

Hydrogen sulfide tolerance in Amazon fish

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Hydrogen sulfide (H_2S) is a highly toxic compound for aerobic organisms due to the binding of sulfide to cytochrome *c* oxidase. It is naturally produced in the Amazon floodplain by the anaerobic decomposition of organic matter and bacterial reduction of sulfates during the high water season. During this period, there is a phenomenon known as "friagem" (cold front), where the rapid cooling of the surface layer disintegrates the thermal stratification causing total mixing in the water column. The impact of the "friagem" results in fish mortality in this type of lakes. Besides oxygen deficit, the increase of hydrogen sulfide in the surface water layer has been also presumed to be one of the causes for mortality (Kramer, 1978; Santos, 1979; Junk *et al.*, 1983).

Some studies have demonstrated that H_2S is an important environmental factor affecting the distribution of animals in different aquatic habitats (Powell *et al.*, 1980; Bagarinao & Vetter, 1989; Vismann, 1991; Bagarinao, 1994). Bagarinao & Vetter (1989; 1992) and Brauner *et al.* (1995) showed that fish living in sulfide-rich habitats are more tolerant to hydrogen sulfide because they present biochemical and/or morphological adaptations to survive in these habitats.

The Amazon floodplain lakes has an abundant fish fauna that lives under extreme hydrochemicals conditions. In general, these fish have survival strategies to live in these lakes, such as: behavioral, morphological, physiological, biochemical, and genetic (Val & Almeida-Val, 1995). These adaptations are often attributed mainly to the effect of hypoxia/anoxia, temperature fluctuations, or low/high pH. But it almost likely that Amazon fish have become adapted to H_2S .

The objectives of this study were to describe the seasonal variation of hydrogen sulfide, its influence on the distribution of fish species in Lago Grande, a Central Amazon floodplain (várzea) lake, and to determine the tolerance level of some fish species to H_2S exposure.

Lago Grande is a lake of the Marchantaria island located about 15 Km above the confluence of the Rio Negro and Solimões River. It is connected to the Solimões River during the high water level when it becomes floodable. The terrestrial vegetation dies, decomposes and aquatic and semi-aquatic vegetation develop in great quantities covering a considerable area of the lake. Maximum water levels occurred in mid-June and begun to decline in August. The dry season (September - December) was the lowest in the last 30 years occurred in the Amazon region. Many lakes dried completely and others had a depth of 20-70 cm. The sediment banks were colonized by terrestrial and semi-aquatic plants, occupying a big area of the island. We made eight excursions along the year during high and low water seasons. We selected four stations: one out of the lake (station A), one at the lake entrance (station B), one in open waters in the lake (station C) and other in the flooded forest (station D). During the low water season, stations A and C dried completely, station B had water only until September, and C had a depth of 20 - 40 cm in the places where the samples were taken.

We measured dissolved oxygen and hydrogen sulfide concentrations and temperature at different depth in all stations (Table 1). The dissolved oxygen concentration was extremely low or absent during high water season (May - August), and it was high during low water season (3.4 - 6.0 mg/l). The low dissolved oxygen concentration during high water season is a common feature of Marchantaria island and probably in Amazon floodplain (Junk *et al*, 1983). During high water season, the decomposition of terrestrial vegetation and the great quantities of aquatic and semi-aquatic plant communities in Lago Grande that covered a large area of lake, were the main causes of dissolved oxygen depletion and increase of H₂S production (Figure 1). We could smell a strong odor of H₂S in all places of the island. H₂S concentrations were similar among stations. Its concentration varied with depth: traces in the surface layer, low concentration in the middle and higher concentrations near the bottom (Table 1). The biological oxidation, principally by microbial activity, and physical process such as gas exchange and thermal stratification probably are the main factors that maintained the gradient of H₂S in the lake. There was no clear diary fluctuation in concentration of H₂S. During low water season only traces of H₂S were detected (0.6 - 0.96 μM).

The “friagem” occurred on June 21st for one day, in contrast to the other years when it persisted for 2 to 3 days. During this day the H₂S concentration was temporally more pronounced near the surface than in the “normal days”. However, we did not observed fish mortality. Probably, even species with low H₂S tolerance survived because of the short duration of the phenomenon. We collected a high number of specimens of *Mylossoma aurium* (pacu) in stations C and D during this day (Table 2). This could indicate that this species was affected by that phenomenon.

During the study we collected approximately 1.050 specimens of 40 species from 15 families (Tables 2 and 3). Stations B and D showed a bigger number of species and specimens than the other stations in the presence of H₂S. Once H₂S was presented almost in the same concentrations in the different stations, we supposed that species and specimens abundance detected in these stations can be related with the food diversity and reduction in predation pressure. These results agree with Junk *et al* (1983) when they studied the importance of oxygen dissolved on the distribution and abundance of fish

Table 1. Hydrogen sulfide, dissolved oxygen and temperature water were determined in different stations and depth during high and low water season on Lago Grande. These parameters were measured in the surface, middle and bottom at each station.

Stations	Months				Fis.-chem. Parameters
	May	June	July	August	
A	-	1.53	0.31	1.23	H ₂ S (μM)
	-	1.84	0.92	3.37	
	-	6.43	11.03	6.43	
B	0.61	1.23	0.46	0.61	
	1.23	1.84	0.61	0.86	
	21.44	8.88	9.19	4.59	
C	0.61	3.06	0.15	0.58	
	0.92	3.66	0.92	1.84	
	13.78	5.82	7.96	7.35	
D	1.84	2.76	1.53	0.4	
	2.45	3.06	0.61	0.4	
	8.88	4.9	8.88	3.12	
A	-	0.9	2.2	1.5	O ₂ (mg/l)
	-	0.8	0.8	1.1	
	-	0.6	0.6	1.0	
B	1.5	1.0	1.7	1.4	
	0.1	0.9	0.3	0.97	
	0.0	0.5	0.2	0.97	
C	0.5	0.6	1.2	1.5	
	0.2	0.4	0.4	1.1	
	0.0	0.2	0.3	0.97	
D	0.5	0.5	1.2	1.7	
	0.1	0.3	0.35	1.6	
	0.0	0.1	0.33	1.3	
A	-	27.3	30.1	30.2	T°C
	-	27.3	28.4	29.2	
	-	27.2	28.0	28.7	
B	30.4	27.2	29.7	30.3	
	29.2	27.2	28.7	28.3	
	28.7	27.1	28.3	28.3	
C	29.8	27.3	29.6	30.3	
	29.5	27.3	28.6	28.7	
	29.3	27.2	28.3	28.4	
D	29.6	27.2	29.6	30.3	
	29.0	27.2	28.8	30.0	
	28.7	27.2	28.4	29.7	

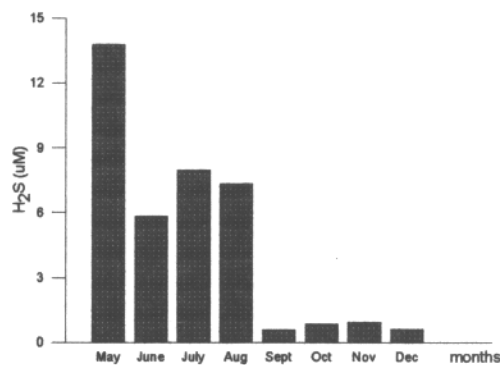


Fig. 1. Seasonal variation of hydrogen sulfide (H₂S) during the high and low water level in a Central Amazonian floodplain lake (Lago Grande). These dates were obtained from station C near the bottom.

species in the Amazon floodplain. However, the highest species number found in June can be justified by high H₂S concentrations detected near the surface. During the low water periods, when the H₂S was almost absent, occurred abundance of specimens. Almost all species were detected in both periods but some of them were more frequent during the low water season (Table 3). It was observed a great concentration of fish species into the lake when the water level diminished. Probably they migrated from flooded forest and other places that had connection to the lake. Comparing the different months, stations and periods, in which this study were done, we concluded that all fish species inhabiting the Marchantaria island are susceptible to H₂S and of course they have adaptations to avoid the H₂S poisoning.

Table 2. Frequency of fish species captured at the different stations during the high water season.

Families Species	Station A			Station B				Station C				Station D			
	J	J	A	M	J	J	A	M	J	J	A	M	J	J	A
OSTEOGLOSSIDAE															
1. <i>Osteoglossum bicirhaosum</i>	-	-	-	-	-	-	3	-	-	-	-	-	-	-	3
2. <i>Arapaimas gigas</i>	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-
CUPLEIDAE															
3. <i>Pellona castelnaena</i>	-	-	-	-	-	1	-	-	-	-	-	-	-	-	-
ERYTHRINIDAE															
4. <i>Hoplias malabaricus</i>	-	-	-	-	1	-	-	-	-	-	-	-	1	1	-
5. <i>Hoplerythrinus unitaeniarus</i>	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
CURIMATIDAE															
6. <i>Potamorhyna altamazonica</i>	2	-	12	2	-	-	4	-	-	-	-	-	5	5	3
7. <i>Potamorhyna latior</i>	-	-	-	1	3	-	-	-	-	-	-	-	1	1	-
PROCHILODONTIDAE															
8. <i>Semaprochilodus insignis</i>	-	-	-	-	5	2	-	-	-	-	-	-	-	-	3
9. <i>Prochilodus nigricans</i>	-	-	-	-	1	-	1	-	-	-	-	2	1	-	4
ANOSTOMIDAE															
10. <i>Schyzodon fasciatus</i>	-	1	-	10	2	-	3	-	-	-	-	3	1	4	-
11. <i>Leporinus trifasciatus</i>	-	-	-	4	-	-	2	-	-	-	-	2	-	2	-
12. <i>Rhytiodus sp</i>	-	-	1	12	5	-	-	-	-	-	-	3	-	-	-
SERRASALMIDAE															
13. <i>Serrasalmus spilopleura</i>	1	1	-	-	2	-	1	-	-	-	-	-	-	-	-
14. <i>Serrasalmus sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
15. <i>Serrasalmus rumbus</i>	-	-	-	-	1	-	1	-	-	-	-	-	-	-	-
16. <i>Pygocentrus nattereri</i>	-	-	-	-	4	-	11	-	4	-	-	3	4	-	-
17. <i>Metynnis sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	1	-	-
18. <i>Mylossoma duriventris</i>	1	-	-	1	1	1	-	-	1	-	-	9	8	-	-
19. <i>Mylossima aurium</i>	1	-	-	-	-	1	-	-	26	-	-	-	32	-	-
20. <i>Colossoma macropomum</i>	-	-	-	4	10	-	2	-	-	-	-	1	3	-	12
21. <i>Piaractus brachypomum</i>	-	-	-	-	-	-	1	-	-	-	-	-	-	-	-
CHARACIDAE															
22. <i>Acestrorhynchus microlepis</i>	-	-	-	-	2	-	-	-	-	-	-	-	-	-	-
23. <i>Rhaphiodom vulpinus</i>	-	1	-	-	-	-	-	1	-	-	-	-	-	-	-
24. <i>Triportheus elongatus</i>	-	-	-	-	1	-	-	-	-	-	-	1	1	-	-
25. <i>Triportheus angulatus</i>	-	-	-	-	-	-	2	-	-	-	-	-	-	-	-
26. <i>Brycon sp.</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	4	2
SCIANIDAE															
27. <i>Plagioscion cf. montei</i>	-	-	-	-	1	1	-	-	-	-	-	-	1	-	-
CHICLIDAE															
28. <i>Cichla ocellaris</i>	1	1	1	-	1	-	1	-	-	1	-	3	4	-	2
29. <i>Geophagus jurupari</i>	-	-	-	-	-	-	1	-	-	-	-	2	-	-	-
30. <i>Astronotus ocellatus</i>	-	-	-	-	1	1	1	-	-	-	-	3	1	1	1
31. <i>Chaetobranchius flavescens</i>	-	-	-	-	1	-	1	-	-	-	-	5	-	-	-
PIMELODIDAE															
32. <i>Pimelodus blochii</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3
CALLICHTHYIDAE															
33. <i>Haplosternum littorale</i>	-	-	1	-	-	-	1	-	-	-	-	-	-	-	-
LORICARIIDAE															
34. <i>Liposarcus pardalis</i>	-	-	2	4	-	-	56	-	-	-	19	12	2	-	24
35. <i>Loricariichthys nudirostris</i>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
HYPOPHTALMIDAE															
36. <i>Hypophtalmus marginatus</i>	-	3	-	-	3	-	-	-	-	-	-	-	-	-	-

Table 3. Frequency of fish species captured only in stations C and B during high and low water season.

Families Species	High water	Low water
OSTEOGLOSSIDAE		
1. <i>Osteoglossum bicirhaosum</i>	3	40
2. <i>Arapaimas gigas</i>	1	16
CUPLEIDAE		
3. <i>Pellona castelnaena</i>	1	
4. <i>Pellona flavipinnis</i>		1
ERYTHRINIDAE		
5. <i>Hoplias malabaricus</i>	1	54
6. <i>Hoplerythrinus unitaenarius</i>	1	
CURIMATIDAE		
7. <i>Potamorhyna altamazonica</i>	6	8
8. <i>Potamorhyna latios</i>	4	4
PROCHILODONTIDAE		
9. <i>Semaprochilodus insignis</i>	7	5
10. <i>Prochilodus nigricans</i>	2	13
ANOSTOMIDAE		
11. <i>Schyzodon fasciatus</i>	15	4
12. <i>Leporinus trifasciatus</i>	6	
13. <i>Rhytiodus sp</i>	17	1
SERRASALMIDAE		
14. <i>Serrasalmus spilopleura</i>	3	31
15. <i>Serrasalmus sp.</i>		25
16. <i>Serrasalmus rumbus</i>	2	5
17. <i>Pygocentrus nattereri</i>	19	61
18. <i>Mylossoma duriventris</i>	4	
19. <i>Mylossima aurium</i>	27	
20. <i>Colossoma macropomum</i>	16	3
21. <i>Piaractus brachypomum</i>	1	4
CHARACIDAE		
22. <i>Acestrorhynchus microlepis</i>	2	36
23. <i>Rhaphiodom vulpinus</i>	1	
24. <i>Triportheus elongatus</i>	1	1
25. <i>Triportheus angulatus</i>	2	1
26. <i>Brycon sp.</i>		8
27. <i>Chalcheus sp</i>		5
SCIANIDAE		
28. <i>Plagioscion cf. montei</i>	2	7
CHICLIDAE		
29. <i>Cichla ocellaris</i>	3	15
30. <i>Geophagus jurupari</i>	1	2
31. <i>Astronotus ocellatus</i>	2	
32. <i>Chaetobranchius flavescens</i>	2	30
DORADIDAE		
33. <i>Pseudodoras niger</i>		5
PIMELODIDAE		
35. <i>Pimelodus blochii</i>		7
36. <i>Pseudoplatystoma fasciatum</i>		5
CALLICHTHYIDAE		
37. <i>Hoplosternum littorale</i>	1	2
LORICARIIDAE		
38. <i>Liposarcus pardalis</i>	79	242
39. <i>Loricariichthys nudirostris</i>		7
HYPOPHTALMIDAE		
40. <i>Hypophtalmus marginatus</i>	3	12

However, the sulfide tolerance limits may differ among the several species living in the same habitat.

Sulfide tolerance limits of some fish species that live in Lago Grande (white water) and Anavilhanas (black water) are showed in table 4. Within the same habitat, the fish species more resistant to hypoxia (*C. macropomum* and *H. littorale*) were more sulfide tolerants. Air breathing in *Hoplosternum littorale* and aquatic surface respiration in *Colossoma macropomum* are important adaptations for surviving hypoxia (Affonso, 1990; Val, 1993) and probably to hydrogen sulfide. Biochemical and physiological mechanisms can be also involved to cope H₂S. As example we have the mitochondrial sulphide oxidation in the California killifish *Fundulus parvipinnis* (Bagarinao & Vetter, 1994) and the option to the anaerobic metabolism (Powell & Somero, 1986, Affonso, unpublished results). The species *Myleus sp* had lower sulfide tolerance than the others above. This can be a characteristic of some species of pacus (e.g. *Mylossoma aurium*) that live in this type of lake. Future experiments will permit to answer this question. The sulfide tolerance limits of two fish species from black water (Anavilhanas) free of H₂S showed that these ones were less sulfide tolerant than that of white water. Even though ones have a low number of fish species studied, these results indicate that the ones living in presence of H₂S might develop different or more efficient adaptive mechanisms to confront hydrogen sulfide than that living in the black water.

Table 4. Sulfide tolerance of five fish species from white water (Lago Grande) and black water (Anavilhanas). n= number of fish in test. Equal number of fish of similar weights were used as controls; none of these died. Weight values are means of fish analyzed.

Species	n	Conc. of H ₂ S at 50% mortality (µM)	Time to 50% mortality (h)	Weight (g)	Source habitat
<i>Hoplosternum littorale</i>	6	50µM	96	99.75	White water
<i>Colossoma macropomum</i>	6	57µM	72	86.29	White water
<i>Myleus sp</i>	6	10µM	72	11.05	White water
<i>Serrasalmus sp</i>	6	7µM	12	186.25	Black water
<i>Leporinus asffinis</i>	5	3µM	6	315.86	Black water

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