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## **Brain acetylcholinesterase as a marine pesticide biomarker using Brazilian fishes**

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## Abstract

Brain acetylcholinesterase (AChE) of some fishes from the coast of Rio de Janeiro state was studied as a possible pesticide biomarker in marine environmental monitoring. AChE specific activity in brain varied from 145 to 530 U/g of proteins and the Michaelis-Menten constant ( $K_M$ ) for acetylthiocholine varied from 104 to 291  $\mu\text{M}$  among the twenty species studied. The enzyme sensitivity to methyl paraoxon, evaluated by the inhibition kinetic constants, shows that some species (*Paralichthys brasiliensis* and *Genidens genidens*) are more sensitive ( $\text{IC}_{50-30\text{min}} = 455$  and  $468$  nM, respectively). The less sensitive *Merluccius hubbsi* and *Percophis brasiliensis* ( $\text{IC}_{50-30\text{min}} = 3,339$  and  $3,259$  nM, respectively) belong to the super-order Paracanthopterygii, which includes the more ancient species. On the other hand, more susceptible species belong to the super-order Acanthopterygii, which includes more recent species. These results suggest a possible evolutionary linkage for AChE sensitivity to methyl paraoxon. The application of inhibition kinetic constants for fish brain AChE in phylogenetic studies is still being investigated. The results have shown that a fish sentinel species should have the highest brain AChE level among the more sensitive ones.

**Keywords:** acetylcholinesterase, organophosphate, pollutant, biomarker.

## 1. Introduction

Pesticides are used in agriculture to control insects and other pests, which contaminates rivers and, eventually, the sea. In addition to traditional chemical analysis, activity of the enzyme acetylcholinesterase (AChE) has been used for environmental monitoring programs (Burgeot, Bocquéné, Porte, Dimet, Santella, Garcia de la Parra et al., 1996; Huang, Obih, Jaiswal, Hartley, & Thiyagarajah (1997); Minier, Levy, Rabel, Bocquéné, Godefroy, Burgeot et al., 2000) because AChE inhibition in tissues of fish and other animals can be used as biomarker for organophosphorous and carbamates exposure (Escartin & Porte, 1996; Sturn, Silva de Assis, & Hansen, 1999). Inhibition of AChE is not necessarily fatal, but using a fish with a highly sensitive AChE as a sentinel species will allow the detection of lower contamination levels.

The proper use of enzymatic biomarkers requires a fundamental understanding of its biochemistry for the species to be utilized. Vertebrate cholinesterases have been proposed to exist alternatively as globular forms or asymmetric forms associated with a collagen-containing tail (Massoulié, Pezzementi, Bon, Krejci, & Vallette, 1993). Soluble globular forms may be nonamphiphilic or amphiphilic (Bon, Toutant, Méflah, & Massoulié, 1988). Because asymmetric forms of vertebrate cholinesterases require high-salt buffers for solubilization, whereas amphiphilic forms require detergent for solubilization, inaccurate measurements of ChE activity may result from the inability to consistently and thoroughly extract the enzyme from tissue samples.

In addition, it is important to consider how environmental history and seasonal conditions affect the physiology of the sentinel species (Monserrat, Bianchini & Bainy, 2002; Rodriguez-Fuentes & Gold-Bouchot, 2004).

The present work is part of a marine environmental monitoring program (MOMAM) undertaken by the Instituto de Estudos do Mar Almirante Paulo Moreira (IEAPM) of the Brazilian Navy. The aim of this work was to assess the potential of brain AChE of some coastal marine fish in Rio de Janeiro State as a biomarker for organophosphates and carbamates.

## 2. Materials and Methods

The oceanographic studies were carried out along the coast of Rio de Janeiro State, in Southeastern Brazil, as shown in Table 1 and Figure 1.

Seawater samples were collected with a Nansen bottle and assayed for dissolved oxygen, nitrate, ammonia and phosphate according to Strickland & Parsons (1972). The salinity was measured with a density-meter model RS-23C (Beckman). The temperatures were measured with thermometers on the Nansen bottle.

The ocean specimen captures were carried out using an otter-trawl in an IEAPM boat, dragging for 20 min at 3 knots. Fish from the Sepetiba Bay were caught by fishermen with rod, line and hook. The animals were identified according to Figueiredo & Menezes (1980) and were sacrificed *in situ*. Their brains were removed, rapidly bathed in a cold 0.9% NaCl solution and wrapped in parafilm. Each small bag was thrown in liquid nitrogen and then kept frozen at -20 °C in a freezer until homogenization. After being defrosted, brains were mopped up quickly with filter paper, weighed, finely chopped and suspended in a phosphate buffer (10 mL of sodium phosphate buffer 0.1 M, pH 7.5, per gram of wet tissue) for homogenization (30 strokes using a Potter-Elvehjem equipment) at 5 °C.

The brain AChE activity was measured in the homogenates at room temperature (25-28 °C) according to Ellman, Courtney, Andres, & Featherstone (1961) with some modifications. The final concentrations in the enzyme assay medium were: dithio-bis-nitrobenzoic acid (DTNB) 0.25 mM, TRITON X-100 0.1% and substrate 1 mM in phosphate buffer 0.1 mM, pH 7.5. The substrate was acetylthiocholine iodide (AceScol) and, when specified, replaced by propionyl (ProScol) or butyrylthiocholine iodides (ButScol) in the same concentration. The extinction coefficient of the reaction product (thionitrobenzoate ion) was  $14,150 \text{ M}^{-1}\text{cm}^{-1}$  in the spectrophotometer UV-160A (Shimadzu) at 412 nm. The activities are expressed in Units (1U = 1  $\mu\text{mol}$  of product formed per min) per gram of wet tissue or per gram of total proteins (specific activity). Five replicated assays carried out with each brain homogenate produced a coefficient of

variation lower than 5%. The total proteins were determined as described by Peterson (1977), with no precipitation step, using bovine serum albumin as standard.

The soluble AChE was determined in supernatants obtained by diluting the homogenates with phosphate buffer to 500 U/mL and centrifuging (30,000 x g/30 min/4 °C). The activity of AChE remaining in the supernatant was considered as soluble AChE and expressed as percentage (%) of the total activity. The pellets of these centrifugations were suspended in phosphate buffer with 0.1% of TRITON X-100, homogenized as described and centrifuged (100,000 x g/90 min/4 °C). The supernatants were collected and pooled by species for Michaelis-Menten constant ( $K_M$ ) and IC50 determinations. The  $K_M$  was calculated with non-linear regressions (Graph Pad Software, Inc.) with acetylthiocholine from 0.1 to 2 mM. The IC50-30min for methyl paraoxon was evaluated by the inhibition kinetic constants according to Johnson & Wallace (1987) and Kemp & Wallace (1990).

Phylogenetic correlations of the brain AChE specific activity among the collected species were assessed by hierarchic cluster analysis (De Gheff, 1978). The non-parametric cluster analysis of brain AChE specific activity and the Mann-Whitney test (U test) with the differences among the groups were carried out with Statistica 5.1 (Statsoft Inc.).

### 3. Results

The properties of the seawater sampled are shown in Table 2, except for station F where no samples were collected. Overall, 133 fishes were collected and classified into 11 families and 20 species (Table 3). The level of cerebral AChE in these species varied from 145 to 530 U/g of protein. The final results were normalized to total protein to compensate for water loss during storage. The soluble AChE varied from 21 to 42% of total activity calculated in the homogenates (Table 4).

The IC50-30 min for methyl paraoxon varied from 455 to 3,339 nM and the Michaelis-Menten constant ( $K_M$ ) for acetylthiocholine varied from 104 to 291  $\mu$ M for some of the species collected. The best substrate for the insoluble cerebral AChE was acetylthiocholine. Using propionyl or butyrylthiocholine values of 13 to 39% and of 1 to

16%, respectively, were obtained in relation to the results for acetylthiocholine (P% and B% in Table 5).

Figure 2 shows the result of a cluster analysis of the collected species which were separated in two groups at the level of super-order.

#### 4. Discussion

Brain AChE specific activity was not influenced by the seawater physicochemical parameters. Variations of physicochemical parameters were very small and even in station B, where the greatest variations were observed, there was no correlation between the physicochemical parameters and AChE activity (Table 2). Similarly, Olson & Christensen (1980) studied the effect of many chemicals in muscle AChE activity in fathead minnow (*Pimephales promelas*) and found that nitrate above 0.01 M caused a slight inhibition of the enzyme while phosphate and ammonium had no effect, even in concentrations above 0,01 M. In the present work, the concentrations of nitrate, phosphate and ammonium did not exceed 0.01 M in any water sample analyzed.

However, brain AChE activity levels varied according to fish length. *Pagrus pagrus*, *Porichthys porosissimus* and *Percophis brasiliensis* showed the greatest correlation (Correlation Coefficient =  $-0.6333$ ,  $-0.7470$  and  $-0.80134$ , respectively), all negatives, indicating that older animals have less AChE in the brain. Reportedly, this ordinary variation in AChE activity relates to the biology and ecology of each species (Bocquené, Galgani, & Truquet, 1990). So, possible intraspecific variation in the brain AChE levels has to be evaluated to correctly interpret low results for brain AChE activity.

The variation among species, or interspecies AChE differences, might be ascribed to phylogeny according to Magnotti, Zaino, & McConnell (1994). The authors separated the species in two groups, one of high enzymatic activity and the other of low enzymatic activity. The group with the highest activities included families of the super-order Acanthopterygii. This super-order includes the more developed species. Our results were similar. A hierarchic cluster analysis (Figure 2) made with the brain AChE specific activities of the collected fish separated them into two groups: one with two species with

the lowest brain AChE specific activity which belong to super-order Paracanthopterygii and other including the rest of the samples. The species in this latter group, except *Genidens genidens*, belong to super-order Acanthopterygii, with the highest brain AChE levels. Another two species, with only one sample, were not included in this analysis (Table 3). The super-order Paracanthopterygii includes species of primitive descent, according to Nelson (1984). Our results confirm a possible linkage between the brain AChE activity levels and the phylogenetic origin of the fish.

The low soluble AChE activity found in brain of the fishes we studied (21 to 44%) support the hypothesis that the principal activity of this enzyme in brain, like in muscle, is particulate (Massoulié, Pezzementi, Bon, Krejci & Vallette, 1993). Our results showed that in the majority of the species studied a centrifugation step discarded up to 60 to 70% of the AChE activity (Table 4). Although several results were published using soluble AChE only (Bocquené et al, 1990; Magnotti et al, 1994; Boone & Chambers 1997; Sturn, da Silva de Assis, & Hansen, 1999; Flammarion, Noury & Garric, 2002; Varò, Navarro, Amat & Guilhermino, 2003), our findings reinforce the importance of using an AChE assay method without including a prior centrifugation step to discard pellets. So, in this report the brain AChE activity was assayed directly in the homogenates guaranteeing the detection of both enzyme activities (soluble and particulate).

The sensitivity of particulate brain AChE to methyl paraoxon varied from species to species (Table 5) and all brain AChE activities from the Neotropical fishes we studied were less sensitive to methyl paraoxon than AChE from other animals (Table 6). Moreover, less sensitive fish species, like *Merluccius hubsi* and *Percophis brasiliensis* (Table 5) exhibited low brain AChE levels (Table 4). The Correlation Coefficient between brain AChE sensitivity (IC50-30min) and brain AChE levels (AChE) for some species (Table 4) was -0.75423. These results indicate that by selecting a sentinel species with low AChE activity levels one might risk biomarker's efficiency by selecting a species also with less sensitivity to the toxic substances.

The amount of enzyme activity is the common parameter when brain AChE is used as biomarker to pesticide exposure. A 50% reduction in specific activity in regard to non-

exposed individuals suggests that these toxics are present in the environment (Burgeot, Bocquéné, Porte, Dimeet, Santella, Garcia de la Parra, Pfol-Leszkowicz, Raoux & Galgani, 1996; Kozlovskaya & Mayer, 1984). Bocquéné, Galgani, & Truquet (1990), working with several species of marine animals, suggested the utilization of the fish *Pleuronectes platessa* and the crustacean *Palaemon serratus* which, in comparing with others marine organisms, had the highest AChE specific activities. Besides, these species were the most sensitive organisms to the inhibitors, increasing the biomarker's efficiency.

For the Brazilian fishes studied, the sensibility to the pesticide was higher for enzymes with high  $K_M$  or with low affinity for the natural substrate. The efficiency of hydrolysis of substrates with crescent alkyl chain also demonstrated that the more sensitive cerebral AChE is less specific for the natural substrate, as shown by the high values for P% and B% and the high  $K_M$  for acetylthiocholine (Table 5). Just the inverse was observed by Kemp & Wallace (1990) for other animals. These authors suggested that the enzyme catalytic center should be more open in more sensitive animals, making substrate and inhibitor catalysis easier. They worked with widely different groups of animals: mammals, birds and fish, but our results, just for fish, do not encompass this latter broad grouping. These results indicate that the sensitivity to the pesticides and the specificity for the substrate can involve different mechanisms in different species. Temperature can influence the  $K_M$  and IC50 for the brain AChE in rat with little influence in changing the kinetic constants for this enzyme in channel catfish (Carr and Chambers, 1996), which substantiate the concept that the higher resistance of AChE to organophosphate pesticide in some fishes can involve sites other than those involved in natural substrate and pesticide catalysis.

## 5. Conclusion

In conclusion, the brain AChE levels and its sensitivity to organophosphorus in fishes of the Rio de Janeiro coast are quite variable. Further *in vitro* and *in vivo* studies on the properties of the brain AChE present in each fish species will make it easier to choose marine sentinel species as a biomarker. However, our results have shown that a sentinel Neotropical fish species should have the highest brain AChE level among the more

sensitive ones.

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**Table 1**

Specifications of oceanographic expeditions (letters) and littoral sampling sites (numbers) organized by expedition dates

Station	Date	City	Longitude - Latitude	Depth (m)
A1	06/17/97	Arraial do Cabo	22° 58.690' S - 042° 05.660' W	44.0
A2	06/17/97		23° 00.910' S - 042° 05.970' W	60.0
B1	06/30/97	Macaé	22° 21.769' S - 041° 43.630' W	7.5
B2	07/01/97		22° 24.642' S - 041° 38.103' W	20.0
B3	07/02/97		22° 26.273' S - 041° 46.680' W	31.0
B4	07/03/97		22° 26.145' S - 041° 40.000' W	30.0
C1	08/20/97	Arraial do Cabo	22° 58.260' S - 042° 03.560' W	30.0
C2	08/20/97		22° 58.690' S - 042° 05.660' W	45.0
C3	08/20/97		23° 00.910' S - 042° 05.970' W	60.0
D1	08/21/97	Cabo Frio	22° 55.103' S - 042° 00.642' W	30.0
D2	08/21/97		22° 56.738' S - 041° 58.523' W	45.0
D3	08/21/97		23° 00.102' S - 041° 53.091' W	60.0
E1	09/29/97	Guanabara Bay	23° 05.189' S - 043° 08.910' W	48.8
E2	09/30/97	Guaratiba	23° 07.012' S - 043° 32.310' W	45.0
E3	10/03/97	Ilha Grande	23° 08.160' S - 044° 23.480' W	29.0
F1	08/24/98	Sepetiba Bay	23° 00.000' S - 043° 45.000' W	6.0
F2	03/28/99		23° 00.000' S - 043° 45.000' W	6.0

The geographic position of the stations A-E were determined by GPS and station F was from a navigation map.

**Table 2**

Physical-chemical parameters of seawater in sampling sites

Stations *	Temperature °C	Salinity ppm	Oxygen mL/L	Nutrients (µM)		
				PO <sub>4</sub>	NO <sub>3</sub>	NH <sub>3</sub>
A1	15.64	35.66	4.58	0.63	6.82	1.02
A2	15.54	35.74	4.56	0.67	7.26	1.05
B1	22.50	36.16	6.30	0.46	0.07	1.17
B2	20.92	36.36	5.72	0.57	1.55	1.96
B3	21.86	36.22	5.89	0.34	0.38	0.86
B4	19.80	36.37	5.56	0.57	3.94	1.11
C1	15.88	35.62	4.32	0.70	8.22	0.89
C2	15.68	35.58	4.60	0.65	8.14	1.06
C3	15.54	35.56	4.20	0.65	8.39	1.10
D1	17.48	35.85	4.96	0.67	7.18	1.22
D2	17.60	35.85	4.78	0.64	6.46	1.53
D3	16.92	35.75	4.84	0.66	7.79	1.07
E1	21.84	nd	4.99	0.66	3.73	1.20
E2	17.28	nd	4.36	0.54	3.56	1.09
E3	21.12	nd	4.52	0.33	0.12	0.99

nd - not determined

\*Seawater was not collected in expedition F

**Table 3**

Taxonomic and distribution of the studied fishes

<b>Family</b>	<b>Species</b>	<b>Common names</b>	<b>N</b>	<b>Stations</b>
Ariidae	<i>Genidens genidens</i>	bagre-guri	6	F1 F2
Batrachoididae	<i>Porichthys porosissimos</i>	mamangá	7	B3 C2 C3 D3 E1 E2
Bothidae	<i>Paralichthys patagonicus</i>	linguado	6	C1 C2 C3 D2 E2
Merluccidae	<i>Merluccius rubbsi</i>	merluza	12	A1 A2 C2 C3
Mullidae	<i>Mullus argentinae</i>	trilha	4	A2 E2
Percophidae	<i>Percophis brasiliensis</i>	tira-vira	6	A1 A2 C2 E2
Pomadasydae	<i>Ortopristis ruber</i>	corcoroca	4	E3
Pomadasydae	<i>Haemulon steindachneri</i>	corcoroca-boca-larga	6	F1 F2
Pomatomidae	<i>Pomatomus saltatrix</i>	enchova	1	D3
Sciaenidae	<i>Cynoscion striatus</i>	pescadinha	8	A1 A2 D2
Sciaenidae	<i>Macrodon ancylodon</i>	pescada-foguete	7	B3 B4 E3
Sciaenidae	<i>Umbrina canosai</i>	castanha	5	A1 D1
Sciaenidae	<i>Stellifer rastrifer</i>	cangoá	10	B1 E3
Sciaenidae	<i>Micropogonias furnieri</i>	corvina	3	A1 D2
Sciaenidae	<i>Paralonchurus brasiliensis</i>	maria-luíza	14	B1 B2 B3
Sciaenidae	<i>Cynoscion jamaicensis</i>	goete	3	B3 B4
Sciaenidae	<i>Menticirrhus americanus</i>	papa-terra	8	B4 E3 F1 F2
Sciaenidae	<i>Ctenociaena gracilicirrhus</i>	cangauá	1	D2
Serranidae	<i>Dules auriga</i>	mariquita	12	B1 C1 D2 E1
Sparidae	<i>Pagrus pagrus</i>	pargo-róseo	10	A1 A2 D3 E1
<b>Total</b>			<b>133</b>	

**Table 4**  
Brain AChE specific activities in the studied species (in brackets the number of animals tested)\*

Species**	Fish length (cm)	Brain weight (mg)	Total proteins (%)	Acetylcholinesterase		
				Soluble (%)	U/g wet weight	U/g proteins
<i>Porichthys porosissimos</i> (7)	23 ± 5	61 ± 32	7.9 ± 3.4	32 ± 2	11.9 ± 8.0	145 ± 39
<i>Merluccius rubbsi</i> (12)	22 ± 7	141 ± 54	7.8 ± 0.8	38 ± 3	13.0 ± 2.1	186 ± 30
<i>Percophis brasiliensis</i> (6)	27 ± 2	107 ± 87	12.5 ± 9.2	27 ± 2	28.6 ± 11.2	270 ± 70
<i>Cynoscion striatus</i> (8)	10 ± 2	102 ± 28	6.8 ± 1.1	30 ± 5	19.8 ± 4.0	297 ± 56
<i>Macrodon ancylodon</i> (7)	22 ± 5	96 ± 46	11.8 ± 6.3	36 ± 4	33.8 ± 17.2	299 ± 41
<i>Mullus argentinae</i> (4)	17 ± 3	141 ± 9	10.4 ± 2.0	32 ± 2	31.0 ± 8.1	299 ± 60
<i>Umbrina canosai</i> (5)	12 ± 1	65 ± 27	5.7 ± 1.4	33 ± 9	20.8 ± 8.5	355 ± 104
<i>Stellifer rastrifer</i> (10)	21 ± 2	117 ± 33	7.6 ± 3.3	30 ± 4	26.7 ± 10.9	355 ± 39
<i>Micropogonias furnieri</i> (3)	21 ± 3	160 ± 27	7.4 ± 0.6	36 ± 11	26.5 ± 4.8	360 ± 94
<i>Genidens genidens</i> (6)	28 ± 8	572 ± 97	8.9 ± 0.7	32 ± 5	34.1 ± 3.4	376 ± 15
<i>Paralanchurus brasiliensis</i> (14)	23 ± 2	140 ± 80	4.9 ± 1.4	32 ± 6	19.1 ± 7.3	389 ± 89
<i>Cynoscion jamaicensis</i> (10)	18 ± 2	125 ± 34	4.9 ± 1.1	30 ± 0	19.5 ± 4.5	392 ± 63
<i>Pagrus pagrus</i> (10)	13 ± 5	248 ± 129	8.9 ± 2.0	27 ± 5	34.4 ± 8.3	392 ± 90
<i>Ortopristis ruber</i> (4)	27 ± 1	109 ± 5	13.6 ± 2.9	40 ± 0	57.4 ± 25.2	411 ± 106
<i>Dules auriga</i> (12)	12 ± 2	70 ± 40	7.7 ± 2.5	35 ± 7	31.2 ± 7.5	427 ± 94
<i>Haemulon steindachneri</i> (6)	22 ± 2	214 ± 75	9.4 ± 1.5	27 ± 6	42.1 ± 13.9	443 ± 93
<i>Menticirrhus americanus</i> (8)	27 ± 6	223 ± 67	8.3 ± 1.6	21 ± 2	38.5 ± 9.6	466 ± 103
<i>Pomatomus saltador</i> (1)	16	88	4.9	44	23.8	482
<i>Paralichthys patagonicus</i> (6)	22 ± 8	62 ± 35	10.6 ± 5.5	33 ± 0	53.1 ± 29.2	493 ± 54
<i>Ctenociaena gracilicirrhus</i> (1)	19	197	7.2	41	38.2	530

\*the values are expressed as mean ± standard deviation of individual replicated assays in the brain of each collected fish.

\*\*common names, number of fish collected and catch localisation are shown in Table 3; 1U = 1 µmol of product / min

**Table 5**

Brain AChE kinetic characteristics of species collected in this work

Species	IC50-30min	K <sub>M</sub>	P%	B%	AChE
<i>Genidens genidens</i>	455	236	39	3	376
<i>Paralomchurus brasiliensis</i>	468	228	32	16	389
<i>Haemulon steindachneri</i>	1035	273	18	2	443
<i>Pagrus pagrus</i>	1087	115	15	1	392
<i>Menticirrhus americanus</i>	1579	291	20	1	435
<i>Cynoscion striatus</i>	1595	206	17	1	297
<i>Dules auriga</i>	1624	164	18	2	427
<i>Merluccius hubbsi</i>	3259	107	28	nd	186
<i>Percophis brasiliensis</i>	3339	104	13	nd	270

nd - not detected

IC50-30min -Pesticide concentration which inhibits 50% of AChE activity after 30 min of incubation expressed in nanomol/liter (nM)

K<sub>M</sub> - Michaelis-Menten constant with AceScol (μM)

P% [(activity with ProScol/activity with AceScol) \* 100] (%)

B% [(activity with ButScol/activity with AceScol) \* 100] (%)

AChE - Specific activity of AChE in the brain (U/g protein, from Table 4)

**Table 6**

Brain AChE sensitivity to organophosphates in various animals

Organism	Pesticide	IC50-30min (nM)	Reference
rat (37°C)	Ethyl paraoxon	13	Johnson & Wallace, 1987
	Methyl paraoxon	29	Kemp & Wallace, 1990
	Ethyl paraoxon	27	Carr & Chambers, 1996
hem (37°C)	Ethyl paraoxon	37	Kemp & Wallace, 1990
fish ( <i>Limanda limanda</i> )	Ethyl paraoxon	312	Sturn <i>et al.</i> , 1999
fish ( <i>Serranus cabrilla</i> )	Ethyl paraoxon	1,925	Sturn <i>et al.</i> , 1999
fish (rainbow trout at 11°C)	Methyl paraoxon	3,310	Johnson & Wallace, 1987

IC50 - Pesticide concentration which inhibit 50% of AChE activity after 30 min of "in vitro" incubation expressed in nanomol/liter (nM)

## Legends of Figures

### Figure 1

Sampling sites of the expeditions (A to F) in the Rio de Janeiro state coast

### Figure 2

Diagram of the hierarchic clusters analysis (Statistica 5.0<sup>A</sup>, StatSoft, Inc.) for brain AChE activity of fish collected (simple linkage distance).

(1) *Porichthys porosissimos*; (2) *Merluccius rubbsi*; (3) *Percophis brasiliensis*; (4) *Cynoscion striatus*; (5) *Macrodon ancylodon*; (6) *Mullus argentinae*; (7) *Umbrina canosai*; (8) *Stellifer rastrifer*; (9) *Micropogonias furnieri*; (10) *Genidens genidens*; (11) *Paralonchurus brasiliensis*; (12) *Cynoscion jamaicensis*; (13) *Pagrus pagrus*; (14) *Ortopristis ruber*; (15) *Dules auriga*; (16) *Haemulon steindachneri*; (17) *Menticirrhus americanus*; (19) *Paralichthys patagonicus*. The species *Pomatomus saltador* (18) and *Ctenociaena gracilicirrus* (20) were not included because only one sample was collected. The number in parenthesis represents the order of these species in relation to their AChE specific activity shown in the Table 4.

Fig 1

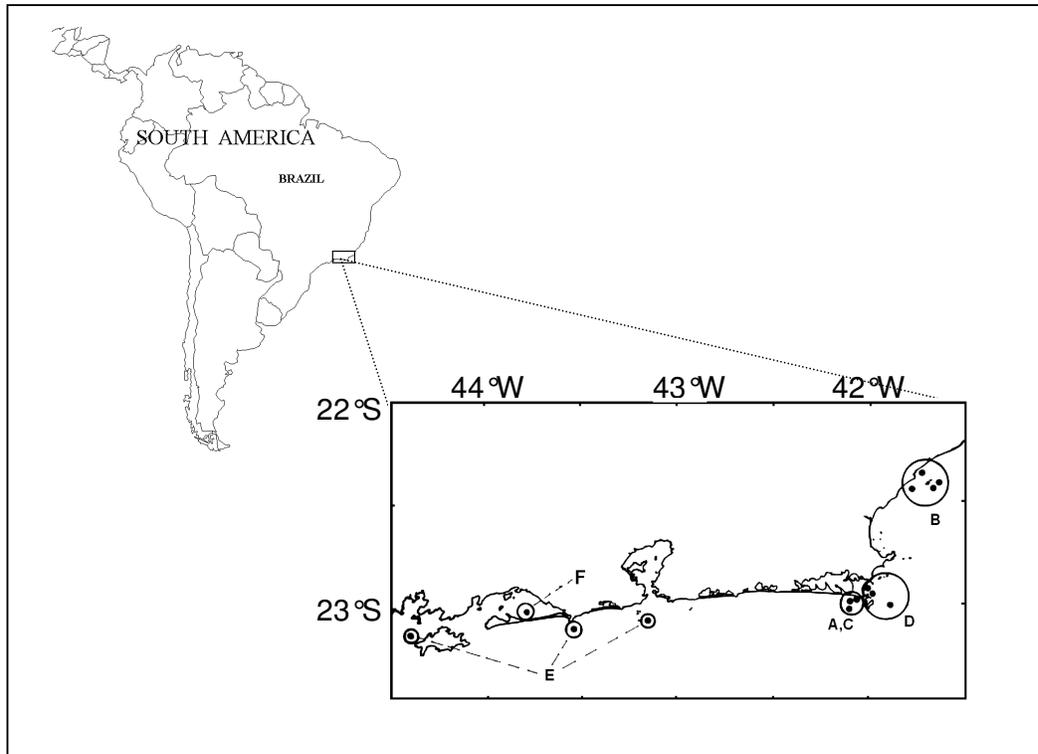


Fig 2

