



## Influence of seasonality on the natural modulation of oxidative stress biomarkers in mangrove crab *Ucides cordatus* (Brachyura, Ucridae)



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### ABSTRACT

The estuaries are characterized by the formation of salinity gradients and are frequently associated with anthropic activities. Studies that show the condition of organisms against the natural environmental variations are fundamental for future evaluation of environmental change due to anthropic impacts. Biomarkers are useful tools defined as biological changes in low levels of organization induced by stressors and considered as predictive responses of more severe effects. The aim of this study was to characterize the modulation of biochemical biomarkers in *Ucides cordatus* against the natural variation a pristine mangrove forest in an Amazonian estuary (Japerica Bay). The samplings were carried out throughout one year at rainy-dry transitional period (June/2013), dry period (September/ 2013), dry-rainy transitional period (November/ 2013) and rainy period (February/ 2014) in the lower, middle and upper estuary. The biochemical biomarkers evaluated were glutathione-S-transferase (GST), glutamate cysteine-ligase (GCL), total antioxidant capacity (ACAP), reduced glutathione (GSH) concentration and the lipid peroxidation (LPO). There was a decrease in the activity of both enzymes, GSH concentration and lower LPO in the rainy-dry transitional period for the organisms of all sites, pointing to this period as a season of greater metabolic stability. The organisms, especially those of the middle estuary, presented physiological plasticity against the variations of the environment which they are submitted. Seasonality, more than salinity influence due to position in estuary, is the main modulator of biomarkers. Also, our results indicate that the species presents high viability for biomonitoring studies.

### 1. Introduction

The mangrove is an estuarine environment, which provides important social-economical services and presents great ecological and biological relevance (Barbier et al., 2011). This ecosystem maintains the balance within marine and coastal ecosystem (Chen et al., 2010) and stand out by presenting gradients of its physical-chemical parameters, especially salinity. This parameter may vary from a 100% of freshwater to a 100% of seawater as a result of tidal cycle (Urbina and Glover, 2015) imposing severe environmental conditions for animal's survival. The salinity variation is a critical environmental peculiarity once affects the organisms physiology (Anger, 2001). Such variation causes responses that ensure the maintenance of cellular homeostasis,

such as osmoregulation, which is the ability to maintain the extracellular ion concentration stable in relation to environmental osmotic change (Monserrat et al., 2007). Osmotic imbalances may injure others physiological processes and, in extreme situations, possibly cause death (Urbina and Glover, 2015).

Among the benthic species that live in the mangrove, the crustaceans are the most abundant. The mangrove crab *Ucides cordatus* (Linnaeus, 1763) is one of the main representative of the benthic fauna in the mangrove of Brazilian coast. It also has high socioeconomic value and ecological characteristics that are essential to the maintenance of the ecosystem in which they live (Pinheiro et al., 2005). Their activities in the mangrove promote drainage and bioturbation of soil (Nordhaus et al., 2006; Pinheiro et al., 2012), besides acting in the predation of the

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propagules of mangrove plants. This predation breaks the seed dormancy through its chew and enables the constant renewal of vegetable biomass (Nordhaus et al., 2006), which influences directly the dynamic of these forests (Lindquist et al., 2009).

*Ucides cordatus* is an hyper-hypo-osmoregulator species, and the gill tissues are the main responsible for ion exchanges (Lignot et al., 2000). It is known that osmoregulation is a cellular mechanism that demands energy expenditure (Boeuf and Payan, 2001), therefore implies in ATP (adenosine triphosphate) consumption mainly through  $\text{Na}^+/\text{K}^+$  ATPase activity (Pham et al., 2016) which creates important gradients and favors other secondary mechanisms (such as  $\text{Na}^+/\text{H}^+$  antiporter) (Kirschner, 2004). Because of the massive consumption, ATP synthesis is enhanced. This process culminates in the production of endogenous reactive compounds arising from oxidative metabolism, in the mitochondrial electron transport chain through partial reduction of oxygen (Burdon, 1999) which are called reactive oxygen species (ROS). When produced in excess and without an adequate antioxidant interception, these species may lead to the oxidative stress (Gutteridge and Halliwell, 2018) causing oxidation of macromolecules and deregulating homeostatic processes (Halliwell and Whiteman, 2004).

Adding to the severe conditions of the environment, the estuarine organisms are subjected to the effects of anthropogenic activities that directly affect the environmental health (Smith et al., 2003). In this context, the biomarkers are used as predictors that are sensitive to environmental stressors (Carajaville et al., 1995). They are defined as changes in biological responses of molecules, cellular components, structures or functions (Depledge et al., 1995). These changes occur when an organism is out of its normal physiological condition caused by the exposure to pollutants or physical-chemical modifications in its habitat (Moore et al., 2004). The biomarkers analyses are able to inform if there was exposure to a stressor source and to identify the level of this condition (exposure biomarkers), and to elucidate the harmful consequences (effect biomarkers) (Sogorb et al., 2014). Therefore, biomonitoring programs that use these resources provide a holistic view of the homeostatic situation of the organisms, being useful in field studies (Monserrat et al., 2007). Many studies has demonstrated that biomarkers are sensitive to environmental changes, whether they are anthropogenic or natural (such as seasonality) (Paital and Chainy, 2013; Zanette et al., 2015; Chan and Wang, 2018).

The main purpose of this study was to characterize the natural seasonal modulation of oxidative stress biomarkers in the crab *Ucides cordatus* over a year in a pristine Amazonian estuary, which is subjected to intense seasonal rainfall variations. In addition, it aims to analyze the feasibility of using the species as biomonitors.

## 2. Material and methods

### 2.1. *Ucides cordatus* sampling

The mangrove crab *Ucides cordatus* were collected in a pristine area called Japerica Bay. The Japerica bay (Fig. 1) is an estuary located in the northeast seaboard of Pará, in the Amazonian coastal zone called the “Saltwater littoral”, a portion of the coast of Pará most affected by Atlantic Ocean. The mangroves begin near Primavera County, extending along the bay on both banks, where there is the water supply of several rivers until its outfall into the Atlantic Ocean.

Three different sites have been chosen according to salinity gradient: A) Upper estuary (00°49' 54.9"S, 47°04'29.7"W): area under conditions of lower salinity and closer to Primavera County; B) Middle estuary (00°51'52.1"S, 47°04'29.4"W): intermediate area characterized by intense mixing of kinds of water; C) Lower estuary (00°55'12.7"S, 47°05'47.2"W): area under condition of higher salinity and greater distance from Primavera County (Fig. 1). It is noted that during the sampling period for this study, near to the estuary of Japerica Bay, a cement factory was in implementation phase, inoperative at that time, but it was inaugurated in April 2016. Therefore, the selection of the

sampling sites also considered an influence gradient related to an epicenter of future anthropic activities in Primavera County.

The four surveys in all points were planned aiming to verify the seasonal variation during a year of study, thus covering: Transitional Period I (rainy-dry transition, June 2013), Dry Period (September 2013), Transitional Period II (dry-rainy transition, November 2013) and Rainy Period (February 2014). The months of sampling period were selected based on rainfall data taken from Instituto Nacional de Meteorologia (INMET) located in Salinópolis City, which is about 56 Km from Primavera County.

*U. cordatus* (20–36 male subjects per point) were collected manually during low tide and immediately cryoanesthetized and slaughtered in ice (0 °C) in order to minimize the stress of the animals and to preserve its metabolic characteristics. Priority was given to larger animals (~ 5.5–7.0 cm of carapace width) within the sampling effort for the capture of adult males of *U. cordatus*. This minimum size is a guarantee of sexual mature individuals and support that they are in the same development stage, reducing possible physiological influences to study.

At the beginning and at the end of the sampling in each site of collection the following physical-chemical water parameters were recorded using a multiparameter equipment (HACH HQ40D): salinity, pH range, dissolved oxygen, conductivity and temperature.

On the same day of sampling, the animals were measured with a caliper (0.1 mm), analyzing the following dimensions: the greater width of the carapace in dorsal view, length of carapace (from de front to posterior margin) and height of body (from the highest point of carapace to lower point at thoracic sternum). Shortly thereafter, the animals were also weighted in semi-analytical balance (0.01 g) so that the gills were removed and packed in eppendorf tubes for ultra-freezer (− 80 °C) storage until the time of biochemical analyses.

### 2.2. Oxidative stress biomarkers analyses

The gill samples were homogenized (1:4; p/v) in buffer with pH set to 7.6, according to Bainy et al. (1996) methodology. The homogenates were centrifuged at 20,000 x g per 20 min at − 4 °C. The supernatant was removed, aliquoted and packed at − 80 °C until the moment of the dosages.

The analyses of total proteins was performed with commercial kit (Doles LTDA, Brazil), based on the biuret test (citratesodium 114 mmol.L<sup>−1</sup>, sodium carbonate 210 mmol.L<sup>−1</sup> and copper sulphate 10 mmol. L<sup>−1</sup>) for proteins, the readings were performed in multimodal microplate reader (Victor X3, Perkin Elmer) at 550 nm. The results were expressed in milligrams of proteins/mL.

The total antioxidant capacity against peroxy radicals was analyzed according to Amado et al. (2009) method. The readings were performed in fluorescence microplate reader (485 and 530 for excitation and emission respectively) during one hour (Victor X3, Perkin Elmer). The results were expressed as the inverse of the relative area.

The activity of Glutathione-S-transferase was analyzed based on works of Habig et al. (1974) and Habig and Jakoby (1981). The readings were expressed in UGST/mg of protein that represents the amount needed of the enzyme to conjugate 1 μMol of CDNB/min/mg of protein, at 25 °C and pH 7.0.

Determination of glutamate cysteine-ligase (GCL) activity and glutathione (GSH) concentration followed the protocol of White et al. (2003). The readings were performed in a spectrofluorimeter (Victor 2, Perkin Elmer) with plate reader (472 for excitation and 528 for emission). The results were expressed in nmol of GSH and activity of GCL (nmol/min/mg of protein).

The lipoperoxidation was determined according to Hermes-Lima et al. (1995) adapted for microplates according to Monserrat et al. (2003). The samples were homogenized (1:6 m/v) in methanol 100% in the cold (4 °C). The homogenates were centrifuged at 1000 × g, during 10 min at 4 °C. The readings were performed in spectrophotometer in the length of 550 nm. The content of lipid peroxides was expressed as

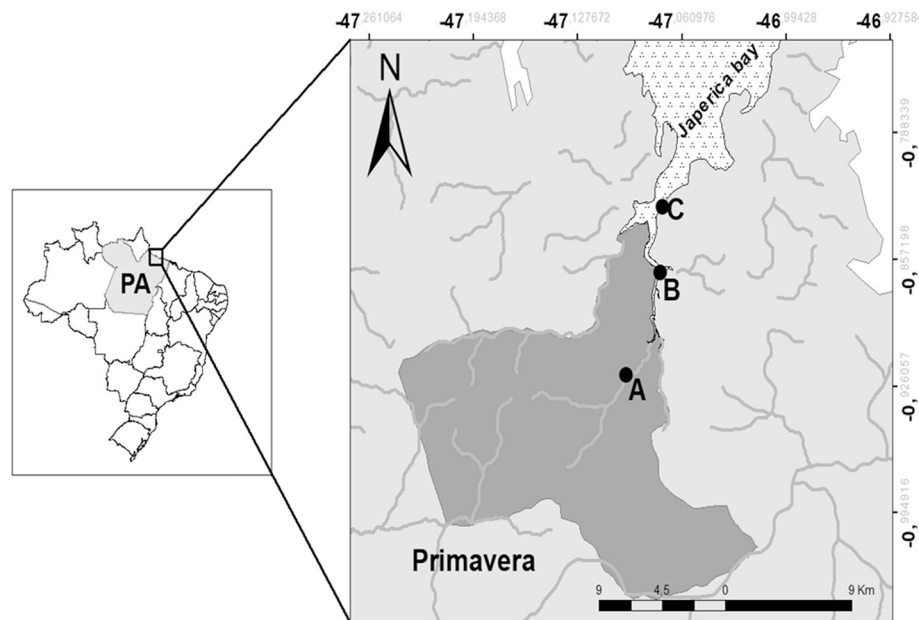


Fig. 1. Map with the geographical location of the Japerica Bay area (to the north of Primavera County), indicating the three sampling sites in Japerica Bay: A) Upper estuary –  $00^{\circ}49'54.9''$  S,  $47^{\circ}04'29.7''$  W; B) Middle estuary  $-00^{\circ}51'52.1''$  S;  $47^{\circ}04'29.4''$  W and C) Lower estuary–  $00^{\circ}55'12.7''$  S;  $47^{\circ}05'47.2''$  W.

equivalent of nM CHP/g of wet tissue.

### 2.3. Statistical analyses

The assumptions of normality were tested (Shapiro-Wilks Test) and homoscedasticity (Levene Test) in the data of biomarkers for later application of variance analysis (ANOVA). Once the assumptions have not been reached, Kruskal-Wallis' nonparametric test was applied for comparison of biomarkers between sampling sites and between climatic periods with a subsequent test of multiple comparison of average ranks. For comparison between climatic locations and periods of biometrics data, it was also tested the assumptions of normality (Shapiro-Wilk Test) and homoscedasticity (Levene Test). Then it was performed a two-way variance analysis (ANOVA) with Tukey test after. The level of significance was 5% (Zar, 1984).

## 3. Results

### 3.1. Rainfall and physical-chemical parameters of water

The rainfall over the periods of sampling and the fluctuation of water salinity in the beginning and the end (low and high tides, respectively) of *Ucides cordatus* sampling in the different sites of the estuary (lower, middle and upper estuary) are shown in Fig. 2.

Among the periods in which the sampling occurred, the highest values of rainfall was in the period of February 2014 to April 2014 (rainy period: cumulative rainfall 500 mm), in contrast the period of September 2013 (August 2013 to October 2013) presented a lower value of 0 mm (dry period: cumulative rainfall 2 mm). The periods of June 2013 (May 2013 to July 2013- transitional period I: cumulative rainfall 141 mm) and November 2013 (November 2013 to January 2014 - transitional period II: cumulative rainfall 117 mm) presented values of 1 mm and 2 mm respectively. It is worth noting that the sampling performed during transitional period were made after the rainy period (June 2013, transitional I rainy-dry) and after the dry period (November 2013, transitional II dry-rainy). It is observed that in chronological order of the months, there was an increase in rainfall.

The salinity also varied greatly between the points for all periods sampled, in which the lower estuary presented the highest values, the middle estuary presented intermediate values during transitional I, dry

and rainy periods and upper estuary presented the lowest values in all periods (Fig. 2). The salinity peak occurred during transitional II period in all sites (34–32; 36–34 and 21–34 respectively), when the lowest rainfall was recorded. The lowest values of salinity were recorded during the rainy season in the lower and middle estuaries, concomitantly it was recorded the highest rainfall during this period (Table 1).

It was noted a ratio inversely proportional between the salinity values and rainfall data, in which the period of higher rainfall presented lower average values of salinity, in contrast to periods of low rainfall, which presented an increase in salinity values (Fig. 2).

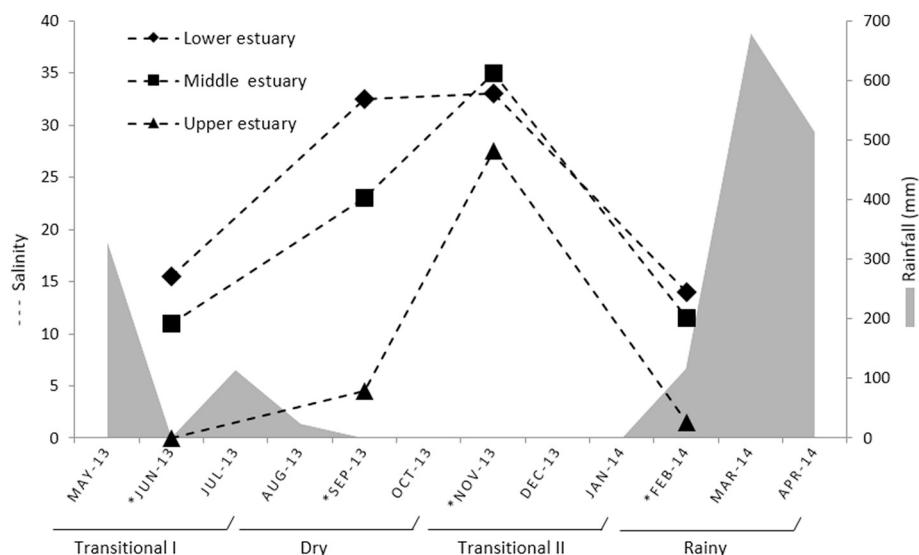
The transitional I period (rainy-dry) presented an average salinity value of 8.83 in all sampling sites, and in rainy season presented average of 8.98, showing that freshwater injection into the estuary promotes its dilution (Table 1). The transitional period I, although it has presented low rainfall value, shown to be influenced by the history of physico-chemical conditions of the past months. Therefore being, in fact, a transitional season between the high freshwater intakes in estuaries during the past rainy months and beginning of rainfall reduction (Fig. 2). The dry period presented an average salinity value of 20 and the transitional II period presented an average of 31.83, showing the process of salinization of the environment with the rainfall reduction.

Water temperature among points or seasons was very similar, keeping its value around  $25^{\circ}\text{C}$  –  $30^{\circ}\text{C}$  and the pH was remained close to neutrality (Boeuf and Payan, 2001; Burdon, 1999) in all the sites during all the periods.

The dissolved oxygen remained around 5–8 mg/L in all points and the upper estuary had the highest values during the dry and transitional I periods. The conductivity varied greatly among the points and periods, in which all the sites presented peaks during the transitional II (59.5–59.4; 60.7–58.9 and 49.3–59.8  $\mu\text{S}/\text{cm}$  respectively) and the lowest values during transitional I (33–37.8; 27.8–27.4 and 1.78–1.90  $\mu\text{S}/\text{cm}$  respectively) with high similarity of values.

### 3.2. Biometry

No differences were found for carapace length of sampled *Ucides cordatus*. The animals carapace width from middle estuary presented differences between transitional I and rainy periods, in which this one presented lower values ( $6.85 \pm 0.13$  and  $6.17 \pm 0.15$  cm,



**Fig. 2.** Distribution of cumulative monthly rainfall in a year (May 2013 to April 2014; INMET, 2018) and salinity fluctuation for each sampling month (\*) and sites (lower, middle and upper estuaries) along four seasonal periods in Japerica bay, PA.

respectively). There were differences only for *U.cordatus* carapace height among sampling sites during the dry period. In this period, the animals from lower estuary presented lower values in comparison to organisms from upper estuary ( $3.43 \pm 0.06$  and  $6.46 \pm 2.7$  cm, respectively). The animals collected from lower estuary in dry period were the smallest in height ( $3.43 \pm 0.06$  cm). For weight the animals from middle estuary collected in transitional I and dry periods showed differences for weight ( $145.86 \pm 67$  and  $106.51 \pm 5.82$  g, respectively) (Table 2).

### 3.3. Oxidative stress biomarkers

#### 3.3.1. Total antioxidant capacity against peroxy radical

There were significant differences ( $p = .004$ ) between the antioxidant capacity of the organisms from lower estuary during dry ( $0.08 \pm 0.48$ ) and rainy ( $5.16 \pm 3.24$ ) periods, occurring an induction in the last one. The same pattern was seen in organisms from upper estuary, in which rainy season ( $6.93 \pm 3.52$ ) presented induction in relation to the dry period ( $0.84 \pm 0.51$ ) ( $p < .0001$ ). There were no differences between sites for any period sampled nor between periods for middle estuary animals (Fig. 3a).

#### 3.3.2. Glutathione-s-transferase activity

The transitional I ( $216.50 \pm 206.26$  UGST/mg of protein;  $p < .0001$ ) and dry ( $251.25 \pm 215.33$  UGST/mg of protein;

$p < .0001$ ) periods are different from transitional period II ( $135.35 \pm 110.87$  UGST/mg of protein) in which there was a decrease in enzyme activity for lower estuary organisms. For organisms in the lower estuary there were differences between transitional period I ( $156.77 \pm 138.26$  UGST/mg of protein  $p = .009$ ) and II ( $146.30 \pm 118.80$  UGST/mg of protein;  $p < .0001$ ) which presented low activity in relation to dry period ( $229.29 \pm 168.71$  UGST/mg of protein). The same pattern was observed for upper estuary animals, in which transitional periods I and II ( $137.68 \pm 89.62$  and  $109.94 \pm 94.19$  UGST/mg of protein;  $p < .0001$  for both) presented inferior activities in relation to dry period ( $220 \pm 179.63$  UGST/mg of protein). There was difference between sampling sites only during transitional period I, in which lower estuary crabs presented greater GST activity in relation to the crabs of other sites ( $p = .029$  for middle estuary and  $p < .0001$  for upper estuary) (Fig. 3 b).

#### 3.3.3. Reduced glutathione (GSH) content

There was difference, for lower estuary crabs, between transitional I ( $94.70 \pm 67.57$  nMol of GSH) in relation to dry ( $33.89 \pm 20.56$  nMol of GSH;  $p < .0001$ ) and transitional II ( $34.08 \pm 24.69$  nMol of GSH;  $p < .0001$ ) periods, which the latter presented lower concentrations. The same pattern was observed for middle estuary animals, therefore the transitional I period had higher concentration ( $110.33 \pm 73.69$  nMol of GSH) in relation to dry and transitional II periods ( $40.72 \pm 31.24$  and  $33.29 \pm 25.95$  nMol of GSH;  $p = .015$  and

**Table 1**

Rainfall along sampling periods and physical-chemical parameters of water from sites sampled along four seasonal periods in Japerica bay, PA. The data are displayed as the values recorded at low tide and high tide (Temp. = Temperature; DO = Dissolved oxygen; Condu. = Conductivity; Sal. = Salinity; Lower = Lower estuary; Middle = Middle estuary; Upper = Upper estuary).

Period	Site	Temp. (°C)	pH	DO (mg/L)	Condu. (µS/cm)	SAL.
Transitional I	Lower	29.2–28.4	7.18–6.82	5.34–0.68	33–37.8	15–16
	Middle	27.4–30.1	7.51–7.54	5.79–6.01	27.8–27.4	12–10
	Upper	26.9–28.7	7.62–7.55	6.59–6.48	1775–1902	0–0
Dry	Lower	28.4–30.5	7.6–7.58	5.52–5.58	49.7–49.5	33–32
	Middle	29.3–28.7	7.46–7.6	5.28–6.58	44.4–38.2	24–22
	Upper	32.1–33.4	7.72–7.56	8.68–8.83	8.67–21.42	0–9
Transitional II	Lower	26.9–29.1	7.71–7.84	6.55–7.55	59.5–59.4	34–32
	Middle	29.4–29.3	7.66–7.72	6.56–6.69	60.7–58.9	36–34
	Upper	28.9–29.8	7.59–7.54	8.11–5.77	49.3–59.8	21–34
Rainy	Lower	28.7–27.6	7.31–7.33	5.66–6.28	30.9–27.95	16–12
	Middle	27.7–29.2	7.26–7.38	4.87–6.51	22.1–26.2	11–12
	Upper	25–25.7	6.5–7.19	5.5–5.0	33.2–33.7	1.1–1.8

**Table 2**

Biometry of *Ucides cordatus* from sites sampled along four seasonal periods in Japerica bay, PA ( $n = 30\text{--}35$  subjects). Data are expressed as mean  $\pm$  standard error. Different capitals letters represent differences between periods for the same place, and different lowercase letters represent differences between places in the same period.

Period	Site	Total length (cm)	Width (cm)	Height (cm)	Weight (g)
Transitional I	Lower	4.88 $\pm$ 0.09 Aa	6.57 $\pm$ 0.13 Aa	3.88 $\pm$ 0.06 Aa	130.1 $\pm$ 8.07 Aa
	Middle	5.24 $\pm$ 0.09 Aa	6.85 $\pm$ 0.13 Aa	3.8 $\pm$ 0.06 Aa	145.86 $\pm$ 7.67 Aa
	Upper	4.94 $\pm$ 0.07 Aa	6.58 $\pm$ 0.11 Aa	3.64 $\pm$ 0.07 Aa	114.1 $\pm$ 5.77 Aa
Dry	Lower	4.69 $\pm$ 0.1 Aa	6.12 $\pm$ 0.13 Aa	3.43 $\pm$ 0.06 Ba	99.68 $\pm$ 6.52 Aa
	Middle	4.81 $\pm$ 0.09 Aa	6.28 $\pm$ 0.12 ABab	3.52 $\pm$ 0.06 Aab	106.51 $\pm$ 5.81 Ba
	Upper	5.12 $\pm$ 0.09 Aa	6.77 $\pm$ 0.12 Ab	6.46 $\pm$ 2.7 Ab	120.3 $\pm$ 7.71 Aa
Transitional II	Lower	4.88 $\pm$ 0.08 Aa	6.28 $\pm$ 0.11 Aa	3.56 $\pm$ 0.05 ABa	113.58 $\pm$ 5.99 Aa
	Middle	4.83 $\pm$ 0.08 Aa	6.3 $\pm$ 0.12 ABa	3.53 $\pm$ 0.06 Aa	108.51 $\pm$ 5.67 ABa
	Upper	5.13 $\pm$ 0.07 Aa	6.7 $\pm$ 0.11 Aa	3.72 $\pm$ 0.04 Aa	136.68 $\pm$ 6.82 Aa
Rainy	Lower	4.91 $\pm$ 0.18 Aa	6.4 $\pm$ 0.14 Aa	3.76 $\pm$ 0.07 Aa	131.9 $\pm$ 8.38 Aa
	Middle	4.82 $\pm$ 0.11 Aa	6.17 $\pm$ 0.15 Ba	3.6 $\pm$ 0.08 Aa	120.56 $\pm$ 7.94 ABa
	Upper	5 $\pm$ 0.08 Aa	6.54 $\pm$ 0.11 Aa	3.69 $\pm$ 0.06 Aa	131.58 $\pm$ 6.93 Aa

$p < .0001$ , respectively). The upper estuary organisms presented the lowest concentration of GSH ( $24.17 \pm 19.77$  nMol of GSH) during the transitional period II in relation to the other sampling periods ( $p = .040$  for transitional I;  $p < .0001$  for dry and  $p = .018$  rainy season). As for GST, there was a difference between the sampling sites only during the transitional period I in which the lower and middle estuaries organisms presented higher concentrations ( $94.70 \pm 67.57$  and  $110.33 \pm 73.60$  nMol of GSH;  $p = .043$  and  $p = .043$  respectively) in relation to upper estuary organisms ( $42.81 \pm 33.90$  nMol of GSH) (Fig. 3 c).

### 3.3.4. Glutamate cysteine ligase activity

The lower estuary animals presented lower activities during dry and transitional II periods ( $18.40 \pm 11.47$  and  $10.35 \pm 4.24$  nMol/min/mg of protein;  $p = .023$  e  $p < .0001$  respectively) in relation to transitional period I ( $108.87 \pm 77.21$  nMol/min/mg of protein). While the middle estuary crabs presented significant differences ( $p < .0001$ ) between transitional period I ( $102.35 \pm 43.11$  nMol/min/mg of protein) with high activity and transitional II ( $6.53 \pm 3.47$  nMol/min/mg of protein) with low GCL activity. There was no difference between periods for upper estuary animals or sites in any sampling periods (Fig. 3 d).

### 3.3.5. Lipoperoxidation content

The lower estuary crabs presented low LPO content during transitional period II ( $15.22 \pm 12.23$  nMol of CHP/g of wet tissue) in relation to rainy season ( $26.82 \pm 20.70$  nMol of CHP/g of wet tissue) ( $p = .011$ ). The upper estuary animals also presented increased amount of oxidized lipids during the rainy season ( $26.99 \pm 21.46$  nMol of CHP/g of wet tissue) in relation to other climatic periods ( $p = .006$  for transitional I;  $p < .0001$  for transitional II and for dry). There was no difference between periods for middle estuary animals nor sites in any sampled period (Fig. 3 e).

## 4. Discussion

### 4.1. Physical-chemical parameters of water

The stable temperature around 27 °C throughout all climatic periods is typical of tropical regions, directly influenced by low variation in insolation in the Amazon (Monteiro et al., 2015). The neutral pH is characteristic of Amazonian estuaries of white waters rich in nutrients (Vieira et al., 2018). The highest levels of dissolved oxygen recorded in upper estuary during transitional I and dry periods are expected since the oxygen dissolution is higher at low salinities (García and Gordon, 1992). This situation demonstrates that even in decreasing rainfall conditions or water instability the upper estuary region is more weakly influenced by the ingress of sea water than other portion of the estuary.

The higher conductivity at points of large and medium estuarine

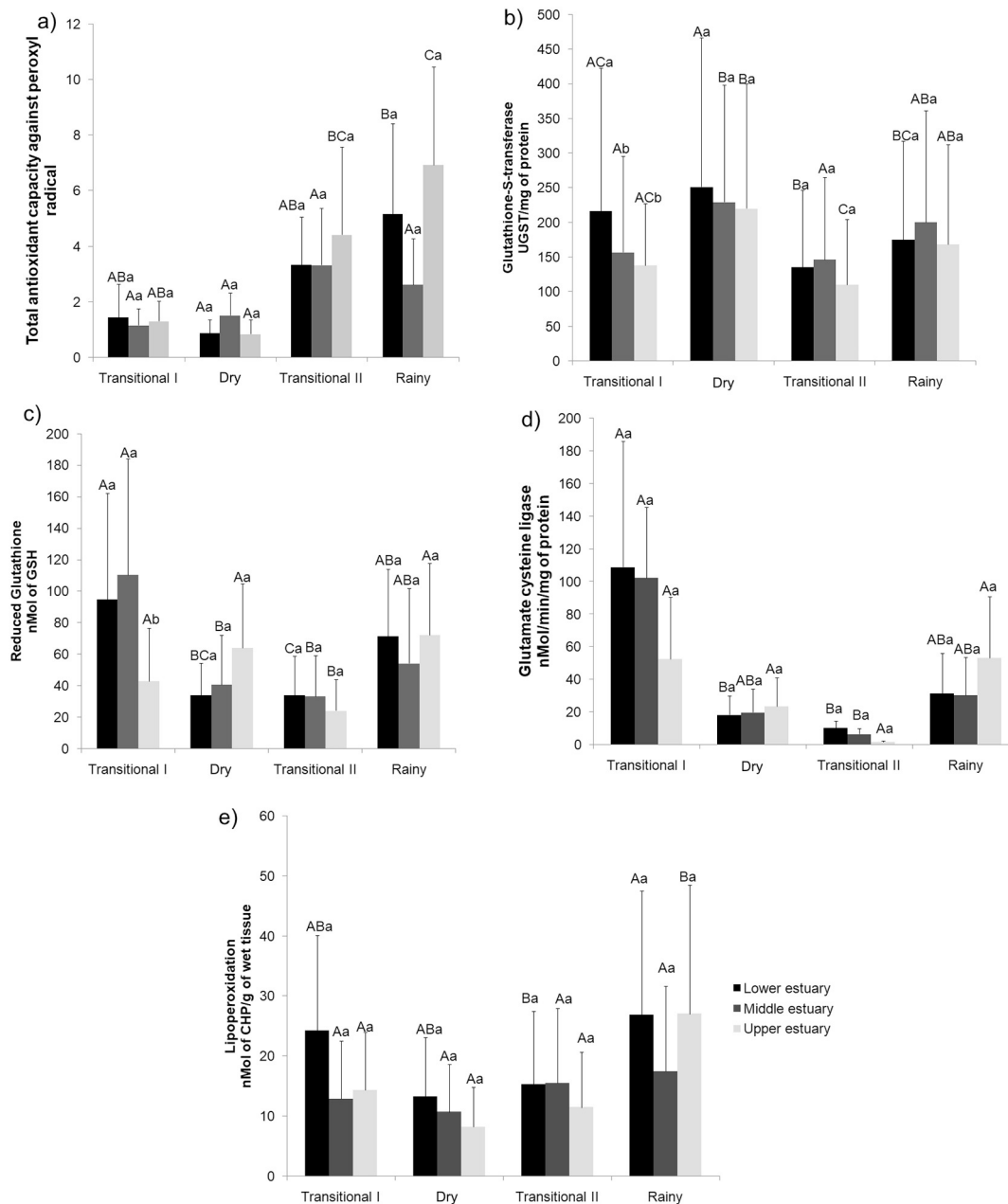
influence along with higher salinity levels, especially during transitional II in which there was a peak of both parameters, demonstrate that there is the formation of a gradient throughout the estuary according to the distance from the outfall. Such a gradient is evident in salinity measurements of the three points during transitional I, dry and rainy periods where is clearly visualized that the point located in lower estuary has higher values, the point located in middle estuary has intermediate values and the point located in upper estuary has very low values around 0. Therefore, this estuary presents the horizontal stratification model proposed in the classic work of Dionne (1963) which divides estuaries into three sectors: a lower estuary, region in free connection with the sea and high salinity levels; a middle estuary, a strong salt and freshwater mixing region characterized by great salinity variation; and an upper estuary, a fresh water region with low salinity levels but subject to daily tidal action. These specific physical-chemical conditions of each region impose different physiological challenges to the organisms, leading to different adaptive responses.

The salinity peak observed during dry and transitional II periods and the low values during rainy season are related to rainfall oscillations that occur during the year, since the physical-chemical parameters are highly dependents on rainfall. Therefore, the rain influenced directly the ionic composition of water, changing this panorama seasonally (Nascimento et al., 2006) and ratifying the division into four distinct climatic periods as occurred in the present study.

### 4.2. Biometry

It was observed that the biometric patterns of the *Ucides cordatus* specimens appear not to be influenced by the physiological demand imposed by the environment, since the lower estuary animals presented lower height during the dry period and high values during low salinity periods such as rainy and transitional I. Such results reinforce the eurihaline characteristic already known of this species that has powerful osmoregulatory capacity being able to either hypo or hyper-osmoregulate (Martinez et al., 1999) adapting successfully in a wide range of salinity (Santos and Salomão, 1985).

The middle estuary animals presented higher values of weight and width during transitional period I in relation to dry and rainy periods, respectively. The transitional periods are characterized by inconstancy of water conditions. These organisms suffer great influence both of the marine waters entering the estuary and of the waters coming from the rains because they are located at the middle estuary, configured as a zone of instability and mixtures of waters (Dionne, 1963). The establishment of strong chemical, physical and biological gradients (Day et al., 1989) characterizes this zone and it can also be subdivided according to the proximity to the tidal or outfall regions (Dionne, 1963). The survival in unstable environments as mangroves favored the appearance of physiological mechanisms that minimize the abiotic



**Fig. 3.** (a) Total antioxidant ( $n = 14\text{--}34$ ); (b) Activity of Glutathione-S-transferase ( $n = 18\text{--}36$ ); (c) Reduced Glutathione concentration ( $n = 7\text{--}36$ ); (d) Activity of Glutamate cysteine ligase ( $n = 4\text{--}28$ ) and (e) Lipoperoxidation ( $n = 14\text{--}36$ ) in gills of *Ucides cordatus* from lower, middle and upper estuary along different seasonal periods. Values are expressed in median  $\pm$  first quartile. The different capital letters represent differences ( $p < .05$ ) between periods for the same place and different small letters represent differences ( $p < .05$ ) between places for the same period.

oscillations (Bamber and Depledge, 1997), therefore, they are physiologically adapted to expressive and constant changes in the physical-chemical parameters of water.

It is worth mentioning that, in general, major changes between climatic periods were not observed in animals from any sampling site. Thus suggesting that the physiological plasticity of the species previously mentioned prevents the damage to the energy allocation to growth and other vital functions. Normant et al. (2012) in a study of the effects of salinity on the bioenergy of the crab *Eriocheir sinensis* affirms that the eurihalinity implies in energetic costs that affect other activities such as oxygen rate, consumption and assimilation of food and excretion. However, it was concluded that this species, which is eurihaline, did not have its feeding and assimilation of food affected by salinity (contrary to what is widely described for marine species such as *Callinectes sp* in studies like Wang and Stickle, 1987 and Guerin and Stickle,

1992) and the same may be occurring with *Ucides cordatus* in the present study.

#### 4.3. Biomarkers

*Ucides cordatus* is a strong hypo-hyper-osmoregulator, in other words, is able to increase or decrease osmotic concentrations of its extracellular fluid in a wide range of salinity, usually 2–33 (Martinez et al., 1999). This species is very well adapted to the estuarine environments, reaching its isosmotic point in salinity around 20 (Leite and Zanotto, 2013). In light of that, we have noted different patterns of biomarkers responses related to the salinity conditions of the sampled sites.

The lower estuary organisms presented a decrease in ACAP, GSH, GCL and LPO during dry and transitional II (salinity peak) periods.

These organisms are best adapted to hypertonic medium in relation to its internal environment because they are continuously exposed to salinities close to sea level, therefore there was lower osmoregulatory and metabolic demand. Thus, it is valid to say that larger salinities provide situations energetically less costly for animals from this location. A contrary behavior of the biomarkers was observed during water dilution period, especially in the rainy season, in relation to periods of salinity increase for lower estuary animals. There was induction of antioxidant defenses and of oxidative damage suggesting that the low salinities generate increase in metabolic demand. Osmotic concentration adjustments require a lot of energy in decapod crustaceans generating metabolic changes (Chen and Chia, 1997; Luvizotto-Santos et al., 2003) and increasing energy expenditure that is directly related to generation of reactive oxygen species (Gutteridge and Halliwell, 2018). Paital and Chainy (2010) demonstrate that salinity alterations may potentialize the oxidative stress and unbalance the antioxidants defenses of crabs as *Scylla serrata*. During transitional period I lower estuary animals presented higher GST activity than upper estuary, suggesting induction of antioxidants defenses against hydrodynamic instability of transitional period. As the lower portion is located near the outfall of the ocean the animals would suffer less variations with the tidal cycles that occur daily, while the animals from the upper portion would be more susceptible to daily changes imposed by the cycles because their resident waters are hyposaline. Freire et al. (2011a) in an experimental study with the swimming crabs *Callinectes* submitted to saline stress concluded that *C. ornatus* activates its antioxidants defenses more prominently as the environmental conditions change, than when it's compared to *C. danae*, which is more widely distributed in different range of salinity. Such situation may be occurring with the lower estuary animals in the present study.

In the upper estuary there was the same pattern: decrease of both biomarkers of exposure and the effects during saltier periods, and increase during periods of more dilute waters. Although the organisms of this site are more exposed to low salinities, this species, like most crustaceans, originate in marine water with subsequent invasion of freshwater (Schubart et al., 1998) carrying on itself genetic factors that lead its metabolism to adaptations to saline water. Besides that, the tidal cycles, that promote the continuous mixing of the watersheds, may have a key role in strategic direction of the biomarkers making the spatial differences inside the estuary not so physiologically visible. The tidal variations along with the flood and the ebb currents are the main responsible by the estuary characteristics (Vaz and Dias, 2008) affecting all physical-chemical parameters of water, especially salinity (Phlips et al., 2002).

It is worth mentioning that *Ucides cordatus* occurrence in low salinities in the Amazon points to a local physiological adaptation. This may be connected to its osmoregulatory capacity over a wide salinity range of 2–33 (Martinez et al., 1999) giving it euryhaline character and physiological potential for the establishment in various environments. For Lucu et al. (2000) many crustaceans have successfully invaded freshwater and brackish environments through a set of physiological mechanisms that they developed during their evolutionary history. The GST activity and GSH concentration increased during dry period and this result may be associated to combined effect of hyposmotic conditions of the environment (that naturally increase the animal osmoregulatory demand) concomitantly with the molt events that usually occur in this season.

According to Xu et al. (2017) during the molt period occur an increase of water accumulation in the tissues leading to a decrease in hemolymph osmolality. The transport of water through the membrane intensifies the regulation of cellular volume (Chen et al., 2012) and the cells of decapodes of freshwater present high metabolic machinery to deal with hyposmotic stresses, from the external environment or by dilution of hemolymph (Foguesatto et al., 2017). It should also be noted that the increase in GST activity during dry period occurred in all locations of our study, pointing to an important role of this enzyme in the

mitigation of damages arising from the energy costs of molt. The GST besides acting as a biotransformation enzyme can play the role of an antioxidant (combining products of pro-oxidant processes) providing cell protection (Prohaska, 1980). Some studies already pointed to enzymatic variation during different stages of molt in brachyuran crabs (Zou and Fingerman, 1999).

Hotard and Zou (2008), in a study about the variation of GST in hepatopancreas of the crab *Uca pugilator* during the molt cycle, did not find changes in the activity and concluded that this enzyme can be used as biomarker in any stage of molt. This ambiguity of response may be related to the fact that the modulation of the antioxidant defense system varies according to the species and to the analyzed tissue (Crawford et al., 2000). Thereby, this increase of the GST activity verified during the dry and rainy period in animals from upper estuary seems to be related to the great effectiveness of its metabolic apparatus against the changes in continuous conditions imposed by seasonality. While sharp changes, however discontinuous (as that ones that occur in transitional periods) seem to be more easily assimilated by the antioxidant defense system. Leite and Zanotto (2013) concluded that animals from freshwater environments presented more effective transport rate of calcium ion (higher affinity and maximum reaction rate) in relation to estuarine animals, thus presenting more efficient osmoregulatory machinery.

The middle estuary crabs presented pattern responses similar to other points. However, the non-differentiation between seasons for lipid damage points to the physiological plasticity and euryhalinity of the animals at this location as they are located in an area of the estuary characterized by high mixing of marine and freshwater. Therefore, are able to maintain the energetic balance between the basal functions and the redox system, and it is known that more euryhaline species naturally possess high basal patterns of antioxidant enzyme activity (Freire et al., 2011b).

In this way the present study in generally points the seasonally as a fundamental factor in the behavior of the analyzed biomarkers, since they are affected by the natural physiological cycle of organisms (Vidal-Linán et al., 2010). In addition, the specie presented high viability to be a good biomonitor of the area since: it presented physiological plasticity establishing itself along the salt gradient with success; it demonstrated a responsive metabolic apparatus to environmental changes without, however, compromising its physiological functions; and a homogeneous response of biomarkers for seasonal changes making the interpretation of data, in conditions of anthropogenic stress, clearer.

## 5. Conclusion

Biomarkers modulation is seasonal, but physical-chemical conditions seem to direct the sensitivity of the organisms to the seasonal changes. Besides, during seasonal dry-rainy transition period (Transitional II) there was a decrease in all biomarkers, especially for lower and middle estuaries organisms demonstrating metabolic stability. It is prudent to point out that this decrease in antioxidant defenses may lead to two different consequences in the case of future exposure to anthropogenic contaminants: (Amado et al., 2009) may represent the increased vulnerability to toxic compounds, which are more bioavailable in low salinities or (Anger, 2001) may allow energy investment in detoxification, since the environment is not requiring high levels of physiological compensation.

Additionally, all physiological plasticity presented by *Ucides cordatus* enables this species to be an interesting biomonitor in biomonitoring programs.

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