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Pollutants and biomarker responses in two reef fish species (*Haemulon aurolineatum* and *Ocyurus chrysurus*) in the Southern Gulf of Mexico

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ABSTRACT

The environmental quality differences between two groups of reefs in the Veracruz Reef System were evaluated. The North group of reefs is very close to Veracruz, an urban and port zone, whereas the South group is more isolated, with minor anthropogenic disturbances. To prove the hypothesis that the North group is more affected by anthropogenic activities, the concentrations of hydrocarbons in liver, metals and metalloids such as Se, As, Ba, Cd, Hg and V in muscle, and PAH metabolites in bile were evaluated, and related to biomarkers (transcript abundance of cytochrome P4501A, Vitellogenin, and Glutathione-S-transferase) in two species of fish: *Haemulon aurolineatum* and *Ocyurus chysurus*. *H. aurolineatum* presents the highest concentrations for many pollutants, but *O. chysurus* shows the most significant differences in pollutant concentrations and biomarkers between the two reef groups, suggesting that this species could be used as a sentinel in future studies in the Gulf of Mexico.

1. Introduction

The coastal environment is one of the most susceptible to the effects of environmental contaminants. Many ecosystems such as coral reefs and estuaries have been altered to levels beyond possible recovery (Métais et al., 2012; Celander, 2011). Marine species are increasingly exposed to chemical pollutants (heavy metals, oil, polycyclic aromatic hydrocarbons, halogenated compounds, etc.) as a result of anthropogenic activities. In field studies where complex contaminant mixtures are likely to occur it is important to include an appropriate multiparametric approach to evaluate the effects of these pollutants on sentinel organisms. The assessment of marine ecosystems is an important aspect of environmental protection, and the use of biomarkers has been incorporated to establish connections between concentrations of pollutants and their effects in organisms. Sensitive biological responses such as biomarkers can help to analyze an environment as an early response about the conditions that are deteriorating this ecosystem (Gusso-Choueri et al., 2015). In this study we measure biochemical responses in fish tissue to determine the health status as possibly affected by the presence of pollutants in the Veracruz Reef System. The early response biomarkers we use are 1) induction of the hepatic cytochrome P-450 1A (CYP1A), which is related to organic xenobiotic biotransformation phase I system, used to evaluate the sub-lethal toxic effects of oil and hydrocarbons (PAHs) (Shirani et al., 2012); 2) Glutathione-S-transferase (GsT) which are enzymes used for conjugation of electrophilic compounds during phase II of xenobiotic metabolism. Hepatic GsT induction and activity have been related to significant alterations by metals and metalloids, PAHs and halogenated hydrocarbons in fish (Anderson and Lee, 2006); 3) induction of Vitellogenin (VTG), which has been used to assess exposure to estrogenic chemicals (Huang et al., 2012); and 4) presence of PAH metabolites in fish bile as biomarkers of recent exposure to hydrocarbons and oil (Anderson and Lee, 2006).

The Veracruz Reef System (VRS) is located near the port city of Veracruz, on the western Gulf of Mexico (Fig. 1). The port of Veracruz is the main cargo port in Mexico and a main tourist destination. The VRS was designated a National Park in 1992, and it was included in 2004 as a wetland of international importance in the Ramsar list. It functions as a reservoir, bridge and spread point of species between Caribbean reef

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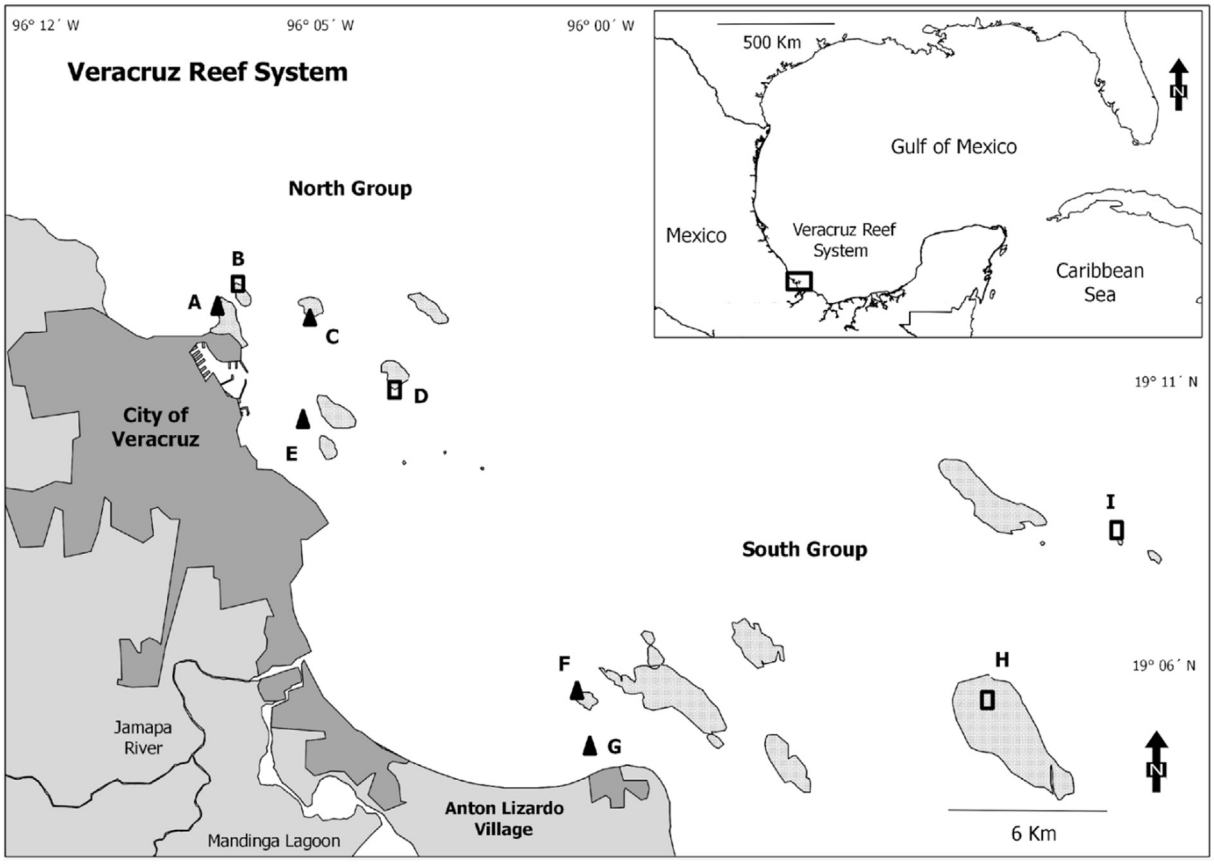

Fig. 1. Location of the Veracruz Reef System (VRS), Southern Gulf of Mexico, study area and collection sites of sentinel organisms (▲*Haemulon aurolineatum*, and □*Ocyurus chrysurus*). North group: (A) Gallega, (B) Galleguilla, (C) Blanquilla, (D) Verde, and (E) Mersey. South group: (F) Blanca, (G) Giote, (H) Cabezo Norte, and (I) Santiaguillo.

areas and Florida (Jiménez-Hernández et al., 2007). One special characteristic of the VRS for this study is that it is composed of two groups of reefs, separated by the plume of the Jamapa River. The Northern Group is composed of smaller reefs, very close to shore near the Veracruz City and port. The Southern group is composed of larger reefs, farther from shore, and near to the town Antón Lizardo, with apparently better environmental conditions (Tunnell et al., 2007).

Since 1991, studies in this area have been conducted to establish the natural concentrations of trace metals as nickel and vanadium, and the presence of petroleum hydrocarbons from natural oil seeps, and input by anthropogenic activities such as oil spills and products related with the oil industry (Vazquez et al., 1991). Given the importance of this reef system a study was conducted to evaluate its environmental condition, within the framework of the Environmental Monitoring and Assessment pilot project of the GEF-funded Gulf of Mexico Large Marine Ecosystem project between the United States and Mexico (García-Ríos et al., 2013). To assess the environmental condition of the VRS, we selected two fish species: *Haemulon aurolineatum* and *Ocyurus chysurus* as target species, because they are found mainly around coral reefs, are commercially important, and benthic feeding (Alpuche-Gual and Gold-Bouchot, 2008). Both species follow the criteria to be used as a sentinel species, such as: easy identification in the field, easy to catch, abundant population, they are not under any legal protection, and are not migratory (Lingo and Szedlmayer, 2006).

The yellowtail snapper (*O. chrisurus*) is a snapper found in the Western Atlantic Ocean, mostly around coral reefs, mostly at depths from 10 to 70 m. They feed at night, mainly on plankton and benthic organisms. Juveniles eat mainly plankton (Allen, 1985; Froese and Pauly, 2000). The tomtate grunt (*Haemulon aurolineatum*) is a grunt found mainly on the Western Atlantic, on sea grass beds, sand flats and reefs. Feeds on benthic invertebrates, algae and plankton (Robins and Ray, 1986).

The goals of this study are to assess the health status of the VRS using the concentrations of pollutants (metals, metalloids and hydrocarbons) and gene expression in the two species of fish selected, to compare the health status of the North group of reefs to the South group of reefs, and to evaluate the possible use of these two fish species as sentinel species for reef environmental monitoring programs.

2. Materials and methods

Sampling was performed on April 2013; nine reefs in the VRS were sampled, five reefs opposite the port of Veracruz conform the North group and four reefs in front of the town Antón Lizardo conform the South group. Five fish from both fish species (*H. aurolineatum* and *O. chysurus*) were sampled in each of the selected sites. Once collected, fish were measured, weighed and dissected. Bile was removed with a hypodermic syringe and placed in an Eppendorf vial. The liver was divided in two parts, one for hydrocarbon analysis and one for molecular biomarkers, and frozen until analysis. The muscle was sectioned and placed in plastic bags for the analysis of metals and metalloids.

2.1. Metals and metalloids analysis

Metals and metalloids (Se, As, Ba, Cd, Hg and V) were analyzed by atomic absorption spectrophotometry with a Perkin Elmer SIMAA 6100 spectrometer with Zeeman correction, after reflux digestions with nitric acid and hydrogen peroxide, as described by Pascual-Barrera et al. (2004). Quality control was ensured by analysis of blanks and certified reference material (Standard Reference Material 2676 from NIST) in parallel with the samples.

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