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The deal with diel: Temperature fluctuations, asymmetrical warming, and ubiquitous metals contaminants

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ABSTRACT

Climate projections over the next century include disproportionately warmer nighttime temperatures ("asymmetrical warming"). Cool nighttime temperatures lower metabolic rates of aquatic ectotherms. In contaminated waters, areas with cool nights may provide thermal refugia from high rates of daytime contaminant uptake. We exposed Cope's gray tree frogs (*Hyla chrysoscelis*), southern leopard frogs (*Lithobates sphenocephalus*), and spotted salamanders (*Ambystoma maculatum*) to five concentrations of a mixture of cadmium, copper, and lead under three to four temperature regimes, representing asymmetrical warming. At concentrations with intermediate toxicosis at test termination (96 h), temperature effects on acute toxicity or escape distance were evident in all study species. Asymmetrical warming (day:night, 22:20 °C; 22:22 °C) doubled or tripled mortality relative to overall cooler temperatures (20:20 °C) or cool nights (22:18 °C). Escape distances were 40–70% shorter under asymmetrical warming. Results suggest potentially grave ecological impacts from unexpected toxicosis under climate change.

1. Introduction

Climate change is altering thermal patterns with potentially important repercussions for ectotherms simultaneously stressed by contaminants. Average global temperatures are projected to rise by 2.5–5.5 °C by 2099 (IPCC, 2014). Over most continental regions, including the United States, nighttime air temperatures are rising more quickly than daytime temperatures, resulting in reduced diurnal temperature ranges, so called "asymmetrical warming" (Li et al., 2012; IPCC, 2014). Nighttime air temperatures have increased by three to nearly seven times (Millett et al., 2009) faster than daytime temperatures over the past 50–100 years. Warming air temperatures appear to be driving current warming trends in freshwater ecosystems (Kaushal et al., 2010). In general, average increases in air temperature of 2, 4, and 6 °C can cause freshwater systems to warm by 1.3, 2.6 and 3.8 °C, respectively (van Vliet et al., 2011). Models predict that warming in surface waters will follow the asymmetrical warming patterns of air temperatures with evidence of this already occurring in the San Francisco Estuary (Brooks et al., 2012).

Whether diel temperature fluctuations will stimulate or suppress physiological responses depends on the range of temperatures and where those temperature fluctuations fall within the thermal envelope of an ectotherm (Kingsolver et al., 2013). For example, when reared with diel temperature fluctuations from 32 to 18 °C the Australian marsh frog (*Limnoidynaste peronei*) exhibited faster growth, shorter time to metamorphosis, and increased post-metamorphic jumping distance. Their performance was compared to controls reared under a constant temperature of 24 °C (Niehaus et al., 2006). In contrast, Arrighi et al. (2013) reported that developmental stage and tail length of Korean fire-bellied toad tadpoles (*Bombina orientalis*) increased in temperatures held steady at 20, 24, and 28 °C. Both metrics declined significantly when tadpoles were exposed to diel temperature fluctuations of 13 or 20 °C (Arrighi et al., 2013).

Warming temperatures and toxicants act as compounding stressors particularly for ectotherms. An impressive number of studies have investigated how increasing temperature can heighten the effects of toxicants (Heugens et al., 2001). Recently, this effect has been termed climate-induced toxicant sensitivity (CITS) based on the realization that when elevated temperatures increase the metabolic rates of aquatic ectotherms, animals increase their

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respiration rates and food uptake to meet metabolic costs. Their increased contaminant uptake per unit time is greater than the key processes that mitigate toxicity—biotransformation and excretion—resulting in greater toxicity. On the other hand, toxicant-induced climate susceptibility (TICS) is the growing recognition that, independent of temperature, chronic contaminant exposure can also stimulate metabolism. Higher metabolism occurs because detoxification and elimination mechanisms must continually function at a higher rate. Chronically higher metabolism, from contaminants alone, occurs in fish (Beyers et al., 1999), larval amphibians (McDaniel et al., 2004), and bivalves (Lannig et al., 2008). Thus, researchers are raising the concern that organisms living in chronically polluted waters might be at greater risk from global warming if they cannot rally the additional energy needed to cope with compounding thermal stress (Lannig et al., 2008; Sokolova and Lannig, 2008; Hooper et al., 2013). Whether either the TICS or CITS hypothesis holds sway, cumulative stressors that increase risk in general (Landis et al., 2013) can divert energy resources from growth and reproduction; effects that can potentially cascade to impacts on populations and communities (Moe et al., 2013).

The worldwide distribution of sublethal contaminants makes their potentially cumulative effects important to wildlife conservation (Viers et al., 2009; Landers et al., 2010). Violations of the criteria that are protective of freshwater organisms, which are subject to site-specific exemptions, are not compiled nationwide (USEPA, 2009). Drinking water criteria, however, are monitored, and if appropriate, designated as legally impaired (i.e. 303(d) listed) under the United States Clean Water Act (United States Congress, 1972). Drinking water criteria are 10–100-fold less stringent than the criteria that apply to freshwater life. Over 42,000 individual waterways in the United States exceed drinking water criteria. Twenty-eight percent of these waterways are impaired because of metals contamination (USEPA, 2014). As discussed above, diel temperature fluctuations alone can have important impacts on biological responses (Caissie, 2006). To our knowledge, no studies examine the combined effects of asymmetrical warming and contaminant exposure. A few have examined the effects of contaminants when compounded by abrupt shifts from one acclimation temperature to another (Kimberly and Salice, 2014) and references therein Moreover, laboratory studies conducted at constant mean temperatures are not always predictive of the results found under gradual diel temperature fluctuations (Niehaus et al., 2012). Herein we present the biological responses of aquatic organisms when asymmetric nighttime warming compounds effects of contaminants.

We investigated the effects of temperature regimes, which varied in level of diel temperature fluctuation, on the lethality and behavioral effects of metals mixtures in three amphibian species. We tested three hypotheses: 1) Nighttime cooling can provide significant thermal relief to aquatic ectotherms from effects of contaminated waters; 2) Nighttime temperatures and the effects of metals contaminants are significantly correlated; and 3) The response to elevated nighttime temperature is consistent among the distinct amphibian taxa tested herein.

2. Methods

2.1. Test Species

Cope's gray tree frogs (*Hyla chrysoscelis*), southern leopard frogs (*Lithobates sphenocephalus*), and spotted salamanders (*Ambystoma maculatum*) were chosen to represent a variety of amphibian taxa whose ranges encompass extensive portions of the Eastern United States. According to the International Union for Conservation of Nature's “Red List of Threatened Species”, all three species have widespread distributions and conservation ratings of “least concern” (www.iucnredlist.org). Adult Cope's gray tree frogs have a critical thermal maximum (CT_{max}) of 38.0–41.5 °C (Blem et al., 1986; Lutterschmidt and Hutchison, 1997). Larval spotted salamanders have a CT_{max} of 28.1–37.9 °C (Keen and Schroeder, 1975). To our knowledge, the thermal sensitivity of southern leopard frogs has not been quantified. Larval southern leopard frogs, however, prefer temperatures of 17.5–30.4 °C, depending on their developmental stage (Dupre and Petranka, 1985), and larvae from the closely related northern leopard frog (*Lithobates pipiens*) have a CT_{max} above 37.0 °C (Noland and Gordon, 1981). We collected frog egg masses from ponds at Southern Illinois University and salamander eggs in the Shawnee National Forest, Illinois.

2.2. Experimental design

We ran toxicity tests with a full factorial experimental design of three replicates, five metals treatments, and three or four temperature regimes for salamander and frog species, respectively. Test duration was 96 h. This full factorial type of research design consists of experimental treatments from all combinations of the levels of two or more distinct treatment factors. For example, this study was a 5 × 3 or 5 × 4 factorial arrangement, meaning all five levels of metals appear in combination with all 3 or 4 temperature regimes. Temperature regimes varied by day:night temperatures, representing a range of diel temperature variation, meant to encompass possible effects of asymmetrical nighttime warming (Fig. 1). The test matrix consisted of filtered (1.0 μm ashed, glass fiber filters) lake water plus four additions of a metals mixture of Cd, Cu, and Pb, for a total of five treatments (Table 1). Upon hatch, five larval salamanders or ten larval frogs were placed in 800 ml tri-pore beakers with 600 ml of test matrix per replicate. Beaker locations were randomized within incubators (Thermo incubators, model 818). All tests ran for 96 h with 14:10 day–night light cycles. Temperatures were adjusted by 0.5 °C every half hour after a change in light until daytime or nighttime test temperature was reached. Test solutions were renewed daily. During renewals, dead larvae were counted, and dead larvae were removed.

Temperature treatments had nominal targets of 22:22, 22:20, and 22:18 °C for all species. For Cope's gray tree frogs and southern leopard frogs, we added a constant temperature regime of 20:20 °C (Fig 1). These temperature regimes are biologically conservative because temperatures are well below the CT_{max} or within the preferred temperature range of all three species, particularly the larval southern leopard frog (see section 2.1. Test Species, above).

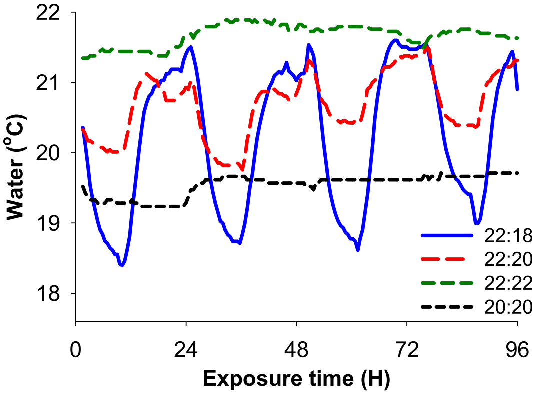

Fig. 1. (Single column image) Average temperatures in bioassays. Target values for temperature regimes are listed as day:night. The 20:20 treatment was not present in the spotted salamander experiments.