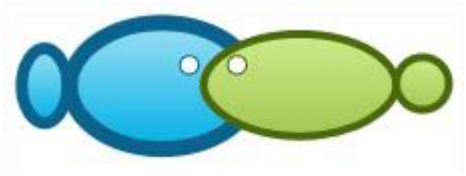

Influence of water from apparently contaminated sources, upon activation time and sperm motility of native fish species from Colombian Orinoquia

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Abstract. Environmental pollution grows significantly throughout the world due to human activity. The present study has been conducted in order to evaluate the effect of water from different natural sources of Meta River basin (Colombian Orinoquia) influenced by agricultural, hydrocarbon extraction and urban activities that could influence activation time and sperm motility of three native characid fish species of the basin: *Piaractus brachypomus*, *Brycon amazonicus* and *Prochilodus mariae*. Semen samples of the related species were collected and activated in laboratory with water collected from the Orotoy, Ocoa, Upía and Meta Rivers, and with groundwater from the fish station. Concentration of cyanide and lead was high in all sampling sites. Mercury was at the highest concentration in Ocoa River and iron was higher in Meta and Upía river. Activation time and sperm motility did not showed significant differences for each species analyzed with different sampling sources of water, presumably because this study was carried out in the high water season and their large volumes of water can dilute contaminating agents that could have an influence on reproductive cells.
Key Words: reproduction, ichthyofauna, effluents, semen*, P. brachypomus, B. amazonicus, P. mariae.*

Introduction. Industrial and economic development has led to a significant and uncontrolled increase in pollution worldwide. Environmental contaminants are varied and include metals, pesticides, insecticides, herbicides, pharmaceuticals, and wastewater from different sources, which for various reasons end up in the water bodies (Daza et al 2005). In Colombia, the activities with the greatest impact on the environment are deforestation, mineral extraction, and agricultural extension, and in recent years these activities have had intensified, even more in the Meta department (Ramirez & Navarro 2015). Extensive deforestation precedes the preparation of land for livestock and agricultural use, and the latter complements its activities with the excessive use of pesticides and chemical fertilizers. Intensification of the exploratory and extractive activity of hydrocarbons with increasingly invasive technologies and of greater environmental impact intoxicate ecosystems full of diversity; while the activities of the growing urban areas generate the dumping of waters with all kinds of substances of industrial waste, domestic and commercial that increase the environmental damage when thrown into rivers and streams that disperse these substances at great distances.

Although modifications to environmental regulations have been implemented in Colombia to establish adequate water policies, poor overall management of water resources is evident (Rodríguez 2012). Studies of water contamination by substances are scarce, and in some cases, the results of these analyzes are not of general consultation.

Aquatic ecosystems can easily lose their balance due to the toxicity generated by pesticides, heavy metals and hydrocarbons. These substances can generate lethal or sub-lethal effects, which in the first case will lead to the death of sensitive organisms and in the second cause competition and dynamics of the communities, altering the trophic networks and the function of the ecosystem (Fleeger et al 2003). Fish represent an

important link in the aquatic environment, since they are part of trophic networks, perform a function in the ecosystem (Jackson et al 2000) and they are also an important source in the diet and economy of diverse populations (Barbarino et al 1998; FAO 2018).

The processes that guarantee the reproduction of the fish can be affected when the contaminants appear in the bodies of water and enter the regulating and executing organs of the reproduction, being able to alter the hormonal behavior, the gametogenesis, the spawning, the fertilization, the incubation and the embryonic development of aquatic species (Denslow & Sepulveda 2008). Among environmental contaminants, a large group of molecules are labeled endocrine disruptors, and it has been reported that in fish captured in contaminated areas or exposed in vivo to chemicals, the cascade of events that involves the activation of sensory receptors to environmental stimuli and the sequence of events in organs such as the hypothalamus, pituitary and gonads have been altered, and therefore presents a decrease in the reproductive parameters (Daza et al 2005).

Meta department is part of the Colombian Orinoquia Region, which extends through the eastern cordillera through plains and savannahs to the east of the country. This region has a high water and fish richness, hydrographically it belongs to the central zone of the Llanero piedmont, and in turn to the upper basin of the Meta River; the Upía river is one of the most important hydric bodies, and the Orotoy and Ocoa rivers are two of the main secondary ones (Caro-Caro et al 2011). All these water bodies belong to the great Orinoco River Basin, habitat of the Characiformes, rheophilic fish species that makes large reproductive group-migrations during the rainy season also called the high water season (Daza et al 2005). The Characiformes order comprises 261 species, 45% of Meta River Basin fish species (Trujillo et al 2016), with a variety of ecological and food specializations, carnivorous, omnivorous and detritivorous (Daza et al 2005). Additionally, this group is represented by species of economic importance for the riverine communities of the Colombian Orinoquia.

Meta river basin a strong anthropic influence due to hydrocarbon extraction and agricultural activities, and also water discharge near the main population centers (Correa et al 2005).

The objective of the present study was to evaluate the effect of water sources influenced by anthropogenic activity, such as the cultivation of African palm and agricultural activities, the exploitation of hydrocarbons, urban settlements and the dumping of wastewater, in the basins of the Orotoy, Meta, Ocoa and Upía rivers, on activation time and sperm motility of the endemic species of the Colombian Orinoquía.

Material and Method

Study site localization. The water samples were collected in the Orotoy rivers (N 04º 04.323 "W 072º 35.29"), Meta (N 04º 22.1364 "W 072º 03.30"), Ocoa (N 04º 06.658 "W 073º 36.85") and Upía (N 04º34. 157 "W 072º 57.51") during the high water season in the month of April 2016. Water analyzes were carried out at the facilities of the Fish Station and in the Reproduction Laboratory of Aquatic Organisms of Aquaculture Institute - University of the Llanos (IALL), located in Villavicencio - Colombia (4º07'35 "N 73º58'24" W), at a height of 440 m.a.s.l, average temperature of 27ºC and average annual precipitation of 4,383 mm.

Biological material. Sexually mature male fish of the UPE were selected from the species: *Piaractus brachypomus* (Cachama blanca), *Brycon amazonicus* (Yamú) and *Prochilodus mariae* (Coporo), at the rate of one individual per species. To be selected, male fish were previously evaluated by abdominal pressure expecting to observe the output of a whitish and viscous liquid characteristic of semen in the urogenital papilla. Later, they were transferred to handling pools where they were weighed and identified. To obtain the seminal samples in sufficient quantity, hormonal induction was carried out, injecting intramuscularly Carp Hippophysis Extract (CHE) diluted in saline, in a single dose of 2.5 mg kg^{-1}, eight hours before squeezing.

At the time of semen collection, the broodstock was calmed by immersion in a 2-phenoxyethanol solution (300 ppm) until the loss of the swim axis was observed. After that, each individual was removed from the solution, afterwards the fins and abdomen were dried with a towel, a gentle pressure was applied on the urogenital papilla in order to extract remains of urine and feces that could contaminate the samples. The semen was obtained by abdominal massage in the cranio-caudal direction, collected directly in sterile centrifuge tubes of 2 mL, and immediately taken to the laboratory for evaluation.

Semen evaluation. The collected material was analyzed in the laboratory by observation under a microscope, depositing a sample of 20 µL of semen from each of the selected species on a specimen slide. When the immobile state of the spermatozoa was confirmed, 180 µL of the water sample to be evaluated was added and the estimated percentage of sperm mass that was in motion (Spermatic motility), and the duration of that movement (Activation time) were recorded quantified manually.

For the activation of sperm, the water was used from five different water sources in the department of Meta-Colombia, as described below:
Site 1: Orotoy River (N 04º 04.323 "W 072º 35.29") with incidence of the oil industry Site; 2: Meta River (N 04º 22.1364 "W 072º 03.30") natural environment where the study species reproduce;
Site 3: Ocoa River (N 04º 06.658 "W 073º 36.85") with incidence of human settlements in Villavicencio city;
Site 4: Upía River (N 04º34.157 "W 072º 57.51") with incidence of palm crops;
Site 5: IALL (N 04º 04.404 "W 073º 34.94") reproduction laboratory, with underground water supply.

Sperm activation in *P. brachypomus*, *B. amazonicus* and *P. mariae* was evaluated in triplicate for each of the five water sources, which were previously subjected to physical, chemical and microbiological tests.

Statistical analysis. Values of the variables, activation time and sperm motility, were expressed as mean ± standard deviation (SD). Data distribution for normality hypothesis were analyzed by means of the Shapiro-Wilk test. One-way ANOVA and Tukey Test were performed in cases where the assumption of normality was not met, nonparametric tests of Kruskall-Wallis and Mann Whitney were used. The statistical analysis was performed with the SPSS software version 21 for mac.

Results. The results of the physicochemical and microbiological analysis performed on the water samples from the different sampling sites are shown in Table 1. The physicochemical parameters conductivity, turbidity, total iron and hardness, showed greater variation between the different sampling sites compared to the other parameters; Likewise, the total coliform and fecal microbiologicals (*Escherichia coli*) showed notable variations, being less abundant in the samples of water coming from the groundwater of the IALL.

As shown in Table 2, in the evaluation of the time of seminal activation no significant differences were found ($P<0.05$) between the activation time of any of the species. Similarly, sperm motility (Table 3) did not show significant differences between the water samples of different study sites.

Table 1

Physico-chemical and microbiological parameters of the water of the five sampling sites

Physico-chemical analysis							
Parameters	*Units*	*Method*	*Water samples*				
			Orotoy River	*Meta River*	*Ocoa River*	*Upía River*	*IALL*
Total cyanide	mg CN L^{-1}	ASTM D7511-09	<0.012	<0.012	<0.012	<0.012	<0.012
Real color	PCU	SM 2120 C	24	20	<5	43	<5
Conductivity	µS cm^{-1}	SM2510 B	20	23	110.4	130.4	83
Total hardness	mg $CaCO_3$ L^{-1}	SM 2340 C	11	10	32	65	17
Fluorides	mg F L^{-1}	SM 4500- FD	<0.3	<0.3	<0.3	<0.3	<0.3
Total hydrocarbons	Mg L^{-1}	NTC 3362F: 2011	<0.5	<0.5	0.8	1	<0.5
Total iron	mg Fe L^{-1}	SM 3500 Fe B	0.3	3.8	0.7	4.9	<0.1
Total magnesium	mg Mn L^{-1}	SM 3030 F SM 3111 B	0.03	0.07	0.17	0.16	0.02
Total mercury	mg Hg L^{-1}	EPA 2008	<0.0003	<0.0003	0,002	<0.0003	<0.0003
Nitrate	mg N-NO_3 L^{-1}	SM 4500 NO_3 B	< 0.3	< 0.3	<0.3	<0.3	<0.3
Nitrite	mg N-NO_2 L^{-1}	SM 4500 NO_2 B	<0.02	<0.02	<0.02	<0.02	<0.02
Dissolved oxygen	mg O_2 L^{-1}	SM 4500 O C	7.3	5.8	5.7	6.6	6.7
pH	UNT	SM 4500 H. B	6.99	7	6.68	7.59	7.19
Organochlorine pesticides	mg L^{-1}	EPA 8081 B	<0.00001	<0.00001	<0.00001	<0.00001	<0.00001
Organophosphorus pesticides	mg L^{-1}	EPA 8141 B	<0.00003	<0.00003	<0.00003	<0.00003	<0.00003
Total lead	mg Pb L^{-1}	SM 3030 F SM 3111 PB	<0.04	<0.04	<0.04	<0.04	<0.04
Turbidity	NTU	SM 2130 B	3.1	120	4.8	650	<1
Microbiologic analysis							
Total coliforms	MPN 100 mL^{-1}	SM 9223 B	24196	14830	24196	54750	327
Escherichia coli	MPN 100 mL^{-1}	SM 9223 B	85	1550	24196	1870	86

MPN - most probable number, NTU - nephelometric turbidity unit, PCU - units of platinum-cobalt, UNT – units.

Table 2

Sperm activation time of the studied fish species from the Colombian Orinoquia, treated with water from five different water sources

Species	Sperm activation time (seconds)				
	Orotoy River	*Meta River*	*Ocoa River*	*Upía River*	*IALL*
Piaractus brachypomus	71.67±10.50[a]	69.67±4.51[a]	69.33±7.09[a]	57.67±9.29[a]	57.67±15.82[a]
Brycon amazonicus	49.67±3.79[a]	52.33±1.15[a]	51.67±3.79[a]	47.00±3.00[a]	45.67±3.51[a]
Prochilodus mariae	60.67±9.29[a]	61.00±2.00[a]	60.33±3.51[a]	53.67±9.07[a]	50.67±4.04[a]

Values represent mean ± standard deviation. Values in the same row did not show significant differences ($p>0.05$).

Table 3

Sperm motility of the studied fish species from the Colombian Orinoquia, treated with water from five different water sources

Species	Sperm motility (%)				
	Orotoy River	*Meta River*	*Ocoa River*	*Upía River*	*IALL*
Piaractus brachypomus	83.00±6.08[a]	80.00±1.00[a]	73.66±5.50[a]	83.66±5.50[a]	70.00±1.00[a]
Brycon amazonicus	80.00±1.00[a]	73.66±5.50[a]	76.67±6.08[a]	70.00±10.00[a]	73.66±5.50[a]
Prochilodus mariae	70.00±1.00[a]	70.00±1.00[a]	80.00±1.00[a]	80.00±1.00[a]	63.33±5.50[a]

Values represent mean ± standard deviation. Values in the same row did not show significant differences ($p>0.05$).

Discussion. Water is the main natural resource with importance for the maintenance of all forms of life on earth; in addition, this resource is fundamental for the all humankind development and all its deeds. Dependence on water in industrial activities and in extensive agricultural and livestock, has made this resource to be manipulated and its original physical-chemical and microbiological conditions to be altered frequently in ecosystems. In the search to preserve aquatic ecosystems and their functionality, environmental and political organisms have sought to provide technical tools that facilitate the establishment of regulatory mechanisms that propitiate the sustainable management of water resources. The environment assembly of the united nations environment program (UNEP), in 2016, formulated the International Water Quality Guidelines for Ecosystems (IWQGES 2016), which contains a set of guidelines required to establish water quality guidelines in ecosystems. To classify ecosystems according to their intervention, IWQGES (2016) established 4 categories :I.) High ecosystem integrity, II.) Moderately disturbed, III.) Highly disturbed and IV.) Extreme impairment. Some parameters of water quality in present study were compared with IWQGES (2016) reference values. The pH of the water in each of the sampling sites was found within the ranges that characterize the high integrity of the ecosystem (pH 6.5 to 9.0). Dissolved oxygen concentration in the Orotoy River was 7.3 mg L^{-1}, which is in the reference range of category I (7.3-10.9 mg L^{-1}), other sampling sites showed lower concentrations of oxygen, which could suggest that there is a moderate level of intervention in these places. It is important to clarify that temperature plays an important role in dissolved oxygen conditions (Boyd 2015). Reference values of the IWQGES (2016) establish oxygen concentrations for freshwater at 20ºC and height at sea level, however water of the Meta River basin presents temperature ranges of 20-32ºC and height between 200-1,600 m.a.s.l (Caro-Caro et al 2011; Salinas-Jiménez et al 2017); these factors can influence dissolved oxygen concentrations at the study site. Concentration of mercury in

Orotoy, Meta, Upía and IALL were higher than Category I (0.05 µg L^{-1}) and lower than Category IV (1.0 µg L^{-1}), and also Ocoa River presented concentrations that exceed the last one. Lead concentration in the five sampling sites was also higher (Category IV 5.0 µg L^{-1}) than the established by the IWQGES (2016).

Three parameters were compared with the values reported in the natural ecosystems of the study species in the Boconó River, the Venezuelan orinoquía (Barbario et al 1998). Values of pH, turbidity and dissolved oxygen showed similarity in both studies. Jackson et al (2000) showed that diversity, fish abundance is an indicator of ecosystem welfare and the availability, and concentration of dissolved oxygen is a fundamental parameter that allows knowing the integrity of the water ecosystem.

Connell et al (1981) conduced a review about hydrocarbons in aquatic environments, in which they report that concentrations between 1 and 100 mg L^{-1} have a lethal effect on a variety of adult organisms. Juveniles and larvae show mortality at hydrocarbon concentrations between 0.1 and 1 mg L^{-1}, and finally exposure to concentrations between 0.01 and 0.001 mg L^{-1} generate sublethal effects in organisms, which can modify their physiology and behavior, including reproduction. Concentration of hydrocarbons reported in water sources of this study are within the range in which it can generate lethal and sublethal effect in fish, depending on the sensitivity and exposure time of these organisms (Connell et al 1981; Jackson et al 2000). The main routes of hydrocarbon intake in fish are respiratory and digestive ways, where absorption of this compounds occur in body parts exposed to them directly or by biotransformation, which is possible because of mechanisms of enzymatic oxidation that transform foreign compounds into metabolites and then on endogenous substrates more hydrophilic; however excretion of molecules can occur without conjugation (Connell et al 1980). Oxidation of aromatic hydrocarbons and olefins, usually leads to electrophilic metabolites formation that may interact chemically with important molecules at cellular level such as DNA, RNA or with functional substrates in the cell generating mutagenic, cytotoxic and carcinogenic effects (Connell et al 1980).

Hydrocarbons can also be formed in the aquatic environment naturally from organic molecules, the composition and chemical structure of these is much simpler than that of petroleum hydrocarbons and are easily degraded by the local microbiota (Connell et al 1980); however, there is no report of biogenesis of this type of substance in the study area. Importantly, some petroleum compounds can be solubilized in water and cause lethal or sublethal effects in fish and other organisms, which also magnify their cytotoxic, carcinogenic and mutagenic effects as they accumulate in the tissues of aquatic organisms through trophic networks to reach humans (Connell et al 1981). At present, it is difficult to find reports of the amount of oil that enters the bodies of water, Connell et al (1981) reported that about 11 million tons of oil per year enter the oceans, however, the increase in extractive dynamics and accidental events in this activity worldwide must undoubtedly have increased this amount not only in the oceans but also in wetlands, lakes and rivers, significantly affecting the function of plants, animals and humans.

The inappropriate use of agrochemicals also causes a negative impact on the microbiota of soil and water, turning them into reservoirs of toxic substances (Önder et al 2011). The use of pesticides that cause specific damage in metabolic pathways not only eliminates the target organism but also affects a variety of harmless organisms. Chemical fertilizers used inappropriately to improve the performance of crops can cause pollution, nitrogen is one of the main components of these substances and in high concentrations can alter the balance of ecosystems and accumulate carcinogenic substances such as nitrosamines (Önder et al 2011). Phosphorus is the second most important nutrient in the eutrophication processes of aquatic ecosystems that occur in urban as well as in agricultural production areas. In the first, phosphates come from detergents of industrial and domestic use and in agricultural production areas phosphorus comes from chemical enrichers (Singh & Gupta 2017). Fertilizers, pesticides, herbicides and insecticides cause water acidity, especially in ecosystems such as the Colombian Oriental plains characteristic of their acidic soils and waters, diminishing their productivity and generating a toxic effect for plants (Singh & Gupta 2017).

According to the United States Environmental Protection Agency (EPA), Guidelines for protection of freshwater aquatic ecosystems, establish Criterion Continuous Concentration (CCC) of cyanide (0.005 mg L^{-1}), iron (1 mg L^{-1}), lead (0.002 mg L^{-1}) and mercury (0.0008 mg L^{-1}). In water samples of the present study cyanide and lead had higher concentrations than those imposed by EPA, and mercury concentration was highest in Ocoa River. Iron concentration in the present study was variable for the different sampling sites. IALL, Orotoy and Ocoa Rivers showed concentrations lower than those established by EPA (1 mg L^{-1}); however, Meta and Upía Rivers presented concentrations well above the benchmark. Conductivity, hardness, color, nitrate, nitrite, dissolved oxygen and pH were within the permissible ranges for human consumption water (EPA 2018). Total and fecal coliform microorganisms presented the highest concentration in Meta, Ocoa and Upía tributaries. In particular, the presence of these bacteria was observed in water from the deep well of the fish station (IALL), this can demonstrate the contamination of aquifers with groundwater. Iron and manganese are rarely present in water and were detected in the Meta, Ocoa and Upía Rivers at concentrations higher than those recommended to avoid adverse effects in humans and animals (0.3 mg iron L^{-1} and 0.05 mg manganese L^{-1}) (Boyd 2015). It is of considerable importance to highlight, that in aquatic ecosystems with a tendency to acid pH, heavy metals can become toxic, with risk upon the life of the aquatic organisms that inhabit these water sources (Boyd 2015). Colombian Orinoquía Rivers, whose white and clear waters can vary according to the physical and chemical characteristics of the soil with which they interact, may have pH that oscillates between slightly acid and neutral (Lasso et al 2014), being fragile ecosystems for effect of heavy metal concentrations.

The hydrologic pattern in the Orinoquía River Basin, marks one seasons of high water in the year, since April to June principally, and seasons of low water mainly during December, January and February (IDEAM 1981-2010). These natural episodes of fluctuation in affluent flows of the Meta River Basin, could magnify the effect of contaminants presents during low water months, generating adverse effects in various physiological parameters of species that inhabit the basin.

Many teleost fishes have external fecundation, which causes female and male gametes to be exposed to the aquatic environment, being vulnerable to environmental variables, such as pH, the concentration of dissolved oxygen, among others, including polluting agents (Lahnsteiner et al 2004). Environmental pollutants such as cadmium, copper, mercury, nickel, nitrite, lead, zinc, cyclohexane and dichlorophenol in different concentrations, can generate alterations in sperm function, which can be altered differentially depending on the sensitivity of the species (Lahnsteiner et al 2004). Some of the alterations that sperm can suffer include damage to the plasma membrane, the alteration of energy metabolism, the suppression of motility and DNA damage (Hatef et al 2013).

Melo-Maciel et al (2015) report the percentage of sperm motility in relation to the activation time in Characids, finding very similar values among the study species, which remain stable up to 30 seconds after their activation (70.69-96.17%); after 60 seconds of activation, significant differences in motility was observed. The sperm motility reported in the present study for *P. brachypomus, B. amazonicus* and *P. mariae* showed values similar to those reported by Melo-Maciel et al (2015). The activation of sperm leads to a decrease in the concentration of adenosine triphosphate (ATP), resulting in a decrease in its speed and motility (Alavi & Cosson 2006). In the natural ecosystem, the interaction of sperm with water during the reproduction process is determinant of sperm motility; alterations in the ionic composition and osmolarity of the seminal plasma, environmental factors such as temperature, osmotic pressure, pH, among others, can affect this biological phenomenon (Melo-Maciel et al 2015).

The percentage of sperm motility and activation time of *P. brachypomus* activated with water from different sources of the Meta river basin reported in the present study, varied between 70-83% and 57-71 seconds respectively and did not present significant differences between treatments. Other studies reported a slightly higher percentage of motility (91-92%) and activation time (72-100 sec) (Fresneda et al 2004). Melo-Maciel et

al (2015) reported motility percentages closer to those reported in the present study (70-82%) during a 30-second activation time in *P. brachypomus*.

B. amazonicus showed activation time and percentage of motility for the different sampling sites in ranges between 45-52 sec and 70-80% respectively, without finding significant differences between sampling sites. Similar values were reported by Cruz-Casallas et al (2007) (activation time 35-41 sec. and motility percentage 85-90%).

Finally, *P. mariae* showed activation times and sperm motility very similar to those of the two characid species previously mentioned. Given the insufficient reporting of of reproductive parameters in characid species, the present study provides relevant information about the sperm characteristics of *P. brachypomus*, *B. amazonicus* and *P. mariae* from Colombian Orinoquia.

Although the Colombian Orinoquia is a region of great biodiversity and water richness, factors such as human intervention, accumulation of pollutants and global warming, can exert a significant negative effect on the species stocks of the region. It is advisable to follow the conditions of these ecosystems including a greater number of physical, chemical, macro and microbiological parameters to have a clearer picture of the state of Colombian Orinoquia Rivers.

Conclusions. It was found that the concentration of iron, mercury, lead, manganese, fecal and total coliforms showed higher ranges than those established in different water quality guidelines and reports principally in Ocoa, Meta and Upía Rivers. The water of the Upía River was the one where higher concentration of heavy metals and microbiological parameters were measured, demonstrating an important risk of pollution. Interaction of factors can be decisive in the effect that fish and other organisms that inhabit the Upía River can suffer, since the decrease in the water level and dissolved oxygen, high temperatures and increase in pollutant concentration may cause adverse effects on the ecosystem biota. It is possible that rainy season increases the water level in the Orinoquia rivers and favors reproductive period of fish species of the region, decreasing or dissolving concentrations of substances that may have an influence on the reproduction of fishes. Although no significant differences were found in activation time and sperm motility between characid species *P. brachypomus, B. amazonicus* and *P. mariae*, It is advisable the monitoring of the natural populations affecting fish and implement actions in the management of chemical substances, pesticides and agrochemicals, residues of hydrocarbons and wastewater, avoiding detrimental effects on species with greater sensitivity to pollutant substances.

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