Using Liquid Chromatography-Mass Spectrometry to assess physiological stress in fish

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Background

- Water pollution can cause physiological stress in fish.
- Physiological modifications can alter the demographic performance because energy used for growth, survival and reproduction is diverted towards internal maintenance.
- Therefore, determine levels of stress can lead to a better understanding of local population dynamics.
- Previous studies used immunoassay analyzes to determine the levels of stress based on cortisol (Fig. 1) in fish, but these methods lacks selectivity.

Goals

- Develop a selective and sensitive method to measure levels of cortisol in the Eastern Mosquitofish (*Gambusia holbrooki*, Fig.2) based on liquid chromatography-mass spectrometry (LC/MS).

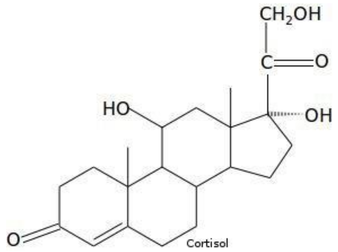


Figure 1: Cortisol structure. Cortisol is often called the "stress hormone" because of its connection to the stress response, but it can also help control blood sugar levels, regulate metabolism, help reduce inflammation, and assist with memory formulation.

Figure 2. Model used for experiment: Mosquitofish

Research Methodology

1. Fish sample homogenization and ultrasonic extraction using methanol and addition of cortisol d4 as internal standard.
2. After the sample extraction, the vials were placed in a centrifuge to separate the phases. Fish tissues are compacted in the bottom of the vial.
3. To conserve the samples, they were placed in a -20 freezer. It helped separate the lipids (fat) from the solution.
4. The supernatant was transferred to 1 mL LC vials.

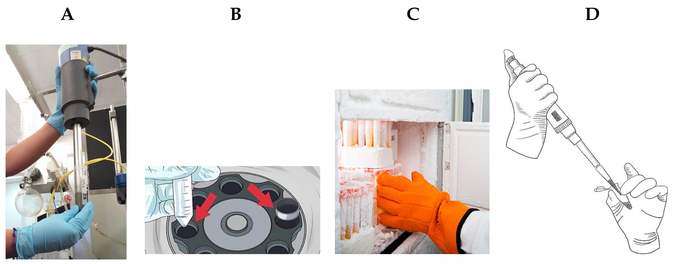


Figure 3. (A) Fish homogenization and ultrasonic extraction. **(B)** Samples were centrifuged for better separation of the fish tissue from the organic extract. **(C)** Lipids (fat) was consider as a problem since it could affect the analyses (matrix), so samples were placed in a freezer (-20 °C) overnight. **(D)** A aliquot of the supernatant was transferred to 1mL vials.

- Samples were analyzed by LC-TSQ – Liquid Chromatography Triple Quadrupole Mass Spectrometer (Fig. 4 & 5). Parameters like ionization, polarity, spray voltage and capillarity temperature were set for the analysis. Water and methanol were used as mobile phase in a gradient through a total of 9 minute run.

Figure 4. LC-TSQ. Parameters – Ionization: ESI; Polarity : Negative; Spray Voltage: 5 KV; and Capillarity temperature: 350°C

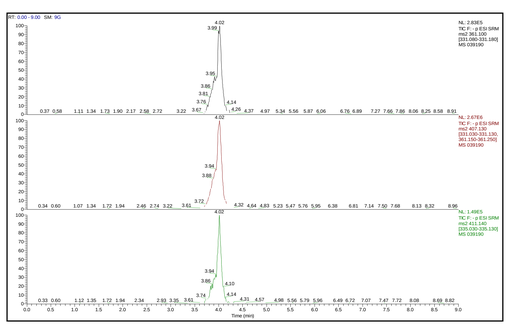


Figure 5. Chromatogram (5 ppb) showing the three transitions monitored. Black line (361.1->331.1 m/z); Red line (407.1->331.1 m/z) both for cortisol and Green line for cortisol d4 (411.1->335.1 m/z).

Results

- The method was successfully validated in terms of linearity (Fig.6), limit of detection, accuracy, precision and matrix effects. MDL= 4 ng/g fish (0.87 ng/mL) (Fig. 7)

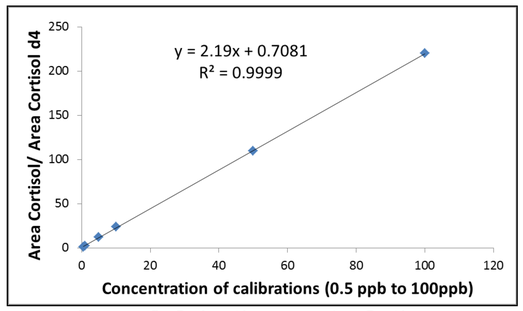


Figure 6. Calibration curve for Cortisol

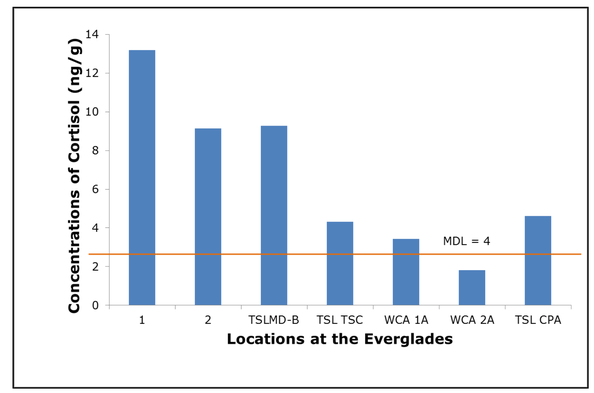
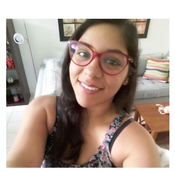


Figure 7. Concentration of cortisol found in fish samples from the Everglades

Table 1. Validation parameters.

Concentration	Recoveries	Intra-Precision	Inter-Precision
1ppb	73.87-120.41	6.683904	21.88
10ppb	105.82-126.29	5.072599	9.64
50ppb	95.85-126.52	3.198059	8.02

- The matrix effects was calculated as : [(concentration found in the spiked sample-concentration found in the unspiked sample)/concentration found in a spiked blank– 1]*100: with negative values indicating ion suppression.
- For the concentrations tested (5 and 50 ppb) (Table 1), the matrix effects ranged from -4 to -21%.



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