10-2023

Warmer Temperature Overrides the Effects of Antidepressants on Amphibian Metamorphosis and Behavior

Peter Fong
Gettysburg College

Aylin Doganoglu
Gettysburg College

Eleanor Sandt
Gettysburg College

Sierra Turbeville
Gettysburg College

Recommended Citation

Fong, Peter & Doganoglu, Aylin & Sandt, Eleanor & Turbeville, Sierra. (2023). Warmer temperature overrides the effects of antidepressants on amphibian metamorphosis and behavior. Environmental Science and Pollution Research. 10.1007/s11356-023-30607-4.

Warmer Temperature Overrides the Effects of Antidepressants on Amphibian Metamorphosis and Behavior

Abstract

Climate change can exacerbate the effects of environmental pollutants on aquatic organisms. Pollutants such as human antidepressants released from wastewater treatment plants have been shown to impact life-history traits of amphibians. We exposed tadpoles of the wood frog Lithobates sylvaticus to two temperatures (20°C and 25°C) and two antidepressants (fluoxetine and venlafaxine), and measured timing of metamorphosis, mass at metamorphosis, and two behaviors (startle response and percent motionless). Antidepressants significantly shortened time to metamorphosis at 20°C, but not at 25°C. At 25°C, tadpoles metamorphosed significantly faster than those at 20°C independent of antidepressant exposure. Venlafax- ine reduced body mass at 25°C, but not at 20°C. Temperature and antidepressant exposure affected the percent of tadpoles showing a startle response. Tadpoles at 20°C displayed significantly more responses than at 25°C. Exposure to fluoxetine also increased the percent of tadpoles showing a startle response. Venlafaxine reduced the percent of motionless tadpoles at 25°C but not at 20°C. While our results showed that antidepressants can affect the timing of metamorphosis in tadpoles, warmer temperatures overrode these effects and caused a reduction in an important reaction behavior (startle response). Future studies should address how warmer global temperatures may exacerbate or negate the effects of environmental pollutants.

Keywords

Ecotoxicology, Pharmaceuticals, Climate change, Aquatic, Development

Disciplines

Animal Sciences | Aquaculture and Fisheries | Biology | Developmental Biology

Warmer temperature overrides the effects of antidepressants on amphibian metamorphosis and behavior

Peter P. Fong[1]*, Aylin Doganoglu[1], Eleanor V. Sandt[1], and Sierra D. Turbeville[1]
[1]Department of Biology, Gettysburg College, Gettysburg, PA, 17325, USA

Abstract
Climate change can exacerbate the effects of environmental pollutants on aquatic organisms. Pollutants such as human antidepressants released from wastewater treatment plants have been shown to impact life-history traits of amphibians. We exposed tadpoles of the wood frog *Lithobates sylvaticus* to two temperatures (20°C and 25°C) and two antidepressants (fluoxetine and venlafaxine), and measured timing of metamorphosis, mass at metamorphosis, and two behaviors (startle response and percent motionless). Antidepressants significantly shortened time to metamorphosis at 20°C, but not at 25°C. At 25°C, tadpoles metamorphosed significantly faster than those at 20°C independent of antidepressant exposure. Venlafaxine reduced body mass at 25°C, but not at 20°C. Temperature and antidepressant exposure affected the percent of tadpoles showing a startle response. Tadpoles at 20°C displayed significantly more responses than at 25°C. Exposure to fluoxetine also increased the percent of tadpoles showing a startle response. Venlafaxine reduced the percent of motionless tadpoles at 25°C but not at 20°C. While our results showed that antidepressants can affect the timing of metamorphosis in tadpoles, warmer temperatures overrode these effects and caused a reduction in an important reaction behavior. Future studies should address how warmer global temperatures may exacerbate or negate the effects of environmental pollutants.

Compliance with Ethical Standards

The collection and use of all animals was approved by the Institutional Animal Care and Use Committee (IACUC) of Gettysburg College.

Keywords: ecotoxicology, climate change, amphibians, antidepressant, tadpole, metamorphosis, aquatic

Acknowledgements

This work was supported by the Cross-Disciplinary Science Institute (X-Sig) at Gettysburg College. Additional funding was provided by the John McCrea and Marion Ball Dickson Professorship and Science Fund, the Eric E. Kolbe'65 Student-Faculty Research Fund, and the Randall S. Alberte Fund for Student/Faculty Research in Biology.

*Corresponding author, pfong@gettysburg.edu; 717-337-6154 (phone); 717-337-6157 (fax)

Introduction

Global climate change has been reported to have serious negative effects on amphibian populations world wide (Zhao et al, 2022, Grant et al., 2020; Li et al., 2013, Rohr and Raffel, 2010, Corn, 2005). While amphibian populations have been in decline for decades, and the causes varied and complex, climate change has been reported to be a possible cause for their decline in combination with other factors such as physiological water loss (Lertzman-Lepofsky et al. 2020), thermohaline circulation (Velasco et al. 2020), and diseases such as chytridiomycosis (Cohen et al. 2019; Bosch et al. 2006). In combination with environmental stressors, warmer temperatures have been reported to shift the timing of egg laying such that tadpoles may be more vulnerable to road salt (Buss et al. 2021) and negatively affect survival of tadpoles exposed to the Chytrid *Batrachochytrium dendrobatidis* (Bosch et al., 2006).

Because of their sensitivity to environmental stressors, amphibian larval stages have been the focus of many toxicological studies. Tadpoles of different species have been subjected to a variety of environmental toxicants such as pesticides (Relyea, 2005), heavy metals (Lefcort et al. 1998), oil and gas operations (Robert et al., 2018) and see a reviews by Pinelli et al. (2019) and Andres Egea-Serrano et al. (2012).

Among emerging aquatic contaminants, human pharmaceuticals such as antidepressants are commonly detected in wastewater treatment plants and in their receiving streams (Metcalfe et al. 2010), and a large number of studies have shown that antidepressants disrupt reproduction, locomotion, feeding, and other physiological functions in aquatic vertebrates and invertebrates (Sehonova et al. 2018, Fong and Ford, 2014, for reviews). These antidepressants have been shown to have negative impacts on amphibians (Aliko et al. 2021; Blahova et al. 2021; Sehonova et al. 2019, Carfagno and Fong 2014, Conners et al. 2009). But, no study to date has tested the combined effects of antidepressants and temperature on amphibian development or behavior.

Wood frogs (*Lithobates sylvaticus*) have a broad geographic distribution in the U.S. from Alaska to Georgia (Wilbur, 1977), lay egg masses in temporary pools, and metamorphose before the pool dries up (Seale, 1982). Their tadpole larval stages have a known sensitivity to environmental toxicants such as road salt (Sanzo and Hecnar, 2006), pesticides (Robinson et al. 2017), agricultural stressors (Ruso et al. 2021), gold nanoparticles (Fong et al. 2016), and antidepressants (Carfagno and Fong 2014). Recently, Larsen et al (2021) reported that due to warmer temperatures and decreases in snow and frost cover in Alaska, the timing of wood frog calls has changed since the 1990's. Since calling is associated with breeding, the timing of reproduction and thus larval development could be modified. Given the continuing warming of both air and water from climate change, and the persistent threat of environmental contamination, we tested the combined effects of temperature (25 and 20^{o}C) and antidepressant (fluoxetine and venlafaxine) exposure to wood frog tadpoles, measuring two life-history traits: time to metamorphosis and mass at metamorphosis, and two behaviors: startle response and percent motionless.

Materials and methods

Collection and handling of tadpoles

Wood frog egg masses were collected from vernal pools in Michaux State Forest (39° 56' N, 77° 27'W), Adams County, PA, USA on March 26th, 2022. They were placed in buckets of pool water and dechlorinated tap water, and placed in the refrigerator at 4^{o}C for 4 days. Thereafter, they were taken out of the refrigerator and kept at room temperature until hatching on April 15th. Upon hatching, they were fed Xenopus brittle powder (Nasco) or algal discs (PlecoWafers, Tetra Corporation), then placed into individual cultures on April 27th at Gosner stage 24-28.

Wood frog tadpole culture experiments

We established 10 groups of tadpoles (5 drug concentrations x 2 temperatures). Sample sizes were n=20/group for wood frogs ~~and n=15/group for toads~~. Tadpoles were maintained individually in plastic dishes (4.5” x 3.0”, Joshsfrogs.com) with 300 ml of solution at room temperature (20^{o}C) or in incubators at 25^{o}C. The antidepressants, fluoxetine HCl (Sigma, CAS # 54910-89-3) and venlafaxine HCl (AK Scientific, CAS # 93413-69-5) were solubilized in dechlorinated tap water (pH 7.5, DO 7.1 ppm, conductivity 770 µS/cm) and serially diluted to achieve the desired concentrations. Solutions were changed 2-3 times per week and tadpoles were fed *Xenopus* brittle powder at each solution change. We monitored tadpoles daily for signs of metamorphosis. Upon metamorphosis we recorded the number of days tadpoles had spent in culture and their fresh mass. Dry mass was attained by anesthesia in MS-222, fixation in 70% ETOH, then oven drying overnight at 50^{o}C before weighing. Differences in time to metamorphosis and body mass between groups was analyzed by two-way ANOVA (temperature x drug).

Wood frog behavior

We used the methods of Fong et al. (2018) slightly modified from Fraker and Smith (2004) to measure two behaviors: startle response and motion. Wood frog tadpoles (Gosner stage 28-30) were placed into 3-liter plastic tanks (6 tadpoles per tank) containing 2000 ml of solution. They were separated into three groups (dechlorinated tap water control, fluoxetine 100 nM, and venlafaxine 100 nM) at two temperatures (20 and 25^{o}C) with n=8 tanks per group (48 total tanks). Tadpoles were fed *Xenopus* brittle and solutions changed two times per week. Behavior measurements began after 10 days exposure to these conditions. Over a three-day period, we counted the number of tadpoles in each tank that responded to a single tap on the top of each tank with a sharp jerk as a startle response and the number that were motionless (lying on the bottom or in the water column without tail movement). We measured these behaviors at three random times each day. Data on percent startle response and percent motionless in each tank were arcsine square-root transformed, then analyzed using 3-way ANOVA (temperature x drug x day) of mean percent startle response and mean percent motionless.

Results

Survivorship, and effect of temperature and antidepressants on metamorphosis and body mass

In general, survivorship was excellent with only three deaths out of 200 cultured tadpoles. There was a significant effect of temperature on the timing of metamorphosis of wood frog tadpoles. Tadpoles at 25^{o}C metamorphosed significantly sooner than those at 20^{o}C (mean=16.8 days for 25 degree tadpoles vs. 29.1 days for 20 degree tadpoles; one-way ANOVA of mean days in culture, $F(9, 184)=89.18$, $p << 0.00001$; Fig. 1). Each pair-wise comparison of similar groups at different temperatures (e.g. control-25 vs. control-20) showed a significant difference (Tukey's $p<<0.00001$for all comparisons, Fig. 1). Furthermore, at 20^{o}C, tadpoles exposed to fluoxetine (10 and 100 nM) and venlafaxine (10 nM) metamorphosed significantly sooner than the control, but at 25^{o}C, there was no effect of antidepressants on the timing of metamorphosis (Fig. 1). We also found a significant combined effect of temperature x drug (2-way ANOVA, $F(4,184)=4.17$, $p=0.0029$) with temperature being the most important factor ($p<0.05$).

Temperature also affected body mass. Since tadpoles at 25^{o}C metamorphosed sooner, they were also significantly lighter in weight than those at 20^{o}C (mean=206.89 grams for 25^{o}C tadpoles vs. 259.55 grams for 20^{o}C tadpoles; one-way ANOVA of mean fresh body mass, $F(9, 184)=8.53$, $p=1.22$ x 10-10; Fig. 2). For fresh mass, pair-wise comparisons of similar groups at different temperatures (Fig. 2, gray bars vs. open bars) showed significant differences between tadpoles exposed to fluoxetine (10 and100 nM) and to venlafaxine (10 nM), (Tukey's $p<0.0008$ for all three comparisons, Fig. 2). Furthermore, at 25^{o}C, tadpoles exposed to venlafaxine (10 nM) were significantly lighter than the control (Fig. 2). We also found a significant combined effect of temperature x drug (2-way ANOVA, $F(4,184)=3.34$, $p=0.01$) with temperature being the most important factor ($p<<0.05$). For dry mass, tadpoles at 25^{o}C were significantly lighter mean=18.48 grams for 25^{o}C tadpoles vs. 25.02 grams for 20^{o}C tadpoles; one-way ANOVA of mean dry body mass, $F(9, 184)=12.24$, $p=3.66$ x 10-15; Fig. 2). Pair-wise comparisons of similar groups at different temperatures, (Fig. 2, striped bars vs. stippled bars) showed significant differences between tadpoles exposed to fluoxetine (10 and100 nM) and to venlafaxine (10 nM), (Tukey's $p<0.001$ for all three comparisons, Fig. 2). We found a marginally significantly ($p=0.07$) difference in dry mass at 25^{o}C between the control and venlafaxine (10 nM).

Effect of temperature and antidepressants on startle response and percent motionless

Exposing wood frog tadpoles to two different temperatures (20^{o}C or 25^{o}C), two different antidepressants (fluoxetine or venlafaxine 10-7 M) on three consecutive days had a strong effect on startle response. The percent of tadpoles showing a startle response was affected by all three single factors, but especially temperature and antidepressants (Table 1). Tadpoles at the lower temperature (20^{o}C) showed significantly more startle responses than those at 25^{o}C independent of drug or day tested (Tukey's $p<0.05$ for all comparisons of 25 vs. 20^{o}C groups, Fig. 3). In addition, tadpoles at 20^{o}C in fluoxetine showed significantly more startle responses than the controls (Tukey's $p< 0.05$ Fig. 3). The strongest two-way effect was the combination of temperature x antidepressant (Table 1). By contrast to the startle response, temperature did not affect the percent of tadpoles that were motionless (Table 2), however exposure to the antidepressant venlafaxine at 25^{o}C, significantly reduced the percent of motionless tadpoles compared to the control at 25^{o} C (Tukey's $p <0.05$, Fig. 4).

Table 1. ANOVA table for mean percent of wood frog tadpoles showing a startle response when exposed to different temperatures (25 and 20^{o}C), day tested (1, 2, or 3), and antidepressant (fluoxetine 100 nM or venlafaxine 100 nM). Percentage data were arcsine-square root transformed.

Source	SS	df	MS	F	p-value
A (temp)	4.843436622	1	4.84343662	113.601949	**2.659E-19**
B (day)	0.327691496	2	0.16384575	3.84297302	**0.02398512**
C (antidep)	0.820121398	2	0.4100607	9.61790112	**0.00012963**
A x B	0.05572769	2	0.02786385	0.65354156	0.52195442
A x C	0.799776004	2	0.399888	9.37930232	**0.0001595**
B x C	0.52921595	4	0.13230399	3.10316661	**0.01786786**
A x B x C	0.062700183	4	0.01567505	0.36765543	0.83129808
Within	5.37202945	126	0.04263515		
Total	12.81069879	143	0.08958531		

Table 2. ANOVA table for mean percent of motionless wood frog tadpoles exposed to temperature (25 or 20^{o}C), day tested (1, 2, or 3), and antidepressant (fluoxetine 100 nM or venlafaxine 100 nM). Percentage data were arcsine-square root transformed.

Source	SS	df	MS	F	p-value
A (temp)	0.019344183	1	0.019344183	0.657642434	0.418922653
B (day)	0.194626866	2	0.097313433	3.308356002	**0.039779031**
C (antidep)	0.647969467	2	0.323984733	11.01447977	**3.90394E-05**
A x B	0.145705903	2	0.072852951	2.476775221	0.088095932
A x C	0.080189785	2	0.040094892	1.363102437	0.25961245
B x C	0.11780765	4	0.029451913	1.001274015	0.4095569
A x B x C	0.121319107	4	0.030329777	1.031118684	0.393909259
Within	3.706219202	126	0.029414438		
Total	5.033182161	143	0.035197078		

Discussion

Climate change has had important effects on the physiology and behavior of both aquatic and terrestrial organisms (Almeida et al. 2021; Domenici and Seebacher, 2020, Noyes et al. 2009). Warmer temperatures have also been shown to exacerbate the toxicity of a number of pollutants on a variety of aquatic organisms (Rodgers, 2021; Brown et al. 2015; Manciocco et al. 2014). Of the large number of human pharmaceutical pollutants detected in wastewater, antidepressants have been shown to have salient effects on aquatic animals (Lopes et al. 2020; Sehonova et al. 2018; Fong and Ford, 2014).

Our major finding was that at 20°C, exposure to the antidepressants fluoxetine and venlafaxine significantly accelerated metamorphosis. But at 25°C, the effect was greatly diminished. Thus, the antidepressants only caused a difference in the timing of metamorphosis at the lower temperature. Similar results were recently reported by Aulsebrook et al (2022) in the water flea *Daphnia magna*, where several life-history traits (e.g. fecundity, body size) were impacted by exposure to fluoxetine at 20°C, but not at 25°C. Previous studies in which amphibian tadpoles were exposed to antidepressants, but not to temperature, have found accelerated time to metamorphosis (Conners et al., 2009), delayed development (Aliko et al. 2021, Foster et al. 2010) and growth inhibition (Carfagno and Fong, 2014). Interestingly, a recent study by Leeb et al. (2022) found that tadpoles of the European frog (*Rana temporaria*) and the green toad (*Bufotes viridis*) were more sensitive to fungicides when exposed to coldest temperatures.

We also found that temperature affects larval life span and thus body size at metamorphosis. At 25°C, tadpoles metamorphosed from 11-15 days sooner than at 20°C, but at a smaller body size. Earlier metamorphosis could be an advantage for species like *L.*

sylvaticus which spend their larval life in temporary ponds and must metamorphose before the pond dries up. On the other hand, metamorphosis at smaller body size would make them more susceptible to predation. We also found that the lowest concentration of venlafaxine (10 nM) caused a significant reduction in body mass compared to the control.

Tadpole behavior was also significantly affected by both temperature and antidepressant exposure. Startle response was reduced in tadpoles at 25^{o}C compared to those at 20^{o}C under both drug-exposed and control conditions. In addition, fluoxetine increased the percent of tadpoles responding to the startle stimulus at 20^{o}C, but not at 25^{o}C. Temperature alone did not affect general tadpole motion, however. Tadpoles at 25^{o}C were also more active in venlafaxine than in controls. Thus, fluoxetine induced more startle responses and venlafaxine caused more general activity. Previous studies on *L. sylvaticus* showed that the antifouling chemical medetomidine increased startle response but reduced general tadpole motion at room temperature (Fong et al. 2018).

Warmer temperatures have been shown to reduce bioconcentration of the psychotropic pharmaceutical temazepam but increase the biotransformation of temazepam to oxazepam at warmer temp in the European perch, but no effect by temperature on the same variables in dragonfly larvae (Cerveny et al. 2021) thus the effect of climate change could have variable consequences between aquatic species.

Cerveny et al. (2021) reported that at higher temperature (20^o C), the psychotropic pharmaceutical temazepam had reduced bioaccumulation in the European perch than at 10^{o}C. But, the transformation of temazepam to its metabolite oxazepam was two times higher at 20^o C compared to 10^{o}C. By contrast, in dragonfly nymphs, these authors found no significant effect of temperature on either bioaccumulation or of bio transformation of temazepam. Thus, accumulation and transformation of pharmaceuticals may be not only dependent upon temperature, but upon taxonomic group.

Our finding that in amphibians, warm temperature overrides the effect of antidepressants in measured life-history traits is a similar result to that of Aulsebrook et al. (2022) in a taxonomically distant organism, *Daphnia magna,* but one which lives in a similar environment (fresh water lakes, streams, etc.), argues for further studies testing broader temperature ranges in different taxonomic groups where life-history traits like age and body size at metamorphosis, fecundity, age at first reproduction, and antipredator behaviors are measured.

Literature Cited

Almeida A., Esteves, V.I., Figueira, E. and Freitas, R. 2021. Impacts of climate change-abiotic factors on the effects caused by pharmaceutical residues to marine organisms. In: Pharamaceuticals in Marine and Coastal Environments (pp. 591-624), Impacts of climate change-abiotic factors on the effects caused by pharmaceutical residues to marine organisms https://doi.org/10.1016/B978-0-08-102971-8.00012-3

Aliko V, Korriku RS, Pagano M, Faggio C (2021). Double-edged sword: Fluoxetine and ibuprofen as development jeopardizers and apoptosis' inducers in common toad, *Bufo bufo*, tadpoles. Sci Total Environ 776. https://doi.org/10.1016/j.scitotenv.2021.145945

Aulsebrook, L.C., Wong, B.M, and Hall, M.D. 2022. Warmer temperatures limit the effects of antidepressant pollution on life-history traits. Proc. Biol. Sci. 289: 20212701. doi: 10.1098/rspb.2021.2701

Blahova J, Doubkova V, Plhalova L, Lakdawala P, Medkova D, Vecerek V, Svobodova Z, Faggio C (2021) Embryotoxicity of Selective Serotonin Reuptake Inhibitors—Comparative Sensitivity of Zebrafish (Danio rerio) and African Clawed Frog (Xenopus laevis) Embryos. Applied Sciences 11(21): 10015, https://doi.org/10.3390/app112110015

Brown, A.R., Owen, S.F., Peters, J. and Tyler, C.R. 2015. Climate change and pollution speed declines in zebrafish populations. PNAS 112(11): E1237-E1246. d**oi.org/10.1073/pnas.1416269112**

Bosch, J., Carrascal, L.M., Duran, L., Walker, S. and Fisher, M.C. 2006. Climate change and outbreaks of amphibian chytridiomycosis in a montane area of central Spain; is there a link? Proc. Biol. Sci. 274(1607): 253-260.

Buss, N., Swierk, L. & Hua, J. 2021. Amphibian breeding phenology influences offspring size and response to a common wetland contaminant. *Front Zool* 18, 31. https://doi.org/10.1186/s12983-021-00413-0

Campbell Grant, E.H., Miller, D.A.W., Muth, E. 2020. A synthesis of evidence of drivers of amphibian declines. Herpetologica 76(2): 101-107

Carfagno GLF, Fong PP (2014) Growth inhibition of tadpoles exposed to sertraline in the presence of conspecifics. Journal of Herpetology 48(4): 571-576.

Cerveny D, Fick J, Klaminder J, McCallum ES, Bertram MG, Castillo NA, Brodin T (2021). Water temperature affects the biotransformation and accumulation of a psychoactive pharmaceutical and its metabolite in aquatic organisms. Environment International 155: 106705, https://doi.org/10.1016/j.envint.2021.106705

Cohen, J.M., McMahon, T.A., Ramsay, C., Roznik, E.A., Sauer, E.L., Bessler, S., Civitello, D.J., Delius, B.K., Halstead, N., Knutie, S.A., Nguyen, K.H., Ortega, N., Sears, B., Venesky, M.D., Young, S. and Rohr, J.R. 2019. Impacts of thermal mismatches on chytrid fungus *Batrachochytrium dendrobatidis* prevalence are moderated by life stage, body size, elevation, and latitude. Ecology Letters 22(5): 817-825. https://doi.org/10.1111/ele.13239

Conners DE, Rogers ED, Armbrust KL, Kwon J-W, Black MC (2009) Growth and development of tadpoles (Xenopus laevis) exposed to selective serotonin reuptake inhibitors, fluoxetine and sertraline, throughout metamorphosis. Env. Tox. Chem. 28(12): 2671-2676.

Corn, P.S. 2005. Climate change and amphibians. Animal Biodiversity and Conservation 28(1): 59-67.

Domenici P, Seebacher F. (2020). The impacts of climate change on the biomechanics of animals *Conservation Physiology* 8(1), https://doi.org/10.1093/conphys/coz102

Egea-Serrano, A., Relyea, R.A., Tejedo, M., and Torralva, M. 2012. Understanding the impact of chemicals on amphibians: a meta-analytical review. Ecology and Evolution 2(7): 1832-1397.

Fong PP, Ford AT (2014) The biological effects of antidepressants on the molluscs and crustaceans: a review. Aquatic Toxicology 151: 4-13, doi: 10.1016/j.aquatox.2013.12.003.

Fong PP, Thompson LB, Carfagno GLF, Sitton AJ (2016) Long-term exposure to gold nanoparticles accelerates larval metamorphosis without affecting mass in wood frogs (*Lithobates sylvaticus*) at environmentally relevant concentrations. Env. Tox. Chem. 35(9): 2304-2310.

Fong PP, Lambert OJ, Hoagland ML, Kurtz ER (2018) Differential sensitivity to the antifouling chemical medetomidine between wood frog and American toad tadpoles with evidence for low-dose stimulation and high-dose inhibition of metamorphosis. Env. Sci. Pollut. Res. 25: 19470-19479.

Foster HR, Burton GA, Basu N (2010). Chronic exposure to fluoxetine (Prozac) causes developmental delays in *Rana pipiens* larvae. Environmental Toxicology and Chemistry 29:2845–2850

Fraker SL, Smith GR (2004) Direct and interactive effects of ecologically relevant concentrations of organic wastewater contaminants on *Rana pipiens* tadpoles. Environ Toxicol 19(3):250–256

Larsen, A.S., Schmidt, J.H., Stapleton, H., Kristenson, H., Betchkal, D., and McKenna, M.F. 2021. Monitoring the phenology of the wood frog breeding season using bioacoustics methods. Ecological Indicators 131, 108142, doi.org/10.1016/j.ecolind.2021.108142

Leeb C, Schuler L, Bruhl CA, Theissinger K (2022). Low temperatures lead to higher toxicity of the fungicide folpet to larval stages of *Rana temporaria* and *Bufotes viridis*. e0258631. doi: 10.1371/journal.pone.0258631.

Lefcort, H., Meguire, R.A., Wilson, L.H., Ettinger WF (1998) Heavy metals alter the survival, growth, metamorphosis, and antipredatory behavior of Columbia spotted frog (Rana luteiventris) tadpoles. Arch Env Contam Toxicol 35(3): 447-456.

Lertzman-Lepofsky, G.F., Kissel, A.M, Sinervo, B., amd Palen, W.J. 2020. Water loss and temperature interact to compound amphibian vulnerability to climate change. Global Change Biology 26(9): 4868-4879.

Li, Y., Cohen, J.M., and Rohr, J.R. 2013. Review and synthesis of the effects of climate change on amphibians. Integrative Zoology 8: 145-161. Doi: 10.1111/1749-4877.12001

Lopes, D.G., Duarte, I.A., Antunes, M., Fonseca, V.F. 2020. Effects of antidepressants in the reproduction of aquatic organisms: a meta-analysis. Aquatic Toxicology 227: 105569. DOI: 10.1016/j.aquatox.2020.105569

Manciocco, A., Calamandrei, G. and Alleva, E. 2014. Global warming and environmental contaminants in aquatic organisms: The need of the etho-toxicology approach. Chemosphere 100: 1-7, DOI: 10.1016/j.chemosphere.2013.12.072

Metcalfe CD, Chu, S, Judt C, Li H, Oakes KD, Servos MR, Andrews DM (2010) Antidepressants and their metabolites in municipal wastewater, and downstream exposure in an urban watershed. Env. Tox. Chem. 29(1): 79-89.

Noyes PD, McElwee MK, Miller HD, Clark BW, Van Tiem LA, Walcott KC, Erwin KN, and Levin ED (2009). The toxicology of climate change: environmental contaminants in a warming world. Env. Int. 35(6): 971-986.

Pinelli, C., Santillo, A., Baccari, G.C., Falvo, S, and Di Fiore, M.M. 2019. Effects of chemical pollutants on reproductive and developmental processes in Italian amphibians. Mol. Rep. Dev. 86(10): 1324-1332. doi.org/10.1016/j.ecolind.2021.108142

Relyea, R.A. 2005. The lethal impacts of Roundup and predatory stress on six species of North American tadpoles. Arch. Env. Contam. Tox. 48: 351-357.

Robert R, McGuire CC, Kim F, Nagel SC, Price SJ, Lawrence BP, De Jesus Andino F (2018) Water contaminants associated with unconventional oil and gas extraction cause immunotoxicity to amphibian tadpoles. Tox. Sci. 166(1): 39-50. **doi.org/10.1093/toxsci/kfy179**

Rodgers EM (2021) Adding climate change to the mix: responses of aquatic ectotherms to the combined effects of eutrophication and warming. Biology Letters 17(10): 20210442, doi: 10.1098/rsbl.2021.0442

Rohr, J.R. and T.R. Raffel. 2010. Linking global climate change and temperature variability to widespread amphibian declines putatively caused by disease. PNAS 107(18): 8269-8274, doi.org/10.1073/pnas.0912883107

Robinson SA, Richardson SD, Dalton RL, Maisonneuve F, Trudeau VL, Pauli BD, Lee-Jenkins SSY (2017) Sublethal effects on wood frogs chronically exposed to environmentally relevant concentrations of two neonicotinoid insecticides. Env. Tox. Chem. 36(4): 1101-1109. doi: 10.1002/etc.3739

Ruso GE, Hogan NS, Sheedy C, Gallant MJ, Jardine TD (2021) Effects of Agricultural Stressors on Growth and an Immune Status Indicator in Wood Frog (*Lithobates sylvaticus*) Tadpoles and Metamorphs. Env. Tox. Chem. 40(8): 2269-2281. doi: 10.1002/etc.5107

Sanzo D, Hecnar SJ (2006) Effects of road de-icing salt (NaCl) on larval wood frogs (*Rana sylvatica)*. Environmental Pollution. 140 (2): 247–256. doi:10.1016/j.envpol.2005.07.013.

Seale DB (1982). Physical factors influencing oviposition by the woodfrog, *Rana sylvatica*, in Pennsylvania. Copeia 1982(3): 627–635. doi*:*10.2307/1444663

Sehonova P, Svobodova Z, Dolezelova P, Vosmerova P, Faggio C. 2018. Effects of waterborne antidepressants on non-target animals living in the aquatic environment: a review. Sci Tot Env, 631-632: 789-794.

Sehonova P, Hodkovicova N, Urbanova M, Orn S, Blahova J, Svobodova Z, Faldyna M, Chloupek P, Briedikova K, Carlsson F (2019) Effects of antidepressants with different modes of action on early life stages of fish and amphibians. Environ Pollut 254(A): 112999, doi: 10.1016/j.envpol.2019.112999.

Velasco, J., Estrada, F., Calderon-Bustamante, O., Swingedouw, D., Ureta, C., Gay, C., and Defrance, D. 2021. Synergistic impacts of global warming and thermohaline circulation collapse on amphibians. Nature Communications Biology 4, 141 (2021). https://doi.org/10.1038/s42003-021-01665-6

Wilbur HM (1977). Interactions of food level and population density in Rana sylvatica. Ecology. 58(1): 206–209. doi.org/10.2307/1935124

Zhao, C., Jiang, J., Xie, F., Li, C., and Zhao, T. 2022. Assessement of amphibians vulnerability to climate change in China. Frontiers Ecol. Evol. 10: doi.org/10.3389/fevo.2022.826910

Figure Captions

Figure 1. Days to metamorphosis (mean ± S.E.) for wood frog tadpoles exposed to fluoxetine and venlafaxine at 20^{o} C (gray bars) and 25^{o} C (open bars). *: $p<0.05$, **: $p<0.01$, Tukeys post-hoc test after one-way ANOVA between groups at 20^{o} C. Differing letters above bars indicate significant differences between 20^{o} and 25^{o} C temperatures. Sample sizes are n=19-20 per group.

Figure 2. Mean fresh and dry mass (± S.E.) of wood frog tadpoles exposed to fluoxetine and venlafaxine at 20^{o} C and 25^{o} C. *: $p<0.05$, Tukeys post-hoc test after one-way ANOVA between groups. Differing letters above bars indicate significant differences between 20^{o} and 25^{o} C temperatures for both fresh and dry mass. Bars without letters above are not significantly different.

Figure 3. Proportion (mean ± S.E.) of wood frog tadpoles showing a startle response in two temperatures and two antidepressants (100 nM each) over a 3-day period (e.g. Control-20 = control group at 20^{o} C). Solid brackets indicate significant differences between groups (e.g. 20^{o} C vs. 25^{o} C). Dotted brace indicates a significant difference between fluoxetine (100 nM) and control at 20 degrees. *: Tukeys $p< 0.05$. n=8 tanks per group.

Figure 4. Proportion (mean ± S.E.) of motionless wood frog tadpoles in two temperatures and two antidepressants (100 nM each) over a 3-day period. (e.g. Control-20 = control group at 20^{o} C). Brackets indicate a significant difference between and the control at 25^{o} C and venlafaxine at 25^{o} C.
*: Tukeys $p< 0.05$. n=8 tanks per group.

Statements and Declarations

Funding

This work was supported by the Cross-Disciplinary Science Institute (X-Sig) at Gettysburg College. Additional funding was provided by the John McCrea and Marion Ball Dickson Professorship and Science Fund, the Eric E. Kolbe'65 Student-Faculty Research Fund, and the Randall S. Alberte Fund for Student/Faculty Research in Biology. The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

Competing Interests

Financial interests: The authors have no relevant financial or non-financial interests to disclose.

Author Contributions

The corresponding author contributed to the study conception and design. Data collection and analysis were performed by Peter Fong, Aylin Doganoglu, Eleanor Sandt and Sierra Turbeville. The first draft of the manuscript was written by Peter Fong and all authors commented on previous versions of the manuscript.

Additional declarations

1. Ethical approval. We have received approval from the Gettysburg College IACUC (Institution Animal Care and Use Committee) to do work with amphibian tadpoles.
2. Consent to participate. The study was done with animals not humans, so there is no consent to participate statement.
3. Consent to publish. The study was not done with humans so there is no consent to publish statement.
4. Availability of data. The data are available on Figshare.com: 10.6084/m9.figshare.22232647

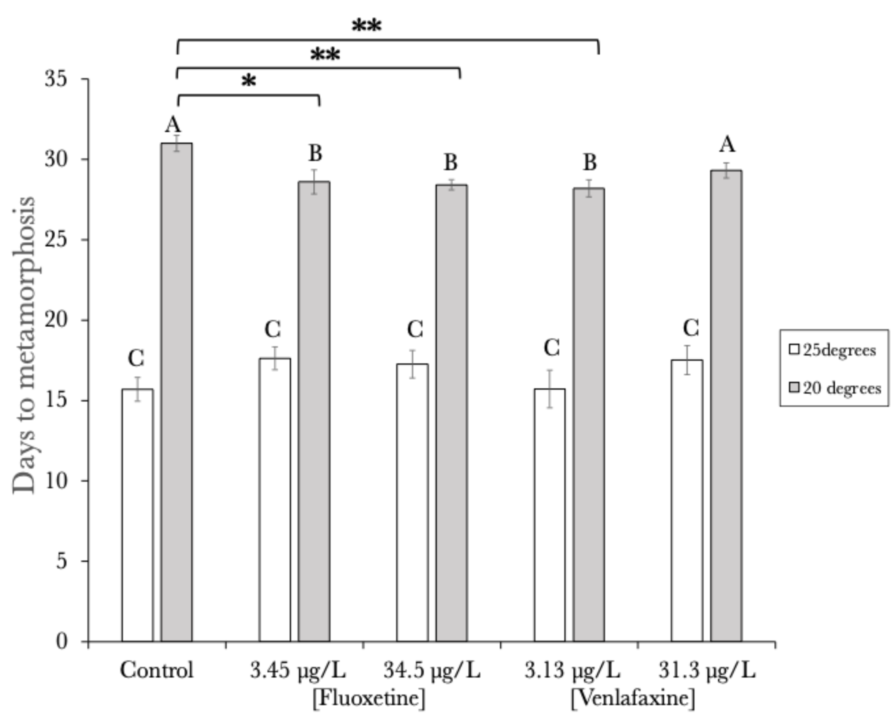
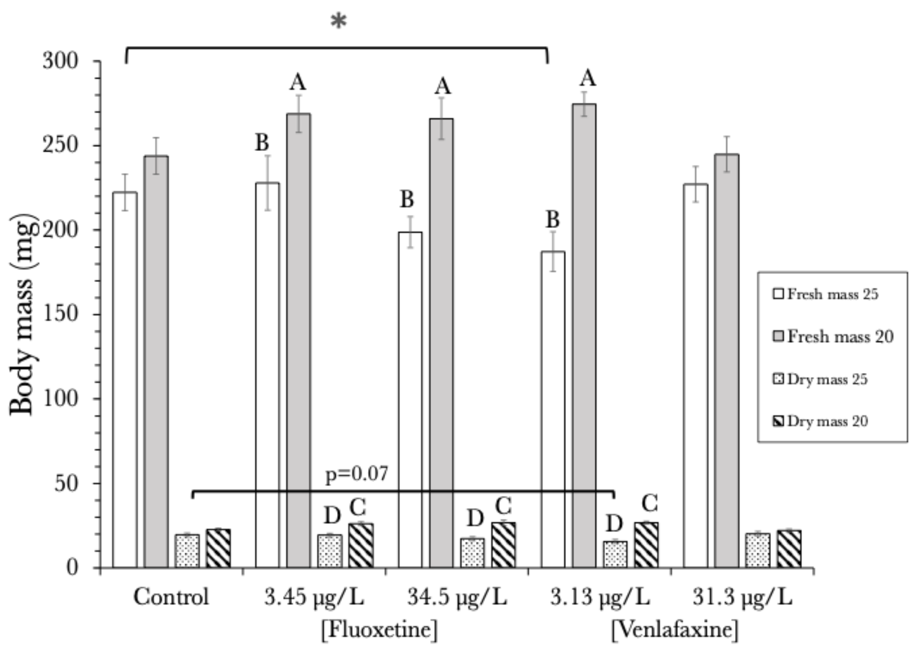
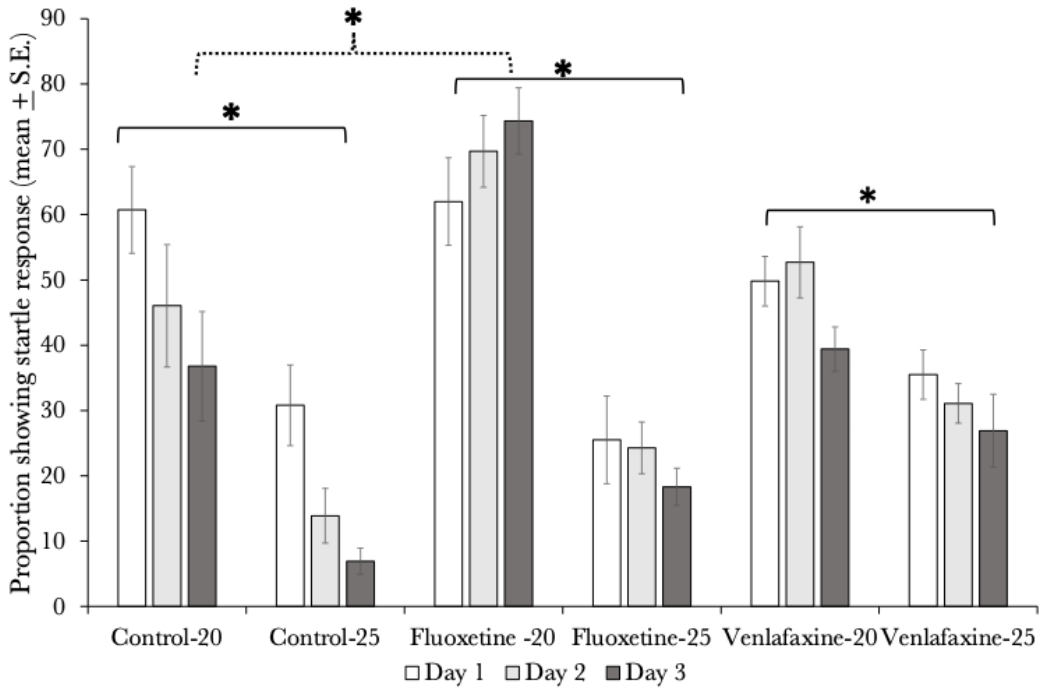


Fig. 1



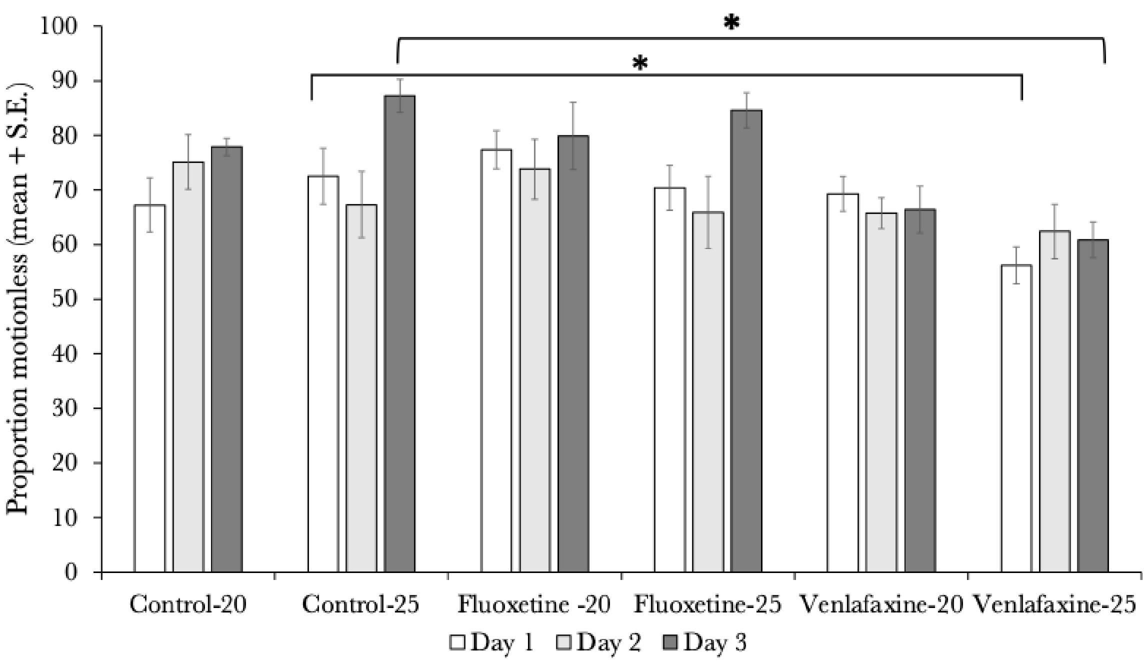
*
300
250
200
150
100
50
0
Body mass (mg)
A
B
p=0.07
D
C
Fresh mass 25
Fresh mass 20
Dry mass 25
Dry mass 20
Control
3.45 µg/L
34.5 µg/L
3.13 µg/L
31.3 µg/L
[Fluoxetine]
[Venlafaxine]

Fig. 2



Proportion showing startle response (mean + S.E.)
Control-20
Control-25
Fluoxetine -20
Fluoxetine-25
Venlafaxine-20
Venlafaxine-25
Day 1
Day 2
Day 3

Fig. 3



Proportion motionless (mean + S.E.)
Control-20
Control-25
Fluoxetine -20
Fluoxetine-25
Venlafaxine-20
Venlafaxine-25
Day 1
Day 2
Day 3

Fig. 4