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VARIATIONS IN LETHAL AND SUBLETHAL EFFECTS OF CYPERMETHRIN AMONG AQUATIC STAGES AND SPECIES OF ANURAN AMPHIBIANS

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Abstract: Despite the use of model species to predict the effects of chemicals in the environment, unpredicted variation in levels of risk to organisms from xenobiotics can be observed. Physiological and morphological differences between species and life stages may lead to differences in sensitivity, while seasonal and spatial variation in pesticide concentrations may affect the level of risk faced by organisms in the environment. Because anurans breed in aquatic habitats subject to contamination by runoff and spraying, they are particularly vulnerable to pesticides. In the present study, embryos, newly hatched larvae, and larvae with limb buds of 3 anuran amphibian species—*Pseudacris regilla*, *Rana cascadae*, and *Rana aurora*—were exposed for 48 h to either 0.5 μg/L or 5.0 μg/L cypermethrin under laboratory conditions. The authors monitored hatching success, larval survival, and measured growth. Additionally, they assayed avoidance behavior 2 wk after exposure or 2 wk after hatching for individuals exposed as embryos. Hatching and survival were not affected in animals of any species exposed as embryos. After exposure as embryos and as newly hatched larvae, however, *P. regilla* displayed behavioral abnormalities in response to prodding. Cypermethrin increased mortality in *P. regilla* exposed in both larval stages. Cypermethrin also increased mortality in larval *R. cascadae* when exposed at the early stage. These results indicate variation in sensitivity to environmentally relevant concentrations of cypermethrin among anuran species and life stages. *Environ Toxicol Chem* 2013;32:2855–2860. © 2013 SETAC

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INTRODUCTION

Due to the 5 billion–plus pounds of active ingredient used globally each year in agricultural, residential, commercial, industrial, and forest settings [1], pesticides have become ubiquitous in the environment. Much effort has been made to understand the human health impacts and ecological effects of environmental pollutants and pesticides in particular. Yet a great degree of variation in effects of contaminants not predicted by dose–response tests with model species can be observed in natural systems [2]. This may be due in part to the use of models that neglect certain ecologically relevant characteristics of different species [3,4]. For example, sensitivity to chemicals may differ with life-history strategies or across developmental stages [5–7]. Additionally, chemicals are not homogenous in the environment; they vary over temporal and spatial scales [8]. Thus, we should expect to see variation in risk to animals exposed to environmental contaminants when spatial and temporal considerations are included. Additional work is needed to understand differences in responses to contaminants across species and stages.

Among pesticides, environmental contamination by pyrethroid insecticides is of growing concern. The restriction of the organophosphate insecticides chlorpyrifos and diazinon for residential pest control has turned users to synthetic pyrethroids as a class over the past 2 decades [9–11]. Pyrethroids are favored over the insecticides that they are replacing for their low persistence in the environment and relatively low toxicity to mammals [12,13]. Pyrethroids are present in over 3500 registered products in the United States alone [14]. Approximately 1 million pounds of active ingredient of the cyano-pyrethroid insecticide cypermethrin are used annually in US agricultural and nonagricultural settings [15], and it is used extensively in other countries as well [16–18]. Agricultural uses include the treatment of insect pests of cotton, pecans, sweet corn, lettuce, and broccoli as well as pests of cattle and other livestock [15]. A wide range of nonagricultural uses—including control of ants, cockroaches, fleas, and termites in indoor and outdoor structural and perimeter applications—make up the majority (750 000 pounds annually) of cypermethrin use [15]. Although 75% of cypermethrin use is nonagricultural, due to the difficulties posed in modeling these uses, risk assessments by the US Environmental Protection Agency (USEPA) have only included uses on agricultural crops [15]. These assessments, therefore, do not include the potential effects of runoff from impervious surfaces after application in urban and industrial settings.

Pyrethroids are neurotoxins that disrupt the sodium channels of nerve cells, leading to repetitive firing of neurons [19]. Despite their relative insolubility and low persistence in water, all pyrethroids are sufficiently soluble to cause adverse effects to aquatic organisms; and their lipophilicity allows pyrethroids to be readily absorbed by biological membranes and tissues, leading to high toxicity in nontarget organisms [20]. For example, cypermethrin is considered by the USEPA to be very highly toxic on an acute basis to marine and freshwater invertebrates and fishes and to honeybees [15]. Moreover, evaluations in California after runoff events indicate that pyrethroids, including cypermethrin, are found at concentrations acutely toxic to invertebrates (test species *Hyalella azteca*) in urban streams [21]. Despite its relatively low water solubility (4–10 μg/L), cypermethrin has been detected at levels ranging from 100 μg/L to 1010 μg/L in surface water [22–24] and at lower levels (0.02–2.6 μg/L) in subsurface waters [16,25].

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Understanding the toxicological effects of pesticides in animals is particularly urgent as conservation biologists are documenting population declines in birds, reptiles, amphibians, and other taxa [26,27]. As aquatic breeding organisms, amphibian eggs and larvae are particularly vulnerable to chemicals in their environments [28]. Moreover, they are among the most threatened taxa in the current biodiversity loss, with approximately 1910 of 6312 amphibian species in danger of extinction [29–31], and some of these declines have been associated with pesticide use [32,33]. Environmentally relevant concentrations of pesticides cause adverse effects in amphibians, including altered growth and development, anatomical deformities, behavioral abnormalities, and mortality (reviewed in Mann et al. [34]). However, differences in morphology and life history among species may make amphibians differently sensitive to pollutants. For example, breeding phenology and rate of development can influence the chance of exposure to a pesticide and exposure period.

In most taxa, the earliest life stage is considered the most sensitive; though in organisms with protective eggs, like amphibians, the earliest free-living (larval) stage is often more sensitive to environmental stressors [12]. Traditionally, the egg stage of aquatic animals has been considered robust, because the jelly protects them from a broad range of external disturbances [35]. However, the extent to which the jelly coat surrounding amphibian eggs protects the developing embryo from a chemical is strongly dependent on both the chemical and the species examined [36]. Embryos are likely exposed to environmental pollutants as the jelly is filled with water shortly after being laid [35]. Moreover, uptake of waterborne contaminants has been observed in anuran eggs [36].

In a comparative study using 3 species of anuran amphibian (*Pseudacris regilla*, the Pacific treefrog; *Rana cascadae*, the Cascades frog; and *Rana aurora*, the northern red-legged frog), we tested the effects of cypermethrin exposure on embryos and larvae. We first assessed the effects of environmentally relevant concentrations of cypermethrin on individuals exposed as embryos and then tested its effects on larval stages. We monitored hatching success, larval survival, and sublethal effects including growth and abnormal avoidance behaviors. We chose to assay avoidance behavior because lacking the appropriate avoidance response may reduce antipredator and foraging success. Additionally, we measured growth because slowed growth may impair an individual's ability to metamorphose or could result in smaller size at metamorphosis. We made several predictions for this experiment. First, we predicted that species would differ in sensitivity to cypermethrin. We anticipated that *P. regilla* would be least sensitive, because it is a generalist species that has persisted in urban and agricultural landscapes, compared with *R. aurora* and *R. cascadae*, species with smaller ranges that have experienced population declines. Second, we predicted that sensitivity would vary depending on the timing of exposure, with newly hatched larvae exhibiting the greatest degree of sensitivity and embryos and larvae with limb buds exhibiting less sensitivity. Finally, we predicted that sublethal effects of environmentally relevant concentrations of cypermethrin would impact ecologically relevant characteristics like behavior and size.

MATERIALS AND METHODS

Test species

We conducted experiments using 3 anuran species (*P. regilla*, *R. cascadae*, *R. aurora*) from egg masses collected within 48 h after they were laid in ponds in the Willamette Valley, Coast Range, and Cascade Mountains of Oregon, USA, respectively, during the spring and summer of 2009 and 2010. In Oregon, *R. cascadae* breed explosively in montane areas in March through July, metamorphosing within 1 mo to 3 mo [37]. *Rana aurora* breed in December through February along the coast and up to some western midelevation sites, with metamorphosis usually occurring in 6 mo to 8 mo [37]. *Pseudacris regilla* are widespread throughout the western United States and Canada, breeding primarily in January and February (though later for higher-elevation populations); and larvae typically metamorphose within 2 mo to 3 mo [37].

Embryos were brought into the laboratory for rearing in 38-L tanks of aerated, dechlorinated water. After hatching, larvae were fed a 3:1 mixture (by weight) of rabbit chow and fish flakes until 48 h prior to initiation of the experiment. Animals were maintained in a controlled laboratory environment at 14 °C, the average ambient temperature for test species, on a natural light–dark photoperiod. To examine variation in sensitivity to cypermethrin, animals were tested at 3 stages [38]: embryo (stages 10–12), larvae <1 wk after hatching (stages 24–25, hereafter "early larvae"), and larvae with limb buds (stages 28–30, hereafter "late larvae"). At the conclusion of the experiment, animals were anesthetized with buffered MS-222 and preserved in 95% ethyl alcohol. Due to differences in breeding phenology, species were not tested at the same time. However, all species were tested using the same methods in the same laboratory.

Cypermethrin exposure

Immediately before each experiment, fresh 100-mL stock solutions of 100 ppm cypermethrin (nominal concentration) were prepared by dissolving cypermethrin (99.5% standards-grade; ChemService) into a carrier solution of 10 mL high-performance liquid chromatography–grade acetone and 90 mL deionized water. Though analytical chemistry was not performed on the stock solutions or exposure solutions used in the present study, cypermethrin stock solutions made with the same method in the same laboratory from an earlier experiment were analyzed at the Mississippi State Chemical Laboratory using gas chromatography/electron capture detection. Actual concentrations of those stock solutions were 53.0%, 62.1%, and 67.2% of nominal concentrations. Serial dilutions (10 ppm, 1.0 ppm, and 0.10 ppm) were made from the stock solution, and nominal test concentrations of 5.0 μg/L and 0.5 μg/L cypermethrin (hereafter "high" and "low," respectively) were made by adding 5 mL of the appropriate dilution to test beakers containing 1000 mL dechlorinated water. Acetone in the cypermethrin treatments did not exceed a concentration of 0.05 mL/L, well below the limit of 0.1 mL/L recommended by the International Organization for Standardization [39] for tests involving invertebrates. This level of acetone had no effect on amphibian embryos and larvae in our pilot experiments and consequently was not added to the controls. Exposure to cypermethrin occurred for 48 h; treatments were randomly assigned to experimental units, and each treatment was replicated 5 times.

Embryo exposure

Embryos were exposed to cypermethrin for 48 h in groups of 10 in 1-L glass beakers containing 1 L of test solution. After exposure, embryos were transferred to 1-L glass beakers containing fresh dechlorinated water. They were maintained in these containers until hatching. As individuals hatched, they were transferred to 600-mL glass beakers containing 500 mL fresh dechlorinated water, where they were maintained individually as larvae for 2 wk after hatching. These individuals

were not fed as they maintain their yolk as a food source for some time after hatching [40].

Both hatching success and timing of hatching were determined. After larvae hatched, we monitored mortality of larvae daily for 14 d. Avoidance behavior was assessed on the final day of the experiment by prodding each larva gently on the side of the base of its tail. Behavioral abnormalities indicative of cyano-pyrethroid poisoning were recorded as present when we witnessed inactivity, twisting, trembling, or weak movement over a short distance (<2 cm) in response to prodding [12], while darting away (>2 cm) was considered a normal response.

Larval exposure

Early larvae and late larvae were exposed to cypermethrin in 1-L glass beakers containing 1 L of test solution for 48 h. Early larvae were exposed in groups of 10. As the result of a limited number of animals, late larvae were exposed in groups of 5. After exposure, each larva was transferred individually to a 600-mL glass beakers containing 500 mL fresh dechlorinated water. Animals were maintained individually for 2 wk after exposure and fed a 3:1 mixture (by weight) of rabbit chow and fish flakes.

We monitored mortality of larvae daily during exposure and for 14 d after exposure. Avoidance behavior was assessed on the final day of the experiment using the same methods as in the embryo exposure. At the conclusion of the experiment, body length and mass were measured.

Statistical analyses

We performed statistical analyses in the R statistical computing environment (Ver 2.15.0; R Foundation for Statistical Computing). Statistical tests were performed within species and within developmental stage. We analyzed both survival and behavior with generalized liner mixed models using a logit link function to determine the effects of cypermethrin treatments. Individuals were nested by exposure group (beaker) for all analyses to avoid pseudoreplication. To test for differences in growth, we performed multivariate analysis of variance to allow quantitative partitioning of effects among experimental factors and their interactions.

RESULTS

Embryo exposure

There was no effect of cypermethrin exposure on survival of animals exposed as embryos for any species (Figure 1A, D, and G). All *R. cascadae* hatched and survived for the duration of the experiment (Figure 1A, D, and G), although some mortality (<10%) was seen in the 2 other species. Additionally, there was no effect of cypermethrin on hatching success for any of the species ($p > 0.05$ for all species). Embryos hatched into larvae 9 d to 13 d (*P. regilla*), 12 d to 16 d (*R. aurora*), and 2 d to 7 d (*R. cascadae*) after exposure began. None of the embryos hatched prior to completion of the 48-h exposure, and time to hatching was not affected by exposure to cypermethrin for any of the species.

Exposure to the high treatment of cypermethrin in *P. regilla* embryos led to a 19% increase in behavioral abnormalities in response to prodding when compared with controls ($\chi^2 = 6.57$, $df = 2$, $p = 0.037$). Abnormalities included inactivity, twisting, trembling, or weak movement over a short distance (<2 cm) all in response to prodding and were consistent with cyano-pyrethroid poisoning. Mass and body length were not affected by either cypermethrin treatment in any species ($p > 0.05$, Table 1).

Larval exposure

There was greater mortality of animals in cypermethrin treatments than in controls (Figure 1B, C, and E), but the effects of cypermethrin differed among species and among stage of exposure. *Pseudacris regilla* were the most sensitive to cypermethrin, while *R. aurora* were the least sensitive. The high cypermethrin exposure increased mortality of *P. regilla* at the early and late larval stages (Figure 1B and C). In *R. cascadae*, exposure to the high level of cypermethrin increased mortality due to early larval stage exposure but not exposure in the late larval stage (Figure 1E). Effects of cypermethrin exposure on survival in *R. aurora* were not statistically significant. However, there was a trend toward decreased survival with cypermethrin exposure in the early larval stage (Figure 1H; $\chi^2 = 5.61$, $df = 2$, $p = 0.06$) and no effect of cypermethrin exposure on survival in the later larval stage.

Cypermethrin exposure in the low treatment led to a 7% increase in abnormal behavioral responses to prodding in *P. regilla* exposed as early larvae compared with controls ($\chi^2 = 7.19$, $df = 2$, $p = 0.027$) but did not affect behavior in the other species when exposed as larvae ($p > 0.05$). All individuals of all 3 species that were exposed as late larvae exhibited normal responses to prodding. Mass and body length were not affect by either the high or the low treatment in any species ($p > 0.05$; Table 1).

DISCUSSION

The present study demonstrated that the amphibian species assayed differ in their sensitivity to cypermethrin, that the degree of sensitivity varies with the stage in which exposure occurred, and that cypermethrin exposure of 5.0 μg/L can lead to sublethal effects on ecologically important characteristics of these species. The effects of cypermethrin exposure, particularly at the 5.0-μg/L level, were detected in each of the 3 species tested and at each of the 3 developmental stages tested. However, these effects varied by species and life stage. Sublethal effects of cypermethrin exposure were observed for *P. regilla* exposed as embryos, whereas individuals of the other species appear to have been unaffected by their exposure as embryos. *Pseudacris regilla* exhibited the greatest sensitivity to cypermethrin compared with the other species, with effects present after all 3 exposure time points (behavioral abnormalities for embryo exposure and mortality for both larval stages). *Rana cascadae* and *R. aurora* demonstrated increased mortality at only the early larval stage, and no sublethal effects were observed in these species at any stage.

Differences in sensitivity to cypermethrin varied strongly with stage. We observed cypermethrin-induced mortality in the early and later larval stages but not in the embryonic stage. This increased sensitivity in the larval stages over the embryonic stages may be due to cypermethrin's action as a neurotoxin; the more developed nervous system of larval individuals may have increased their vulnerability to its effects [12].

Although mortality was not observed in individuals exposed as eggs, sublethal effects were observed due to this exposure that were not present in older anurans. *Pseudacris regilla* exposed as embryos displayed behavioral abnormalities (such as inactivity or twisting, trembling, or weak movement over a short distance in response to prodding) after hatching. The differences in tolerance to exposure in the embryonic stage may reflect the protective effects of the jelly coat surrounding anurans that others have demonstrated [41,42]. This coat, composed of glycoproteins, mucoproteins, carbohydrates, and

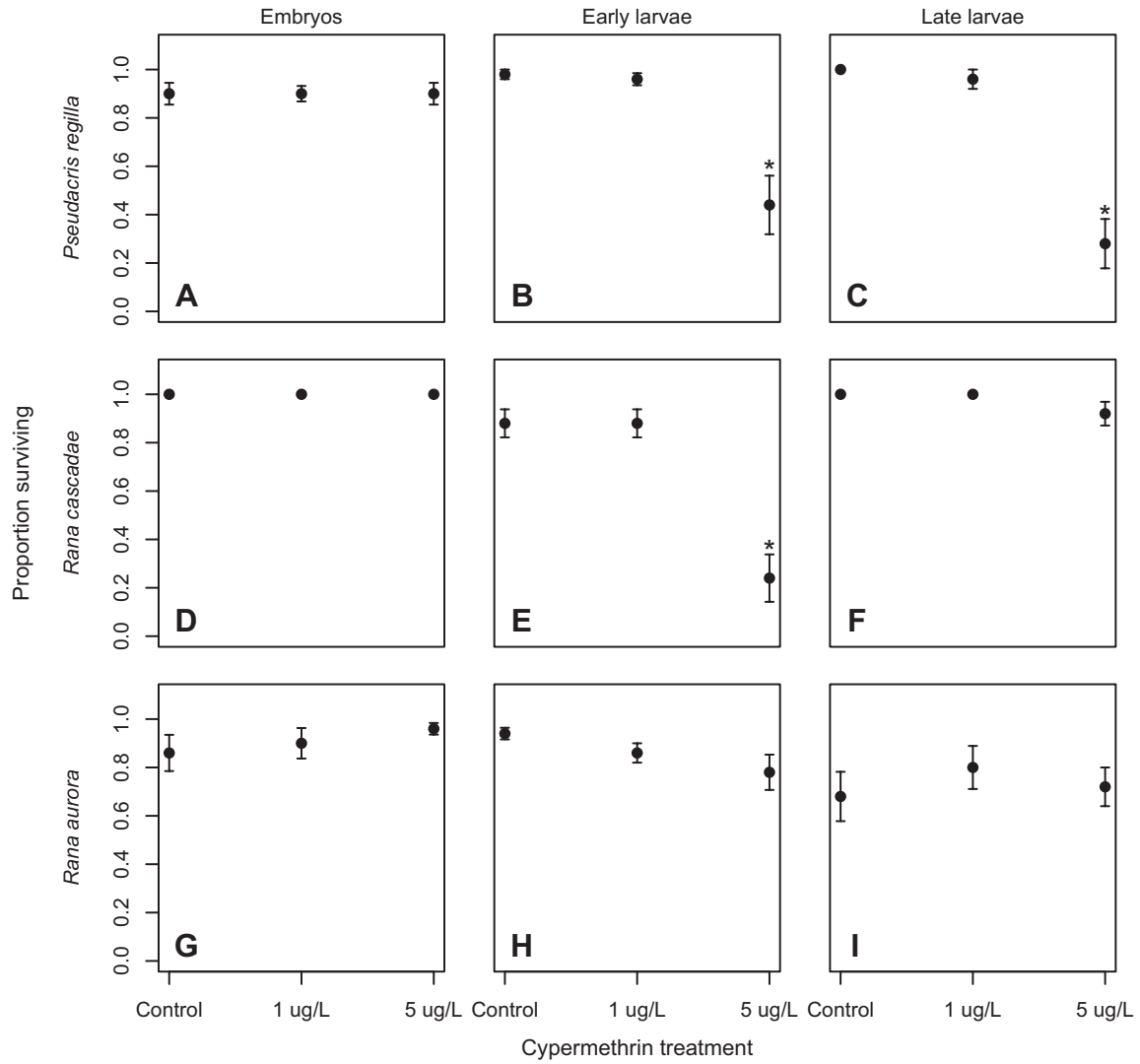

Figure 1. Survival of cypermethrin-exposed and control groups of amphibians of 3 species—*Pseudacris regilla* (top row), *Rana cascadae* (middle row), and *Rana aurora* (bottom row)—exposed at 3 distinct developmental time points: embryos (first column), newly hatched larvae (second column), and larvae with limb buds (third column). Asterisk (*) indicates treatments that are significantly different ($p < 0.05$) from controls. Values plotted are means ± 1 standard error.

Table 1. Summary of morphometric data from amphibian embryos and larvae exposed to cypermethrin treatments for 3 species[a]

	Control		Low cypermethrin treatment (0.5 μg/L)		High cypermethrin treatment (5.0 μg/L)	
Stage and species	Length ± SE (mm)	Mass ± SE (g)	Length ± SE (mm)	Mass ± SE (g)	Length ± SE (mm)	Mass ± SE (g)
Embryo						
Pseudacris regilla	5.3 ± 0.3	0.032 ± 0.005	5.1 ± 0.2	0.028 ± 0.005	5.0 ± 0.2	0.025 ± 0.003
Rana cascadae	9.2 ± 0.1	0.094 ± 0.003	9.2 ± 0.1	0.093 ± 0.004	9.4 ± 0.2	0.112 ± 0.014
Rana aurora	8.8 ± 0.3	0.095 ± 0.007	8.8 ± 0.2	0.098 ± 0.007	8.8 ± 0.2	0.092 ± 0.006
Newly hatched larvae						
Pseudacris regilla	11.7 ± 0.2	0.269 ± 0.006	12.0 ± 0.1	0.282 ± 0.007	12.2 ± 0.2	0.289 ± 0.013
Rana cascadae	13.3 ± 0.2	0.246 ± 0.011	13.3 ± 0.2	0.241 ± 0.008	12.8 ± 0.7	0.223 ± 0.031
Rana aurora	13.1 ± 0.1	0.298 ± 0.006	12.8 ± 0.3	0.282 ± 0.014	12.6 ± 0.4	0.266 ± 0.022
Larvae with limb buds						
Pseudacris regilla	13.7 ± 0.2	0.460 ± 0.009	14.1 ± 0.4	0.457 ± 0.040	13.4 ± 0.6	0.402 ± 0.051
Rana cascadae	16.8 ± 0.3	0.537 ± 0.021	16.2 ± 0.4	0.504 ± 0.038	16.3 ± 0.2	0.515 ± 0.017
Rana aurora	15.4 ± 0.2	0.502 ± 0.031	15.6 ± 0.3	0.493 ± 0.028	15.7 ± 0.5	0.486 ± 0.043

[a]Cypermethrin exposure did not affect growth in any species at any stage ($p > 0.05$).
SE = standard error

mucopolysaccharides, differs among amphibian species in regard to the number of layers and unique molecular composition [35]. It follows that protection by the jelly coat may vary as well. Sensitivity of embryos to exposure likely varies not only by species, as seen in the present study, but also by chemical as a chemical's ability to penetrate the jelly coat depends on its composition as well as the morphology of the jelly coat [36].

Further work is needed to understand how cypermethrin might affect amphibians in the field. Others have observed effects of insecticides that appeared to be detrimental to amphibians in the lab but did not find correlating negative long-term consequences in subsequent mesocosm experiments. For instance, Relyea and Mills [43] documented an increase in toxicity of pesticides in the presence of predators in the lab, yet this effect has not been demonstrated in mesocosms or in the field, to our knowledge. However, a commercial formulation of permethrin, a synthetic pyrethroid insecticide with the same mode of action as cypermethrin, resulted in 98% mortality of amphibian larvae in a mesocosm experiment, indicating that direct effects of pyrethroids in aquatic systems may be severe [44]. Consequently, the sublethal effects we documented in the laboratory could have serious long-term consequences for individuals suffering similar effects in the field. Although we did not test pond water from embryo collection sites for cypermethrin, the levels tested in the present study have been observed in the environment in several studies [16,22–25]. The behavioral effects we observed, including inactivity, twisting, trembling, or weak movement over a short distance in response to prodding, were obvious signs of cyano-pyrethroid poisoning [45]. When prodded, the initial response of anuran larvae is typically to dart away [46]. The inability to dart away when prodded may likely render larvae more vulnerable to predation [12]. Additionally, if a behavior is associated with foraging, these behavioral abnormalities could inhibit growth and contribute to reduced reproductive fitness. However, extrapolation to population-level effects is inherently challenging, adding another layer of complexity to understanding the full impact of chemicals in the environment [47].

Despite being one of the most widely used pesticides, the ecological impacts of cypermethrin are not well understood [17]. The results of the present study highlight the importance of multispecies toxicity testing and of evaluating sublethal effects to better understand these impacts. We demonstrated that, at environmentally relevant concentrations, cypermethrin induces behavioral abnormalities and death but that toxicity of cypermethrin varies among amphibian species and among life stages during which exposure occurs. Cypermethrin was more toxic to *P. regilla* than *R. aurora* and *R. cascadae*. Additionally, cypermethrin toxicity was strongest when exposure occurred at the early larval stage. Our results suggest that environmentally relevant concentrations of cypermethrin are capable of causing adverse effects in anurans.

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REFERENCES

1. Grube A, Donaldson D, Kiely T, Wu L. 2011. Pesticide industry sales and usage: 2006 and 2007 market estimates. EPA 733/R-11/001. US Environmental Protection Agency, Washington, DC.
2. Boone MD, James SM. 2005. Aquatic and terrestrial mesocosms in amphibian ecotoxicology. *Appl Herpetol* 2:231–257.
3. Stark JD, Banks JE, Vargas R. 2004. How risky is risk assessment: The role that life history strategies play in susceptibility of species to stress. *Proc Natl Acad Sci U S A* 101:732–736.
4. Relyea RA, Hoverman J. 2006. Assessing the ecology in ecotoxicology: A review and synthesis in freshwater systems. *Ecol Lett* 9:1157–1171.
5. Berrill M, Bertram S, McGillivray L, Kolohon M, Pauli B. 1994. Effects of low concentrations of forest-use pesticides on frog embryos and tadpoles. *Environ Toxicol Chem* 13:657–664.
6. Rogers JM. 1997. Life stage and its impact on risk of environmentally-induced adverse effects. *Environ Toxicol Pharmacol* 4:299–300.
7. Yu S, Wages MR, Cai Q, Maul JD, Cobb GP. 2013. Lethal and sublethal effects of three insecticides on two developmental stages of *Xenopus laevis* and comparison with other amphibians. *Environ Toxicol Chem* 32:2056–2064.
8. Bridges CM, Semlitsch RD. 2000. Variation in pesticide tolerance of tadpoles among and within species of Ranidae and patterns of amphibian decline. *Conserv Biol* 14:1490–1499.
9. Amweg E, Weston D, Ureda N. 2005. Use and toxicity of pyrethroid pesticides in the Central Valley, California, USA. *Environ Toxicol Chem* 24:966–972.
10. Weston DP, Holmes RW, You J, Lydy MJ. 2005. Aquatic toxicity due to residential use of pyrethroid insecticides. *Environ Sci Technol* 39: 9778–9784.
11. Saha S, Kaviraj A. 2008. Acute toxicity of synthetic pyrethroid cypermethrin to some freshwater organisms. *Bull Environ Contam Toxicol* 80:41–52.
12. Berrill M, Bertram S, Wilson A, Louis S, Brigham D, Stromberg C. 1993. Lethal and sublethal impacts of pyrethroid insecticides on amphibian embryos and tadpoles. *Environ Toxicol Chem* 12:525–539.
13. Moore A, Waring CP. 2001. The effects of a synthetic pyrethroid pesticide on some aspects of reproduction in Atlantic salmon (*Salmo salar* L.). *Aquat Toxicol* 52:1–12.
14. US Environmental Protection Agency. 2012. Pyrethroids and pyrethrins. Washington, (DC). [cited 2013 June 17]. Available from: http://www.epa.gov/oppsrrd1/reevaluation/pyrethroids-pyrethrins.html
15. US Environmental Protection Agency. 2008. Reregistration eligibility decision for cypermethrin (revised 1/14/08). OPP/2005/0293. Washington, DC.
16. Jergentz S, Mugni H, Bonetto C, Schulz R. 2005. Assessment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. *Chemosphere* 61:817–826.
17. Kim Y, Jinyong J, Sorin OH, Kyungho C. 2008. Aquatic toxicity of cartap and cypermethrin to different life stages of *Daphnia magna* and *Oryzias latipes*. *J Environ Sci Health B* 43:56–64.
18. Ghodageri MG, Pancharatna K. 2011. Morphological and behavioral alterations induced by endocrine disrupters in amphibian tadpoles. *Toxicol Environ Chem* 93:2012–2021.
19. Soderlund DM, Bloomquist JR. 1989. Neurotoxic actions of pyrethroid insecticides. *Annu Rev Entomol* 34:77–96.
20. Oros DR, Werner I. 2005. Pyrethroid insecticides: An analysis of use patterns, distributions, potential toxicity and fate in the Sacramento-San Joaquin delta and Central Valley. White Paper for the Interagency Ecological Program. SFEI Contribution 415. San Francisco Estuary Institute, Oakland, CA, USA.
21. Weston DP, Holmes RW, Lydy MJ. 2009. Residential runoff as a source of pyrethroid pesticides to urban creeks. *Environ Pollut* 157:287–294.
22. Crossland NO. 1982. Aquatic toxicology of cypermethrin. II. Fate and biological effects in pond experiments. *Aquat Toxicol* 2:205–222.
23. Crossland NO, Shires SW, Bennett D. 1982. Aquatic toxicology of cypermethrin. III. Fate and biological effects of spray drift deposits in fresh water adjacent to agricultural land. *Aquat Toxicol* 2:253–270.
24. Marino D, Ronco EA. 2005. Cypermethrin and chlorpyrifos concentration levels in surface water bodies of the Pampa Ondulada, Argentina. *Bull Environ Contam Toxicol* 75:820–826.
25. Day KE. 1989. Acute, chronic and sublethal effects of synthetic pyrethroids on freshwater zooplankton. *Environ Toxicol Chem* 8: 411–416.
26. Lawton JH, May RM, eds. 1995. *Extinction Rates*. Oxford University Press, Oxford, UK.

27. Wake DB, Vredenburg VT. 2008. Are we in the midst of the sixth mass extinction? A view from the world of amphibians. *Proc Natl Acad Sci U S A* 105:11466–11473.
28. Blaustein AR, Romansic JM, Kiesecker JM, Hatch AC. 2003. Ultraviolet radiation, toxic chemicals and amphibians population declines. *Divers Distrib* 9:123–140.
29. Stuart SN, Chanson JS, Cox NA, Young BE, Rodrigues ASL, Fishman DL, Waller RW. 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306:1783–1786.
30. McCallum ML. 2007. Amphibian decline or extinction? Current declines dwarf background extinction rate. *J Herpetol* 41:483–491.
31. International Union for Conservation of Nature. 2012. IUCN Red List of Threatened Species. Ver 2012.2. [cited 2013 June 20]. Available from: http://www.iucnredlist.org.
32. Sparling DW, Fellers GM, McConnell LL. 2001. Pesticides and amphibian population declines in California, USA. *Environ Toxicol Chem* 20:1591–1595.
33. Davidson C. 2004. Declining downwind: Amphibian population declines in California and historical pesticide use. *Ecol Appl* 14: 1892–1902.
34. Mann RM, Hyne RV, Choung CB, Wilson SP. 2009. Amphibians and agricultural chemicals: Review of the risks in a complex environment. *Environ Pollut* 157:2903–2927.
35. Marquis O, Millery A, Guittonneau S, Miaud C. 2006. Toxicity of PAHs and jelly protection of eggs in the common from *Rana temporaria*. *Amphib-Reptilia* 27:472–475.
36. Greulich K, Pflugmacher S. 2004. Uptake and effects on detoxification enzymes of cypermethrin in embryos and tadpoles of amphibians. *Arch Environ Contam Toxicol* 47:489–495.
37. Jones LLC, Leonard WP, Olson DH. 2005. *Amphibians of the Pacific Northwest*. Seattle Audubon Society, Seattle, WA, USA.
38. Gosner KL. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. *Herpetologica* 16:183–190.
39. International Organization for Standardization. 2012. Water quality—Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea)—Acute toxicity test. ISO 6341. Geneva, Switzerland.
40. Stebbins RC, Cohen NW. 1995. *A Natural History of Amphibians*. Princeton University Press, Princeton, NJ, USA.
41. Berrill M, Coulson D, McGillivray L, Pauli B. 1998. Toxicity of endosulfan to aquatic stages of anuran amphibians. *Environ Toxicol Chem* 17:1738–1744.
42. Pauli BD, Coulson DR, Berrill M. 1999. Sensitivity of amphibian embryos and tadpoles to MIMIC 240LV insecticide following single or double exposures. *Environ Toxicol Chem* 18:2538–2544.
43. Relyea RA, Mills N. 2001. Predator-induced stress makes the pesticide carbaryl more deadly to grey treefrog tadpoles (*Hyla versicolor*). *Proc Natl Acad Sci U S A* 98:2491–2496.
44. Boone MD. 2008. Examining the single and interactive effects of three insecticides on amphibian metamorphosis. *Environ Toxicol Chem* 27:1561–1568.
45. David M, Marigoudar SR, Patil VK, Halappa R. 2012. Behavioral, morphological deformities and biomarkers of oxidative damage as indicators of sublethal cypermethrin intoxication on the tadpoles of *D. melanostictus* (Schneider, 1799). *Pestic Biochem Physiol* 103:127–134.
46. Caldwell JP, Thorp JH, Jervey TP. 1980. Predator–prey relationships among larval dragonflies, salamanders, and frogs. *Oecologia* 46:285–289.
47. Blaustein AR, Han BA, Relyea RA, Johnson PTJ, Buck JC, Gervasi SS, Kats LB. 2011. The complexity of amphibian population declines: Understanding the role of cofactors in driving amphibian losses. *Ann N Y Acad Sci* 1223:108–119.