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Assessment of ecosystem health disturbance in mangrove-lined Caribbean coastal systems using the oyster *Crassostrea rhizophorae* as sentinel species

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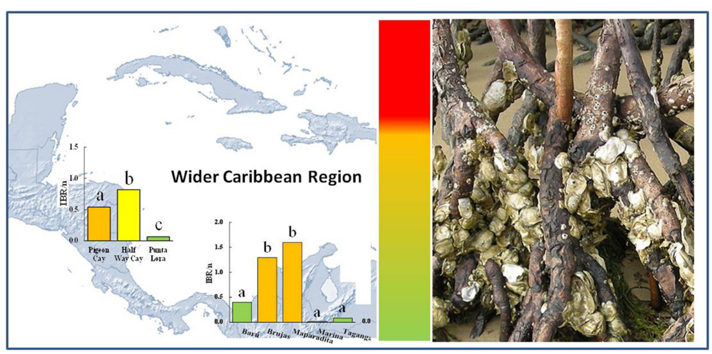
HIGHLIGHTS

- Health status of wild oysters, determined in Caribbean mangrove conditions
- A toolbox of non-sophisticated biological effects endpoints was applied.
- Signs of ecosystem health disturbance, related to contaminants and sewage
- Approach suitable for assessing health disturbance in mangrove-lined coastal ecosystems
- Mangrove cupped oysters, proper sentinels for tropical coastal biomonitoring

GRAPHICAL ABSTRACT



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ABSTRACT

This investigation was aimed at contributing to develop a suitable multi-biomarker approach for pollution monitoring in mangrove-lined Caribbean coastal systems using as sentinel species, the mangrove cupped oyster, *Crassostrea rhizophorae*. A pilot field study was carried out in 8 localities (3 in Nicaragua; 5 in Colombia), characterized by different environmental conditions and subjected to different levels and types of pollution. Samples were collected in the rainy and dry seasons of 2012–2013. The biological effects at different levels of biological complexity (Stress-on-Stress response, reproduction, condition index, tissue-level biomarkers and histopathology) were determined as indicators of health disturbance, integrated as IBR/n index, and compared with tissue burdens of contaminants in order to achieve an integrative biomonitoring approach. Though modulated by natural variables and confounding factors, different indicators of oyster health, alone and in combination, were related to the presence of different profiles and levels of contaminants present at low-to-moderate levels. Different mixtures of persistent (As, Cd, PAHs) and emerging chemical pollutants (musk fragrances), in combination with different levels of organic and particulate matter resulting from seasonal oceanographic variability and sewage discharges, and environmental factors (salinity, temperature) elicited a different degree of disturbance in ecosystem health condition, as reflected in sentinel *C. rhizophorae*. As a result, IBR/n was correlated with pollution

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indices, even though the levels of biological indicators of health disturbance and pollutants were low-to-moderate, and seasonality and the incidence of confounding factors were remarkable. Our study supports the use of simple methodological approaches to diagnose anomalies in the health status of oysters from different localities and to identify potential causing agents and reflect disturbances in ecosystem health. Consequently, the easy methodological approach used herein is useful for the assessment of health disturbance in a variety of mangrove-lined Caribbean coastal systems using mangrove cupped oysters as sentinel species.

1. Introduction

Mangrove ecosystems are threatened by a combination of natural disasters, tourism, aquaculture, deforestation and chemical pollution (Bayen, 2012; Lewis et al., 2011). In mangroves of the Wider Caribbean Region (WCR, UNEP Regional Seas Programme), pollution monitoring programmes have been carried out since the 1970′s to determine the concentrations of pesticides and other persistent organic pollutants (POPs), PAHs and metals in seawater, sediments, seafood and in biomonitor species (Fernandez et al., 2007; Sericano et al., 1995). Particularly, mangrove cupped oysters (*Crassostrea rhizophorae*) have been proposed as biomonitors for pollution monitoring in mangrove ecosystems (Aguirre-Rubí et al., 2017; Silva et al., 2006).

Marine pollution monitoring cannot be based solely on chemical data because these do not provide any indication of the magnitude of deleterious effects exerted to biota and ecosystems by combinations of pollutants in multiple stress scenarios (Allan et al., 2006; Cajaraville et al., 2000). Consequently, it is recommended to include both chemical and biological effects endpoints in pollution monitoring programs (ICES, 2011, 2012). Thus, biomarkers are "early warning signals" commonly used to assess the biological effects exerted by mixtures of chemicals on sentinel species in complex environmental conditions (Cajaraville et al., 1993; Marigómez et al., 2013); which have recently become an integral component of environmental monitoring programmes in several countries (Schettino et al., 2012).

"Biological effects" monitoring based on the biomarker approach is not currently being carried out in the Caribbean coastal zone. Although information about the biological effects exerted by chemical pollutants on mangrove oysters is gaining attention (Alves et al., 2002; Maranho et al., 2012; Rebelo et al., 2003; Zanette et al., 2008), the monitoring capacities and facilities in many WCR countries can be far away from those required to conduct sophisticated and most-advanced biomarker-based monitoring using oysters as sentinels. However, as very recently stated by Blaise et al. (2016), effortless and low cost biomarkers can provide basic knowledge on animal health and water quality and are technically achievable everywhere. Accordingly, and aware of the technical and logistic limitations (e.g., dealing with limited accessibility and difficulties for secure sample transportation and quality in situ processing), a toolbox of biological effects endpoints was selected to assess the potential of the mangrove cupped oyster, *C. rhizophorae*, as sentinel for pollution monitoring in Caribbean mangroves and coastal zones. This toolbox included undemanding measurements of responses at population and individual level, and histopathological analyses; these latter allow scoring responses at systemic and tissue levels on the basis of low-cost, solid and straightforward technology.

The Stress-on-Stress (SoS) response has been recommended by ICES (2012) for monitoring programmes as an indicator of mussel health status (Hellou and Law, 2003). The successful application of SoS response as biomarker for environmental monitoring in mussels led to its subsequent wider application to other bivalve species, especially in subarctic and temperate regions (Blaise et al., 2016). SoS response is a cost-effective test, in which the capacity of bivalves to survive on air is scored as a measure of resilience (Smaal et al., 1991; Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995). It has been applied in the field to detect effects of urban discharges to estuarine and coastal waters using both native (Hellou and Law, 2003) and transplanted mussels (Moles and Hale, 2003), as well as for assessing oil spill impact (Thomas et al., 1999) and in laboratory experiments (Eertman et al., 1995; Veldhuizen-Tsoerkan et al., 1991; Viarengo et al., 1995). It is not as sensitive as some core biomarkers of general stress (e.g. lysosomal membrane stability) but is more sensitive than others and its methodology is simple, rapid and low-cost (Viarengo et al., 1995). Flesh Condition Index (FCI) reflects the physiological status of bivalves and it has been reported that it is reduced on exposure to chemical pollutants (Mubiana et al., 2006). SoS response and FCI are considered low-cost biomarkers that are easy to be applied with little monetary or logistic investment (Blaise et al., 2016).

Gamete development and gonad histopathology were included in the toolbox of biological effects endpoints because the gametogenic cycle is a backbone reference to understand health condition and the biological effects of pollutants, and because changes in normal gametogenic cycle and reproduction disturbances (e.g. intersex) are well-known biological effects of chemical pollutants (Ortiz-Zarragoitia and Cajaraville, 2010; Ortiz-Zarragoitia et al., 2011). Finally, digestive gland histopathology was also incorporated into the toolbox, because a wide range of contaminants, including metals, pesticides and PAHs, is known to provoke histopathological alterations in this tissue (Au, 2004; Bignell et al., 2012; Garmendia et al., 2011; Kim and Powell, 2007; Kim et al., 2008).

For the purpose of contributing to develop a suitable multi-biomarker approach for pollution monitoring in Caribbean mangroves and coastal zones using as sentinel species the mangrove cupped oyster, *C. rhizophorae*, a pilot field study was carried out in 2012–2013 in 8 localities (3 in Nicaragua and 5 in Colombia) characterized by different levels and types of pollution. Samples were collected in the rainy and dry seasons of 2012–2013. Results describing the tissue levels of pollutants were published in a preceding paper (Aguirre-Rubí et al., 2017), in which the suitability of mangrove cupped oysters as biomonitors for Caribbean mangroves and coastal zones was confirmed. In the present investigation, the biological effects at different levels of biological complexity (stress on stress response, reproduction, condition index, tissue-level biomarkers and histopathological condition) were determined, integrated as IBR/n index (Beliaeff and Burgeot, 2002; Broeg and Lehtonen, 2006; Marigómez et al., 2013), and compared with tissue burden of chemical pollutants (Aguirre-Rubí et al., 2017) in order to achieve an integrative biomonitoring approach.

2. Material and methods

2.1. Sampling sites and sample collection

Different representative scenarios of mangrove ecosystems from the Caribbean were selected. In Nicaragua, subtidal (<1 m depth) oyster reefs were sampled in two localities (Fig. 1a): Bluefields (sampling sites: Punta Lora and Half Way Cay) and Pearl Lagoon (sampling site: Pigeon Cay). Punta Lora was considered as a prospective reference site (remote from urban settlements) whilst Half Way Cay and Pigeon Cay were selected as potentially polluted areas influenced by aquatic transport and urban discharges (GEF-REPCar, 2011; Ebanks-Mongalo et al., 2013). In Colombia, intertidal prop roots of red mangrove trees (*Rhizophorae mangle*) were sampled in Cartagena Bay and Barbacoas Bay (Fig. 1b) and in Taganga Bay (Fig. 1c), and intertidal rocky habitat