Par, Brazil, showing the epaxial (e) and hypaxial (h) musculature parasitized by *Kudoa* sp. (scale bar = 2 cm); (B) Photomicrograph of fresh pseudocyst *Kudoa* sp. (arrowhead) in the skeletal musculature of the host (scale bar = 100 μ m); (C) Pseudocyst of *Kudoa* sp., showing the arrangement of the spores (*) within the muscle fiber (scale bar = 40 μ m); (D) Spores of *Kudoa* sp. showing the four polar capsules (arrowhead) (scale bar = 10 μ m); (E) Photomicrograph of the valvogenic elongations (arrowhead) in *Kudoa* sp. (scale bar = 10 μ m).

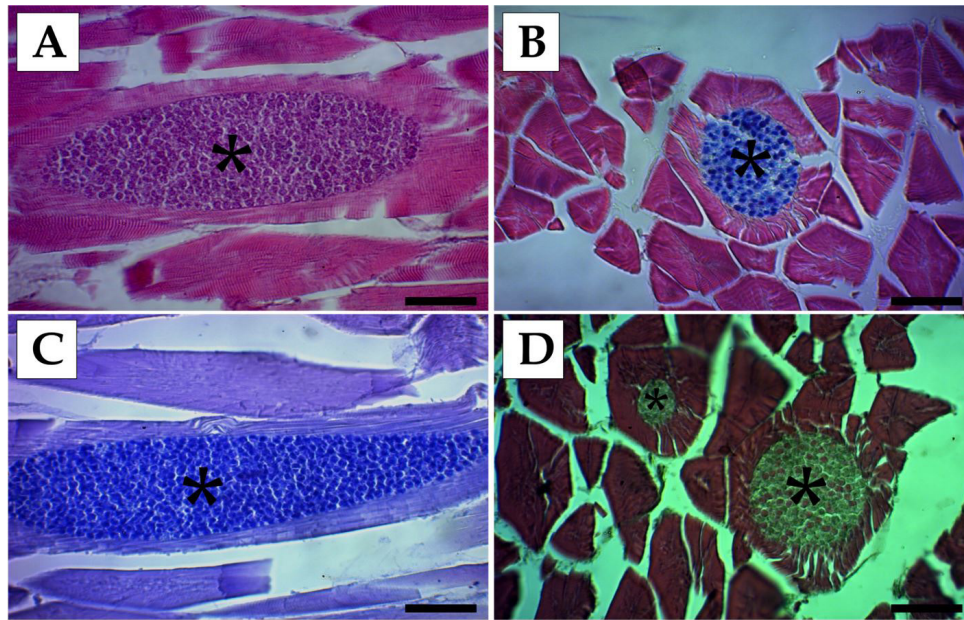


Figure 3. Cysts of *Kudoa* sp. (*) parasitizing the skeletal musculature of *Cathorops spixii* (A and B) and *C. agassizii* (C and D) hosts collected in the Guajar-mirim estuary in Par, Brazil. Histological sections stained by: (A) Hematoxylin-Eosin; (B) May-Grunwald-Giemsa; (C) Ziehl-Neelsen; (D) Gomori. Scale bar = 20 μ m.

In the apical view, the *Kudoa* sp. spores were somewhat star-shaped, with elongated extremities of the four valves, containing four piriform/rounded polar capsules of equal size, arranged regularly within the spore. In the lateral view, the spores were oval or piriform, with the elongation of the valves visible in the inferior part of the spore, and a slight increase in the dimensions of the polar capsules in comparison with the apical view.

The host presented *post mortem* myoliquefaction, and the histological analyses revealed damage to the host organism with substitution of the muscle tissue for parasite pseudocysts.

DISCUSSION

More than 100 *Kudoa* species have been described, although only one, *Kudoa orbicularis*, occurs in a freshwater host, the cichlid fish, *Chaetobranchopsis orbicularis* (Azevedo et al., 2016). Despite this, *Kudoa* has been described in fish from estuaries, where there is no geographic barrier between the river and the sea, and salinity may fluctuate considerably over the year. Examples include *Kudoa* spp. recorded in *Plagioscion squamosissimus* in the district of Outeiro, in Par, Brazil (Oliveira et al., 2015), and *Kudoa aequidens* observed in *Aequidens plagiozonatus* in the municipality of Peixe-Boi, also in Par (Casal et al., 2008).

One species, *Kudoa shkae*, has been recorded in a fish host of the order Siluriformes, *Ariopsis felis*, being found in specimens collected from saline and brackish waters on the coast of the American state of Mississippi, in the northern Gulf of Mexico (Dyková et al., 1994). The present study provides the first record of the infection of a host of the genus *Cathorops* (family Ariidae,

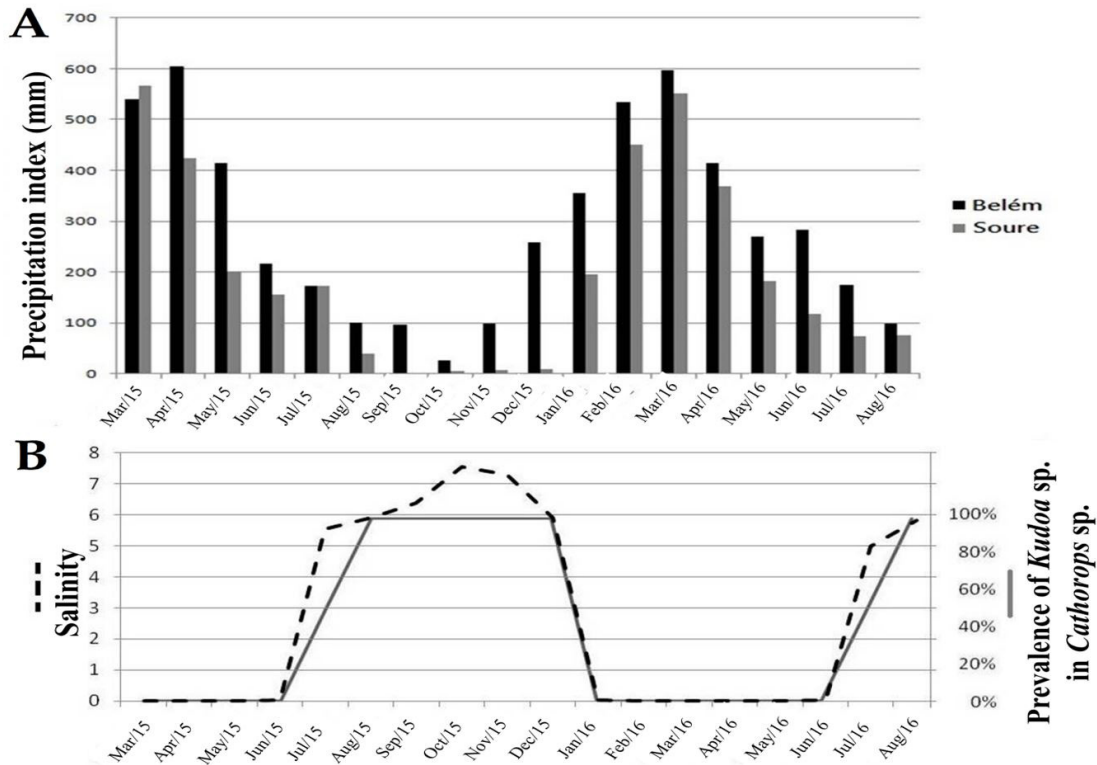
order Siluriformes) by *Kudoa* sp. in Brazil, from the Amazon region. The spores found in these fish were compared with those of *Kudoa shkae*, and other *Kudoa* species recorded in the Amazon region (Table 1), although in all cases, considerable differences were found, which left the identification of the species observed in the present study uncertain.

The occurrence of *Kudoa* sp. in *Cathorops* on the Amazon coast was highly seasonal, with no records being obtained during the first half of the year, that is, the rainy season, followed by a prevalence of 100% during the last five months of the year, coinciding with the peak of the dry season (Reboita et al., 2010). This general pattern was confirmed for the period of the present study (Figure 4A) by the data obtained from the Brazilian National Institute of Meteorology (INMET, 2016).

In Amazonian estuaries, rainfall levels are the primary determinant of the physical-chemical and microbiological parameters of the water, and nutrient concentrations, through their influence on river discharge, current speeds, and the penetration of the saline wedge (Rosa Filho and Aviz, 2013). Low salinity, in particular, was likely the principal factor determining the absence of the parasite during the rainy season, when salinity was at its lowest levels (Figure 4B), i.e., mean of 0.01 (range: 0.00-0.02). During the dry season, by contrast, mean salinity increased to 7.71 (7.3-8.41), and it was during this period that the *Kudoa* sp. infections were observed. As *Kudoa* is known to infect primarily marine fish (Abdel-Ghaffar et al., 2012; Eiras et al., 2014; Lom and Dyková, 2006; Moran et al., 1999; Swearer and Robertson, 1999; Yurakhno et al., 2007), the occurrence of *Kudoa* sp. in the *Cathorops* specimens during this period appears to be dependent on salinity levels.

Table 1. Comparison of the measurements (in μm) of the spores of different *Kudoa* species that infecting fish in Brazilian Amazonia and a siluriform species from the Gulf of Mexico.

Species/Author	<i>Kudoa</i> sp. (Present study)	<i>K. orbicularis</i> (Azevedo et al., 2016)	<i>K. aequidens</i> (Casal et al., 2008)	<i>Kudoa</i> sp. (Oliveira et al., 2015)	<i>K. shkae</i> (Dyková et al., 1994)
Host	<i>Cathorops</i> sp.	<i>Chaetobranchopsis orbicularis</i>	<i>Aequidens plagiozonatus</i>	<i>Plagioscion squamosissimus</i>	<i>Ariopsis felis</i>
Habitat	Estuary	Freshwater	Freshwater/ Estuary	Freshwater/ Estuary	Marine/ Estuary
Location		Brazilian Amazonia			Mexico
Length of spore (Apical view)	15.9 \pm 0.4 (15.3-16.4)	4.3 (3.6-5.0)	3.2 (2.9-3.5)	9.70 \pm 0.14 (9.6-9.85)	6.2 (6.1-6.2)
Width of spore (Apical view)	12.0 \pm 0.6 (11.3-12.8)	5.1 (4.2-5.8)	6.8 (6.2-7.1)	9.33 \pm 0.25 (9.15-9.5)	7.5 (7.0-8.1)
Thickness of the spore (Lateral view)	8.5 \pm 0.3 (7.9-9.0)	4.3	-	-	-
Length of polar capsule (Apical view)	2.5 \pm 0.2 (2.4-2.8)	-	2.2	4.05 \pm 0.07 (4.00-4.10)	2.5
Width of polar capsule (Apical view)	2.1 \pm 0.1 (2.0-2.2)	1.3	1.2	1.28 \pm 0.32 (1.05-1.5)	2.0
Length of polar capsule (Lateral view)	2.8 \pm 0.2 (2.4-3.1)	2.1	-	-	-
Width of polar capsule (Lateral view)	2.2 \pm 0.1 (2.0-2.4)	-	-	-	-

**Figure 4.** (A) Monthly precipitation (mm) recorded at the Belém and Soure (Pará) meteorological stations during the present study period (March 2015-August 2016). Data obtained from the Brazilian National Institute of Meteorology (INMET, 2016); (B) Monthly salinity of the Guajará-mirim estuary in Pará and the prevalence of *Kudoa* sp. in the skeletal musculature of *Cathorops* sp.

The salinity recorded in the Guajará-mirim estuary during the rainy season is consistent with its classification as a freshwater environment, based on CONAMA resolution 357/2005 (salinity ≤ 0.5). The Guajará-mirim would also be classified as a limnetic estuary (salinity ≥ 0.5) in the Venice System (1958). In the dry season, the estuary would be classified as brackish (salinity 0.5-30) in the CONAMA (Brasil, 2005) scheme, and as a mesohaline estuary (salinity 5-18) in the Venice System (1958).

The increase in salinity observed during the dry season was associated with a reduction in the input of river discharge, which permitted an increased penetration of oceanic waters into the Guajará-Mirim estuary (MRC, 2005). This favored the occurrence of *Kudoa* sp. in the catfish (*Cathorops* spp.) caught in the estuary during this period.

CONCLUSION

The results of the present study indicate clearly that the infection of the striated skeletal musculature of *Cathorops spixii* and *C. agassizii* by *Kudoa* sp. in the Guajará-mirim estuary was dependent on the salinity of its waters. The parasite was not recorded under freshwater conditions, but was found in all the fish specimens when the waters of the estuary were brackish.

The histological analyses revealed that *Kudoa* sp. had deleterious effects on the health of the host, which emphasizes the need for further research on the potential impact of this infection on the health of consumers, given that both *Cathorops spixii* and *C. agassizii* are targeted by commercial fisheries.

Given the high rate of infection recorded during the dry season, there is a clear need for more systematic monitoring of the fishery produce by the local public health authorities. A number of precautions can also be taken by the consumers of these catfish, such as cooking at temperatures of 95 °C for 10 minutes or freezing the fish overnight at -80 °C to eliminate the parasites and avoid potential intoxication (Kawai et al., 2012; Ohnishi et al., 2013; Sugita-Konishi et al., 2014).

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REFERENCES

- Abdel-Ghaffar, F.; Morsy, K.; Mehlhorn, H.; Bashtar, A.-R.; Shazly, M.A.; Saad, A.-H.; Abdel-Gaber, R. 2012. First report of *Kudoa* species (Myxozoa: Kudoidae) infecting the spotted coral grouper *Plectropomus maculatus* from Red Sea: a light and ultrastructural study. *Parasitology Research*, 111(4): 1579-1585. <http://dx.doi.org/10.1007/s00436-012-3011-x>. PMID:22740296.
- Azevedo, C.; Rocha, S.; Matos, E.; Oliveira, E.; Matos, P.; Al-Quraishy, S.; Casal, G. 2016. Ultrastructural and phylogenetic description of *Kudoa orbicularis* n. sp. (Myxosporea: Multivalvulida): a parasite Infecting the muscle of the fish *Chaetobranchopsis orbicularis* (Teleostei: Cichlidae) in the amazon region. *The Journal of Eukaryotic Microbiology*, 63(1): 27-36. <http://dx.doi.org/10.1111/jeu.12244>. PMID:26095978.
- Brasil. CONAMA – Conselho Nacional do Meio Ambiente, 2005. Resolução n°. 357, de 17 de março de 2005. Dispõe sobre a classificação dos corpos de água e diretrizes ambientais para o seu enquadramento, bem como estabelece as condições e padrões de lançamento de efluentes, e dá outras providências. *Diário Oficial da União, Brasília*, 18 de março de 2005, n°. 053, p. 58-63.
- Burger, M.A.A.; Adlard, R.D. 2010. Four new species of *Kudoa* Meglitsch, 1947 (Myxosporea: Multivalvulida) from Australia with recommendations for species descriptions in the Kudoidae. *Parasitology*, 137(5): 793-814. <http://dx.doi.org/10.1017/S0031182009991557>. PMID:20025820.
- Casal, G.; Matos, E.; Matos, P.; Azevedo, C. 2008. Ultrastructural description of a new myxosporean parasite *Kuoda aequidens* sp. n. (Myxozoa, Myxosporea) found in the sub-opercular musculature of *Aequidens plagiozonatus* (Teleostei) from the Amazon liver. *Acta Protozoologica*, 47(2): 135-141.
- Dyková, I.; Lom, J.; Overstreet, R.M. 1994. Myxosporean parasites of the genus *Kudoa* Meglitsch, 1947 from some Gulf of Mexico fishes: description of two new species and notes on their ultrastructure. *European Journal of Protistology*, 30(3): 316-323. [http://dx.doi.org/10.1016/S0932-4739\(11\)80078-7](http://dx.doi.org/10.1016/S0932-4739(11)80078-7).
- Eiras, J.C.; Lima, J.T.; Cruz, C.F.; Saraiva, A. 2014. A note on the infection of *Scomberomorus brasiliensis* (Osteichthyes, Scombridae) by *Kudoa* sp. (Myxozoa: Multivalvulida). *Brazilian Journal of Biology = Revista Brasileira de Biologia*, 74(3, suppl. 1): 164-166. <http://dx.doi.org/10.1590/1519-6984.23712>. PMID:25627380.
- Eiras, J.C.; Pereira Júnior, J.; Saraiva, A.; Cruz, C.F. 2016. Observations on the Infection by *Kudoa* sp. (Myxozoa, Multivalvulida) in fishes caught off Rio Grande, Rio Grande do Sul State, Brazil. *Acta Scientiarum. Biological Sciences*, 38(1): 99-103. <http://dx.doi.org/10.4025/actascibiols.v38i1.30492>.
- INMET – Instituto Nacional de Meteorologia, 2016. Variação do índice pluviométrico (mm) mensal acumulado, março de 2015 à agosto de 2016. Brasília: INMET. Disponível em: <<http://www.inmet.gov.br/portal/index.php?r=bdmep/bdmep>> Acesso em: 10 dez. 2016.
- Kawai, T.; Sekizuka, T.; Yahata, Y.; Kuroda, M.; Kumeda, Y.; Iijima, Y.; Kamata, Y.; Sugita-Konishi, Y.; Ohnishi, T. 2012. Identification of *Kudoa septempunctata* as the causative agent of novel food poisoning outbreaks in Japan by consumption of *Paralichthys olivaceus* in raw fish. *Clinical Infectious Diseases*, 54(8): 1046-1052. <http://dx.doi.org/10.1093/cid/cir1040>. PMID:22281845.

- Lom, J.; Dyková, I. 2006. Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitologica*, 53(1): 1-36. <http://dx.doi.org/10.14411/fp.2006.001>. PMID:16696428.
- Luna, L.G. 1968. Manual of histologic staining methods of the armed forces institute of pathology. 3rd ed. New York: MacGraw-Hill Book Company. 258p.
- Moran, J.D.W.; Whitaker, D.J.; Kent, M.L. 1999. A review of the myxosporean genus *Kudoa* Meglitsch, 1947, and its impact on the international aquaculture industry and commercial fisheries. *Aquaculture*, 172(1): 163-196. [http://dx.doi.org/10.1016/S0044-8486\(98\)00437-2](http://dx.doi.org/10.1016/S0044-8486(98)00437-2).
- MRC – Mekong River Commission, 2005. Overview of the hydrology of the Mekong BASIN. Vientiane: Mekong River Commission. 73p.
- Ohnishi, T.; Kikuchi, Y.; Furusawa, H.; Kamata, H.; Sugita-Konishi, Y. 2013. *Kudoa septempunctata* invasion increases the permeability of human intestinal epithelial monolayer. *Foodborne Pathogens and Disease*, 10(2): 137-142. <http://dx.doi.org/10.1089/fpd.2012.1294>. PMID:23373474.
- Oliveira, J.C.; Velasco, M.; Santos, P.F.S.; Silva, J.M.V.; Clemente, S.C.S.; Matos, E. 2015. *Kudoa* spp. (Myxozoa) infection in musculature of *Plagioscion squamosissimus* (Sciaenidae) in the Amazon region, Brazil. *Revista Brasileira de Parasitologia Veterinária*, 24(2): 235-240. <http://dx.doi.org/10.1590/S1984-29612015023>. PMID:26154967.
- Reboita, M.S.; Gan, M.A.; Rocha, R.P.; Ambrizzi, T. 2010. Regimes de precipitação na América do Sul: uma revisão bibliográfica. *Revista Brasileira de Meteorologia*, 25(2): 185-204. <http://dx.doi.org/10.1590/S0102-77862010000200004>.
- Rosa Filho, J.S.; Aviz, D. 2013. Macrobenthic communities of an Amazonian estuary (Guajará Bay, Brazil): temporal and spatial changes. *Journal of Coastal Research*, 65(2): 123-128. <http://dx.doi.org/10.2112/S165-022.1>.
- Sugita-Konishi, Y.; Sato, H.; Ohnishi, T. 2014. Novel Foodborne Disease Associated with Consumption of Raw Fish, Olive Flounder (*Paralichthys olivaceus*). *Food Safety*, 2(4): 141-150. <http://dx.doi.org/10.14252/foodsafetyfscj.2014026>.
- Swearer, S.E.; Robertson, D.R. 1999. Life history, pathology, and description of *Kudoa ovivora* n. sp. (Myxozoa, Myxosporea): an ovarian parasite of Caribbean labroid fishes. *The Journal of Parasitology*, 85(2): 337-353. <http://dx.doi.org/10.2307/3285645>. PMID:10219318.
- Venice System. 1958. Symposium on the classification of brackish waters, Venice April 8-14. *Archives Oceanography and Limnology*, 11(1): 1-248.
- Yahata, Y.; Sugita-Konishi, Y.; Ohnishi, T.; Toyokawa, T.; Nakamura, N.; Taniguchi, K.; Okabe, N. 2015. *Kudoa septempunctata*: induced gastroenteritis in humans after flounder consumption in Japan: a case-controlled study. *Japanese Journal of Infectious Diseases*, 68(2): 119-123. <http://dx.doi.org/10.7883/yoken.JJID.2014.027>. PMID:25420663.
- Yurakhno, V.M.; Ovcharenko, M.O.; Holzer, A.S.; Sarabeev, V.L.; Balbuena, J.A. 2007. *Kudoa unicapsula* n. sp. (Myxosporea: Kudoidae) a parasite of the Mediterranean mullets *Liza ramada* and *L. aurata* (Teleostei: Mugilidae). *Parasitology Research*, 101(6): 1671-1680. <http://dx.doi.org/10.1007/s00436-007-0711-8>. PMID:17846792.