

# Effects of Fluoride on Crayfish

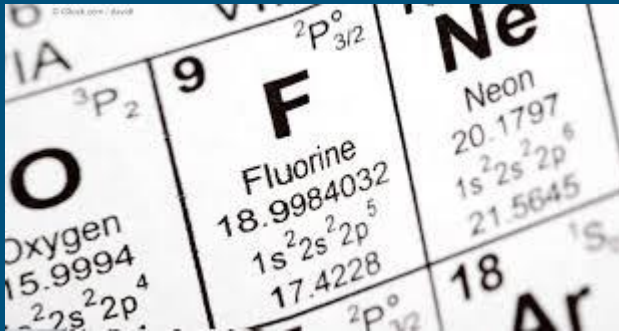
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Ashley Burke, Lauren Law, Prachi Patel



# Introduction

- Fluoride



WATER WITH FLUORIDE BUILDS A FOUNDATION FOR HEALTHY TEETH.

Brush with fluoride toothpaste. Visit the dentist regularly. Drink water with fluoride.

Fluoride in water. 70 years and going strong. At a faucet near you. [www.cdc.gov/fluoridation](http://www.cdc.gov/fluoridation)

# THERE IS POISON IN THE TAP WATER.

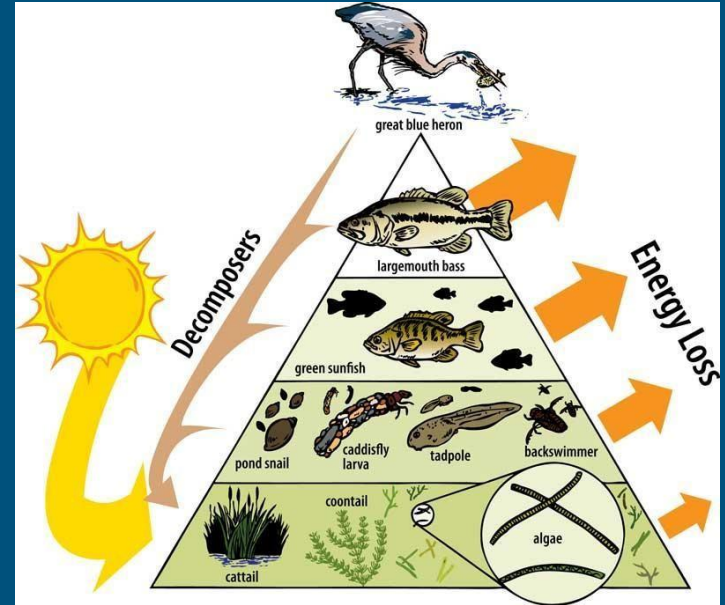
**FACT:** Fluoride is a dangerous substance and the active ingredient in most insecticides! If ingested, as little as 1/10 an ounce of fluoride can kill a 100 lbs adult and 1/100 of an ounce can kill a 10 pound infant. Studies have shown that exposure to fluoride can cause neurological damage, and an increased risk of bone cancer.

**FICTION:** Fluoride added to the public water supply strengthens teeth and helps prevent cavities.

DEMAND SAFE WATER. DEMAND THE TRUTH. [VISIT INFOWARS.COM](http://VISIT INFOWARS.COM)

# Introduction

- Fluoride levels too high?
- How does Fluoride affect freshwater organisms?  
Crayfish ?
- What will happen to crayfish treated with fluoride?



# Hypothesis

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- *Pacifastacus Leniusculus* treated with fluoride will show degrading behavioral activity, lower frequency action potentials, and increase concentration of the protein mitogen-activated protein kinase, MAPK, Jun N Terminal Kinase, JNK.

# Materials

- *Pacifastacus leniusculus*, Carolina Bio supply
- Sodium Fluoride (NaF)
- Anti-MAPK8/JNK1
- WesternBreeze anti-Rabbit Western Blot Kit
- Note: Highest level of Fluoride detected in tap water in 2016 has been 0.70 PPM (Fulton County Annual Water Quality Report 2016)



# Methods

- Dependent Variables

- Lack of energy
- loss of sensorimotor skills
- frequency decrease of neuronal spiking in electrophysiology
- Amount of protein (expressing mitogen-activated protein kinase, MAPK/JNK)

- Independent Variables- Levels of Fluoride in Water

- Controls

- All male subjects
- same feeding
- same size
- same lighting
- same temperature water

# Methods - Behavioral

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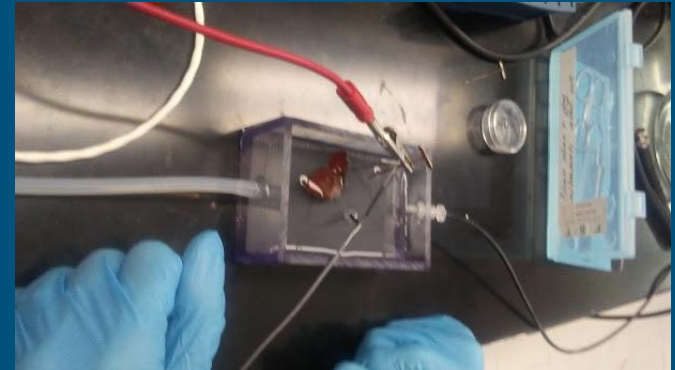
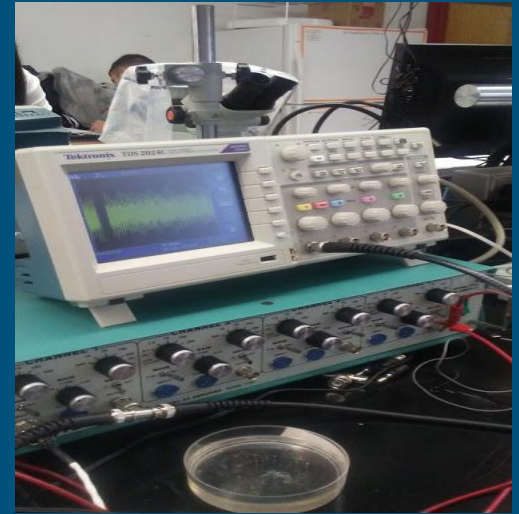
- 4 groups
  - Control group
  - Fluoride concentration of 3 ppm
  - Fluoride concentration of 5 ppm
  - Fluoride concentration of 7 ppm
- 3 crayfish in each group
- Observed for 2 weeks each class
- Behavior assessed using an Ethogram
  - Test group vs. Naive group



# Methods- Electrophysiology

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- 8 *Pasifastacus Leniusculus* subjects
  - Anaesthetized for 10 minutes
  - Dissect nerve cord
  - Saline (2min.), 10.5 mg/L NaF in saline (2min.), saline (2min.)
  - Drained in between each run: 5 mins. and 8 mins, respectively.
  - Repeat 8 times
  - Analyze and spike sort using DataView



# Methods- Western Blot

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- 7 *Pasifastacus Leniusculus* subjects (same subjects as behavioral)
- Will do Western Blot to analyze amount of protein
  - Primary antibody: Anti-MAPK8/JNK1

# Expected Results

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- Behavioral: Lethargic behavior and decrease in sensorimotor functioning skills
- Electrophys: Neuronal spiking decrease in electrophysiology analysis
- Western Blot: increased amount of protein in crayfish treated with fluoride

# Alternative Results

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- Behavioral: possible neuronal death if [x] of NaF is too potent
- Electrophysiology
  - If no change in spike frequency, fluoride effects could be more long term.
  - Fluoride concentration could kill nerve cord → no spikes.
  - Spike frequency increase → fluoride interference with transmission
- Western Blot
  - No change in protein amount → fluoride didn't activate the ER Stress Response

# Discussion

- Hypothesis supports the experiment
  - Causes abnormal behavior
    - Toxic bioaccumulation build up
    - Decrease in food consumption
    - Increase in mortality rate
  - Decrease in neuronal activity
    - Competition between  $\text{Cl}^-$  and  $\text{Fl}^-$
  - Changes in Calcium level
    - Triggers ER response that activating MAPK/ JNK
      - Visualized with the help of antibodies

# Discussion

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- If the Hypothesis is supported, then toxic levels of fluoride interfere with survival of Crayfish, *Pacifastacus Leniusculus*.
  - Attack on Central Nervous system
- Crayfish and other freshwater organisms are important to the ecosystem.
- Research moves toward discovering safe levels of Fluoride in areas affected by fluoride pollution.

# References

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