Journal of Invertebrate Pathology 212 (2025) 108386

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

A protocol for assessing histological changes and biometry in *Ucides cordatus* (Crustacea, Decapoda, Ocypodidae) using linear indices

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ARTICLE INFO

Keywords:
Biomonitoring
Invertebrate
Pathology
Sanitation
Variables
Xenobiotics

ABSTRACT

For decades, histological analysis has been used to assess the acute and chronic effects of xenobiotics in vertebrates. However, there are few studies discussing the role of histological alterations and their relationship with biometry in the health of invertebrates, such as crustaceans. Furthermore, there are no mathematical indices in the literature to assess histological or biometric findings in crustaceans. This study aimed to develop a protocol for assessing histological alterations in organs affected by xenobiotic exposure and correlations with biometry in crustaceans using linear indices. The research was guided by the need to create a specific scoring table and classification system for histological changes and biometrics in crab organs. The developed protocol is based on linear mathematical indices comprising three variables: (α) pathological factor, referring to the relationship between histological alteration and organ impairment; (β) alteration score, defined from lesion extent and/or number of alterations; and (χ) biometric factor, determined based on the relationship between body weight and carapace width, which is directly related to the occurrence of histological changes in *Ucides cordatus*. Sensitivity analysis showed that the sensitivity of alteration, reaction pattern, and total indices to a specific change is directly influenced by the lesion's pathological factor or score. By presenting a standardized scoring table for histological changes in crustaceans, the proposed protocol contributes to a global and weighted assessment of crab health, with potential application in other crustaceans.

1. Introduction

Crustaceans are invertebrates found in planktonic and benthic communities of marine ecosystems and freshwater environments (Pinheiro et al., 2013). These organisms play essential roles in maintaining ecological balance, in addition to having great economic importance for human communities (Oliveira et al., 2023). Crustaceans contribute to fundamental processes, such as nutrient recycling, and serve as a source of livelihood through fishing and marketing (Jesus et al., 2021).

Despite the ecological and economic importance of crustaceans, natural populations that inhabit marine ecosystems have become increasingly threatened by xenobiotics derived from human activities, such as industrial, domestic, and port activities (Jesus et al., 2021; Oliveira et al., 2023). These pollutants can cause deleterious effects on aquatic biota, compromising the sustainability of ecosystems and the health of resident species (Jesus et al., 2021). One of the crustacean species threatened by xenobiotic contamination is the crab *Ucides cordatus* (Linnaeus, 1763).

U. cordatus is a semi-terrestrial crab that is crucial for the maintenance of mangrove environments (Duarte et al., 2016; Jesus et al., 2021). This species is highly sensitive to chemical pollutants, serving as an indicator of environmental quality in these fragile ecosystems (Jesus et al., 2020a; Oliveira et al., 2023). A study carried out by Jesus et al. (2021) demonstrated the presence of heavy metals in muscle tissues of *U. cordatus* in regions with industrial and port enterprises. Thus, the species can bioaccumulate heavy metals, which is a useful characteristic for the monitoring of environmental pollution (Duarte et al., 2016).

Research examining the effects of xenobiotics on crustaceans, particularly *U. cordatus*, has gained relevance in Brazil (Duarte et al.,

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https://doi.org/10.1016/j.jip.2025.108386
Received 2 January 2025; Received in revised form 27 May 2025; Accepted 15 June 2025
Available online 17 June 2025

2016; Carvalho Neta et al., 2019; Oliveira et al., 2019; Jesus et al., 2020a; Jesus et al., 2021; Oliveira et al., 2023). These studies underscored the importance of biomonitoring in economically and environmentally sensitive areas, such as mangroves, which are particularly vulnerable to anthropogenic stressors. Furthermore, the findings of these investigations contribute to advancing the diagnosis of environmental conditions in mangrove areas. For instance, serious histological changes have been described in different organs of *U. cordatus*, such as the hepatopancreas and gills (Carvalho Neta et al., 2019; Jesus et al., 2021; Oliveira et al., 2023).

Histological techniques offer invaluable information for pathological studies. The assessment of histological biomarkers in crustacean organs exposed to xenobiotics (e.g., gills, hepatopancreas, ovaries, testes, muscles, heart, and intestines) has emerged as a promising method for identifying recurrent diseases (Cheng et al., 2019; Carvalho Neta et al., 2019; Jesus et al., 2020a, 2020b, 2021; Li et al., 2023; Cheng et al., 2023; Oliveira et al., 2023). These analyses, when combined with the assessment of biometric parameters, are essential for understanding the health of animals throughout their life cycle (Jesus et al., 2020a, 2020b, 2021; Li et al., 2023; Silveyra et al., 2023). Histological analysis of crustaceans also finds application in ecotoxicology, environmental resource management, and biomonitoring (Li et al., 2023; Oliveira et al., 2023; Silveyra et al., 2023).

Alterations in a given organ are commonly expressed as percentages and frequencies. Additionally, weighting methods can be used to reflect the importance and severity of alterations (Kubrusly, 2001). Studies commonly apply linear indices to express correlations among variables through summation and/or multiplication (Turkman et al., 2000). Semi-quantitative scoring systems are widely used for the evaluation of histopathological alterations in fish (Bernet et al., 1999; Rāskovíc and Poleksíc, 2017).

Fish scoring systems are occasionally adapted for use in crustaceans (Jerome et al., 2017; Oliveira et al., 2023), as exemplified by the studies of Carvalho Neta et al. (2019) and Oliveira et al. (2023), which adapted the scoring system of Bernet et al. (1999) to classify and score histological lesions in *U. cordatus*. The lack of a standardized scoring or classification system results in the use of distinct terminologies for describing alterations among studies. This variability hinders the accurate analysis of histological and biometric findings in crustaceans. Additionally, for crustaceans, there are no mathematical indices that correlate histological changes with biometric parameters, which are necessary for assessing crustacean health in a global and quantitative manner. Thus, it is crucial to develop a scale for measuring the degree of organ impairment and, consequently, the health of crustacean populations inhabiting areas impacted by xenobiotics. This approach can support accurate monitoring and effective management of the mangrove ecosystem. To fill these gaps in the literature, this study aimed to create a specific scoring table and classification system for histological changes and biometric parameters in *U. cordatus* organs.

2. Material and methods

2.1. Histological scoring protocol for crabs

2.1.1. Histological description

A survey of the literature was carried out to better understand the histological description of crab organs exposed to xenobiotics (Lei et al., 2011; Mao et al., 2012; Vasanthi et al., 2014; Jerome et al., 2017; Carvalho Neta et al., 2019; Cheng et al., 2019; Oliveira et al., 2019; Jesus et al., 2020a, 2020b; Jesus et al., 2021; Cheng et al., 2023; Li et al., 2023; Oliveira et al., 2023; Silveyra et al., 2023; Yu et al., 2023). Histological descriptions, scoring systems, classifications, and definitions of the degree of severity of histological changes are available in the literature for fishes (Bernet et al., 1999; Rāskovíc and Poleksíc, 2017).

A protocol was developed comprising a simple and practical scoring table, descriptions, and a classification system for histological changes (Table S1, Supplementary Material) in crabs, focusing on *U. cordatus*. Detailed descriptions of histological alterations were adapted from the findings of Bernet et al. (1999). Histopathological changes were classified into six reaction patterns: circulatory disorders or changes, regressive changes, progressive changes, inflammatory changes, neoplastic changes, and pathogenic changes (Table S1, Supplementary Material) (Bernet et al., 1999).

Histological changes were scored, described, and classified according to crab organ (Table S1, Supplementary Material). The scoring and classification of alterations were based on and modified from the studies of Bernet et al. (1999), Jerome et al. (2017), and Oliveira et al. (2023). A pathological factor (α) ranging from 1 to 3 was proposed to evaluate the severity of histological changes and the impacts of the change on organ function, crab health, and survival.

Reaction pattern 1 (rp1)

Circulatory disorders or changes: a pathological condition characterized by the abnormal flow of hemolymph or tissue fluids into tissues. This reaction pattern includes the following findings:

1. ***Edema* (Fig. S1C, Supplementary Material): *Edema in the marginal canal region of the secondary lamellae of the gills* (Fig. S1L):** Inflammation due to fluid leakage into the tissue. *Telangiectasia* (Fig. S1G): Presence of hemolymph vessels in the tissue. *Clavate-globate lamellae:* A tissue-specific reaction to toxic substances in water or food that causes tissues to club or become congested with blood fluid. *Intramuscular edema:* Fluid accumulation in muscles. *Enterocyte inflammation:* Pathological enlargement of enterocytes (specialized epithelial cells of the small intestine responsible for nutrient absorption)

Reaction pattern 2 (rp2)

Regressive changes: changes capable of inducing functional reduction or organ loss. This reaction pattern includes the following findings:

1. **Architectural and structural changes:** changes in tissue structure, cell shape, and cell arrangement. *Pilaster cell rupture* (Fig. S1E)*:* rupture of the *T*-shaped cells that connect the two sides of the epithelial wall of the secondary lamella. *Disorganization of secondary lamellae* (Fig. S1F). *Epithelial lifting or epithelial lamella lifting: the epithelium of the secondary lamellae is loose or displaced. Displaced cuticle or detached cuticle or lamellar cuticle lifting* (Fig. S1L)*:* the cuticle that covers the basement membrane of the epithelium is loose or displaced. *Cuticle rupture or cuticle breakage:* cuticle rupture caused by cell death. *Desquamated epithelium* (Fig. S1A)*:* Loose epithelium caused by cell death. *Marginal canal deformation or tip of the marginal canal damaged or tip of the marginal canal deformed* (Fig. S1J): changes in the structure or shape of marginal canal cells and tissues. *Tip of the marginal canal bent:* loss of shape and structure of the tip of the marginal canal. *Lamellar fusion or fused lamellae:* increase in lamellar epithelium leading to partial or total fusion of lamellae. *Thickening of the lamellar epithelium:* enlargement of the epithelium. *Shortening of secondary lamellae* (Fig. S1B): shrinking of lamellae, leading to changes in cell size. *Ruptured gill lamellar stem*: cell rupture and tissue loss. *Damaged myoepithelial layer* (Fig. S1P) *or degeneration of the epithelial layer or degenerated tubules:* changes in cells and tissue structures, leading to necrosis. *Disintegrated lumen or abnormal lumen* (Fig. S1N) *or dilation of the tubular lumen or enlarged lumen:* changes in cells and tissue structures, altering the normal shape of the lumen (star shape). *Loss of lobular structure:* cell rupture or decrease in cell density, leading to tissue loss. *Basal lamina detachment* (Fig. S1R), *displacement of the epithelial layer into the lumen* (Fig. S1S): displacement of the epithelium from the basal lamina. *Cell lysis:* cell breakdown or disruption. *Unclear cell boundaries:* cell borders or contours not well defined or indistinguishable. *Loss of microvilli:* decrease or absence of microvilli on the cell surface. *Disordered lamina epithelialization:* a condition in which the epithelium (cell layer lining

surfaces and cavities) grows irregularly or chaotically onto the lamina, which is the underlying layer of tissue. *Extended lamina propria:* abnormally thick or expanded lamina propria (layer of connective tissue lining the epithelium in several mucous membranes). *Epithelial cell exfoliation:* a process in which epithelial cells, which normally line the body's inner and outer surfaces, detach from the basal layer. *Irregular and abnormally arranged epithelial cells or epithelial cell disorder:* abnormalities in the structure, function, or organization of epithelial cells. *Ruptured muscle bundles or myofibrils:* destruction or rupture of myofibrils, which are the fundamental contractile units of skeletal and cardiac muscles. *Fusion of the muscle bundle:* pathological change in which bundles of muscle fibers, which are normally separate and distinct, fuse or group abnormally.

2. **Plasma changes:** caused by hyaline droplets (*granular degeneration*), colloidal droplets (*colloidal degeneration*), fatty vacuolization degeneration or hydropic glycogen droplets (*glycogen degeneration*), and calcareous degeneration and thickening of the thin fibers of connective tissue (*hyaline degeneration*). *Large vacuoles*, *vacuolation of tubules* (Fig. S1Q) *and vacuolated B cells* (Fig. S1N): B cells increased in number or size. *Granuloma:* chronic inflammatory cells, with the presence of epithelioid cells and multinucleated giant cells. *Loose connective tissue*, *loss of tubular structure:* presence of loose tissue in the epithelium. *Apparent vacuoles in microvilli:* presence of spaces or bubbles within microvilli (small finger-like projections on the surface of epithelial cells). *Enterocyte vacuolization:* pathological changes in enterocytes.
3. **Deposits:** intercellular accumulation of substances caused mainly by degenerative processes.
4. **Nuclear changes:** changes in the nucleus, shape, and structure of chromatin (e.g., karyopyknosis and karyorrhexis). *Pyknotic nuclei* (Fig. S1U): enlargement of the cell nucleus.
5. **Atrophy:** reduction in cell number and volume and/or a decreasing amount of intercellular fluid. *Atrophied lamella:* a condition in which the lamella, which is a thin, blade-like structure in the gills, reduces in size or development. *Cell swelling or lamellar swelling or lamellar collapse with swelling* (Fig. S1E): intracellular accumulation of water due to failure in active transport, hypoxia, or damage caused by toxic substances. *Lamellar collapse* (Fig. S1D)*:* thin lamellae due to the disruption of pilaster cells. *Atrophied epithelial tubules* (Fig. S1M): increased lumen and reduced thickness of epithelial cells. *Atrophy*, *focal atrophy of muscle fibers:* decrease in muscle size and mass due to a reduction in the number of muscle fibers or a decrease in the size of existing fibers.
6. **Necrosis:** morphological state of cells or tissues following irrevocable loss of cellular function. Necrosis in the primary lamellae of the gills (Fig. S1K). Necrosis in the tubules of the hepatopancreas (Fig. S1T).

Reaction pattern 3 (rp3)

Progressive changes: processes leading to increased activity in cells or tissues. The alterations included in this reaction pattern are as follows:

1. **Hypertrophy:** an increase in cell or tissue volume, without an increase in the number of cells.
2. **Hyperplasia:** enlargement of tissues or organs due to an increase in the number of cells without changes in cell volume. *Hyperplasia in secondary lamellae* (Fig. S1H) *or hyperplasia in primary lamellae* (Fig. S1H). *Hyperplasia* (Fig. S1M).

Reaction pattern 4 (rp4)

Inflammatory changes: associated with processes belonging to other reaction patterns (e.g., *edema*). Alterations are classified as follows:

1. **Exudate:** protein-rich fluid containing a large quantity of cellular debris exuded from the hemolymph and lymphatic vessels.
2. **Activation of the reticuloendothelial system:** hypertrophy of the reticuloendothelial system, which consists of endothelial cells and macrophages lining small lymph vessels.
3. **Infiltration:** leukocytes infiltrating lymph vessels and surrounding tissues. *Epithelial cell cytoplasm infiltrate:* leukocytes penetrating the vessel walls of the hemolymph and infiltrating the surrounding tissues. *Basophilic depositions:* focal accumulation of degranulated hemocytes. *Hemocytic infiltration* (Fig. S1B): accumulation of hemocytes in tissues. *Eosinophilic deposit:* accumulation of eosinophils, causing inflammation.

Reaction pattern 5 (rp5)

Neoplastic alterations: proliferation of cells within tissues (*autonomic proliferation*), producing tumors that can be divided into two classes:

1. **Benign tumors:** differentiated cells that replace or displace the original tissue. Tumor cells resemble normal tissue cells. Benign papilliform hamartoma (*tumor*). *Fibrotic nodules.*
2. **Malignant tumors:** poorly differentiated, rapidly multiplying cells that invade and destroy resident tissues. Metastases may be observed. *Neoplasm in the posterior and anterior intestine*: cells in the colon and rectum undergo genetic mutations that lead to uncontrolled multiplication, generating a *malignant tumor*.

Reaction pattern 6 (rp6)

Pathogenic changes: presence of parasites or parasite cysts (Fig. S1I) in tissues. *Metacercarial cysts. Cercariae. Acanthocephalan parasites.* Parasitic cyst in the tubules of the hepatopancreas (Fig. S1O).

2.1.2. Pathological factor (α)

This weighting factor considers the degree of histological alteration in each organ exposed to xenobiotics, as well as the impacts of the alteration on organ function and crustacean health and survival, as shown in Table 1.

A pathological factor (α) ranging from 1 to 3 was proposed to evaluate the severity of histological changes and the impacts of these changes on organ function, as well as on the health and survival of crustaceans. The criterion was based on the description of histological changes found in *U. cordatus* and other species of crustaceans.

2.1.3. Alteration score (β)

Histological changes were scored according to their extent using a cardinal score of 0 to 4. Progressive scoring levels and mean percentages are shown in Table 2.

2.2. Biometric factor (χ)

Crabs were assessed for body weight (BW) in grams and carapace width (CW) in centimeters. These biometric measurements were then used to calculate the biometric factor (χ) of *U. cordatus*. This factor, which represents the importance of biometric measurements for the health of *U. cordatus*, was developed based on prior knowledge of the ecology of the species by sex. Although males are larger than females, the biometric scoring table features weight intervals that also apply to female biometrics. The index can be adapted to other crab species.

The biometric factor was assigned a cardinal score of 0 to 4, according to the correlation between the BW and CW of *U. cordatus*

Table 1
Pathological factor (α) for evaluating the severity of histological alterations.

α	Alteration
1	Mild pathological alteration, generally reversible
2	Moderate pathological alteration, generally reversible
3	Severe pathological alteration with partial or total loss of organ function, generally irreversible

Table 2
Alteration score (β) used to assess the extent of histological changes in *Ucides cordatus* and other crustacean species.

β	Extent of alteration	Percentage[a]
0	Absent, normal condition	0–10 %
1	Scarce	11–25 %
2	Moderate	26–40 %
3	Abundant	41–55 %
4	Highly abundant	>55 %

[a] Mean percentage of four fields at 100 × magnification.

Table 3
Biometric factor (χ) based on the correlation between body weight (g) and carapace width (cm) in *Ucides cordatus*.

χ	BW < 70	70 ≤ BW ≤ 90	90 < BW ≤ 100	100 < BW ≤ 200	BW > 200
CW > 8.0	4	3	2	1	0
7.0 < CW ≤ 8.0	3	2	1	0	1
5.0 < CW ≤ 7.0	2	1	0	1	2
3.0 ≤ CW ≤ 5.0	1	0	1	2	3
CW < 3.0	0	1	2	3	4

BW, body weight; CW, carapace width.

(Table 3). The χ score can be interpreted as follows: 0, ideal BW and CW; 1, slight deviation from the normal range of BW and CW; 2, moderate deviation from the normal range of BW and CW; 3, significant deviation from the normal range of BW and CW; and 4, severe deviation from the normal range of BW and CW. This mathematical interpretation was based on BW and CW values reported in previous studies on *U. cordatus* sampled over 10 years in port and preserved regions (Carvalho Neta et al., 2019; Jesus et al., 2020a, 2020b; Jesus et al., 2021; Oliveira et al., 2023).

2.3. Linear indices

Indices related to the quantification of histological alterations that correlate with biometry in *U. cordatus* were based on the sets organs = {gills, hepatopancreas, intestine, muscle, testis, ovary, and heart} and reaction patterns = {circulatory disorders or changes (I_{rp1}), regressive changes (I_{rp2}), progressive changes (I_{rp3}), inflammatory changes (I_{rp4}), neoplastic changes (I_{rp5}) and pathogenic changes (I_{rp6})} (Table S1, Supplementary Material). A schematic diagram of the proposed indices is shown in Figs. 1–3.

2.3.1. Organ alteration index (I_{alt})

This index represents the sum of all changes in each reaction pattern for a specific organ (Fig. 1A, B). Mathematically, it is expressed as follows (Eq. (1)):

$$I_{alt} = \sum_{i=1}^{alt} \alpha_{org\{k\},rp\{j\},alt\{i\}} \times \beta \quad (1)$$

where org {k} is the k-th element of the organ (org) set, with $k = 1, \ldots, 7$; rp {j} is the j-th element of the reaction pattern (rp) set, with $j = 1, \ldots, 6$; alt {i} is the number of changes that depend on the organ and reaction pattern; and β is the lesion score.

2.3.2. Organ index (I_{org})

This index is the sum of all I_{alt} values of a specific organ. Mathematically, it is expressed as follows (Eq. (2)):

$$I_{org} = \sum_{j=1}^{rp} I_{alt,rp\{j\}} \quad (2)$$

where rp{j} is the j-th element of the rp set ($j = 1, \ldots, 6$).

2.3.3. Total reaction index (I_{rp})

This index (Eq. (3) is the sum of the I_{org} values of a specific reaction pattern for all organs (Fig. 2).

$$I_{rp} = \sum_{k=1}^{org} I_{org\{k\},rp} \quad (3)$$

where rp{j} is the j-th element of the rp set ($j = 1, \ldots, 6$), and org{k} is the k-th element of the org set ($k = 1, \ldots, 7$).

2.3.4. Total index

The total index (I_{total}) is a measure of the overall health status of *U. cordatus* based on histological changes and biometry (Fig. 3). The total index is the sum of the I_{org} values of all organs plus the biometric factor, as follows (Eq. (4)):

$$I_{total} = \left(\sum_{k=1}^{org} I_{org\{k\}} \right) + \chi \quad (4)$$

where org{k} is the k-th element of the org set ($k = 1, \ldots, 7$), and χ is the biometric factor.

The I_{total} developed in this study correlates histological changes in different organs with biometric factors of *U. cordatus* males. This index can also be applied to females of the same species.

2.3.5. Sensitivity analysis

Sensitivity analysis of biological models is a crucial approach for understanding how variations in input parameters affect model predictions. Such an analysis allows identifying which variables are most influential or critical for the dynamics of the studied system. This approach is especially useful in complex models, where multiple factors interact in a nonlinear manner (Zi, 2011). Consider I_p (Eq. (5), a generic index that depends on the factors α_1, α_2, α_3, …, α_n and β_1, β_2, β_3, …, β_n, as follows.

$$I_p = \sum_{i=1}^{n} \alpha_i \times \beta_i \quad (5)$$

If the value of α_i varies by $\alpha_i \pm \Delta\alpha_i$, then the index I_p will vary by $I_p \pm \Delta I_p$. Thus, the sensibility $S_{\alpha_{i_{I_p}}}$ relative to the initial variation of the index I_p regarding the change in α_i can be expressed as follows (Eq. (6):

$$S_{\alpha_{i_{I_p}}} = \frac{\Delta I_p}{\Delta \alpha_i}, i = 1, 2, 3, \cdots, n \quad (6)$$

If $\Delta\alpha_i \to 0$, the equation can be written in terms of the partial derivative (Eq. (7).

$$S_{\alpha_{i_{I_p}}} = \frac{\Delta I_p}{\Delta \alpha_i} = \frac{\partial I_p}{\partial \alpha_i}, i = 1, 2, 3, \cdots, n \quad (7)$$

Therefore, the sensitivity of I_{alt}, I_{org}, I_{rp}, and I_{total} regarding the parameters α_i, β_i, and χ is given by Eqs. (8), (9), (10, and (11), respectively.

$$S_{\alpha_{i_{I_{alt}}}} = \frac{\partial I_{alt}}{\partial \alpha_i} = \beta_i; S_{\beta_{i_{I_{alt}}}} = \frac{\partial I_{alt}}{\partial \beta_i} = \alpha_i; S_{\chi_{I_{alt}}} = \frac{\partial I_{alt}}{\partial \chi} = 0 \quad (8)$$

$$S_{\alpha_{i_{I_{org}}}} = \frac{\partial I_{org}}{\partial \alpha_i} = \beta_i; S_{\beta_{i_{I_{org}}}} = \frac{\partial I_{org}}{\partial \beta_i} = \alpha_i; S_{\chi_{I_{org}}} = \frac{\partial I_{org}}{\partial \chi} = 0 \quad (9)$$

$$S_{\alpha_{i_{I_{rp}}}} = \frac{\partial I_{rp}}{\partial \alpha_i} = \beta_i; S_{\beta_{i_{I_{rp}}}} = \frac{\partial I_{rp}}{\partial \beta_i} = \alpha_i; S_{\chi_{I_{rp}}} = \frac{\partial I_{rp}}{\partial \chi} = 0 \quad (10)$$

$$S_{\alpha_{i_{I_{total}}}} = \frac{\partial I_{total}}{\partial \alpha_i} = \beta_i; S_{\beta_{i_{I_{total}}}} = \frac{\partial I_{total}}{\partial \beta_i} = \alpha_i; S_{\chi_{I_{total}}} = \frac{\partial I_{total}}{\partial \chi} = 1 \quad (11)$$

Eqs. (8)–(11) show that the sensitivity of indices in relation to a specific change depends directly on the pathological factor or score of the lesion.

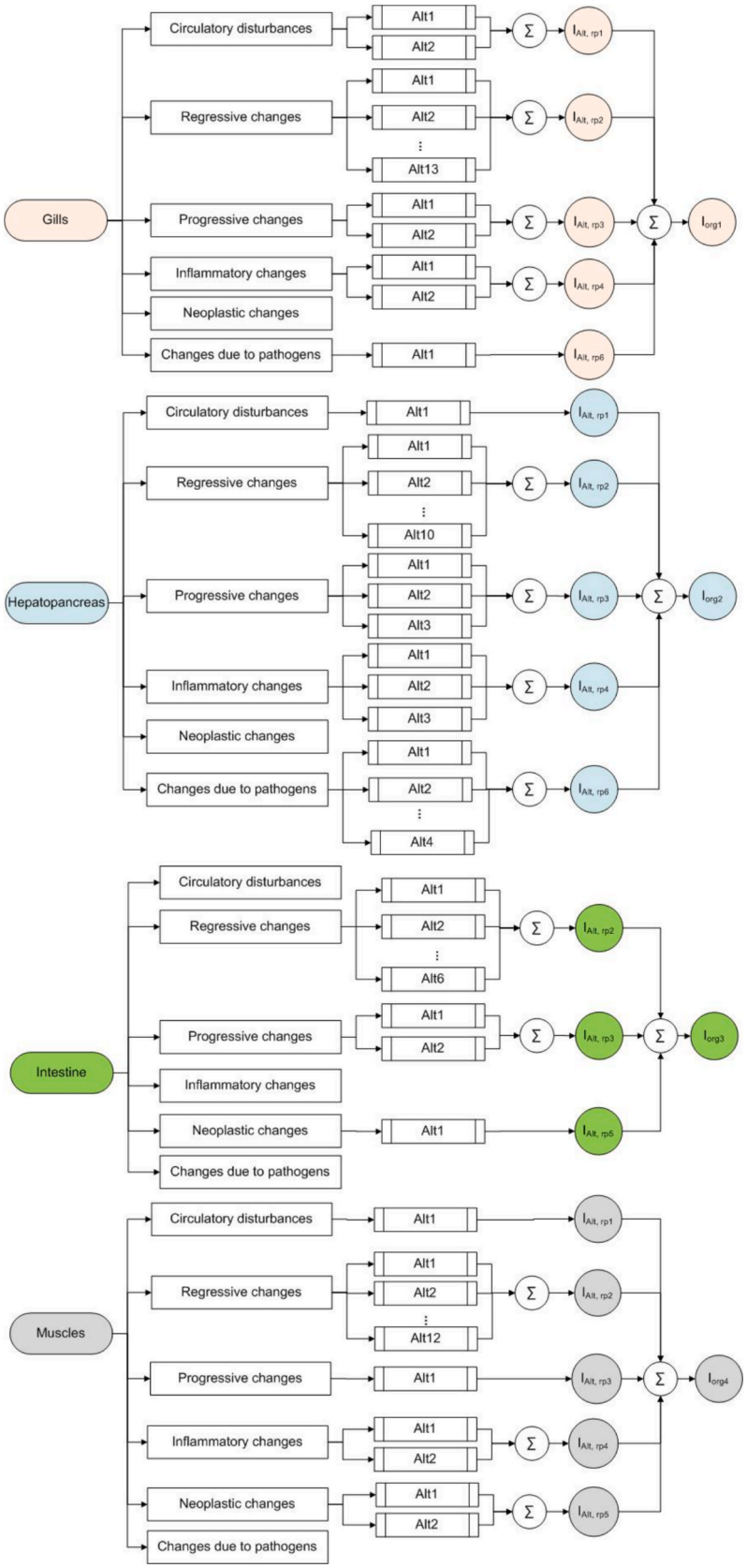

Fig. 1. Composition of the alteration index (I_{alt}) for the (A) gills, hepatopancreas, intestine, and muscles and (B) testes, ovaries, and heart of crabs.

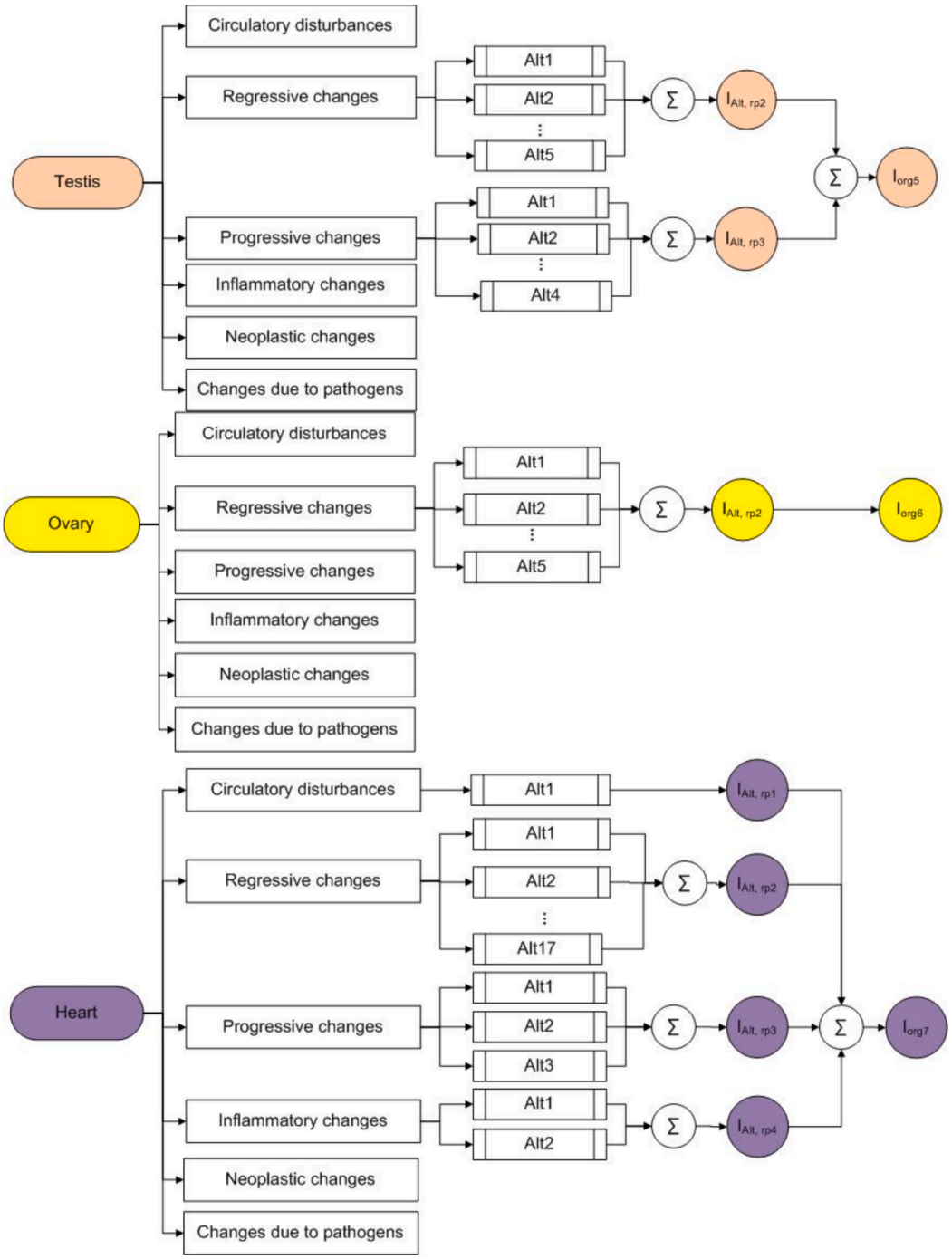

Fig. 1. (*continued*).

3. Discussion

The study of histological changes in the context of environmental impacts is a reliable, fast, and low-cost method widely used to diagnose the health of aquatic organisms. It allows for the early identification of the effects of emissions and other environmental pressures. Such an approach may provide valuable insights into the state of ecosystems and facilitate decision-making (Oliveira et al., 2019). This field of study examines microscopic changes in animal tissues, affording detailed information about changes and diseases that can be caused by environmental contaminants (Jaber et al., 2021).

In this context, the correlation between histology and biometry was found to be reliable, given that *U. cordatus* biometry is affected by anthropogenic factors. These variables provide a comprehensive view of impacts at different levels of biological organization, helping to understand how environmental or internal factors affect the organism as a whole (Kakwi et al., 2021).

Previous studies conducted in São Marcos Bay, Maranhão State, Brazil (Carvalho Neta et al., 2019; Oliveira et al., 2019; Jesus et al., 2020a; Jesus et al., 2021; Oliveira et al., 2023) revealed that pollution, seasonality, and the closed season are some of the preponderant factors leading to an increase in histological and biometric alterations in crabs. In *U. cordatus*, statistical analyses showed differences in histological changes and biometry between collection sites in the rainy and dry seasons (Oliveira et al., 2019; Jesus et al., 2020a, 2020b, 2021; Oliveira et al., 2023). Oliveira et al. (2019) identified a higher frequency of gill

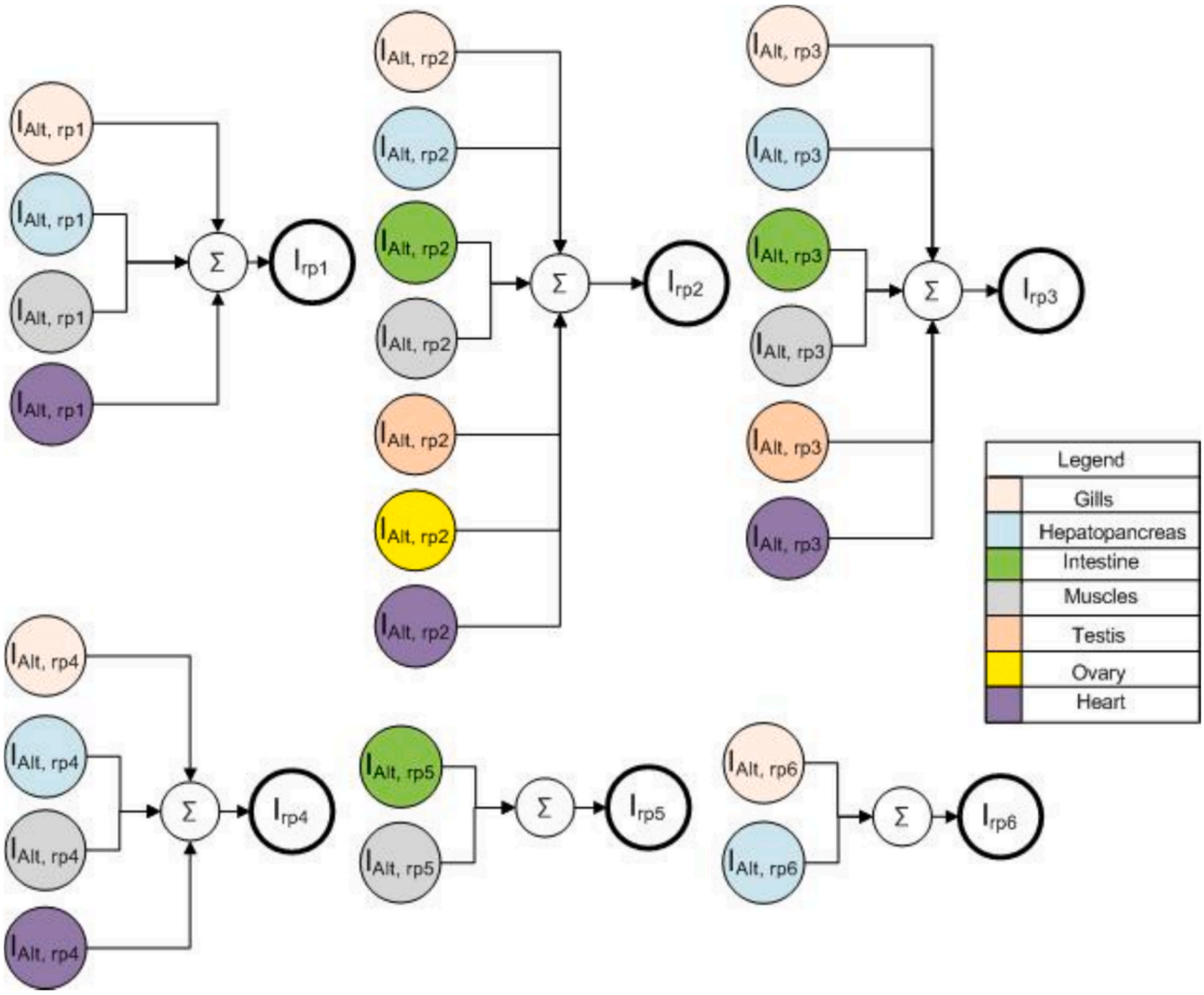

Fig. 2. Schematics of the calculation of Irp for reaction patterns. Legend. circulatory disorders or changes (Irp1), regressive changes (Irp2), progressive changes (Irp3), inflammatory changes (Irp4), neoplastic changes (Irp5) and pathogenic changes (Irp6). Reaction patterns (rp). Organ alteration index (IAlt).

alterations in *U. cordatus* captured close to an industrial hub with high levels of trace elements in sediments. The cited authors found that crabs of smaller sizes showed an increase in gill changes. Jesus et al. (2021) assessed the bioaccumulation of heavy metals in *U. cordatus* and the presence of trace elements in sediments from four locations in São Marcos Bay, off the Brazilian Amazon coast. The authors observed that, in industrial and port regions, the presence of heavy metals in tissues was associated with a higher frequency of gill and liver alterations in small crabs from heavily impacted areas.

Thus, exposure to different xenobiotics can impact growth quality in aquatic organisms. Exposed organisms need to redirect energy from essential processes such as growth and reproduction to detoxification and defense mechanisms against toxins (Truchet et al., 2023). This energy diversion impairs metabolism and gradually compromises healthy development (Santos et al., 2019).

The development of a linear model assessing the degree of histological alterations in invertebrate tissues may contribute to elucidating the gaps in the research field of aquatic organism health. However, there are no studies that have proposed linear models and standardized scoring protocols to assess histological alterations in invertebrates. Such approaches generally focus on fish and other vertebrates (Bernet et al., 1999; Răskovíc and Poleksíc, 2017). Bernet et al. (1999) proposed a protocol to standardize the histopathological evaluation of fish, improving the comparability of data between studies and facilitating the identification of patterns of damage related to aquatic pollution. Similarly, other studies developed protocols for scoring and classifying changes in aquatic animals (Torres et al.2024; Macedo et al., 2024).

Regarding crustaceans, Jerome et al. (2017) described the histopathological changes in the gills and hepatopancreas of *Callinectes amnicola* using a scoring protocol adapted from Bernet et al. (1999). Oliveira et al. (2023) applied the same classification and histological index used by Jerome et al. (2017) and Bernet et al. (1999) to evaluate *U. cordatus*. Given the lack of a standardized scoring and classification system for crustaceans, we developed a protocol for the global quantification of histological changes in *U. cordatus* with a focus on biometric correlations.

The protocol developed here is based on the calculation of the indices I_{alt}, I_{org}, and I_{rp}, which represent the degree of impairment of an organ and, consequently, of an organism. Of note, the indices can be applied to any type of crustacean. As described in Table S1 (Supplementary Material), the pathological score (α) ranges from 1 to 3 for each histological alteration in different organs.

The total index (I_{total}) correlates the occurrence of histological alterations with changes in biometry, taking into account past knowledge of the ecology of *U. cordatus*. Furthermore, biometric measurements are influenced by several isolated factors related to crab development, such

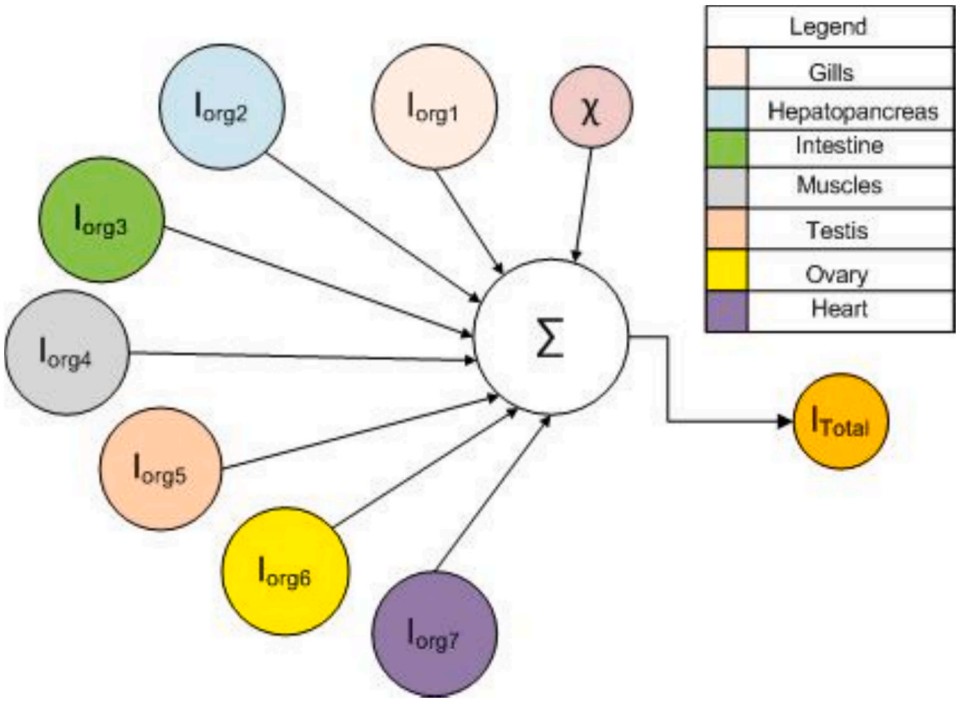

Fig. 3. Composition of the total index (I_{total}) of adult male *Ucides cordatus*, based on alterations in organs and crab biometrics.

as genetics, age, sex, and stage of gonadal maturation, as well as by external agents, such as seasonality, abiotic agents, and environmental stress from anthropogenic activities.

In *U. cordatus*, males are usually larger and heavier than females, as their larger size is associated with mating behavior, particularly in the search and defense of females (Pinheiro et al., 2013). The total index developed here for *U. cordatus* cannot be directly applied to other crustacean species, as crustaceans typically exhibit highly distinct characteristics, such as exoskeleton, body divided into cephalothorax (head and thorax) and abdomen, and different types of appendages. However, the I_{total} can be modified and adapted to other species of crustaceans, according to their specific particularities.

Sensitivity analysis revealed that the relationship between I_{total} and χ is 1 (unit), that is, a unit variation in χ results in a unit variation I_{total}, maintaining the other constant variables. I_{alt}, I_{rp}, and I_{org} do not depend on χ, so they have null sensitivity to χ. Thus, with the analysis of I_{alt}, I_{rp}, and I_{org}, and I_{total}, it is possible to statistically treat data to evaluate the significance of biological factors that influence the health of *U. cordatus* from different populations and localities. According to Sirri et al. (2018), histological investigative methods are applicable to various experimental scenarios and paraphysiological adaptations. Furthermore, pathological findings and changes should be compared, and statistical analyses should be performed to interpret the results. Using these data and a weighted score for the biometric factor, it is possible to correlate the degree of impact of changes at each stage of crab development.

In view of the lack of mathematical models for assessing histological and biometric changes in *U. cordatus*, the protocol developed here may contribute to a more accurate global and weighted evaluation of histological and biometric alterations in *U. cordatus*. This information can help environmental managers better understand the health status of crabs potentially exposed to pollutants from environmental matrices.

Finally, the model is a starting point for evaluating the health of crustaceans, especially *U. cordatus*, in environmental monitoring, species management, and breeding programs. However, it is necessary to develop more robust nonlinear models to evaluate the influence of environmental, xenobiotic, and ecological factors on the biological responses of *U. cordatus*.

CRediT authorship contribution statement

Wanda Batista de Jesus: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Raimunda Nonata Fortes Carvalho Neta:** Writing – review & editing, Writing – original draft, Resources, Methodology. **Raimundo Nonato Diniz Costa Filho:** Writing – review & editing, Resources, Methodology, Formal analysis. **Débora Batista Pinheiro Sousa:** Writing – review & editing, Writing – original draft, Resources, Methodology, Formal analysis.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We especially thank Laboratório de Biomarcadores em Organismos Aquáticos (UEMA) and Laboratório de Ecotoxicologia e Biotecnologia Ambiental (LEBiA) for their logistic support in the field and laboratory analyses, respectively. We thank the staff of the Maranhão State Research Foundation (FAPEMA, grant No. 001/2022), Programa de Pós-Graduação em Biodiversidade e Biotecnologia (REDE BIONORTE), and Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES) for the financial support.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jip.2025.108386.

Data availability

Data will be made available on request.

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