SHORT COMMUNICATION

Acetylcholinesterase and ethoxyresorufin-o-deethylase in the surgeonfish *Acanthurus bahianus* around Martinique Island (French West Indies)

F. Galgani, G. Bocquené and T. Burgeot

The biochemical indicators of biological effects of pollutants, ethoxyresorufin-o-deethylase (EROD) and acetylcholinesterase (AChE), were measured in *Acanthurus bahianus* from 10 stations around Martinique Island (French West Indies). Strong induction of EROD was demonstrated in the bay of Fort de France in relation to organic pollution. Depression of AChE may suggest that neurotoxic compounds are having some effects along the east coast.

Keywords: *pollution, Martinique,* Acanthurus bahianus, *acetylcholinesterase, ethoxyresorufin-o-deethylase.*

Introduction

Tropical and coral reef fauna have both been suggested to be sensitive to the effects of contaminants (Stegeman *et al.* 1990). However, only a few studies have been performed and these are restricted to some species and biomarkers such as ethoxyresorufin-o-deethylase (EROD) in *Haemulon* spp. *Holocentrus rufus, Abudedduf saxatilis* and *Chaetodon* spp. (Stegeman *et al.* 1990, Vrolick *et al.* 1994) for field experiments, and some reared species in the case of acetylcholinesterase inhibition. The analysis of effects on these biochemical indicators for environmental contamination has focused on fish from temperate and cold water regions. In addition, no information is available concerning biochemical indicators in fish from the West Indies. We therefore describe the response of the biochemical indicators EROD and cholinesterase from the surgeon fish *Acanthurus bahianus*, a sedentary and ubiquitous member of the coral reef communities collected from different sites on Martinique Island where contamination by organic pollutants occurs in the bay of Fort de France and where pesticides are widely used, especially on the east coast (Cooper 1992, Kempf 1992).

METHODS

Adult specimens (15–25 cm) were collected live from fishermen's traps. The depth of immersion ranged from 5 m (onshore stations) to 30 m (station one). For EROD measurements, fresh livers were suspended in a buffer (Tris 50 mM, pH 7.4; KCl 150 mM; EDTA 1 mM and glycerol 20%) and then minced (5 ml tissues) for 5–10 s in an Ultraturrax homogenizer. Centrifugation was performed at 9000 *g* for 15 min at 4 °C. The supernatant was used as enzyme solution. The protein assay was performed using the Bradford method (1976) with bovine serum albumin as the standard. Measurements were done on a spectrophotometer plate reader at 595 nm. Assays were performed in a buffer (Tris 0.1 M, pH 8; NaCl 0.1 M) containing 2 μM of 7-ethoxyresorufin and 0.25 mM NADPH. Activity was determined by kinetic measurements at 20 °C on post-mitochondrial supernatant extracts diluted prior to the assay. The quantity of resorufin, the specific product of EROD activity, was measured according to a modified Burke and Mayer method (1974) using a fluorimetric plate reader as described previously (Eggens and Galgani 1992). Results are expressed as pmoles of resorufin formed per minute and per mg of protein.

For acetylcholinesterase measurements, fresh lateral muscles were suspended in 0.1 M Tris buffer, pH 8.0 (2/1 w/v), and homogenized for 1 min using an Ultraturrax. Extracts were centrifuged at 10 000 *g* for 10 min and the supernatants were analysed for cholinesterase activity. As for EROD measurements, proteins were determined using the Bradford method (1976). Acetylcholinesterase was determined using acetylthiocholine as a substrate. The principle of Ellman *et al.* (1961) was used as modified for microtitration plate reading (Galgani and Bocquené 1991). For each microplate well 0.3 ml of Tris buffer (0.1 M, pH 8), 20 ml of dithiobios nitrobenzoic acid (DTNB, 0.01 M) and 10 ml of enzyme suspension were added successively. Substrate (10 ml, 0.1 M) was added before enzyme reaction was started and absorbance was monitored on a microplate reader at 405 nm. One unit of AChE activity is the variation of 0.001 OD. Results are given as units min^{-1} mg $protein^{-1}$ (specific activities). ANOVA and Duncan tests were performed using Statistica software (Statsoft Inc.)

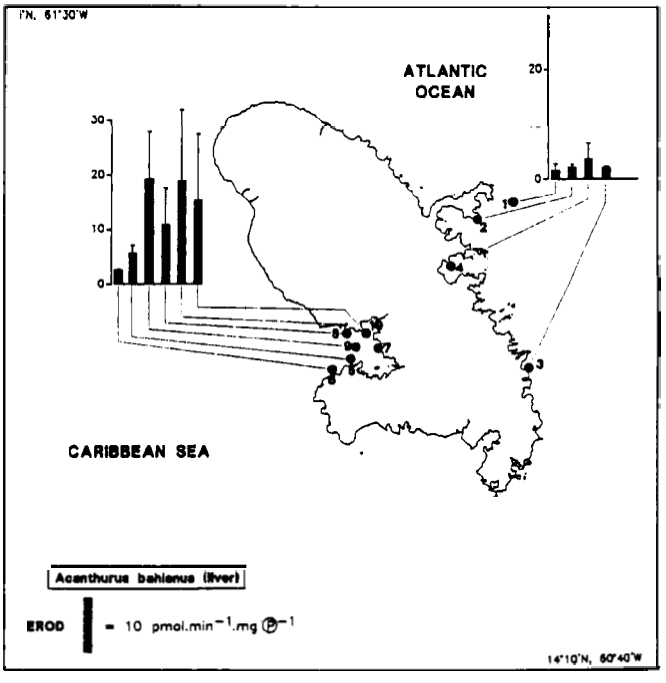

Figure 1. Specific activity of ethoxyresorufin-o-deethylase (EROD) from the liver of *Acanthurus bahianus* from 10 sampling locations around Martinique Island (mean value ± SD, $p < 0.05$). Number of sampled fishes were respectively 9, 5, 7, 4, 9, 9, 6, 8, 9 and 9 for stations 1–10.

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Results

Figure 1 presents the levels of EROD activities in fish collected from 10 stations including sites from the bay of Fort de France, the main town on the island, and sites situated on the east coast. The enzyme activity was found to range from 1.4 ± 1.20 pmoles min^{-1} mg^{-1} in the reference station situated offshore on the east coast to 19.31 ± 8.68 pmoles min^{-1} mg^{-1} in the middle of the bay of Fort de France. In addition onshore stations outside the bay exhibited low activity with values under 3.68 pmoles min^{-1} mg^{-1}, while all sites within the bay were found with values from 5.61 to 19.31 pmoles min^{-1} mg^{-1}. The effect of sampling location was tested using ANOVA. An '*F*' value of 2.46 (10 stations, 75 samples) was observed, indicating significant differences within stations. This was confirmed by Duncan's test.

Results concerning cholinesterase (Figure 2) are different with a decrease of activity at onshore stations on the east coast where values were found to range from 962 ± 163 to 1659 ± 424 specific units. This corresponds to 62.5% of the activity found in the reference offshore station (2559 ± 229 units) and 68% of the activity found outside of the bay of Fort de France (2993 ± 616).

As for EROD, significant effect of sampling location was found with an '*F*' value of 8.97 after ANOVA analysis (9 stations, 66 samples), this was also confirmed using Duncan's test.

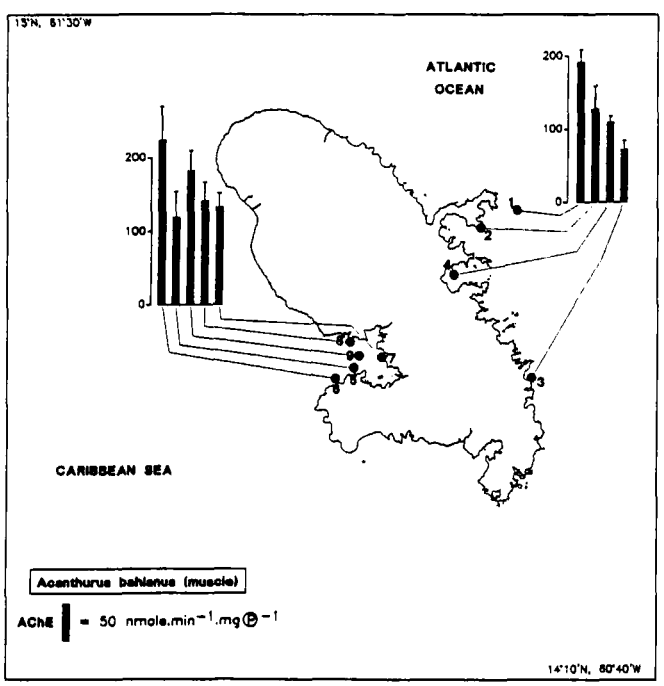

Figure 2. Specific activity of acetylcholinesterase (AChE) from the muscle of *Acanthurus bahianus* from nine sampling locations around Martinique Island (mean value ± SD, $p < 0.05$). Number of sampled fishes for stations 1–9 were as in Figure 1.

Discussion

The surgeon fish *Acanthurus bahianus* is a sedentary species from the reef community. No migrations occur during the adult phase of the life cycle. Its wide distribution around the Caribbean waters makes it a good candidate for monitoring the effects of pollutants.

The cytochromes P450 are a family of haem proteins catalysing monooxygenase reactions that metabolize many endogenous molecules as well as xenobiotic compounds. Different isozymes with distinct catalytic, structural and regulatory properties have been described in fish. One form of cytochrome P450, CYP IAI, is induced by several classes of contaminants, including environmental pollutants (Stegeman and Kloepper-Sams 1987). This P450 form catalyses the monooxygenase reactions aryl-hydrocarbon-hydroxylase and ethoxyresorufin-*o*-deethylase, both of which are induced by polyaromatic hydrocarbons (PAH) and polychlorobiphenyls (PCB). The use of EROD activity to monitor the effect of contaminants is a new approach in assessing pollution in marine ecosystems which has been already widely validated in fish from temperate areas (Addison and Edwards, 1988, Spies *et al.* 1982, Eggens *et al.* 1992, Burgeot *et al.* 1993). Moreover, effects of natural factors such as temperature do not affect EROD to the same extent as pollutant induction since a temperature change of at least 8 °C is necessary for significant changes in EROD activity (Sleiderink *et al.* 1995) and the scale of the sampled area does not show such variations. Therefore, the observed results in *Acanthurus bahianus* suggest the presence of cytochrome p450 IAI inducers in the bay of Fort de France. The presence of urban effluents in Fort de France, together with a refinery in the north of the bay, may account for these biological effects.

The major role of acetylcholinesterase is the regulation of nerve impulse transmission by hydrolysis of the neurotransmitter acetylcholine. Inhibition of the enzyme has been used for years in monitoring the effects of pesticides such as organophosphorus and carbamate compounds in both terrestrial and freshwater organisms. The study of cholinesterase in marine fishes has focused on biochemical characterization (Habig *et al.* 1988, Bocquené *et al.* 1996, Galgani and Bocquené 1991) or toxicity tests (Coppage and Mathews 1974, Habig *et al.* 1988, Bocquené *et al.* 1990, 1993). Recently, the benefits of using enzyme measurements as a tool for monitoring pollution effects in marine animals have been established (Galgani *et al.* 1992, Bocquené *et al.* 1993). Sea water temperature around the island remains constant at a value of 26 ± 2 °C (Dereynal, L., IFREMER/Martinique, personal communication). As for EROD activity, the strong depression of the enzyme activity may not be related to natural variability such as water temperature but to the effects of each of the following organophosphorus compounds: Terbufos, Ethoprophos, Izasophos, Phenamiphos, Methyl-parathion, and the carbamates Aldicarb and Carbofuran, are used, mainly along the east coast of the island (Cooper 1992) where agriculture occurs. These may account for the depression of cholinesterase activity.

In conclusion, this study suggests that non-natural changes occur in the biomarkers EROD and cholinesterase in

Acanthurus bahianus from the waters of Martinique and gives both a scientific and a technical basis for biological monitoring of Caribbean waters. Identification of compounds involved in biological effects is now in progress.

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