# The effects of two antihypertensives on the locomotion of juvenile American horseshoe crabs, *Limulus polyphemus*

Johnathan R. Martineau[1], Amber R. Lauchner[2], Tyler N. Thomas[2], Chris C. Chabot[2]
Plymouth State University [1]Department of Interdisciplinary Studies and [2]Department of Biological Sciences

## Introduction

**Pharmaceutical compounds are found in the aquatic environment**

- Not fully broken down in the human body or wastewater treatment plants (Cunningham et al., 2006)
  - 80 – 90% of dose passes through the human body (Walle et al., 1985; Walmeier et al., 1997)
  - 0 – 96% rate of elimination in wastewater treatment plants (Maurer et al., 2007; Bayer et al., 2014)
  - Thus, some of these enter worldwide aquatic environments (Kostich et al., 2014)
- Propranolol: found up to 0.26 µg/L in aquatic environments
  - Environmentally relevant levels affect locomotor behavior and reproductive success in mussels, sea urchins, and seabream (Capolupo et al., 2018)
- Valsartan: found up to 5.3 µg/L, 20-times that of propranolol but its effects on aquatic life are currently unknown

**Effects on American Horseshoe Crabs, *Limulus polyphemus*, are unknown**

- Commonly found keystone species in east coast estuaries,
- If affected it may impact many other estuarine organisms as well
- Carries economic significance in Atlantic ecotourism and fishing, and the biomedical industry

## Methods

**Animals & Environmental Conditions**

- Animals used in experiment were 72 second-instar *Limulus polyphemus*
- Crabs housed individually in twelve 6-well microplates
  - Each well filled with 12 mL of artificial salt water (21-25° C, 28-33 PSU)
- Kept in constant darkness

**Experimental Procedure**

- Environmentally relevant concentrations of propranolol and valsartan (Sigma-Aldrich, Saint Louis, MO) were administered at the beginning of the experiment:
  1. Propranolol: 2.6 ng/L, 26 ng/L, and 260 ng/L (@n=9)
  2. Valsartan: 53 ng/L, 530 ng/L, and 5300 ng/L (@n=9)
  - Two controls: artificial salt water (0 ng/L) and 1000 nL DMSO
  - 11 day exposure
- Activity was recorded continuously with infrared video cameras at 30 fps
  - Activity data were quantified with EthoVision XT 13 (Noldus; Wageningen, Netherlands)
  - Calculated linear velocity, angular velocity, and determined rhythm patterns

**Analyses**

- ANOVA were used to determine significance ($p < 0.05$)
- Lomb-Scargle Periodograms were used to determine statistically significant peaks of activity ($p < 0.001$)
- 17 crabs died before end of experiment
  - Data prior to death were not excluded from analyses
  - Propranolol – 1 death; DMSO & Valsartan – 16 deaths
  - Main causes were dehydration and potential distress from DMSO and Valsartan

## Propranolol and valsartan do not alter rhythmic behaviors

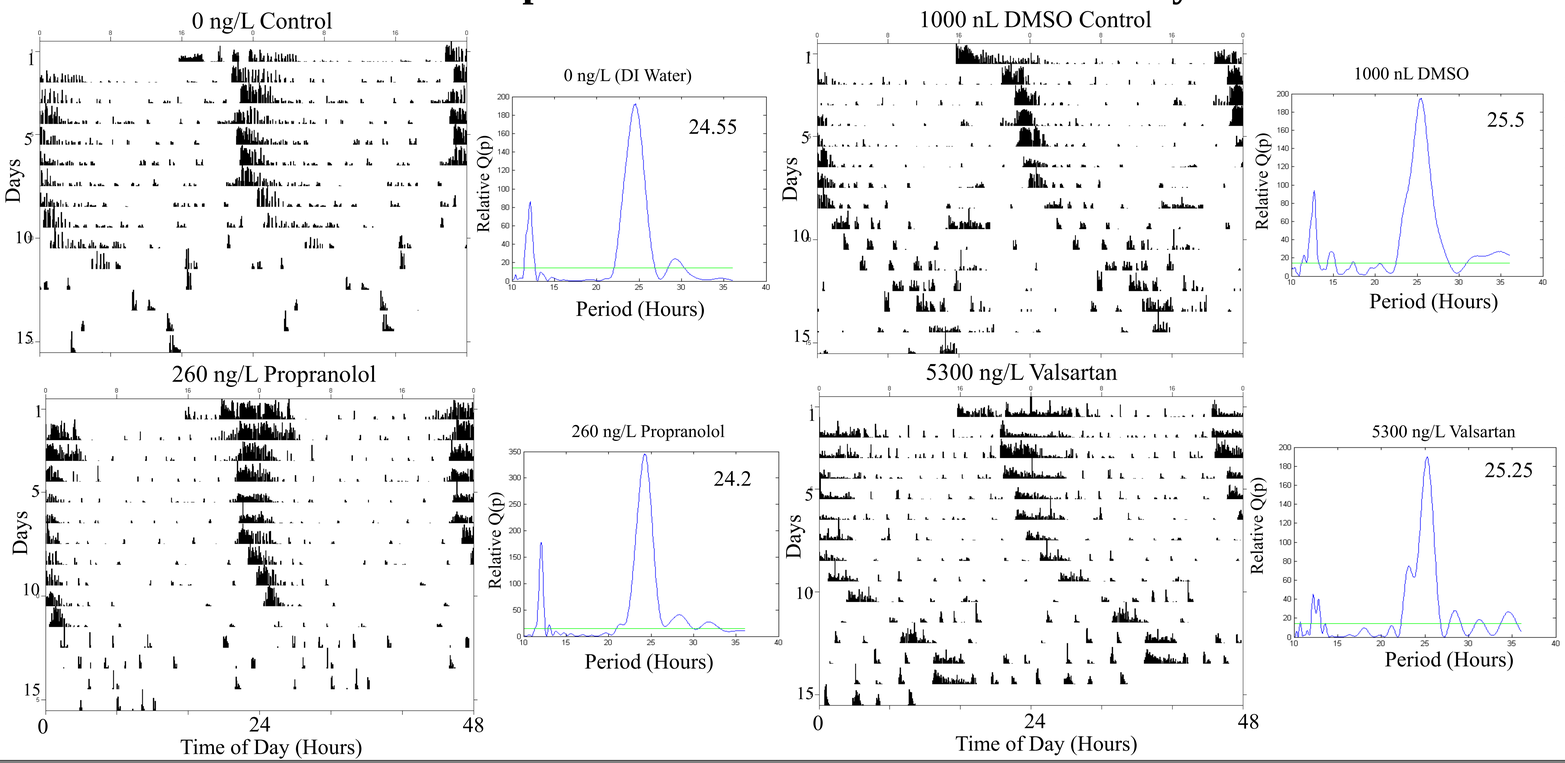


Figure 1. The effects of propranolol and valsartan on *Limulus* locomotor rhythm in total darkness. *Large panels:* representative double-plotted actograms propranolol and valsartan utilizing the highest dose. S*maller line graphs:* Lomb-Scargle periodograms. Peaks above the green horizontal line indicate statistical significance ($p < .001$). Values - highest peaks in the circalunidian range.

- Neither Propranolol ($F$ (3, 30) = 1.8, $p$ = 0.17) nor Valsartan ($F$ (4, 35) = 0.22, $p$ = 0.92) had significant effects on rhythmic behaviors

## No effects on linear velocity

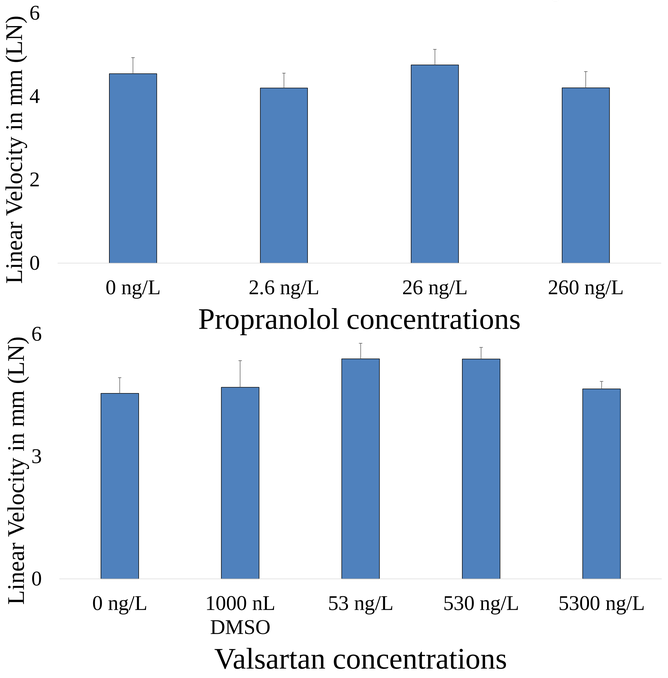


Figure 2. The effects of propranolol and valsartan on *Limulus* linear velocity. Data were transformed via natural log.

- Neither Propranolol ($F$ (3, 32) = 0.66, $p$ = 0.582) nor Valsartan ($F$ (4, 39) = 1.10, $p$ = 0.371) had significant effects on linear velocity

## No effects on angular velocity

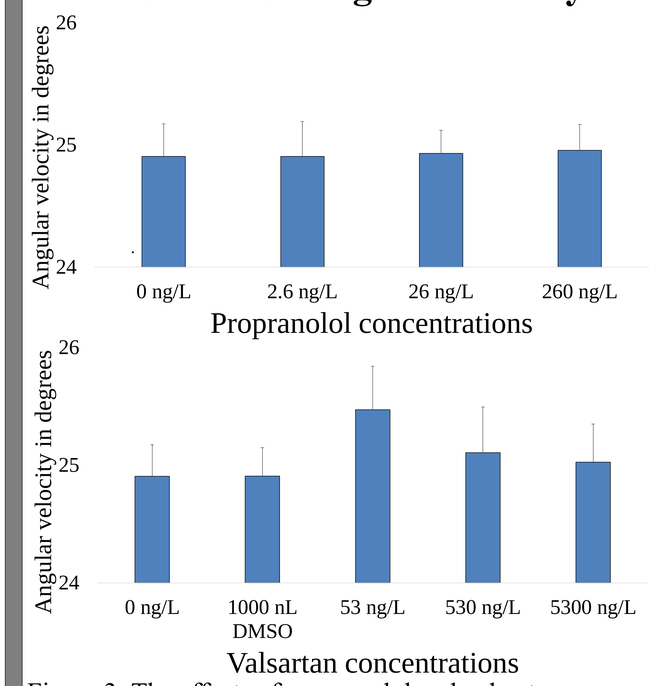


Figure 3: The effects of propranolol and valsartan on *Limulus* angular velocity. Data were transformed via the Johnson Transformation, but raw data are shown instead.

- Neither Propranolol ($F$ (3, 32) = 2.00, $p$ = 0.134) nor Valsartan ($F$ (4, 40) = 1.71, $p$ = 0.166) had significant effects on angular velocity

## Conclusions

**There were no statistically significance effects in all measured behaviors across all concentrations**

- This differs from mussels, seabream, sea urchins, and several species of fish that were affected by propranolol (Capolupo et al., 2018; Matus et al., 2018; Mitchell & Moon, 2015; Oliveira et al., 2018)
- Interestingly enough these crabs' ability to maintain circalunidian rhythms were not affected, further corroborating *Limulus'* persistent endogenous rhythm behaviors (Chabot & Watson, 2010)
- Further evidence that Limulus is a highly resilient species against environmental pollutants, such as cadmium and mercury (Itow, 1998; Botton, 2000 )
- Future directions for further research
  - Longer exposure—chronically from fertilization development to second in-star stage
  - Use a more sensitive species

## Acknowledgements

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