Published in final edited form as:

Environ Toxicol Chem. 2011 July ; 30(7): 1618–1620. doi:10.1002/etc.539.

Juvenile Green Frog (*Rana clamitans*) Predatory Ability not Affected by Exposure to Carbaryl at Different Times During Larval Development

Melanie J. Davis*, Peter Kleinhenz, and Michelle D. Boone

Department of Zoology, Miami University, Oxford, Ohio, USA

Abstract

Larval exposure to pesticides can occur at different times during development, and can negatively impact amphibian fitness. We examined the effects of larval green frog (*Rana clamitans*) exposure to carbaryl at 2, 4, 8, or 16 weeks of development on juvenile predatory ability. We did not find evidence that predatory ability was affected by exposure to carbaryl, which suggests that carbaryl does not have latent effects on the predatory performance of green frogs in subsequent life stages.

Keywords

Carbaryl; *Rana clamitans*; Larval development; Predatory ability

Introduction

Amphibian populations have been declining at an alarming rate worldwide, especially in areas where agricultural pesticides are commonly used, such as the United States [1]. Because pesticides are widespread, can move through global processes, and are found in nearly all habitats, understanding their impacts on non-target wildlife represents a basic ecological question. Herbicides and insecticides may be applied at multiple times and at different points during the growing season depending on crop type, which means that acute exposure to these contaminants can occur to amphibians at different stages of larval development. With amphibian declines on the rise, it is important to determine the long-term effects of exposure to these contaminants, and whether exposure at different points in larval development may have greater negative impacts on subsequent life stages.

Carbaryl (1-naphthyl-N-methyl carbamate), a neurotoxic insecticide, is one of the most commonly used pesticides in the United States, and it can be found at environmental concentrations of ≤ 3.7 mg/L [2]. Carbaryl enters aquatic environments via direct application or runoff from agricultural applications, where it may affect nontarget species such as amphibians. It tends to be short lived in aerobic aquatic environments [3], but degradation and toxicity vary widely and may depend on pH, oxygen levels, temperature, and presence of ultraviolet light [3–5], and application may occur at several times during the agricultural season.

Carbaryl has been shown to directly impact the survival of larval amphibians. A broad range of LC50s from 1 to 10 mg/L has been found for varying amphibian species [6,7], and

*Corresponding author: Melanie J. Davis, Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80523, 9 (614) 519-3107, melanie.davis@colostate.edu).

The present address of M. Davis is: Colorado State University, Fort Collins, Colorado, USA

ultraviolet radiation has been shown to increase mortality due to carbaryl exposure in the laboratory [7]. Besides increasing mortality through direct exposure, carbaryl may also indirectly affect survival through complex food web and competitive interactions. These indirect effects may positively or negatively impact size [8,9], feeding behavior [10], activity levels [10, 11], and responses to predators [12].

Larval amphibians could be exposed to pesticides at different life stages and may be exposed more than once. Exposure at different developmental stages may vary the extent of the pesticide's effect. Previous research has shown that tadpoles exposed to carbaryl later in development may exhibit faster development and greater mass [8]. Contrastingly, tadpoles dosed on multiple occasions generally weigh less [8], but may still experience early metamorphosis [13]. Tadpoles exposed at different stages of development exhibit variation in overall survival, mass, or time to metamorphosis [8].

At short durations of exposure, there is evidence that carbaryl acts on the endocrine system of fish by elevating the levels of triiodothyronine (T_3) and decreasing levels of thyroxine (T_4) [14]. There may be implications for growth and development of amphibians as well. The receptor genes for thyroid hormone, TR-alpha and TR-beta, grow in prevalence as a tadpole develops, peaking just before metamorphosis [15, 16]. The more receptor genes that are available, the more a tadpole's endocrine system responds to circulating thyroid hormones. Hormones such as T_3 are responsible for initiating many aspects of metamorphosis, such as changes in the intestine, development of the nervous system, tail resorption, limb bud growth, and muscle development [16, 17]. Besides affecting physiology, these factors may also contribute to the behavioral characteristics of an adult amphibian.

After metamorphosis, a juvenile amphibian's ability as a predator will significantly impact its likelihood of reaching reproductive maturity. Thus, a reduction in predatory ability due to an environmental contaminant such as carbaryl may significantly decrease individual or species fitness. Little research has been performed concerning juvenile activity and motor ability after exposure to carbaryl as larvae; however, Boone [18] found that while larval carbaryl exposure resulted in smaller size at metamorphosis, these size differences were eliminated after less than a year of terrestrial growth. Bridges [10] also found that when adult newts were exposed to carbaryl, they consumed fewer prey items during exposure.

To test the effects of larval exposure to carbaryl on amphibian predatory ability, we utilized green frogs as our experimental species. Green frogs commonly breed in water systems close to agricultural landscapes. The breeding season occurs from May to July, and tadpoles may overwinter, meaning that exposure to environmental contaminants likely occurs at different stages of larval development. Previous studies have also indicated that green frogs will exhibit precocious metamorphosis with carbaryl exposure [8, 13], which could result from changes in physiology.

The objective of the present study was to determine the effects of carbaryl on the predatory ability of juvenile green frogs, and if these effects differed based on the timing of larval exposure. We predicted that exposure to carbaryl would decrease predatory ability regardless of timing of exposure.

Materials and Methods

Four egg masses were collected from Boesel Pond in the Miami University Natural Areas, Oxford (Butler County), Ohio, USA, on June 25, 2008. Tadpoles were reared individually in 2-L glass beakers filled with 1.5 L water beginning on July 8, with a 12:12 light:dark regime

at 20°C. Water was changed every 2 to 3 d, and tadpoles were fed ground Tetramin® fish food after each water change.

Forty Tadpoles were exposed to each treatment group: a control group (dechlorinated water), or 1-mg/L carbaryl at approximately 2, 4, 8, or 16 weeks of development. Exposure lasted until the next water change. We searched for metamorphs (defined as the emergence of one forelimb) daily and they were held individually in a small, plastic sandwich container filled with approximately 1 cm dechlorinated tap water until tail resorption.

Feeding trials occurred on the day of complete tail resorption (Gosner stage 46 [19]). Feeding arenas consisted of one 5.39-L plastic Rubbermaid® shoebox container lined with two damp paper towels. Juveniles were weighed, and placed in the feeding arena with 20 10-d old house crickets (*Acheta domesticus*; approximately 0.2–0.5 cm). The feeding trials started between 1500 and 1600 EST and lasted 24 h, after which, the number of crickets remaining in the feeding arena was counted to determine juvenile feeding success.

We tested for the effect of treatment type on juvenile predatory ability using a one-way analysis of covariance (ANCOVA), using mass at metamorphosis as a covariate, with SAS version 9.2. Predatory ability was analyzed as the proportion of crickets eaten out of 20. We used a Dunnett's test to determine if carbaryl treatments differed from the water control.

Results

Administering carbaryl dosage at different times during larval development had no overall effect on the predatory ability of juveniles ($F_{4,135}=0.94$, $p=0.4452$, Fig. 1). Juvenile mass (the covariate) did not significantly affect feeding ability ($F_{1,135}=2.03$, $p=0.1570$).

Discussion

The goal of the present study was to determine how exposure to an environmental contaminant (i.e., carbaryl) at different periods of green frog larval development affected juvenile predatory ability. We expected that juvenile predatory ability would be compromised by carbaryl exposure, regardless of timing of developmental exposure, based on past studies of the effects of carbaryl on larval amphibians [8, 13].

Overall, carbaryl did not significantly affect juvenile predatory ability. Previous studies have found that although carbaryl exposure early in development can have effects on mass at metamorphosis or larval survival, it did not have latent effects on overwinter survival or growth in the terrestrial environment [18, 20]. For example, Boone [18] found that carbaryl exposure reduced mass at metamorphosis for American toads, but after overwintering there were no differences between carbaryl-exposed and control toads. This suggests that carbaryl-exposed toads were able to compensate for small mass with increased foraging in the terrestrial environment. The present study does not indicate that carbaryl-exposed frogs had an increased feeding rate over a 24 h period, suggesting that increased size in previous studies may be more related to differences in metabolic costs associated with small versus larger frogs.

Research that was performed concurrently with this experiment found that while tadpoles that were exposed to carbaryl at 16 weeks of development did not differ in mass at metamorphosis or time to metamorphosis, they did show changes in thyroid hormone receptor-alpha levels in brain tissue (M. Boone, unpublished data). This suggests that there is still the possibility for latent effects beyond the scope of our experiment, but that despite evidence of endocrine disruption, predatory ability did not appear to be affected.

Pesticides have been shown to cause endocrine problems in amphibian species, even at relatively low doses [21, 22]. Such damage to the endocrine system can have long-lasting impacts on the survival and reproduction of adult amphibians. More research is needed to elucidate the mechanisms by which agricultural contaminants such as pesticides affect the development and reproductive potential of amphibian species, and how exposure during crucial periods of larval development might lead to below-average functioning as adults. An understanding of such processes would aid in clarifying contaminants' roles in amphibian declines, and would support improved amphibian conservation practices worldwide.

Acknowledgments

The authors are grateful for the laboratory assistance of L. Phillips, M. Youngquist, and H. Puglis. This research was made possible with the funding from NIH AREA grant (0717088). The experiment described here complied with current U.S. laws and was approved under animal care protocol 749 by the Institutional Animal Care and Use Committee at Miami University.

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Fig. 1. Bar chart showing predatory ability (number of crickets eaten) for each carbaryl treatment. Error bars represent one standard error.