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The use of biomarkers for assessing effects of pollutant stress on fish species from a tropical river in Southeastern Brazil

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ABSTRACT. Biomarkers are measurements within an organism that respond to environmental effects and are used as tools in bioassessment programs since they reflect physiological changes induced by exposure to pollutants. The hepatosomatic index (HSI) and the condition factor (CF) in three fish species *Geophagus brasiliensis, Hypostomus affinis* and *Hypostomus auroguttatus* were used to assess effects of pollutant stress in a tropical river during two seasons (dry and wet). Fish from the least disturbed sites had the lowest condition factor (CF) and the lowest HSI, whereas those from sites near pollutant discharges had the highest CF and HSI. Higher HSI and lower CF occurred during the dry season. It is suggested that species adapted to stressful conditions take advantage on food availability from organic loads or unoccupied niches by lesser tolerant species, increasing CF. We concluded that higher HSI values were directly associated with environmental stress whereas the higher CF values are related to availability of food resources derived from organic loads or other sources. CF and HSI are simple and cost-effective, thus suitable as routine screening tools in pollution monitoring, but caution should be taken in relating changes in physiological measurements and any specific pollutant to prevent misinterpretation.

Keywords: condition factor, hepatosomatic index, fishes, monitoring, rivers.

O uso de biomarcadores para avaliar efeitos de estresses de poluentes sobre espécies de peixes em um rio tropical no sudeste do Brasil

RESUMO. Biomarcadores são medidas dentro de um organismo que respondem a efeitos ambientais e são utilizadas em programas de bioavaliação, já que refletem alterações fisiológicas induzidas pela exposição a poluentes. O índice hepatossomático (IHS) e o fator de condição (FC) das espécies *Geophagus brasiliensis, Hypostomus affinis* e *Hypostomus auroguttatus* foram calculados em um rio tropical durante duas estações (seca e cheia). Peixes de locais menos alterados apresentaram menores FC e IHS, enquanto os de locais próximos de descargas de poluentes apresentaram maiores FC e IHS. Maiores IHS e menores FC ocorreram durante a estação seca, comparados com a estação chuvosa. É sugerido que espécies adaptadas às condições estressantes levam vantagem da disponibilidade de alimentos próxima às descargas orgânicas ou nichos não ocupados por espécies menos tolerantes, aumentado o FC. Os valores mais elevados do IHS foram diretamente associados ao estresse ambiental, enquanto os valores mais elevados FC estão relacionados com a disponibilidade de recursos alimentares derivados de descargas orgânicas ou outras fontes. FC e IHS são simples e de baixo custo, portanto, adequados como ferramentas no controle da poluição, mas cuidados devem ser tomados ao relacionar suas mudanças às medições fisiológicas ou a qualquer poluente específico para evitar efeitos de interferência.

Palavras-chave: fator de condição, índice hepatossomático, peixes, monitoramento, rios.

Introduction

Fish are particularly sensitive to environmental contamination of waters and, therefore, pollutants may significantly interfere with several of their biochemical processes (Ribeiro et al., 2013). Biomarkers measured in different organs of an organism at the individual, population, or other higher levels can represent responses to environmental effects and have been used as tools in bioassessment programs since they reflect physiological changes induced by exposure to pollutants (Adams, Crumby, Greeley, Shugart, & Saylor, 1992; Barton, Morgan, & Vijayan, 2002; Bervoets et al., 2009). Biomarkers at the level of organs, namely the hepatosomatic index (HSI) are frequently related to exposure to contaminants (Goede & Barton, 1990; Viarengo et al., 2000; Khan, 2010; Ribeiro et al., 2013). The HSI is expressed as the relative weight of the liver as percent of the total body mass and is supposed to increase under stress conditions (Facey et al., 1999; Liebel, Tomotake, & Ribeiro, 2013). The HSI is one of indices most often associated with exposure to contaminants (Grady, Mclaughlin, Caldwell, Schmitt, & Stalling, 1992; Hismayasari, Marhendra, Rahayu, Saidin & Supriyadi, 2015). Likewise, the liver is a primary detoxification organ in fish (Hinton & Laurén, 1990; Liebel et al. 2013) and an important target organ related to important metabolic mechanisms. According to Casarett, Amdur, Klaassen, and Doull (1991), the liver is the main organ related to biotransformation process of xenobiotics, by increasing in size (hypertrophy), increasing the number of hepatocytes (hyperplasia) or both in polluted areas (Goede & Barton, 1990; Hinton & Lauren; 1990; Sudarshan & Kulkarni, 2013).

The condition factor (CF) is another organism-level response to variables such as nutritional status, pathogen effects, and exposure to toxic chemicals causing greater-than-normal or less-than-normal weights. The condition factor shows the degree of well-being of the fish in their habitat is expressed by 'coefficient of condition' also known as length – weight factor. When condition factor value is higher it means that the fish has attained a better condition. The condition factor of fish can be affected by a number of factors such as stress, season and availability of food (Nehemia, Maganira, & Rumisha, 2012). Condition factor is also a biomarker at the individual level (Le Cren, 1951) being frequently used to assess the physiological condition, based on the principle that individuals exhibit higher weight when they have better condition associated with food resources in a given area (De Silva, Gunasekera, Austin & Graeme, 1998; Vila-Gispert & Moreno-Amich, 2001; Chakrabarti & Kundu, 2015). It is assumed that heavier fish for a given length is in better condition and able to indicate fish fitness under metabolic stress caused by pollution; as trade-off it is required deal to detoxification and the energy available for growth may thus be reduced. CF in fish is known to decline upon exposure to heavy metal (Roussel et al., 2007; Bervoets et al., 2009), polycyclic aromatic hydrocarbon (PAHs) and polychlorinated biphenyls (PCBs) (Khan, 2003). Furthermore, condition factor can vary seasonally, being directly associated with food availability (Le Cren, 1951; Bolger & Connolly, 1989; Braga & Andrade, 2005; Sudarshan & Kulkarni, 2013).

The CF furnishes information related to fish physiological status (Lima-Junior, Cardone & Goitein, 2002). A fish that is heavier for a given length (higher CF) is considered to be a healthier fish, because extra weight means extra energy reserve, whereas a lighter fish lack energy reserves and therefore, tend to be more susceptible to environmental stressors. A low body condition may also suggest muscle wasting (proteolysis) indicating a starvation response (Barton et al., 2002; Alam et al., 2013). Increase in both CF and HSI can signal deleterious effects from a given stressor although most interpretation of the CF indicates that a relative high weight for a given length is an indication of health condition for the individual. Moreover, the presence of few larger and more robust individuals can be an indication of abnormal condition (Wege & Anderson, 1978; Lehtonen & Jokikokko, 1995). Conversely, the highest CF in species at disturbed sites has also been associated with chronic exposure (Gibbons, Munkittrick, & Taylor, 1998; Galloway et al., 2003; Khan, 2006; Hamid, Mansor, & Nor, 2015).

The Paraíba do Sul river supplies 90% of water to the city of Rio de Janeiro and metropolitan area. It has a long history of pollution and human interference that last for more than 400 years. The river watershed is composed of large urban areas, pasture, sugar cane, steel and textile, metallurgic, cellulose, petrochemical industries (Carvalho & Torres, 2002; Linde-Arias, Inacio, Novo, Albuquerque, & Moreira, 2008; Parente et al., 2015). Furthermore, rice crops and other agricultural activities that require pesticides are taking place in flooding areas of the medium course of the river. Despite the high load of pollution, the river basin still has some protected areas nearby the headwaters and some tributaries drain comparatively well preserved areas. Rainfall is a main force changing river flow and consequently water quality, by carrying into the river pollutants and organic inputs from the catchment area during the wet season. On the other hand, during the dry season, the river has comparatively lower dilution capacity compared to the wet season.

The aim of this study was to test two unspecific physiological biomarkers (hepatosomatic index - HSI and condition factor – CF) in three fish species, *Geophagus brasiliensis* (Quoy & Gaimard, 1824), *Hypostomus affinis* (Steindachner, 1876) and *Hypostomus auroguttatus* (Steindachner, 1876) to assess eventual influence of diffuse source of pollutants at 10 sites throughout the Paraíba do Sul river basin in two seasons (summer/wet and winter/dry). The tested hypothesis is that sites near large urban-industrial and agricultural effluents discharges (diffuse pollution) are prone to present more xenobiotics and their presence is indicated by the high values of HSI compared with least disturbed sites. We also expect that polluted sites have opportunistic fish with high CF, especially during the wet season when most organic loads are carried into the river.

Material and methods

The Paraíba do Sul river basin (Figure 1) is 1150 km long and encompasses the three most industrial and urban developed states of Brazil (São Paulo, Rio de Janeiro and Minas Gerais). Its headwaters are located in Bocaina Mountains, at 1800 m altitude and the estuary is in the Atlantic Ocean to the north of the State of Rio de Janeiro. The basin is situated between the coordinates 20°26' and 23°39'S, and 41° and 46°30'W, covering an area of 55.500 km^2.

Ten sites were selected along the river length (Figure 1): (1) Paraitinga town (latitude 23°9′02″S; longitude 45°28′07″W), a least disturbed site at the headwaters in Bocaina mountains); (2) São José dos Campos (23°09′28″S; 45°53′10″W), an altered site by the presence of a huge industrial and urban city with 530,000 people; (3) Barra Mansa/Volta Redonda (22°29′23″S; 44°05′13″W), probably the most altered site (Pinto, Peixoto, & Araujo, 2006) due to the presence of a huge steel factory, the National Steel Company; (4) Preto river (22°05′02″S; 43°33′35″W), a least disturbed site in a well-preserved tributary); (5) Paraibuna river (22°01′46″S; 43°12′25″W), an intermediate altered site; (6) Piabanha river (22°08′05″S; 43°09′37″W), an altered site that receives large organic loads from cities with poor or no treatment of discharges; (7) Três Rios/Sapucaia/Anta (22°02′13″S; 42°59′37″W), an intermediate altered site; (8) Grande river (21°36′17″S; 41°47′38″W), an intermediate altered site; (9) Muriaé river (21°37′06″S; 41°24′57″W), an intermediate altered site; and (10) Campos/São João da Barra (21°42′11″S; 41°09′25″W), an altered site due to the presence of a city and intense agricultural (sugar cane) activities.

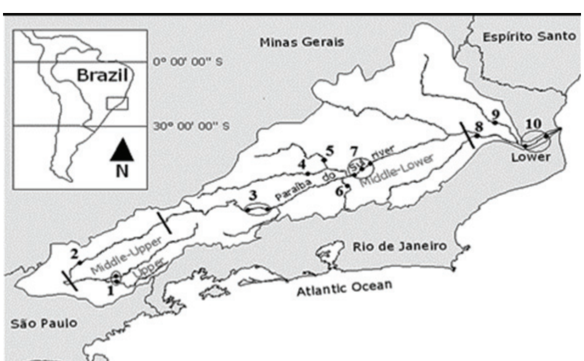

Figure 1. The Paraíba do Sul river basin with indication of the 10 sampling sites. Fish from encircled sites were pooled. Sampling sites: 1, Paraitinga; 2, São José dos Campos; 3, Barra Mansa/Volta Redonda; 4, Preto river; 5, Paraibuna river; 6, Piabanha river; 7, Três rios/Sapucaia/Anta; 8, Grande river; 9, Muriaé river; 10, Campos/São João da Barra.

Fish were collected in two seasons: summer 2002 (wet season) and winter 2003 (dry season) using gill nets and cast nets in a standardized effort. Three well-adapted species native to the Paraiba do Sul river basin (Araújo, Fichberg, Pinto, & Peixoto, 2003; Pinto, Peixoto, & Araújo, 2006) were selected: a cichlid, *G. brasiliensis* and two silurids, *H. affinis* and *H. auroguttatus,* ranging from 130 to 370 mm Total Length (TL). All fish were adults to minimize physiological variation during early growth period. Immediately after collection, fish were anesthetized in ice, and the total length was measured. Then, fish were dissected to remove the liver, which was weighed to 0.01 g accuracy. We also weighed the eviscerated fish to calculate the condition factor. The number of each examined fish species per season and sites is shown in Table 1. Voucher specimens were deposited in the fish collection of the Laboratory of Fish Ecology, *Universidade Federal Rural do Rio de Janeiro* under number: LEP-UFRRJ#533, 585, 850, 851, 852.

The hepatosomatic index (HSI) was calculated as the percentage of the liver weight related to eviscerated body weight, according with the following equation: HSI = $(W_l \times W_t^{-1}) \times 100$, where W_l, liver weight (g) and W_t, total eviscerated weight (g).

The Fulton condition factor (CF) was expressed by the equation: CF = $(W_t/L_t^3) \times 100$ where W, total eviscerated body weight (g) and L^3, total length (mm). A Permutational Analysis of Variance (PERMANOVA) was applied to compare both HSI and CF (response variables) between the sites and seasons (fixed factors) at 95% confidence level ($p < 0.05$) and to assess interactions between sites and seasons. When we found significant interactions, we compared sites separately for each season (dry and wet).

Results

Hepatosomatic Index

Significant differences in HSI for *Geophagus brasiliensis* were detected between sites, but not between seasons, according to PERMANOVA. Moreover, significant sites vs. seasons interactions were also found. The highest HSI was found at site 3, Volta Redonda/Barra Mansa (2.2±0.9) compared with the other sites during the wet season. Further, significant differences were also detected for the highest HSI values between site 3 (disturbed), 9 (intermediate disturbed) and 10 (disturbed) compared with those in sites 1 (least disturbed) and 8 (intermediate disturbed) in the dry season (Table 2).

Hypostomus affinis had significantly higher HIS values at site 2 (disturbed site) (1.5±0.2), compared with the remaining sites, with the lowest values recorded at sites 5 (intermediate disturbed) and 10 (disturbed site), in both seasons (Table 2). This species had higher HSI values in the dry season compared with the wet season.

Table 1. Number of examined fish species per season and sites. Ha, *Hypostomus affinis;* Hau, *Hypostomus auroguttatus;* and Gb, *Geophagus brasiliensis*.

Sites	Wet			Dry		
	Ha	Hau	Gb	Ha	Hau	Gb
1	4	-	10	10	-	18
2	-	-	3	4	-	-
3	4	8	21	13	-	15
4	-	8	-	-	6	10
5	10	7	-	-	7	14
6	7	-	12	13	-	-
7	-	-	-	2	7	17
8	-	-	6	-	-	7
9	-	-	6	-	-	10
10	7	-	6	7	-	7
Total	25	23	64	49	20	98

Table 2. Results of PERMANOVA testing for differences in fish species Hepatosomatic Index, in response to sites and seasons (fixed factors) and interaction effects. Significant differences also shown for site *versus* season interaction. Means ± SD in brackets. MS, mean square; df, degree of freedom.

Source	df	MS	Pseudo-F	P(perm)	Significant differences
Geophagus brasiliensis					
Site	4	4510.7	11.6	0.001	Wet: 3 > 1 > 8; 9; 10;
Season	1	959.6	2.5	0.103	(2.2±0.9) (1.4±0.7) (0.6±0.1–1.0±0.2)
Site *vs*. Season	4	2043.9	5.2	0.001	Dry: 3, 9, 10 > 1, 8
Residual	105	390.1			(1.2±0.4 –1.7±0.9) (0.9±0.4–1.0±0.4)
Total	114				
Hypostomus afinnis					
Sites	6	882.4	3.7	0.002	Sites: 2; > 1; 3; 6; 7; > 5; 10;
Season	1	2549.9	10.6	0.	(1.5±0.2) (0.9±0.2 –1.2±0.1) (0.6±0.1–0.7±0.1)
Site *vs*. Season	1	444.07	1.8	0.165	Seasons: Dry > Wet
Residual	63	241.34			(1.3±0.2) (0.8±0.3)
Total	71				
Hypostomus auroguttatus					
Site	3	644.1	3.6	0.008	Sites: 4; 7; > 3; 5;
Season	1	4797.5	27.0	0.001	(0.7±0.1 – 0.9±0.2) (0.5±0.2 – 0.6±0.1)
Site *vs*. Season	1	369.1	2.1	0.125	Seasons: Dry > Wet
Residual	42	177.9			(1.1±0.2) (0.6±0.1)
Total	47				

Hypostomus auroguttatus similarly to *H. affinis*, also changed HSI between sites and between seasons. The highest HSI in both seasons was recorded at sites 4 (least disturbed) and 7 (intermediate disturbed) and the lowest at sites 3 (disturbed) and 5 (intermediate disturbed). Seasonally, the highest HSI was recorded in the dry season (Table 2).

Condition factor

Geophagus brasiliensis had significant higher CF values at sites 3, 8, 9 and 10 compared with site 1 (headwaters, least disturbed site) during both the wet and dry seasons. No significant differences in CF were detected between the two seasons (Table 3).

For *H. affinis* no significant differences in CF was found between sites or between seasons. On the other hand, *H. auroguttatus* has significant differences in CF between sites and between seasons and no significant interaction site vs. season. The highest CF was found at sites 4, 5 and 7 compared with site 3 (disturbed site). Seasonally, higher values of CF were found during the wet season (Table 3).

Discussion

The highest HSI values were consistently recorded in fish from sites under diffuse pollution, namely those located near large urban-industrial plants. The site 3 is located nearby the largest South American Steel plant, the Brazilian National Siderurgic Plant (BNSP), where a large amount of pollutants are discharged into the river (Carvalho & Torres, 2002). According to Pfeiffer, Fiszman, Malm, and Azcue (1986), the highest level of pollution in the entire Paraíba do Sul river basin is located upstream site 3. Large amounts of coal have been used since the start of operation of the BNSP in 1942. It is well known that coal combustion generates a complex mixture of organ chloride pollutants particularly rich in heavy PAHs (Edwards, 1983). The BNSP discharges about 55 tons of effluents per day, comprising the largest pollutant load introduced in the Paraiba do Sul river (Gruben, Lopes, & Johnsson, 2002).

The site 6 also receives large organic loads mainly from textiles and brewery industries in the municipality of Petropolis. Comparatively higher HSI was found in this site for *G. brasiliensis* during the dry season. Two small hydropower plants damming the Piabanha river can also contribute to increased pollution in this site by polychlorinated biphenyls (PCBs), which are used in closed systems like electric transformers as a dielectric fluid.

Table 3. Results of PERMANOVA testing for differences in fish species Condition Factor, in response to sites and seasons (fixed factors) and interaction effects. Significant differences also shown for site *versus* season interaction. Means ± SD in brackets.

Source	df	MS	Pseudo-F	P(perm)	Significant differences
Geophagus brasiliensis					
Site	4	873.7	20.8	0.001	Sites: Wet & Dry: 3; 8; 9; 10; > 1
Season	1	109.4	2.6	0.113	(1.8±0.2–2.2±0.2) (1.5±0.1)
Site *vs*. Season	4	96.7	2.3	0.063	
Residual	105	42.0			
Total	114				
Hypostomus afinnis					
Site	6	104.7	1.6	0.146	No significant differences
Season	1	12.7	0.2	0.700	
Site *vs*. Season	1	11.2	0.2	0.718	
Residual	63	66.0			
Total	71				
Hypostomus auroguttatus					
Site	3	340.9	6.4	0.002	Sites: 4; 5; 7; > 3
Season	1	446.9	8.4	0.007	(1.2±0.2–1.3±0.1) (1.0±0.1)
Site *vs*. Season	1	40.6	0.8	0.436	Seasons: Wet > Dry
Residual	42	53.5			(1.3±0.1) (1.1±0.1)
Total	47				

Although their release to the environment is not frequent, accidents involving leakage and direct contamination of surface water and groundwater, especially near electrical facilities and large foundries, do occur (Carvalho & Torres, 2002). Indications of large amounts of xenobiotics in the area were registered by Parente et al. (2015) by using the biomarker ethoxyresorufin-*O*-deethylase (EROD) activity in *G. brasiliensis*. The site 2 is another altered site located nearby the greatest industrial city in the Paraiba do Sul watershed, the municipality of São José dos Campos, with high industrial and agricultural developments that discharge large pollutant loads into the river. The highest HSI detected in *H. affinis* at site 2 is likely to be associated with such pollutants.

The values of HSI as indicator of environmental stress have been observed elsewhere. Wilhelm-Filho, Torres, Tribess, Pedrosa, and Soares, (2001) found increased HSI values in polluted sites compared to least disturbed sites using *G. brasiliensis* as biomarker. Facey, Blazer, Gasper, and Turcotte (2005) compared HSI values in rock bass, *Ambloplites rupestris* (Rafinesque, 1817) before and after improvements in sewage treatment and found significantly ($p < 0.05$) lower values after the treatment, suggesting that physiological stress in fish population decreased with reduction in exposure to pollutants. Carrola, Fontaínhas-Fernandes, Matosm, and Rocha (2009) studying a histopathology in brown trout, *Salmo trutta* Linnaeus, 1758 from the Tinhela river, in Portugal, found highest HSI in fish collected at polluted sites compared to a reference site. Khan (2010), working with flatfish *Pleuronectes americanus* (Walbaum, 1792), and plaice, *Hippoglossoides platessoides* (Fabricius, 1780), also found higher HSI in more disturbed sites. However, departure from this pattern can be found, as the case of Sheahan et al. (2002), who reported the highest HSI at references sites compared with sites influenced by sewage treatment in the Aire river, UK, by using rainbow trout, *Oncohynchus mykiss* (Walbaum, 1792). Fang et al. (2009), studying the use of physiological indices in the rabbitfish *Siganus oramin* (Bloch & Schneider, 1801) in the Victoria Harbour, coast of Hong Kong, found that spatial changes in CF and HSI were generally in accordance with the integrated pollution pattern in the harbour, and seemed more specific to the effects of heavy metals.

Overall, the highest HSI values were verified in the dry season for *Hypotomus* species, which suggest a consistent seasonal trend in HSI values. Because the Paraiba do Sul river drains a highly industrialized and populated region and receives continuously organic and industrial pollutant loads, it is expected a lower capacity for pollutant dilution in the dry season, when the river water level reaches its lowest levels. This is likely to be associated with the increases in HSI in the winter (dry season) when rainfall is at the lowest levels (Carvalho & Torres, 2002). Scherer and Strohschoen (2013) using Comet assay, frequency of micronuclei and nuclear abnormality in erythrocytes of tilapia *Oreochromis niloticus* (Linnaeus, 1758), a cichlid species, for assessing pollution in the Paraíba do Sul river near site 2 found indication of higher pollutants concentration in the dry season.

CF seems to be inversely associated with water quality in this study, since the lowest values were recorded in least disturbed sites, mainly site 1, whereas site 3 which, have an opposite condition, had the highest CF values, as indicated for *G. brasiliensis*. According to Tyler and Dunn (1976), CF varies directly with nutrient availability. Condition Factor may vary in either direction outside the normal range in response to exposure to chemicals. High CF has been found for white sucker, *Catostomus commersoni* (Lacepède, 1803) and redbreast sunfish, *Lepomis auritus* (Linnaeus, 1758) in sites polluted with pulp mill effluents (McMaster et al., 1991; Adams et al., 1992). Rypel and Bayne (2010) evaluated growth rates of six fish species correlated with PCB concentrations in a moderately-to-heavily polluted freshwater ecosystem, and concluded that at the range of concentrations investigated, these PCBs do not exert negative impacts on growth.

Conversely, decreased CF has been recorded in several disturbed sites, such as, for the Atlantic cod, *Gadus morhua* Linnaeus, 1758 exposed to petroleum (Kiceniuk & Khan, 1987), for white sucker (*C. commersoni*) in sites with high concentration of metals Cu and Zn (Munkittrick & Dixon, 1988; Miller, Munkittrick & Dixon,1992). In accordance with Liebel et al. (2013), CF is an indicative of the health status and may be used to assess field contamination. Alterations in growth rates are induced by contaminants and the explanation is the reallocation of energy to detoxification mechanisms, depleting reserves that were originally destined for growth. Linde-Arias et al. (2008) reported that significant higher CF values were verified in reference sites than in other areas, with evidences of less environmental stress in fish from the reference site. Additionally, fish inhabiting chronically contaminated habitats can develop resistance to pollutant (Elskus, Monosson, Mcelroy, Stegman, & Woltering, 1999; Nacci et al., 1999; Brammell, Price, Birge, & Elskus, 2004). Our finding seems to point to an increased CF with increased pollution, which, in turn can also have increased organic matter and other nutrients that increase food availability that is used by tolerant and opportunistic species. Such species, including *G. brasiliensis*, take advantage of this situation in polluted sites of the Paraiba do Sul river and increase its condition in areas where others species cannot tolerate.

Additionally, CF varies seasonally associated with food and nutrient availability as reported by Jenkins (2004). According to Teixeira et al. (2005), the highest rainfall during the wet season carry larger amounts of allochthonous material, increasing food availability in areas under strong human disturbance. Alam et al. (2013) studying freshwater fish from Bangladesh reported that fish showed maximum robustness during the summer (rainy season) due to availability of food and suitable environmental condition, which confirm our findings.

Both CF and HSI increased in polluted sites, but seasonally there was an inverse situation, with comparatively higher CF in the wet season and higher HSI in the dry season. A decrease in condition factor (CF), hepatosomatic index (HSI), or both indicate depletion in energy reserves because these indices are positively related to total muscle and liver energy content (Lambert & Dutil, 1997). There is then a logical relationship between this depletion of energy reserves and potential health problems in fish. On the other hand, an increase in liver size is directly associated with pollutant biotransformation processes when exposed to stress. Our results corroborate the hypothesis that an increased in both HSI and CF signalizes effects of stressors as reported by Wege and Anderson (1978) and Lehtonen and Jokikokko (1995). The most impacted sites had the highest HSI and CF, with the HSI being a better indicator of pollution, while the CF was more influenced by food availability, which used to be higher in polluted sites. Despite CF and HSI being simple and cost-effective, thus suitable as routine screening tools in pollution monitoring, caution should be taken in relating changes in physiological measurements and any specific pollutant in water quality monitoring studies to prevent misinterpretation.

Conclusion

The biomarkers HSI and CF indicated effects of pollutant stress along the Paraiba do Sul river. Higher HSI values were detected near large discharges of organic or industrial effluents such as the industrial steel plant of Barra Mansa-Volta Redonda or sites with large organic loads. Low HSI values were found in more preserved sites. CF was higher in sites altered by organic loads, where tolerant species take advantage of food availability. Low values of both indices suggest acceptable conditions. Higher HSI and lower CF occurred in the dry season. CF and HSI are simple and cost-effective, thus suitable as screening tools in pollution management.

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