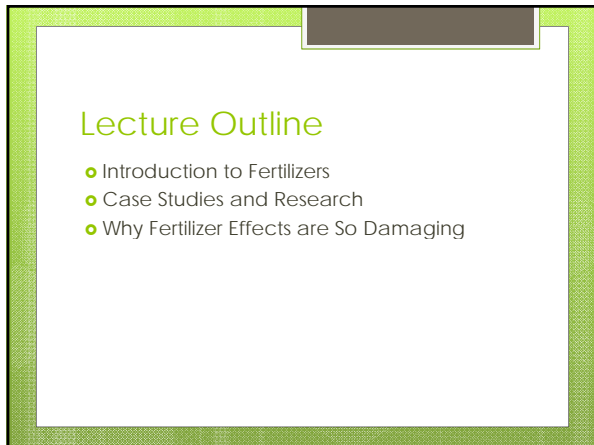


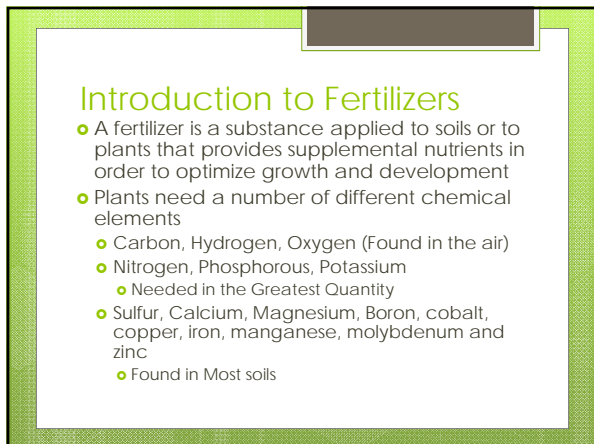
Fertilizers and the Effect They Have On Amphibian Populations

Glen Searcy
WFS 433
Amphibian Ecology and Conservation



Lecture Outline

- Introduction to Fertilizers
- Case Studies and Research
- Why Fertilizer Effects are So Damaging



Introduction to Fertilizers

- A fertilizer is a substance applied to soils or to plants that provides supplemental nutrients in order to optimize growth and development
- Plants need a number of different chemical elements
 - Carbon, Hydrogen, Oxygen (Found in the air)
 - Nitrogen, Phosphorous, Potassium
 - Needed in the Greatest Quantity
 - Sulfur, Calcium, Magnesium, Boron, cobalt, copper, iron, manganese, molybdenum and zinc
 - Found in Most soils

The Problem With Fertilizers

- Misuse and over application
 - Results in runoff of chemicals
 - Leaching of chemicals into water sources
- "Waterbodies situated within agricultural areas receive run off from surrounding land where fertilizers and pesticides are applied, and concentrations of nitrogen and phosphorus in these often exceed levels encountered in non-agricultural areas." (Hamer-2004)

The Problem With Fertilizers

- Fertilizers are extremely common
- Used on both large and small scales
 - Commercial and Residential



Problems With Fertilizers

- Direct application to water is often overlooked
- Used both for Agriculture and Aquaculture
 - Farm ponds connected to nearby drainages



The Research



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 Agriculture, Ecosystems and Environment 102 (2004) 299–303



Amphibian decline and fertilizers used on agricultural land in south-eastern Australia

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The Research

- Compared historical records of fertilizers with current and past frog populations
 - golden bell frog (*Litoria aurea*)
 - common eastern froglet (*Crinia signifera*)
 - Striped marsh frog (*Limnodonastes peronii*)

The Research

- Total Tonnage of fertilizers peaked between 1964–1973
- “All three species apparently increased from 1990 onwards when the issue of globally declining amphibian populations was first raised...” (Hamer-2004)

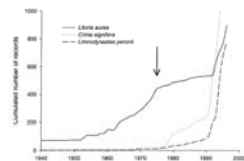


Fig. 1. Total record numbers (cumulative over the years 1940–2000) for *Crinia signifera*, *Limnodonastes peronii*, and *Litoria aurea* in the NSW database. Arrow indicates time of major construction for J. series.

The Research

- The experimental evidence showed differential sensitivity to fertilizers between *L. aurea* and two non-declining frog species.
- Significantly fewer *L. aurea* tadpoles survived to metamorphosis in the two highest concentrations of ammonium nitrate (76 and 78% survival, 10 and 15 mg/l, respectively; $F_{3,20} = 6.32$, $P = 0.003$) and in 15 mg/l of calcium phosphate (82% survival; $F_{3,20} = 3.44$, $P = 0.04$) compared to the control (Fig. 3).

The Research

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ORIGINAL PAPER

Sublethal effects of nitrite on eastern tiger salamander (*Ambystoma tigrinum tigrinum*) and wood frog (*Rana sylvatica*) embryos and larvae: implications for field populations

Karen E. Griffis-Kyle

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Abstract Ephemeral ponds, which can have high nitrite levels and low dissolved oxygen, are the sites of amphibian reproduction. As such, it is important to understand how exposure to nitrite might affect development and growth of amphibian embryos/larvae in ephemeral ponds. Wood frog (*Rana sylvatica*) and eastern tiger salamander (*Ambystoma tigrinum tigrinum*) embryos and tadpoles and young larvae were exposed to elevated concentrations of nitrite derived from an

amphibian development, growth and hatching and how nitrite exposure in amphibians differs in ephemeral environments. Potential measures to identify on field populations caused by sublethal effects of nitrite are discussed.

Keywords Metamorphosis · Growth · Development · Ephemeral ponds · Nitrogen · Amphibian decline

Introduction

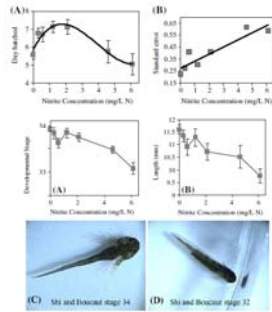
-This study explores the indirect effects of Nitrites and Nitrates on Amphibian Populations.

- "Nitrite can cause direct lethal effects in amphibians but the sublethal effects, especially on amphibians that breed in ephemeral ponds in agricultural regions, need to be explored." (Griffis-Kyle-2005)

The Research

- Three wood frog egg masses were collected from three ponds and 18 eastern tiger salamander egg masses were collected from six ponds in 2004.
- Early tadpole and larval survival were tested at nitrite concentrations of 0, 0.3, 0.6, 1.2, 2.1, 4.6, and 6.1 mg l⁻¹ NO₂-N.
- "For the eastern tiger salamander, nitrite has a significant negative effect on developmental stage at hatching controlling for the size of the hatchling" (Griffis-Kyle-2005)

The Research

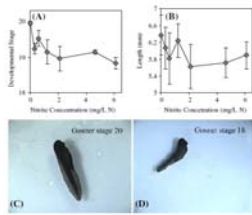


Results from Nitrite Exposure to tiger salamanders

-*Exposed salamanders hatched without branched gills- (reduces oxygen uptake)

- Pictures taken of tiger salamanders 24 hours after hatching
- (C) 0 mg l-1 NO₂-N and
- (D) 6.1 mg l-1 NO₂-N

The Research



Results from nitrite exposure to wood frogs

- wood frog embryos exposed to higher concentrations of nitrite hatched at Gosner (1960) stages 18 and 19, before circulation begins in the gills
- Photographs taken 24 hours after hatching
- (C) 0 mg l-1 NO₂-N and
- (D) 6.1 mg l-1 NO₂-N

The Research



- "Nitrite may enter organisms with food, diffuse through dermal layers as HNO₂, or enter by active diffusion through the gills of aquatic organisms through the chloride uptake mechanism." (Griffis-Kyle-2005)
- Ephemeral pools dry and can cause full mortality in the cohort of amphibians within the pool if they have not completed metamorphosis (Berven 1990)

The Research

- Many public water supplies in the United States contain levels of nitrate that routinely exceed concentrations of 10 mg N/L [8]. In the Willamette Valley, average nitrate concentrations of 17.8 and 21.9 mg N/L were recorded in water samples from some crop soils receiving recommended rate of nitrogen fertilization.* (Brandt-Dohrn-1997)

Table 3. Median lethal concentrations (LC50 \pm SE) of nitrite (mg N-NO₂-L) for aquatic larvae of five amphibian species at 4, 7 and 15 days of exposure. Standard errors for LC50 are in parentheses.

Day	<i>R. pretiosa</i>	<i>R. aurora</i>	<i>R. boreas</i>	<i>H. regilla</i>	<i>A. gracile</i>
4	6.82 (0.615)	5.59 (1.446)	>7.0	3.50 (0.742)	1.90 (0.737)
7	1.30 (0.345)	4.00 (1.021)	5.38 (0.646)	2.60 (0.650)	1.54 (0.593)
15	0.57 (0.033)	1.19 (0.288)	1.75 (0.612)	1.23 (0.312)	1.01 (0.279)

Marco-1999

The Research

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THE EFFECTS OF NITRITE ON BEHAVIOR AND METAMORPHOSIS IN CASCADES FROGS (*RANA CASCADA*)

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(Received 26 May 1998; Accepted 27 July 1998)

Abstract—Amphibian metamorphosis is a period of drastic morphologic reorganization, during which larvae experience a decrease in locomotor ability and are more vulnerable to predation. Our results indicate that exposure to sublethal concentrations of nitrite in the water induces behavioral and morphologic changes in the Cascades frog (*Rana cascada*). Tadpoles exposed to a nitrite concentration of 0.50, at 1 mg/L, transformed more slowly than control tadpoles exposed to dechlorinated tap water. No difference was found in time at emergence and snout-vent length at emergence between experimental and control tadpoles, but development was retarded in tadpoles exposed to nitrite and they emerged at an earlier developmental stage. Also, tadpoles exposed to nitrite occupied shallow water more frequently than did control tadpoles.

Keywords—Amphibian, Pesticides, Metamorphosis, Nitrite, *Rana cascada*

The Research

- Egg masses of Cascades frog (*Rana cascadae*) collected and exposed to nitrites in water
- When nitrites contact blood plasma, they transform hemoglobin to methemoglobin, decreasing the oxygen carrying capacity of the blood* (Marco-1998)
 - Methemoglobinemia
- Tadpoles exposed to nitrite were less developed and were observed in shallower water with heads out of the water.
 - Shows lack of ability to efficiently get oxygen
 - Ponds that experience algal blooms
 - Creates chances for increases in predation



The Research



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Agriculture
Ecosystems &
Environment

Short communication

The effect of ammonium nitrate fertiliser on frog (*Rana temporaria*) survival

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Accepted 17 June 1996

Abstract

The toxicity of ammonium nitrate fertilizer to common frogs (*Rana temporaria*) was tested in the laboratory and field. Considerable ammonium nitrate is the most commonly used fertilizer in Britain, especially during the spring, when adult frogs migrate most fast. Ammonium nitrate was acutely toxic to frogs at concentrations well below those recommended for field application. However, it lost its acute effect when dissolved in the soil and even on relatively dry soil (7% moisture), toxicity declined to less than 3 hours. A potentially high mortality rate owing to ammonium nitrate is probably mitigated by the fortuitous asynchrony between fertilizer application during daylight, and frog migration at night. It remains to be determined whether there are sublethal effects and whether fertilizers that dissolve most slowly are implicated in the widespread amphibian declines in agricultural areas observed since the Second World War.

The Research

- Common frogs (*Rana temporaria*) were exposed to multiple levels of concentration of ammonium nitrate
- All subjects exposed to concentrations over 3 g/m² showed significant signs of effect

The Research

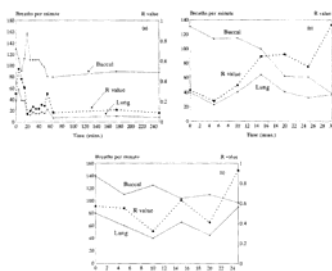


Fig. 1. (a) Ventilatory rates and R-values of frog held in laboratory apparatus on damp filter paper at 20°C. (b) Ventilatory rates and R-values of a frog held in an incubator on damp filter paper with 15.0 g m⁻² of ammonium nitrate granules at 20°C. (c) Ventilatory rates and R-values of a frog held in an incubator on a bed of pot grass immediately after the application of ammonium nitrate granules at a concentration of 15.0 g m⁻² at 17°C.

Fertilizers: A Widespread Problem

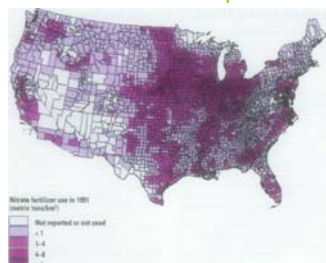


Figure 1. Nitrogen fertilizer use in the continental United States, 1991 (29).

Rouse et. al -1999

Country	Production (thousand tons of wheat)	Production (thousand tons of wheat)
	1990	1991
Algeria	247.0	247.0
Argentina	1,000.0	1,000.0
Australia	285.0	285.0
Brazil	79.0	79.0
Canada	1,043.0	1,043.0
China	1,000.0	1,000.0
France	1,000.0	1,000.0
Germany	100.0	100.0
India	1,000.0	1,000.0
Italy	1,000.0	1,000.0
Japan	1,000.0	1,000.0
South Korea	1,000.0	1,000.0
Spain	1,000.0	1,000.0
Sweden	1,000.0	1,000.0
Switzerland	1,000.0	1,000.0
Taiwan	1,000.0	1,000.0
Thailand	1,000.0	1,000.0
United Kingdom	1,000.0	1,000.0
United States	1,000.0	1,000.0
USSR	1,000.0	1,000.0
Yugoslavia	1,000.0	1,000.0

Average commercial fertilizer
purchased by states per year
(EPA)

	2000	2001	2002	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025	2026	2027	2028	2029	2030	2031	2032	2033	2034	2035	2036	2037	2038	2039	2040	2041	2042	2043	2044	2045	2046	2047	2048	2049	2050	2051	2052	2053	2054	2055	2056	2057	2058	2059	2060	2061	2062	2063	2064	2065	2066	2067	2068	2069	2070	2071	2072	2073	2074	2075	2076	2077	2078	2079	2080	2081	2082	2083	2084	2085	2086	2087	2088	2089	2090	2091	2092	2093	2094	2095	2096	2097	2098	2099	2100	2101	2102	2103	2104	2105	2106	2107	2108	2109	2110	2111	2112	2113	2114	2115	2116	2117	2118	2119	2120	2121	2122	2123	2124	2125	2126	2127	2128	2129	2130	2131	2132	2133	2134	2135	2136	2137	2138	2139	2140	2141	2142	2143	2144	2145	2146	2147	2148	2149	2150	2151	2152	2153	2154	2155	2156	2157	2158	2159	2160	2161	2162	2163	2164	2165	2166	2167	2168	2169	2170	2171	2172	2173	2174	2175	2176	2177	2178	2179	2180	2181	2182	2183	2184	2185	2186	2187	2188	2189	2190	2191	2192	2193	2194	2195	2196	2197	2198	2199	2200	2201	2202	2203	2204	2205	2206	2207	2208	2209	2210	2211	2212	2213	2214	2215	2216	2217	2218	2219	2220	2221	2222	2223	2224	2225	2226	2227	2228	2229	2230	2231	2232	2233	2234	2235	2236	2237	2238	2239	2240	2241	2242	2243	2244	2245	2246	2247	2248	2249	2250	2251	2252	2253	2254	2255	2256	2257	2258	2259	2260	2261	2262	2263	2264	2265	2266	2267	2268	2269	2270	2271	2272	2273	2274	2275	2276	2277	2278	2279	2280	2281	2282	2283	2284	2285	2286	2287	2288	2289	2290	2291	2292	2293	2294	2295	2296	2297	2298	2299	2300	2301	2302	2303	2304	2305	2306	2307	2308	2309	2310	2311	2312	2313	2314	2315	2316	2317	2318	2319	2320	2321	2322	2323	2324	2325	2326	2327	2328	2329	2330	2331	2332	2333	2334	2335	2336	2337	2338	2339	2340	2341	2342	2343	2344	2345	2346	2347	2348	2349	2350	2351	2352	2353	2354	2355	2356	2357	2358	2359	2360	2361	2362	2363	2364	2365	2366	2367	2368	2369	2370	2371	2372	2373	2374	2375	2376	2377	2378	2379	2380	2381	2382	2383	2384	2385	2386	2387	2388	2389	2390	2391	2392	2393	2394	2395	2396	2397	2398	2399	2400	2401	2402	2403	2404	2405	2406	2407	2408	2409	2410	2411	2412	2413	2414	2415	2416	2417	2418	2419	2420	2421	2422	2423	2424	2425	2426	2427	2428	2429	2430	2431	2432	2433	2434	2435	2436	2437	2438	2439	2440	2441	2442	2443	2444	2445	2446	2447	2448	2449	2450	2451	2452	2453	2454	2455	2456	2457	2458	2459	2460	2461	2462	2463	2464	2465	2466	2467	2468	2469	2470	2471	2472	2473	2474	2475	2476	2477	2478	2479	2480	2481	2482	2483	2484	2485	2486	2487	2488	2489	2490	2491	2492	2493	2494	2495	2496	2497	2498	2499	2500	2501	2502	2503	2504	2505	2506	2507	2508	2509	2510	2511	2512	2513	2514	2515	2516	2517	2518	2519	2520	2521	2522	2523	2524	2525	2526	2527	2528	2529	2530	2531	2532	2533	2534	2535	2536	2537	2538	2539	2540	2541	2542	2543	2544	2545	2546	2547	2548	2549	2550	2551	2552	2553	2554	2555	2556	2557	2558	2559	2560	2561	2562	2563	2564	2565	2566	2567	2568	2569	2570	2571	2572	2573	2574	2575	2576	2577	2578	2579	2580	2581	2582	2583	2584	2585	2586	2587	2588	2589	2590	2591	2592	2593	2594	2595	2596	2597	2598	2599	2600	2601	2602	2603	2604	2605	2606	2607	2608	2609	2610	2611	2612	2613	2614	2615	2616	2617	2618	2619	2620	2621	2622	2623	2624	2625	2626	2627	2628	2629	2630	2631	2632	2633	2634	2635	2636	2637	2638	2639	2640	2641	2642	2643	2644	2645	2646	2647	2648	2649	2650	2651	2652	2653	2654	2655	2656	2657	2658	2659	2660	2661	2662	2663	2664	2665	2666	2667	2668	2669	2670	2671	2672	2673	2674	2675	2676	2677	2678	2679	2680	2681	2682	2683	2684	2685	2686	2687	2688	2689	2690	2691	2692	2693	2694	2695	2696	2697	2698	2699	2700	2701	2702	2703	2704	2705	2706	2707	2708	2709	2710	2711	2712	2713	2714	2715	2716	2717	2718	2719	2720	2721	2722	2723	2724	2725	2726	2727	2728	2729	2730	2731	2732	2733	2734	2735	2736	2737	2738	2739	2740	2741	2742	2743	2744	2745	2746	2747	2748	2749	2750	2751	2752	2753	2754	2755	2756	2757	2758	2759	2760	2761	2762	2763	2764	2765	2766	2767	2768	2769	2770	2771	2772	2773	2774	2775	2776	2777	2778	2779	2780	2781	2782	2783	2784	2785	2786	2787	2788	2789	2790	2791	2792	2793	2794	2795	2796	2797	2798	2799	2800	2801	2802	2803	2804	2805	2806	2807	2808	2809	2810	2811	2812	2813	2814	2815	2816	2817	2818	2819	2820	2821	2822	2823	2824	2825	2826	2827	2828	2829	2830	2831	2832	2833	2834	2835	2836	2837	2838	2839	2840	2841	2842	2843	2844	2845	2846	2847	2848	2849	2850	2851	2852	2853	2854	2855	2856	2857	2858	2859	2860	2861	2862	2863	2864	2865	2866	2867	2868	2869	2870	2871	2872	2873	2874	2875	2876	2877	2878	2879	2880	2881	2882	2883	2884	2885	2886	2887	2888	2889	2890	2891	2892	2893	2894	2895	2896	2897	2898	2899	2900	2901	2902	2903	2904	2905	2906	2907	2908	2909	2910	2911	2912	2913	2914	2915	2916	2917	2918	2919	2920	2921	2922	2923	2924	2925	2926	2927	2928	2929	2930	2931	2932	2933	2934	2935	2936	2937	2938	2939	2940	2941	2942	2943	2944	2945	2946	2947	2948	2949	2950	2951	2952	2953	2954	2955	2956	2957	2958	2959	2960	2961	2962	2963	2964	2965	2966	2967	2968	2969	2970	2971	2972	2973	2974	2975	2976	2977	2978	2979	2980	2981	2982	2983	2984	2985	2986	2987	2988	2989	2990	2991	2992	2993	2994	2995	2996	2997	2998	2999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Table 15—Percentage of cotton acreage receiving nitrogen fertilizer, selected States, 1964-2010 (USDA)

- Studies have shown that 10-25% of nitrogen fertilizers run off into adjacent running water. (Maitland-1984)
- In areas where there are intensively fertilized lands, this is a huge problem

Fertilizers: Residential Use



- Increasing impermeable surfaces results in higher amounts of runoff
- Must consider the watershed approach

Fertilizers: The Big Picture

- Its Both Residential and Commercial Use



Aquaculture and Fertilizers

- Application of Fertilizers to ponds
 - Common practice is to add nitrogen and phosphorous
 - This spike in nutrients results in high amounts of zooplankton and can result in an algal bloom
 - Without constant fertilization, these algae will die.
 - Bacteria will decompose and consume all of the oxygen in the water.
 - This application of fertilizers is not always intentionally



Summary

- Exposure to Nitrates and Nitrites have devastating effects on early development that result in mortality
 - Effects predation rates, oxygen uptake efficiency, risk of desiccation etc...
- Adult Anuran Species can also be affected (Need more research to be done)
- Fertilizers are a problem from misuse and over-application
- Fertilizers are the significant cause for amphibian declines because it effects a wide range of distributions and causes many complications that eventually lead to mortality

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Questions?
