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LUANA BARBOSA SEIXAS

Potencial de uso de *Orthopristis ruber* (Pisces, Haemulidae) como espécie sentinela em uma baía costeira poluída do Estado do Rio de Janeiro

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POTENCIAL DE USO DE *ORTHOPRISTIS RUBER* (PISCES, HAEMULIDAE) COMO ESPÉCIE SENTINELA EM UMA BAÍA COSTEIRA POLUÍDA DO ESTADO DO RIO DE JANEIRO

LUANA BARBOSA SEIXAS

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Seixas, Luana Barbosa

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RESUMO

A Baía de Guanabara está inserida em uma zona altamente urbanizada, onde os impactos antropogênicos afetam a diversidade e funcionamento de seus habitats, além de provocarem mudanças na composição, abundância e riqueza da ictiofauna. Em especial, a exposição ambiental aos contaminantes orgânicos e inorgânicos, e algumas características ambientais pode influenciar nas estruturas corpóreas de peixes, determinando alterações morfológicas e fisiológicas. A cocoroca *Orthopristis ruber* (Cuvier, 1830), espécie com ampla distribuição geográfica ao longo de toda a Baía de Guanabara, apresenta atributos de história de vida peculiares, relacionado com sua alimentação, reprodução e crescimento, que confere a espécie um potencial para ser utilizada em estudos como sensor biológico de alterações ambientais de sistemas estuarinos tropicais, como é o caso da Baía de Guanabara, pois podem sofrer alterações morfológicas, estruturais e fisiológicas em decorrência dos efeitos subletais da contaminação por atividades estressoras de origem antropogênica ou natural. Espera-se que os agentes estressores naturais ou de contaminação ambiental influenciem negativamente não somente os atributos morfológicos, bem como alguns descritores fisiológicos e no hábito de vida de *O. ruber*. Também se espera que os efeitos sobre a morfologia e fisiologia da espécie sejam inferiores nos locais com menores níveis de contaminação e melhores condições ambientais. Neste contexto, o objetivo principal é avaliar o potencial do *O. ruber* como espécie sentinela na Baía de Guanabara, através de análise de atributos morfológicos, fisiológicos, dos níveis de contaminação por mercúrio, da qualidade da água e da ecologia trófica. Os locais para amostragem foram selecionados para abranger o gradiente ambiental ao longo do principal canal central da Baía de Guanabara. As coletas foram realizadas trimestralmente, contemplando todas as quatro estações do ano, entre 2011 e 2015. Para análises morfológicas de simetria, foram realizadas contagens e/ou medições dos caracteres morfométricos e merísticos das principais estruturas pareadas de *O. ruber*. Amostras do tecido muscular da região dorsal de cada indivíduo foram retiradas para realização das análises de contaminação por mercúrio, e removido o estômago para identificação da dieta no menor nível taxonômico possível. O presente estudo se destaca por ser pioneiro em utilizar uma espécie de peixe marinha Neotropical comparando atributos morfológicos, fisiológicos e ecologia trófica em relação a ação de agentes estressores ambientais entre as áreas com diferentes características ambientais na Baía de Guanabara.

Palavras-chave: Perciformes, assimetria flutuante, ecomorfologia, mercúrio, ecologia trófica, costões rochosos.

ABSTRACT

The Guanabara Bay is located in a highly urbanized zone where anthropogenic impacts affect the diversity and functioning of its habitats, as well as causing changes in the composition, abundance and richness of the ichthyofauna. In particular, environmental exposure to organic and inorganic contaminants, and some environmental characteristics may influence the body structures of fish, determining morphological and physiological changes. The cocoroca *Orthopristis ruber* (Cuvier, 1830), a species with a wide geographic distribution throughout the Guanabara Bay, presents attributes of a peculiar life history, related to its feeding, reproduction and growth, which confers the species a potential to be used in studies as a biological sensor of environmental alterations of tropical estuarine systems, such as the Guanabara Bay, as they may undergo morphological, structural and physiological changes due to the sublethal effects of contamination by stressing activities of anthropogenic or natural origin. It is expected that natural stressors or environmental contaminants negatively influence not only morphological attributes, but also some physiological descriptors and *O. ruber's* life habit. The effects on the morphology and physiology of the species are also expected to be lower at sites with lower levels of contamination and better environmental conditions. In this context, the main objective is to evaluate the potential of *O. ruber* as a sentinel species in Guanabara Bay, through the analysis of morphological and physiological attributes of levels of mercury contamination, water quality and trophic ecology. Sampling sites were selected to encompass the environmental gradient along the main central channel of Guanabara Bay. The collections were performed quarterly, covering all four seasons of the year, between 2011 and 2015. For morphological analyzes of symmetry, counts and/or measurements of the morphometric and meristic characters of the main paired structures of *O. ruber* were performed. Samples of the muscular tissue of the dorsal region of each individual were removed to carry out the mercury contamination analyzes, and the stomach was removed to identify the diet at the lowest possible taxonomic level. The present study stands out for being a pioneer in using a species of Neotropical marine fish comparing morphological, physiological and trophic ecology attributes in relation to the action of environmental stressors among the areas with different environmental characteristics in Guanabara Bay.

Keywords: Perciformes, floating asymmetry, ecomorphology, mercury, trophic ecology, rocky shores.

INTRODUÇÃO GERAL

Em todo o mundo, existem aproximadamente 32.000 espécies de peixes descritas e isso representa mais de 50% dos vertebrados conhecidos (Nelson, 2016). A maior parte desta riqueza de espécies de peixes encontra-se nas regiões tropicais, com mais de 7.000 espécies na região Neotropical (Albert e Reis, 2011)). A ictiofauna das regiões tropicais apresenta uma variada diversidade em suas características morfológicas, fisiológicas e ecológicas (Lowe-McConnell, 1987).

Baías costeiras são ambientes semifechados, que compõem diferentes ecossistemas, e comumente exibem uma estreita relação com a variabilidade das condições oceanográficas adjacentes e com a ação de suas bacias hidrográficas (Franco et al., 2016; Silva et al., 2018). Esses ambientes suportam uma grande biodiversidade e atuam como um importante papel no ciclo de vida de muitas espécies de peixes marinhos (Castro et al., 2005; Silva-Júnior et al., 2013). Devido à ampla gama de funções ecológicas e econômicas, as baías costeiras são ambientes cada vez mais estudados (Vasconcelos et al., 2007; Franco e Santos, 2018).

A Baía de Guanabara é um grande estuário tropical que apresenta alta e severa pressão ambiental em praticamente toda sua extensão (Fistarol et al., 2015), onde os impactos antropogênicos afetam negativamente a diversidade e funcionamento de seus habitats. Estes impactos provocam mudanças na composição, abundância e riqueza de organismos, em especial a ictiofauna associada aos substratos consolidados (Kehrig et al., 2002; Silva et al., 2003). Estas espécies de peixes, especialmente aquelas que apresentam uma íntima associação com os habitats consolidados, podem funcionar como indicadores da integridade do ecossistema (Facey et al., 2005). Assim, compreender e monitorar as respostas biológicas na ictiofauna é uma importante ferramenta para a avaliação das condições da qualidade do ambiente (Allenbach, 2011).

A cocoroca *Orthopristis ruber* (Cuvier, 1830) apresenta grande abundância e frequência nas regiões Sul e Sudeste da costa brasileira (Vianna e Verani, 2002). Na Baía de Guanabara, esta espécie é uma das mais representativas associadas aos costões rochosos (Chaves et al., 2018). A espécie possui atributos de história de vida peculiares, relacionado com seu crescimento rápido, comportamento sedentário e demersal e íntima associação com substratos consolidados, que lhe conferem um potencial interessante para ser utilizada em estudos como sensor biológico de alterações ambientais de sistemas estuarinos tropicais.

Em geral, os peixes não respondem somente aos efeitos de contaminação aguda, mas também, podem sofrer alterações morfológicas, estruturais e fisiológicas mais sutis, em decorrência dos efeitos subletais da contaminação por atividades de origem antropogênica e condições ambientais (Seixas et al., 2013; 2016), especialmente as espécies de peixes com hábitos residentes, que tendem a refletir fortemente a variabilidade ambiental local, podendo ser considerados bons indicadores das condições ambientais (Allenbach, 2011).

Entre as ferramentas para o monitoramento das alterações ambientais desponta a assimetria flutuante, que são variações sutis aleatórias da simetria bilateral perfeita (Palmer e Strobeck, 1992), inferindo sobre o estresse ambiental a que a população está submetida (Seixas et al., 2016). Assimetria flutuante está relacionada às alterações antropogênicas e ambientais, sendo maior em ambientes degradados do que em áreas pristinas. Apresenta uma relação inversa, entre a sua magnitude e o desempenho das funções biológicas do indivíduo (Trokovic et al., 2012; Beasley et al., 2013). Desta forma, a assimetria flutuante pode fornecer dados para estudos de conservação de uma população antes que, contaminações agudas afetem toda a comunidade ou ecossistema (Palmer, 1992; 1994).

Diversas atividades antropogênicas atuam como fontes poluidoras na Baía de Guanabara. A contaminação por mercúrio (Hg), por exemplo é um grande problema devido à sua toxicidade, biomagnificação, altas taxas de absorção e baixa excreção (Kehrig et al., 2010; Seixas et al., 2013). O monitoramento da presença, localização e determinação das concentrações de Hg exibe as condições ambientais locais. Com base nisso, alguns questionamentos se tornam importantes: O Hg na Baía de Guanabara pode agir como um agente estressor capaz de ocasionar uma instabilidade no desenvolvimento no *O. ruber*? Quando o nível de concentração de mercúrio pode afetar a simetria bilateral no *O. ruber*? E essa instabilidade pode ser detectada através da aplicação da assimetria flutuante?

Outra ferramenta que pode ser usada para indicação de qualidade ambiental é o estudo da ecologia trófica de peixes. O conhecimento sobre a dieta de peixes é parte importante e fundamental para o aumento do conhecimento dos processos que regulam os ecossistemas aquáticos tropicais e o uso sustentável da diversidade biológica (Pessanha e Araújo, 2014). As diferenças morfológicas do aparelho bucal nas espécies de peixes, e consequentemente os diferentes mecanismos alimentares, permitem que a obtenção de alimentos reflita em adaptações na captura de potenciais presas permitindo a especialização das espécies (Labropoulou e Eleftheriou, 1997; Lowe-McConnell, 1999). Apesar de sua ampla distribuição por todo o Atlântico Ocidental, há poucos estudos sobre sua ecologia trófica de *O. ruber* (Aguiar e Filomeno, 1995; Amaral, e Migotto, 1980), principalmente quando relacionada com as condições ambientais e oceanográficas em Baías costeiras.

A hipótese da presente tese é que atributos morfológicos, fisiológicos e ecologia trófica de *O. ruber* sejam indicadores de qualidade ambiental na Baía de Guanabara. A tese está dividida em quatro capítulos em formato de artigos científicos:

Capítulo 1: Fluctuating asymmetry: A tool for impact assessment on fish populations in a tropical polluted bay, Brazil.

No capítulo 1 abordou o padrão espacial da presença da assimetria flutuante em *O. ruber* nas áreas da baia de Guanabara, investigando qual a melhor estrutura morfológica e índice para a validação da presença da assimetria flutuante, e se o gradiente ambiental encontrado ao longo do canal principal da Baía pode influenciar na presença da assimetria. Este capítulo foi publicado na

Seixas, L.B., Santos, A.F.G.N., Santos, L.N. 2016. Fluctuating asymmetry: a tool for impact assessment on fish populations in a tropical polluted bay, Brazil. Ecological Indicators, 71:522–532. http://dx.doi.org/10.1016/j.ecolind.2016.07.024.

Capítulo 2: Fluctuating asymmetry and organosomatic indexes in fish: the Corocoro grunt *Orthopristis ruber* (Haemulidae) as a case study

No capítulo 2 relacionamos assimetria flutuante usando apenas o índice composto (CFA1) com os índices gonadossomático (IGS), hepatossomático (IHS) e repleção (IR) e fator de condição (K) em juvenis, adultos, machos e fêmeas. Este capítulo foi submetido para Regional Studies in Marine Science, abril de 2019.

Capítulo 3: How much fluctuating asymmetry in fish is affected by mercury concentration?

No capítulo 3 demonstramos o potencial de *Orthopristis ruber* como espécie biondicadora de contaminação por Hg ao longo da Baía de Guanabara. Este capítulo foi submetido para Regional Studies in Marine Science, setembro de 2018.

Capítulo 4: Nearshore upwelling events mediating the diet of Corocoro grunt *Orthopristis ruber* (Cuvier, 1830) in a eutrophicated tropical bay

No capítulo 4 analisamos se mudanças na dieta de *Orthopristis ruber* está relacionada principalmente aos efeitos das massas de água predominantes na região oceânica adjacente a Baía de Guanabara, e em menor grau a variações entre juvenis e adultos e entre períodos seco e chuvoso. Este capítulo será submetido para Neotropical Ichthyology.

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Fluctuating asymmetry: A tool for impact assessment on fish populations in a tropical polluted Bay, Brazil

Luana Barbosa Seixas[a,c], Alejandra Filippo Gonzalez Neves dos Santos[c], Luciano Neves dos Santos[a,b,*]

[a] Laboratory of Theoretical and Applied Ichthyology (LICTA), Av. Pasteur, 458-R314A, CEP 22290-240 Rio de Janeiro, RJ, Brazil

[b] Federal University of Rio de Janeiro State (UNIRIO), Graduate Course in Neotropical Biodiversity (PPGBIO), Av. Pasteur, 458-R509, CEP 22290-240 Rio de Janeiro, RJ, Brazil

[c] Department of Zootechny and Sustainable Socioenvironmental Development, Fluminense Federal University, Niterói, Rio de Janeiro, Brazil

ABSTRACT

Fluctuating asymmetry (FA), which can be defined as random morphologic changes on bilateral symmetry plan of paired morphometric and meristic characters in response of environmental disturbances, is an alternative tool to traditional methods of environmental health assessment that has an interesting potential to appraise the state of adaptation of a population before that acute contaminations affect the whole community or ecosystem. This study aimed to assess the occurrence of FA in three morphometric and three meristic characters of the Corocoro grunt *Orthopristis ruber* (Cuvier, 1830), and compare the deviations in bilateral symmetry of this reef-associated and omnivorous in Guanabara Bay (Brazil), the venue for various outdoor aquatic sports during the 2016 Olympic Games. Five indexes of FA were tested over the six characters of 66 *O. ruber*, which were caught during the dry and wet seasons of 2011 and at three areas Guanabara Bay of different environmental characteristics. In addition to validate the existence of FA for *O. ruber*, our findings revealed significant deviations in the bilateral symmetry according to the FA indexes and fish characters. Together with the FA1 index, the number of gill rakers and the number of rays of the pectoral fin provided the best methodological approach to address the levels of FA in *O. ruber* as a response to environmental stressors in each region. The levels of FA in *O. ruber* was significantly lower in the Urca region (less impacted) than for individuals caught near the Paquetá Island and Rio-Niterói Bridge sites (more degraded). Partial Redundancy Analysis also shows that the fish charcters are affected in different ways by environmental stressors, but especially in response to the levels of dissolved oxygen and, secondarily, to water transparency, and that Paquetá Island is apparently less impacted than the site near the Rio-Niterói Bridge. Our results confirm the potential of FA to be used as a tool to detect environmental

effects on a reef-associated fish species in a tropical polluted Bay, but further studies are necessary to validate our findings for other species and ecosystems.

Keywords: Fluctuating asymmetry, Haemulid, Gill rakers, Morphometric traits, Antropogenic disturbances, Guanabara Bay, Brazil

1. INTRODUCTION

Fluctuating asymmetry (FA) is defined as random morphological deviations in the bilateral symmetry planes of an organism due to the effects of adverse environmental conditions (Van Vallen, 1962; Palmer, 1994; Hermita et al., 2013). The general prediction of this theory is that FA increases with the instability of an organism through its development (Van Vallen, 1962; Palmer, 1994), decreasing its adaptative fitness (Gonçalves et al., 2002; Almeida et al., 2008; Hermita et al., 2013). Since the degree of FA also reflects the adaptive ability of the entire population, allowing inferences on the health of the whole ecosystem (Øxnevad et al., 2002; Kristoffersen and Magoulas, 2009), FA studies are increasingly used over traditional methods for environmental and biomonitoring assessments (Palmer and Strobeck, 1992; Kitevski and Pyron, 2006; Graham et al., 2010 Graham et al., 2010). Increased levels of FA were correlated with decreased fertility, growth, reproductive success, egg size, and survival rates of some species (Somarakis et al., 1997; Morris et al., 2012), but the responses can differ with the taxa and the paired traits chosen for FA analyses (Lens et al., 2002). For example, traits of high functional importance are highly conservative during ontogeny, showing generally low FA values (Gonc¸alves et al., 2002; Trokovic et al., 2012). Since the differential response of each body structure to environmental disturbances can result in varied levels of FA (Palmer, 1994; Graham et al., 1998; Ayoade et al., 2004), the use of multiple traits is largely recommended to assess the FA at a population level, minimizing the potential bias of a particular trait (Leary and Allendorf, 1989; Palmer and Strobeck, 1992; Palmer, 1994).

Fish is the richest group among the vertebrates and spread in virtually all aquatic habitats, being thus not only susceptible to influences of environmental conditions throughout their development but also interesting candidates to test the potential of FA as an indicator of environmental health (Allenbach, 2011). Most endogenous stressors related to FA in fish are the level of inbreeding, heterozygosity, hybridization, and genetic based diseases (Palmer and Strobeck, 1986, 2003; Fries et al., 2004; Hermita et al., 2013), while commom biological stressors are the density of offspring and the degrees of competition predation, and parasitism (Palmer, 1994; Palmer and Strobeck, 2003; Allenbach, 2011). Exogenous stressors range from changes on physical habitat and variations in water salinity, pH, and temperature to the effect of pesticides, heavy metals, and even climatic changes (Palmer, 1994; Øxnevad et al., 2002; Kitevski and Pyron, 2006; Allenbach, 2011; Jawad et al., 2012). Despite the growing number of experiments demonstrating the relationships of bilateral asymmetry with fish traits (censu Allenbach, 2011), there are surprisingly few field studies that addressed how FA in fish is associated with habitat degradation, and similar studies for tropical marine fish are virtually unknown (Ayoade et al., 2004; Mamry et al., 2011; Jawad et al., 2012).

The Corocoro grunt *Orthopristis ruber* (Cuvier, 1830) is a Haemulid fish commonly distributed throughout the South Atlantic Ocean and widespread in several marine and estuarine systems along the Brazilian coast (Vianna and Verani, 2002). This sedentary species is one of the most abundant fish associated with rocky shores in Guanabara Bay (LNS; unpublished data), a tropical estuary located in Southeastern Brazil (Rio de Janeiro city) that historically undergoes high levels of contamination and habitat degradation (Kjerfve et al., 1997; Fonseca et al., 2013; Fistarol et al., 2015). Despite the greater abundance of *O. ruber* in the outer zones of this Bay, which are less degraded and more influenced by adjacent oceanic waters (Vianna and Verani, 2002; Rodrigues et al., 2007), there is no study that addressed the effects of environmental conditions on the bilateral symmetry of this species, despite the interesting potential of resident fish species closely associated with submerged habitats to be used as indicator of the integrity of whole ecosystem (Allenbach, 2011).

In this paper, the deviation from bilateral symmetry in six morphometric and meristic characters of *O. ruber* captured in three areas of different environmental characteristics in Guanabara Bay was investigated. The objectives of this work are: (1) to test whether the deviations in bilateral symmetry can be attributed to FA or other kinds of asymmetry; (2) to test for the levels of FA among the six morphological characters of *O. ruber* and the five FA indexes; and (3) to compare the levels of FA among the three areas of Guan- abara Bay and correlate them with physical and chemical water variables that are proxies of environmental conditions. The potential of applied studies using FA and fishes as indicator of the health of tropical marine system is also discussed.

2. MATERIAL AND METHODS

2.1. Study area

Guanabara Bay (22◦24′–22◦57′S; 42◦33′–43◦19′W) is a ∼380 km2 estuarine environment that accounts for the final destination of 50 rivers and streams that drain the Rio de Janeiro (the larger and most urbanised metropolis) and other 15 adjacent cities toward the sea (Fistarol et al., 2015). It is one of the most degraded coastal environments in Brazil, receiving high loads of organic matter and inorganic nutrients, and large amounts of industrial and agricultural effluents daily. Long term effects of disordered use of watershed and diffuse pollution, which started early in the XVI century but escalated especially from 1930 with the industrialization process, have increased significantly the concentration of inorganic nutrients (mainly phosphorus), heavy metals (including Pb, Cr, Cu and Ni), and several refractory organic pollutants (such as PCBs) at the water column and in the sediment (Kjerfve et al., 1997; Fonseca et al., 2013). As consequence, the current environmental and sanitary conditions of Guanabara Bay are so critical that is adversely affecting not only the entire aquatic community, from microbes to higher trophic levels of the marine life, but also human health (Fistarol et al., 2015). Currently, attention toward Guanabara Bay pollution increased significantly, because the city of Rio de Janeiro is going to host the 2016 Olympic Games and Guanabara Bay will be the venue for various outdoor aquatic sports during the games.

Water circulation in Guanabara Bay is complex, depending on the combining effects of tidal regime, freshwater runoff, and depth countour, which affect directly the gradients of water qualities in the Bay (Kjerfve et al., 1997; Fistarol et al., 2015). Most of the Bay (84%) has <10 m depth, ranging from a maximum depth of 58 m on its central channel to less than 1.0 m in the inner areas (Mayr et al., 1989). The presence of this deep channel allows the intrusion of more saline (34–36), oxygenated, and oligotrophic waters from the adjacent ocean, which positively affect the southeast parts of the Bay and also the areas more directly affected by the central channel (Mayr et al., 1989; Paranhos et al., 1998; Valentin et al., 1999). The influence of marine water is largely controlled by tide (mainly semidiurnal), with a maximum amplitude of 1.4 m and decreasing current velocities from the mouth of the Bay (80–150 cm/s^{-1}), toward the central areas (30–50 cm/s^{-1}), until to attain a minimum of less than 30 cm s−1 in the inner zones. Freshwater runoff follows, however, the opposite pattern, with the greater discharges (up to 186 m^3/s^{-1} in the rainy austral summer) largely affecting the innermost regions of the Bay (due to the greater amount of rivers, lower depths, and the lower influence of marine waters), in contrast to lower effects of freshwater discharges on the outer zones of the Bay, which are largely under marine influence and less affected by river drainages (Kjerfve et al., 1997). Therefore, there is an environmental gradient from the entrance of the Bay to its internal areas, in which more saline, oxygenated, transparent (up to 5.0 m), and clean waters, and high levels of marine biodiversity are generally found in the southeastern part and in areas near the central channel, in contrast to the gradual decrease of water quality and species richness toward the inner zones of the Bay (Mayr et al., 1989; Kjerfve et al., 1997). Recently, Fistarol et al. (2015) also demonstrated the presence of a lateral gradient in the Bay, with the worst water quality on its northern and northwestern parts (as a result of synergistic interactions of greatest loads of wastewater and lowest rates of water circulation) and better conditions on the central channel and the eastern part of the Bay (as consequence of higher dilution by seawater through tidal mixing).

2.2 Sampling design

Three sites were selected to encompass the environmental gradient along the main central channel in Guanabara Bay (Figure 1), following to the general classification of Mayr et al. (1989) but also taking into account the recent contributions of (Fistarol et al., 2015): (1) Urca (at Fora Beach); (2) Rio-Niterói Bridge (near the Botafogo cove); and (3) Paquetá Island (the southward area). Fora Beach is located near the entrance of the Bay and undergoes a strong influence of the high transparency (i.e. up to 5 m) and salinity (i.e. 34–36) waters from the adjacent ocean. Although the best environmental conditions are expected to occur in this region, as result of the high water circulation that dilutes the contaminants (Mayr et al., 1989), the Urca region is often subjected to high organic loads from large urbanised areas at the land, especially during the rainy season and stormwaters (Fistarol et al., 2015). The site located near the Rio-Niterói Bridge is at the central part of the Bay and also undergoes the circulation effects of the main central channel, which allows the intrusion of marine waters from the ocean (Mayr et al., 1989). Despite the potential for pollutants dilution by sea- water through tidal mixing, this site are also close to deteriorated areas at the western part of the Bay (such as the Cunha Canal and Rio de Janeiro seaport), which are under strong influence of urban and industrial contaminants (Fistarol et al., 2015). The site situated near the Paquetá Island is at the northward limit of the main channel of water circulation (Mayr et al., 1989). This area is still under influence of marine waters but water circulation is not as high as in the southeastern parts because of the decreased velocities in tidal currents. Although the relative low rates of water exchange, even so this site undergoes good water quality conditions according to Mayr et al. (1989) and Fistarol et al. (2015).

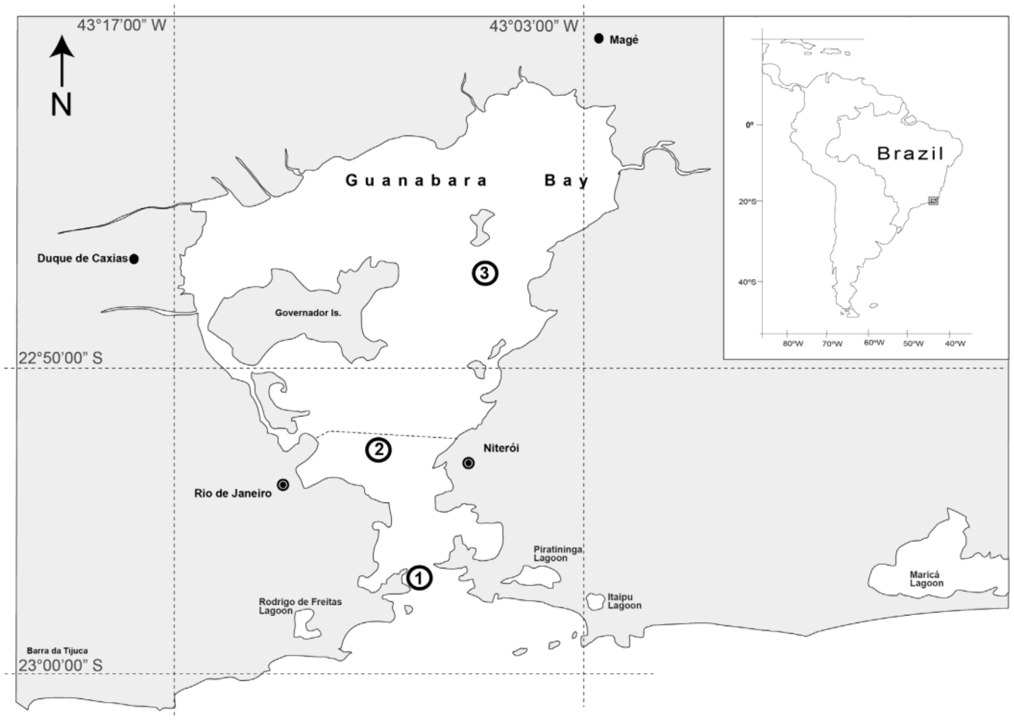

Figure 1. Geographic location of Guanabara Bay (Brazil), showing the sites where *Orthopristis ruber* were caught. 1 = Urca; 2 = Rio-Niterói Bridge; 3 = Paquetá island. (Map was gently provided by Áthila Bertoncini).

Monofilament gillnets (20 m) of three different mesh (15, 30 and 45 mm between adjacent knots) were tied together to form a gillnet set (60 m x 1.5 m) that was used to capture fish in the three sampling sites. Fish were caught in dry (September; winter) and rainy (December; spring) periods of 2011. Gillnet sets (three replicates per site) were installed, perpendicularly to the shore, over the rocky substrates of the three sampling sites and recovered 24 h later. Rocky substrates were chosen not only to standardize the habitat for sampling but also because of the high relationship of *O. ruber* with hard substrates. Concurrently to fish samplings, some physical and chemical water variables, such as temperature (°C), dissolved oxygen (mg/L), pH, salinity and transparency (i.e. Secchi depth; cm) were measured (at 1.0 m below surface; except for transparency) through a multiparameter probe (Hanna HI9828) to appraise the influence of environmental conditions on the bilateral symmetry of *O. ruber*.

2.3. Data treatment and analysis

All the 66 *O. ruber* captured were euthanised in ice in the field and them transferred to the Laboratory of Theoretical and Applied Ichthyology (LICTA) at Federal University of Rio de Janeiro State (UNIRIO), Rio de Janeiro city, Brazil. The right (R) and left (L) sides of six bilateral body structures of *O. ruber* were inspected by the same single researcher, using the same binocular stereomicroscope (Zeiss Stemi DV4; 8 magnification) and digital calliper (for morphometric attributes), in order to minimise the possible effects of methodological artifacts on asymmetry results. The three morpho metric traits assessed were the diameter of the eye, the length of the pectoral fins, and the length of ventral fins, while the three meristic traits evaluated were the number of gill rakers, the number of pectoral fin rays, and the number of ventral fin rays. Damaged structures that prevented measurements or counts were removed from the dataset.

Since other types of bilateral asymmetry (e.g. directional and antisymmetry, which are more related to a genetic basis than to developmental instability; Leary and Allendorf, 1989; Van Dongen et al., 1999; Palmer and Strobeck, 2003) might counfound our results, the Student t-test and distribution histograms were applied to evaluate the prevailing type of asymmetry in *O. ruber*. Effects of directional asymmetry (DA) are negligible if the mean differences between the right and left sides for each attribute were not significantly ($P > 0.05$) different from zero. The FA4 index was applied whenever the means differed significantly from zero ($P < 0.05$), following the procedure proposed by Palmer and Strobeck (2003) to control for the effects of DA. Histograms were also applied to appraise data distribution patterns, since a normal distribution of the measurements indicates the prevalence of fluctuating asym- metry (FA) whereas a bimodal distribution denotes antisymmetry (AS) (Palmer, 1994; Øxnevad et al., 2002). STATISTICA 8.0 was used to perform the Student t-tests and build the histograms on data distribution.

The patterns of FA might be also biased by the effects of the trait size (Palmer and Strobeck, 1986; Palmer, 1994). Therefore, Spearman and Pearson correlation tests were performed on the averaged measurements of the right and left sides for all six structures in *O. ruber* to test the dependence of the FA with the size of the analysed trait (Palmer and Strobeck, 2003; Kristoffersen and Magoulas, 2009). The FA2 index was thus applied whenever significant correlations ($P < 0.05$) were found, following the procedure proposed by Palmer (1994). This index was applied not only on attributes with significant FA trait size correlations, but also on all the analysed traits, in order to evaluate the potential of FA2 index to be used in studies on FA.

Three individual indexes, namely FA1, FA2, and FA5 as in Palmer (1994), were used to calculate the levels of FA in *O. ruber*. These three were the most widespread indexes that are commonly used in FA studies (Palmer and Strobeck, 1992; Palmer 1994). Although their positive qualities, such as easy application and understanding, and low vulnerability to data biases, these three individual indexes do not have the same sensitivity to drawn the levels of FA (Somarakis et al., 1997; Øxnevad et al., 2002; Kristoffersen and Magoulas, 2009). Therefore, the composite indexes CFA1 and CFA2 were also calculated to analyse the combined effects of FA levels from all ($N = 6$) the traits together, which are much less sensitive to sampling and measurement biases than individual indexes (Leung et al., 2000; Lens et al., 2002). The levels of FA were thus calculated for the six attributes of *O. ruber*, using the three individual (FA1, FA2, and FA5) and two composite indexes (CFA1 and CFA2).

Thirty *O. ruber* were ramdomly chosen and their six attributes were measured twice (i.e. independent measures) to evaluate the importance of measurement errors on FA levels, according to Palmer and Strobeck (1986). Permatutional Multivariate Analysis of Variance (PERMANOVA) was performed (Euclidean distance; 4999 permutations per analysis) on data matrix to test for possible differences between the first and second measurements for each trait. As in Palmer and Strobeck (1986), the side of the structure in *O. ruber* was considered as fixed factor and each fish as random factor in all PERMANOVA analyses. Significant ($P < 0.05$) trait side fish interactions denote negligible effects of measurement errors on the FA levels. PERMANOVA was also used (Euclidean distance; 4999 per- mutations per analysis) to compare the FA levels in *O. ruber* among the three studied areas of Guanabara Bay. PERMANOVA post-hoc tests (Euclidean distance; 4999 permutations per analysis) were applied whenever significant differences ($P < 0.05$) were detected to identify which areas differed significantly to each other. All PERMANOVA analyses were performed with PAST 3.10 (Hammer et al., 2001). Multivariate ordination analyses were also applied to assess the relationship of the FA levels in *O. ruber* with some physical and chemical water variables of the three studied areas of Guanabara Bay. Firstly, Principal Component Analysis (PCA), an indirect gradient technique based on correlation matrices (Tabachnick and Fidell, 2001), was applied on the environmental matrix to identify which water variables best explained the three sampling areas. A constrained technique was also applied on abiotic (i.e. water variables) and biological (i.e. FA values) matrices to assess their relationship in a single ordination diagram. Partial Redundancy Analysis (RDA) was thus applied, using pH, salinity and temperature as covariables (i.e. to control for the effects of ocean influence), to detect whether the levels of FA in *O. ruber* were related to the proxies (i.e. dissolved oxygen and transparency) of anthopogenic disturbances in each sampling area. RDA was performed only for individual and composite FA1 index, since it is the most widespread used in FA studies. All multivariate ordination analyses were performed in CANOCO 4.5 software (Leps and Smilauer, 2003).

3. RESULTS

3.1. Validating fluctuating asymmetry

Data distribution of all the six traits in *O. ruber* fitted satisfactorily to the expected assumptions of the normal curve (Figure 2), suggesting thus that AS is absent or negligible.

Except for the diameter of the eyes (mean = 2.02 mm; Student t-test P = 0.009), the mean differences between the right and left sides for the other five attributes did not depart significantly (P 0.08) from zero.

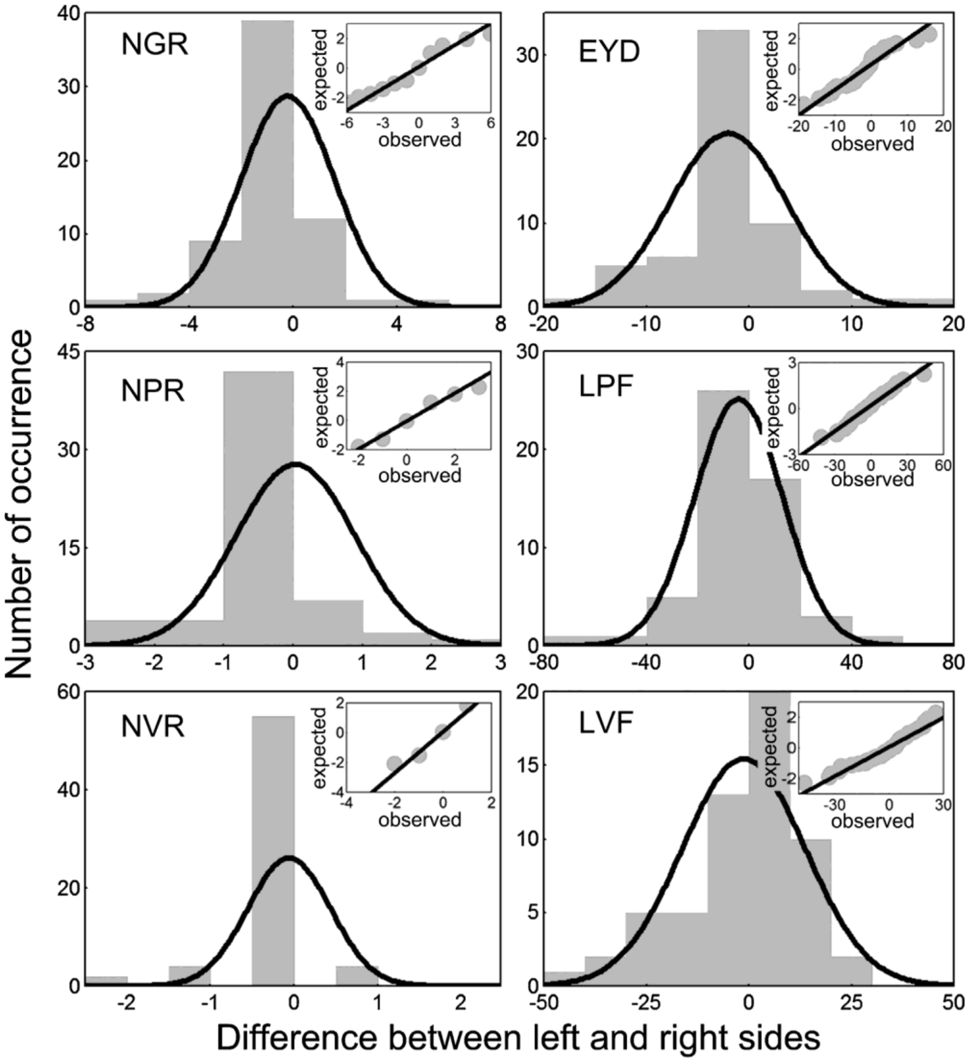

Figure 2. Frequency histograms of the differences (measurements or countings) between the right and left sides of each trait and the degree of adjustment of values to the expected normal curve distribution (figure at the top right corner of each histogram) for each of the six *O. ruber* traits. NGR: number of gill rakers; NPR: number of pectoral fin rays; NVR: number of ventral fin rays; EYD: diameter of the eyes; LPF: length of pectoral fin; LVF: length of ventral fin.

This difference persisted even when the procedure proposed by Palmer (1994) to control for this type of artifact was applied, indicating the presence of DA for the diameter of the eye. However, according to Palmer and Strobeck (2003), the effects of DA were considered less important than those of FA, since the value found by calculating the FA4 index (i.e. 4.54 mm) was higher than the mean difference (2.02 mm) found between the right and left sides for this attribute. Mean differences between right and left sides were positively correlated with *O. ruber* length for the number of pectoral fin rays (Pearson's r = 0.27; P < 0.05), the number of gill rakers (Pearson's r = 0.24; P = 0.05) and the diameter of the eye (Spearman's r = 0.35; P < 0.05). Except for the diameter of the eye (PERMANOVA; $F_{3,27}$ = 1.31; P = 0.20), significant trait side fish interactions were recorded for the other ve attributes analysed (PERMANOVA; P 0.02). Since the FA2 index was also calculated and generally applied to control for the effects of fish size on FA levels (Palmer, 1994), all the six attributes of *O. ruber* were considered for spatialanalysis of FA.

3.2. Spatial variations of fluctuating asymmetry

The levels of FA in *O. ruber* varied significantly with the areas of Guanabara Bay, but the patterns are quite different among the indexes of FA (Table 1). The number of gill rakers was the only attribute that showed significant spatial differences in the FA levels for all the three indexes, whereas no significant difference were found for the diameter of the eyes and the number of ventral fin rays.

Table 1: PERMANOVA results for individual and composite indexes of fluctuating asymmetry applied on the six morphological traits of *O. ruber* caught in Guanaba Bay. NGR: number of gill rakers; NPR: number of pectoral fin rays; NVR: number of ventral fin rays; EYD: eye diameter; LPF: length of pectoral fin; CNV: length of ventral fin; CFA: composite index; Ur: Urca; Br: Rio-Niterói Bridge; Pa: Paquetá. * P < 0.05. ** P < 0.01.

O. ruber traits	Fluctuating Asymmetry Indexes					
	FA1		FA2		FA5	
	F	Post-hoc	F	Post-hoc	F	Post-hoc
NPR	0.86		**3.21***	Br > Ur	0.47	
NVR	0.10		0.08		0.09	
NGR	**4.46***	Pa > Ur	**4.69***	Pa > Ur	**4.45***	Pa> Ur
DO	0.64		0.48		0.36	
LPF	**4.16***	Pa = Br > Ur	1.36		**5.72***	Br > Ur
LVF	**3.75***	Pa = Br > Ur	1.98		1.98	
CFA	**9.29****	Pa = Br > Ur	**4.51***	Pa > Ur		

Overall the levels of FA in *O. ruber* were consistently lower at the Urca region than in the other two areas, but especially if the number of gill rakers and the results of FA1 index were taken into account (Figure 3). Differences of the FA levels in *O. ruber* were generally lower between Rio-Niterói Bridge and Paquetá Island, but *O. ruber* was significantly more asymmetric in

Paquetá Island in relation to the number of the gill rakers (irrespectively of the individual index used) and the FA2 composite index. Nevertheless, *O. ruber* was significantly more asymmetric in Rio-Niterói Bridge in relation to the pectoral fin, more specifically according to the FA2 index for the length of this attribute and the FA5 index for the number of fin rays.

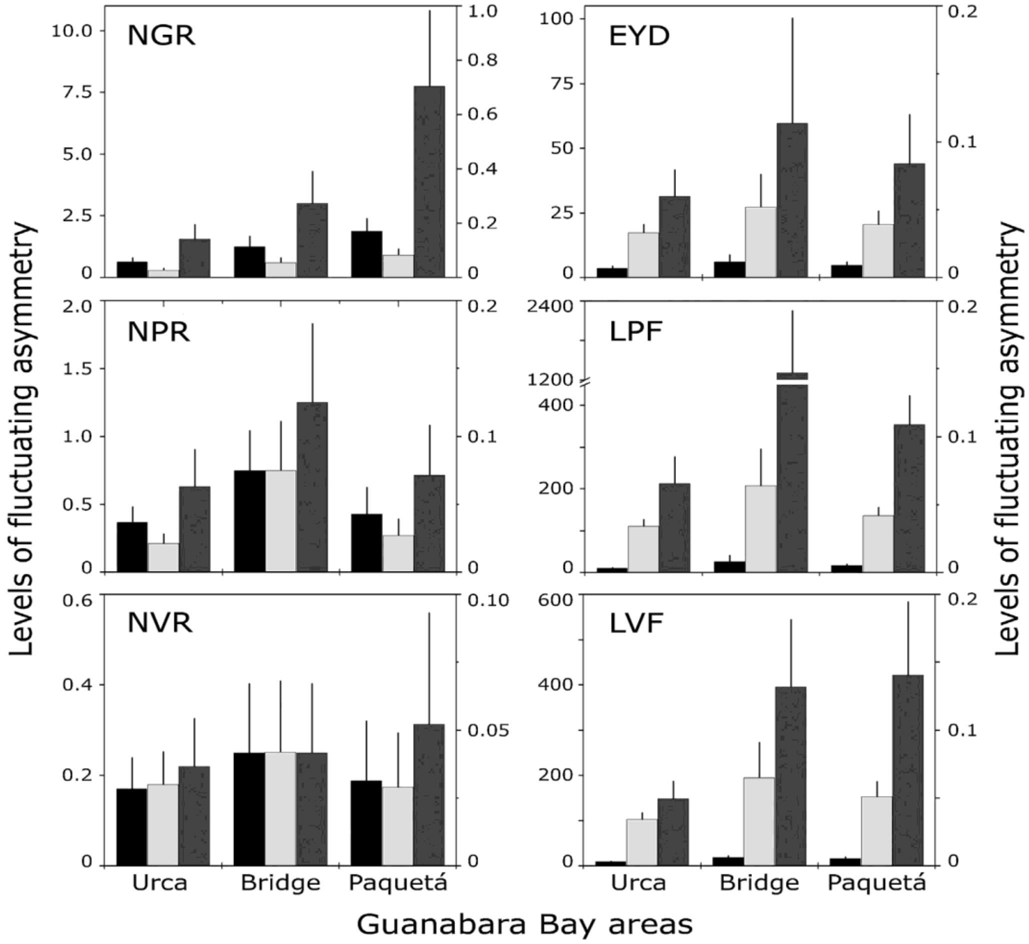

Figure 3. Variations in the levels of fluctuating asymmetry calculated through the three individual indexes for the six traits of *O. ruber* caught in Guanabara Bay. Vertical lines represent the standard error. NGR = number of gill rakers; NPR = number of pectoral fin rays; NVR = number of ventral fin rays; EYD = diameter of the eyes; LPF = length of pectoral fin; LVF = length of ventral fin; black column = FA1 index; light gray column = FA2 index; dark gray column = FA5 index. Values for FA1 and FA5 indexes are showed in the left Y-axis, while values for FA2 index are showed in the right Y-axis.

Composite indexes overall followed the general trend observed for individual indexes (Figure 4), but differences were stronger for CFA1 ($P < 0.01$) than in CFA2 ($P < 0.05$). Both indexes indicated that *O. ruber* was less asymmetric in Urca region, but, in contrast to the CFA1 results, the FA levels in *O. ruber* of Rio- Niterói Bridge were not clearly separated from the other two areas according to CFA2 index.

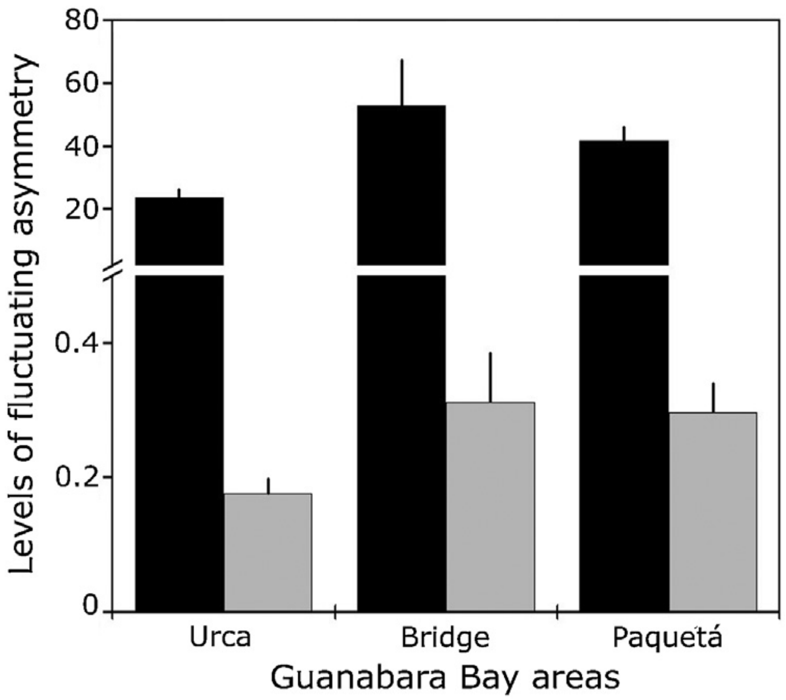

Figure 4. Variations in the levels of fluctuating asymmetry calculated through the two composite indexes for the six traits of *O. ruber* caught in Guanabara Bay. Vertical lines represent the standard error; black column = CFA1 index; light gray column: CFA2 index.

3.3. Effects of environmental variables on fluctuating asymmetry

Subsurface waters along the main central channel in Guanabara Bay are typical of coastal marine systems for most of the environmental variables, showing averaged temperatures above 20ºC, alkaline pH values, salinity of 33.2, and high levels (>7.0 mg/L) of dissolved oxygen (Table 2). However, the low averaged values recorded for water transparency (<2.0 m) and the broad spatial variation observed for dissolved oxygen also indicated the influence of estuarine and of lower quality waters in the Bay. Overall more saline, transparent, and alkaline waters prevailed in the outer area (i.e. Urca), in contrast to the dominance of warmed and turbid waters near to Paquetá Island, the innermost site that is also at the end of influence of marine waters from the major central channel. The region close to the Rio-Niterói Bridge showed, in general, intermediate environmental conditions in relation to the two other sites.

Table 2: Mean values and range (between parentheses) of temperature, salinity, dissolved oxygen (mg/L), pH, and transparency (m) recorded on the sampling sites at Guanabara Bay where *O. ruber* was caught. Values were provided for the entire dataset (pooled) and for each sampling site.

Environmental variable	Pooled values	Guanabara Bay Sampling Sites		
		Urca	Rio-Niterói Bridge	Paquetá Island
Temperature (◦C)	22.09	20.00	22.42	23.86
	(17.2–25.2)	(17.2–22.2)	(22.1–23.4)	(23.4–25.2)
Salinity	33.23	34.45	33.10	32.15
	(29.1–35.4)	(32.0–35.4)	(30.9–34.0)	(29.1–33.3)
Dissolved oxygen (mg/L)	7.45	6.94	6.83	8.59
	(5.6–9.6)	(5.6–8.5)	(6.6–7.0)	(6.0–9.6)
pH	8.44	8.50	8.45	8.39
	(7.7–9.0)	(7.9–9.0)	(8.3–8.6)	(7.7–8.7)
Transparency (m)	1.59	2.02	1.93	0.82
	(0.6–3.4)	(0.9–3.4)	(0.7–2.6)	(0.6–1.0)

The spatial and seasonal changes in the physical and chemical conditions of the subsurface waters of the Guanabara Bay are summarized by the first two PCA axes, which together accounted for 71.5% of total variance (Figure 5). PCA axis 1 was highly significant (Monte Carlo test; P = 0.01) and explained 43.2% of the data variation, clearly separating the study sites. This axis was positively correlated with temperature (r = 0.89) and the samples of Paquetá Island, in both dry and rainy periods, and Rio-Niterói Bridge, during the rainy season. In contrast, PCA axis 1 was negatively correlated with salinity (r = 0.87) and transparency (r = 0.52), and the samples of Urca region, particularly in the dry season. PCA axis 2 was marginally significant (Monte Carlo test; P = 0.06) and explained 28.3% of the data variance, clearly separating samples of the rainy season, at the positive quadrant, from those of the dry period, at the lower portion of the diagram. Samples of the dry period were also associated with high values of dissolved oxygen (r = 0.74) and transparency (r = 0.71).

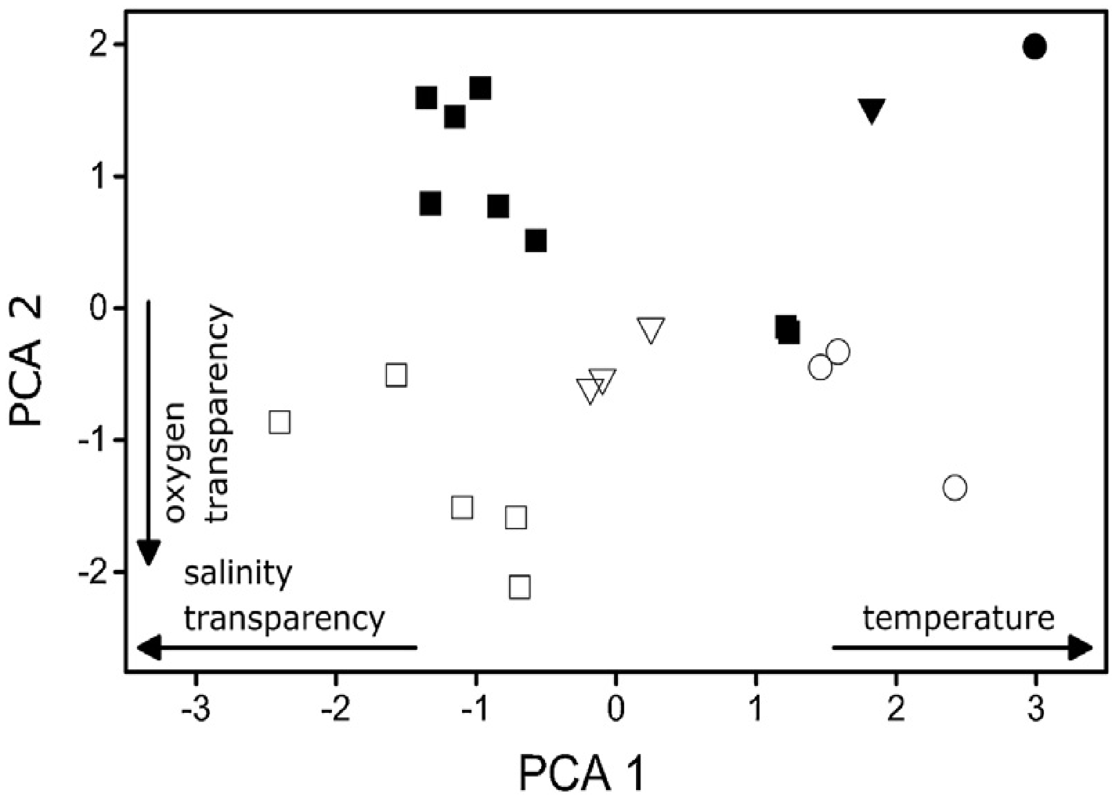

Figure 5. Ordination diagram (the first two axes) of the Principal component analysis (PCA) applied on the environmental matrix obtained for the three sampling sites of *O. ruber* at Guanabara Bay. The directions of the arrows indicate which variables showed greater correlations to the distribution of the samples along each axis. Season-site legend: □ = dry-Urca; ■ = rainy-Urca; ▽ = dry-Bridge; ▼ = rainy-Bridge; ○ = dry-Paqueta; ● = rainy-Paquetá.

The relationship of the FA levels in *O. ruber* with environmental variables, sampling sites, and seasons was summarized in Figure 6. The first two RDA axes were only marginally significant, either when individual FA1 (Monte Carlo test; P = 0.11; Figure 6A) or composite CFA1 (Monte Carlo test; P = 0.06; Figure 6B) indexes were computed in the analysis. However, there were similar and complementary patterns shared by the two partial RDAs, after controlling for the influence of marine waters, which were also quite different from the PERMANOVA (Table 1; Figs. 3 and 4) and PCA results (Figure 5). The increased FA values found for the length of pectoral fin were mostly related to the samples of Rio-Niterói Bridge and to low values of dissolved oxygen and water transparency, but, despite their inverse relationship with the samples of Urca and Paquetá Island, this attribute did not clearly separate the samples of these latter two sites (Figure 6A).The high FA values found for the number of gill rakers and the length of ventral fin were largely related to the samples of Rio-Niterói Bridge during the dry season, and secondarily to those of Paquetá Island, but inversely associated with dissolved oxygen and the samples of Urca region.

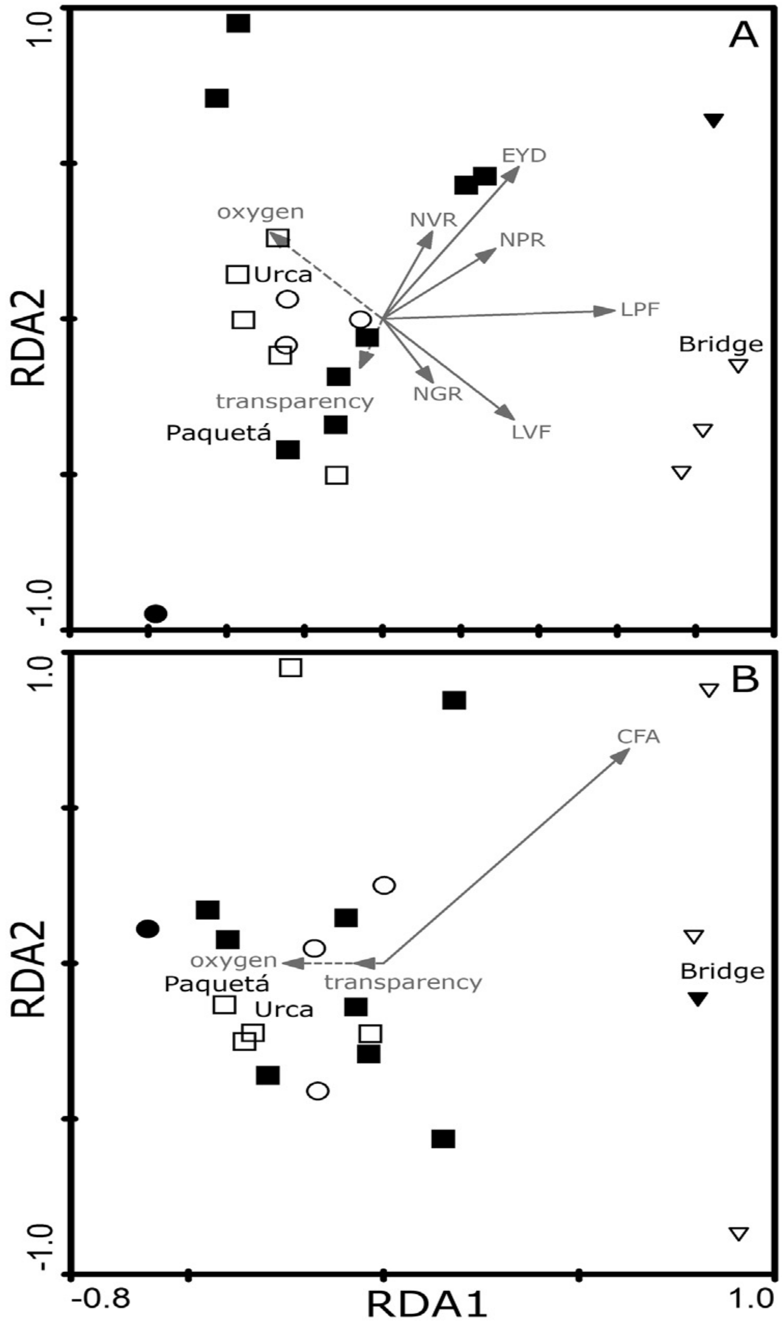

Figure 6. Partial Redundancy Analysis (RDA) showing the relationship of the levels of fluctuating asymmetry (FA) in *O. ruber* with dissolved oxygen and water transparency after the control for seawater influence (i.e. salinity, pH, and temperature usead as covariables). Top panel (A) = Partial RDA of the levels of fluctuating asymmetry calculated for each individual trait (FA1 index); bottom panel (B) = Partial RDA of the levels of fluctuating asymmetry calculated for the composite index (CFA1). NGR = number of gill rakers; NPR = number of pectoral fin rays; NVR

= number of ven- tral fin rays; EYD = diameter of the eyes; LPF = length of pectoral fin; LVF = length of ventral fin length. Season-site legend: □ = dry-Urca; ■ = rainy-Urca; ▽ = dry-Bridge; ▼ = rainy-Bridge; ○ = dry-Paqueta; ● = rainy-Paquetá.

Interestingly, the other three attributes that showed no significant spatial differences for FA (as in Table 1; Figure 3) were negatively associated with the samples of Paquetá Island and water transparency, and secondarily related to the samples of Rio-Niterói Bridge and some samples of Urca region during the rainy season. The partial RDA computed with the values of composite CFA1 index showed a similar pattern of spatial separation of the samples of Rio-Niterói Bridge, also associated with high FA levels, but did not clearly discriminate the samples of Urca and Paquetá Island, more associated with low FA levels and high values of dissolved oxygen.

4. DISCUSSION

4.1. Confirmation of fluctuating asymmetry

The presence of FA was confirmed for *O. ruber*, but not at the same degree among the six biological features or the FA indexes tested. Among the biological attributes, the number of gill rakers and the diameter of the eyes showed, respectively, the best (i.e. spatial differences in FA recurrently recorded for all the three indexes) and the worst (i.e. no spatial difference) potential for detecting FA in *O. ruber*. These differences appeared to be mostly related to the synergetic effects of measurement errors and the interference of other types of asymmetry, since none of these potential biases were detected for the number of gill rakers while the opposite trend (i.e. significant measuring errors and DA) was found for the diameter of the eye. The other individual traits showed intermediate levels of sensitivity to detect FA, except for the length of pectoral fins, to which spatial differences were detected by two different indexes. Our findings agree thus with previous studies that demonstrated species-specific abilities of fish traits to detect FA (Øxnevad et al., 2002; Allenbach, 2011; Mamry et al., 2011; Jawad et al., 2012).

The number of gill rakers is one of the features most widespread used in studies on FA and fish (Øxnevad et al., 2002; Allenbach, 2011; Jawad et al., 2012). Agreeing with our results, significant levels of environmentally related asymmetry were also recorded in gill rakers of the striped bass *Morone saxatilis* (Fries et al., 2004), Europen perch *Perca fluviatilis* (Øxnevad et al., 2002), and African carp *Labeo parvus* (Ayoade et al., 2004). The potential of fish gills anomalies to be used as biomarkers of environmental quality in tropical freshwater systems were also addressed by Santos et al. (2011), Gomes et al. (2012), and Nascimento et al. (2012), although those studies did not specifically addressed the effects of FA. Taking into account our findings and the results from previous studies, the number of gill rakers is thus an interesting candidate to be used as a key attribute to validate the presence of FA in fish.

Despite the high potential found for the number of gill rakers, the sensitivity of meristic traits to detect FA in fish is generally controversial, because biological changes resulting from adverse environmental conditions are expected be fixed mostly in the early life stages than, except under uncommon levels of environmental disturbances, during the ontogenetic development (Kristoffersen and Magoulas, 2009; Mamry et al., 2011). The FA values also apparently increased with *O. ruber* size for some traits, including two meristic (i.e. the number of gill rakers and the number of pectoral fin rays) and one morphometric (i.e. the diameter of the eyes). However, the FA2 index (i.e. an approach designed especially to control for the confounding effects of fish size) was useful only to detect FA for the number of pectoral fin rays, with few changes on the results of the other traits tested. Since these findings could be also affected by the greater difficulty to measure accurately FA in small-sized individuals than to purely the aggravation of assimetry with fish size, further experimental studies should be performed to assess how meristic and morphometric traits would respond to cumulative effects of environmental degradation throughout fish ontogeny.

Surprisingly, the morphometric traits played at most a secondary role as indicators of FA in *O. ruber*, constrasting to mostly results from similar studies. Even the length of the pectoral fins, a morphometric trait applied to detect FA in the peacock blenny *Salaria pavo* (Gonc¸alves et al., 2002), mosquito fish *Gambusia affinis* (Allenbach et al., 1999), goldfish *Carassius auratus* and common carp *Cyprinus carpio* (Almeida et al., 2008), was apparently less effective than the number of gill rakers to detect FA in *O. ruber*. In addition to its lower sensitivity to environmental disturbances (Jawad et al., 2012), the negligible importance of the diameter of the eyes to detect FA in our study might be also related to the notfully understood effects of DA, to which no genetic or ecological causes can be found to explain the hypothetical differences for the eye sizes in *O. ruber*. Therefore, other traits, such as the measurements of the head and otoliths (Lens et al., 2002; Almeida et al., 2008; Kristoffersen and Magoulas, 2009; Allenbach, 2011), should be further tested to provide a better knowledge on the potential of morphometric attributes to detect FA in tropical marine fishes.

4.2. Spatial changes of fluctuating asymmetry

The levels of FA in *O. ruber* differed significantly among the areas of Guanabara Bay, and despite the variations in the sensitivity between biological traits, corocoro grunts of the Urca region were less bilaterally asymmetric than those at the other two inner areas of the Bay. No clear difference was found, through univariate PERMANOVAs, between Rio-Niterói Bridge and

Paquetá Island for the FA levels in *O. ruber*, suggesting these two areas were more similar in relation to environmental conditions. Those results contrast partially with the scores of our first PCA axis (Figure 5) and the previous hypothesis that the FA levels would increase progressively toward the inner zones of Guanabara Bay (i.e. Urca < Rio-Niterói Bridge < Paquetá Island), as a response to the gradual decrease of marine influence, lowering the potential of pollutant dilution by clean seawater (Mayr et al., 1989).

However, the influence of water quality on FA levels was surprisingly more complex, since the potential confounding effects of seawater dilution was controlled and the load of variables (i.e. dissolved oxygen and transparency) considered better proxies for environmental degradation was highlighted through partial RDAs (Figure 6). These new findings revealed that the worst water conditions (i.e. low values of oxygen and transparency) and high FA levels in *O. ruber*, considering not only the contribution of all traits separately but also the account of composite CFA1 index, occurred in Rio-Niterói Bridge. In addition, the differences between Urca and Paquetá Island for the levels of FA in *O. ruber* were not as high as predicted, being mostly related to the individual contribution of each trait and to its differential responses to oxygen and salinity. The emerging patterns from RDAs might be, however, largely explained by the spatial gradient proposed by Fistarol et al. (2015), in which the ultimate environmental scenario is a result of the combined effects of marine water influence (i.e. north-south gradient) with land use patterns and freshwater runnof in the vicinity of each zone of the Bay.

In this sense, the lower FA levels recorded for *O. ruber* at Urca were greatly influenced by the number of gill rakers and length of ventral fins, and secondarily by the length of pectoral fins, the traits that invidually weighted more to the spatial pattern found for asymmetry (see Table 1) and to which the values of FA were the lowest in this region. Despite the high levels of dissolved oxygen, especially during the dry season, either envinronmental conditions or the FA levels were more variable during the wet season, when high loads of raw sewage are carried into the Bay by frequent and intense rainfalls (Fistarol et al., 2015), also adversely affecting water transparency. However, the overall lower FA levels recorded for *O. ruber* at Urca suggest that this species was not much adversely affected by those periods of low water transparency and high loads of organic waste, probably because of counterbalanced positive effects of the increased richness and availability of benthonic preys (Lavrado et al., 2000; Rodrigues et al., 2007; Omena et al., 2012). Therefore, *O. ruber* was apparently affected by both direct (i.e. sea-water dilution of contaminants) and indirect (i.e. availability of feeding resources) positive effects of the prevalent marine conditions at Urca, which can be supported not only by the low FA levels found but also by the high abundance of corocoro grunts in this region (Chaves, 2013).

The FA levels in *O. ruber* of the other two studied sites at Guanabara Bay were higher than those recorded at Urca, but due to quite different reasons that are closely related to the prevalent environmental conditions of each site. Paquetá Island is at the edge of the direct influence of seawater intrusion from the main channel (Mayr et al., 1989), as supported by our records of lower salinities and higher temperatures (Table 2; Figure 5). Despite the general prediction on decreased environmental conditions toward the inner areas of the Bay, which is partially influenced by the low rates of water exchange, even so Paquetá Island undergoes good water quality conditions (Mayr et al., 1989; Fistarol et al., 2015). In addition, our results revealed that the FA levels in *O. ruber* and the values of water transparency and dissolved oxygen were not much different from those at Urca after controlling for the influence of marine waters (i.e. partial RDAs; particularly Figure 6B). Therefore, the FA levels recorded for *O. ruber* at Paquetá Island were probably more associated with the decreased influence of seawater and its adverse influence on physiology and competitive abilities of corocoro grunt to deal with other fish species more adapted to estuarine conditions (i.e. such as the Guri sea catfish *Genidens genidens* and whitemouth croaker *Micropogonias furnieri*; Santos et al., 2007), than to degradation of the surrounding shoreline, which is expected to be only moderate in this region (Fistarol et al., 2015). Interestingly, the FA levels of *O. ruber* at Paquetá Island were, however, more sensitive to traits different from those that contributed more for Urca, raising mostly due to contributions of the number of gill rakers and the length of ventral fins (i.e. the two traits that invidually weighted more to the spatial FA pattern), but receiving few influence from the diameter of the eye, number of the pectoral fin rays, and number of the ventral fin rays (i.e. traits that invidually led little to the spatial FA pattern).

Located at the central part of the Bay, the site near the Rio-Niterói Bridge is under influence of heavy subsurface currents, which enhance the effects of water exchange with the adjacent ocean and lead to seawater conditions similar to those found at Urca, particularly during the dry season, according to our PCA results. However, the FA levels in *O. ruber* associated with Rio-Niterói Bridge were high and not significantly different than those recorded for Paqueta Island. Partial RDAs (Figure 6A and B) also revealed that emergent underlying factors, apparently more associated with environmental degradation (i.e. low values of dissolved oxygen and water transparency) than to the influence of seawater circulation, were the key causes of the FA levels recorded for *O. ruber* at the Rio-Niterói Bridge. This site is close to the most deteriorated areas of Guanabara Bay, undergoing a strong influence of domestic sewage and industrial contaminants, which are primarily carried to the region near to Rio-Niterói Bridge through the Canal do Cunha drainage basin (Kjerfve et al., 1997). Another potential but virtually unknown source of fish stress and contamination at this site is the traffic of shipping vessels, which are particularly intense due to proximity to the Rio de Janeiro seaport, the major local centre of domestic and international routes of cargo, touristic, and people transportation by the sea. The deviations found in bilateral symmetry of *O. ruber* near the Rio-Niterói Bridge can be thus mostly attributed to the levels of pollutants and environmental stressors in this zone, such as the high concentrations of heavy metals (particularly Pb, Cu, Cd, Zn and Ni) at water column and bottom sediments (Borges et al., 2014). A similar trend were observed by Kitevski and Pyron (2006) for the banded killifish *Fundulus diaphanus* in Lake Erie, which showed a more asymmetrical banding colour pattern at contaminated sites with heavy metals and pesticides. However, the adverse effects of environmental conditions on *O. ruber* symmetry only became more evident after the control for influence of marine waters, being supported by all the FA indexes tested (Figure 6A and B).

4.3. Implications for impact assessment

Our findings indicate that the deviations in bilateral symmetry of *O. ruber* have an interesting potential to be applied as proxy for changes of environmental conditions in Guanabara Bay. They further reveal that applying a proper methodological approach, which comprises the sequential selection and fitness evaluation of a suite of metrics, is of critical importance to validate the use of FA as an accurate indicator of anthropogenic disturbances in field non-experimental studies. According to our results, the procedure followed here, which encompassed from the proper selection of target species to the control for potential artifacts from environmental covariables, fulfilled the major requisites to tie the changes of environmental conditions in Guanabara Bay with the levels of FA in *O. ruber*. Therefore, if similar studies were performed, it would be an interesting opportunity to test whether the methodology used here could be applied to environmental assessment of other ecosystems. The corocoro grunt *O. ruber* was primarily targeted to perform this pioneering FA study because it is one of the most abundant and broadly distributed fish species throughout the Guanabara Bay (Rodrigues et al., 2007; Chaves, 2013). In addition, this fish species has a close relationship with bottom-consolidated substrates (i.e. rocky reefs) to obtain food (i.e. mostly invertebrates and organic detritus) and refuge, and has a moderate to strong site fidelity within the size range (85–256 mm of total length) in which the individuals were assessed (Santos et al., 2007; Santos et al., 2010). Since the levels of FA recorded in *O. ruber* were well correlated to the spatial changes of environmental conditions in Guanabara Bay, our findings suggest that the suite of ecological attributes showed by this species was key to detect environmental disturbances at local scales and should be thus taken into account by similar studies using fish as ecological indicators of tropical polluted Bays.

Apart to our study, *O. ruber* was priorly used to assess mercury bioaccumulation and food web transference within Guanabara Bay (Kehrig et al., 2010, 2011) and to compare mercury biomagnification and biochemical indicators of contaminants among the coastal Bays in Rio de

Janeiro State (Ventura et al., 2002; Bisi et al., 2012). Besides *O. ruber*, other fish species, such as *G. genidens, M. furnieri*, the white mullet *Mugil liza*, and the Atlantic cutlassfish *Trichiurus lepturus*, were previously tested as bioindicators of marine contamination, especially by mercury and selenium, not only in Guanabara Bay but also in nearby Bays at Rio de Janeiro State (Kehrig et al., 1998, 2001, 2010, 2011; Ventura et al., 2002; Bisi et al., 2012; Seixas et al., 2007, 2012, 2013). Surprisingly, except for our paper, none of these previous studies addressed the potential of a single fish species to detect spatial changes of environmental conditions within the same Bay, probably due to the inadequacy of these other fish species to meet the ecological attributes (i.e. benthonic and non-migratory habits) required in studies of such nature. Other two studies stressed the potential of the catfish *G. genidens* (Silva-Junior et al., 2013) and the pond perch *Diplectrum radiale* (Silva-Junior et al., 2012) to be used as sentinel species of environmental disturbances among Brazilian estuarine systems, but failed to detect responses to site-specific degradation within the Guanabara Bay, agreeing with our hypothesis. Therefore, further studies would be interesting to test whether the FA approach could be broadened to deal with fish species other than *O. ruber* and with varied ecological requirements, in order to evaluate their potential to be used as bioindicators of spatial degradation along environmental gradients in Guanabara Bay.

Finally, our findings indicate that, through the deviations of bilateral symmetry recorded in *O. ruber*, the environmental conditions are overall better near the entrance of Guanabara Bay (=lower FA levels), decreasing toward the inner areas of this ecosystem (=higher FA levels), aggreing thus with previous studies using other inorganic or biochemical markers. Our results also confirm that environmental quality do not decreased linearly toward the inner zones of the Bay, but respond probably to complex interactions among seawater dilution of pollutants, land use patterns and fresh-water runnof in the vicinity of each site, and the presence and accumulation over time of refractory contaminants, such as heavy metals and pesticides. In addition to indicate the need of more novel, accurate, and complementary approaches to precisely evaluate the spatial changes of environmental conditions in Guanabara Bay, our study also can be used to, on the basis of a long-term assessment programme that is currently being conducted in Guanabara Bay (http://www.lncc.br/peldguanabara), check whether the government measures launched to reach the goals set by the Olympic Committee will successfully restore water quality and the surrounding shoreline of Guanabara Bay.

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Fluctuating asymmetry and organosomatic indexes in fish: the Corocoro grunt Orthopristis ruber (Haemulidae) as a case study

Luana B. Seixas[a], Luciano Neves dos Santos[b], Alejandra Filippo Gonzalez Neves dos Santos[c]

[a]Fluminense Federal University (UFF), Graduate Course in Ocean and Earth Dynamics, Av. General Milton Tavares de Souza, s/n, 4° andar, Campus da Praia Vermelha, Niterói, RJ, Brazil, CEP: 24210-346.

[b]Federal University of Rio de Janeiro State (UNIRIO), Av. Pasteur, 458-R314A, Rio de Janeiro, RJ, Brazil, CEP: 22290-240.

[c]Department of Zootechny and Sustainable Socioenvironmental Development, Fluminense Federal University (UFF), Rua Vital Brasil, 64, Niterói, RJ, Brazil, CEP: 24230340.

ABSTRACT

Fluctuating asymmetry (FA) is regarded as random changes on bilateral symmetry plan of paired morphological characters in response of environmental disturbances. The relationship of FA with gonadossomatic (GSI), hepatosomatic (HSI), and repletion (RI) indexes and condition factor (K) for juveniles, adults, males and females of Corocoro grunt *Orthopristis ruber* (Cuvier, 1830) were evaluated in this paper. The composite fluctuating asymmetry (CFA1) was used to calculate the combined effects of FA over these four physiological traits of 66 *O. ruber* caught during 2011 in Guanabara Bay, Brazil, one of the most eutrophicated coastal Bay in the world. Redundancy Analysis (RDA) confirmed a significant relationship between FA and the physiological descriptors (HSI, RI, K), but without clear differences among juveniles, adults, males or females. Our results support the potential of FA to be used as proxy of environmental effects over reef associated fish species in a tropical Bay, but the relationship between FA and physiological descriptors are complex, requiring further studies, especially experimental trial, to elucidate it.

Keywords: Haemulid, bioindicator, Guanabara Bay, Brazil

1.INTRODUCTION

Fluctuating asymmetry (FA) is defined as random morphological deviations in the bilateral symmetry planes of an organism due to the effects of adverse of exogenous stressors in habitats, either of natural or anthropogenic origin (Van Vallen,1962; Palmer, 1994). Since FA rises with the increasingly instability of an organism through its development (Van Vallen, 1962), the levels of FA are thus correlated with individual fitness, which is the ability to survive, flourish, and pass these successful qualities through genes to offspring (Palmer, 1994; Oxnevad et al., 2002). Therefore, the levels of FA reflect the adaptive ability of the entire population, allowing inferences on the health of the whole ecosystem (Øxnevad et al., 2002). Previous studies have addressed the relationship between FA levels and adaptive fitness, reproductive success (Bakker et al., 2006), egg size (Hechter et al., 2000) and survival rates of fishes (Lajus et al., 2015). However, whether FA levels are associated with physiological descriptors if fish, especially from tropical marine ecosystems, are virtually unknown.

Guanabara Bay (GB), southeastern Brazil, is one of the most degraded coastal environments in the world, undergoing long term effects of organic and chemical diffuse pollution and disordered use of watershed (Seixas et al., 2016). Environmental disturbances started early in the XVI century but escalated especially since 1930 with the aggravation of industrialization process, which have increased significantly the concentrations of inorganic nutrients (mainly phosphorus), heavy metals (including Pb, Cr, Cu and Ni), and several refractory organic pollutants (such as PCBs) both in the water column and bottom sediments (Kjerfve et al., 1997; Kehrig et al., 2010; Silveira et al., 2017). The second largest industrial site of Brazil, located in Rio de Janeiro city and surroundings, is also found in this area. There are more than 12.000 industries in the drainage basin, accounting for 60% of the state's facilities, accounting for 25% of the organic pollution released to the Bay (Soares-Gomes et al., 2016; Baptista-Neto et al., 2017). Approximately 500 tons of raw sewage is discharged daily through river inflow, responding for 80% of biochemical oxygen demand (BOD) and including a complex mixture of nutrients and toxic chemicals loaded, in the water column and bottom sediments (Silveira et al., 2017). Therefore, Guanabara shows a complex pattern of water quality, of considerable spatial and temporal variability, depending on the combining effects of river inflow, watershed use, and seasonal regime of rainstorms, this latter harshening the input of seawage and chemical contaminants to the system (Kjerfve et al., 1997).

The Corocoro grunt *Orthopristis ruber* (Cuvier, 1830) is a Haemulid fish commonly near rocky and reef substrates over the South Atlantic coast and widespread in several marine and estuarine systems along the Brazilian coast (Vianna and Verani, 2002). *Orthopristis ruber* preys mainly on invertebrates and small fish (Kehrig et al., 2010) and spawns throughout the year, but peaking during spring and summer (Garcia-Junior et al., 2010). This species is one of the most abundant fish associated with rocky shores in Guanabara Bay (Seixas et al., 2016), showing apparently high site-fidelity when smaller than 300mm total length and greater abundances in the outer zones of this Bay (=lower estuary) (Chaves et al. 2018). The potential of using FA levels of *O. ruber* as indicator of the ecological integrity of zones of Guanabara Bay was evaluated by Seixas et al. (2016). In this paper, the relationship between FA levels and four physiological descriptors of *O. ruber* was addressed and its implications of being applied as proxies of ecological integrity of tropical Bays were discussed.

2.MATERIALS AND METHODS

2.1.Study area

Guanabara Bay (22º24'– 22º57'S; 42º33'– 43º19'W) is one of the most degraded coastal environments in the world, covering approximately 384 km^2 of surface area and yielding 12 million inhabitants living in the surrounding, wherein 74.3% are of urbanised areas (IBGE, 2013). The drainage basin accounts for receptor of most of the effluents produced by industrial plants, two international airports, and two harbours landing approximately 2.000 commercial ships every year (Baptista-Neto et al., 2017). The Bay also has two naval bases, 20 shipyards, thousands of ferries, fishing boats, and yachts, and a Petrochemical Complex that respond by 7% of the national oil refining (Kjerfve et al., 1997). Sedimentation rates in the Bay ranges from 0.60-2.2 cm per year, and their growing levels are attributed to the increased urbanization process (Soares-Gomes et al., 2016). Its fish assemblage is composed by approximately 174 marine or estuarine species (Vianna et al., 2012), which, despite the environmental disturbances, continue to use this ecosystem as feeding and nursery grounds (Castro et al., 2005; Franco et al., 2016; Silva et al., 2018).

2.2.Fish sampling and data analysis

Fish was sampled from inner to outer zones of Guanabara Bay, Rio de Janeiro, Brazil, encompassing most of its environmental gradient (Figure 1). Monofilament gillnets (20 m) of three different mesh (15, 30 and 45 mm between adjacent knots) were tied together to form set (60 m × 1.5 m) that was used to capture fish in the three sampling sites. Fish were caught in dry (September; winter) and rainy (December; spring) periods of 2011. Gillnet sets (three replicates per site) were deployed, perpendicularly to the shore, over the rocky substrates of the three sampling sites and recovered 24h later. Rocky substrates were chosen not only to standardize the habitat for sampling but also because of the high fidelity of *O. ruber* with hard substrates.

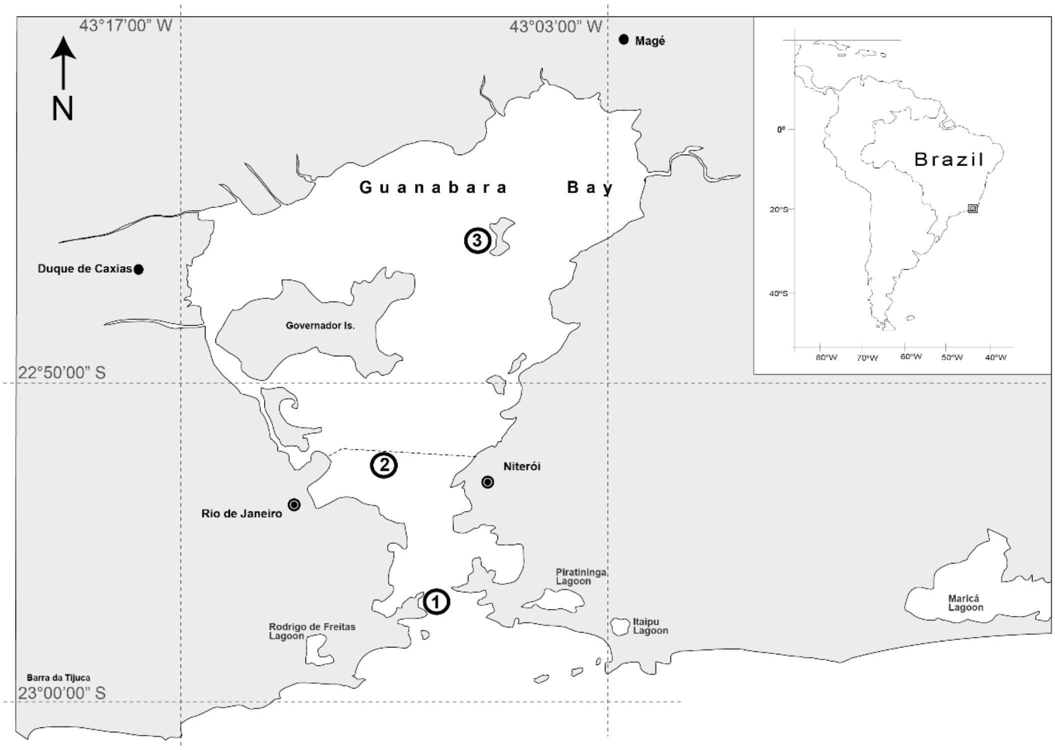

Figure 1. Geographic location of Guanabara Bay (Brazil), showing the sites where *Orthopristis ruber* were caught.

A total of 66 *O. ruber* was captured and euthanized in ice in the field, and then transferred to the Laboratory of Theoretical and Applied Ichthyology (LICTA) at Federal University of Rio de Janeiro State (UNIRIO), Rio de Janeiro, Brazil. The right (R) and left (L) sides of six bilateral body structures were inspected by the same single researcher, using the same binocular stereomicroscope (Zeiss Stemi DV4; 8× magnification) and digital caliper for morphometric measurements, in order to minimize possible effects of methodological artifacts on asymmetry results. Diameter of the eye, length of the pectoral fins, the length of ventral fins were the three morphometric traits assessed, while number of gill rakers, number of pectoral fin rays, and number of ventral fin rays were the three meristic traits evaluated (Figure 2).

The composite fluctuating asymmetry (CFA) was used to calculate the combined effects of FA from all the six morphological traits according to Leung et al. (2000). The CFA can be computed by summing of absolute FA values for all traits for each individual ($CFA=\sum|D-E|$). The CFA is regarded as less sensitive to sampling and measurement biases than individual indexes (Leung et al., 2000).

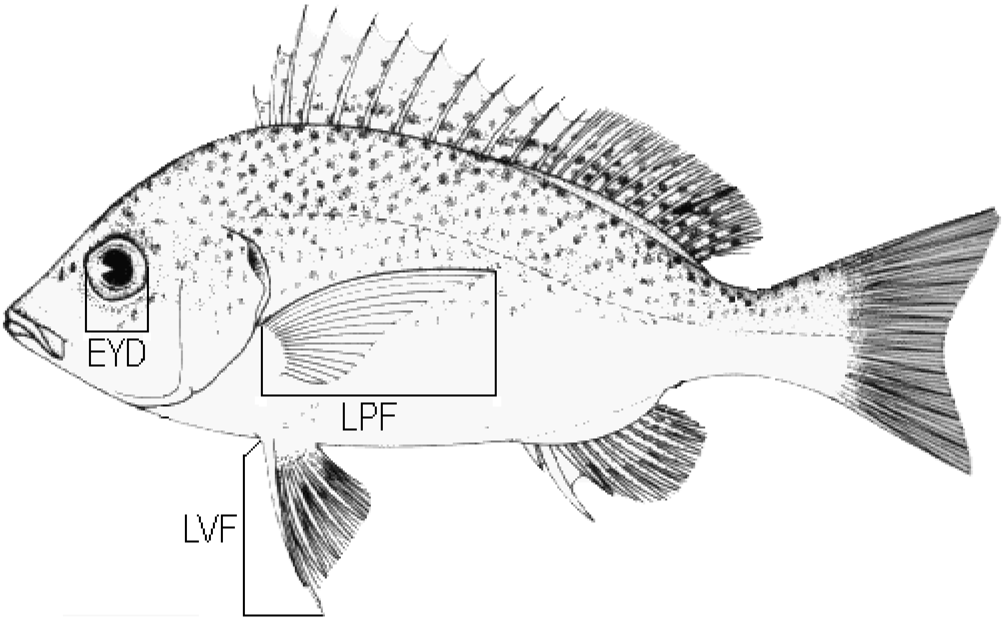

Figure 2. The locations of the traits used for the fluctuating asymmetry analyses.

The same procedure developed by Seixas et al. (2016) was applied to validate the measurements of FA in *O. ruber*. Since other types of bilateral asymmetry (e.g. directional and antisymmetry, which are more related to a genetic basis than to developmental instability) might interfere with our results, the Student t-test and distribution histograms were applied to evaluate the prevailing type of asymmetry in *O. ruber* (Palmer and Strobeck, 2003). Effects of directional asymmetry are negligible if the mean differences between the right and left sides for each attribute were not significantly ($p>0.05$) different from zero. Histograms were also applied to appraise data distribution patterns, since a normal distribution of the measurements indicates the prevalence of FA, whereas a bimodal distribution denotes antisymmetry (Palmer, 1994). Pearson Correlation tests were performed on the averaged measurements of the right and left sides for all six structures in *O. ruber* to test the correlation between FA and the size of the traits (Palmer and Strobeck, 2003). Significant correlations ($p<0.05$) indicate effects of structure size on asymmetry (Palmer, 1994). STATISTICA 12.0 was used to perform the Student t-test, build the histograms on data distribution and perform Pearson Correlation tests.

All morphological attributes were measured twice (i.e. independent measures) to evaluate the importance of measurement errors on FA levels, according to Palmer and Strobeck (1986). Permatutional Multivariate Analysis of Variance (PERMANOVA) was performed (Euclidean distance; 4.999 permutations per analysis) on data matrix to test for possible differences between the first and second measurements for each trait. The side of the structure in *O. ruber* was considered as fixed factor and each fish as random factor in all PERMANOVA. Significant analyses ($p<0.05$) trait side × fish interactions denote negligible effects of measurement errors on the FAs.

Four descriptors of physiological condition of *O. ruber* were used: condition factor (K), and gonadosomatic (GSI), hepatosomatic (HSI), and repletion (RI) indexes. GSI, HSI and K were determined as in Vazzoler (1996): GSI= Gw/Ew; HSI=Lw/Ew; K=Tw/Tl3. The IR index was calculated by Hyslop (1980): RI=Sw/Ew. Where, Gw: gonad weight; Lw: liver weight; Sw: weight stomach; Ew: eviscerated weight; Tw: total weight; and Tl: Total length. IR values was multiplied by 100. The CFA index, K, and GSI, HGI, and RI indexes were compared between 14 males and 33 females, and between 53 adults (161-256 mm) and 13 juveniles (85-157 mm). Fish was classified as juvenile or adult after comparing the length of each individual with the size of first maturation (L50=160 mm total lenght) proposed by Vianna and Verani (2002).

Multivariate ordination analyses were also applied to assess the relationship of the FA in *O. ruber* with the four physiological descriptors. Firstly, Partial Redundancy Analysis (RDA) was applied, using total length as covariable (i.e. to control for the effects of size fish), and a $p\leq0,10$ was considered as significant. RDA was performed for comparisons between juvenile and adult, and between female and male. Generalized additive models (GAMs) were used to define tendencies of the relationships between the CFA index and scores the RDA axis. The step-by-step selection procedure with the Akaike information criterion (AIC) was used to determine the complexity of the model. AIC considers not only the goodness of fit, but also parsimony, penalizing more complex models (Burnham and Anderson, 1998). All multivariate ordination analyses were performed in CANOCO 4.5 software (Leps and Smilauer, 2003). PERMANOVA was performed on data matrix to test for possible differences of FA and the four physiological descriptors between age class (juvenile versus adult) and gender (male versus female). The Euclidean distance was used and data was permuted 4.999 at $p<0.05$ per analysis. All PERMANOVA analyses were performed with PAST 3.10 (Hammer et al., 2001).

3.RESULTS

The composite index (CFA) was applied to 66 specimens, composed by 14 males and 33 females; 53 adults and 13 juveniles. The FA values for the six structures are shown in table 1, only for the description of the FA, since the CFA that involved the total of these structures was tested for the present study.

Table 1. Mean values and range (between parentheses) of results for individual and composite indexes of fluctuating asymmetry applied on the six morphological traits of *O. ruber* caught in Guanaba Bay. CFA: composite index; NPR: number of pectoral fin rays; NVR: number of ventral fin rays; NGR: number of gill rakers; EYD: eye diameter; LPF: length of pectoral fin; and LVF: length of ventral fin.

	n	**CFA**	**NPR**	**NVR**	**NGR**	**EYD**	**LPF**	**CNV**
Adult	53	31.47	0.44	0.19	1.10	4.65	13.32	11.88
		(1.2-86.4)	(0-3)	(0-2)	(0-6)	(0-18.7)	(0-60.2)	(0-47)
Juvenile	13	22.3	0.44	0.15	0.67	1.72	11.96	8.35
		(7-46)	(0-1)	(0-1)	(0-3)	(0-5.4)	(0-18.7)	(0-26.7)
Male	14	25.91	0.14	1.62	3.01	9.19	12.85	25.91
		(8.8-77.8)	(0-2)	(0-6)	(0-10.6)	(0-22.1)	(0-47)	(0-77.8)
Female	33	34.70	0.51	0.16	0.88	4.59	17.18	10.87
		(7.6-86.4)	(0-3)	(0-2)	(0-6)	(0-18.7)	(0-60.2)	(0-26.7)

It was observed higher mean HSI (PERMANOVA; F=12.95; p=0.002) and GSI PERMANOVA; F=4.02; p=0.05) for adults, whereas higher mean K (PERMANOVA; F=4.79; p=0.04) and RI (PERMANOVA; F=2.19; p=0.11) were recorded for juveniles (Table 2). Males exhibited higher mean GSI (PERMANOVA; F=2.88; p=0.09) and higher mean females of RI (PERMANOVA; F=0.14; p=0.71). Males and females did not exhibit any significant difference of the K (PERMANOVA; F=0.09; p=0.77) and IHS (PERMANOVA; F=0.009; p=0.94).

Table 2. Mean values and range (between parentheses) results for composite index of fluctuating asymmetry and physiological indexes applied of *O. ruber* caught in Guanaba Bay. CFA: composite index; K: condition factor; HSI: hepatosomatic index; GSI: gonadosomatic index; and RI: repletion index.

	n	**CFA**	**K**	**HSI**	**GSI**	**RI**
Adult	53	31.47	0.01	10.05	18.49	8.39
		(1.2-86.4)	(0.002-0.015)	(1.02-20.54)	(0.83-69.82)	(0.94-35.22)
Juvenile	13	22.3	0.013	9.72	4.28	9.35
		(7-46)	(0.012-0.015)	(5.07-15.87)	(1.09-13.08)	(3.2-26.47)
Male	14	25.91	0.013	10.4	21.83	6.16
		(8.8-77.8)	(0.011-0.014)	(6.25-13.84)	(1.64-69.82)	(0.94-15.13)
Female	33	34.70	0.013	10.85	14.30	8.55
		(7.6-86.4)	(0.01-0.02)	(4.99-20.54)	(1.09-69.29)	(3.20-30.23)

The RDA was statistically significant (Monte Carlo test; F=3.1; p=0.05) between the relationship CFA and physiological descriptors, which were summarized by the two first RDA axes, which explained 98.1% and 1.9% of the variation, respectively. The first axis clearly separating the adults and juveniles, and showing stronger relationship the adults with the GSI and HSI (Figure 3). However, these descriptors were not affected by the presence of FA. The second axis revealed an inverse relationship between asymmetry and physiological descriptors (K and RI; Figure 3).

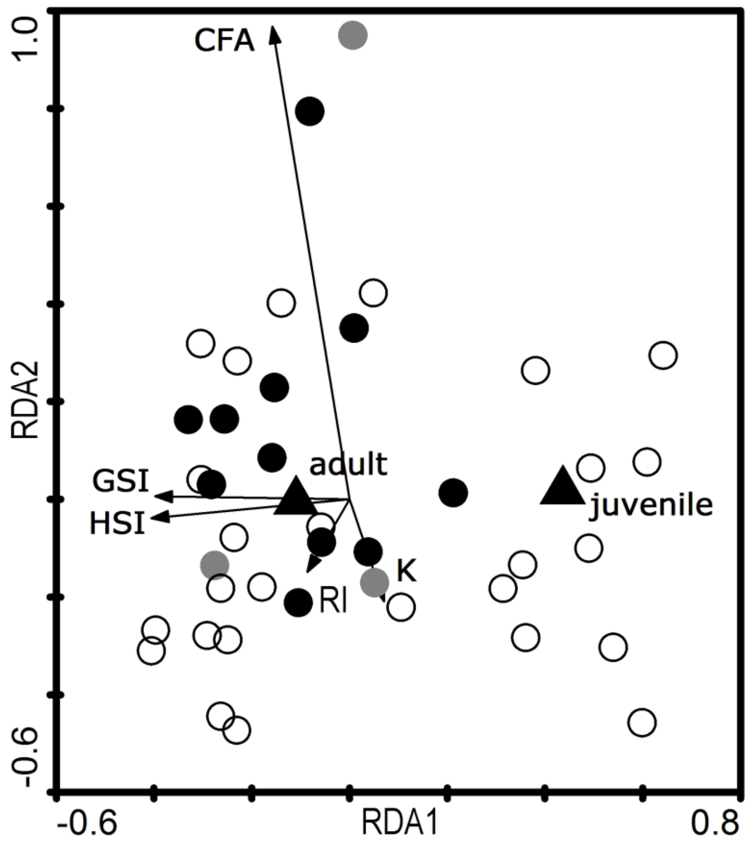

Figure 3. Partial Redundancy Analysis (RDA) showing the relationship of fluctuating asymmetry (FA) in *O. ruber* with physiological index (GSI, HSI, RI and K) for adults and juveniles. ○ =Urca; ● =Rio-Niterói Bridge; ● =Paquetá.

Due to the lack of ecological significance in juveniles with small amplitude of their physiological descriptors (Table 2), we have tested RDA, on a separate basis, only in the adult data matrix. RDA exhibit the same standard, but showed no significant difference for the adults of *O. ruber* (Monte Carlo test; F=0.2; p=0.83).

The relationship of the FA of males and females with the physiological descriptors were statistically significant (Monte Carlo test; F=7.68; p=0.02). The first axis RDA explaining 98.2%

of the data variance, while the second axis explained 1.8% total variance. The first axis RDA showed no relationship between of the effect of asymmetry between the sexes (Figure 4). The second axis showed an inverse relationship between CFA and descriptors K, RI and HSI, regardless of sex (Figure 4).

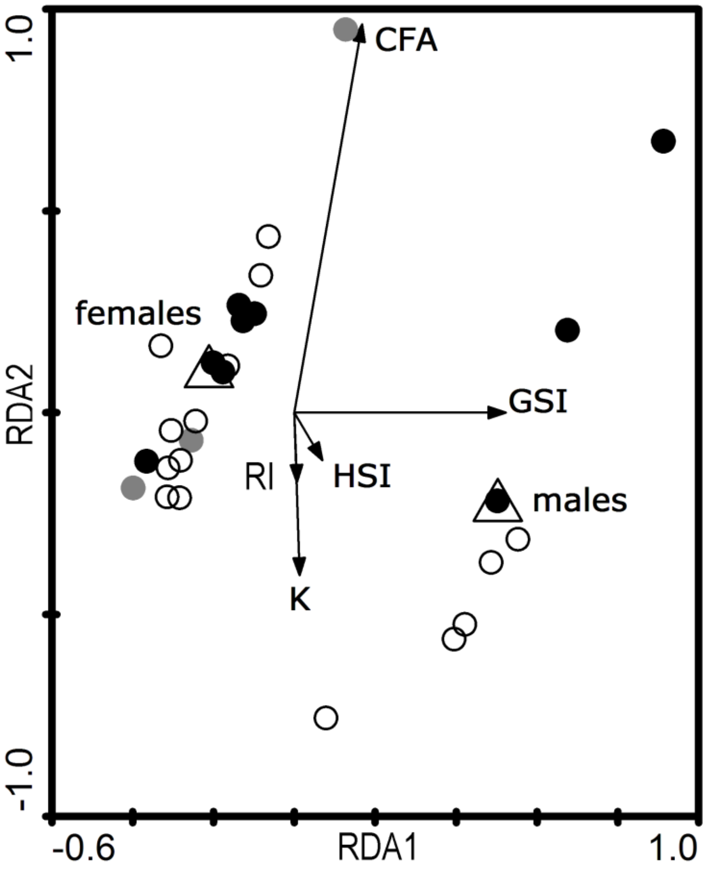

Figure 4. Partial Redundancy Analysis (RDA) showing the relationship of fluctuating asymmetry (FA) in *O. ruber* with physiological index (GSI, HSI, RI and K) for male and female. ○ =Urca; ● =Rio-Niterói Bridge; ● =Paquetá.

Because the IGS did not present a better predictor to validate the FA between the sexes, we considered this index as covariate, along with the total length. However, despite presenting the same relationship, they did not differ significantly (Monte Carlo test; F=0.96; p=0.69).

The AIC revealed relationship between FA and K for males (non-linear; F=16.46; p=0.006), with increased of FA and decreased of K (Figure 5). Thus, FA is interfering the physiological condition of males. Other relationships with the physiological descriptors between males and females were not found by GAM.

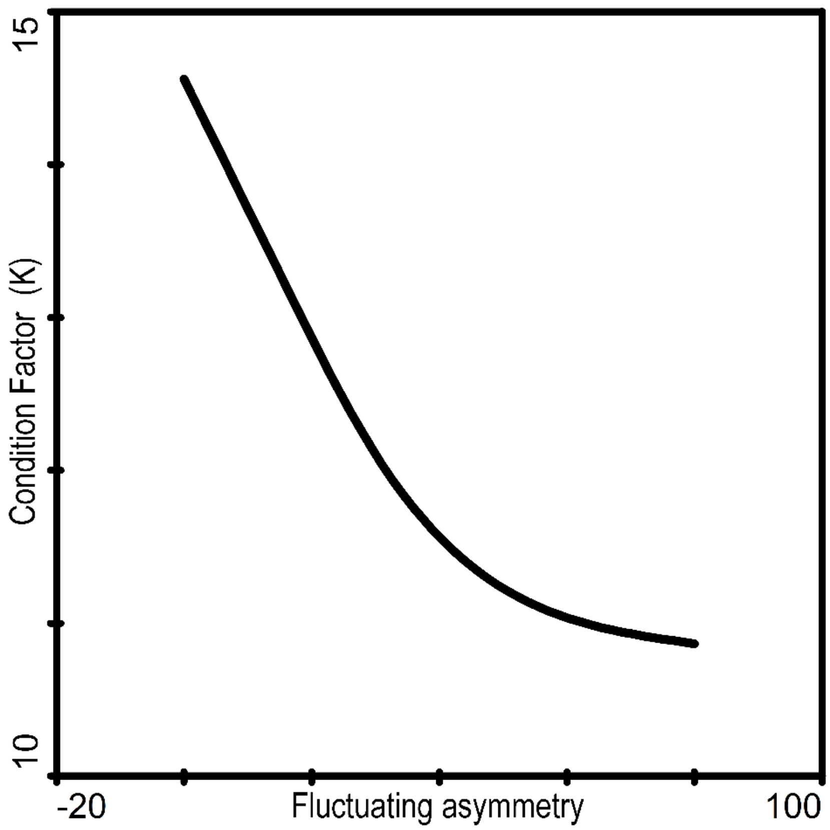

Figure 5. Generalized Additive Model (GAM) showing the relationship of fluctuating asymmetry (FA) in *O. ruber* with physiological index Condition Factor (K).

4.DISCUSSION

Changes in the symmetry of *O. ruber* may be negatively influencing the process of well-being and foraging of the species in the Guanabara Bay. Our results corroborate with the works of Somarakis et al. (1997) and Ayoade (2004), where they mention that effects of stress caused by the environment can cause changes in fitness and alter the homeostasis of the normal development of the species.

Among the physiological descriptors, the condition factor (K) is a qualitative physiological tool pointing out the body condition of the fish and that can be used to compare the health status of the species (Le Cren, 1951). This index has been used as an additional datum to study reproduction and feeding processes, being possible to relate it to the environmental conditions and behavioral aspects of species (Vazzoler, 1996).

Environmental conditions along with the life strategy of *O. ruber* may limit the penetration of the species in the inner zones of the Guanabara Bay. This same distribution pattern obtained for *O. ruber* was similar to that found for the species in Sepetiba Bay (Vasconcelos et al., 2007).

This agonistic behavioral pattern is widely reported for other rocky shore species, corroborating benefit theory in area defense (Ceccarelli et al, 2001).

The condition factor provides important information about the physiological state of the fish, assuming that individuals with greater body mass in a given length are in better physiological conditions (Vazzoler, 1996). However, K can be influenced by the age, since younger fish have different foraging rates and metabolic activity associated with rapid growth relative to older fish, and generally presenting lower conditions than the latter (Pyle et al., 2005), which explains in the present study the highest values of K observed in adults (Table 2).

The subtle tendency observed in the inverse relationship of FA with RI can be a strong indication that the asymmetry may be compromising the food function and integrity of the organism in food consumption, protein and glycogen storage, and may be insufficient to guarantee the body condition and the body mass of *O. ruber* in Guanabara Bay. The energy reserve affected by the asymmetry may reflect low energy stock, entailed by loss of appetite or excessive use of energy resources to compensate the detoxification mechanisms (Ramirez et al., 2012). This explains the increase of asymmetry in males resulting in the decrease of K, indicating their difficulty in maintaining the body condition, more than females (Figure 3). Probably, the morphological alteration in males is interfering in the intraspecific interactions that occur with *O. ruber*. Possibly, when males cannot satisfactorily maintain their territorial physical space and end up compromising their foraging actively, which explains their lower IR. Thus, the differentiated RI between the sexes indicates a greater energetic need by the females, with a better alimentary condition. The foraging strategy is in line with the reproductive strategy, reflecting that the intensity of feeding activity is the period preceding spawning, which may have occurred with *O. ruber* in the GB in the present study, when the reproductive period for Haemulidae occurs in the seasons of spring and summer (Garcia-Junior et al., 2010).

The highest values of HSI in adults of *O. ruber* may be related to K and GSI, reflecting that the liver is working in the mobilization of reserves to synthesize sex hormones (Querol et al., 2002) and responding to variations in foraging. However, higher values of asymmetry with lower HSI values indicate that morphological changes are affecting the energy reserve and metabolic activity of the liver of this species.

FA did not affect the reproductive success of *O. ruber*, despite the presence of a series of contaminants (Kehrig et al., 2010; Soares-Gomes et al., 2016; Baptista-Neto et al., 2017) contributing to aggravate the process of environmental degradation of the GB ecosystem and may negatively affect the reproductive process of fish. Other species such as *Cyprinodon pecosensis* (Kodric-Brown, 1997), guppies (Sheridan and Pomiankowski, 1997), *Salaria pavo* (Gonçalves et al., 2002) and *Perca fluviatilis* (Oxnevad et al., 2002) also exhibited that the presence of FA did not affect the reproductive success and/or the GSI of these species.

In short, the descriptors K, RI and HSI were subtly affected by the fluctuating asymmetry. This is possibly due to diverse environmental conditions and/or biotic and genetic stressors that negatively influenced the fitness of the species. There is no direct relationship between asymmetry of juveniles and adults, but the asymmetry negatively interferes with the physiological condition of *O. ruber* males, indicating their difficulty in keeping K higher than females (Table 2). Thus, fluctuating asymmetry can be considered an effective tool to infer the understanding of the instability of *O. ruber* development in Guanabara Bay, which makes this species a possible indicator of environmental quality in this ecosystem.

5.CONCLUSION

This work stands out for being pioneer in the comparative analysis of the FA with different physiological descriptors indicators of fitness using a tropical marine species. The results corroborate that FA can be considered an effective tool to diagnose small bilateral differences in *O. ruber* inferring about the understanding of the instability of the development and the body condition of the species in the Guanabara Bay.

Our results showed that the physiological descriptors K, RI and HSI of *O. ruber* were subtly affected by FA. Some issues, however, still need to be raised, such as the time at which FA is determined during the development of the species; and whether the bilateral deviations are corrected during growth, or if there is a natural variation of the species, in order to better understand the biology of the species and how it responds to possible stressors.

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How much fluctuating asymmetry in fish is affected by mercury concentration?

Luana B. Seixas[a,], Carlos A. Conte-Junior[b] Luciano Neves dos Santos[c], Alejandra Filippo Gonzalez Neves dos Santos[d]

[a]Fluminense Federal University (UFF), Graduate Course in Ocean and Earth Dynamics, Av. General Milton Tavares de Souza, s/n, 4° andar, Campus da Praia Vermelha, Niterói, RJ, Brazil, CEP: 24210-346.

[b]Department of Food Technology, Fluminense Federal University (UFF), Rua Vital Brasil, 64, Niterói, RJ, Brazil, CEP: 24230340.

[c]Federal University of Rio de Janeiro State (UNIRIO), Av. Pasteur, 458-R314A, Rio de Janeiro, RJ, Brazil, CEP: 22290-240.

[d]Department of Zootechny and Sustainable Socioenvironmental Development, Fluminense Federal University (UFF), Rua Vital Brasil, 64, Niterói, RJ, Brazil, CEP: 24230340.

ABSTRACT

This study aims to analyze if the Fluctuating asymmetry (FA) of the Corocoro grunt *Orthopristis ruber* is affected by the concentration of mercury in a polluted Tropical Bay. The fishes were caught during 2012-2014 in two areas of the Guanabara Bay, Brazil: Vermelha Beach, of strong influence of ocean waters; and Paquetá Island, of greater freshwater loads of the rivers of the region and the little influence of marine waters. It was used FA in nine characters of 162 *O. ruber*: number of pectoral and ventral fin rays, and of gill rakers; eye diameter and height; length of pectoral and ventral fin; pectoral and ventral fin base length. In addition, 130 samples were analyzed for THg. The presence of FA was confirmed for all characters of the *O. ruber*. The absence of relationships, however, of the FA between juvenile and adult ($F_{109.1}$=0.06; p=0.80), and males and females ($F_{45,1}$=1.15; p=0.30), both on the Vermelha Beach and on the Paquetá Island ($F_{125.1}$=0.02; p=0.90) suggests that the FA acts indistinctly among *O. ruber* individuals. Possibly availability of food resources, exposure to other pollutants and harmful agents, environmental and oceanographic factors may be reflecting on the FA detected for the *O. ruber* population. Our results do not report the influence of THg on FA. In turn, the THg was significantly different between areas ($F_{124.1}$=4.32; p=0.04). Likely higher THg on the Vermelha Beach occurred due to hydrodynamic factors, increasing the bioavailability of THg from the sediment and incorporated into the food web.

Keywords: Haemulid; mercury; bioindicator; Guanabara Bay; Brazil

1.INTRODUCTION

Several anthropogenic activities have been as polluting sources, emitting a variety of pollutants in the environment, modifying marine coastal ecosystems and causing several contamination issues. One of the pollutants at the most relevance to ichthyofauna is mercury (Hg), due to its toxicity, biomagnification, high absorption rates and low excretion (Kehrig et al., 2010; Seixas et al., 2013). Monitoring the presence, location and determination of Hg concentrations becomes important because it shows local environmental conditions and often benefits the environment quality.

Fishes are sensitive to environmental influences and are considered good indicators of environmental conditions (Seixas et al., 2013), especially those species with resident habits that tend to reflect strongly the local environmental variability. Some species of fish were used as bioindicators of Hg contamination in surveys at USA, as spotted seatrout *Cynoscion nebulosus* (Adams et al., 2010); *Lophius americanus* (Johnson et al., 2011); and goliath grouper *Epinephelus itajara* (Adams and Sonne, 2013). In the Guanabara Bay, there is a record of Hg contamination in *Trichurus lepturus* (Kehrig et al., 2010), *Micropogonias furnieri, Mugil liza, Sardinella brasiliensis, Genidens genidens* and *Ortopristis ruber* (Kehrig et al., 2011).

Environmental disturbances can affect individual and populations causing morphological alterations in paired structures (Seixas et al., 2016). These alterations are known as fluctuating asymmetry (FA), which is defined as random morphologic changes on bilateral asymmetry plan of paired characters, is commonly used as a measure of the developmental stability (Palmer and Strobeck, 1992). FA is widely promoted as a useful bioindicator of exogenous stressors in habitats, which has an interesting potential to appraise the state of adaptation of a population before that acute contaminations affect the whole community or ecosystem (Palmer, 1994).

The Guanabara Bay is one of the coastal regions of the State of Rio de Janeiro, Brazil, that has been historically modified by anthropogenic activities. The quality of the water in the Bay exhibits considerable spatial and temporal variability, governed by its hydrology, pollutant hotspots and rainy periods (Kjerfve et al., 1997). The highest accumulation of mercury in the Guanabara Bay is found in the northwest region (Kehrig et al., 2010). According to Kjerfve et al. (1997), the variation of the Hg concentration is indicative of the local environmental conditions, and can be used as a method for environmental assessment and biomonitoring in the region.

The cocoroca grunt *Orthoprister ruber* (Cuvier, 1830) is a specie distributed in the western Atlantic, very frequent and abundant in the southeastern/southern coast of the Brazilian coast (Menezes and Figueiredo, 1980). In Guanabara Bay is one of the most representative species of the fish assemblage associated to the rockyshores. The species shows rapid growth, resident behavior, demersal and intimate association with rocky substrates. Thus, *O. ruber* presents good potential as a biological sensor of mercury contamination in the ecosystems of this region (Kehrig et al., 2010; Guerrieri et al., 2015). There is no study, however, that addresses the relationship of fluctuating asymmetry and total mercury concentrations to *O. ruber*.

In this context, the main goals of this study were to answer questions as: How much FA in *O. ruber* is affected by mercury concentration in a polluted Tropical Bay – Guanabara Bay? Is *O.ruber* a bioindicator species of stress environmental?

2.MATERIALS AND METHODS

Guanabara Bay (22º24'- 22º57'S; 42º33'- 43º19'W) is considered one of the most degraded coastal environments in Brazilian coast, with approximately 384 km^2, it its surroundings live circa 16 million inhabitants (Fistarol et al., 2015). The drainage basin receives toxic effluents from the more than 16.000 industries, two airports, two commercial ports, 20 shipyards of the Bay and a large number of ferries, yachts and fishing boats, added to a complex petrochemical pole that processes 7% of the national oil (Kjerfve et al., 1997, Soares-Gomes et al., 2016). It presents an estimated sedimentation rate of 0.65-4.5 cm year^{-1} as a result of deforestation of the drainage basin and channelization of rivers (Fistarol et al., 2015). The fish assemblage in the Guanabara Bay is formed by approximately 174 marine or estuarine species (Vianna et al., 2012). Despite all the negative impacts, Guanabara Bay has an important role as a nursery site for larvae of several species of fish (Castro et al., 2005). The water circulation pattern in the Guanabara Bay is complex and strongly influenced by its topography, freshwater runoff, circulation of tidal currents and the influence oceanic water (Fistarol et al., 2015). Thus, Guanabara Bay exposes an environmental gradient from water quality is worse on inner Bay and better conditions on outer Bay (Mayr et al., 1989; Kjerfve et al., 1997).

2.1.Sample design

Two sites were selected to encompass the environmental gradient along the main central channel in Guanabara Bay (Mayr et al., 1989; Kjerfve et al., 1997), were they (Figure 1): 1) Vermelha Beach (22°95'S-43°15'W), is located near the entrance of the Bay and undergoes a strong influence of the influence of adjacent ocean waters, with transparency above 5 m and salinity between 34-36. This region presents better environmental conditions as a result of the high water circulation that dilutes the contaminants present inside the Bay (Mayr et al., 1989); 2) Paquetá Island (22°79'S-43° 10'W), is at the northward limit of the channel of water circulation (Mayr et al., 1989). It presents a complex of rocks of varied sizes and shapes, sometimes forming small islands (Itacolomis and Currais) of low hydrodynamic energy. This area presents synergistic interactions of the higher freshwater loads of the rivers of the region and the under influence of marine waters due to the decrease of the velocity of the tidal currents. Despite the low water exchange, this place has good water quality conditions.

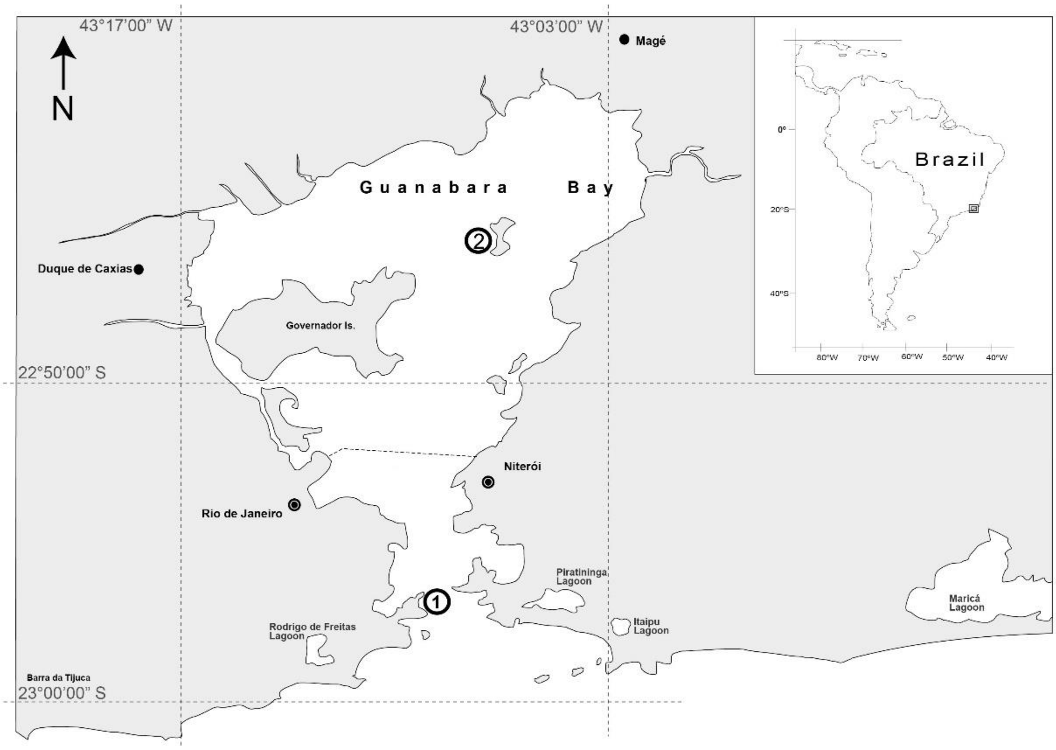

Figure 1. Geographic location of Guanabara Bay (Brazil), showing the areas where *Orthopristis ruber* were caught. 1= Vermelha Beach; 2= Paquetá Island.

Monofilament gillnets (20m) of three different meshes (15, 30 and 45 mm between adjacent knots) were used, forming a gillnet set with 60 m × 1.5 m. The gillnet sets (three replicates per site) were installed, perpendicularly to the shore, over the rocky substrates of the two sampling sites and recovered after 24h later. Rocky substrates were chosen not only to standardize the habitat for sampling but also because of the high relationship of *O. ruber* with hard substrates. Sampling was conducted quarterly between September 2013 and June 2014. In addition, some physical and chemical water variables were measured in situ: temperature (ºC), pH and salinity, measured at a depth of approximately 1 m by means of a probe multiparameter model Hanna HI 9828. The transparency was measured with Secchi depth (cm).

2.2.Validation of fluctuating asymmetry

All *O. ruber* captured were euthanized in ice in the field and then transferred to the laboratory of Theoretical and Applied Ichthyology of the Federal University of Rio de Janeiro

State (UNIRIO), Rio de Janeiro, Brazil. During dissection, the fish were measured in total length and the gonads analyzed according to Vazzoler (1996) for identification of the sex. The individuals were classified as immature and adult according to the length of first maturation (L50=160 mm) defined by Vianna and Verani (2002).

The right (D) and left I sides of nine characters were measured using binocular stereomicroscop stereomicroscope (Zeiss Stemi DV4, 8x magnification) and digital caliper (resolution 0.001 mm). All measurements were made by one operator. The evaluated characters were: diameter of the eye, height eyes, length of the pectoral fins, length of ventral fins, length base of the pectoral fins, length base of ventral fins, number of gill rakers, number of pectoral fin rays and number of ventral fin rays.

To validate the presence of fluctuating asymmetry (FA) in *O. ruber* (directional and antisymmetry, which are more related to genetic basis than to developmental instability, Leary and Allendorf, 1989), we tested for the protocol developed by Seixas et al. (2016), and the Student t-test and the distribution histogram were applied. Effects of directional asymmetry are negligible if the mean differences between the right and left sides for each attribute were not significantly ($p>0.05$) different from zero. The FA4 index ($FA4=0.798\sqrt{(var\ D-E)}$) was applied whenever the means differed significantly from zero ($p<0.05$; Palmer and Strobeck, 2003). Histograms were also applied to appraise data distribution patterns, since a normal distribution of the measurements indicates the prevalence of fluctuating asymmetry whereas a bimodal distribution denotes antisymmetry (Palmer, 1994). We performed Pearson correlation test on the averaged measurements of the right and left sides for all nine structures in *O. ruber* to test the correlation between FA and size traits of analyzed. ($p<0.05$) indicate possible effects of the size of the structure on the asymmetry, thus, the FA2 index ($FA2=|D-E|\div[(D+E)\div2]$) was applied (Palmer, 1994). This index was applied not only to attributes with significant correlations of asymmetry × character size, but also to all analyzed characters in order to calculate asymmetry. All univariate tests were performed in the Statistica 12.0 program (Statsoft Inc. USA).

We calculated the second composite fluctuating asymmetry index (CFA2) to analyze the combined effects of FA levels from all the traits together (Leung et al., 2000). For the calculation of the CFA2 index, the sum of the index FA2 ($CFA2=\Sigma|D-E|\div[(D+E)\div2]$) was applied. The CFA is recommended because of the less risk of the possible effects of the measurement error on the calculations of FA (Leung et al., 2000), are also considered more powerful for data with normal distribution among heterogeneous characters (Seixas et al., 2016). The CFA was applied in 43 adult and 119 juveniles, and 37 males, 27 females and 98 fish had no sex determined by the impossibility of visual inspection of the gonads.

2.3.Determination of Total Mercury (THg)

A total of 130 samples of muscle of the *O. ruber* were analyzed for THg concentration. From this total, 114 were collected at rockyshores in Vermelha Beach and 16 in Paquetá Island. Dorsal muscle tissue samples of 0.27 gr were used on the left side of the *O. ruber*. The analysis were made in triplicate. The analyses were performed of the Laboratory of Chemical Control (Faculty of Veterinary, Fluminense Federal University). Analytical determinations of total Hg (THg) were performed by Direct Mercury Analyzer (DMA-80, Milestone, Sorisole, Italy), following the manufacture´s recommendations and following the procedure proposed by Guimarães et al. (2015).

DMA is a analyzer of solid sample not requiring presentation or other wet chemical preparation prior to the analysis (Ipolyi et al., 2004). DMA analyzer's principle is based on thermal decomposition, amalgamation, and atomic spectrometry detection (Boylan and Kingston, 1998). Muscle samples were placed in quartz or nickel boat and are transferred from the analytical balance to the DMA. The samples were dried and thermally decomposed in the furnace with 99.9% pure oxygen (White Martins, São Paulo, Brazil) at a pressure of 4 $kgfcm^{-1}$. The sample evaporation was carried out in three steps as in the following: 160ºC for 1 min for drying, decomposition for 650ºC for 2 min and amalgamation of the decomposition residues by 650ºC for 1 min. The analytical signal obtained was measured at atomic absorbance at 253.65 nm, subsequently converted to a quantified total Hg concentration using the calibration curve (R^2=0.99), taking into account sample weight and peak height. The results were expressed in $mgkg^{-1}$ (Guimarães et al., 2015). The by-products of combustion were all eliminated.

2.4.Data analysis

The non-parametric multivariate PERMANOVA was applied to test significant differences between the composite index (CFA2) and the individual index (AF2). The distance from Bray-Curtis was used in all analyzes. Significant comparisons with the value of $p<0.05$ revealed interactions between the indices and the characters analyzed. The greater the measure of dissimilarity, the lower the similarity between the individuals studied. Post-hoc tests were also applied whenever significant differences were detected to identify, which zones differed significantly from each other. The PERMANOVA was applied through the PAST statistical program (Hammer et al., 2001).

Analysis of Covariance (ANCOVA) was used to compare THg concentration and FA among for the two areas. A significance level of 95% was adopted. Separately, ANCOVA was used for sexual maturity, males and females. The values of FA and the THg were considered as dependent variables. The total length was considered as covariate (i.e. to control the effect of the influence of the size of individuals). Log-transformation was applied when necessary. The analyses were conducted using Statistica 12.0 (Statsoft Inc., USA).

Multivariate Redundancy Analysis (RDA) was applied using salinity and temperature as a covariate to detect the relationship between the FA and THg. The multivariate RDA analyzes was performed in software CANOCO 4.5 (Leps and Smilauer, 2003).

Principal Component Analysis (PCA) was applied on the environmental data matrix to identify which abiotic variables best explained the pattern of separation of the Guanabara Bay areas. It was performed in PC-ORD6 software (Tabachnick and Fidell, 2001).

3.RESULTS

From the total of 162 fish caught (Table 1), 73.46% of the individuals were classified as juveniles (81-143mm) and 26.54% as adults (160-261mm); females represented 16.67% of the fish, 22.84% males and 60.49% did not have sex determined by the impossibility of visual inspection of the gonads.

3.1.Fluctuating asymmetry in *Orthopristis ruber*

Data distribution of all nine traits in the *O. ruber* fitted satisfactorily to the expected assumptions of the normal curve suggesting thus that antisymmetry is absent. The differences between the sides for most of the characters did not differed significantly from zero (Student t-test; $p \geq 0.3$), except for the length ventral fin (Student t-test; $p<0.005$) and height eye (Student t-test; $p=0.007$). This difference did not persist when the procedure to control this type of asymmetry was applied, the value found when calculating the AF4 index for length ventral fin (FA4=0.29) and for height eye (FA4=0.18) was greater than the mean difference between the right and left sides for length ventral fin (FA4=0.18) and height eye (FA4<0.001). In this way, no structure was removed from subsequent analyzes.

Following the validation of the presence of FA and the calculation of the composite index analyzed, the differences between the right and left sides were positively correlated with the characters eye diameter (Pearson; $r=0.38$; $p=0.005$) and length base of the pectoral fin (Pearson; $r=0.37$; $p=0.003$). We opted to maintain the structures and apply the FA2 index, which revealed the existence of FA for the structures previously masked by the effect of the size of the fish.

The FA values were significantly different (PERMANOVA; $F=111.1$; $p<0.0001$) between the composite index and the individual indexes, with the composite index showing higher asymmetry values. Significant differences were found between the individual indexes (PERMANOVA; $F=70.52$; $p<0.0001$) for all characters, with the base of the ventral fin presenting the highest value for FA, followed by the length ventral fin (Table 1). The character of the ventral fin had lower asymmetry values.

No significant differences were recorded for the FA between the Vermelha Beach and the Paquetá Island (ANCOVA; $F_{125,1}$=0.02; p=0.90). The *O. ruber* collected on the Vermelha Beach, although showing a greater amplitude of the FA values (Figure 2), when analyzing these data separately, the same pattern was observed without significant differences between juveniles and adults (ANCOVA; $F_{109.1}$=0.06; p=0.80) and females and males (ANCOVA; $F_{45,1}$=1.15; p=0.30).

Table 1. Mean values and range (between parentheses) of results for the second composite index (CFA2) and for the second individual index of fluctuating asymmetry (FA2) applied on the nine morphological traits of *O. ruber* caught in Guanaba Bay. CFA: composite index; NPR: number of pectoral fin rays; NVR: number of ventral fin rays; NGR: number of gill rakers; EYD: eye diameter; EH: height eye; LPF: length of pectoral fin; BPF: pectoral fin base length; CNV: length of ventral fin; BVF: ventral fin base length.

	n	CFA	NPR	NVR	NGR	EYD	EH	LPF	BPF	LVF	BVF
Adult	43	0.57	0.03	0.02	0.06	0.05	0.04	0.09	0.07	0.09	0.13
		(0.21-1.90)	(0-1.77)	(0-0.18)	(0-0.24)	(0-0.14)	(0-0.14)	(0-1.41)	(0-0.21)	(0-0.32)	(0-0.35)
Juvenile	119	0.66	0.04	0.01	0.04	0.05	0.07	0.08	0.08	0.13	0.15
		(0.06-2.02)	(0-1.67)	(0-0.4)	(0-0.24)	(0-0.26)	(0-0.29)	(0-0.51)	(0-0.46)	(0-0.91)	(0-0.47)
Male	37	0.60	0.03	0.004	0.06	0.05	0.07	0.07	0.07	0.13	0.12
		(0.22-1.28)	(0-0.12)	(0-0.18)	(0-0.24)	(0-0.26)	(0-0.28)	(0-0.33)	(0-0.27)	(0-0.91)	(0-0.32)
Female	27	0.56	0.02	0.03	0.04	0.04	0.05	0.06	0.07	0.07	0.16
		(0.27-1.04)	(0-0.13)	(0-0.18)	(0-0.20)	(0-0.12)	(0-0.17)	(0-0.12)	(0-0.21)	(0-0.30)	(0-0.45)
Total	162	0.64	0.04	0.02	0.04	0.05	0.06	0.08	0.08	0.12	0.14
		(0.06-2.02)	(0-1.67)	(0-0.4)	(0-0.24)	(0-0.26)	(0-0.29)	(0-1.41)	(0-0.46)	(0-0.91)	(0-0.47)

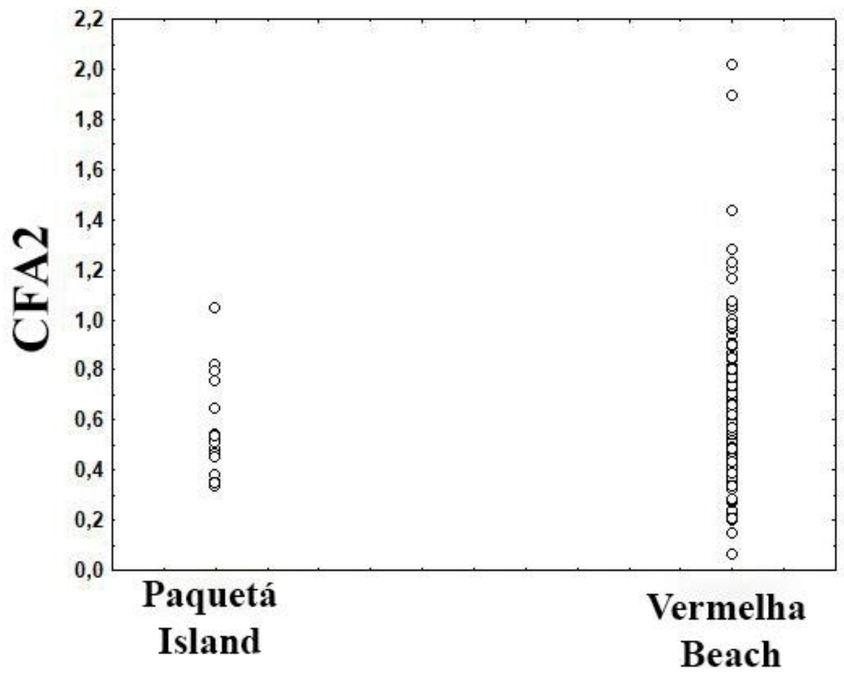

Figure 2. Distribution of fluctuating asymmetry values in *Orthopristis ruber* in Vermelha Beach and Paquetá Island.

3.2.THg concentrations in Guanabara Bay

Among the 130 fish that were analyzed for THg concentrations (Table 2), 67% of the individuals were classified as juveniles (81-115 mm) and 33% as adults (116-261 mm). The females represented 24.09%, males with 20.44% and 55.47% did not have the sex determined due to the impossibility of the visual determination of the gonads.

Our results demonstrated that mean of THg of 0.072 $mgkg^{-1}$in muscle of *O. ruber* in the Guanabara Bay. Significant differences were found (ANCOVA; $F_{127.1}$=5.72; p=0.004) among adults and juveniles, with adults accumulating higher concentrations of THg (0.11 $mgkg^{-1}$) in muscle tissues than juveniles (0.06 $mgkg^{-1}$). However, juveniles showed greater variation in THg (Table 2), indicating that environmental pressures, physiological or behavioral mechanisms acted differently during growth. There were also significant differences between male and female *O. ruber* in Guanabara Bay (ANCOVA; $F_{126,1}$=2.93; p=0.04). Females (Table 2) showed a higher variation in THg (0-0.52 $mgkg^{-1}$) than males (0-0.41 $mgkg^{-1}$).

Table 2. Mean values, ± standard deviation and range (between parentheses) of total Mercury (THg) between sex, adult and juveniles recorded on the sampling areas at Guanabara Bay where

O. ruber was caught. Values were provided for the entire dataset (pooled) and for each sampling site.

Sampling Sites	n	HgT $mgkg^{-1}$
Guanabara Bay	130	0.07 ± 0.11 (0-0.98)
Male	33	0.07 (0-0.41)
Female	29	0.08 (0-0.52)
Adult	40	0.11 (0-0.52)
Juvenile	90	0.06 (0.01-0.98)
Vermelha Beach	114	0.08 ± 0.12 (0-0.98)
Male	28	0.08 (0-0.41)
Female	20	0.09 (0-0.52)
Adult	26	0.12 (0-0.52)
Juvenile	88	0.06 (0.01-0.98)
Paquetá Island	16	0.05 ± 0.04 (0-0.11)
Male	5	0.05 (0-0.08)
Female	9	0.06 (0-0.11)
Adult	14	0.05 (0-0.11)
Juvenile	2	0.05(0.03-0.06)

Following the same pattern, we recorded significant differences for the concentrations of THg between Vemelha Beach and Paquetá Island (ANCOVA; $F_{124,1}$=4.32; p=0.04). Higher concentrations of THg were found in *O. ruber* populations in the Vermellha Beach (Table 2) with mean values of 0.08 HgT $mgkg^{-1}$ in relation to the Paquetá Island with 0.05 HgT $mgkg^{-1}$. No significant differences were found between juvenile and adult (ANCOVA; $F_{108.1}$=3.83; p=0.53) and between males and females (ANCOVA; $F_{44,1}$=0.57; p=0.81) in the Vermelha Beach.

3.3.Relation between fluctuating asymmetry and THg in *O. ruber*

The RDA showed a significant relationship between the THg and the FA (Monte Carlo test; F=2.60; p=0.05), which were summarized by the first two axes of the RDA, which explained 93.7% and 6.3% of the variation of the data, respectively. The first axis clearly separated the population of Vermelha Beach and Paquetá Island, and showed a strong relationship between the salinity with *O. ruber* of Vermelha Beach and the temperature with the individuals of Paquetá Island (Figure 3). The concentrations of THg in *O. ruber*, however, are not related to the presence of FA.

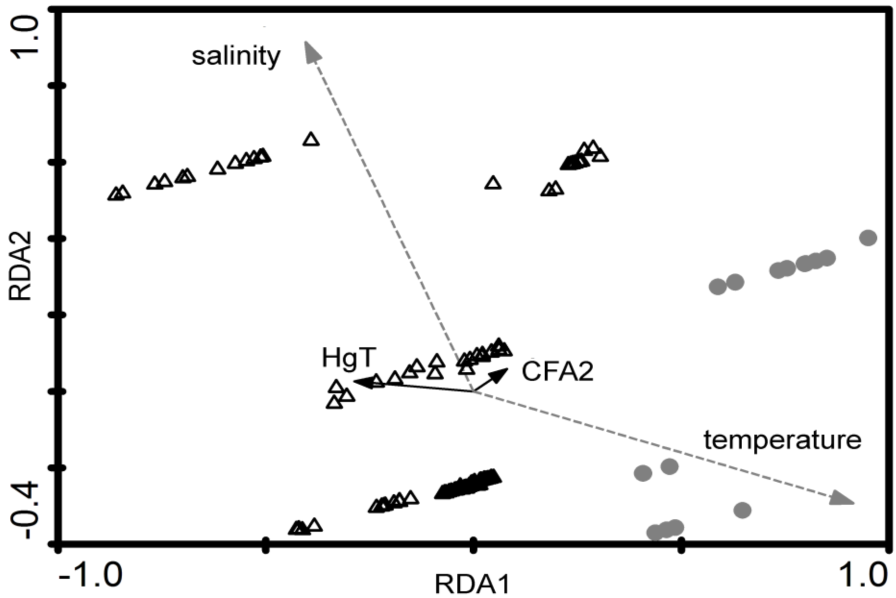

Figure 3. Partial Redundancy Analysis (RDA) showing the relationship of the levels of fluctuating asymmetry (CFA) in *Orthopristis ruber* with total length, salinity and temperature. Site Legend: Δ= Vermelha Beach; ●= Paquetá Island.

3.4.Physical-chemical parameters of the Guanabara Bay water

Guanabara Bay is characterized as an environment in which the most superficial strata of water present low transparency, with pH, temperature, salinity and oxygen level (Fistarol et al., 2015; Seixas et al., 2016) close to those expected for estuarine environments (Table 3).

Table 3. Mean values and range (between parentheses) of temperature, salinity, pH, and transparency (m) recorded on the sampling areas at Guanabara Bay where *O. ruber* was caught. Values were provided for the entire dataset (pooled) and for each sampling site.

Environmental variable	Pooled values	Sampling Sites	
		Vermelha Beach	Paquetá Island
Temperature (°C)	21.09 (15.70-25.82)	20.65 (15.70-21.83)	25.05 (24.62-25.82)
Salinity	27.95 (16.42-36.37)	28.13 (20.91-36.37)	26.28 (16.42-28.36)
pH	8.01 (8.42-9.70)	9.04 (8.42-9.70)	8.75 (8.53-9.56)
Transparency (m)	4.07 (0.55-7.00)	4.41 (3.4-7.00)	0.95 (0.55-1.05)

We observe considerable spatial variation in the physical and chemical characteristics of the water, due to the alternating influence of ocean and estuarine waters to which the Bay is submitted (Figure 4). The spatial variations in the physical and chemical conditions of the Guanabara Bay surface waters were summarized by the first two axes of the PCA, which together explained 89.13% of the data variance, with axis 1 explaining 67.11% and the axis 2 with 22.02% of the total data variability. Axis 1 at temperature (r=0.57) was elevated for both the Paquetá Island and Vermelha Beach to the right of the axis (positive correlation), while higher transparencies (r=-0.58) and salinity (r=-0.51) were more representative in Vermelha Beach to the left of the axis (negative correlation), with significant differences between the two locations (Monte Carlo test; p=0.001).

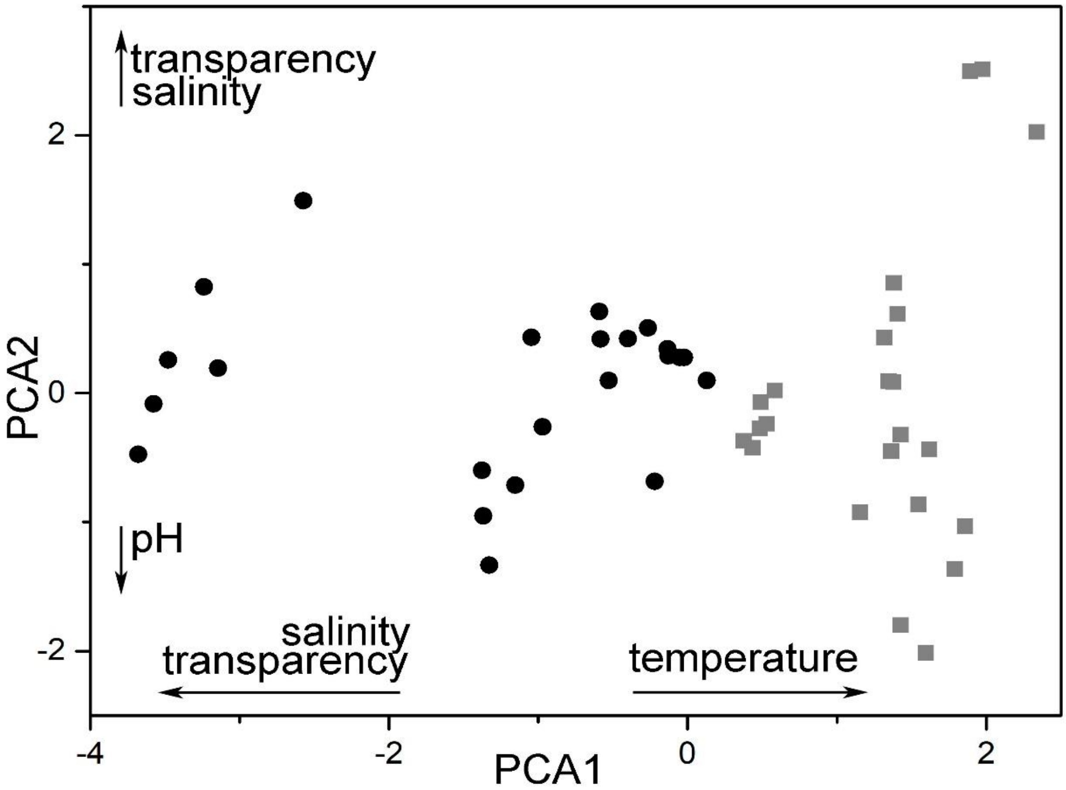

Figure 4. Ordination diagram of the Principal Component Analysis (PCA) applied on the environmental matrix obtained for sites at Guanabara Bay. The directions of the arrows indicate which variables showed greater correlations to the distribution of the samples along each axis. Site legend: ●= Vermelha Beach; ■ = Paquetá Island.

4.DISCUSSION

4.1.Validation of fluctuating asymmetry

The presence of FA was confirmed for all characters of the *O. ruber*. Nevertheless, our results do not report the influence of THg on asymmetry. The absence of relationships of the FA between juveniles and adult and males and females, both on the Vermelha Beach and Paquetá Island of suggests that the asymmetry acts indistinctly among *O. ruber* individuals, although the adults remain exposed to stressors by one longer period of its life cycle. According to Vasconcellos et al. (2007), the species presents the same behavior in the choice of habitat and feeding habit continuously throughout the year in Guanabara Bay, which may explain the difference in the FA of *O. ruber* in the two areas of this study. Possibly other effects related to environmental issues may be reflecting on the FA detected for the *O. ruber* population, such as availability of food resources, exposure to other pollutants and harmful agents, environmental and oceanographic factors, but for this, experimental studies should be prepared.

4.2.THg in *O. ruber* of the Guanabara Bay

The distribution of *O. ruber* in Guanabara Bay may be related to the behavior of the species, with greater abundance in the outermost zone of the Bay (Guerrieri et al., 2015), confirming its adaptation to the environment closest to the oceanic conditions (Pessanha and Araujo, 2003). However, concentrations of THg in muscle of *O. ruber* did not explain the presence of FA, although the contaminants already reported for the Guanabara Bay act as possible stressors causing asymmetry (Kehrig et al., 2011; Seixas et al., 2013; Fistarol et al., 2015).

According to Botaro et al. (2012), an increase in THg concentrations with the size and time of fish exposure to THg is expected, as in the case of adult individuals (Covelli et al., 2012; Seixas et al., 2013). However, although juveniles accumulate THg gradually, they more accurately reflect the THg levels of the site, due to the lower displacement, which explains the greater amplitude of variation of the THg data observed in this study. This pattern is corroborated by some studies reporting the fish length pattern at THg concentrations in Guanabara Bay (Kehrig et al., 2001; 2011; Seixas et al., 2013) in other Brazilian coastal Bays (Kehrig et al., 1998; 2011; Seixas et al., 2013), and in the Montevideo Bay (Corrales et al., 2016) and La Plata Bay (Marcovecchio, 2004).

The difference in the accumulation of THg between males and females may be associated with the process of dilution and slower growth for females or the greater metabolic activity of males (Gewurtz et al., 2011; Jankovská et al., 2014), as well as the contribution differentiated fish at different levels of gonadal development, with gonads having a role in the elimination of THg.

The concentration of THg in fish muscles does not depend exclusively on the main pathways of assimilation and biotic factors (Botaro et al., 2012). The influence of some environmental factors is also important for the understanding of THg, acting as agents in the bioavailability and accumulation of THg in fish (Kehrig et al., 2001; 2011). According to Kehrig et al. (2001), the main physico-chemical parameters of water for the evaluation of THg contamination are pH, salinity, dissolved oxygen, temperature, precipitation, turbidity, sedimentation and organic matter. Most of the THg in Guanabara Bay is associated with the sediment, making it immobilized or with reduced availability to organisms (Kehrig et al., 2001; Covelli et al., 2012). In addition, the water quality gradient in the Guanabara Bay (Baptista-Neto et al., 2005; Kehrig et al., 2011, Seixas et al., 2013) shows that it is not possible considered as a unique homogeneous environment (Fistarol et al., 2015). Although the concentration of THg is higher in the inner areas of the Guanabara Bay, its bioavailability is lower (Baptista-Neto et al., 2016), which explains the lower concentration of THg in *O. ruber* on the Paquetá Island. In this region, we can find a reducing environment generated by the high level of organic contamination, with the THg interacting with the fine sediment (silt) (Catanzaro et al., 2004; Covelli et al., 2012) in suspension that acts as the recipient environmental compartment (Kehrig et al., 2011). According to Lacerda and Malm (2008), THg in the inner of the Guanabara Bay is also complexed by sulfides and microbial biofilm, which leads to a decrease in its residence in the water column, consequently decreasing its bioavailability to the environment (Kehrig et al., 2010; 2011).

On the other hand, our results showed the increase in THg in *O. ruber* muscle in the outermost area of Guanabara Bay – Vermelha Beach. This pattern may be due to hydrodynamic factors acting on the Vermelha Beach that provide better conditions and water circulation, come back Hg more available in areas with greater water turnover (Catanzaro et al., 2004). The water turnover found in the Vermelha Beach region decreases the ability of the system to sequester THg due to the lower amount of suspended particulate matter (Fistarol et al., 2015), with the same being removed from the sandy sediment (Fistarol et al., 2015) influenced by adjacent ocean waters and made available in surface waters (Ansari et al., 2016). Another factor that may be acting on the bioavailability of THg on Vermelha Beach is the increase in water salinity (Silva et al., 2011). The region has a clear marine contribution (Kehrig et al., 2011; Fistarol et al., 2015), which decreases inner due to the influence of river discharges. At the entrance of Guanabara Bay (Moser et al., 2016) we find coastal waters (CW) and the adjacent shelf presents shelf waters (SW) with an increase of chlorophyll a (Kehrig et al., 2011). A little further away from Vermelha Beach, about 36 km away from the mouth of the Bay, we find Atlantic Central Southern Water (SACW) which in summer approaches the coast by the baroclinic effect (Moser et al., 2016). The marine freshwater currents that operate in the region of the Vermelha Beach bring the nutrients providing increased productivity in the region (Moser et al., 2016). These marine currents function with a natural Hg reactor increasing their bioavailability, with phytoplankton blooming, which may render the THg of the sediment bioavailable and incorporated into the food webs (Lacerda and Malm, 2008; Silva et al., 2011). Similarly, in the Sepetiba and Grande Island (RJ) Bays, higher concentrations of THg in fish muscles were found in the outermost area (Paraquetti et al., 2007).

Similar results were reported by Lacerda and Malm (2008) who demonstrated higher concentrations of THg and methylmercury in coney *Cephalopholis fulva* caught in the oceanic banks than in the coastal population, localities with high and low circulation, respectively. A study by Ansari et al. (2016) with the mussel *Perna perna* pointed out the same pattern, with a lower concentration of THg in the inner of the Guanabara Bay and higher concentrations in individuals from the Cagarras archipelago (RJ), when THg becomes more bioavailable due to hydrographic conditions such as high salinity, pH and high influence of oceanic waters and resurgence.

Our study indicates that the highest THg concentrations in *O. ruber* on the Vermelha Beach, in the outermost area of the Guanabara Bay can directly influence the bioaccumulation of this metal in the commercially important species present in this region, through the trophic chain due to *O. ruber* constitute an important part of their diet.

5.CONCLUSION

The THg concentration present in the outermost and inner area of the Guanabara Bay had no direct relation with FA in *O. ruber*. The FA present in the *O. ruber* population (regardless of the male or female, juvenile or adult) may be related to the availability of food resources, exposure to other pollutants and toxic agents, environmental and oceanographic factors, but for this, experimental studies should be developed.

O. ruber is a bioindicator species of THg contamination along the Guanabara Bay. The species presented higher levels of THg in the Vermelha Beach (outermost area) due to the hydrodynamic factors of the area, which provide better water circulation, which act as a natural THg reactor increasing its bioavailability with the phytoplankton bloom, which can make THg of the bioavailable sediment and incorporated into the food webs. This indicates that the higher THg concentrations in the outermost area of the Guanabara Bay can directly influence the bioaccumulation of this metal in the commercially important species present in this region through the trophic chain because *O. ruber* constitutes an important part of the diet of the themselves.

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Capítulo 4 – a ser submetido para Neotropical Ichthyology.

Nearshore upwelling events mediating the diet of Corocoro grunt *Orthopristis ruber* (Cuvier, 1830) in a eutrophicated tropical Bay

Luana B. Seixas[1], Cintia Silva[2], Luciano Neves dos Santos[2], Alejandra F. G. N. Santos[3*]

[1]Fluminense Federal University (UFF), Graduate Course in Ocean and Earth Dynamics, Ave. General Milton Tavares de Souza, s/n, 4° andar, Campus da Praia Vermelha, Niterói, RJ, Brazil, CEP: 24210-346.

[2]Federal University of Rio de Janeiro State (UNIRIO), Ave. Pasteur, 458-R314A, Rio de Janeiro, RJ, Brazil, CEP: 22290-240.

[3]Department of Zootechny and Sustainable Socioenvironmental Development, Fluminense Federal University (UFF), St. Vital Brasil Filho, 64, Niterói, RJ, Brazil, CEP: 24230340.

*Corresponding author at: Laboratory of Applied Ecology, St. Vital Brasil Filho, 64, Niterói, RJ, Brazil, CEP: 24230340. Email: alejandrafilippo@hotmail.com

The Corocoro grunt (*Orthopristis ruber*), a medium-sized fish species distributed throughout the Western Atlantic, has a benthic habit and is associated with rocky coral reefs, but little is known about the species' diet. The aim of this study was to characterize the diet and feeding strategy of the Corocoro grunt at the entrance of a Brazilian Southeastern eutrophicated Bay, and to evaluate how the occurrence offshore upwelling events influences the observed patterns. The fish were collected between December 2011 and June 2015 at Praia Vermelha. The stomachs of 167 specimens were analyzed. Seven food categories were identified which included 13 items: *Crustaceans* (amphipods, anomura, brachyura, shrimp, copepods and isopods); *Molluscs* (bivalves); *Teleostei* (fish scales); *Plant material* (seagrass); *Sediment* (sand particles); *Inorganic allochthonous material* (nylon filaments); *Worms* (nemathelminths and polychaetas). The diet was predominantly invertebrate, with a tendency to specialize in crustaceans, according to the Index of Relative Importance (Pinkas et al., 1971) and the Amundsen Diagram et al. (1996), showing little variation between juveniles versus adults. The non-metric Multidimensional Scale (nMDS, stress=0.09) and Simper analyses confirmed the absence of significant differences in the diet of juveniles and adults between dry and wet seasons. Three distinct groups were identified by Canonical Correlation Analysis (CCA; Monte Carlo test; $F=17.60$; $p<0.001$), representative of the differentiated consumption of food items with the water temperature ranges in the region. In periods of lower water temperatures, indicative of the occurrence of upwelling events, the diet was more diversified with species such as: crabs, fish and amphipod, and associated with higher values of salinity and total length of the individuals. At intermediate temperatures (21-22ºC), the food preference was for inorganic material, copepods and anomura. At warmer temperatures (>22ºC), shrimp, polychaete, plant material and sand were more consumed, coinciding with records of higher rainfall indexes. The changes in the temperature of the water influenced actions of the Coastal Water (CW) and of the South Atlantic Central Water (SACW), which influenced the diet of the Corocoro grunts, probably through variations in marine productivity and, therefore, the availability of food resources for the species.

Keywords: Haemulidae, trophic ecology, invertivorous, water mass

1.INTRODUCTION

Coastal Bays constitute a particular estuary. They are considered semi-enclosed environments, highly dynamic and able to reconcile different ecosystems. They are regions of great biological, cultural and economic importance (Fistarol et al., 2015). The proximity of estuarine systems to sites with high populations and human disturbances results in a vulnerable environment and a high risk of eutrophication (Soares-Gomes et al., 2016). These environments support high productivity and diversity of species and ecological resources, including shelter, protection and availability of food, and play an important role in the life cycle for many species of marine teleostes (Castro et al., 2015; Silva-Júnior et al., 2016).

Coastal Bays are governed by the interaction between connectivity with the marine environment and the morphology of adjacent hydrological basins. Tidal currents are the main determinants of environmental conditions, which vary sharply along the ocean-to-earth gradient (Neves et al., 2011). Tropical Bays, in particular, exhibit well-defined temporal and spatial patterns in the variations of abiotic and biotic factors, which affect the behavior and distribution of fish assemblage (Franco et al., 2016; Souza et al., 2018). Among the abiotic factors described as main for these ecosystems, we highlight the temperature, salinity, depth, current velocity, and diversity of submerged habitats (Araújo and Azevedo, 2001).

Guanabara Bay is a large estuary located in the metropolitan region of Rio de Janeiro, Southeastern Brazil. This Bay has high levels of environmental disturbance in practically all its extent (Fistarol et al., 2015), in which anthropogenic impacts have adversely affected the integrity of its habitats and organisms, and in particular the fish assemblage (Franco and Santos, 2018). Despite the anthropic impacts, Guanabara Bay still plays an important role on feeding, spawning and as nursery areas for various species of fish (Castro et al., 2015; Franco et al., 2016; Seixas et al., 2016; Silva-Júnior et al., 2016; Franco and Santos, 2018).

In regions near the entrance of this Bay, where Praia Vermelha is also located, greater abundances and riches of fish species are found. Seasonal variations of the ichthyofauna are also recorded, due to the greater influence of more oceanic and cold waters (salinity ~35; temperature <20ºC), especially during the rainy season (November to March), which coincides with events of low intensity upwelling in the region of the Brazilian Southeast coast (Silva-Júnior et al., 2016). In the other periods, in which the upwelling events are attenuated, the contribution of the Coastal Water (CW) (Paranhos and Mayr, 1993; Silva-Júnior et al., 2016) is observed. On the other hand, more internal areas of the Guanabara Bay predominate eutrophic, polluted waters and mixohaline (Castro et al., 2005; Fistarol et al., 2015; Seixas et al., 2016; Franco and Santos, 2018).

The Corocoro grunt *Orthoprister ruber* (Cuvier, 1830) is fairly abundant and frequent specie in the South and Southeast regions of the Brazilian coast (Menezes and Figueiredo, 1980). It is found throughout Guanabara Bay, appearing as one of the species associated with rocky substrates most representative of this ecosystem (Chaves et al., 2018). Its distribution along the entire Guanabara Bay seems to be associated with the transparency, salinity and temperature of the water (Chaves et al., 2018). Despite the importance of studies on trophic ecology, the promotion of subsidies for conservation policies, the rational use of available fish resources and the sustainable use of biological diversity (Pessanha and Araújo, 2014; Santos et al., 2007), information on the *O. ruber* diet is scarce and its variations is not well-known in the environmental and oceanographic conditions in coastal Bays.

In this context, the aim of this study was: i) to characterize the diet and trophic strategy of the Corocoro grunt in Praia Vermelha, located at the entrance of the Guanabara Bay; ii) to test whether the food habit of the species varied between size classes (juveniles *versus* adults) and between dry and wet periods; and iii) to evaluate how the occurrence offshore upwelling events influence the observed patterns. The main hypothesis is that changes in the Corocoro grunt diet are more related to the effects of the predominant water masses in the adjacent ocean region, than variations between juveniles and adults and between dry and wet periods.

2.MATERIAL AND METHODS

2.1.Study area

Guanabara Bay (22º24'– 22º57'S; 42º33'– 43º19'W) is considered one of the most degraded coastal environments of the Brazilian coast. Covering approximately an area of 384 km^2, this Bay is densely populated, with more than 12 million inhabitants living in its environment (Fistarol et al., 2015). The fish assemblage in Guanabara Bay is formed by approximately 174 species of marine or estuarine origin (Vianna et al., 2012). Despite the growing picture of environmental degradation, Guanabara Bay still plays an important role in the nursing and growth of several species of fish (Castro et al., 2015; Seixas et al., 2016; Silva-Júnior et al., 2016).

Precipitation in the region shows a marked seasonality. The periods with the highest rainfall (rainy period) correspond to summer and austral spring, and contrast considerably with those with lower precipitation (dry period), which coincide with winter and austral autumn (Paranhos and Mayr, 1993; Chaves et al., 2018; INMET, 2018). Following this pattern, the river flow into the Bay varies from 186m^{3s-1}1 in the rainy season to 33m^{3s-1} in the dry season (Kjerfve et al., 1997).

The combined effects of fluvial water with the cyclical intrusion of oceanic water through the tidal regime explain much of the hydrological characteristics in Guanabara Bay (Mayr et al., 1989; Fistarol et al., 2015; Seixas et al., 2016; Chaves et al, 2018). Explained in part by the influence of cold fronts, precipitation patterns, and tidal regime, hydrological conditions follow two interconnected gradients: i) ocean-to-land, ranging from typically marine conditions at the entrance of the Bay to estuarine and eutrophic waters in more internal areas; ii) bathymetry, in which greater depth (~ 60 m) are generally found along the main central channel and gradually decrease in the east and west directions of the Bay. The combined influence of these factors leads to strong variability of environmental conditions reflecting the increase in biological productivity (Muto et al., 2014; Lopez-Lopez et al., 2017; Oliveira-Souza and Lavrado, 2017).

The salinity is lower in the internal areas in response to the water supply and the effluents. On the other hand, higher salinity levels predominate in areas of greater depth, especially in the region along the main channel, with great influence of marine and oceanic waters, mainly in the wet period (Seixas et al., 2016). The temperature, on the other hand, follows the inverse pattern. During the wet season, the influence of the South Atlantic Central Water (SACW) intensifies, contributing to more subtropical and temperate waters (T<20ºC) to the waters closest to the entrance of the Bay, as a more tropical characteristic, typical of the greater influence of Coastal Water (CW), predominate in the other seasons of the year (Moser et al., 2016; Silva-Júnior et al., 2016; Oliveira-Souza and Lavrado, 2017; Franco and Santos, 2018).

Praia Vermelha (22º57'S; 43º09'W) is an urban ecosystem, located near the entrance of Guanabara Bay (Figure 1). This region is influenced by the adjacent ocean area, where transparent, saline and cold waters predominate. It is also periodically affected by eutrophic, turbid and estuarine waters carried by tides and coming from the interior of the Bay (Franco et al., 2016; Seixas et al., 2016; Rodrigues et al., 2017). There are no legal restrictions for bathing, fishing, or other sporting activities at Praia Vermelha, resulting in its intensive use for recreation by the local population (Franco et al., 2016).

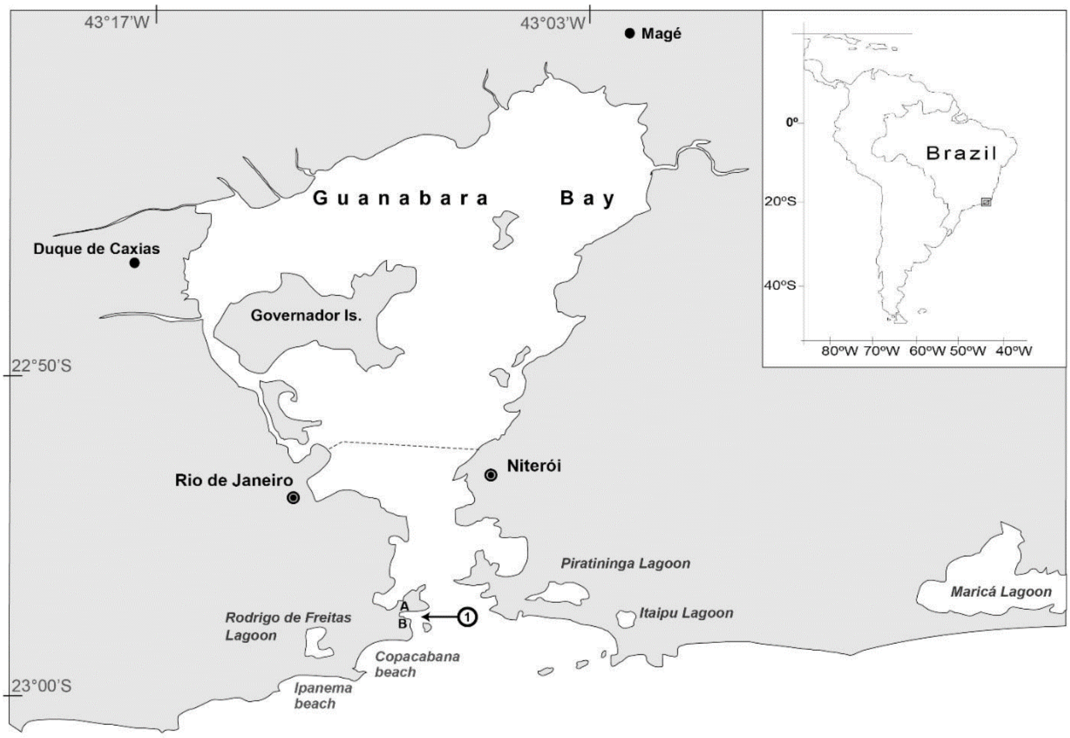

Figure 1. Geographic location of the studied site showing Guanabara Bay, Rio de Janeiro, Brazil, with the location of Praia Vermelha (1) at the outer zone of the Bay and its rocky reefs (A: left, and B: right).

The fish were captured in the rocky shores on the left and right sides of Praia Vermelha, through quarterly collections made between December 2011 and June 2015. Monofilament gillnets were used in three different meshes (20m of 15, 30 and 45 mesh mm between adjacent nodes, joined without sequencing), forming a battery with 60 m × 1,5 m. The network batteries (three replicates for each shore) were installed on the rocky substrates, perpendicular to the coastline, and removed after 24 hours. The environmental variables of the water temperature (ºC) and salinity were measured by a multiparameter probe model Hanna HI 9828.

The dry and wet periods were considered for evaluation of seasonal variations of the Corocoro grunt's diet in the present study (Paranhos and Mayr, 1993; Chaves et al., 2018; INMET, 2018). The water temperature ranges (cold 16-20ºC; intermediate 21-22ºC; and warm >22ºC) were established according to seasonal variations of river flow and marine currents, representing cyclical changes in oceanographic characteristics (cold waters = greater influence of SACW; warm water = a bigger influence for the CW) for the region near the entrance of Guanabara Bay (Silva-Júnior et al., 2016; Oliveira-Souza and Lavrado, 2017; Franco and Santos, 2018).

2.2.Diet and Feeding Strategy

All fish were identified, weighed, measured and gutted to remove the stomachs. Stomach contents were identified at the lowest possible taxonomic level, using a stereoscopic microscope and specialized bibliography. Then the food items were counted and weighed on a digital scale.

The diet was characterized according to the Index of Relative Importance (IRI; Pinkas et al., 1971), calculated for each category and food item, where: IRI=%OF x (%NF + % WF) x 100; %OF = number of stomachs in which a food item was found; expressed as the percentage of the total number of non-empty stomachs; %NF = percentage contribution of the numerical abundance of each item in relation to the total of food items; %WF = percentage contribution of the biomass of each item in relation to the total biomass of food items.

The trophic strategy of the species was estimated using the Amundsen Diagram et al. (1996). In the x-coordinates were plotted the values %OF and in the ordinate the values of the specific biomass of the prey (WF), that is, percentage of the weight of a certain type of alimentary item in relation to the weight of the other alimentary items, in the stomachs of fish in which this determined type of food item occurred. Through the relationship between specific biomass of the prey (%WF) and its frequency of occurrence (%OF), it is possible to predict information about the importance of the prey and the predator feeding strategy, which were obtained by the distribution of the points along the diagram.

To evaluate eventual ontogenetic changes in the diet, the individuals were classified as juveniles and adults according to the length of the first maturation (L50=160 mm), defined by Vianna and Verani (2002). For the analysis of the seasonal variation of the diet, the stomachs were analyzed considering the dry and wet periods.

2.3.Data treatment and analysis

The non-metric multidimensional ordering (nMDS; Clarke and Warwick, 2001) was performed to evaluate dietary changes between juveniles and adults and dry and wet periods. nMDS is a multivariate ordering technique in which dietary weight values are used to calculate the similarity matrix. Then, the Similarity Percentage Analysis (SIMPER) was applied to determine which food items presented the highest similarity within the diet of adults and juveniles and between dry and wet periods. Bray-Curtis distance was applied in all analyses.

Possible relationships between environmental variables and the corocoro grunt diet were analyzed through a Canonical Correlation Analysis (CCA). The significance of the axes was tested through the Monte Carlo simulation (9999 permutations). CCA is a widely used ordering method to evaluate species-environment relationships, especially when unimodal species responses are expected on abiotic gradients. One of the characteristics of the CCA is that the ordering diagram displays data along the canonical axes according to their ecological optimization, allowing easy interpretation (Borcard et al., 2011). Multivariate statistical analyses were performed using the CANOCO software (Leps and Smilauer, 2003).

3.RESULTS

3.1.Diet

A total of 167 *O. ruber* stomachs were analyzed. Out of this total, 134 stomachs were derived from juvenile fish (8-147 mm) and 33 from adults. Seventy-eight juveniles and 13 adults (162-279 mm) were analyzed from the dry period and 56 juveniles and 20 adults from the wet season. In general, the diet of the species consisted of seven categories, which comprised 13 food items (Table 1). *Crustaceans* were represented by amphipods, anomura, brachyura, shrimp, copepods and isopods; *Worms*, consisting of nemathelminths and polychaetas; *Mollusks*, represented by bivalves; Teleostei, represented by fish scales; *Plant material*, characterized by seagrass; *Sediments*, consisting of grains of sand; and *Inorganic allochthonous material*, consisting of nylon filaments.

Table 1. Occurrence (%OF), numeric (%NF), weight (%WF) frequencies and index of relative importance (%IRI) of the diet items of *O. ruber* in the PraiaVermelha, Guanabara Bay, Southeastern Brazil. Values expressed as a percentage.

Diet categories and items	OF	NF	WF	IRI
PLANT MATERIAL	0.12	0.12	0.65	0.11
Plant material	15.46	0.13	0.65	0.05
WORM	0.02	0.06	0.03	<0.01
Polychaeta	0.48	0.04	<0.01	<0.01
Nemathelminth	1.45	0.02	0.02	<0.01
SAND	0.03	0.49	0.35	0.03
Sand	3.87	0.49	0.35	0.05
CRUSTACEA	0.43	99.12	97.27	99.57
Crab	1.45	0.01	0.57	<0.01
Isopoda	0.48	<0.01	1.54	<0.01
Amphipod	3.38	0.22	1.49	0.02
Shrimp	43.96	93.31	88.69	99.27
Copepoda	3.86	5.56	4.95	0.52
Anomura	1.45	0.01	0.03	<0.01
DEBRIS	0.16	0.18	1.27	0.27
Debris	20.77	0.18	1.27	0.09
MOLLUSCS	0.02	0.02	0.21	<0.01
Bivalvia	2.42	0.02	0.21	<0.01
TELEOSTEI	<0.01	<0.01	0.21	<0.01
Scale	0.97	<0.01	0.21	<0.01

In general, the Corocoro grunt presented a food strategy specialized in crustaceans throughout the ontogenic development (Figure 2). However, differences in the proportion and use of food items were observed between juveniles and adults. Juveniles mainly consumed shrimp (99.13%IRI), followed by reduced fractions of copepods (0.43%IRI), isopods, amphipodan and anomura (<0.01%IRI, for each item). Among adults, the diet was also based on shrimp (48.27%IRI) consumption, but with important participations of amphipod (21.14%IRI), plant material (14.68%IRI), brachyura (5.52%IRI) and copepods (4,98%IRI). Brachyura, polychaeta and fish scales were unique items in the adult diet.

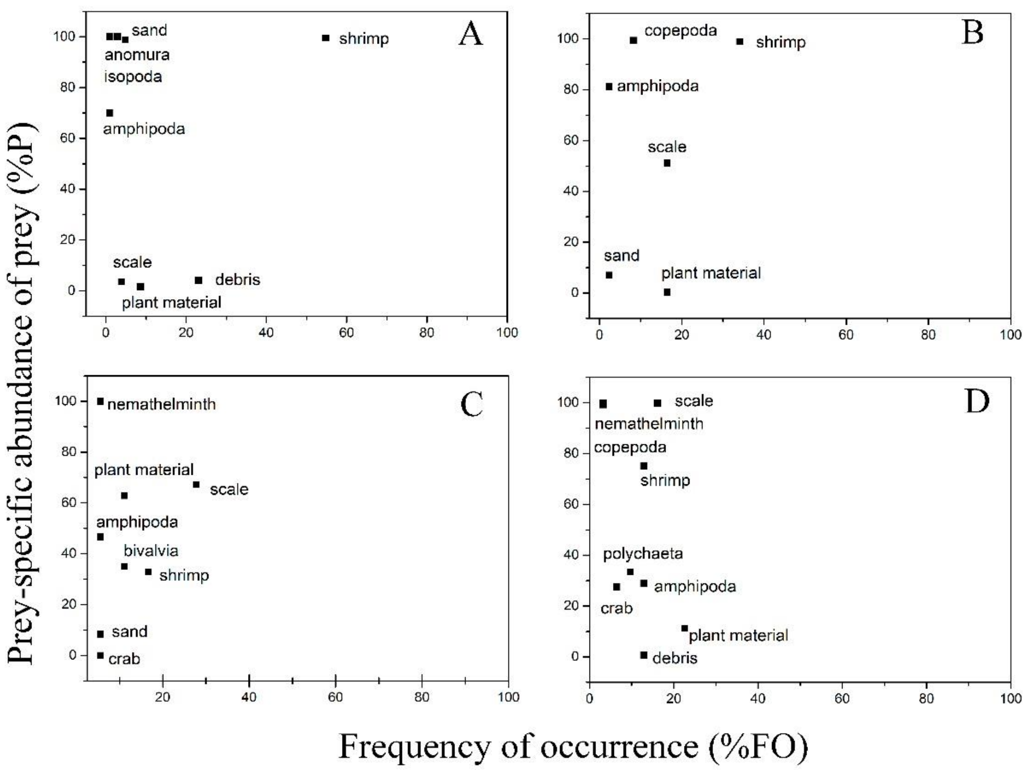

Figure 2. Feeding strategy diagram of *O. ruber* in the PraiaVermelha, Guanabara Bay, Brazil. (A) juveniles/dry period; (B) juveniles/wet period; (C) adult/dry period; (D) adult/wet period.

In the wet season, the most important items in the juvenile diet were shrimp (95.81%IRI), followed by copepods (3.98%IRI). At the same time, the adult diet consisted of shrimp (48.01%IRI), followed by amphipodan (21.02%IRI), plant material (14.59%IRI), copepods

(4.95%IRI) and polychaeta (0.32%IRI). No unique items were recorded in the juvenile diet during the rainy season, whereas polychaetas occurred exclusively in the adult's stomach during the same period. A similar pattern was recorded for juveniles in the dry period, in which shrimp (99.47%IRI) was a predominant item in the diet, followed by vegetal material (0.43%IRI). Sand grains, isopoda, amphipods, anomura and inorganic allochthonous material had a small contribution in the feeding of juveniles (<0.02% IRI, for each item) for this period. Isopods were an exclusive item for the juvenile diet. During the dry period, the most important items in the diet of adults were shrimp, followed by fish scales (9.91%IRI), bivalve (9.25%IRI), plant material (8.26%IRI) and anomura (4.61%IRI).

The ordering by nMDS (stress=0.09) based on the weight values of the food items consumed by Corocoro grunt did not reveal well defined groupings for juvenile and adult diets in the dry and wet periods, indicating a high overlap in the consumption of prey between size classes (Figure 3). SIMPER (Table 2) indicated a greater dissimilarity in the diet of adults between the dry and wet periods (92.46%), with greater representativeness of shrimp (32.66%), scale (30.82%) and amphipods (10.41%). For juveniles, 65% of dissimilarity in the diet was recorded between the periods, being shrimp (67.57%), copepod and anomura (10.75%, for each item), the major responsible for this differentiation. In this context, adults presented a more diversified diet and juveniles had a more homogeneous diet between periods.

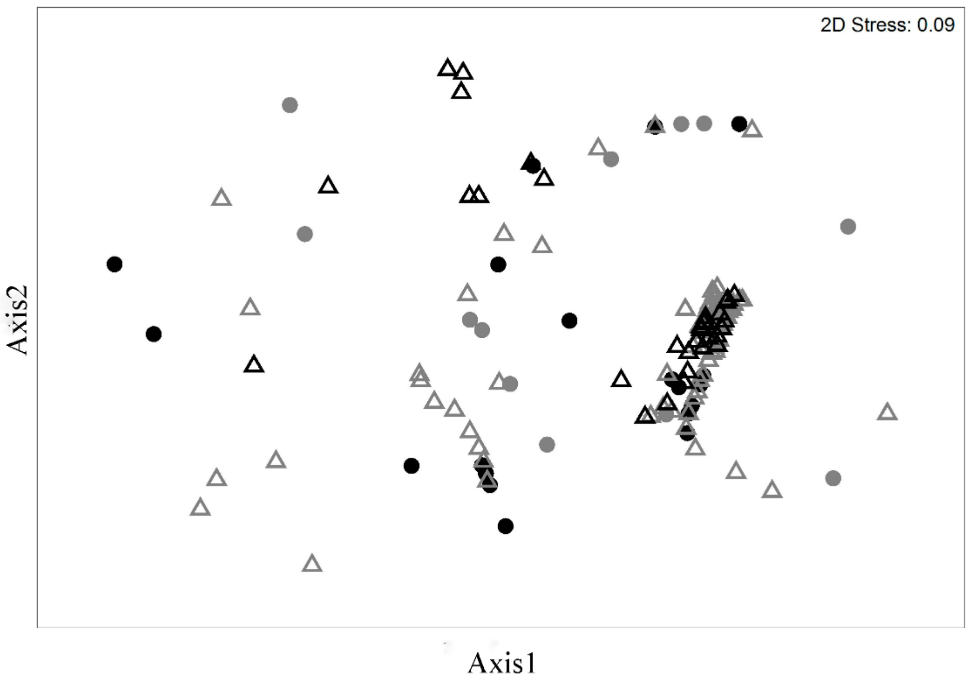

Figure 3. Nonmetric multidimensional scaling (NMDS) ordination plot showing from the diet distribution of *O. ruber* according to ontogenic development and sampling periods. ● = adult-dry; ○ = adult-wet; △ = juvenile-dry; △ = juvenile-wet.

Table 2. SIMPER analysis of similarity between juveniles and adults of the *O. ruber* captured in the dry and wet periods in the PraiaVermelha.

	Food itens	Dissimilary contribution %	Contribution %	Contribution cumulative %
Adult dry	Plant material	0.31	53.49	53.49
	Scale	0.24	28.20	81.68
	Shrimp	0.23	10.88	92.56
Adult wet	Shrimp	0.41	70.94	70.94
	Scale	0.24	23.90	94.83
Adult dry x Adult wet	Shrimp	0.79	32.66	32.66
	Scale	0.74	30.82	63.48
	Amphipoda	0.40	10.41	73.89
Adult dry x Juvenile dry	Shrimp	1.57	66.19	66.19
	Scale	0.57	16.14	82.62
Adult wet x Juvenile dry	Shrimp	1.50	53.63	53.63
	Scale	0.63	22.48	76.11
Adult dry x Juvenile wet	Shrimp	1.53	63.73	63.73
Adult wet x Juvenile wet	Shrimp	1.46	48.25	48.25
	Scale	0.57	17.55	65.80
Juvenile dry	Shrimp	0.88	97.19	97.19
Juvenile wet	Shrimp	0.96	91.76	91.76
Juvenile dry x Juvenile wet	Shrimp	1.37	67.57	67.57
	Copepoda	0.49	10.75	78.31
	Anomura	0.49	10.75	89.06

3.2.Effects of oceanographic events

In the period of warmer water temperatures (>22ºC), high abundances of juveniles (n=65) were recorded in relation to adults (n=4). This period coincided with higher mean values of precipitation (129 mm) and salinity (30). During the colder water temperature period (16-20ºC), abundance of juveniles (n=15) and adults (n=14) was similar and mean rainfall was 111 mm, with salinity of 32. At intermediate water temperature (21-22ºC) 55 juveniles and 14 adults were recorded, with mean rainfall of 62 mm and salinity of 29.

The multivariate canonical correspondence analysis (CCA) evidenced a correlation (F=17.60; p<0.001) between food items and water temperature ranges, with the formation of three distinct groups (Figure 4). The first two canonical axes explained 91.3% of this variance, with a contribution of 62.6% for axis 1 and 28.7% for axis 2. In waters with higher temperatures (>22ºC), the Corocoro grunts preferably consumed shrimp, polychaeta, plant material and sand. Under these conditions, rainfall was highly correlated with the main items consumed by the species. At intermediate temperature (21-22ºC), the diet was based on inorganic allochthonous, copepods and anomura. In the period of cooler water (16-20ºC), crabs, fish and amphipod were the most consumed items, which were related to the total length of the individuals. The salinity was directly associated with the items consumed in the period of cooler waters, and to a lesser extent, those consumed in waters of intermediate temperatures.

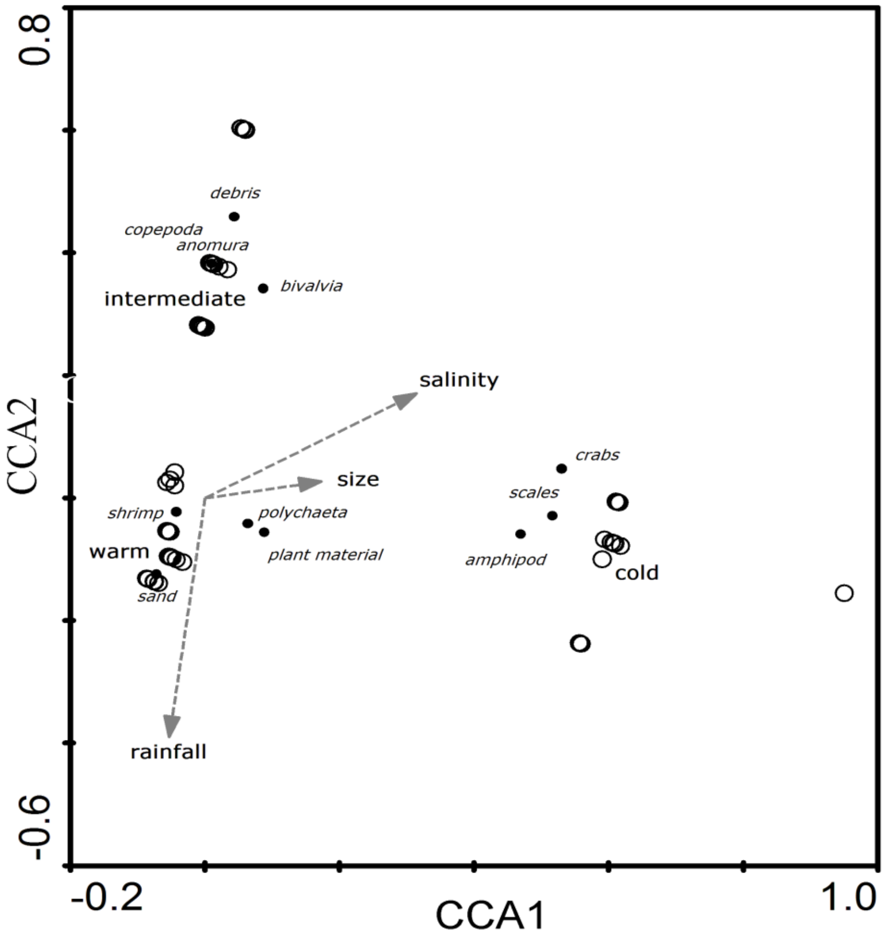

Figure 4. Canonical Correspondence Analysis (CCA) between diet and water temperature in the Guanabara Bay.

4.DISCUSSION

The present work confirmed the hypothesis that, although the Corocoro grunt *O. ruber* presented predominantly invertivorous food habit, with a tendency of specialization in the consumption of crustaceans, the alternation of oceanographic events near the entrance of the of Guanabara Bay influenced the trophic strategy of the species, and its effects were more pronounced than environmental variations between dry and wet periods or changes in prey consumption between juveniles and adults. The diet of this Corocoro grunt species, which is medium in size, is widespread throughout the Western Atlantic and is generally associated with rocky substrates and reefs. It has also been studied in other coastal Bays, where the preferential consumption of crustaceans, mainly shrimp (Muto et al., 2014; Soares et al., 2018) and amphipod (Aguiar and Filomena, 1995). The predominance of shrimp seems to be associated with their availability and the characteristics of the preferred habitat (i.e., consolidated substrates) of occurrence of both prey and their predators in the case, *O. ruber* (Batista et al., 2001; Souza et al., 2008). Other species of Haemulidae have also used crustaceans as the main food resource, such as *Pomadasys incises* (Fehri-Bedoui and Gharbi, 2008), *Haemulon* sp. (Pereira et al., 2015), *Haemulon flavolineatum* (Burke, 1995) e *Orthopristis. chrysoptera* (Howe, 2001).

In Guanabara Bay, the preference for crustacean consumption and the specialization in shrimp ingestion prevailed in both juveniles and adults of *O. ruber*. According to Pereira et al. (2015) and Vianna and Verani (2002), changes in preferential feeding sites and in habitat use among Haemulidae are common, where juveniles forage shallow areas and warm areas (22-25ºC), while adults in deeper and cooler areas (19-22ºC). This pattern of habitat use is also expected for the Guanabara Bay as a whole, however, on Praia Vermelha the rocky shores rarely exceed 5-6 m maximum depth. In this sense, both juveniles and adults of Corocoro grunts probably share similar areas of occupation and habitat use for foraging, which could explain the similarity in diet and the preference in shrimp consumption among the studied size classes.

The diet of the species was more diversified in the wet season, which coincided with the greater similarity in the diet between juveniles and adults. This pattern may be associated with the higher contribution of freshwater to the Guanabara Bay, mainly during the summer months, which could have contributed to the increase of productivity in this period. The precipitation regime is considered the major controlling factor in the seasonal dynamics of estuarine plankton in tropical and subtropical areas (Sassi, 1991). According to Valentin et al. (1999), between the end of spring and throughout the summer an increase in the density of zooplankton is observed, which could be reflecting the higher productivity of Guanabara Bay in this period, and consequently affecting the availability of food items for *O. ruber*.

Although precipitation regimes were curiously similar between wet and dry periods during the study, the period of higher rainfall coincided with the occurrence of colder waters (16-20ºC). This period coincided with the higher food plasticity of *O. ruber* and with higher values of salinity. Cooler and more saline temperatures indicate the intensification of Southern Atlantic Central Water (SACW) upwelling events, which possibly influenced the composition and structure and dynamics of the benthic invertebrate assemblage, and, therefore, the availability of prey for the *O. ruber* (Davies et al., 2008; Kehrig et al., 2011; Moser et al., 2016).

In the wet season, abundances of juveniles and adults were similar, as did the variety of items consumed. However, although they continue to consume shrimps preferentially, the *O. ruber* adults presented more significant proportions of other items in the diet, revealing greater food plasticity in relation to the juveniles. This question can be explained in part by the differential investment between adults and juveniles in the consumption of prey with different sizes and biomasses, where the former consume larger prey, in smaller numbers, but larger biomasses, while juveniles feed on smaller prey, but in larger quantities (Soares et al., 2018).

In general, it is possible to conclude from the present work the Corocoro grunt *O. ruber* presented invertivorous food habit and specialized consumption in benthic crustaceans in the rocky shores of the Praia Vermelha, located in the entrance of Guanabara Bay. During the wet season, there were predominant oceanographic characteristics that indicate the greater influence of upwelling, which coincided with the increase in the overlap in the consumption of prey between juveniles and adults of the species. During the dry period, there was a predominance of warmer and less saline waters, coinciding with the reduction in the diversity of food items consumed and the greater contribution of small prey, favoring larger occurrences of juveniles over adults. In this sense, the alternation of oceanographic conditions, sometimes with more tropical characteristics, sometimes approaching more subtropical and temperate conditions, significantly affects the diet of the Corocoro grunt *O. ruber*, with probable effects on the levels of intraspecific competition and the patterns of use of habitat by juveniles and adults of the species.

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CONCLUSÃO GERAL

- O presente estudo é pioneiro em utilizar uma espécie de peixe marinha Neotropical comparando atributos morfológicos, fisiológicos e ecologia trofica em relação à ação de agentes estressores ambientais na baía de Guanabara;
- Os menores valores de assimetria flutuante registrado em *O. ruber* coletados próximo à entrada da baía de Guanabara, reflete que a região apresenta as melhores condições ambientais e que decresce em direção às zonas internas na baía. No entanto, não podemos afirmar que as variações ambientais da baía de Guanabara foram as únicas a influenciarem nos desvios bilaterais na morfologia da espécie;
- O índice composto CFA1 apresentou melhores resultados na detecção de desvios bilaterais para *O. ruber*;
- Entre os caracteres analisados, o rastro branquial de *O. ruber* mostrou maior vulnerabilidade a agentes estressores;
- Assimetria flutuante em *O. ruber*, infere sobre a compreensão da instabilidade do desenvolvimento e da condição corporal da espécie na baía de Guanabara;
- Os descritores fisiológicos: fator de condição, índice de repleção e índice hepatossomatico de *O. ruber* foram sutilmente afetados pela assimetria flutuante;
- A concentração de HgT presente nas áreas mais externa e interna da baía de Guanabara não teve relação direta com a assimetria flutuante em *O. ruber*;
- *Orthopristis ruber* é uma espécie bioindicadora de contaminação por HgT ao longo da baía de Guanabara;
- A espécie apresenta maiores teores de HgT na praia Vermelha, devido aos fatores hidrodinâmicos da área que proporcionam melhor circulação de água, atuando como um reator natural de mercúrio aumentando sua biodisponibilidade o incorporando nas cadeias alimentares;
- As alterações na temperatura da água expressaram a atuação de massas de Água Costeiras (CW) e Água Central do Atlântico Sul (SACW), as quais influenciaram na dieta bentônica invertivora de *O. ruber* com prováveis efeitos sobre os níveis de competição intraespecífica e nos padrões de uso de habitat por juvenis e adultos da espécie.