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Ecological Risk Assessment
of
Human Impacts on Ecosystems
in
Parque Estadual Turístico do Alto Ribeira
(PETAR),
State of São Paulo, Brazil

Status report from an ERA project
August 1998

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Rosana Moraes

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Abstract

The case study of this ERA-project is about human impacts on ecosystems in a Brazilian reserve of rain forest in the south western part of the State of São Paulo, the Parque Estadual Turístico do Alto Ribeira (PETAR). The reserve is of special interest because of the small amount of relatively undisturbed Atlantic rain forest in Brazil, and overall, because of the fauna, which include several endemic species and several species threatened by extinction e.g. jaguar and otter.

This report summarize some background information, the ecological risk assessment framework, with specific focus on the PETAR ERA-project, and some data from a first field trip to PETAR during June 1998.

Anthropogenic stressors, suspected of affecting PETAR ecosystems, are often stemming from sources related to land-use, mostly outside the reserve, *i.e.* agricultural activities (inorganic nutrients and pesticides used in tomato culture) and closed mining activities (heavy metals), as well as human settlements (sewage water containing oxygen demanding organic compounds and inorganic nutrients). The selected assessment endpoint is the fish community, with focus on two groups of catfishes, the predator catfishes (Fam. Pimelodidae) and the periphyton grazing catfishes (Fam. Loricariidae) which represent important food resources for both otters and humans. Conceptual models, relating sources of stressors to the endpoint and an analysis plan, are presented here. The applicability of indices of biotic integrity complemented by histopathological studies in PETAR as measurement endpoints are under investigation. Water quality analysis and fish sampling results from the first field trip that took place in June 1998 are preliminary discussed at the end of the report.

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1. Background

Considered one of the three most threatened ecosystems on Earth, Brazil's Atlantic Forest has been reduced to less then 8% of its original area. The the Parque Estadual Turístico do Alto Ribeira (PETAR) is of special interest because of the small amount of relatively undisturbed Atlantic rain forest in this area, and because of the fauna, which include several endemic species and several species threatened by extinction, *e.g.* jaguar and otter. The project also wants to address some important methodological issues related to the integration of data on exposure and effects, as well as the use of this integrated data for risk management and communication.

Many studies in the temperate zone have shown that one of the most severe threats to diversity is the release of contaminants in the environment as a consequence of human activities. However, little research has been done on the impact of contaminants in the tropics. Tropical ecosystems contain more than 75% of the global biological diversity, but most of the ecotoxicological research has focused temperate zones. However, environmental degradation in tropical ecosystems has increased over the past decade and new studies are essential. Considering physical, chemical and biological attributes, tropical environments differ ecologically from temperate ecosystems. This means that our understanding of contaminant fate, transport and effects in temperate ecosystems may have limited application in the tropics (Lacher & Goldstein 1997). Thus the project also want to contribute to tropical ecotoxicology.

Two proposals to SIDA were partially approved in 1997. The initial proposals were for a research and a doctoral project respectively. The approval from SIDA was for a doctoral project and funding was provided for one year starting 980101. The doctoral student, Rosana Moraes, has started her doctoral studies working on the ecological risk assessment (ERA) in Parque Estadual Turístico do Alto Ribeira (PETAR).

A second application was submitted in May 1998. This report complements that application and relates what has been accomplished with respect to literature and background studies after about seven months. The report also further develops a detailed and more focused analysis plan.

Further information on PETAR was collected in January/February 1998, which resulted in a better knowledge about viewpoints on the most relevant environmental problems in the area and the present state of available scientific information. During that period, Ms. Moraes visited potential Brazilian collaborators in universities, research institutes, and NGOs. One result of these contacts was the establishment of a Brazilian-Swedish multidisciplinary research group composed of professionals from many disciplines, some with long experience from PETAR. Those collaborators also have provided much information reported here and they are briefly presented below.

Ph.D. **Eleonora Trajano** is docent of the Department of Zoology of University of São Paulo, co-supervisor of Rosana Moraes and supervisor of several masters and doctoral students that are studying or had studied ecology of aquatic fauna of PETAR. Biologist **Sônia Buck** is a doctoral student and has experience in studies of fish communities in Atlantic rain forests. Biologist **Pedro Gerhard** is studying population ecology and social behavior of catfishes in PETAR. Geologist **Hélio Shimada** is a scientific researcher in mining and environment at the Geological Institute (IG) of the Environmental Secretary of the State of São Paulo and a Ph.D. student of the Institute of Geosciences of the University of São Paul.

Sr. **Roberto Bürgi,** biologist and the director of PETAR is an important partner for this project since he represents the responsible authorities. He can also contribute to the project with his professional expertise since he is working with the mapping of vegetation and water resources of PETAR using GIS. Geographer **Viviane Mazini** works in the Cartography Laboratory of the Instituto Socioambiental with land use mapping of the PETAR area using Geographical Information System (GIS) and satellite and aerial images. The **Instituto Socioambiental (ISA)** is a non-governmental organization in Brazil involved in an socio-environmental characterization of the Ribeira Valley. **João Paulo Capobianco,** biologist and executive secretary of the institute coordinates a project that includes identification of environmental, social and economical indicators, and the identification of conflict areas that could give subsidy for planning and governmental and non-governmental actions. The ISA is also working on deforestation indices and maps for the years 1990 to 1995 in the Ribeira Valley, where PETAR is located. An agreed cooperation between Instituto So-

cioambiental (ISA) and the Technical Environmental Planning Department at Chalmers University of Technology was signed.

Environmental chemist **Henrik Kylin**, PhD, is at the Institute of Environmental Analysis at the Swedish Agricultural University, Ultuna. He is involved in that part of the project, which deals with exposure analysis of pesticides as advisor for the sampling program and as analyst of the pesticide samples. **Johanna Lundqvist** is a master student of an international master program at Chalmers University of Technology. She takes part of fieldwork and analysis of water quality and is going to write a master's thesis on anthropogenic impacts on inorganic nutrients and primary production in the streams of PETAR.

Ms. Moraes also attended the Symposium "Extrapolation in Human Health and Ecological Risk Assessments" organized by the US EPA in Cary, North Caroline last April. During the symposium new concepts and methodologies that have recently been applied in Ecological Risk Assessment by American researchers was presented and the project was discussed with Dr. Marshall Adams (Oak Ridge National Laboratory, USA) and Dr. Michael Power (University of Manitoba, Canada). Both researchers have provided important and relevant suggestions for the improvement of the project.

The first field trip took place during 7-17th June. Rosana Moraes, Johanna Lundqvist, Pedro Gerard, Sonia Buck, Helio Shimada and Roberto Bürgi participated in the planning and implementation of a sampling campaign and data collection. The main objective of this trip was to select sampling sites, to test sampling techniques and analysis methods which will be used during the next trip (October-November 1998) when a more detailed and exhaustive sampling will take place. A description of applied methodologies and preliminary results on water quality together with results from sampling of fish communities are presented here.

2. Ecological Risk Assessment

Risk Assessment is the process of assigning magnitudes and probabilities to the adverse effects of human activities or natural catastrophes. It involves identifying hazards, and uses measurements, testing, and mathematical or statistical models to quantify the relationship between the initiating event and the effects (Suter 1993). Risk Assessment is found in many different contexts, from insurance companies over financial concerns in banking to the assessment of risks related to human activities versus the environment. It is a fundamental component of decision-making because the cost of eliminating all possible adverse effects of a human activity is impossibly high. Risk Assessment aids in the rational weighing of various options.

Numerous and diverse risk assessment methods have been developed for protection of human life, health, and property which, in most of the cases, ignore ecological effects. This tendency results partly from anthropocentrism and partly from the common mistake that protection of human health automatically protects non-human organisms, despite the fact that many examples show that non-human-organisms, populations, or ecosystems may be even more sensitive than humans (Suter 1993). The field of risk assessment that is concerned with events in the environment is called Ecological Risk Assessment (ERA) and the focal point is shifted from humans to the entire ecosystem, or certain parts of it like important species, populations, communities or habitats. The objective of risk-based environmental regulation is to balance the degree of accepted risk against the cost of risk reduction and competing risks.

Unites States Environmental Protection Agency have initiated work on ERA and suggests that ERA "evaluates the likelihood that adverse ecological effects may occur or are occurring as a result of exposure to one or more stressors" (US EPA, 1992). They also state that ERA "is a process for organizing and analyzing data, information, assumptions, and uncertainties to evaluate the likelihood of adverse ecological effects. Ecological risk assessment provides a critical element for environmental decision making by giving managers an approach for considering available scientific information along with the other factors they need to consider (e.g., social, legal, political, or economic) in selecting a course of action." (US EPA, 1996)
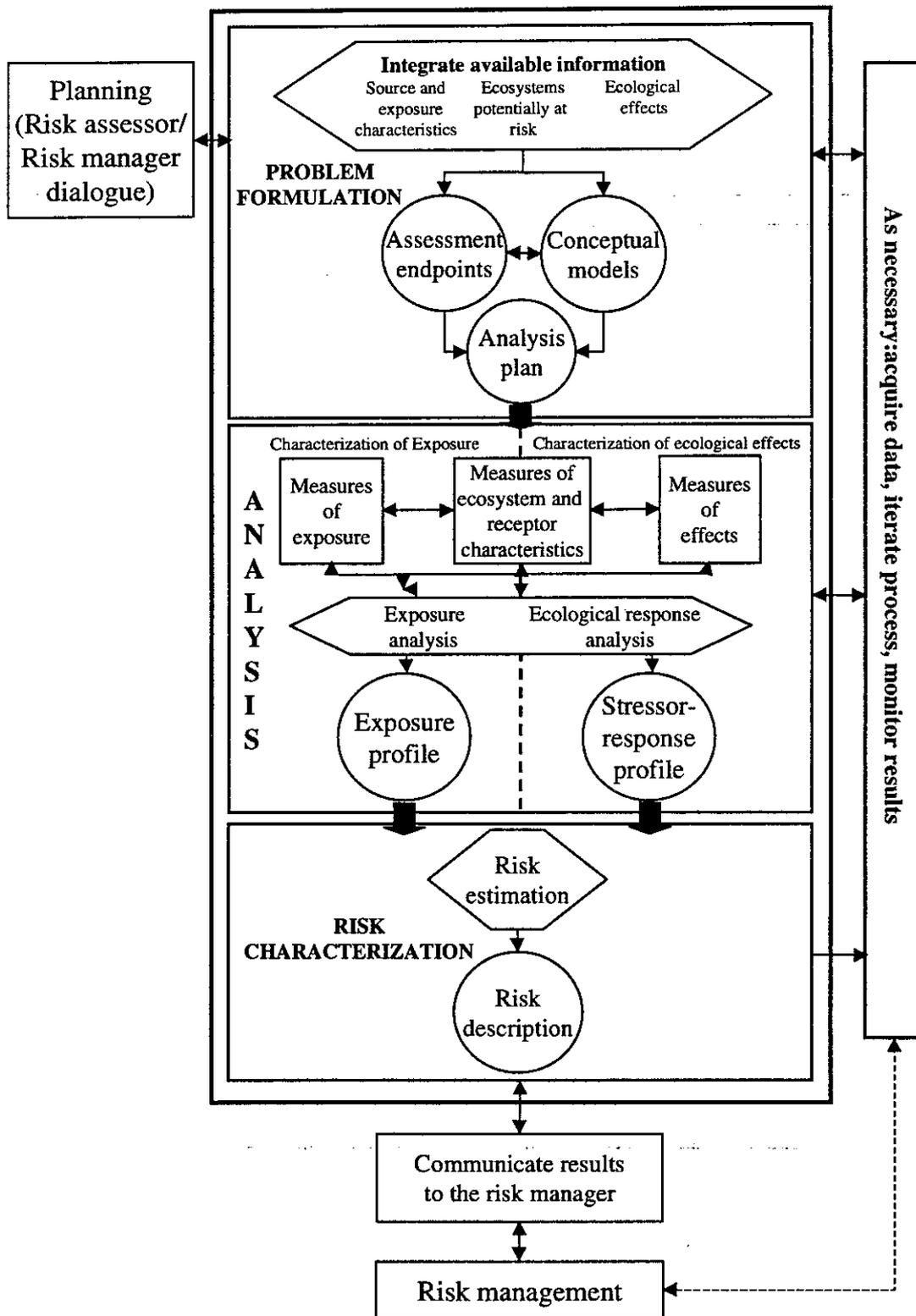
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Figure 1. *The Ecological Risk Assessment Framework*

Source: EPA (1996).

There is relatively little literature specifically on ecological risk assessment (see however, Clifford et al. 1995, Linder et al. 1993, Peters et al. 1997, US EPA 1996, and a very large literature in the related field of ecotoxicology). The field is however rapidly developing and much attention is paid to the proposed ERA guidelines (US EPA, 1996), where several important fields of further investigations is identified.

The proposed framework for ERA (US EPA, 1996) includes three steps: Problem Formulation, Analysis and Risk Characterization (Figure 1). ERA is a methodical and systematic way of gathering and ordering relevant information, to aid in an complex decision-situation, and is thus following a general systems analysis methodology (Miser and Quade, 1985). A stringent identification of the stress-inducing agent(s) or activities and their targets together with a thorough analysis of exposure and potential effects are the information on which the quantitative risk assessment is based.

A quantitative risk value is however not necessarily a realistic prediction of the effect of the exposure. Due to uncertainty, risk assessments generally do not present an absolute estimate of risk; rather most risk assessment establishes plausible upper bounds on risk to indicate the potential for adverse impacts (Anderson et al. 1990). Regulation and a rational decision making, however, would be impossible, without some method of making public decisions facing the scientific uncertainties involved and risk assessment methods are probably better than other methods for preparing these decisions. The benefits of the risk assessment methods are its systematic and generally reproducible method of digesting and analyzing relatively large bodies of complex information. Furthermore, it avoids the arbitrariness of relying upon appeal to authority or faith, and it assists in demonstrating that zero risk is no real option. However, the most important reason to accept the principles of risk assessment may be that, as a practical matter, there are no other feasible alternatives for formulating the input to a dialogue between interested parties and ERA is in this respect similar to other participatory methods like Environmental Impact Assessment.

The ERA-process is also described by the US EPA. It includes the choice of assessment endpoints (the valued ecological entity forming the focus of the assessment) the identification of stressors (chemical, physical or biological agents potentially affecting endpoints), exposure and effect analyses and, finally, a risk characterization step where the risk adverse ecological effects are estimated and interpreted.

3. Parque Estadual e Turístico do Alto Ribeira - Literature Review

3.1. General Characterization

Parque Estadual Turistico do Alto Ribeira (PETAR) was created in 1958 aiming at the conservation of the area for the future. The reserve is located in the southwestern part of São Paulo State (Figure 3), in the Ribeira Valley, between the municipal districts of Apiaí and Iporanga. The Ribeira Valley was originally occupied by indigenous populations but later, starting in 1531, colonized by Europeans. New villages were created in connection to the gold mines and later, due to the development of agriculture, chiefly for cropping of rice. The Ribeira Valley is a good area for mineral exploitation. Since the 17:th century mining companies have affected it not paying attention to the potential risks related to environmental pollution (Kotez, not published).

The altitude of the hilly terrain of the park (Figures 2) varies from about 100 to 1000 m above the sea level. Most of its 35.712 hectares is situated on the Paranapiacaba Mountain Ridge. Together with the other neighbor reserves - Fazenda Intervales State Park, Serra do Mar Environmental Protection Area, Xitue Ecological Station and Carlos Botelho State Park - it constitutes one of the most protected areas of the state of São Paulo (Figure 4). Those five areas represent more than 400.000 hectares of protected Atlantic Rain Forest. Many species threatened by extinction can be founded in the forests (*e.g.* jaguar, *Pantera onca*), in some of the many rivers and streams which cross the area, (*e.g.* otter, *Lontra longicaudis*), or inside caves (*e.g.* blind catfish, *Pimelodella kronei*).

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Figure 2. *Picture of Betari Valley showing the hilly topography of the region.*

(R. Moraes).
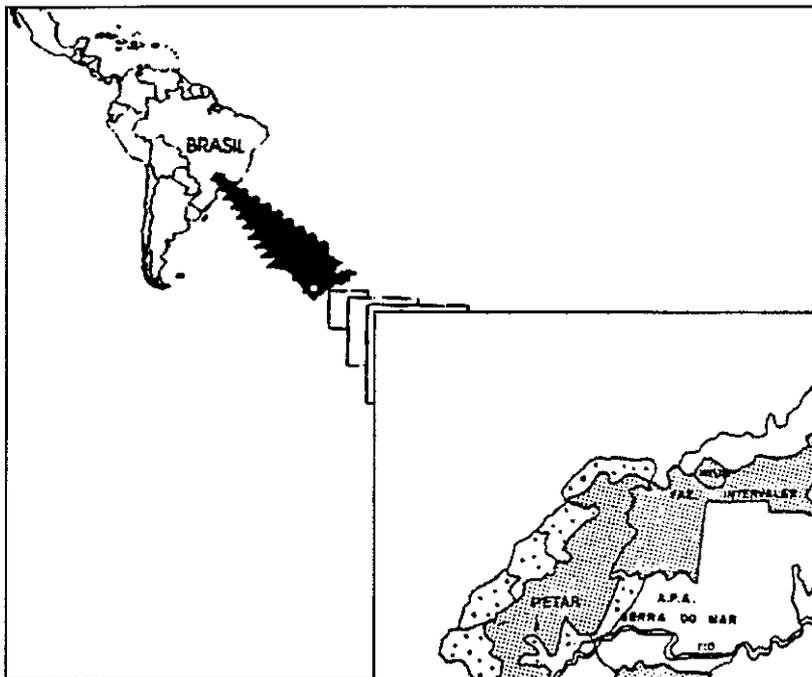
Figure 3. *Localization of Parque Estadual Turistico do Alto Ribeira (PETAR), Sao Paulo state, Brazil.*

(Courtesy of Sr. Roberto Burgi).
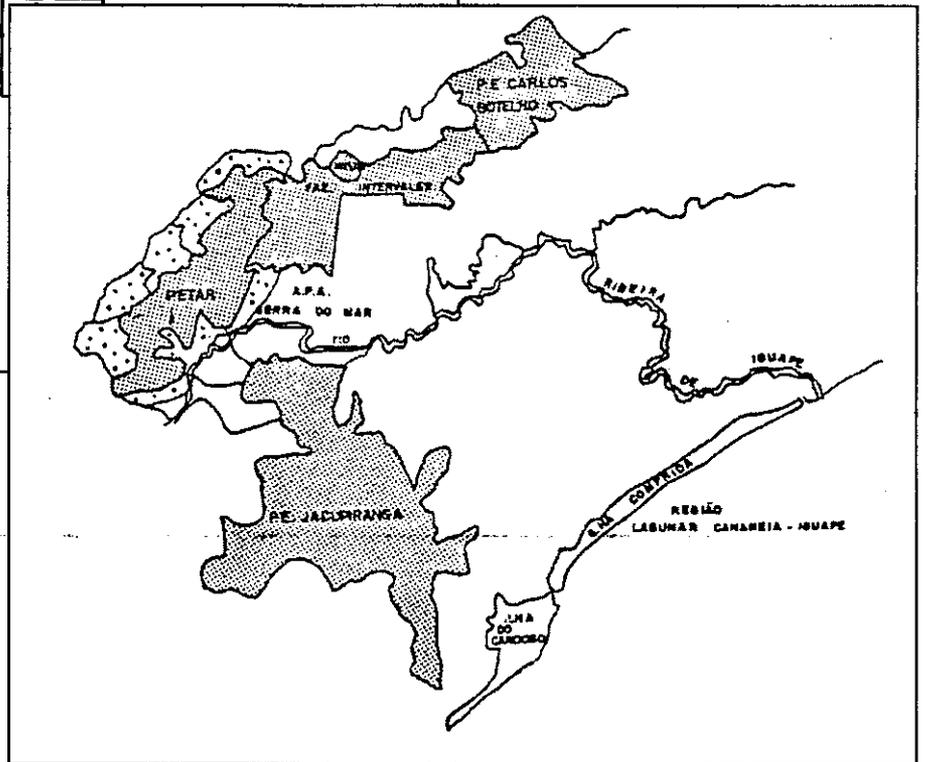
Figure 4. *PETAR and other protected rain forest areas in the Ribeira Valley*

(Source: SMA, 1991)

The climate is classified as mesotermic humid (Cfb), according to Köpper (Lepsh 1988). Two air masses affect the region during a year. The Atlantic Tropical air mass is the most prevalent, and influences the rain distribution. The influence of the Atlantic Polar air mass just lasts for a short period during the winter season. The dry period occurs between April to September. Figures 5 A and B show precipitation (1972-1997) and temperature patterns in the region (1955).
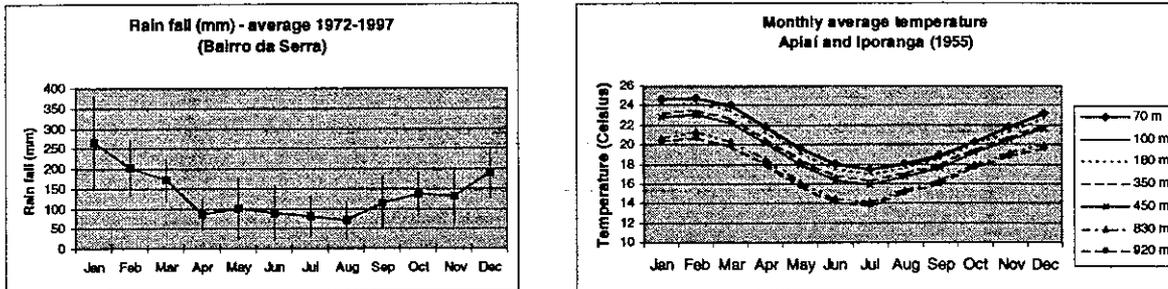
Figure 5. A. *Monthly average and standard deviation of rainfall in Bairro da Serra, Berati valley (1972-1997).*
B. *Monthly temperature average and standard deviation at five different sites in Apiaí and Iporanga region (1955).*

Source: Water and Electrical Energy Department of the State of São Paulo (DAEE).
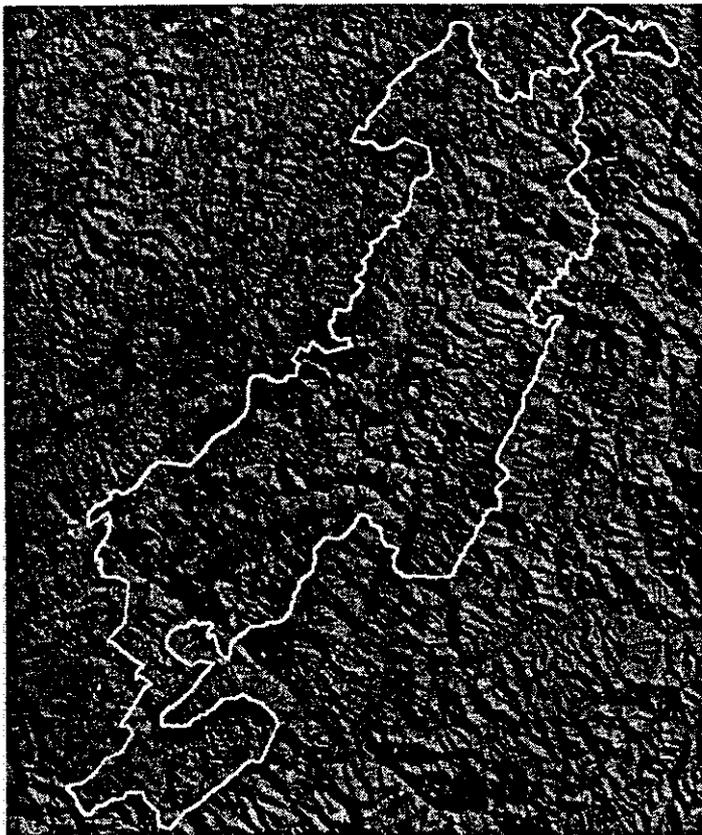
The Ribeira Valley is located on a geological unit called the Açungui Group, dated from the Pre-Cambrian period. Since the area is of interest for mining activities, many geological surveys have been carried out in the region (e.g., DNPM 1981, 1984; IPT 1988, 1989). Calcareous rocks emerge non-continually, forming 1 to 10 km wide strips directed NE-SW. Most of the rivers in this region form caves (Sanchez 1984). PETAR have one of the highest concentrations of caves in Brazil, with more than 180 registered caves. The Santana cave is internationally known due to its diverse and rare ornamentation.

Figure 6. *Satellite image (Landsat 5 TM 1997) of PETAR.*

(Kindly provided by Roberto Bürgi, PETAR).

3.2. Social and economical aspects

The Ribeira Valley is one of the poorest and less developed areas of the state of São Paulo. According to the Environmental Secretary of the State of São Paulo, the social indicators (year 1993) are worse than the average for the state: high illiteracy (22%), high index of infant mortality (4.2%) and precarious house conditions (only 66 % of houses are provided with public water system and only 22% are connected to sewage

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systems). The average values for the State of São Paulo are: 14% illiteracy, 2.7% infant mortality, 95% public water system, 76% sewage systems (SEMA 1997).

Comparing data from Apiaí and Iporanga with the average of the municipalities in the state of São Paulo (Table 1) it is possible to see that most of the population lives in the rural areas and that there is a high level of illiteracy and poverty.

Table 1. *Population data of Apiaí, Iporanga and average of the entire state of São Paulo.*

Source: SEADE. 1991.

	Iporanga	Apiaí	São Paulo State
Population (inhabitants)	4 600	16 700	31 436 273
Rural Population (%)	64	53	7
Demographic Density (inhab./km^2)	3,6	28,3	126,9
Illiteracy (% among adults)	33	23	11
Income lower than US$ 250	83	74	35

Agriculture is the main economical activity in the Ribeira Valley but its expansion faces problems related with land occupation and environment, including soil, topography and climate (IBGE 1981). According to the Environmental Secretary of the State of São Paulo, land ownership matters are one of the main drawbacks to the development of agriculture in the valley. Approximately 40% of the land do not have their juridical situation defined. Due to the complexity and slowness of the juridical process, many conflicts are created and environmental protected areas are frequently invaded (SEMA 1997).

As shown in figure 7, the economy of the Ribeira Valley is mainly based on fishing and agriculture (47% of the population). Fishing and agriculture is even more important for the populations living in Apiaí (51%) and Iporanga (63%), the two towns closest to PETAR. Notice that those data do not include hunting, gathering and subsistence agriculture, which are also very important in the region.
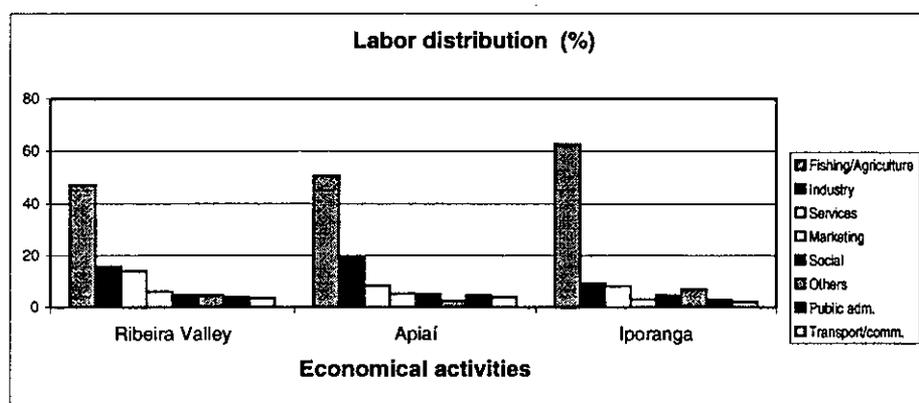
Figure 7. *Labor distribution among different economical sectors in the Ribeira Valley (average of all municipalities) and in Apiai and Iporanga.*

Source: IBGE 1981.

Sixty five percent of the agricultural properties in Apiai (total 99 049 farms) are smaller than 10 ha, and they represent only 6.4% of the cultivated area in the municipality (Figure 8). Most of the farms in Iporanga (56% of the 45 798 farms) have between 10 and 199 ha (IBGE 1987 *apud* Lepsch 1988). In both municipalities, approximately 70% of the land are farms larger than 100 hectares, and they represent less than 10% of the properties. In other words, most of the land is concentrated in the hands of few persons, which reflects not only the situation in Apiaí, Iporanga or Ribeira Valley, but also in the whole country. Furthermore, most of the landowners do not have enough money to buy fertilizer, which has a great effect on land use practices. For instance, after two or three years of tomato plantation, the soil became poor and most of the farmers switch to another kind of culture or clear forest for new fields or just abandon the land (Sr. Modesto, PETAR employee, pers. comm.). Due to this practice, the deforested area around the PETAR has increased during the last years.
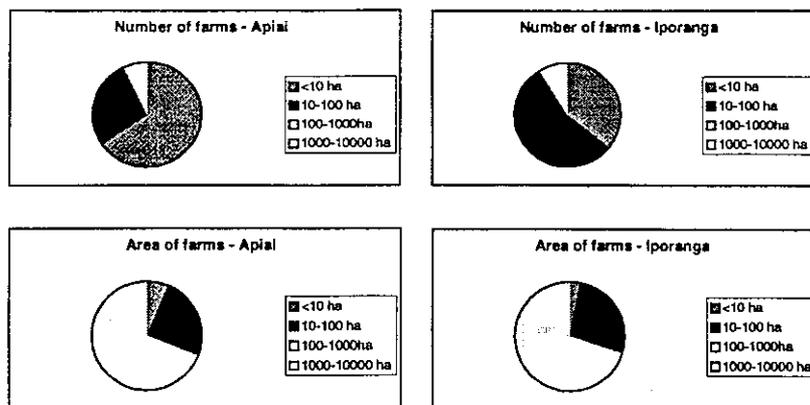
Figure 8. *Number and area of different agriculture propriety sizes in Apiai and Iporanga municipalities.*

(Source: IBGE 1987 apud Lepsch 1988).

In this social and economical context it is easy to understand most of the social problems that the administration of the park has to face: partial deforestation due to illegal logging and subsistence farming, exploitation of palm cabbage, construction of illegal roads, poaching and illegal mining. According to the legislation, all activities, which could affect the ecosystem or landscape integrity of the parks, are illegal. Due to the impacts, from roads, heavy machines and discharges, mining activities are considered completely illegal inside a natural reserve like PETAR. It is however known that in many cases the mining activity in areas outside the park is just an excuse for construction of roads inside the park opening up areas for logging and exploitation of palm cabbage (SDLP 1985). All these conflicts have to be handled by a small number (8) of low-paid, badly equipped guards working in 2 teams (in 1991) and facing the risk of violent conflicts.

4. Ecological Risk Assessment in PETAR

4.1. Source of stressors and stressors characterization

The stressors of this study are related to anthropogenic sources connected to land-use mostly outside the reserve, *i.e.*, lead (heavy metals) and calcarious (particles) mining, agriculture (inorganic nutrients and pesticides used in tomato culture) as well as human settlements inside the reserve (sewage water containing oxygen demanding organic compounds and inorganic nutrients).

Human Settlements – plant nutrients

There are between 10 and 15 small villages inside the park, which are composed by groups of families (e.g. around 20 families). Most of them survive with subsistence agriculture, but their main activity is poaching and gathering. Untreated domestic sewage from small villages located inside the park and in areas near the park is discharged directly-into watercourses. Close-to the settlements-possible-effects are related to the decrease in oxygen concentrations in the water due to the breakdown of organic compounds, while at longer distances the outlets may cause an anthropogenic eutrophication, i.e. a change towards a more nutrient-rich condition in the ecosystem due to an increased input of plant nutrients. This may affect the physico-chemical environment of the freshwater ecosystems and may lead to an increase in algal and macrophytal biomass, if there is no other limiting factor (SEPA 1994). This potential change in species composition and thus in the community structure of the primary producers of the aquatic environments are of great importance for the future of the aquatic ecosystems of the PETAR, and even for the entire ecosystem of the park, if and when connections between aquatic ecosystems and the entire ecosystem turns out to be crucial.

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Table 2 shows some water measurements taken from Betari rivers and some of its tributaries during dry (July, measurements at sites 1 to 15) and rain season (February and October, measurements at sites 16-18). Water temperature ranged from 11 to 22 °C. pH values ranged from 5.2 to 8.5. The lowest value, measured at Furnas River tributary (site 5) is under the value recommended by CONAMA (Brasil 1986 *apud* CETESB 1991) for aquatic life preservation. DO values ranged from 5.44 (Bica do Parque spring, site 10) to 11.0 mg/l (Furnas river, site 18). All values are above the recommended (5.0 g/l) by CONAMA for aquatic life preservation (Brasil 1986 *apud* CETESB 1991). Nitrate values ranged from 0.011 to 0.497 mg/l. Those values are below the recommended by CONAMA for aquatic life preservation, 10 mg/l (Brasil 1986 *apud* CETESB 1991).

Table 2. *Water quality parameters in the Betari river and its tributaries.*
Sources: Measurements 1-15, Nogueira & Negri (1993); 16[1]-18 CETESB (1991), 16[2]-17[2] CETESB (1996).

	Temp. water (°C)	pH	Hardness	DO (mg/l)	Turbidity (U.N.T.)	Nitrate (mg/l -N)	Nitrite (mg/l -N)	Ammonia (mg/l - N)	Total N (mg/l -N)	Total P (mg/l -N)
1	17	7.5	90.80	7.04	0.65	0.242	0.001			
2	18	8.5	105.29	8.00	0.05	0.213	ND			
3	18	8.3	82.11	7.84	0.05	0.165	ND			
4	18	7.5	16.42	6.88	0.06	0.156	ND			
5		5.2	11.59	7.04	37.0	0.147	ND			
6	17	8.2	69.12	8.48	0.55	0.306	ND			
7	15	8.1	147.8	7.36	0.70	0.574	0.001			
8	16	6.4	23.18	7.84	0.50	0.080	0.001			
9	14	7.1	17.38	6.40	2.70	0.165	ND			
10		6.4	21.25	5.44	0.50	0.222	ND			
11	16	7.0	24.15	6.72	2.00	0.263	0.001			
12	16	7.4	83.08	6.08	1.70	0.232	ND			
13	11	5.9	24.15	7.41	0.50	0.011	ND			
14	16	6.8	76.31	8.09	0.65	0.497	ND			
15	16	7.5	66.65		0.90	0.483	ND			
16[1]	19	6.0	75.6	9.00						
17[1]	22	6.1		9.60		0.320	ND	0.12	1.20	0.015
18	19	7.3	95.9	11.0						
16[2]	19	7.6		8.20	3.00	0.400	<0.01		0.61	
17[2]	19	7.4		8.30	3.00	0.420	<0.01		0.83	

1. Sampling period: 1-15 taken in July 1993; 16[1]-17[1] -18 February 1991; 16[2] – 17[2] October 1996
2. Sites: 1 Bica do Quiosque, 2 Santana Cave / Roncador stream, 3 Couto cave, 4 Morro Preto Cave / Morro Preto stream, 5 Furnas river tributary, 6 Agua Suja cave, 7 Laje Branca cave, 8 Biquinha spring, 9 Espalhada spring, 10 Bica do Parque spring, 11 Nhotingueira spring, 12 Ressurgencia das Areias cave, 13 Morro do Tatu spring, 14 Ouro GrossoCave, 15 Alambari de Baixo cave / Alambari cave, 16 Betari river upstream Furnas river confluence, 17 Betari river dowstream Furnas river confluence, 18 Furnas river

Agriculture – plant nutrients and pesticides

Springs of the Pescaria, Iporanga and Betari rivers that are crossing the park are situated in important areas of preserved forest, but headwaters are also found in areas where certain human activities outside the park may affect the aquatic environment inside the park. The park authorities have been trying to expand the park boundaries to include these areas, but without success due to conflicts with landowners. In those areas, farmers cultivate among other crops, tomatoes and peaches. Ppesticides are spread and may leak into watercourses, exposing biota giving the possibility of adverse effects on the fish community. Related to, but still different from, the release of domestic sewage water is the leakage of plant nutrients from different types of agriculture. Both inside and outside of the park different types of agriculture occur giving different effects in the watercourses. The subsistence agriculture illegally practiced by many small farmers inside the park adds nutrients to the streams by increasing the losses from the soil as a consequence of the altered use of the land (from forest to agriculture) while larger farms using fertilizers contribute by this.

Tomato (*Lycoperscon esculentum*) is a typical sub-tropical plant and is susceptible to both frosts and elevated temperatures. Due to these characteristics, production in São Paulo State during the warm season (January to April) is limited to high altitude areas, such as Apiaí and neighbor municipalities. Brazil was the eighth largest world producer of tomato (3% of the world production) and São Paulo State is the most important tomato producer in Brazil (48% of the production in 1979). Of all vegetables produced in Brazil, tomato is the one that requires most pesticides. Carbofuran, chlorothalonil and deltamethrin are three examples of pesticides used in the region. They are used at different stages of tomato cultivation, as shown in Figure 9. Carbofuran and chlorothalonil are used mostly on flourishing plants during September to October and during the beginning of fructification. Delthamethrin is used, together with chlorothalonil, to control fruit pests until harvesting. Harvesting starts 85 days after seeding or 50 days after flourishing, and lasts more or less 3 months, from November to March. The fruit is collected at different stages of ripeness, depending on market requirements.

July	Aug	Sept	Oct	Nov	Dec	Jan	Feb	Mar	Apr	Activity
										Seeding
										Flourishing
										Fructification
										Harvest

										Pesticides
										Carbofuran
										Chlorthalonil
										Deltamethrin
										inorganic copper compounds
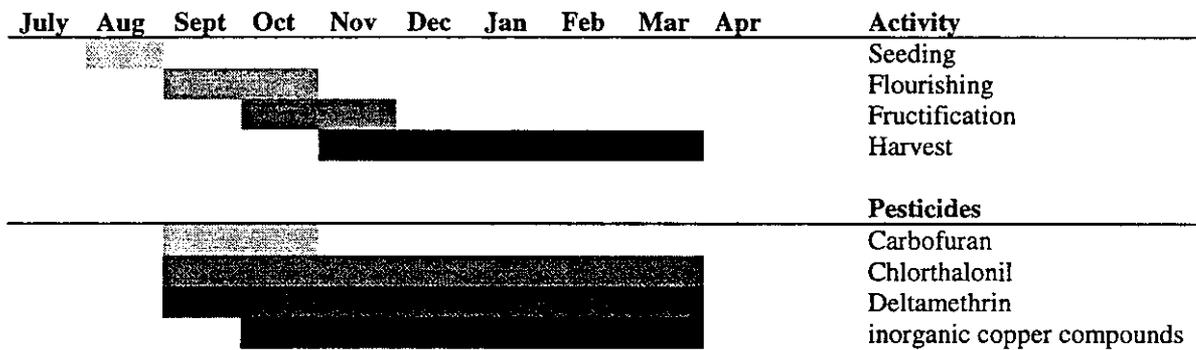
Figure 9. *Stages in tomato cultivation and the associated use of some pesticides.*

In 1997, the Agricultural Secretary of São Paulo performed a inquiry to evaluate which type of pesticides are usually used for different plantations in the state of São Paulo. Figure 10, shows the most used fungicides, insecticides and herbicides used in tomato plantations in Apiaí region.

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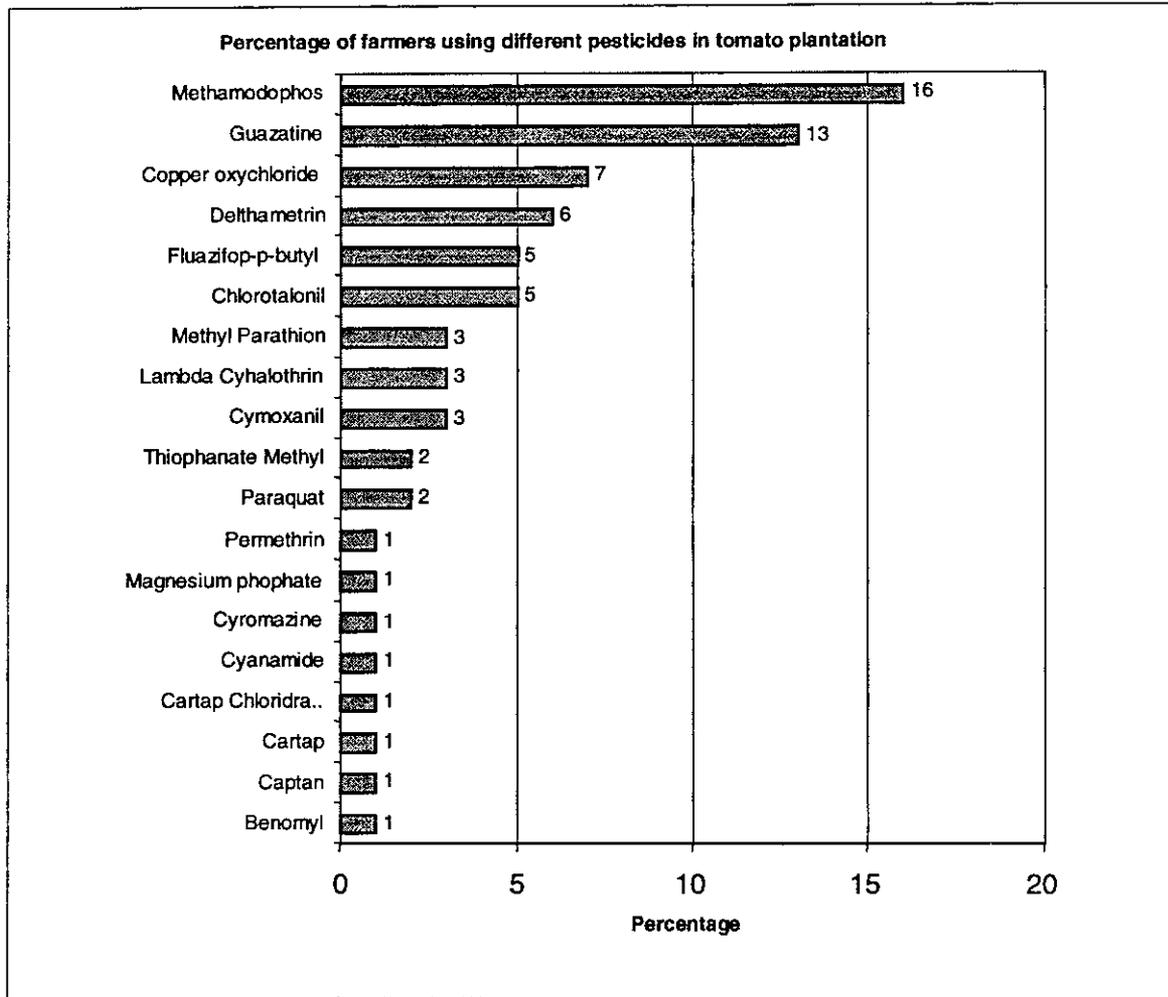
Figure 10. *Percentage of farmers that use the different sort of pesticides in tomato plantation in Apaí area.*

Source: Secretaria de Estado dos Negócios da Agricultura e Abastecimento – Instituto de Economia Agrícola.

Some of those pesticides are very toxic to fishes, for instance methamidorphos, permethrin, fluazifop-p-butyl, clorothalonil and benomyl. Some are very stable in the surface water, such as methamodophos and benomyl. Table & compares physical, chemical, fate and toxicological characteristics of most commonly used pesticides in tomato cultivation in the Apiaí region.

Table 3. *Physical, Chemical and Toxicological Properties of pesticides used in Apiaí region.*

(Source: E X T O X N E T, Extension Toxicology Network).

Group – chemical class	Chemical Class	Name	Chemical Name	CAS	Molecular Weight	Water solubility (mg/l) (ppm*)	Vapor Pressure (mPa) (mmHg*)	Partition Coefficient (log Kow*)	LC 50 (mg/l)	Half-life soil	Half-life surface water
Insecticide/acaricide/	avicide - organophosphate	Methamodophos	OS-Dimethyl-phosphora-mi-dothiolate	10265-92-6	141.12	90000 (20°C)	3E-4 * (30°C)	-1.74	25-51 (rainbow trout, 96 hr); 100 carp; 100 gold fish	1.9 to 12 days	27 days
Insecticide	pyrethroid	Delthametrin	cyano(3-phe-noxy-phenyl)methyl	52918-63-5	505.24	0.002 (20°C)	1.5E-8* (25°C)		1-10 (fish)	1-2 weeks	
Insecticide/acaricide		Lambda Cy-halothrin	(RS)-alpha-cyano-3-phe-noxybenzyl	91465-08-6	449.9	0.005 (20°C)	Negligible (0°C)	10.000.000	210(bluegill), 240 (rainbow trout); 360 D. magna;	4-12 weeks	
Insecticide	Organophos-phorate	Methyl Para-thion	O,O-dimethyl O-4-nitro-phenyl phos-phorothioate	298-00-0	263.21	55-60	1.3 (20°C)	3.5185-3.8388	1. 9-8.9 (fish, 96hr)	1-30 days	8-38 days
Insecticide		Cartap Chlori-dra..									
Insecticide		Magnesium phophate									
Insecticide	pyrethroid	Permethrin	3-phenoxy-benzyl(IRS)-cis,trans-3-(2,2-dichlo-rovinyl)-2,2-dimethylcy-clopropane-carboxylate	52645-53-1	391.30	0.2 (20°C)	0.045 (25°C)	6.1004	0.0125(rainbow trout, 48hr)	30-38 days	<2.5 days
Insecticide		Cyromazine		66215-27-8	166.19	11000 * (20°C)	4.5E-4 (25°C)	0.155*		107-142 days	
Herbicide	phenoxy com-pound	Fluazifop-p-butyl	(R)-2-[4-(5-tri-fluoromethyl-2-pyridy-loxy)phenoxy] propionic acid	79241-46-6	383.4	1.1 (25°C)	0.054 (20°C)	4.5000	0.53 (bluegill sunfish, 96 hr)	<1 week	
Herbicide	quartenary ni-trogen com-	Paraquat	1,1'-dimethyl-4,4'-bipyri-	1910-42-5	257-2	700,000 (20°C)	Negligible	4.4683	32 (rainbow trout, 96hrs),	>1000 days	13.1 hrs

		pound	dinium						1.2-4.0 (Daphnia)		
Herbicide		Cyanamide		420-04-2	42.040	Highly soluble					
Fungicide		Guazatine									
Fungicide		Copper oxychloride									
Fungicide	organochlorine	Chlorotalonil	tetrachloroisophthalonitrile	1897-45-6	265.89	0.6 (25 °C)	1.300 (40°C)	1.32*	0.25 (rainbow trout); 0.3(bluegills) ; 0.43 (channel catfish)	1-3 months	
Fungicide		Cymoxanil									
Fungicide		Thiophanate Methyl		23564-05-8	342.4	3.5 * (20°C)	<9.5E-3	1.5*			
Fungicide	Benzimidazole	Benomyl	Methyl-1-[(butylamino)carbonyl]-H-benzimidazol-2-ylcarbamate	17804-35-2	290.62	2	< 1 (20°C)		0.05-14 (catfish)	3-12 months	2 months
Fungicide	Phthalimide	Captan		133-06-2	300.61	3.3 (25°C)	1.3 (25°C)	2.7853	0.056(trou, 96 hr); 7-10 (D. magna)	1-10 days	23-54 hours
Fungicide		Cartap									

Mining operations - heavy metals and particles

CAF Argentiferas Furnas Mineração was a silver mining company that exploited the Furnas subterraneous mine. The old mine is located nearby the Furnas stream, outside the PETAR, which is a tributary of the Betari River. The water used for extraction was of underground origin and often discharged directly into the Furnas stream, which is crossing the PETAR, without treatment. The area does not belong to PETAR, but is considered an area of environmental protection (Sobral & Oliveira 1989).

Even though most of the lead mining had stopped in the region during the last decades, it may still be threatening the environment due to waste rock with high contents of heavy metals, which are left near the rivers (Shimada, pers. comm.). Figure 11 is a picture of some of the waste rocks next to Furnas river.
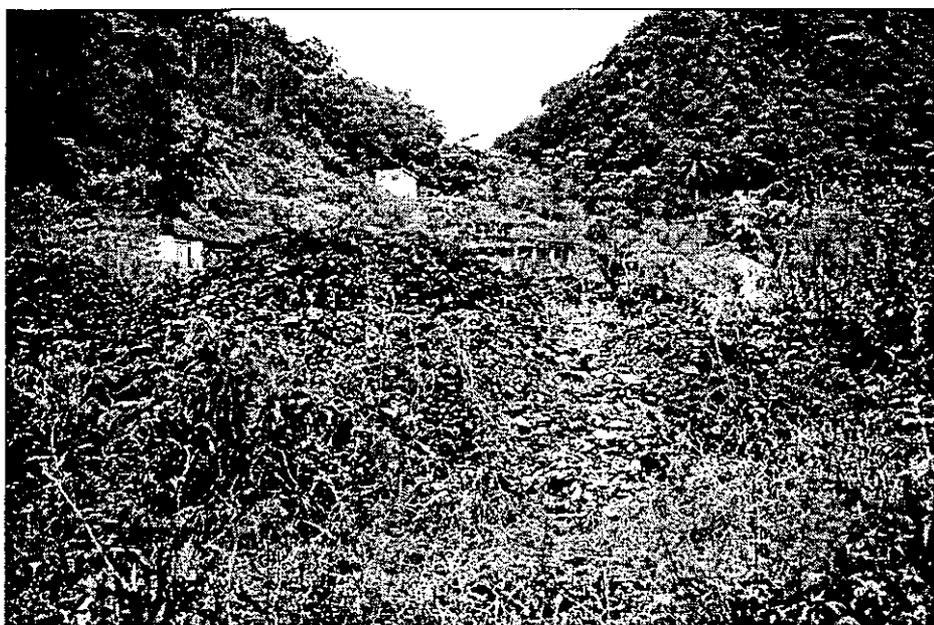
Figure 11. *Waste rock with high contents of heavy metals left near Furnas river*

(R. Moraes).

According to CETESB (1991), heavy metals are found in high concentrations in the region (Table 4). Sediments collected at the bottom of Furnas stream had a concentration of arsenic 124 higher than the limits recommended by CONAMA for the preservation of the aquatic life (8 µg/g). The Betari River also had a high concentration of arsenic in the sediment (68 µg/g).

Table 4. *Heavy metal concentration in sediment and water samples from the Furnas stream and the Betari river together with recommended values.*

Source: CETESB (1991).

Metal	recommended	Sediment (µg/g) Furnas	Betari	recommended	Water (µg/l) Furnas	Betari
Arsenic	8(1)	990	68	50 (2)	n.d.	31
Cadmium	5(1)	2.55	0.3	1(2)	n.d.	n.d.
Copper	25(1)	9	6	2(3)	4	n.d.
Lead	20(1)	300	260	5(3)	n.d.	n.d.
Mercury	0.1(1)	0.5	0.05	0.05(4)	0.01	0.1
Zinc	200(1)	383	65	30(3)	60	n.d.

(1) Bowden

(2) CONAMA(Conselho Nacional do Meio Ambiente)

(3) CWQG (Canadian Water Quality guidelines)

(4) EPA (Environmental Protection Agency, EUA)

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Lead concentration in the Furnas stream (300 µg/g) and the Betari river (260 µg/g) sediment were much higher than the recommended value of 20 µg/g by Bowden (apud Prate & Anderson 1977). Mercury was detected in water from the Betari River (0.1 µg/l) and in sediment form the Furnas stream (0.5 µg/g). Those values are above the recommended values (0.05 µg/l and 0.1 µg/g) by EPA and Bower respectively. Copper concentration in water from the Furnas stream (4 µg/l) was higher than the recommended (2 µg/l). Zinc was detected in sediment (383 µg/g) and water (65 µg/l) from the Furnas stream. Both values are above the recommended values (200 µg/g, 30 µg/l) by Bower and CWQG, respectively. Cadmium was detected in sediment of the Furnas stream (2.55 µg/g). Even though the cadmium concentration in sediments was considered lower than the limits (5.0 µg/g), it was detected in high concentration in muscles and internal organs of the crustaceans *Aegla* sp. from the Ribeira river. Catfishes, which eat benthic organisms, showed high concentrations of heavy metals in their internal organs and bones. Lead, cadmium and mercury have large bioconcentration factors in aquatic organisms, are highly toxic and, unlike many of the transitional elements, have no natural biological function. Copper, used in fungicides in the tomato culture is another possible stressor due to its high toxicity. The most common route into the food chains is from sediment via detritivores or sediment-dwelling species.

Inorganic mercury becomes problematic in aquatic ecosystems when it is converted into organic or methyl mercury via bacterial methylation processes, which can be stimulated under acidic or low alkalinity conditions (which are seldom found in the PETAR area) or by increased availability or organic substrate. Methylmercury is well known to bioaccumulate and bioconcentrate in aquatic food chains. Mercury is a naturally occurring element present in rocks and its concentration varies with location depending on the nature of the bedrock and degree of mineralization. However, even "background" concentration levels of mercury in water, soil or sediment can lead to elevated mercury concentration in fish tissue (Wren et al. 1995). Water hardness and pH influence the rate of uptake and availability of mercury to aquatic biota within a lake. Organic mercury compounds are more toxic (LC_{50}, 96 h: 24-74 µg/l) than inorganic mercury (LC_{50}, 96 h: 33-687 µg/l) in short-term exposures (Wren et al. 1995).

Cadmium acute toxicity is greatly influenced by species and test condition. For instance, 96-h LC_{50} for fathead mirrows range from 11.7 to 7160 µg/l (Wren et al. 1995). Medium bioconcentration factors for cadmium in fishes are less than 100 times. Cadmium is less toxic to freshwater organisms in hard water and at lower pH. Table 5 shows acute toxicity data for heavy metals for some freshwater test fishes.

Table 5. *Acute toxicity (µg/l, 48-96 h LC_{50} or EC_{50}) data for some freshwater fishes.*
Source: Leland & Kuwabara (1985).

Fish	Copper	Lead	Mercury	Zinc
Salmonidae	10-900	1000-500000	3-20000	50-7000
Centrachidae	700-10 000	20000-400000	3000-10000	1000-20000
Cyprinidae	20-2000	2000-500000		400-50000

Some calcareous mining is still active in the region (e.g., Oxical and Purical). They do not release toxic components in the environment, but after explosion of rocks, a considerable amount of particles is released to the air and is deposited on surface water, increasing water turbidity . The increase of turbidity may limit penetration of light into the water, which affects the biological activities. Particles are also responsible for an increasing number of respiratory problems for the population living in the vicinity of the mines.

4.2. Ecosystems Potentially at Risk

Several streams that are tributaries to the Ribeira River, the largest river in the region, drain PETAR. Many of them have their origins outside PETAR, first flowing through areas that are influenced by humans in different ways and then through the park. Others have theirs origins inside the park and flow through essentially undisturbed forest. These watershed characteristics, together with water chemistry of sources and typical changes in streams between headwaters and mouth, yield a diversity of aquatic systems and rich opportunities for a comparative research strategy.

Figure 12. *The Betari river showing its rocky bottom and riparian vegetation.*

(R. Moraes)

In general, the streams of PETAR are transparent, narrow with rocky bottoms (Figure 12). During heavy rain, their volume increases quickly, which also increases turbidity and water flow. Such events even change river bottom topography. Variation in rainfall is also directly responsible for variation in river flow. This is exemplified by the Betari river (Figure 13, compare river flow variation with rainfall, Figure 5). Heavy rains will increase the transport of contaminants from sources to rivers, but on the other hand, increasing water volume in streams will decrease the concentration of contaminants.
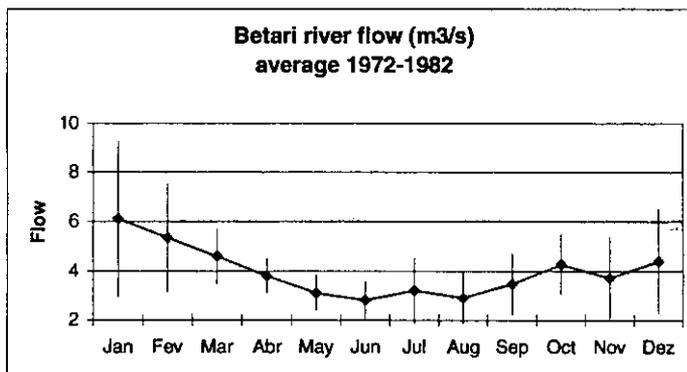
Figure 13. *Monthly average of water flow of Betari river (1972-1982).*

Source: Water and Electrical Energy Department of the State of São Paulo (DAEE).

Variations in stream width as well as height and type of trees along stream banks determine the extent of shading and the quantity and quality of detrital inputs to the stream consumers. Covich & McDowell (1996) studied the stream community of a tropical forest, El Verde, Costa Rica. Their study showed that variability of rainfall and stream discharge is the main factor determining food web structure through its influence on

the availability of detrital resources. Intense storms indirectly alter food webs by exporting nutrients, altering particulate organic matter, eroding channels and causing landslides.

Although food webs in tropical waters are often very complex, food chains are usually short. In these tropical communities many kinds of fishes have specialized on foods at the lowest trophic levels. According to Lowe-McConnell (1987), in river systems the detrital chain is the most important, for instance from bottom detritus, through microorganisms, to detritus-feeding invertebrates or fish, to several levels of piscivores. Food webs are based largely on allochthonus material (aerial insects and vegetal debris) in the headwater streams while in lower reaches detritus comes mainly from decomposition of aquatic macrophytes. The direct influence of the riparian zone on stream ecology is greatest in the small headwater channels. Most of invertebrates feed on benthic algae and this, rather than phytoplankton, is the principal food for aquatic herbivores in streams. Some invertebrates also feed on mosses which grow in stream channels but these plants, like the macrophytes, mostly enter the food chain as detritus (Archibold 1994). Typically, headwater streams carry fewer species than the lower stretches (Lowe-McConnell 1987).

Data about taxonomy and ecology of the fish fauna in PETAR is still limited (but see Appendix). This lack of information is typical for most of Atlantic Rain Forest streams, which usually have a specific fish fauna, with a high level of endemism due to geographic isolation (Sabino 1996). Trajano (1991) and Pardini (1996) found a total of 24 fish species in the Betari River, one of the few rivers in PETAR that has already been studied. The list of species collected is presented below.

Order Characiformes
    Characidae
        Astyanax sp.
        Bryconamericus sp.
        Deuterodon cf iguape
        Deuterodon sp.
        Hollandichthys multifasciatus
        Oligosarcus hepsetus
        Characidium sp.
Order Siluriformes
    Siluroidei
        Callichthyidae
            Corydoras barbatus
        Loricariidae
            Ancistrus brevipinnis
            Harttia kronei
            Hemipsilichthys sp.
            Hypostomus punctatus
            Kronichthys heylandii
            Kronichthys subteres
            Rineloricaria sp1
            Rineloricaria sp2
    Pimelodidae
        Heptapterus sp.
        Imparfinis piperatus (=Rhamdiioglanis frenatus)
        Pimelodella transitoria
        Rhamdia quelen
    Trichomycteridae
        Trichomycterus sp. .
    Gymnotoidei
        Gymnotidae
            Gymnotus sp.
Order Cyprinodontiformes
    Poediliidae
        Poesilia sp.
Order Perciformes
    Cichlidae
        Geophagus sp.

The most common fishes in Betari River are characidae (69.3 % of the individuals in the study of Pardini 1996). However 50 percent of the species collected by Pardini and Trajano were siluriform catfish (Figure 14). They represent a diverse group of fishes mostly found in the Neotropics. Their bodies have no true scales. They can be completely absent (Fam. *Pimelodidae*, Portuguese *"bagres"*) or protected by an osseous plate (Fam. *Loricarridae*, Portuguese *"cascudos"*). *Bagres* are predators with different food habits, ranging from insectivorous (e.g., *Pimelodella transitoria*) to piscivorous (*Rhamdia quelen*). Four species are present in Betari River and they represent 10% of the fish community in terms of abundance. They have a long longevity (average 6 years), compared to other tropical fishes. *Cascudos* eat algae on the bottom of streams and rivers (e.g., *Hypostomos punctatus*) and are rather sedentary. Eight species are present in Betari River and they represent 6% of the fish community in terms of abundance.
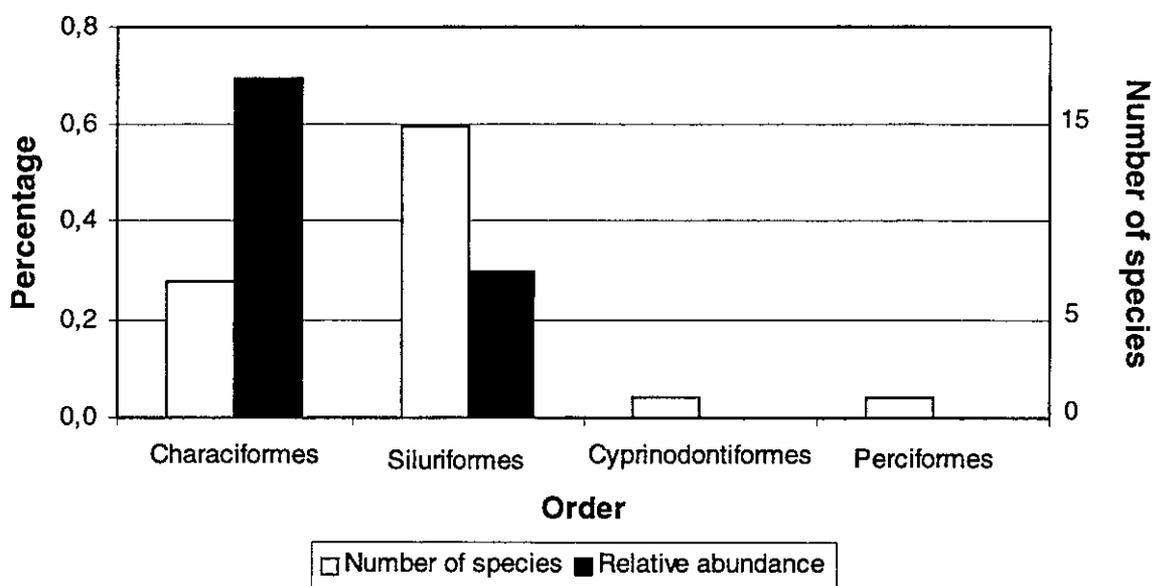
Figure 14. *Number of species and relative abundance of fishes in the Betari River.*

Source: Pardini (1996).

4.3. Selection of Assessment Endpoints

The term endpoint has two distinct uses in ecological assessments: measurement endpoint and assessment endpoint. *Assessment endpoints* are explicit expressions of the environmental value that is to be protected (US EPA, 1996). *Measurement endpoints* are e.g. the output of a toxicity test, series of field measurements, or other attempts to measure the responses of a biological system to a stressor (Suter 1995). The choice of the fish community as an assessment endpoint for this study was based on several considerations related to management, ecological and methodological arguments, which are discussed below. The choice of measurement endpoints will be discussed in the next chapter.

Representation of management goals. Interviews with local inhabitants of Ribeira Valley showed that 91% of them use *cascudos* and 63% uses *bagres* as food resources. *Cascudos* are also an important food item for otter (*Lutra longicaudis*) which is a vulnerable species (according to Diario Oficial 1998.02.05). It has been shown that *cascudos* are the most frequent food item in otter diet (84%) in the Betari River (Pardini 1996). According to Environmental Board of the State of São Paulo (CONSEMA 1987), the creation of parks and natural reserves among other goals, aim to preserve the native fauna and flora for future generations. Therefore the protection of the aquatic fauna from contamination represents one of management goals of PETAR.

Ecological relevance. Loss or reduction of fish species in the community can cause adverse effects in the ecosystem, since they are important food items for terrestrial mammals such as *Chironectes minimus, Lutra longicaudis, Procyon cancrivorus*. One group consists of siluriform fishes (order Siluriformes) that are important predators in stream communities and disturbance of their abundance can result in changes in prey species populations. Around 15% of the total fish abundance in the streams of PETAR are siluriform catfishes. Due to the ecological relevance of this group of fish, ecological effects will also be studied at the fish community level.

Susceptibility to stressors. Ecotoxicological studies with one of the potential stressors (deltamethrin) indicate that other species of fish than catfish are sensitive to exposure in the range of 1 to 10 µg/liter. Catfish are also quite sensitive to transport and changes of environmental conditions (temperature, for instance). The change of nutrient conditions in the streams may also affect the fish community due to influences on availability of food other than allochtonous material.

Exposure. A reasonable assumption is that pesticides will find their way to the streams either in connection to the application as spray drift or later with run-off from the fields. The exposure to organic pollution from sewage outlets, and the following increase in inorganic nutrients will also, directly or indirectly, influence the fish community, but in other ways than the toxicants. Likewise metals and particles from mines will be found in the water. *Cascudos* may be exposed directly to the contamination in water and also by eating sediment and algae. *Bagres* may also be directly exposed but will be, due to their predatory feeding, be exposed also through food-chain transfer of contaminants.

Available information. Studies by Trajano, and her students, on the fish fauna in PETAR and other parts of the Atlantic rain forest, have been performed for many years. These studies cover both specific species and the entire community. Some of these studies may be directly useful for the ERA in PETAR, while others give valuable background information. Work in progress by several researchers in the area will also contribute to the ERA. See also below.

Available methods. Many well-known methods for studies of fish and fish communities are available. Electric fishing is a non-selective, and mostly non-destructive, method to get both smaller and larger items of the species comprising the entire fish community. This method has been, and will be used, for sampling in the streams. Other methods related to both exposure analysis and effects analysis are also available. Fish will *e.g.* be sampled for analyses of pesticides and metals. Several bioindicators at different levels of biological organization, such as the induction of the mixed function oxygenase enzyme system, the activity of acethylcholine esterase, growth rate or lethality may be used as exposure or effect indicators.

4.4. Selection of Measurement Endpoints

Background

Biological communities are sensitive to their chemical environment, although the degree of sensitivity varies among species and communities. Response to chemical stress often involves rapid changes in species composition of aquatic ecosystems that can translate into changes in various aspects of community structure, such as species richness (Ford 1989). In order to evaluate potential stress effects on ecosystems it is necessary to know about the baseline condition of the ecosystem and its natural range of variation. Furthermore, it is necessary to identify the point at which it is possible to say when the system has begun to change. According to Ford (1989), there are several ways to evaluate baseline and natural variability: 1) accessing previous work on that or similar ecosystems; 2) using simultaneous studies of upstream, adjacent nearby ecosystems as controls; or 3) using the stratigraphic record to reconstruct site-specific baseline conditions. Failure to have baseline and natural variability reference data will cast doubt on causal linkage between stress and effect. In this study, simultaneous studies of upstream ecosystems and also nearby streams with no influence of anthropogenic sources will be used as references sites. Data from those sites will be compared with data from sites that are exposed to stressors released from agriculture, mining or human settlements. Sites representing all possible combinations of stressors (pesticides x metals x nutrients) will be selected following the evaluation of the initial chemical analyses. Data will be complemented by data on various stream characteristics; location, stream order, flow, water-quality parameters, (assumed) stressor

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exposure, bottom, riparian zone, date of sampling etc. With this data-base the possibility to pinpoint anthropogenic stressors as causal agents will increase.

The second issue, identifying the point at which a system has begun to deviate from baseline conditions, is essentially the issue of early warning signals of ecosystem deterioration. A major challenge in the issue is to identify diagnostic changes against a complex background rich in spatial and temporal heterogeneity (Ford 1989). PETAR aquatic system represents a good example of such heterogeneity and therefore the understanding of its structure of aquatic ecosystem depends critically on sampling design specially because rivers and streams are temporally variable and can be heavily affected by nature and timing of both upland process and regional climatic factors.

Many different parameters are available as measurement endpoints for studies of the fish community. Below a short review of available parameters are presented before the measurement strategy of the PETAR-project is presented.

Community composition

Community composition (i.e., indicator species and species composition and their relative abundance) is parameters commonly used as indicators of chemical stress. The use of **indicator species** for assessing environmental conditions is based on the idea that the distribution of at least some species or groups of species are constrained to a narrow range of environmental conditions. Changes in chemical conditions will therefore affect the occurrence of those species, eliminating some and encouraging colonization by others. However, the value of the indicator species approach is low in the absence of other supporting data. The usefulness can be enhanced if sets of indicator species are used and particularly if they are chosen from different guilds or trophic levels (Ford 1989). **Species composition and their relative abundance** have also been used as indicators of stress at the community level. Relatively rapid changes in overall community composition have been found in association with, for instance, pesticides (e.g., Hulburt *et al.* 1972 *apud* Ford 1989); heavy metals (e.g., Marshall and Mellinger 1978 *apud* Ford 1989) and organic enrichment (e.g., Pearson and Rosenberg 1978 *apud* Ford 1989). There are, however, several problems with using community composition per se as assessment tool such as taxonomic difficulties, cost and logistics of sampling design, etc. Furthermore, the use of abundance as measurement endpoint may not give a clear indication of whether a stressor caused the observed changes in abundance or whether it is part of natural fluctuations (M. Power, pers. comm.).

Community structure

Several studies suggested that heavily stressed ecosystems tended to have reduced biomass, abundance, species richness and species diversity relative to pristine ecosystems. Those parameters are related with **community structure**, which can be used as bioindicator for ecological effects on the community level. For instance, pesticide applications typically result in temporary intermediate reductions of **biomass and abundance** (Ford 1989). However, stress related changes in biomass or abundance may involve several factors. Decreases may be caused by direct or indirect toxicity effects by disruption of the reproductive process, negative effects on food prey items, or changes in species composition. For plants, *e.g.* increased biomass may result from enhanced growth and reproduction in the altered environment, decreased grazing or predation, etc. Because any combination of these factors can occur, patterns of fluctuation in biomass and abundance may not be useful in assessing the course of ecosystem deterioration. For Suter (1995), the use of species richness as a measurement endpoint is consistent with the maintenance of biodiversity. According to the same author, in several studies of streams and rivers subject to pollutant effluents, **species richness** was more consistently affected by toxicants than other measured parameters. Measurement of species richness are however influenced by sampling effort, and indirectly to biomass, since a higher number of specimens will increase the probability for finding more species and *vice versa*.

Species diversity

At the next level of analysis, needing more data input, **indices of species diversity** differ from simple species richness (number of species found in a specific habitat-sample) in that they typically include information about the partitioning of individuals among species. According to Ford, some indices appear reasona-

bly correlated with stress effects in particular situations, but as a whole, they do not reliably reflect disturbance over time and space. Even more data demanding **biotic indices** are designed to collapse complicated compositional data into concise numerical descriptions that both reflect stress effect on particular ecosystem and permit direct comparisons with other ecosystems. They are measures of community composition that combine pollutant tolerances with relative abundance of species within ecologically sensitive and defined groups. The critical feature of these techniques is the development of species weightings, which requires exacting taxonomic proficiency as well as deep understanding of species ecology (Ford 1989). Another approach is the toxicological risk ranking method, which was presented by Hartwell (1997). It can be used for correlating measures of ambient toxicity and fish community diversity. The method has five components: severity of effect, degree of response, bioassay variability, consistency and number of measured endpoints. The model is supposed to reduce an array of ambient toxicity data into a site-specific metric that is appropriated for comparisons with other metrics, such as biotic indices.

Histopathological and physiological indicators - biomarkers

Besides the measurements at community level, other indicators at several levels of organization can also be used in order to establish linkages or relationships between the contaminants of concern and fish community. Those indicators could be at the histopathological (i.e., changed structure of gills, liver etc.) or physiological (induction of enzyme systems. changed enzymatic function etc.), or individual (gonad size, growth, fecundity, etc.). Schwaiger and collaborators (1997) investigated the use of histopathological characteristics for assessing the effects of contaminated (pesticides, PCBs, PAHs and heavy metals) streams on the health of two fish species (trout and loach). They observed differences in types and severity of organ (kidney, liver and gills) lesions between contaminated and non-contaminated streams. Histopathological studies were recommended by those authors for increasing the probability of identifying cause (stressor) and effect (biomarker) relationships.

Studies of fish physiology have demonstrated the usefulness of various physiological indicators in establishing exposure to industrial effluents and other organic substances (Haux and Förlin 1988, Goksøyr and Förlin 1992). These **biomarkers** are considered early warning signals but establishing firm casual relationships between exposure and effects on higher levels of organization can be difficult due to various confounding factors (Newman 1998).

Index of Biotic Integrity

Steedman (1987) developed an Index of Biotic Integrity (IBI) in order to assess stream quality in Southern Ontario, Canada. The IBI, which was originally developed by Karr (1981 *apud* Steedman 1987) is composed of a number of subindices or metrics related to species richness and composition, local indicator species, trophic composition, fish abundance and fish condition. The indices provide information about a range of structural and organizational aspects of the stream ecosystem, including habitat features of different types and sizes, food sources, productivity, predation and parasitism.

Measurement endpoints in the PETAR-project

We have chosen a gradual approach, starting with the use of fish community composition (number of species or higher taxonomical entities if necessary, and number of specimens) in combination with simple community structure measures (length, weight). This will give the opportunity to use diversity indices and also to perform multivariate statistical analyses on the results and comparing different sites.

We are presently investigating the possibilities to extend these community level measures with measurements on lower organizational levels such as physiological and anatomical measures on the individual level.

The possibility of the development of an index similar to the IBI, modified according to PETAR streams characteristics, has been preliminarily discussed with some of the Brazilian collaborators. These methods may be complemented by biomarkers. The idea will be further developed and presented in the next report.

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4.5. Conceptual Models

Several factors may directly or indirectly influence the structure of the fish community. The following is a presentation of the stressors so far identified as meriting further investigations. The choice of stressors was guided by the general conceptual model for linking the endpoints to possible anthropogenic sources of stressors (Figure 15). They are followed by an example of a more detailed conceptual model (Figure 16) which covers the pathways from sources of the stressor, in this case pesticides, to the endpoints taking into account the characteristics of the ecosystem that may influence the exposure and effect

The general conceptual model (Figure 15) describes the general relationships between land use activities, such as agriculture and human settlement, stressor sources, exposure pathways and ecological receptors presented in the risk hypotheses.
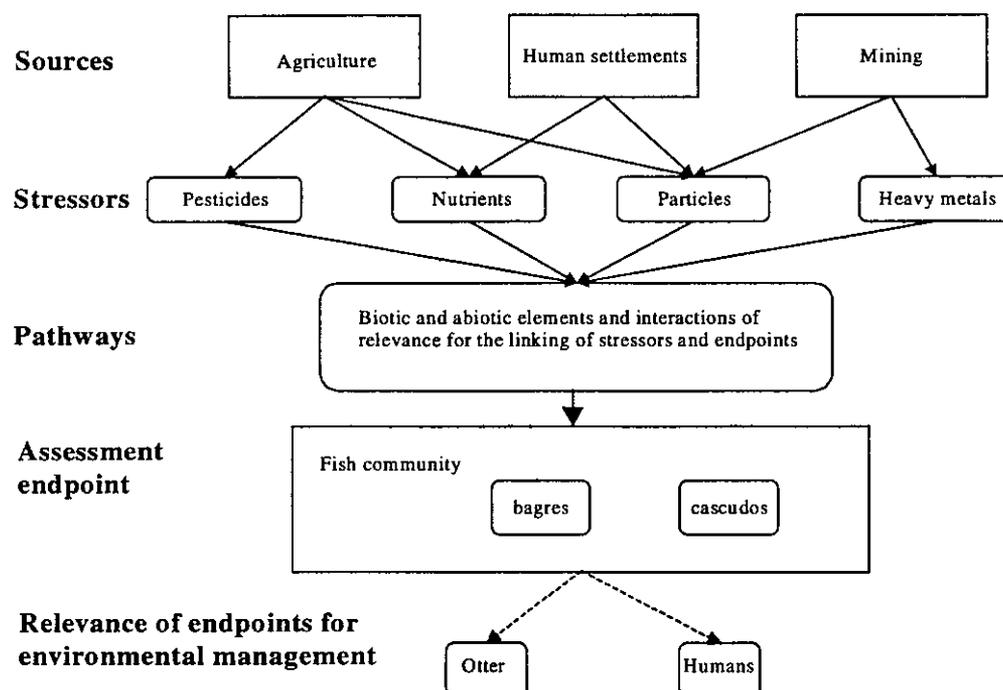
Figure 15. *General conceptual model relating sources of stressors to assessment endpoints.*

Figure 16 also presents some abiotic factors that may affect fate-process of pesticides in terrestrial and aquatic ecosystems. Several factors can be important for each of the links between entities. Fish can absorb pesticides directly from water as well as ingesting contaminated food. The more lipophilic or fat-soluble the pesticide, the more readily it will be taken up by aquatic organisms, especially if the concentration of the pesticide exceeds its water solubility (Nimmo, 1985). The size, age, and sex of the individual organism can affect the absorption rate, which can differ from species to species. After absorption, pesticides are often modified by metabolism, e.g., oxidation, hydrolysis and reduction. Those reactions may be followed by excretion of metabolites.

Figure 16. *Specific conceptual model covering the possible pathways of pesticides from sources to receptors.*

Arrows represent food uptake and plant absorption. The influence of various characteristics of the ecosystem, on the links between boxes, is represented by the open arrows. Direct uptake of pesticides to animals from water and sediments are not represented in the figure.

Temperature can affect the chemical and physical state of the pesticide, the time that it remains in solution or suspension, its persistence and the rate of pesticides uptake by aquatic organisms. Most pesticides are increasingly toxic at higher temperatures (Nimmo, 1985). pH can influence the rate of degradation and therefore the toxicity of the pesticides. According to Nimmo (1985), hardness and alkalinity may influence the effect of pesticides if changes in pH are produced at the same time. Carbon content of sediment may for instance influence the uptake of pesticides by sediment-dwelling insects. Likewise may one factor, such as temperature, affect several of the links between entities.
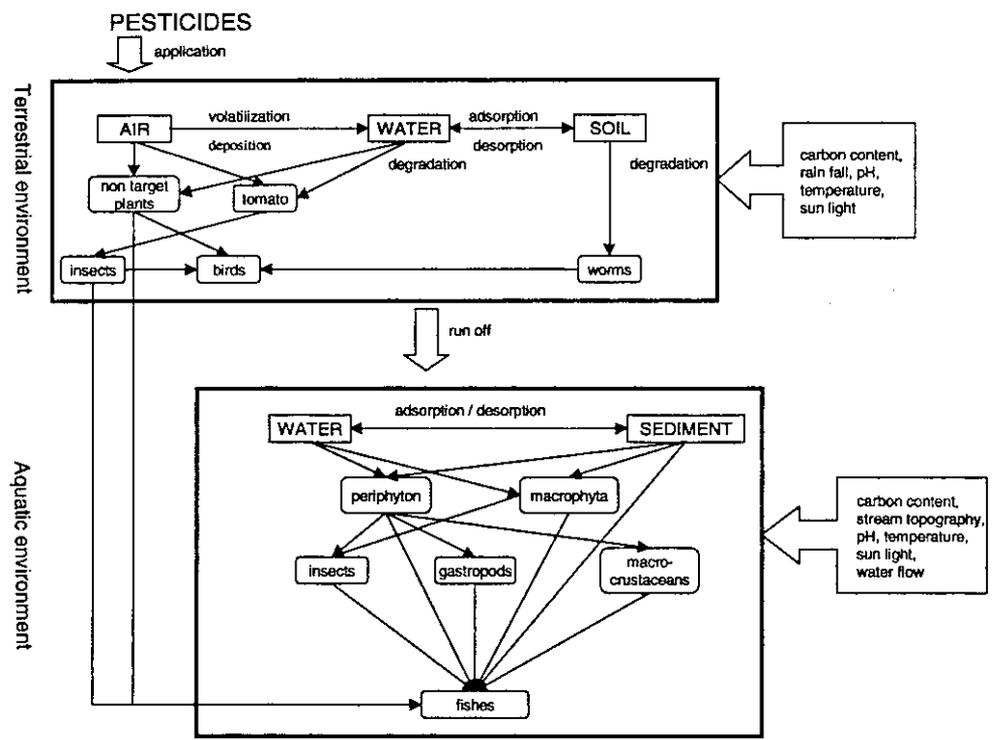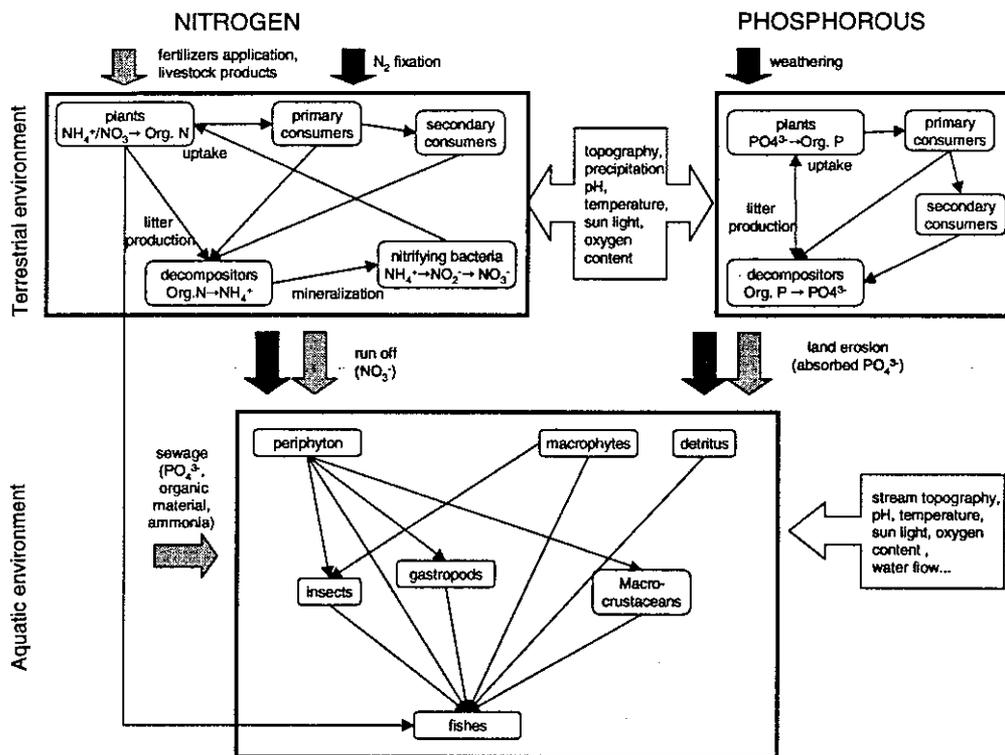
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Figure 17. *Specific conceptual model covering the possible pathways of nutrients from sources to receptors.*

Arrows represent food uptake and plant absorption. Black arrows represent natural sources and gray arrows represent anthropogenic sources. The influence, from various characteristics of the ecosystem, on the links between boxes is represented by the large open arrows. Indirect links through detritus are not represented in the conceptual model.

A further development of the conceptual model is found in figure 17, which exemplifies pathways and fate processes of nutrients from their sources to the assessment endpoints. Nitrogen is considered to be the growth-limiting factor in most terrestrial ecosystems. In order to increase the yield of agricultural crops, addition of N fertilizers is a common practice. The high inputs are followed by large outputs by leaching, gaseous losses and crop removal (Gundersen & Bashkin 1994). Another important anthropogenic source of nitrogen is livestock that releases large amounts of ammonia.

Bacteria that live symbiotically in the roots of legumes and certain blue algae fix nitrogen gas (N_2) from the reservoir in air or water and thereby bring nitrogen to the biosphere. Nitrogen that has been bound in various organisms is regenerated on decomposition of organic material to salt as ammonium ions (NH_4^+). Liberated NH_4^+ ions can be assimilated by plants or, via the intermediate nitrite (NO_2^-), be oxidized (nitrified) by bacteria to nitrate (NO_3^-) and in this form be assimilated by plants. Nitrate can also be denitrified to nitrogen gas or nitrous oxide by bacteria in oxygen free environments and return to the atmosphere (SEPA 1994). Ammonium normally occurs in low concentrations in soil and in water. There it is bound to particles and undergoes bacterial oxidation to nitrate in presence of oxygen. Therefore nitrate is the main inorganic nitrogen species which is transported and taken up in ecosystems.

Ammonia (NH_3) is relatively highly toxic in aquatic systems (48 0r 96-h LC_{50} to fish ranges from 0.49 to 4.6 mg/l NH_3, Russo, 1985). Nitrite (NO_2^-) is extremely toxic to aquatic life (48 0r 96-h LC_{50} to fish ranges from 0.1-13 mg/l NO_2^-, Russo, 1985), but does not usually occur in natural surface water systems. Nitrate (NO_3^-) can be found in relatively high concentrations in surface waters, but it is relatively nontoxic to aquatic organisms (48 0r 96-h LC_{50} to fish ranges 180-2000 mg/l NO_3^-, Russo, 1985). In relatively stable oxygenated natural waters the oxidation of nitrite is rapid, while the conversion of ammonia to nitrite is slower.

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Phosphorous is considered to be the most important limiting factor in freshwater ecosystems (Mason 1990). Phosphorous is largely insoluble and enters water from land mainly by erosion (Mason 1990). Important sources of phosphorous include fertilizer run off (to a lesser extent) and the increased use of phosphorous-containing detergents, which enter the rivers in sewage effluents together with outer phosphorous containing substances.

Phosphorous is not stored in the atmosphere like nitrogen, but in minerals. Inorganic phosphate ions (PO_4^{3-}) are liberated by the weathering of minerals and by decomposition of organic materials. Plants and microorganisms take up phosphate in competition with adsorption that often takes place to iron hydroxide complexes, lime precipitates or aluminum hydroxides. The complex and precipitates often contain negatively charged organic ions (humus). The adsorbed phosphate ions constitute a more important source of plant-available phosphorous in soil, sediment and water than pure minerals (SEPA 1994). The availability of phosphorous is strongly affected by the pH, with maximum binding to iron and aluminum complex at slightly acid conditions and maximum desorption at pH above 9. The oxygen content is also of great importance in that oxygen free conditions result in release of phosphate from ion complexes.

The biological changes that occur as a result of eutrophication can be separated into those which are a direct result of raised nutrient influx and those, which are indirect effects. Direct effects occur when organisms, are released from nutrient-limited growth and increase their population density. Indirect effects can be for instance when growth rates increases in such a way that the population starts competing for one or more other resources with neighboring species. One consequence of this may be the replacement of a species by another, which is more efficient in its use of resources (Golterman & Oude 1991).

Another indirect effect can occur when an increase in production of any one species' population has effects upon the physic-chemical environment in which it lives. This will affect other species sharing that environment but not direct competing for resources within it (Harper 1992). The bloom of algae reduces underwater light penetration. Reduced light can cause the loss of macrophyte vegetation, which affects niches for fish.

Risk Hypotheses

Pesticides. Exposure of stream biota to pesticides typically occurs by a combination of short-term spikes of high concentrations following spraying and rain events, and long-term exposure in stream that drain areas sprayed repeatedly or sprayed with more persistent components. Residue levels of several pesticides in streams are frequently found to be highest a few days after spraying (Li & Magita 1992).

Risk hypothesis I: The presence of pesticides in runoff water from fields immediately after treatment may affect the aquatic ecosystem since aquatic biota will be exposed to high concentrations of pesticides. Sensitive populations of macroinvertebrates and fishes may decline if the concentration of pesticides in water increases to levels higher than the one predicted to cause effects, unless populations have adapted to contaminated conditions. This will cause changes in the structure of the fish community in the exposed streams.

Organic pollution and nutrients. Traditional water pollution by readily biodegradable organic substances influence streams by two mechanisms. Primarily by lowering the oxygen concentration in the water during the degradation of organic matter, and secondarily by contributing to an increased level of inorganic nutrients. The changes give effects on the structure of the aquatic communities that are related to the discharged amount, the distance from the discharge and several characteristics of the receiving water (water-flow, temperature, etc). The outcome of the increased availability of nutrients is even much dependent on other factors like other limiting substances.

Risk hypothesis II: Close to sewage outlets oxygen concentrations will decrease, especially during low water-flow conditions, and thereby altering the conditions for fish in such a way that the fish community will change. Further down-stream, phosphorous and nitrogen concentrations, from both sewage outlets and from tomato plantations, will increase. This increase of nutrients will cause an increase of primary production, which means additional food availability to primary consumers, such as *cascudos*. This will change the fish community, primarily through an increased production of primary consumers and secondarily by and increase in secondary consumers.

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Heavy metals. Sediments are the main sinks for metals entering freshwaters. Toxic metals in the bottom sediments of rivers can cause effects even when water column metal concentrations are low. Direct transfer of metals from sediment to organisms is considered to be a major route of exposure for many species. Effects of metal exposure depend on how much is taken up by organisms, which differs for each metal. Considerations of total metal concentrations alone can be misleading because chemical speciation of trace metals significantly affects availability to aquatic organisms and hence toxicity. Non-complexed metal ions $(Cd^{2+}, Cu^{2+}, Pb^{2+}$ and $Zn^{2+})$ are apparently more readily assimilated by organisms than are complex forms. In general, metals are less toxic to fish in harder water.

Risk hypothesis III: Waste rock with high contents of heavy metals was left near Furnas Rivers. High concentrations of heavy metals are found in sediments of the Furnas River. Those contaminants may be accumulated in the food chains and high concentrations may be detected in the predatory *bagres* close to this area. Increased concentration of metals in fish tissues may be detected in fish. The increased exposure to metals may influence the fish community structure due to different tolerance among species. Likewise increased exposure to copper in the areas close to tomato culture may cause changes in the fish community.

Particles. Particles in water can decrease the input of light in the ecosystem, which may decrease photosynthesis and thereby the primary production of the stream. At extreme occasions the concentration of particles in the water may also mechanically influence organisms. Particles are released in the rivers and streams from different sources, especially agriculture and calcareous mining. High concentration of particles may be found in water after explosions of rock to extract calcareous which can affect aquatic ecosystem primary production.

Risk hypothesis IV: The decrease of light due to particles in water may cause a decrease in primary production close to the point sources, which means a decreased food availability to primary consumers, such as *cascudos,* and a changed fish community.

5. Analysis Plan

The plan for the complex analysis of the ERA is outlined in Figure 18, which illustrates the relationships between different analysis steps, and methods related to the respective step. Characterization of the ecosystem and the receptors, in this case mainly the fish communities, organisms influencing it, and important factors influencing the stressor exposure and effects are important for the further studies. This ERA is aimed at the study of effects that are taking place now, which means that the analysis is mainly retrospective and based much on field observations and sampling. There are however good possibilities to extend the analysis with predictive components *e.g.* related to possible effects related to increasing population (approximately equal to increased outlets of sewage) or increased tomato cultivation. The relative impact of various exposure scenarios can be best evaluated by linking a series of models. Results of GIS manipulations reflecting chemical inputs in watershed can be used to initialize transport models such as CREAMS (Knisel 1980 *apud* Lowrance & Vellidis 1995) and GLEAMS (Leonard et al. 1987 *apud* Lowrance & Vellidis 1995).

For description of *stressor sources* remote sensing techniques (satellite and aerial images) will be used in combination with field sampling in streams. The intention is to identify potentially affected parts of the streams, close to settlements and tomato cultures, and to quantify their areas as a first step to estimate the spatial extent of the exposure. "Ground truth" will be determined at particular sites; concentrations of pesticides and phosphorus, nitrogen and dissolved oxygen levels in waters will be measured, as well as heavy metals in water and sediment, downstream and upstream some stressor sources, in the Betari, Iporanga and Pescaría Rivers and their tributaries. These values will be used in conjunction to GIS models in order to estimate temporal and spatial stressor distribution in the streams, including streams where no measurements take place.
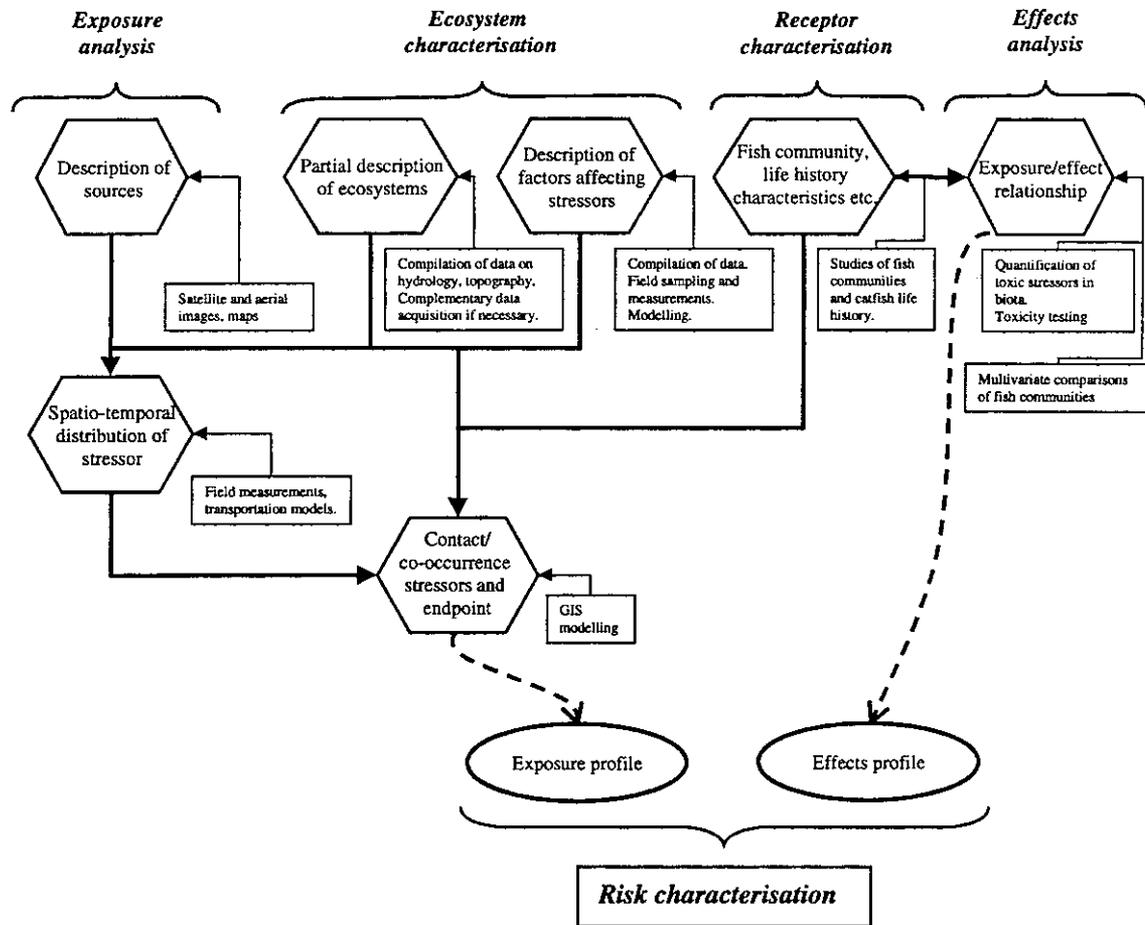
Figure 18. *Analysis plan.*

Hexagonal boxes indicate analysis steps, rectangular boxes indicates methods and oval boxes indicate results.

A description of the *ecosystem characteristics* that influence the behavior and distribution of stressors such as water temperature, water velocity, pH, hardness and alkalinity etc., is a necessary input to the transport models. To reach this compiled data from various sources as well as own field measurements are used.

Receptor characteristics, such as life history characteristics of the assessment endpoint that may affect exposure or response to the stressor (*e.g.* natural siluriforms population structure and distribution in different streams, food habits of selected species) will be studied, if not available in the literature or through the collaboration with Brazilian researchers.

Some studies (e.g., Clifford *et al.* 1995) have showed that GIS is an effective tool for extrapolation information from sample points to a complete presentation of contaminant concentration in the study area, establish relations between stressor concentration in the environment and in the receptor, and also to generate estimations of risk to assessment endpoints. In this study, data will be stored and processed using GIS in order to evaluate *the co-occurrence between stressors and receptors* on overlaid distribution maps.

As an input to the *effects analysis* electrical fishing, which is a non-selective methodology for fish sampling, will be used for sampling of the fish community. The high variability in environmental factors combined with synergistic and/or cumulative interactions of theses factors in aquatic ecosystems complicate the interpretation and evaluation of the effects of contaminant-related stressors on organisms. Some of the areas in PETAR are exposed to more than one stressor. In this context, one of the main challenges for this project will be to assess the influence of multiple stressors on stress responses in biological systems and the deter-

mination of casual relationships between various levels of biological response to the different stressors. Different streams exposed to none or some of the discussed stressors can be assessed and compared for this purpose. A methodology for ecological response analysis in order to evaluate the response of the assessment endpoints (the fish community with emphasis on the siluriform fishes) when exposed to the different stressors will be developed. However, natural variations in fish communities are expected which may make the interpretation of results difficult. Furthermore, there is a possibility that the toxicants had already acted as selection pressures on the biota and excluded sensitive individual and populations. Major factors complicating the establishment of relationships between the effects of stressors on measurement endpoints on the community level are: (1) effects of other stressors on fish community dynamics such as food and habitat availability, competition, etc., (2) the problems related to variability and techniques of measurement, and (3) the slow nature of change in fish communities to environmental perturbations.

The use of multivariate statistical methods, such as multidimensional scaling (MDS) and partial least-square regression on latent variables (PLS) in conjunction to principal components analysis (PCA), will assist in sorting out factors important for understanding the complex situation where both natural factors and several anthropogenic factors simultaneously contribute to the effect on the fish community. The characterization of the ecosystem through measurements and observations will assist in feeding data to this analysis. Another possibility is to use multivariate discriminant analysis, which provides statistical and graphical displays of the fish community at various sites and identifies those variables most responsible for influenceing the fish community. One of the scientific contributions from this project is to develop and carry out such an analysis.

Risk characterisation is the last step of the ERA and will take place when the exposure and effects profiles are completed. This last step will estimate the probability that effects are occurring by comparing exposure estimates and effect measures, while maintaining a clear image of the uncertainty related to the estimates. The results will also contribute to a GIS model intended to show which streams that have fish communities influenced by anthropogenic stressors.

Below is a planning schedule summarizing the time plan for the different stages in the ERA. The work will first focus on the two first risk hypotheses dealing with pesticides and nutrients, since the preliminary results of the first field trip points to detectable changes of various water quality parameters related to organic pollution. In parallel initial investigations regarding metal contamination are taking place.

So far the first field trip (see results), beside the goal of finding sampling points, has provided substantial input to the process. The results have contributed to a better description of the ecosystem, measurements regarding factors affecting the distribution of the stressors, some measures of exposure (nutrients, possibly pesticides) and preliminary data on the fish community from several sampling sites.

Planning schedule for the PETAR ERA-project, 1998-08-13.

	1998	1999	2000	2001
Field trips			?	?!
water parameters				
nutrients		?	?	?!
pesticides			?	?!
fishing	?		?	?!
Ecosystem characterization				
partial description of ecosystem				
description of factors affecting stressors				
Exposure analysis				
distribution of stressors – lit.rev. + measurements				
satellite imaging and mapping				
GIS				
transport modeling				
Effects analysis				
fish community analyses				
toxicity testing			? ? ? ?	
multivariate analyses				
Exposure profile				
Effects profile				
Risk characterization				
Model development and synthesis				
Final report				

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6. Field Trip

Rosana Moraes, Johanna Lundqvist, Pedro Gerard, Sonia Buck and Helio Shimada participated in the first field trip to PETAR that took place during 7-17th June 1998. The main objective of this trip was to select sampling sites and test sampling and analysis methodologies.

6.1. Sampling sites

Sixteen sites were selected for sampling water, sediment and fish. Figure 19 is a map of PETAR showing the watersheds of Betari, Iporanga and Pescaria/Pilões rivers and the sampling sites locations. As shown in the map, watersheds are partially located outside PETAR limits. Since the limits of the agricultural areas are not well defined, they are not presented on the map. A better definition of these areas will hopefully be possible with the use of recent satellite images.

Sites were selected according to river order, suspected presence or absence of contaminant sources and also accessibility by car, which was due to the need for transportation of the electrical fishing equipment. Different streams are exposed to none or some stressors.

Sites B6, B8, B9, I3 and I4 were selected as controls. Site B6 is at a tributary of the Furnas stream and has no human influence except for a non-asphalt road, which is located in the vicinity. Site B8 is at the Couto stream and only is influenced by the presence of a few pigs held by a farmer who lives far upstream. Drawbacks are that is located close to a cave entrance (30 meters) and that the stream is only 50 meters from the cave to the Betari confluence. Site B9 is on Betari River, next to a camping area in Nucleo Santana. Site I4 and I3 are located in the Iporanga River. Site I4 is located downstream to the confluence with a stream that receives particles from a calcareous mine, but it is probably far enough not to be influenced by that. However, further investigations are needed before a final choice of control sites can take place.

Sites P2, P3 and P4 are in streams located next to agricultural areas. Site P2 is also close to a livestock farm. Information given by PETAR employees suggested areas in the vicinity of P2 and P3 to have been tomato plantations, but not during the last 2 years or more. Farms near P3 cultivated tomatoes last year. Sites I1 and I2 receive particles from a calcareous mine located in the vicinity. Sites B4 and B5 are located in the Furnas River downstream a former lead mine. Site B1 is located in the Betari river, downstream the Furnas confluence. Site B7 was taken in the Jaguatirica stream, which is located downstream Bairro da Serra, a small village. Sites P1 and I3 are located next to very small human settlements, just a few houses. Site B3 is located in the Betari River, downstream Bairro da Serra and the Furnas confluence. Table 6 shows the expected stressors at each sampling site in PETAR.

Table 6. *Expected stressors in different sampling sites in PETAR.*

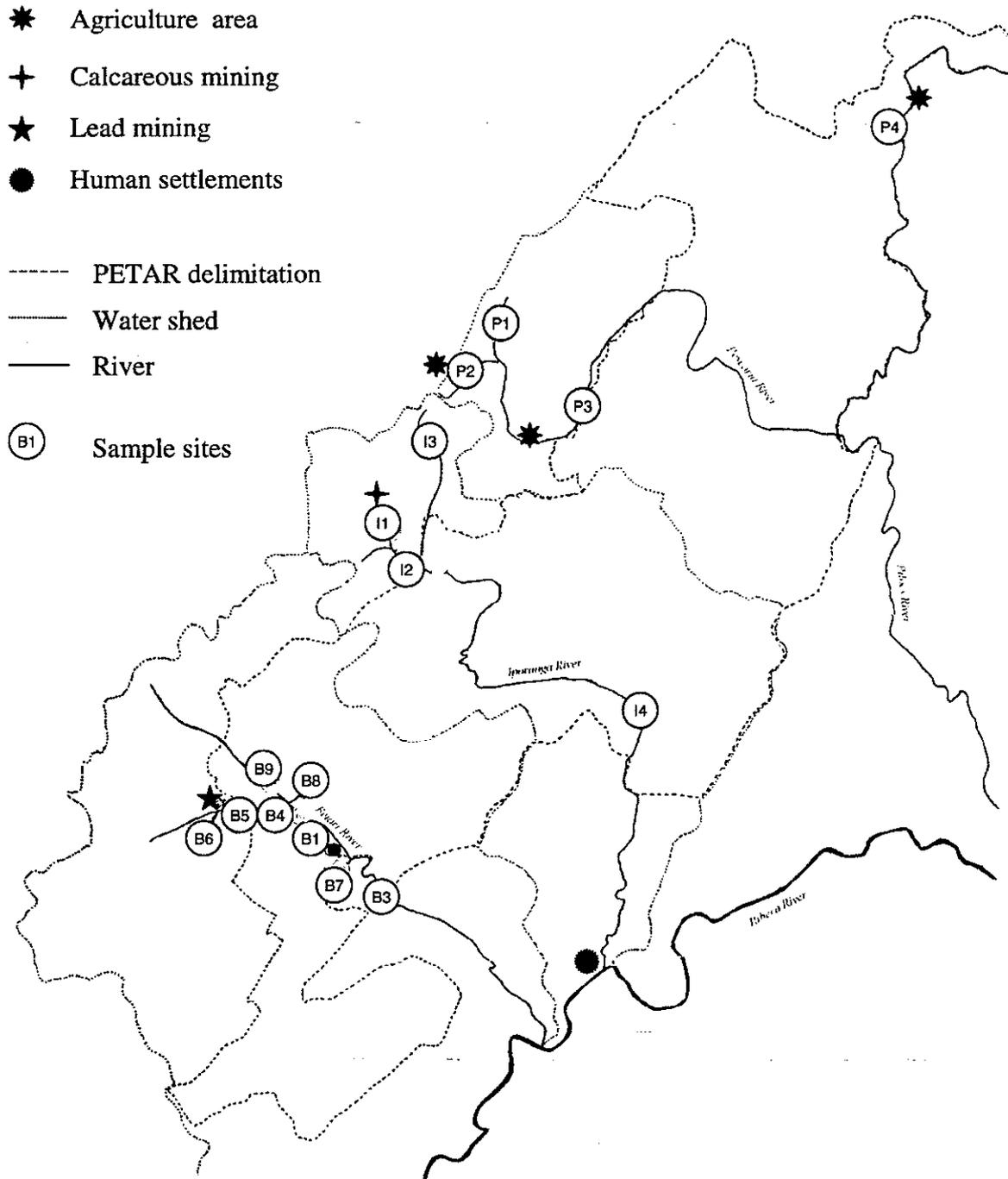|				Sources of Stressors			
Stressors	None	Agriculture	Human Settlement	Calcareous mining	Lead Mining	Human Settlement + Lead Mining	Human Settlement + Agriculture
Pesticides		+					+
Organic pollutants			+			+	+
Inorganic nutrients		+	+			+	+
Heavy metals					+	+	
Particles			+	+		+	+
Sampling sites	B6,B8,B9,I3, I4 (controls)	P2,P3	B7,I3,P1	I1,I2	B1,B4,B5	B3	P4

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Figure 19. Watersheds of Betari, Iporanga and Pescaria/Pilões Rivers, location of stressor sources (mines and main human settlements) and sampling sites.

Table 7 shows sampling sites coordinates, approximated altitude based on topography maps, river depth and breadth based on visual estimation and current speed, measured by a mechanical flowmeter (General Oceans Model 2030R - Standard mechanical). Latitude and longitude was determined using a GPS receiver (Silva GPS Compass XL 1000 Forest).

6.2. Methodology

Conductivity, pH, turbidity, dissolved oxygen, temperature, and salinity were all measured in the field by a multipurpose instrument (Horiba U-10 Water Quality Checker). Nitrate, nitrite and phosphorous were measured in a temporary laboratory at one of the houses in PETAR using a Hach DR/700 Portable Colorimeter. Water samples (3 plastic bottles, 50 ml, from each sampling site) were acidified with 0.2 ml of H_2SO_4 (9 M), frozen and later transported to Sweden for analysis at the laboratory of the Department of Sanitary Engineering at Chalmers University of Technology by Johanna Lundqvist. Table 8 shows methodology and instruments used for analysis of different parameters and number of samples.

Table 7. *Location and description of the sampling sites.*

Current speed is the average of two measurements, unless marked *, which is an average of four measurements).

Site Number	Sampling datum (m/d)	Latitude 48°- S	Longitude 24 ° - W	Altitude (m – approx.)	River depth (m)	River breadth (m)	Current speed $(10^{-2} cm/s)$
B01	06.08	41'58''	31'58''	240	1.0	4.0	88
B03	06.13	40'06''	33'42''	240	0.5	6.0	46
B04	06.11	42'02''	32'06''	260	1.0	2.5	31
B05	06.13	43'10''	32'07''	460	0.3	1.0	29
B06	06.13	43'20''	32'11''	540	0.6	0.5	145
B07	06.14	40'42''	33'09''	180	0.4	1.0	22
B08	06.14	41'58''	32'20''	280	0.5	1.5	00
B09	06.16	42'08''	31'55''	260	0.5	10.0	No meas.
I01	06.09	39'20''	26'43''	700	0.4	2.5	No meas.
I02	06.09	39'15''	26'43''	700	0.4	4.0	No meas.
I03	06.10	39'25''	26'55''	840	0.5	4.0	13
I04	06.11	35'17''	24'44''	160	1.0	6.0	59*
P01	06.10	38'11''	29'47''	840	0.1	0.6	44*
P02	06.10	38'15''	22'49''	860	0.4	1.0	No meas.
P03	06.12	36'13''	23'20''	840	1.0	5.0	00
P04	06.12	30'00''	18'53''	720	1.0	5.0	92

Sediment samples (one 50 ml polypropylene jar from each site) were kept frozen and covered with aluminum foil. Those samples will be used for heavy metals and pesticides analysis.

Water samples were collected from 16 streams using polypropylene bottles (2 L) for pesticides analysis. Samples that presented pH higher than 7 were acidified with approximately 0.1 ml of H_2SO_4 (9M) to avoid hydrolysis of silica in the columns. pH was once again measured after acidification, and values lower than 2 were detected, which may affect the results. After adding 7 µl of internal standard samples were extracted with solid phase columns using a pressure filtration apparatus. On the outlet of the pressure container a prefilter and a silica based solid-phase extraction column (Env⁺ 200 mg or 1 g) were placed. Columns were activated with few milliliters of methanol before extraction started. After extraction, columns and filters were

involved in aluminum foil and kept in plastic bags at approximately 6°C. Tests on tap water were performing adding 7 µl of standard solution 1, 7 µl of ISTD and approximately 0.1 ml of H_2SO_4 (9M).

An electrofishing survey was conducted by Pedro Gerard and Sonia Buck at 10 sites using an electricity generator (1000 watts) Honda. Most of those streams had never been sampled before and since the survey was only a preliminary study, sampling effort was not the same at the different sites. Since Betari river has been their study are for several years, sampling was not carried out there this time. Fishes were identified and sent to *Museu de Zoologia de São Paulo*.

Table 8. *Selected parameters, methods and number of sampling sites and replicates.*

Parameter	Instrument	-Method	Range	Local of analysis	Number of sampling sites	Number of replicates per site
pH	Horiba	Electrode	0-14	Field	16	3
Water temperature	Horiba	Thermistor	0-100mS	Field	16	3
Dissolved oxygen	Horiba	Electrode	0-19.9 mg/L	Field	16	3
Conductivity	Horiba	Electrode	0-50°C	Field	16	3
Salinity	Horiba	Electrode	0-4%	Field	16	3
Turbidity	Horiba	Scat./Trans. Light	0-800 NTU	Field	16	3
Hardness		Titration		PETAR lab	16	1
Nitrate	Hach DR/700	Cadmium reduction, Diazotization	0-0.5 mg/L	PETAR lab	16	3
Nitrite	Hach DR/700	Diazotization	0-0.500 mg/L	PETAR lab	8	1
Phosphate	Hach DR/700	PhosVer 3 (Ascoric Acid)	0-2.50 mg/L	PETAR lab	16	3
Total Nitrogen	Hach DR/2000	Persulfate Digestion	0-25 mg/L	Chalmers lab	16	3
Total Phosphorous	Hach DR/2010	PhosVer 3 with Acid Persulfate Digestion	0-3.50 mg/L	Chalmers lab	16	3
Ammonia	Hach DR/700	Salicylate	0-1.00 mg/L	Chalmers lab	16	1
Chemical Demand of Oxygen	Hach/ DR/700	Reactor Digestion	0-1500 mg/L	Chalmers lab	16	3

6.3. Results and Preliminary Discussion

Water quality

Table 9 shows average values of water temperature, pH, hardness, conductivity, nitrate, nitrite, phosphate, total nitrogen, total phosphorous dissolved oxygen and chemical demand of oxygen for sixteen different sampling sites in the Betari, Iporanga and Pescaria watersheds.

Salinity values at all sites were close to 0% (below the detection limit), excepted for B07, which presented salinity 0.01%. Turbidity was always <10 Fau, (values below detection limit). Salinity and turbidity results are thus not presented on the table.

Sites B6, B8, B9, I3 and I4 were considered to be reference sites, supposedly not being exposed to stressors originating from agriculture, mining or human settlements areas. Samples collected from those streams showed pH values (average 7.9; standard deviation 0.28) slightly higher than samples collected from sites which are exposed to one of the stressors coming from sources mentioned above (average 7.4, standard deviation 0.51). Lowest pH values were found at P02, which was located next to a farm with livestock and old tomato plantations (Figure 20). Other factors like geology and soil characteristics of the area will be affecting pH values and should be further studied in order to reach conclusions.

Table 9. *Water quality parameters at sampling sites in PETAR during June 1998.*

Values represent average of three measurements, excepted for ammonia, hardness and nitrite that were measured only once in each site.

Sites	pH	T (°C)	Hardness (°d)	DO (mg/l)	Conductivity (mS/cm)	NO$_3$-N (mg/l)	NO$_2$-N (mg/l)	PO$_4$ ion-P (mg/l)	Tot-N (mg/l)	Tot-P (mg/l)	COD (mg/l)
B01	7.7	17.8	4	9.87	0.159	0.35	0.00	0.15	0	0.02	3
B03	7.9	18.5	3	9.13	0.159	0.27		0.27	1	0.02	3
B04	7.9	18.0		9.57	0.199	0.31	0.00	0.56	0	0.01	2
B05	7.8	17.7	4	7.86	0.227	0.13		0.20	0	0.02	5
B06	7.9	18.4	4	8.51	0.214	0.21		0.32	0	0.03	8
B07	7.9	17.9	7	8.04	0.297	0.13		0.49	3	0.07	13
B08	8.0	18.6	3	9.05	0.140	0.29		0.21	3	0.03	2
B09	8.2	17.5	4	9.60	0.153	0.35		0.23	0	0.02	3
I01	7.5	16.1	2	11.56	0.075	0.14	0.00	0.31	0	0.02	4
I02	7.5	14.9	3	11.83	0.096	0.11	0.00	0.21	1	0.02	2
I03	6.9	14.2	3	7.71	0.152	0.12	0.00	0.16	1	0.02	7
I04	7.7	16.4	3	9.82	0.124	0.29	0.00	0.27	0	0.03	4
P01	7.1	14.3	2	12.31	0.111	0.15	0.00	0.15	0	0.02	2
P02	6.3	13.8	1	12.71	0.027	0.15	0.00	0.11	1	0.01	3
P03	7.1	14.3	1	8.92	0.041	0.15		0.18	1	0.02	6
P04	7.6	15.4		8.87	0.172	0.10		0.65	1	0.02	4

Figure 20. *pH values from some rivers and streams in PETAR, June 1998.*

(B06 to I04, at the left side of the panel, are considered reference sites, B01 to P04 are sites which are supposedly exposed to anthropogenic stressors).
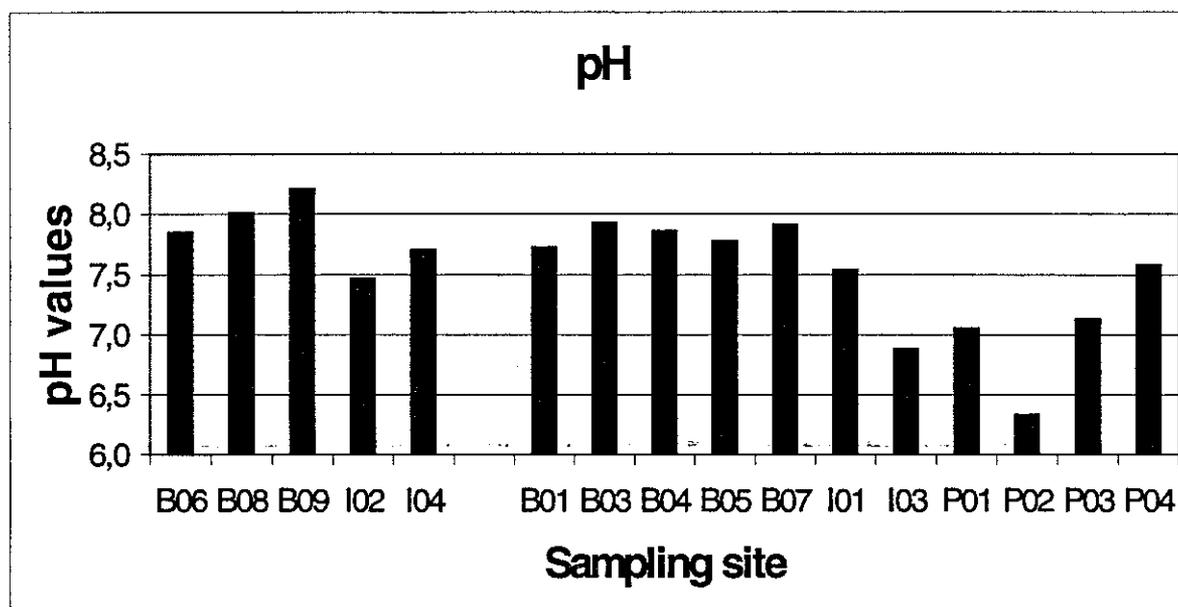
Nitrate and Phosphate

Figure 21. *Nitrate and phosphate values from some rivers and streams in PETAR, June 1998.*

(B06 to I04, at the left of the panel, are considered reference sites, B01 to P04 are sites which are supposedly exposed to anthropogenic stressors).
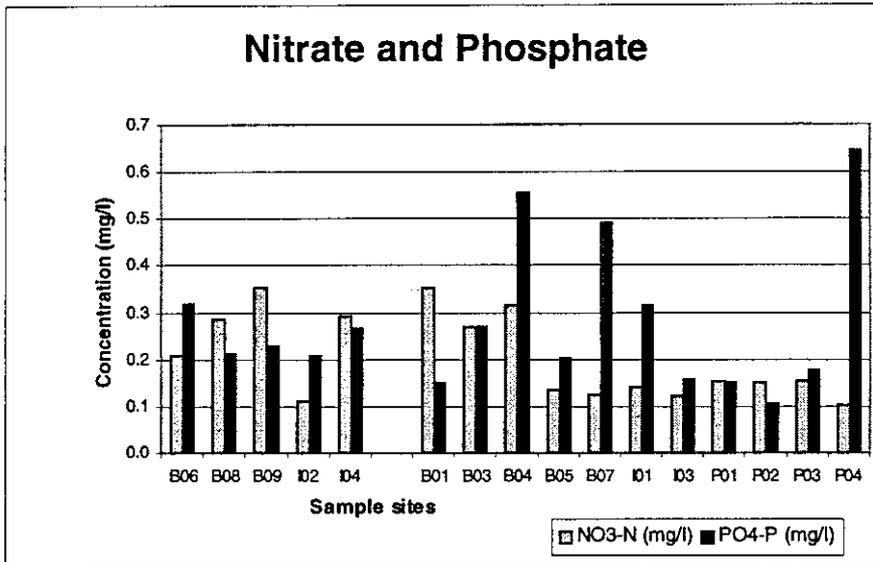
Phosphate values were higher (average 0.3 mg/l, standard deviation 0.19) at sites exposed to anthropogenic stressors than the average (0.2, standard deviation 0.04) of the reference sites. The highest values were found at sites P04 (Figure 21), which is located next to a tomato farm and near some human settlements (0.65 mg/l), B04 at the Furnas river, downstream the mining area (0.56 mg/l) and B07, downstream the small village at Bairro da Serra (0.49 mg/l). As showed in Figure 22, this site (B07) also presented the highest values for chemical oxygen demand (13 mg/l; the average for the possible affected sites was 4.6 mg/l). It also showed one of the lowest dissolved oxygen concentrations (8.04 mg/l, average for possible affected sites was 9.7 mg/l), the highest total phosphorous concentration (0.07 mg/l) and the highest total nitrogen concentration (3 mg/l). At this last site, a bad smell and a high biomass of algae was observed. These effects seem to be the result of the sewage discharge of the village.

DO and COD
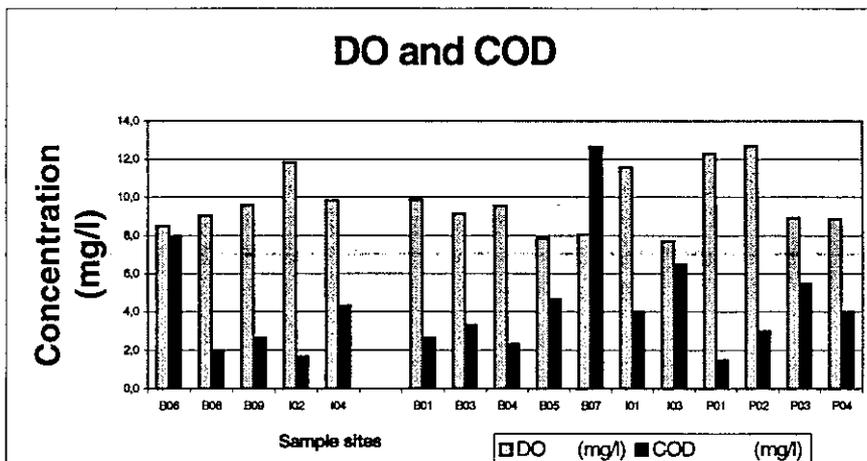
Figure 22. *Chemical oxygen demand and dissolved oxygen values from some rivers and streams in PETAR, June 1998.*

(B06 to I04 are considered reference sites, B01 to P04 are sites which are supposedly exposed to anthropogenic stressors).

Fish community

Table 10 is a list of species together with the number of individuals of each species that were collected at 10 sites during the field trip to PETAR by S. Buck and P. Gerhard. Since Betari River has been studied by them for some years, electrical fishing was not used this time.

The list of species of sites B1, B3 and B9 was prepared by S. Buck based on results from various earlier campaigns using different sampling techniques. Her efforts have been concentrated in two sites in Betari river: next to Nucleo Ouro Grosso and next to the Figueira brigde, that is located near the Aguas Quentes stream confluence with the Betari river.

Table 10. *List of species of fishes and number of individuals collected in Betari, Iporanga, Pescaria rivers and their tributaries during June 1998 and before (sites B1, B3 and B9).*

(Kindly provided by S. Buck and P. Gerhard).

Sampling site \ Collected species	B01	B03	B04	B05	B07	B08	B09	I03	I04	P01	P02	P03	P04
Astyanax ribeirae	*	*					*						
Astyanax scabripinnis	*	*					*	31					
Astyanax janeiroensis	*	*					*						
Astyanax sp.			1	15	15					27		4	
Deuterodon iguape	*	*			1		*		8				2
Bryconamericus microcephalus	*	*				3	*						
Oligosarchus hepsetus	*	*					*						
Characidium pterostictum	*	*					*		7				
Characidium sp.								9		11	11	60	
Phalloceros caudimaculatus	*	*			4		*						
Rhandioglanis frenatus	*	*	10			4	*		2				
Chasmocranus lopezi	*	*					*						
Rhamdia cf. quelen	*	*			1		*						1
Pimelodella transitoria	*	*	8				*		6				
Ancistrus multispinis	*	*					*		3				
Kronicthys heylandii	*	*			2		*						
Rineloricaria jaraguensis	*	*					*		4				
Hypostomus agna	*	*					*						
Hypostomus interruptus	*	*			13		*						
Neoplecostomus ribeirensis	*	*					*						
Pareiorhaphis alipionis	*	*					*						
Pareiorhaphis duseini	*	*					*						
Pareiorhaphis sp.						1							
Parotocinclus maculicauda	*	*					*						
Hisonotus gibbosus	*	*					*						
Corydoras barbatus	*	*					*		2				
Trychomycterus davisi	*	*					*						
Trychomycterus sp.	*	*			14		*						
Ituglanis sp.	*	*					*						
Hollandichthyis multifasciatus													2
Geophagus brasilensis													7
Hartia kronei						5							
Hipoptomatinae n.i.					8								

* Species found during former investigations by S.Buck.

6.4. Further analyses

Since chemical speciation of metals affects their availability to aquatic organisms and also their toxicity, processes such as inorganic complexation, chelation, precipitation, and adsorption should be monitored. However, those studies are complex and very expensive. Most of the analyses are limited by the factor that the transport of samples from Brazil to Sweden would affect the results. Laboratories in Brazil have been contacted but none of them perform metals speciation analysis. Due to these problems, heavy metal analysis will be limited to total metal concentration in sediment.

Columns and sediment samples will be analyzed with regard to pesticides by Henrik Kylin, PhD, at the Institute of Environmental Analysis at the Swedish Agricultural University, Ultuna.

A new sampling campaign will take place during the rain season (October/November 1998) when the same sites, and possibly new ones, will be investigated. At this time the use of pesticides in agriculture is more pronounced due to the tomato growing season, and therefore pesticides are expected to be found in higher concentrations.

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Appendix – Fish fauna in PETAR streams

The text below discusses some details about habitat, diet and feeding behavior of the species collected during the field trip. It was prepared and kindly provided by P. Gerhard and S. Buck based on a literature review (Aranha, Caramaschi, Caramaschi, 1993; Buck & Sazima 1995; Burguess, 1989; Castro & Casatti, 1997; Costa, 1987; Strahler, 1952; Menezes, *et al.* 1990; Sabino & Castro, 1990. Sazima, 1986; Soares-Porto, 1994; Uieda, 1984. Uieda, 1995; Uieda, Buzzato & Kikuchi 1997) and on their ongoing research.

Order Characiformes

Family Characidae

Astyanax spp., *Bryconamericus microcephalus, Deuterodon iguape* and *Hollandichthyis multifasciatus.* Those species swim in middle-water, exploring a broad range of microhabitats, such as pools and places with different water-flow speeds. Apparently, *Bryconamericus* is the species that is more frequently found in faster water-flow areas. They feed on items brought by the water flow. They have been considered omnivorous by some authors. *Characidium* spp. are bottom-feeding fishes. Like other characidae, they are found in different habitats, more frequently in places of faster water-flows. They eat insects living in the subtract both using actively and widely foraging strategies (carnivorous-insectivorous).

Order Siluriformes

Family Trichomycteridae

Trichomycterus sp. 1 are bottom-dwelling fishes found mostly in flowing waters, sometimes even very fast. They occupy, and are probably foraging in, crevices and cracks between rocks and also between roots of riparian vegetation. It is probably insectivorous, but further investigations are needed before conclusions.

Family Loricariidae

Ancistrus multispinis, Harttia kronei, Kronichthys heylandii, Pareiorhaphis spp. e *Rineloricaria jaraguensis.* They are fishes that are associated with river bottoms, where they find shelter and food. Most of loricariids ("cascudos") scrap microalgae on rocks, stems and branches of submerse plants. All species ingest considerable quantities of sediment when grazing. *Ancistrus multispinis* ingest large amounts of detritus (organic material partially decomposed) and may be classified as detritovorous. Some loricariidae species, e.g., de *Pareiorhaphis* spp., *Rineloricaria jaraguensis,* and *Kronichthys heylandii,* are found in fast flowing water. *Ancistrus multispinis* dwell in pools or in places with slow water flow. The mentioned species of loricariids are nocturnal, except by *Kronichthys heylandii,* which also is active during the day.

S. Buck is studying reproductive biology of loricariidae from Betari River. Preliminary results showed that *Kronichthys heylandii* and *Rineloricaria jaraguensis* have relatively small clutches (approximately 50 eggs, which is considered to be small for fishes in general). Males of those two species show parental care behavior and their reproductive season coincide with the dry period (May- August).

Family Callichthyidae

Corydoras barbatus is a bottom-dwelling fish that feeds on invertebrates when grubbing substrate (carnivorous-insectivorous). Algae can be also found in their gut content. They are found in places with slow current, sometimes associated with marginal vegetation.

Family Pimelodidae

Pimelodella transitoria, Rhamdia cf. *quelen* e *Rhamdioglanis frenatus.* These three species occupy a large diversity of microhabitats, although *P. transitoria, Rhamdia* can be more frequently found in calm current places. All species are bottom-dwelling fishes, but

P. transitoria also swims in the middle water. The diet of *Rhamdia* and *R. frenatus* is based on other fishes, but insects and crustaceans are also found in their stomach content (carnivorous-insectivorous, piscivorous). *Rhamdia* may eventually be considered the only piscivorous species in headwater streams, for instance in Betari river headwaters and Jaguatirica stream.

Order Cyprinodontiformes

Family Poecilidae

Phalloceros caudimaculatus is a small size species, found in general next to marginal places, associated with vegetation. Its diet is based on algae that grow on other plants branches as well as small bentic and planktonic invertebrates (omnivorous).

Order Perciformes

Family Cichlidae

Geophagus sp. is associated with river bottoms, but can eventually be found in middle waters as well. They are commonly present in pools or slow current places. They feed by biting pieces of bottom substrate, filtering sediment and then retaining small invertebrates. Larger specimens may also eat small fishes (carnivorous-insectivorous).