

Appendix E-4
**Investigation into fish mortality on the Sao Francisco River,
Minas Gerais, Brazil, September 17 to October 10, 2005**



**Prepared for,
World Fisheries Trust
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Background Information

A significant fish kill occurred in several species of fish in the Sao Francisco River starting in January, 2005 and extending out over several months. The peak mortality that occurred in January occurred in large Surubim (20 kg+). Mortality then started in the smaller Surubim (3kg+) in February and in Mandi and Pacu. Mortality continued at a lower rate through to July, 2005. The total numbers of dead fish and economic value of the fish are unclear at this time but is considered to be significant. During July, August and early September, samples of several species showing signs of sickness were collected by Jason Emert, World Fisheries Trust and sent for analysis for heavy metals. The fish carcasses were stored at -10 degrees C.

The very high mortality in fish stocks in this river system is of great concern both to the community and to the government. This fishery is the main source of economic income for the communities living along the river and many of the species affected are of great economic value. Fish is also one of the main food sources for the local population.

There are many factors that may have contributed to the high level of mortality. To date the main local factors that have been identified as possible contributing factors include,

- a. A major zinc smelter in Tres Marias that historically has discharged untreated water into the river.
- b. Possible pesticide runoff from agriculture on the River Abaete.
- c. Other pollutants including raw sewage from the town and communities that enters untreated directly into the river.

As many of these fish species are migratory to different degrees, waste from other industries and pesticide runoff from agriculture further down river also needs to be considered.

Reported external symptoms in the sick fish and fresh mortalities collected early in 2005 suggest a possible toxicity in the fish. Major external symptoms included pale (anemic) gills and loose flaking skin from the external surface. This investigation was to do an extensive series of tests on fish to try and determine the cause of mortality. These analyses include heavy metals, pesticides, bacterial pathogens, viral pathogens and parasites.

Fish Sampling Program

A sampling program was organized to sample several fish species in several areas in the upper Sao Francisco River. The sampling took place from September 19 to October 2, 2005. The data is presented in Appendix 1, Tables 1 – 5.

Sampling Sites

Sampling sites were chosen based on ease of catching the species required and also based on their location within the 'kill zone'. The sites chosen extended from the dam at Tres Marias downriver to Norbertos Island. Several sampling areas were chosen,

- a. Near the hydroelectric dam: This site is just above the zinc smelter plant
- b. Near the zinc smelter waste water outflow
- c. In the rapids downstream from the zinc smelter
- d. At the mouth of the River Abaete.
- e. Near Norberto's Island.

Fish Species to be Sampled

The plan was to collect as many sick fish as possible. This would include fish that are still alive but in a weakened state or showing abnormal external signs of sickness. The aim was to also collect a minimum of five healthy fish from each of several species. The species chosen represented species where previous mortality had been observed, species of commercial value and species commonly eaten by the local population

Target Fish Species Sampled

The target fish species sampled included Curimba (*Protilodus marggravi*), Pira, Pacu (*Mylopus micans*), Dourado (*Salminus brasiliensis*), Surubim (*Pseudoplatystoma corruscans*), One sample each of a Matricha and a Pacama (*Pseudopimelodus zumgaro*) were also taken although they were not a target species.

A total of 45 fish were sampled over the 3 week period

A minimum of 5 'healthy fish' from each species were sampled except for the Matricha (1 fish) and Pacama (1 fish) which were not in fact target species. Fish showing signs of sickness from each species were sampled where possible. (Unfortunately not a large number of 'sick fish' were caught).

Numbers of Fish Sampled and Health Status

Curimba: 15; 15/15 healthy

Pacu: 9; 1/9 showing external and internal signs of sickness

Pira: 7; 1/7 pale (anemic) gills

Dourado: 7; 1/7 showing abnormal internal signs

Surubim: 5; 1/5 showing abnormal internal signs

Matricha: 1; 1/1 healthy

Pacama: 1; 1/1 healthy

Feeding Type

Curimba: Herbivorous, feed on sediment

Pacu: Herbivorous, feed on sediment

Pira: Herbivorous, feed on sediment

Dourado: Carnivorous (top end)
Surubim: Carnivorous (top end)
Matricha: Carnivorous (mid level)
Pacama: Carnivorous (mid level)

Method of Capture

Curimba: Cast net
Pacu: Cast net and harpoon
Pira: Harpoon
Dourado: Harpoon
Surubim: Harpoon
Matricha: Harpoon
Pacama: Harpoon

The site of capture was recorded as 'approximate location'. GPS coordinates were not recorded for each fish captured as for much of the time the fishermen were out alone in several boats, sometimes fishing at night. It was not possible to accompany the fishermen for each fishing expedition.

Technical Assistance

Technical assistance was provided by Jeremy Hackett (Consultant), Jason Emert (World Fisheries Trust), Gabriel Torquato (University Puc Minas), Ms. Renata Correa (Codevasf), Dr Vince Palace (Fisheries and Oceans Canada), Ms. Lisa Peters (Fisheries and Oceans Canada), Dr. Geraldo Eysink (Consultant)

Weighing and Measuring

Fish were weighed and measured where possible. Measurements were taken for the total length of the fish and for the length from head to fork in tail.

Fish Necropsy

Fish were brought in live except where mortalities were collected for analysis. The eyes and gills were immediately checked for health status on live fish. The external surface was also checked for any lesions or abnormalities. Fish were killed and immediately necropsied to get the required organs for the different analyses. An examination was made of all the organs for abnormalities and noted on the workup sheet. The sex of each fish was also noted.

Samples were collected for the following groups,

- a. Dr. Vince Palace, Fisheries and Oceans, Winnipeg, Canada, (heavy metal and pesticide analysis)
- b. Jeremy Hackett, Consultant, (bacteriology and virology)
- c. Dr. Hugo Godinho, University of Puc Minas, (histology)
- d. FEAM (heavy metals and pesticide analysis) – Note these samples were instead submitted to the Justice Department
- e. Reserva (reserve samples for analysis if required)

Analyses to be Done

The following analyses are to be done on a minimum of five of the target fish species sampled,

- a. Heavy metal analysis
- b. Pesticide analysis
- c. Bacteriology
- d. Virology
- e. Histology
- f. Parasitology

Organs Collected

Liver (Heavy metals, virology, histology)

Gills (Heavy metals, virology, histology)

Muscle (Heavy metals, pesticides, histology)

Brain (Pesticides)

Kidney (Bacteriology, virology, histology)

Spleen (Bacteriology, virology, histology)

Gut (Virology, histology)

Heart (Histology)

Eye (Histology)

Sampling Procedures

Sampling procedures for heavy metal and pesticide analysis were done according to the method provided by Dr. Vince Palace.

Sampling procedures for histology were done according to the method provided by Dr. Hugo Godinho

Sampling procedures for bacteriology and virology were done according to the method provided by Jeremy Hackett

Sample Labeling

A code system was used for labeling samples.

Code: TM (Tres Marias), Day, Month, Species, Sample number, organ

Example:

TM289Pi02

Organ: Liver

Masking tape and a permanent marker were used for labeling as this was found to be the best way of ensuring the label remained fixed to the sample with the storage methods used

Sample Storage and Incubation

All samples for pesticide and heavy metal analysis were wrapped in aluminum foil; samples for pesticide analysis were stored in liquid nitrogen; samples for heavy metal analysis were divided into three groups (Dr. Vince Palace, FEAM, Reserva), labeled and put in labeled, sealed ziploc bags. These were then all stored by date collected at -10 degrees C in large plastic bags that were sealed so that they could easily be identified.

Samples for histology were stored in Bouins fixative for 24 hours and then transferred to 95% ethanol.

Samples for virology had to be stored in ziploc bags at –10 degrees C until a laboratory can be found to process the tissues. (This is not the ideal method but the best option under the current conditions).

Samples of kidney and spleen for bacteriological analysis were plated on Blood agar and BHI agar immediately and incubated at 20 – 25 degrees C for 48 hours. Imprints of the organs including gills were made on microscope slides.

Sample Submission

- a. Justice Department: The samples originally destined for FEAM were instead submitted to the Justice Department for testing for heavy metal and pesticide analysis. The Justice Department contracted the Department of Agriculture to do the analysis for heavy metals and pesticides.
- b. Dr. Vince Palace: We are currently waiting for an export permit to be granted before these samples can be tested in Canada for heavy metals and pesticides.
- c. Jeremy Hackett: Bacteriology was done on site. Selected bacterial isolates were submitted to Dr. Luciene Lima at the Veterinary College in Belo Horizonte for identification. Samples for virology are being stored at –10 degrees C until a suitable laboratory can be found
- d. Dr. Hugo Godinho: Samples for histology were submitted to his lab at the University of Puc Minas for workup.

Results to date (November 6, 2005)

Sampling:

It was very difficult to find 'sick' fish. During the sampling period only one fish in a weakened state was taken. This was a Pacu (TM229Pa01).

Pacu (TM229Pa01):

External Observations: The fish had pale (anemic) gills, opaque eyes.

Internal Observations: The fish was very anemic. Kidney and spleen were granular.

All the other Pacu caught appeared healthy. It should be noted that all the Pacu carried high levels of nematode parasites in the gut, which is not unusual (ref. Renata Correa, Codevasf)

All other fish species taken for analysis appeared healthy at the time of capture. However, several of these fish showed external or internal abnormalities. These included, Dourado, TM249Do04 (pale liver), Surubim, TM0210Su05 (granular/mottled spleen), Pira, TM0210Pi05 (pale gills)

Heavy Metal and Pesticide Analysis:

No results have been obtained to date for heavy metal analysis. Samples were submitted to the Promoter (Justice Dept) on October 13, 2005. The Justice Department is unable to complete the heavy metal analysis or pesticide analysis on the samples submitted as the sample sizes are too small. They require a sample size of 30 grams to 1 kg for analysis.

We are waiting for an export permit to export samples from Brazil to Alberta, Canada for analysis at the Department of Fisheries and Oceans, Winnipeg, Manitoba (Dr. Vince Palace).

Bacteriology

Samples were taken aseptically from the anterior kidney and from the spleen of a minimum of six fish from each species where available and plated on to Blood agar and BHI agar. The Matricha and Pacama were not sampled. Imprints of kidney, spleen and gills were made on slides for gram staining.

Samples were incubated for a minimum of 48 hours at 20 to 25 degrees C. The plates were then observed for bacterial growth and selected isolates were subcultured for identification. Results are presented in Appendix 1, Table 6

Bacterial cultures considered significant were isolated from three species (Curimba, Pacu, Surubim). Dr. Luciene Lima at the veterinary college in Belo Horizonte has identified the bacterial cultures selected.

Kidney: Surubim healthy (TM229Su01) and Curimba (TM219Cu01) *Aeromonas hydrophila*

Kidney: Surubim sick (TM0210Su05) *Staphylococcus epidermidis*

Kidney: Pacu sick (TM229Pa01) *Edwardsiella tarda*

These are all considered as opportunistic fish pathogens and not obligate or primary fish pathogens

Virology

We are still searching for a laboratory to do virus analysis. Once a lab has been identified, samples will be sent for analysis.

Histology

This work is ongoing and is being done in Belo Horizonte at Puc Minas by Dr. Hugo Godinho.

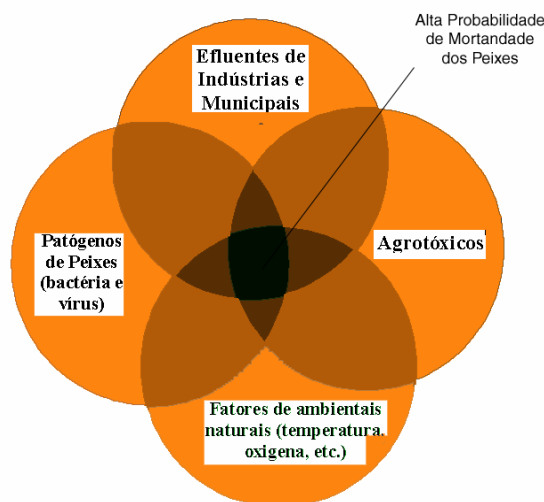
Parasitology

This work is ongoing and being done by Renata Correa at the Codevasf laboratory in Tres Marias. It was noted that in the Pacu there was a high load of nematodes observed in the gut. This was seen in all the fish necropsied including the sick fish. This level of parasite infestation is not unusual (Ref. Renata Correa, Codevasf)

Discussion

Fish kills occur on a fairly regular basis in all parts of the world and in lots of different habitats. Many are caused by natural phenomena such as high temperature, low oxygen, temperature inversions, low water levels, plankton blooms etc. With the reality of climate change these types of mortality are likely to increase. There are also many fish kills that are caused by other phenomena. There are many causes, examples of the main ones being pollution from waste discharge into rivers, eg. heavy metals, toxic liquids, untreated sewage, pesticides and herbicides from agriculture and agriculture waste from farming. The headwaters of the Sao Francisco River lie in the Canastra Hills, in the southern part of the state of Minas Gerais. After running north for about 2,300 km through the states of Minas Gerais and Bahia, the river turns east to run another 400 km between the states of Bahia, Pernambuco, Sergipe and Alagoas to empty into the Atlantic Ocean (ref. Book: Migratory fish of South America). There are many dams on the river that have physically interfered with the natural migratory pattern of many species. There are also many industries along the river and its tributaries and extensive deforestation has been done for agriculture development which has resulted in bank erosion. The industry and agriculture development has resulted in a wide variety of toxic pollutants such as heavy metals and chemical entering the river system.

Each individual factor in itself would have some level of impact upon the different fish species, some species being more sensitive than others. The combined effect of several factors (man-made and natural are enough to cause significant mortality.



It is unfortunate that there are no test results from the fish kill that started in January, 2005 in large Surubim. However, the current investigation from September 17 to October 10, provided the opportunity to collect fish samples from several species to look for levels of heavy metals, pesticides, bacterial pathogens, virus and parasites. The species targeted included some of those affected by the mortality but also those of economic

value and a main food sources of the population. It was hoped that more 'sick' fish could be caught during the investigation; however, the sampling of several fish from five different species in the Upper Sao Francisco provided the opportunity to obtain baseline data for these species for heavy metals, pesticide levels, bacterial pathogens and virus pathogens and may provide some answers with respect to the cause of mortality earlier in the year. To date, pesticide results and bacterial results are complete. No pesticides have been detected in any of the samples analyzed so far. The bacterial results showed that the one sick Pacu fish was infected with *Edwardsiella tarda*, an opportunistic fish pathogen. Other opportunistic pathogens isolated included, *Staphylococcus epidermis* isolated from a Surubim showing internal disease signs and *Aeromonas hydrophila* isolated from several 'healthy' Curimba and from a 'healthy' Surubim. These results are significant in that it demonstrates that bacterial pathogens may have a role in the fish mortality that occurred. Results of heavy metal analysis will be available once an export permit is granted by the Brazilian government for samples to be shipped to Canada for analysis. The results from the analyses to be done in Canada will be of great interest and significance and will provide some excellent baseline data. The significance of the baseline data obtained from this study includes,

Heavy Metal Analysis:

- Will determine the presence or absence of heavy metals in several fish species.
- If present, may demonstrate a possible relationship with fish mortality.
- Will determine the safety of fish to eat (heavy metal levels in muscle)
- Will provide baseline data for database

Pesticide Analysis:

- Will determine the presence or absence of pesticides in several fish species.
- If present, may demonstrate a possible relationship with fish mortality.
- Will determine the safety of fish to eat
- Will provide baseline data for database.

Bacteriology:

- The presence of bacterial pathogens demonstrate a possible relationship with fish mortality.
- Will provide baseline data for database.

Virology:

- If viral pathogens are present, they may demonstrate a possible relationship with fish mortality.
- Will provide baseline data for database

Parasitology:

- If parasites present, they may demonstrate a possible relationship with fish mortality.
- Will provide baseline data for database

Future Work

There is a need to continue this work,

- a. To collect further samples according to Brazilian guidelines and have the samples analysed in Brazil
- b. To develop a lead agency and an ongoing monitoring plan for the fishery which includes the fishermen and the required government sectors
- c. To develop a centralized database
- d. If the mortality is repeated in January, 2006, to have a well organized monitoring plan in place to collect and record fish and collect samples for analysis
- e. To examine the economic impact of the fish mortality
- f. To develop research programs to look at impacts on the fishery
- g. To request funding from government agencies to save and restore the fishery as needed

There are many theories as to why this particular fish kill occurred. The initial mortality was at a time just after the spawning time of several species including the Surubim. Prior to spawning most fish species are not actively feeding and post-spawning fish are in a weakened state. This, combined with a toxic insult and/or infection by an opportunistic pathogen, would certainly be adequate to cause significant mortality.

Summary

In summary, the results of the testing on the fish samples collected from September 17 to October 2, 2005 and analysis of the samples collected earlier during July, August and early September, 2005 will provide answers to some of the theories being put forward as to why this mortality occurred. It will also provide results with respect to the safety of these fish to eat. The data collected will be extremely useful as baseline data for a future extended monitoring program. Results from pesticide testing are to date negative for the presence of pesticides targeted in the analyses. Results from bacteriology testing suggest that bacterial pathogens may have had a role in the fish mortality. *Edwardsiella tarda*, *Aeromonas hydrophila* and *Staphylococcus epidermis* are all recognized as fish pathogens. No results are yet available for virus testing, histology or heavy metal analysis.

This short work term in Brazil was also an opportunity to make a contribution to a conference focused on this fishery and also provided an opportunity to transfer technology to local scientists and the fishermen.

Transfer of Technology to Brazilian Groups and Conference Presentation

Transfer of Technology to Brazilian Groups

A part of this project is to be able to transfer technology to Brazilian groups.

While in Brazil I spent time with Renata Correa, parasitologist at Codevasf laboratory and provided her with some training in bacteriology. I will be corresponding with her by e-mail to provide additional assistance with protocols.

We also identified a laboratory in Belo Horizonte that is interested in taking on the fish health bacteriology work. This new fish health lab is situated at the veterinary college (VFMG) and is being managed by Dr. Luciene Lima. I will be corresponding with her on an ongoing basis with respect to the identification of the bacterial cultures isolated and possible further involvement. I have been told that there is also a plan to start a fish virology laboratory in January, 2006.

It is planned that someone will go to Alberta from Brazil to train with Dr. Vince Palace in their lab when the samples are being analysed for heavy metals and pesticides.

Conference and University Presentations

A presentation was made at a two day conference organized by CIDA in Tres Marias which approximately 200 people attended and was focused on the fishery and the fish mortality. The title of my presentation was 'Investigation into the fish mortality on the Sao Francisco.' It was a tremendous opportunity for different groups to present information and data on various aspects that may be related to the fish mortality on the Sao Francisco. One of the significant outcomes was the development of working groups in a network of cooperation,

Working Groups

1. Fish health monitoring and research.
2. Public health
3. Sanitation
4. Social communication
5. Regulatory

I also made a presentation to a University class at PUC Minas University which also had the title, 'Investigation into the fish mortality on the Sao Francisco.'

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Dr. Geraldo Eysink, Consultant, San Carlos, Brazil

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Dr. Luciene Lima, VFMG, Belo Horizonte, Brazil

Norberto, Tres Marias, Brazil

Bigua, Tres Marias, Brazil



Fig 1. Pacu (*Myleus micans*) (TM229Pa01), showing anemic gills



Fig 2. Pacu (*Myleus micans*) (TM229Pa01), showing pale liver and no food in gut



Fig 3. Pacu (*Myleus micans*) (TM229Pa02), showing healthy gills

APPENDIX 1
Tables 1 to Table 6

APPENDIX 2

References

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