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Can fractal methods applied to video tracking detect the effects of deltamethrin pesticide or mercury on the locomotion behavior of shrimps?

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ABSTRACT

Shrimps can accumulate environmental toxicants and suffer behavioral changes. However, methods to quantitatively detect changes in the behavior of these shrimps are still needed. The present study aims to verify whether mathematical and fractal methods applied to video tracking can adequately describe changes in the locomotion behavior of shrimps exposed to low concentrations of toxic chemicals, such as 0.15 $\mu g\ L^{-1}$ deltamethrin pesticide or 10 $\mu g\ L^{-1}$ mercuric chloride. Results showed no change after 1 min, 4, 24, and 48 h of treatment. However, after 72 and 96 h of treatment, both the linear methods describing the track length, mean speed, mean distance from the current to the previous track point, as well as the non-linear methods of fractal dimension (box counting or information entropy) and multifractal analysis were able to detect changes in the locomotion behavior of shrimps exposed to deltamethrin. Analysis of angular parameters of the track points vectors and lacunarity were not sensitive to those changes. None of the methods showed adverse effects to mercury exposure. These mathematical and fractal methods applicable to software represent low cost useful tools in the toxicological analyses of shrimps for quality of food, water and biomonitoring of ecosystems.

1. Introduction

Shrimps are a specious group of decapod crustaceans with more than 3500 known species; they are very important in aquatic ecosystems and interact with many species in a wide range of both fresh and salt water habitats around the world (De Grave et al., 2015). In addition, shrimp has high nutritional value and pleasant taste for human consumption, making it a very healthy choice of food (Bono et al., 2012). The presence of toxic chemicals in the environment where shrimps live is a growing concern in the scientific community and health institutions, since shrimps can accumulate such toxicants (Gräslund et al., 2003; Nimmo et al., 1970). Exposure to toxic compounds may represent a high risk to the shrimp's health, increasing its level of stress, reducing growth and increasing its susceptibility to disease (Gräslund et al., 2003). These toxicants can also harm the health of humans who eat those animals (Chen et al., 2014). Despite the importance of assessing the exposure of shrimps to hazardous substances, most conventional toxicological methods are expensive, time consuming, require skilled labor, various equipment and high cost materials.

In recent decades, behavioral responses have been used to assess toxicological changes in aquatic animals, including shrimps (ASTM, 1994; Güner, 2013). Behavioral assays can represent an excellent tool for detecting toxicity of contaminants in animals and water effluents (García-de la Parra et al., 2006).

Biological systems are very complex and irregular. Consequently, conventional methods are often insufficient to describe them; in such cases, non-linear mathematical methods can characterize the process and quantify difficult changes in identification (Nimkerdphol and Nakagawa, 2008; Pessoa et al., 2015). The linear method is applied to variables which shown a straight line in a Cartesian graph; for example the linear regression method that allows to relate the shrimp

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mean speed over time. Non-linear methods are applied to variables which follow more complex functions in the Cartesian graph, such as the method of the fractal dimension. The fractal methods quantify structures that cannot be described by Euclidean dimension (integer), which considers straight lines, planes and three-dimensional space. The fractal dimension measures how much of the space is filled by an irregular structure; for example, the distribution of blood vessels in the tissue (Costa et al., 2013). Therefore, fractal methods allow the measurement of non-quantifiable structures by conventional geometry. On the other hand, fractal methods can provide the same dimension value for geometrical different structures (Gould et al., 2011); Because of this, another method such as lacunarity is needed to distinguish these structures. Lacunarity measures the degree of heterogeneity of the structure, evaluating the spaces within and around this structure, as well as how it is dispersed. The multifractal method describes the structure at different scales; therefore, it is more efficient to evaluate certain biological systems which show different characteristics at different scales (Costa and Nogueira, 2015).

The present study aims to verify whether linear and nonlinear mathematical methods applied to video tracking can be used as tools to detect changes in the locomotion behavior of shrimps exposed to low concentrations of toxic chemicals such as deltamethrin pesticide and mercury.

2. Material and methods

2.1. Experimental design

Shrimps were obtained from the hatchery Pirre at 9°39'24"S 35°42'47"W coordinates (Maceió, Brazil). Adult fresh water shrimps *Macrobrachium jelskii* (Miers, 1877) (0,3 ± 0,1 g) were kept in dechlorinated (Dechlor-ease, GTA, USA) tap water (Maceió City), dissolved oxygen (4.95 ± 0.21 mg/L), salinity (0.31 ± 0.01 ppm), temperature (27.02 ± 0.45 °C), pH (6.95 ± 0.29), total ammonia-nitrogen < 0.01 mg/L, total dissolved solids (567 ± 27 mg/L), conductivity (875 ± 66 Ω/m), and photoperiod 10 h light and 14 h dark. Water quality parameters were measured by using a multiparameter probe (HANNA Instruments 9828, Woonsocket, USA). Stock shrimps were acclimated for one week (Wang et al., 2013) in 25 L tanks (50 × 30 × 20 cm) at a density of 15 individuals per tank.

During the experimental period, shrimps were transferred to the video tracking arena and kept in similar environmental conditions as described above for acclimatization. The experiments were performed using a video tracking arena in a 32 cm circular plastic tank (3-liter volume), kept in groups of 3 shrimps per tank and acclimated for additional 48 h in this arena before experimental treatments. Shrimps were fed with 1.1×10^{-3} g/day/shrimp commercial food (MEP 200, Alcon, Brazil) once a day at 4 p.m. The water and toxic chemicals were renewed every 24 h during the experiments by gently siphoning out and replacing the same solution 33% each day (Adapted from Chiodi Boudet et al., 2015).

Shrimps were randomly chosen to compose the following groups: I) control group (n = 6); II) group exposed to 0.15 μg L^{-1} deltametrin (n = 6); III) group exposed to 10 μg L^{-1} mercuric chloride (n = 6). The control group and treatments using deltamethrin or mercury were replicated twice. The concentrations of deltamethrin and mercury were based on Tu et al. (2012) and Eguiraun et al. (2014). All shrimps were video recorded and analyzed at 1 min, 4, 24, 48, 72 and 96 h after the addition of toxic chemicals; a pre-exposure behavior analysis was also performed. Deltametrin (Bayer, France) and mercuric chloride ($HgCl_2$ Nuclear, CAQ, Brazil) were diluted in 1 mL distilled water; 1 mL distilled water was also added to the control tanks, both being applied in the center of the tanks.

All animals were cared for in accordance with the guide for the care and use of laboratory animals (National Research Council - National Academy of Sciences, 2011).

2.2. Video tracking

The locomotion behavior of the shrimps was recorded using a Finepix 4500 digital camera (Fujifilm, Brazil), mounted 36 cm above the water surface. Each video was recorded in 320 × 240 pixels and 30 FPS for 25 min, this resolution was enough to track the shrimps. These videos were transformed into 3 FPS by using the Virtual Dub 1.10.4 software (Avery Lee, 2012) and 3 pictures per second were used to measure movements. 4500 coordinate points X,Y, describing the location of shrimp in the arena, were obtained by using video tracking in Image J 1.49v software (National Institutes of Health – NIH, USA) with plug-in MTrackJ (Meijering et al., 2012; Erasmus University Medical Center, Netherlands). The same plug-in was used to calculate the parameters: length of the track (cm), mean speed (cm/s), mean distance from the first point to the current track point - D2S (cm), mean distance from the current to the previous track point - D2P (cm), mean angle of the in plane components of the track points vector - θ (degrees) and the angular change between the in plane components of the track points vector Δθ (degrees).

2.3. Fractal Dimension Methods

The time series of coordinates X,Y were used to plot a line graph (1 pixel thick) of the locomotion behavior of the shrimp. These graphs were used to calculate the fractal dimension by the box counting (Box), perimeter-area (Per-area), information entropy (Inform) and ruler dimension methods. Fractal dimension was calculated as previously described by Costa et al. (2013). The graph images were fed to software Benoit 1.3 (Fractal Analysis System, Trusoft, St. Petersburg, USA) in order to calculate the fractal dimensions.

In brief, the fractal dimension was obtained by covering up the image of the locomotion behavior with N(r) boxes, where N is the number of boxes and r is the length of one side of the box containing at least one point of the fractal object in the image. This procedure was repeated with boxes of different sizes and plotted in a double log graph of N(r) as a function of r. The slope of this relationship between the inverted signals is the fractal dimension by box counting and Ɛ is the small variation in box size. This can be defined as follows:

$$D_{bc} = -\lim_{\varepsilon \to 0} \left[\frac{\log N(r+\varepsilon) - \log N(r)}{\log(r+\varepsilon) - \log r} \right] \quad (1)$$

The fractal dimension by information entropy (D_{inf}) of the images of shrimp locomotion behavior was also covered by boxes, but this method is related to the frequency in which each box is occupied, as formally defined by:

$$D_{inf} = \lim_{\varepsilon \to 0} \left[\frac{(S(r+\varepsilon) - S(r))}{\log(r+\varepsilon) - \log r} \right] \quad (2)$$

$$S(r) = -\, lim_{N\to\infty} \sum_{i=1}^{N(r)} m_i \log(m_i) \quad (3)$$

where S(r) is the Kolmogorov entropy, $m_i = M_i/M$ (M_i is the number of points in the ith box, and M is the number of points on the object).

Area-perimeter fractal dimension (D_{AP}) was obtained through the slope of regression line obtained using the logarithm of area against the logarithm of its perimeter in different scales, as showed by following relation: $\ln(\text{area}) = b_0 + b_1 \cdot \ln(\text{perim})$. Formally, $D_{AP} = 2/b_1$, in which b_1 is the angular coefficient of the linear relationship.

Ruler fractal dimension measures how the contour length of structure depends on the length of the ruler used for its measurement. Formally, ruler fractal dimension is obtained by the slope of the graphic log L versus log R, in which L is the length and R are the different lengths of the ruler.