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**ECOSYSTEMS**

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

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**Abstract:** Fluctuation asymmetries (FA) are random on the bilateral symmetry plan of paired morphological characters, and other types of symmetry, such as: for instance, translational or rotational symmetry, in response to environmental, and genetic stress. The relationship of FA with gonadossomatic (GSI), hepatosomatic (HSI), and fullness (RI) indexes and condition factor (K) for juveniles (n=8), adults (n=32), males (n=9), and females (n=26) of Corocoro grunt *Orthopristis ruber* were evaluated in this paper. The composite fluctuating asymmetry (CFA) was used to calculate the combined effects of FA over these four organosomatic indexes of 66 Corocoro grunt caught during 2011 in Guanabara Bay, Brazil, one of the most eutrophic coastal bays in the world. Redundancy Analysis (RDA) confirmed a significant relationship between CFA and the physiological descriptors (HSI, RI, K), but without clear differences among juveniles, adults, and sexes. Our results support the potential of CFA to be used as a proxy of environmental effects over reef-associated fish species in a tropical bay, but the relationship between CFA and physiological descriptors is complex, and further studies, such as experimental trials, are needed.

**Key words:** Bioindicator, Brazil, Guanabara Bay, Haemulid.

## INTRODUCTION

Fluctuating asymmetries (FA) are small, random deviations from symmetry that occur in the development of bilaterally symmetrical characters (Van Vallen 1962), and are commonly used as a measure of developmental stability. Thus, a high level of FA is assumed to reflect reduced developmental stability. Considered as a whole-organism indicator of stress, FA theory relies on that deviations in bilateral symmetry will rise with increased instability of an organism along with its development (Sopinka et al. 2017). The levels of FA are thus correlated with individual fitness (i.e. the ability to survive, flourish, and carry on the successful qualities through genes to offspring) and the adaptive ability of the entire population, also supporting inferences on the health of the whole ecosystem (Palmer 1994, Oxnevad et al. 2002, Palmer & Strobeck 2003, Sopinka et al. 2017). Despite its increased application for environmental and biomonitoring assessments, the connections of FA levels with other secondary or tertiary measures of stress are barely known, except for few studies.

Changes in fish growth, condition, and health can be also used to indicate the extent to which stress may affect fish performance and provide a basis for understanding the effects of environmental perturbations on fish populations (Barton et al. 2002). The relationship of FA levels with adaptive fitness, reproductive success (Bakker et al. 2006), egg size (Hechter et al. 2000), and pollution levels in fish (Lajus et al. 2015) has been addressed recently. Other more physiological and condition-related indicators of environmental stress in fish have been proposed, but whether FA levels are associated with organosomatic indexes related to the fish condition, such as the condition factor, and gonadosomatic, hepatosomatic, and fullness indexes are virtually unknown, especially for tropical marine ecosystems (Sopinka et al. 2017).

Guanabara Bay (GB), located in 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 (Kjerfve et al. 1997, Fistarol et al. 2015). Environmental disturbances started early in the XVI century in GB but escalated especially since 1930 with the aggravation of the industrialization process, which have increased significantly the concentrations of inorganic nutrients (mainly phosphorus), heavy metals (particularly 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). This ~380 km$^2$ bay is located in Rio de Janeiro city and its surroundings, the second largest industrial center in Brazil. As consequence, there are more than 12.000 industries in the drainage basin, which accounted for 60% of the state's facilities and 25% of the organic pollution released to the bay (Soares-Gomes et al. 2016, Baptista et al. 2017). Approximately 500 tons of raw sewage (~80% of biochemical oxygen demand) is discharged daily through river inflow, leading to a complex load of nutrients and toxic chemicals in the water column and bottom sediments (Silveira et al. 2017). Therefore, GB shows a complex pattern of water quality, of considerable spatial and temporal variability, depending on the combining effects of river inflow, watershed use, and the seasonal regime of rainstorms, this latter harshening the input of sewage and chemical contaminants to the system (Kjerfve et al. 1997).

The Corocoro grunt *Orthopristis ruber* (Cuvier 1830) is a Haemulid fish commonly found near rocky and reef substrates across the South Atlantic coast and widespread in several marines and estuaries systems along the Brazilian coast (Vianna & Verani 2002). Corocoro grunt preys mainly on invertebrates and small fish (Kehrig et al. 2010) and spawns throughout the year, with peaks in spring and summer (Garcia et al. 2010). This species is associated with rocky shores in GB (Seixas et al. 2016), showing high site-fidelity when < 300 mm total length (TL) and greater abundances in the outer zones of this bay (Chaves et al. 2018).

In this paper, the deviation from bilateral symmetry in six morphometric and meristic characters of *O. ruber* captured GB was investigated. The hypothesize is that the greater the fluctuating asymmetry, the lower the species' physiological indices, with differences between life stages and sexes. The aims of this study are: (1) to test composite fluctuating asymmetry (CFA) among the juvenile and adults, and males, and females, (2) compare the levels of CFA among the four descriptors of the physiological condition of *O. ruber*, and correlate them with life stages and sexes, and (3) to evaluate the implications the fluctuating asymmetry being applied as proxies of the ecological integrity of tropical bays.

## MATERIALS AND METHODS

### Study area

Guanabara Bay (22°24'– 22°57'S, 42°33'– 43°19'W), covering approximately 384 km$^2$ of surface area and yielding 12 million inhabitants living in the surroundings, wherein 74.3% are composed of urbanized areas (IBGE 2017). The drainage basin accounts for the receptor of most of the effluents produced by industrial plants, two international airports, and two harbors landing approximately 2,000 commercial ships every year (Baptista et al. 2017). This bay also has two naval bases, 20 shipyards, thousands of ferries, fishing boats, and yachts, and a large Petrochemical Complex responding by 7% of the national oil refining (Kjerfve et al. 1997, Fistarol et al. 2015). Sedimentation rates range from 0.60-2.2 cm per year, and their growing levels are attributed to the increased urbanization process (Soares-Gomes et al. 2016). A total of 174 species is listed for the marine and estuarine fish assemblages inhabiting Guanabara Bay (Vianna et al. 2012), which persist in using this ecosystem as feeding and nursery grounds, despite the increasingly environmental disturbances (Castro et al. 2005, Franco et al. 2016, Souza et al. 2018).

### Fish sampling and data analysis

*Orthopristis ruber* was sampled from inner to outer zones of Guanabara Bay, Rio de Janeiro - Brazil, encompassing most of its environmental gradient (Fistarol et al. 2015). The areas studied were: Urca, Rio-Niterói Bridge, and Paquetá Island. Seixas et al. (2016) demonstrated that the level of CFA 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). Thus so it was compared the levels of CFA, among the four descriptors of the physiological condition of *O. ruber*, and correlates them with life stages and sexes, in these sites.

Monofilament gillnets (20 m) of three different mesh (15, 30, and 45 mm between adjacent knots) were tied together to form a set (60 m × 1.5 m) that was used to capture fish in the three sampling sites. Fish were caught in dry (September end of winter season) and rainy (December end of spring season) 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 (Chaves et al. 2018).

A total of 66 Corocoro grunts 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, to minimize possible effects of methodological artifacts on asymmetry results. The diameter of the eye (EYD), length of the pectoral fins (LPF), length of ventral fins (LVF) were the three morphometric traits assessed, while the number of gill rakers (NGR), number of pectoral fin rays (NPR), and number of ventral fin rays (NVR) were the three meristic traits evaluated. Specimens with damaged fins were removed from the analysis. PERMANOVA (Permutational Multivariate Analysis of Variance) was applied (Euclidean Distance, 10,000 permutations per analysis) to detect possible measurement errors between the first and second measurements. All morphological attributes were measured twice (i.e. independent measures) to evaluate the importance of measurement errors on FA levels, according to Palmer & Strobeck (1986). The side of the structure in *O. ruber* was considered as a fixed factor and each fish as a random factor in all PERMANOVA tests. Significant trait side × fish interactions ($p<0.05$) denote the negligible effects of measurement errors on the FAs. Non-significant results ($p>0.05$) indicate that measurement errors were negligible.

The composite fluctuating asymmetry (CFA) was used to calculate the combined effects of FA from all of 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 ($CFA=\Sigma|R-L|$). The CFA is regarded as less sensitive to sampling and measurement biases than individual indexes (Leung et al. 2000). The validation of the presence of FA followed the protocol composed by Seixas et al. (2016) that was applied to this same database.

Four descriptors of the physiological condition of *O. ruber* were used: condition factor (K), and gonadosomatic (GSI), hepatosomatic (HSI), and fullness (RI) indexes. GSI, HSI, and K were determined as in Vazzoler (1996): $GSI = Gw \times Ew^{-1}$, $HSI = Lw \times Ew^{-1}$, $K = Tw \times Tl^{-3}$. The RI index was calculated as in Hyslop (1980): $RI = (Sw \times Ew^{-1}) \times 100$, where, Gw = gonad weight, Lw = liver weight, Sw = stomach weight, Ew = eviscerated weight, Tw = total weight, and Tl = Total length.

Fish was classified as male or female through macroscopic inspection of gonads, and as juvenile or adult after comparing the total length of each individual with the size of first maturation (L50 = 160 mm TL) proposed by Vianna & Verani (2002).

PERMANOVA was performed on a data matrix, to test for differences in the four physiological descriptors between juvenile and adult, and sex. The Euclidean distance was used and data was permuted 4,999 per analysis and $p<0.10$ was regarded as significant. 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 CFA in *O. ruber* with the four physiological descriptors between juvenile and adult, and sex. Partial Redundancy Analysis (RDA) was applied, using TL as covariable (i.e. to control for the effects of fish size). Monte Carlo permutation tests were performed to test the significance of the axes ($p \leq 0.05$). The Generalized Additive Models (GAMs) were used to investigate relationships between CFA with the scores of RDA axes.

The step-by-step selection procedure and the Akaike Information Criterion (AIC) were used to determine the complexity of the model. AIC considers not only the goodness of fit but also parsimony, penalizing more complex models (Burnham & Anderson 1998). All multivariate ordination analyses were performed in CANOCO 4.5 software (Leps & Smilauer 2003).

## RESULTS

The mean values and range of the levels of fluctuating asymmetry calculated through individual and composite indexes for the six morphological traits of 66 *O. ruber* caught in GB composed of 13 juveniles and 53 adults, and 14 males and 33 females (i.e. the sexes of nineteen fishes immature, were not determined) (Table I). FA values were more variable for the length of the pectoral fins (LPF) and length of ventral fins (LVF) among morphometric traits, and the number of gill rakers (NGR) among meristic traits (Table I).

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; $F=2.19$; $p=0.011$) were recorded for juveniles (Table II). Males exhibited higher mean GSI (PERMANOVA; $F=2.88$; $p=0.09$) and females

**Table I.** **Mean values and range (between parentheses) of the levels of fluctuating asymmetry calculated through individual and composite indexes for the six morphological traits of *Orthopristis ruber* caught in Guanabara 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 | LVF |
|---|---|---|---|---|---|---|---|---|
| Adult | 53 | 31.47 | 0.44 | 0.19 | 1.10 | 4.65 | 13.32 | 11.88 |
| | | (1-86) | (0-3) | (0-2) | (0-6) | (0-18.7) | (0-60.2) | (0-47) |
| Juvenile | 13 | 22.30 | 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 |
| | | (9-78) | (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 |
| | | (8-86) | (0-3) | (0-2) | (0-6) | (0-18.7) | (0-60.2) | (0-26.7) |

higher mean of RI (PERMANOVA; F=0.14; p=0.071). Males and females did not exhibit significant difference of the K (PERMANOVA; F=0.09; p=0.77) and IHS (PERMANOVA; F=0.009; p=0.94) (Table II).

The RDA was statistically significant (Monte Carlo test, F=3.1, p=0.05) between the relationship of CFA and physiological descriptors, which were summarized by the two first RDA axes, which explained 98.1% and 100% of the variation, respectively. The first axis separating the adults (n=32) and juveniles (n=8), and showing a stronger relationship of the adults with the GSI and HSI (Fig. 2). However, these descriptors were not affected by the presence of CFA. The second axis revealed an inverse relationship between CFA with K, and RI values (Fig. 2).

The RDA of the relationship of the CFA between sexes with the physiological descriptors also was statistically significant (Monte Carlo test; F=7.68; p=0.02). The first axis RDA explained 98.2% of the data variance, but no clear relationship was found between the CFA and sexes (males n =9, and females n=26) (Fig. 3). The second axis showed an inverse relationship between CFA and descriptors K, RI, and HSI, regardless of sex (Fig. 3). With that, AIC was tested between CFA and these three descriptors. The AIC revealed the relationship between CFA (standard error = 23.46) and K (standard error = 0.0012) for males (n=9, non-linear, F=16.46, p=0.006), with an increase of CFA and decreased K (Fig. 4). Thus, FA is interfering with the physiological condition of males. Other relationships with the physiological descriptors between sexes, were not found by GAM.

The physiological indices in juveniles only occurred in the Urca region. GSI, RI, HIS, and GSI in adults, were no significant differences in BG areas (PERMANOVA; F=27; p=0.11).

The physiological indices in females, was no significant difference in BG areas (PERMANOVA; F=1.01; p=0.42), unlike that observed in males (PERMANOVA; F=52.84; p<0.01). GSI, RI, and HSI in males were no statistically significant in BG areas (p>0.50), but K was a significant difference, with higher averages in Urca compared to Paqueta (p<0.01).

Both sexes showed, a proportionally balanced sample in both the dry, and rainy periods with no possibility of the results reflecting seasonality. The number of females analyzed was n=18 in September, and n=15 in December, while the males analyzed were n=6 in September and n=8 in December.

**Table II.** Mean values and range (between parentheses) of the physiological indexes calculated for *Orthopristis ruber* caught in Guanabara Bay. K: condition factor; HSI: hepatosomatic index; GSI: gonadosomatic index; and RI: fullness index. *: significant values ($p < 0.05$).

| | n | K | HSI | GSI | RI |
|---|---|---|---|---|---|
| Adult | 32 | 0.010 | 10.05* | 18.49* | 8.39 |
| | | (0.002-0.015) | (1.02-20.54) | (0.83-69.82) | (0.94-35.22) |
| Juvenile | 8 | 0.013* | 9.72 | 4.28 | 9.35* |
| | | (0.012-0.015) | (5.07-15.87) | (1.09-13.08) | (3.2-26.47) |
| Male | 9 | 0.013 | 10.4 | 21.83* | 6.16 |
| | | (0.011-0.014) | (6.25-13.84) | (1.64-69.82) | (0.94-15.13) |
| Female | 26 | 0.013 | 10.85 | 14.30 | 8.55* |
| | | (0.01-0.02) | (4.99-20.54) | (1.09-69.29) | (3.20-30.23) |

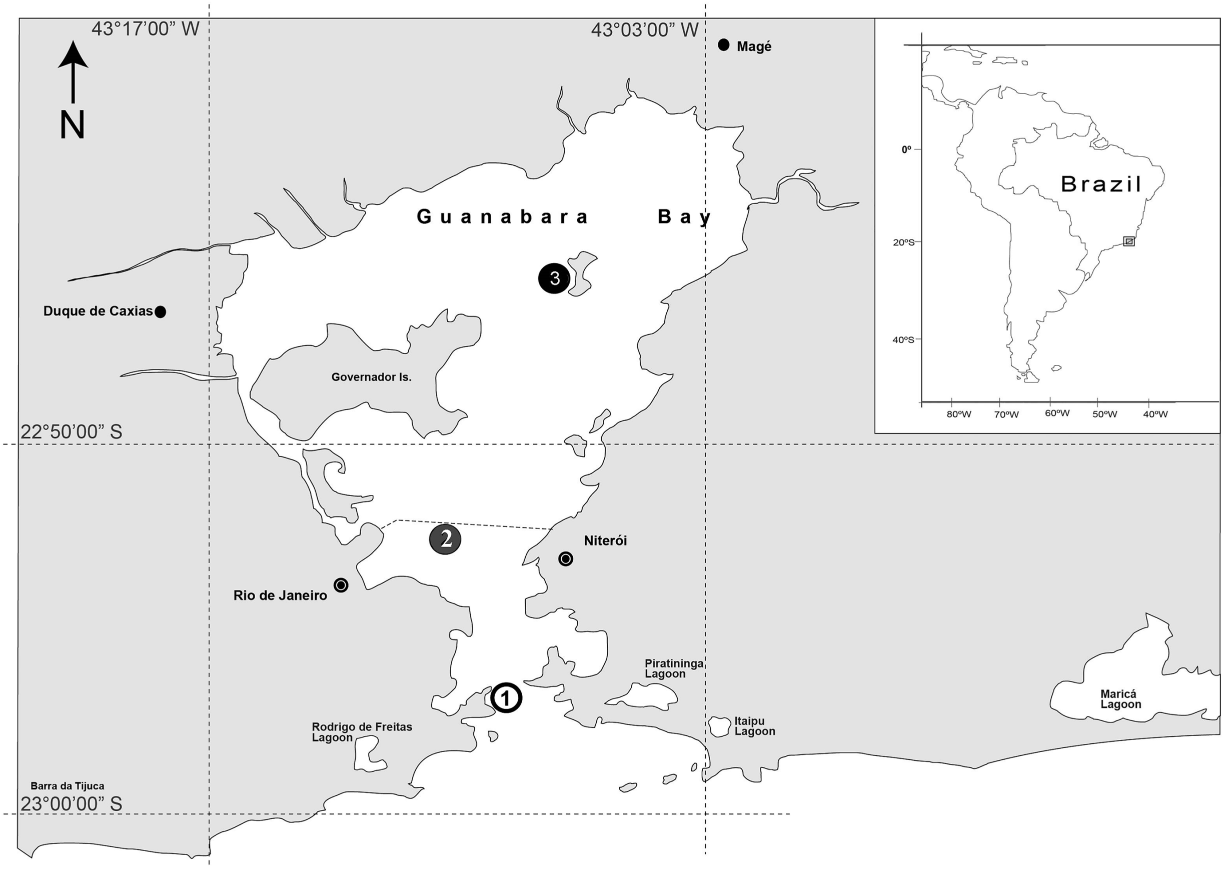

**Figure 1.** The geographic location of Guanabara Bay (Southeastern Brazil), showing the sites where *Orthopristis ruber* were caught. 1 = Urca region, located near the mouth of Guanabara Bay; 2 = site located over the main circulation channel, near the Rio-Niterói Bridge; 3 = site located near the Paquetá Island, the innermost region of Guanabara Bay undergoing direct influence by the main circulation channel.

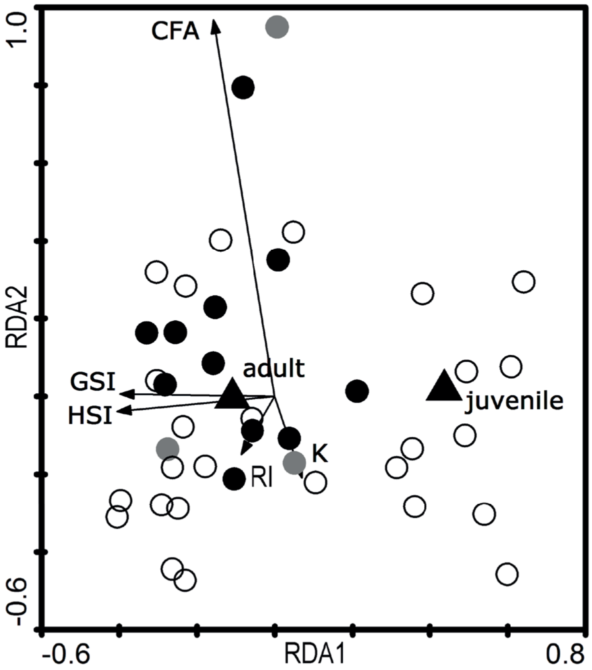

**Figure 2. Partial Redundancy Analysis (RDA) showing the relationship of fluctuating asymmetry (CFA) with four physiological indexes (GSI, HSI, RI, and K) of adult and juvenile *Orthopristis ruber*. ◯ =Urca; ◍ =Rio-Niterói Bridge; ● =Paquetá; ▲ = grouping of adults and juveniles.**

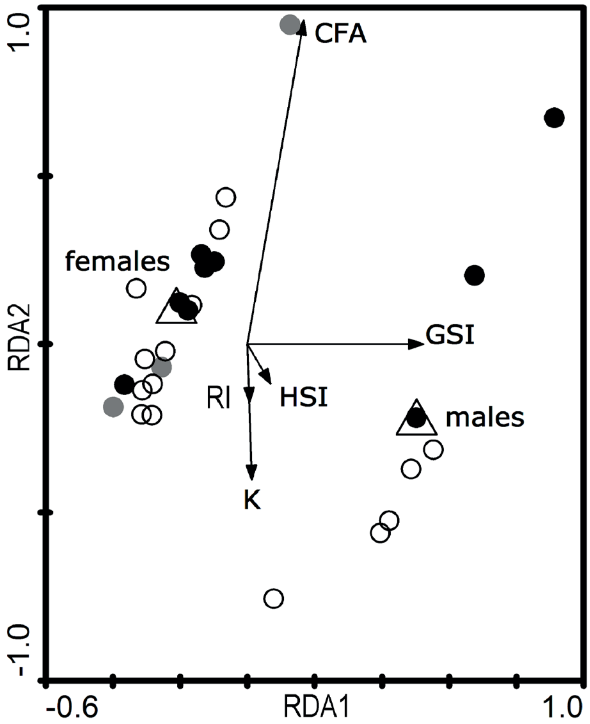

**Figure 3. Partial Redundancy Analysis (RDA) showing the relationship of fluctuating asymmetry (CFA) with four physiological indexes (GSI, HSI, RI, and K) of male and female *Orthopristis ruber*. ◯ =Urca; ◍ = Rio-Niterói Bridge; ● =Paquetá; Δ= grouping of male and female.**

## DISCUSSION

Changes in the symmetry of the *Orthopristes ruber* may be negatively influencing the process of well-being and foraging of the species in Guanabara Bay. Our results are in agreement with the works of Somarakis et al. (1997) and Ayoade et al. (2004), where they mention that the 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).

According to Kjerfve et al. (1997) in the rainy season the higher river-runoff influences the entrance of marine water, thus limiting the extension of the salt wedge. This balance between marine input, regulated by river-runoff, and tide amplitude, results in a characteristic seasonal variation in GB, which presents an extremely wide watershed (Kjerfve et al. 1997). An increase in runoff in tropical bays enhances the input of sediment, organic matter, heavy metals, organochlorates, and other anthropically originated residuals, thereby increasing the bottom bacterial activity (Baptista et al. 2017). During the process of the carbonic chain are degraded, inducing the consumption of dissolved oxygen, both from the sediment and

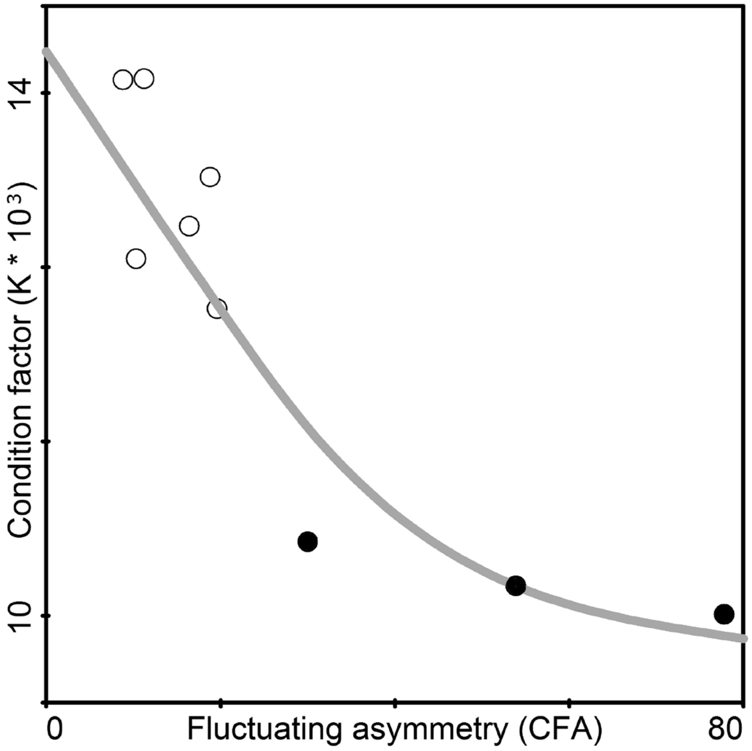

**Figure 4.** **Generalized Additive Model (GAM) selected by Akaike information criterion (AIC) showing the relationship of fluctuating asymmetry (CFA) with condition factor (K) for males of *O. ruber*.**

the water column, resulting in an increase in substrata acidity and a reduction in dissolved oxygen (Gray & Elliot 2009). These disturbances generate changes in the benthic community structure, and the increase in organic matter deposition and river-originated nutrients occurs by the preceding wet seasons. This suggests the existence of distinct regimes within the GB caused by the seasonality of the rainfall regime which influences the abiotic variables of the bottom water and sediment. In this context, the effect of rainfall and water current seasonality in the drainage basin of GB influence the water, sediment, and, consequently, the fluctuating asymmetry and organosomatic indexes of the *O. ruber*.

Environmental conditions along with the life strategy of *O. ruber* may limit the penetration of the species in the inner zones of the GB. In GB, *O. ruber* is found throughout its length, with a greater amount of external GB jumps in waters with oceanic characteristics and, therefore, has a positive correlation with salinity and transparency (Chaves et al. 2018) and with the lowest levels of organic matter (Araújo et al. 2002). The distribution of *O. ruber* over the entire length of the GB, among other factors, may be useful with a single influence of the water from the South Atlantic water since the water signals can be perceived up to the internal limit of the distribution of the central channel (Moser et al. 2016). In other coastal areas (Sepetiba Bay, RJ), the distribution of *O. ruber* is preferably restricted to depths of around 50 m, when in the summer months a probable probability of ACAS can also be seen, which comprises a mass of Coastal Water (Vianna & Verani 2002, Santos et al. 2007). The restriction of *O. ruber* at the entrance to Sepetiba Bay may also be conditioned by interspecific competitions by territories (Vianna & Verani 2002), an unproven standard for statistics in GB, where a species is distributed across all rock costs (Chaves et al. 2018). 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 (K) 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 age, since younger fish have different foraging rates and metabolic activity associated with rapid growth relative to older fish, being able to present higher conditions than the latter (Pyle et al. 2005), which explains in the present study the highest values of K observed in juveniles (Table II).

The subtle tendency observed in the inverse relationship of CFA 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 maybe insufficient to guarantee the body condition and the body mass of *O. ruber* in GB. 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 for the detoxification mechanisms (Ramirez et al. 2012). This explains the increase of asymmetry in males resulting in a decrease of K, indicating their difficulty in maintaining the body condition, more than females (Fig. 4). 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 RI (McFarland & Hilis 1982). 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 at the end of spring and summer seasons (Garcia et al. 2010). Male K was higher in Urca, less impacted area than in Paqueta, more degraded area. However, when we exclude the Paqueta samples and make the relationship of K with CFA in males, we observe the same trend that K decreases with increasing CFA.

The highest values of HSI in adults of *O. ruber* may be related to the lowest K and highest GSI, reflecting that the liver is working positively in the mobilization of reserves to synthesize sex hormones (Querol et al. 2002) and responding negatively 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.

Fluctuating asymmetry 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 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 1997), *guppies* (Sheridan & Pomiankowski 1997), *Salaria pavo* (Risso 1810) (Gonçalves et al. 2005), *Perca fluviatilis* (Linnaeus 1758) (Oxnevad et al. 2002), *Oreochromis niloticus* (Linnaeus 1758) (Budi et al. 2017) and *Pimephales notatus* (Rafinesque 1820) (Simon & Burskey 2016) and Lajus et al. (2019), also exhibited that the presence of fluctuating asymmetry 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 the 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 II). Thus, fluctuating asymmetry can be considered an effective tool to infer the understanding of the instability of *O. ruber* development in GB, which makes this species a possible indicator of environmental quality in this ecosystem.

This work stands out for being a pioneer in the comparative analysis of the CFA with different physiological descriptors indicators of fitness using a tropical marine species. The results corroborate that CFA 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 GB.

Our results corroborate the hypothesis proposed in the paper, showed that the physiological descriptors K, RI, and HSI of *O. ruber* were subtly affected by CFA, with differences between life stages and sexes. Some issues, however, still need to be raised, such as the time at which CFA 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, to better understand the biology of the species and how it responds to possible stressors.

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## Author Contributions

LBS- performed field activities, analyzed the data, and wrote the manuscript. LNS - performed field activities and analyzed the data. AFGNS - analyzed the data and wrote the manuscript.

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