

# Effects of Developmental Exposure to BPA and EE on Neurobehavioral Programming in Painted Turtles (*Chrysemys picta*)



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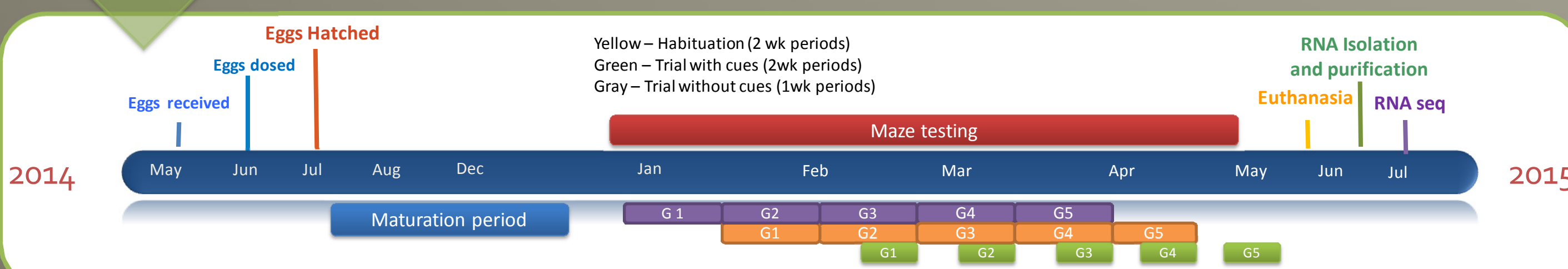
## Background

- Endocrine disrupting chemicals (EDCs) are abundant and widespread throughout the environment. Several, including bisphenol A (BPA) and ethinyl estradiol (EE2), can induce feminization or de-masculinization in many animal species.
- BPA is used in many household items and has been detected in aquatic environments across the US [1]. It is important to identify the possible impacts that exposure to BPA has on human and animal health.
- It has been shown that exposure to BPA and EE2 alters sexual differentiation, with turtles incubated at male producing temperatures (26°C) producing partial to full sex reversal to females in painted turtles (*C. picta*, Figure 1). [2,3]
- Little is known about possible effects of exposure to EDCs on growth, learning, and memory

**Aim: Determine whether developmental exposure to BPA and EE2 disrupts Neurobehavioral programming and affects Growth**

## Methods

- Turtle Eggs**
  - At developmental stage 17, (see Figure 2) painted turtle eggs were randomly assigned to one of 5 treatment groups and dosed.
    - BPA High (100 µg/mL), n=12, BPA Low (0.01 µg/mL) n=12. EE2 (0.2 µg/mL) n=12
    - EtOH (vehicle control) n=12, Control n=12 –The two controls groups were considered together when analyzing the data.
- Growth**
  - Turtles were allowed to mature to juveniles.
  - Growth of turtles was measured every 5 weeks by measuring body weight, carapace length, carapace width, and plastron length.
  - Measurements taken from hatching (July 2014) to May 2015.
- Behavioral Testing**
  - Commenced at 5 months old, for 14 consecutive days per group. Each turtle randomly assigned one of 4 food containers, associated with intra-maze cues (Star, Circle, Square, and Triangle). Shown in Figure 3
  - Tested for 600 seconds or until correct food container was found.
- Video Analysis**
  - Video footage of trials was taken on HD video recorder.
  - ANY-maze and Cleversys tracking programs used to track latency, total distance, and speed. See Figure 4
  - Percent censored was taken into account in data – the number of turtles that failed to locate the correct food container in the allotted 600 seconds.
- Genetic Data**
  - After trials were complete turtles were euthanized.
  - Brains, liver, gonad, and fat tissue were collected.
    - Coding RNA from Brains are currently being examined to determine if there are specific neural transcripts associated with any potential BPA- or EE-induced behavioral disruptions.



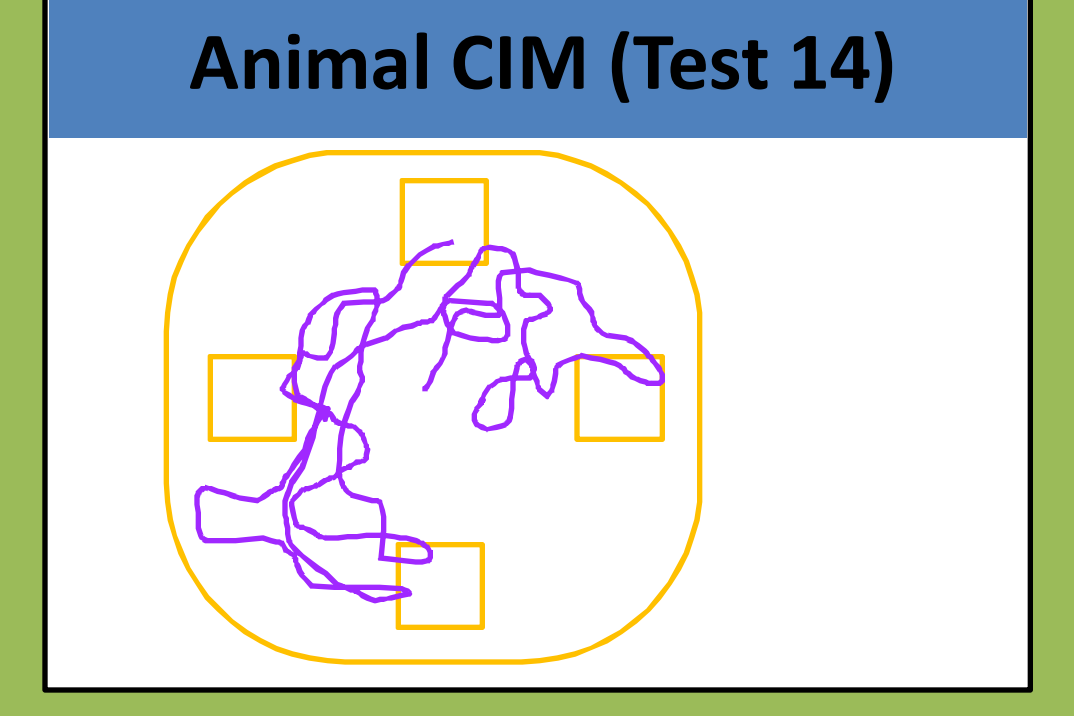
**Figure 1**  
Painted Turtles (*Chrysemys picta*) were used because of their temperature-dependent sex determination and previously seen full or partial sex reversal to female when exposed to BPA and EE2 [2,3].



**Figure 2**  
Painted turtle (*Chrysemys picta*) eggs were candled to determine developmental stage in order to dose animals at appropriate developmental stage.



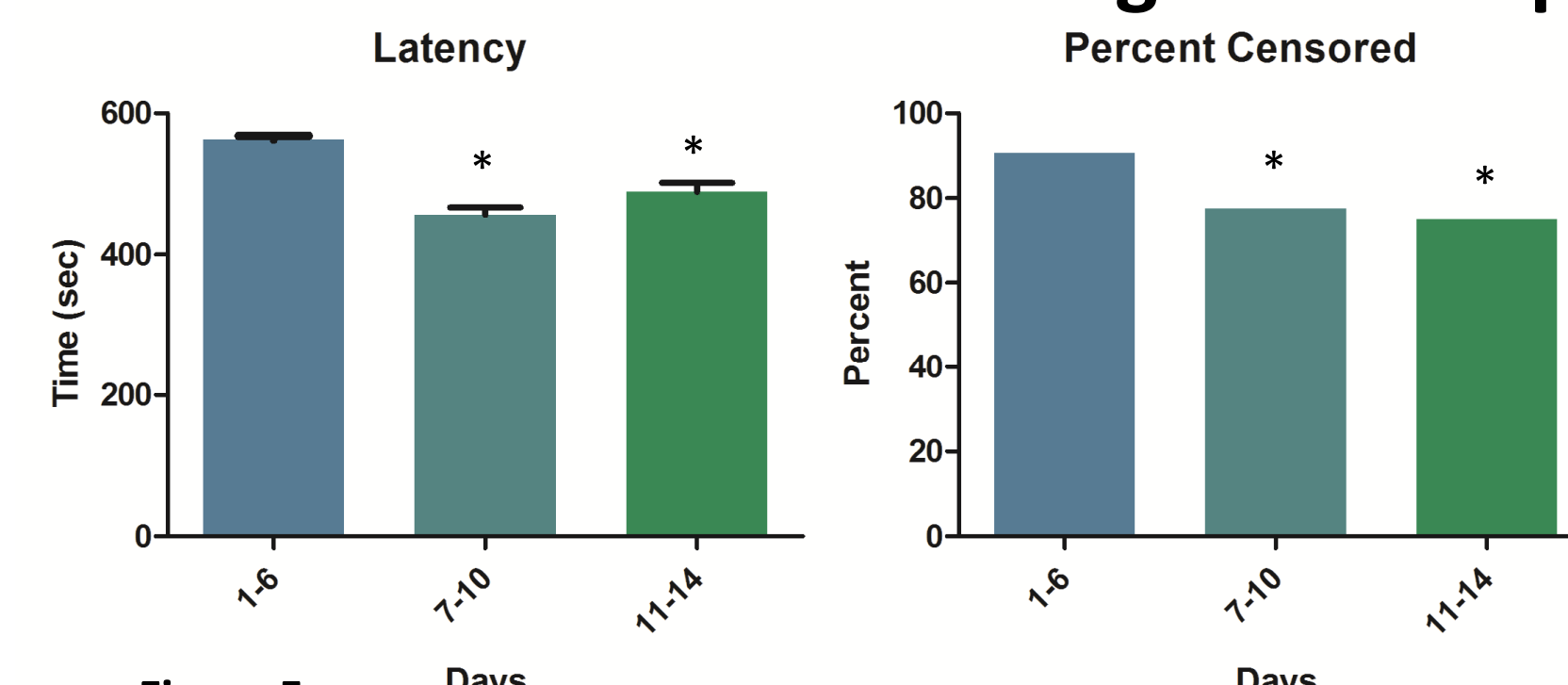
**Figure 3**  
Aquatic Barnes maze was used to test turtles' spatial navigation, learning, and memory. Equipped with removable intra-maze cues.



**Figure 4**  
ANY-maze track plot report from video analysis. Shows distance traveled and target areas entered as well as measures velocity and latency.

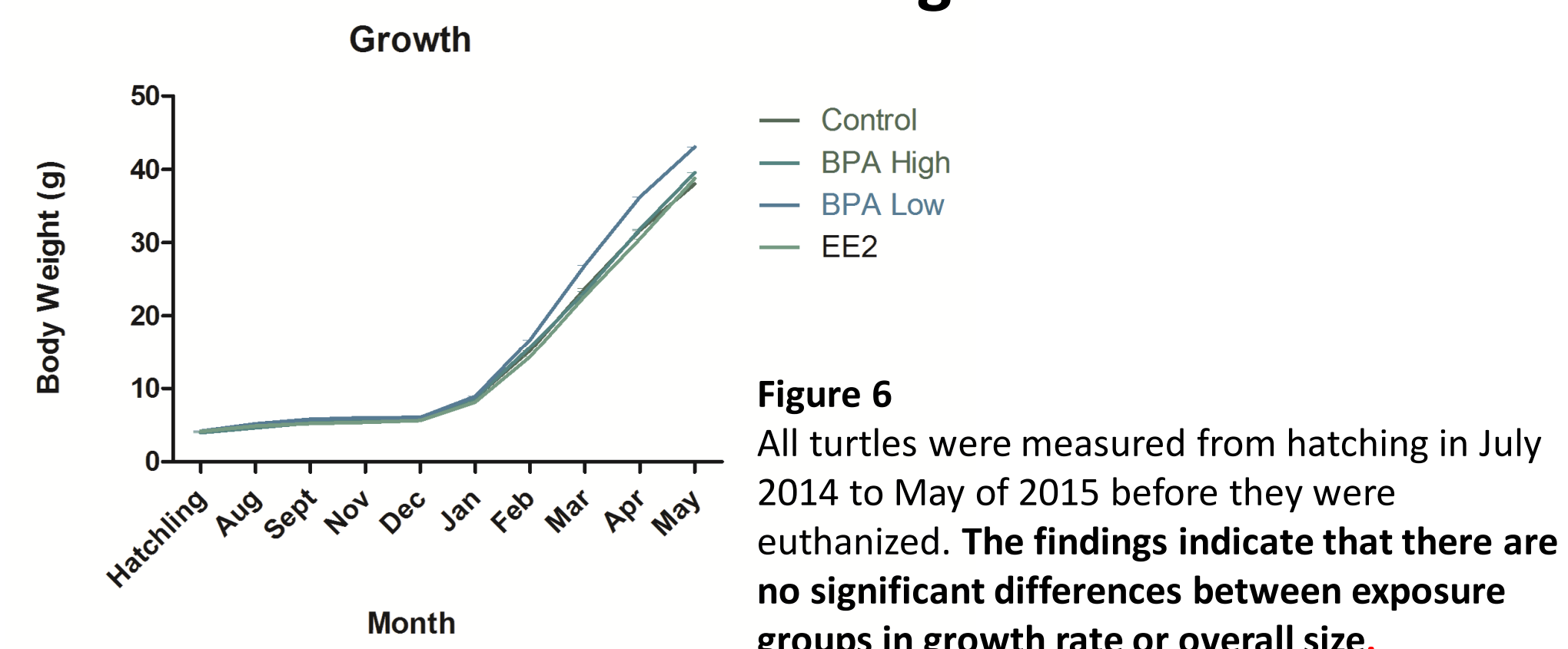
## Results

### Turtles learned the maze throughout trial period



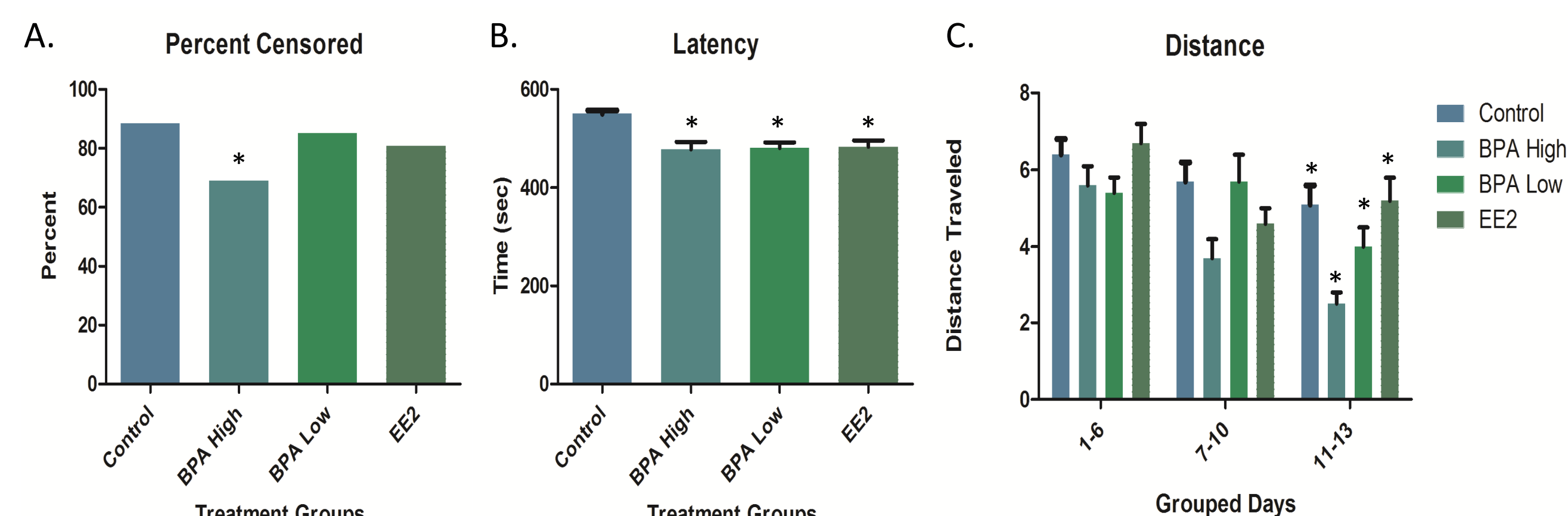
**Figure 5**  
In all groups latency times improved and number of individuals censored decreased over 14 day trial period. (\* indicate significantly different from days 1-5, P value, 0.0001).

### Developmental exposure to BPA and EE2 did not show effects on growth



**Figure 6**  
All turtles were measured from hatching in July 2014 to May of 2015 before they were euthanized. The findings indicate that there are no significant differences between exposure groups in growth rate or overall size.

### Improved Spatial Navigation Seen with Exposure to EE2 and BPA



**Figure 7**  
A. Percent censored is equal to the number of turtles that failed to locate the correct food container in the allotted time (600 sec). Treatment groups were more likely to solve the maze. B. Turtles exposed to one of the treatment groups *in ovo* solved the maze faster than control males. (P values = 0.04 to 0.0001) C. Exposed turtles traveled less distance along with solving the maze faster than control maze. (\* indicate significantly different from Control group, P value = 0.05 to 0.03). Overall the collective findings in the BPA and EE groups suggest potential brain feminization.

## Conclusion

- Growth data show no difference in body weight or growth of individuals exposed to BPA and EE2 compared to the controls. This suggests that there may not be any external signs of exposure to EDCs. Therefore, histology or physiology may need to be considered.
- Surprisingly Spatial Navigation trial results show that individuals with developmental exposure to BPA and EE2 show improved learning and memory compared to controls, who did not show demasculinization. This suggests BPA and EE can induce reversal of neurobehavioral programming in addition to the gonads.

## Ongoing studies

- Currently, we are examining global gene expression patterns (RNAseq) of test subjects to determine if behavioral differences are due to gene alterations in the brain.
- These changes in behavior suggest that exposure EDCs in the environment could result in a wide range of behavioral alterations, which may affect the ability of wild turtles to thrive and reproduce. This needs to be further explored.

## Acknowledgments

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