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Temperature and salinity effects on the toxicity of common pesticides to the grass shrimp, *Palaemonetes pugio*

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This study investigated the effects of increased temperature and salinity, two potential impacts of global climate change, on the toxicity of two common pesticides to the estuarine grass shrimp, *Palaemonetes pugio*. Larval and adult grass shrimp were exposed to the fungicide chlorothalonil and the insecticide Scourge® under standard toxicity test conditions, a 10°C increase in temperature, a 10 ppt increase in salinity, and a combined increased temperature and salinity exposure. Toxicity of the fungicide chlorothalonil increased with temperature and salinity. Toxicity of the insecticide Scourge® also increased with temperature; while increased salinity reduced Scourge® toxicity, but only in adult shrimp. These findings suggest that changes in temperature and salinity may alter the toxicity of certain pesticides, and that the nature of the effect will depend on both the organism's life stage and the chemical contaminant. Standard toxicity bioassays may not be predictive of actual pesticide toxicity under variable environmental conditions, and testing under a wider range of exposure conditions could improve the accuracy of chemical risk assessments.

Keywords: Climate change; pesticides; toxicity; estuaries; shrimp.

Introduction

Ecological risk assessments are, in part, based on results of toxicity tests conducted under standard exposure conditions. Global climate change could have a wide range of effects on aquatic habitats, including changes in water temperature and salinity, which may alter the risk assessment of aquatic pollutants. Variation in water temperature and salinity may impact the toxicity of pollutants, both because of altered chemical fate and transport, and because of changes in physiological response. Estuarine systems are considered especially sensitive to climate change, as they are already subject to considerable anthropogenic stress.[1]

While there is still a great deal of controversy over the magnitude and effects of global climate change, there is a general consensus on a few predictions. Global air temperatures are predicted to increase between 1–6°C over the next 100 years.[2] Rising temperature may cause thermal expansion and ice cap melting, leading to a global rise in sea level.[3–4] These major factors may cause numerous effects in estuarine systems, including increased water temperature, current alteration, increased salinity, increased erosion, and alteration in freshwater runoff patterns.[5–6] Additional alterations of the estuarine environment may follow, including changes in the flooding zones and brackish water boundaries, residence times, and water quality.[5]

Over half (53%) of all U.S. residents now live within 50 miles of the coast.[7] Estuaries provide people with commercial and recreational services, but also provide invaluable ecosystem services such as nutrient cycling, flood control, waste treatment, and species habitat.[8] In the southeastern United States, increased resort and golf course development in coastal areas may increase pesticide runoff into estuaries.[9] Toxicity data are available for many commonly used pesticides based on their effect on organisms under standard test conditions. These test conditions, however, may not reflect actual environmental conditions which fluctuate on a seasonal and even daily basis. Physiological effects of increased temperature and salinity may change how organisms respond to pesticides.[10] Additionally, changes in water quality may change the transport and fate of chemicals within the estuarine system, potentially altering their toxicity.

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This study investigated the effects of increased temperature and salinity, two potential impacts of global climate change, on the toxicity of two common pesticides to adult and larval grass shrimp (*Palaemonetes pugio*). *P. pugio* is widely distributed throughout the Atlantic and Gulf coasts of the Uinted States.[11] Grass shrimp are known to survive in a range of temperatures and are considered halotolerant.[12]

Chlorothalonil (2, 4, 5, 6-tetrachloroisophthalonitrile), a broad spectrum fungicide, is the second most widely used fungicide in the United States.[13] Primarily used for agriculture, golf course maintenance, and lawn care, chlorothalonil is applied via broadcast, band, or foliar spray year round, leading to its chronic presence in water bodies.[13] It is thought to persist up to 10 days in marine waters[13] and has been detected in estuarine surface waters.[14–15]

Scourge® is a common insecticide used primarily to target adult mosquitoes. Its active ingredients are resmethrin (5-(phenyl methyl)-3-furnylmethyl-2, 2-dimethyl-3-(2-methyl-1-propenyl) cyclopropane carboxylate) combined with a synergist, piperonyl butoxide.[16] Resmethrin is a synthetic pyrethroid, which targets the peripheral and central nervous system by interacting with sodium channels. Due to its rapid sunlight degradation and easy sorption, chronic resmethrin exposure is uncommon; however, acute pulses due to active spraying are a concern for aquatic organisms.[16]

Exposures were conducted to examine the toxicity of chlorothalonil and Scourge® under standard conditions compared to toxicity at elevated temperatures and salinities. These toxicity tests under altered conditions will help assess the validity of standard toxicity bioassays under changing natural conditions.

Materials and methods

Adult *P. pugio* were collected from Leadenwah Creek (N 32° 38.850′, W 80° 13.301′), a pristine tidal tributary of the North Edisto River, SC. Shrimp were held in the lab in 72-L tanks (25°C, 20 ppt natural seawater, 16:8 light: dark photoperiod) for 7–14 days prior to testing. Shrimp were fed a mixture of Tetramin® Fish Flakes and newly hatched *Artemia*. Gravid females were placed in brooding traps to allow larvae (zoea) to hatch and escape without interference. Larvae from at least ten females were pooled for testing.

Aqueous static renewal tests were conducted to determine 96 h LC50 (median lethal concentration) values for chlorothalonil and Scourge® under different temperatures and salinities. Larval and adult shrimp tests were conducted in Revco® environmental chambers set at either 25°C or 35°C and a 16 hr light: 8 hr dark cycle. Before each daily media change, water quality parameters (dissolved oxygen, pH, temperature, and salinity) were measured. The final concentration of the acetone carrier was equivalent in the control and all treatments (0.1%).

Larvae used for all tests were 1–2 d old and exposed in 600-mL glass beakers containing 400 mL of media with ten larvae per beaker and three replicates per treatment. Larvae were assessed daily to determine survival and fed newly hatched *Artemia* after each daily media change. Nominal chlorothalonil concentrations for the larval exposures were 15.63, 31.25, 62.5, 125, and 250 μg/L. Nominal Scourge® concentrations were 0.07, 0.22, 0.67, 2, and 6 μg/L.

Adult shrimp (20-30 mm in length) were exposed in 4-L wide-mouth glass jars containing 2 L of media with ten shrimp per jar and three replicates per treatment. Adults were not fed during the exposure. Nominal chlorothalonil concentrations for the adult exposures were 31.25, 62.5, 125, 250, and 500 μg/L. Nominal Scourge® concentrations were 0.51, 1.28, 3.2, 8, and 20 μg/L.

For each pesticide, the test was conducted under standard conditions (25°C and 20 ppt),[17–18] increased temperature conditions (35°C and 20 ppt), increased salinity conditions (25°C and 30 ppt), and both increased temperature and salinity conditions (35°C and 30 ppt).

Mortality data were used to calculate 24 h and 96 h LC50 values with Probit Analysis (PROC PROBIT, SAS V.9.1.3, Cary, NC, USA). Data were expressed as percent of control to account for effects of the different exposure conditions on control shrimp survival. Significant differences ($p < 0.05$) between LC50s under standard exposure conditions versus altered exposure conditions were determined using the LC50 ratio test.[19] Two-factor Analysis of Variance (ANOVA) (PROC GLM, SAS V.9.1.3, Cary, NC, USA) was used to examine the interaction between exposure conditions and pesticide concentration, with exposure condition and pesticide concentration as the independent variables and mortality as the dependent variable.

Results and discussion

Grass shrimp were found to be sensitive to the higher exposure temperature of 35°C, particularly the larval life stage. In the absence of pesticides, increasing salinity to 30 ppt did not alter shrimp survival compared to 20 ppt, whereas elevated temperature (35°C) reduced survival compared to standard conditions (25°C) (Figure 1). The effect of temperature was greater for larval shrimp (Figure 1A) than for adult shrimp (Figure 1B). Adult control mortality was significantly different from standard conditions only after 24 h of increased temperature exposure (24 h $p = 0.0280$, 96 h $p = 0.0988$). Larval control mortality was not significantly different in any of the exposures after 24 h ($p = 0.3524$), but was significantly greater with increased temperature exposures after 96 h ($p = 0.0004$). Control survival for larval shrimp at 35°C was approximately 50% after 96 h,

Table 1. Results of the two-factor analysis of variance tests, with exposure condition and pesticide concentration as the independent variables and mortality as the dependent variable. Significant differences (p value < 0.05) were observed for all treatments and interactions.

Life stage/ pesticide	*Time*	*Exposure conditions*	*Pesticide concentration*	*Interaction between exposure & concentration*
Larval shrimp/ chlorothalonil	24 h	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
	96 h	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Adult shrimp/ chlorothalonil	24 h	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
	96 h	$p < 0.0001$	$p < 0.0001$	$p < 0.0008$
Larval shrimp/ Scourge®	24 h	$p < 0.0001$	$p < 0.0001$	$p = 0.0148$
	96 h	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
Adult shrimp/ Scourge®	24 h	$p < 0.0001$	$p < 0.0001$	$p < 0.0001$
	96 h	$p < 0.0001$	$p < 0.0001$	$p < 0.0138$

therefore only 24 h LC50 values were calculated for increased temperature with the larval life stage.

In preliminary experiments, we attempted to hold the shrimp for two days at the higher temperature before initiating the pesticide exposure. This resulted in even greater control mortality. As evident in the data presented here, the shrimp can tolerate the higher temperature for a short duration, but mortality increases with time. Control survival was not significantly affected by the increase in salinity, which is consistent with the grass shrimp being a halotolerant species.[12] To account for the greater control mortality found at higher temperature, all LC50 values were calculated using control-corrected data. The LC50 values presented could thus be considered conservative estimates of pesticide toxicity under this multi-stressor scenario.

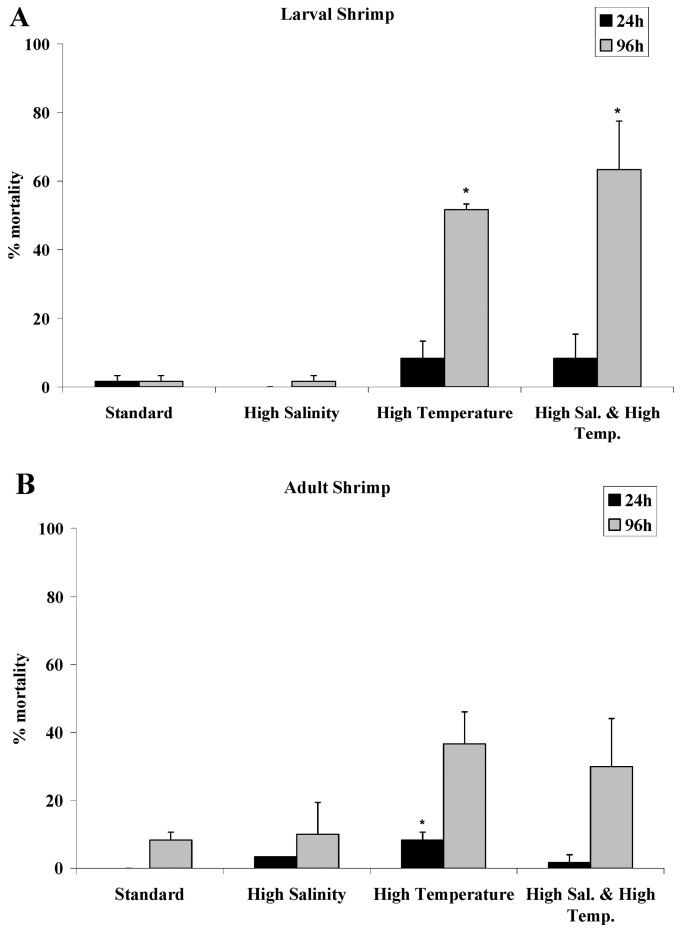

Fig. 1. Control shrimp survival (A = larvae, B = adult) under the different exposure conditions. Bars represent averages and standard deviations of the two sets of experiments (chlorothalonil and Scourge®). Asterisks indicate significant differences in mortality compared to standard conditions.

Scourge® and chlorothalonil are commonly used pesticides in the Southeast U.S. coastal zone and their toxicity to *P. pugio* has been described under standard test conditions of 25°C and 20 ppt.[17–18] Estuarine organisms are exposed to a wide range of temperature and salinity, thus it is important to examine the interaction between temperature, salinity, and pesticide toxicity.

Two-factor analysis of variance (ANOVA) revealed a significant interaction between exposure conditions and pesticide concentration on grass shrimp survival (Table 1). The p-values for each factor individually and for the interaction were all significant (Table 1), indicating that pesticide concentration affects shrimp survival, exposure condition affects shrimp survival, and exposure conditions affect pesticide toxicity.

Figure 2 shows the 24 h percent-effect probability curves calculated with the Statistical Analysis Software (v. 9.1, SAS Institute Inc.; SAS) probit model for chlorothalonil. The curves represent the predicted dose-response under each exposure condition, and the separation of the curves represents differences in toxicity with varying conditions. Chlorothalonil toxicity increased in shrimp exposed at 35°C as compared to those exposed at 25°C in both larval (Figure 2A) and adult life stages (Figure 2B). Chlorothalonil toxicity also increased with increased salinity as compared to standard conditions, but was significant ($p = 0.026$) only after 96 h (Table 2). Time played a significant role in chlorothalonil toxicity, with approximately three times greater toxicity after 96 h than 24 h with adult

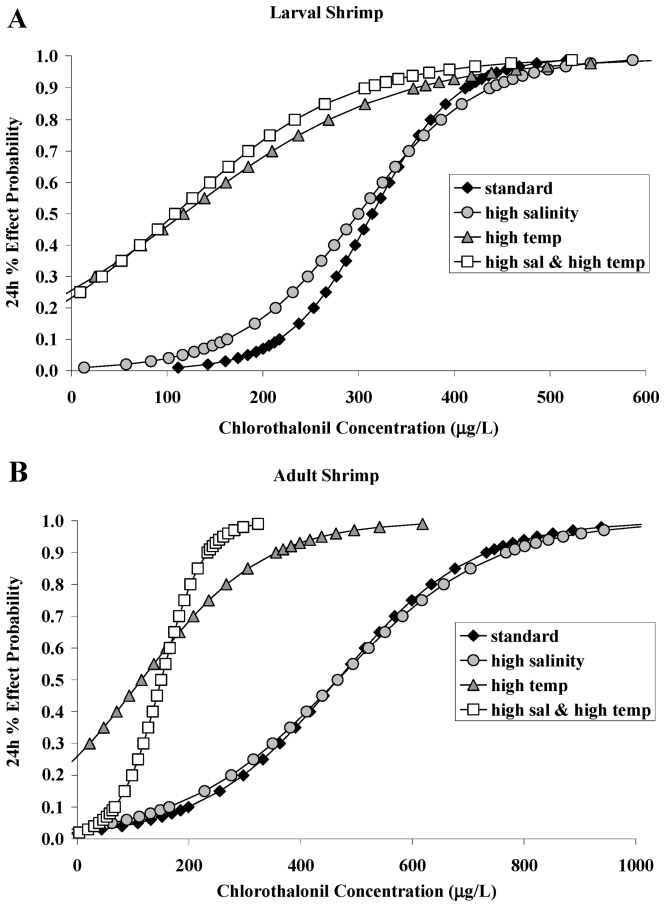

Fig. 2. Predicted 24 h percent effect probability curves from SAS probit analysis for each exposure condition. A = chlorothalonil exposures with larval shrimp; B = chlorothalonil exposures with adult shrimp.

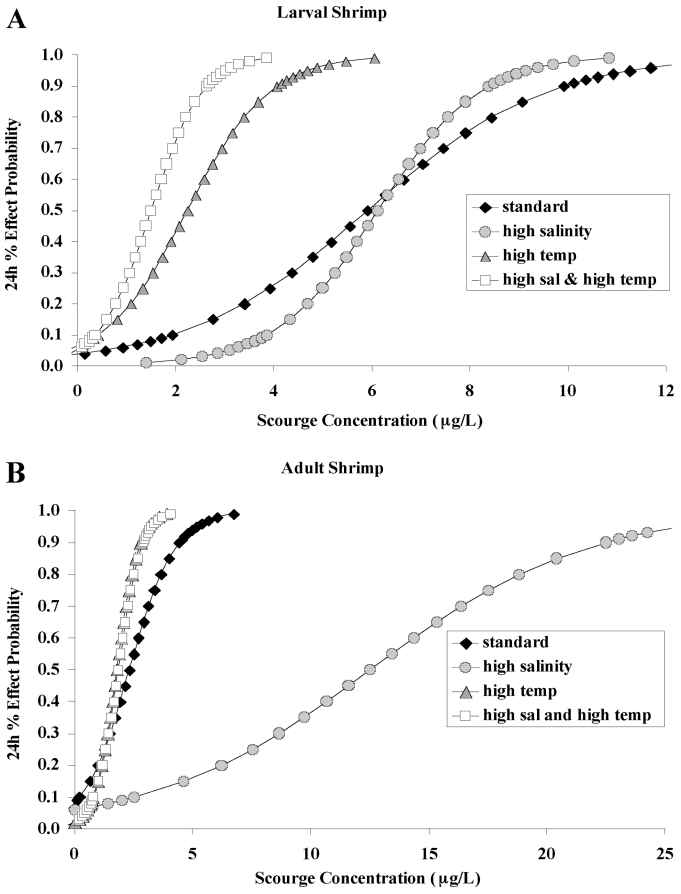

Fig. 3. Predicted 24 h percent effect probability curves from SAS probit analysis for each exposure condition. A = Scourge® exposures with larval shrimp; B = Scourge® exposures with adult shrimp.

shrimp, and approximately six times greater toxicity after 96 h compared to 24 h with larval shrimp. The increase in chlorothalonil toxicity due to increased salinity was also time dependent, with significantly different LC50 values after 96 h, but not after 24 h in both adult and larval shrimp (Table 2).

Increased temperature also increased the toxicity of Scourge® to adult and larval grass shrimp (Figure 3). The effect of temperature was evident after 24 h, but was not significant after 96 h (Table 3). In contrast to chlorothalonil, increased salinity decreased Scourge® toxicity in adult *P. pugio* (Figure 3B). At 24 h, the Scourge® LC50 was approximately five times higher for the increased salinity exposure than standard conditions (Table 3). Larval shrimp toxicity was not significantly different under increased salinity compared to standard conditions (Table 3). The combined exposure of increased temperature and increased salinity resulted in increased Scourge® toxicity to both larval and adult shrimp after 24 h, but there was no significant difference after 96 h (Table 3).

Both pesticides were found to be more toxic at the higher temperature than under standard testing conditions. This may be due to an increased metabolic rate at higher temperatures, resulting in increased water movement across the gills and increased pesticide uptake. The effect of increasing salinity was not consistent across pesticides. Increased salinity increased chlorothalonil toxicity, but decreased Scourge® toxicity. Scourge® may become less bioavailable in higher salinity due to binding. The effect however, was limited to adult shrimp, perhaps indicating a physiological difference between life stages. Combining increased salinity with increased temperature resulted in increased toxicity of both chlorothalonil and Scourge® to *P. pugio*.

Depending on the rate of increase in global temperatures, there may be a selective pressure for more thermotolerant individuals in estuarine populations. It is possible that future grass shrimp populations could gradually adapt to longer durations of elevated temperatures; an aspect of global climate change impacts on grass shrimp survival that could not be addressed with this study. These results do indicate, however, that changing temperature and salinity may alter the toxicity of certain pesticides. The extent and direction of this toxicity alteration will depend on the specific pesticide involved and the life stage of the organism

Table 2. Toxicity values for grass shrimp exposed to chlorothalonil. LC50 values for each test condition are based on percent of control mortality; CI = confidence interval. 96 h LC50 values were not determined (ND) for the larvae in the high temperature exposures due to insufficient control survival after 96 h.

Larval shrimp

Exposure conditions	*Duration*	*LC50 (μg/L)*	*95% CI (μg/L)*	*LC50 ratio test comparison to standard conditions*
Standard	24 h	314.3	276.1–423.5	
	96 h	49.09	42.74–56.26	
High salinity	24 h	299.8	256.4–378.0	p value = 0.7044
	96 h	39.40	34.03–46.43	p value = 0.0260
High temperature	24 h	117.1	78.92–169.1	p value = <0.0001
	96 h	ND	ND	ND
High salinity &	24 h	108.3	76.10–149.1	p value = <0.0001
high temperature	96 h	ND	ND	ND

Adult shrimp

Exposure conditions	*Duration*	*LC50 (μg/L)*	*95% CI (μg/L)*	*LC50 ratio test comparison to standard conditions*
Standard	24 h	465.8	399.5–569.2	
	96 h	156.0	127.8–191.7	
High salinity	24 h	466.2	393.6–586.4	p value = 0.9949
	96 h	116.2	92.62–144.7	p value = 0.0411
High temperature	24 h	115.19	68.76–159.0	p value = <0.0001
	96 h	32.62	4.88–45.86	p value = <0.0001
High salinity &	24 h	150.4	129.1–178.6	p value = <0.0001
high temperature	96 h	44.36	25.34–57.79	p value = <0.0001

Table 3. Toxicity values for grass shrimp exposed to Scourge®. LC50 values for each test condition are based on percent of control mortality; CI = confidence interval. 96 h LC50 values were not determined (ND) for the larvae in the high temperature exposures due to insufficient control survival after 96 h.

Larval shrimp

Exposure conditions	*Duration*	*LC50 (μg/L)*	*95% CI (μg/L)*	*LC50 ratio test comparison to standard conditions*
Standard	24 h	5.91	4.86–7.68	
	96 h	1.35	1.12–1.65	
High salinity	24 h	6.12	5.42–7.19	p value = 0.7831
	96 h	1.64	1.39–1.92	p value = 0.1212
High temperature	24 h	2.24	1.78–3.04	p value = <0.0001
	96 h	ND	ND	ND
High salinity &	24 h	1.49	1.22–1.87	p value = <0.0001
high temperature	96 h	ND	ND	ND

Adult shrimp

Exposure conditions	*Duration*	*LC50 (μg/L)*	*95% CI (μg/L)*	*LC50 ratio test comparison to standard conditions*
Standard	24 h	2.31	2.05–2.61	
	96 h	1.22	0.99–1.56	
High salinity	24 h	12.59	11.37–14.02	p value = <0.0001
	96 h	2.10	1.59–2.74	p value = 0.0008
High temperature	24 h	1.89	1.71–2.10	p value = 0.0125
	96 h	0.99	0.72–1.29	p value = 0.1960
High salinity &	24 h	1.83	1.35–2.04	p value = 0.0036
high temperature	96 h	0.86	0.43–1.20	p value = 0.1113

exposed. This will influence the outcome of toxicity tests used for risk assessment, both now and in the future.

Continuous monitoring data (2007–2008) from Leadenwah Creek, SC, show temperature ranged from 2.79–37.88°C. Salinity for the same time period ranged from 0–36.83 ppt. This suggests that standard toxicology data are not always applicable to southeastern U.S. estuarine systems. These gaps between field conditions and standard toxicology protocols will only widen if climate change continues as predicted. To encompass this variability and account for future changes in climate, toxicology tests should be conducted across a wider range of environmental conditions.

Conclusion

The interactive effects of climate change with anthropogenic stresses, such as pollution, are not well understood. In this study, toxicity of the fungicide chlorothalonil to the grass shrimp (*P. pugio*) increased with temperature and salinity. Toxicity of the insecticide Scourge® also increased with temperature; while increased salinity reduced Scourge® toxicity, but only in adult shrimp. Therefore, changes in temperature and salinity may alter the toxicity of certain pesticides, and the nature of the effect will depend on both the organism's life stage and the chemical contaminant. Standard toxicity bioassays may not be predictive of actual pesticide toxicity under variable environmental conditions, and testing under a wider range of exposure conditions could improve the accuracy of chemical risk assessments.

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