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Multi-level biological responses in *Ucides cordatus* (Linnaeus, 1763) (Brachyura, Ucididae) as indicators of conservation status in mangrove areas from the western atlantic

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ABSTRACT

There is a global lack of knowledge on tropical ecotoxicology, particularly in terms of mangrove areas. These areas often serve as nurseries or homes for several animal species, including *Ucides cordatus* (the uçá crab). This species is widely distributed, is part of the diet of human coastal communities, and is considered to be a sentinel species due to its sensitivity to toxic xenobiotics in natural environments. Sublethal damages to benthic populations reveal pre-pathological conditions, but discussions of the implications are scarce in the literature. In Brazil, the state of São Paulo offers an interesting scenario for ecotoxicology and population studies: it is easy to distinguish between mangroves that are well preserved and those which are significantly impacted by human activity. The objectives of this study were to provide the normal baseline values for the frequency of Micronucleated cells (MN‰) and for neutral red retention time (NRRT) in *U. cordatus* at pristine locations, as well to indicate the conservation status of different mangrove areas using a multi-level biological response approach in which these biomarkers and population indicators (condition factor and crab density) are applied in relation to environmental quality indicators (determined via information in the literature and solid waste volume). A mangrove area with no effects of impact (areas of reference or pristine areas) presented a mean value of MN‰ < 3 and NRRT > 120 min, values which were assumed as baseline values representing genetic and physiological normality. A significant correlation was found between NRRT and MN, with both showing similar and effective results for distinguishing between different mangrove areas according to conservation status. Furthermore, crab density was lower in more impacted mangrove areas, a finding which also reflects the effects of sublethal damage; this finding was not determined by condition factor measurements. Multi-level biological responses were able to reflect the conservation status of the mangrove areas studied using information on guideline values of MN‰, NRRT, and density of the uçá crab in order to categorize three levels of human impacts in mangrove areas: PNI (probable null impact); PLI (probable low impact); and PHI (probable high impact). Results confirm the success of *U. cordatus* species' multi-level biological responses in diagnosing threats to mangrove areas. Therefore, this species represents an effective tool in studies on mangrove conservation statuses in the Western Atlantic.

1. Introduction

Although tropical ecosystems house 75% of the world's biodiversity and generate approximately 60% of the primary productivity of the planet, ecotoxicological studies have been conducted almost exclusively in temperate ecosystems (Peters et al., 1997; Lacher and Goldstein, 1997; Sueitt et al., 2015). Such limited knowledge contrasts with current increasing human impacts in the tropics, impacts which are generally the result of unsustainable activities and the release of xenobiotics into water bodies. These activities ultimately have negative effects on the aquatic

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biota (Peters et al., 1997; Harford et al., 2015).

Different approaches, which consider information ranging from data at the population-community level to sentinel species' sublethal responses, are the current basis for most biomonitoring programs (Marine Strategy Framework Directive EU - MSFD, 2008/56/EC). Short-term responses among lower levels of biological organization have been used to detect the first signs of impairment caused by xenobiotics (Amiard-Triquet et al., 2013; Pereira et al., 2014). These responses have generally been referred to as "biomarkers," which are defined herein as genetic, biochemical, cellular, physiological, or behavioral variations that can be measured in tissue or body fluid samples or at the level of the whole organism and which provide evidence of exposure to and/or effects of one or more chemical pollutants and/or radiations (Depledge et al., 1993).

Genetic and physiological changes are therefore of particular importance, since they are conspicuous in organisms that inhabit impacted areas (Adams, 1990; Dailianis et al., 2003; Otomo and Reinecke, 2010; Amiard-Triquet et al., 2013; Toufexia et al., 2013). Because of their effectiveness and ecological relevance (Bonassi et al., 2000; Dailianis et al., 2003; Neri et al., 2003), two biomarker assays are widely used. The first is the micronucleus test (MN‰), which quantifies the frequency of micronucleated cells and which measures genotoxicity. Genotoxicity is higher in more highly impacted areas (Brunetti et al., 1988; Burgeot et al., 1995; Collier et al., 2013; Pinheiro et al., 2013). The second is the neutral red retention time (NRRT) assay, which evaluates lysosomal membrane stability as a diagnostic biomarker of individual health status (Buratti et al., 2012, Ospar, 2013).

Genetic and physiological changes are considered pre-pathological indicators (Tsarpali and Dailianis, 2012) that can detect biological dysfunction in response to a stressor before population problems occur (Amiard-Triquet et al., 2013). As determined by Adams, (1990) and by Fishelson et al. (1999), such sublethal damages may have more serious effects, such as increases in disease, changes to mortality rates, and population decline. According to these authors, other parameters found at higher levels of biological organization, such as at the population level, may also be relevant bioindicators of environmental impact and quality (Bruner et al., 2001; Barrilli et al., 2015). Therefore, Fulton's condition factor (K) would be a relevant population parameter in these assessments: it can quantify "well-being" as a ratio of size to individual weight (Lima-Junior et al., 2002), with higher values indicating better environmental quality (Viana et al., 2014; Barrilli et al., 2015). Similarly, density is a parameter that can be reduced by contaminants, as seen among crustaceans (Krebs and Valiela, 1974; Krebs and Burns, 1977), although the reverse effect can also occur, leading to an increase in density due the tolerance of contaminants and their indirect effects on the environment (Cannicci et al., 2009).

There is limited knowledge available on links between sublethal damages seen in the lower levels of biological organization and those seen in higher levels (at the population or community level), particularly when a multi-level approach is considered. However, in order for the biological model to be effective, it must consider a species that is both important to the ecosystem and sensitive enough to reveal early environmental disturbances (Beltrame et al., 2010, 2011; Pereira et al., 2012, 2014). Thus, Pinheiro et al. (2012) describe characteristics of the uçá crab (*Ucides cordatus*) that reflect its importance as a sentinel species in mangroves in the Western Atlantic: endemism and reduced mobility, bioturbation of sediments, feeding on leaves and sediments (deposit feeding); reduced growth, a relatively long lifespan, higher abundance, and increased ease of capture. The uçá crab accumulates contaminants in its tissues; there is a high correlation between this bioaccumulation and the genetic impacts observed (Pinheiro et al., 2012, 2013). As a result, these xenobiotics are magnified by the trophic chain of the mangrove (Fiscarelli and Pinheiro, 2002). This species also has a close relationship with three environmental compartments of mangroves with which it interacts and where it absorbs pollutants: (1) water, via contact, ingestion, the respiration process, and metabolite excretion; (2) sediment, via contact when digging burrows and mud feeding; and (3) trees, through their feeding on the senescent leaves and seedlings (Fiscarelli and Pinheiro, 2002; Pinheiro et al., 2013; Christofoletti et al., 2013).

A multi-level biological response approach must include not only the most ideal biological model – the area selected for study must also present a wide regional environmental impact gradient so that the investigation will be more effective and meaningful. Therefore, it is important to highlight the high anthropic impact observed in southeastern Brazil, particularly in coastal areas. However, pristine mangroves also exist and are represented by protected areas (Cetesb, 2001, 2007). It is, therefore, a contrasting scenario that has been mentioned and studied by several authors (Silva et al., 2002; Abessa and Ambrozevicius, 2008; Cordeiro and Costa, 2010; Martins et al., 2011; Pereira et al., 2011; Torres et al., 2012; Pinheiro et al., 2008, 2012, 2013).

Given both the contamination gradient of the mangroves in the region and the possible biological responses at the cellular, physiological, and population levels of a sentinel species (*U. cordatus*), this study aims to: (1) provide normal baseline values for the micronucleated cell frequency (MN ‰) and neutral red retention time (NRRT) in *U. cordatus* at pristine locations; and (2) perform a multi-level assessment using this species as a biological model by analyzing the effectiveness and ecological relevance of two biomarkers (genetic via MN ‰ and physiological via NRRT), their correlation with population parameters (condition factor and density), and their interactions with the conservation status of different mangrove areas (determined via information from the literature and solid waste volume). The validation of this multi-level approach will allow for the conservation statuses of the mangroves to be categorized without the need for contaminant quantification or qualification. High costs are therefore avoided, which will provide more opportunities for diagnosing this ecosystem's conservation status.

2. Materials and methods

2.1. Mangrove areas studied and their conservation statuses

The coast of São Paulo State in Brazil is frequently divided into three sections referred to as the northern coast, the central coast, and the southern coast. These sections are distinguished by their coastal plain areas (6000 km^2, 3200 km^2, and 7800 km^2, respectively) (Gouveia, 2012), by the extent of river basins (194,800 ha, 281,800 ha, and 1,706,800 ha, respectively; see BRASIL (2006)), and by the extent of mangroves (central: 8858 ha; southern: 15,193 ha; see Cunha-Lignon et al. (2011a, 2011b) and Cunha-Lignon (2014)). The present study considered mangroves from only the central coast (8858 ha) and the southern coast (15,193 ha), which represent the vast majority of the state's mangrove area (99%). These sections also have a history of human occupation and impact dating back to the 1500 s when the first Brazilian city (São Vicente) was founded on the central coast of the state (Cunha-Lignon et al., 2011b). Six mangrove areas have been established (Fig. 1), three of which are located on the central coast (Bertioga, or BET, Cubatão, or CUB, and São Vicente, or SAV) and the other three which are located on the southern coast (Iguape, or IGU; Cananéia,