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Rapid communication

The thyroid endocrine disruptor perchlorate affects reproduction, growth, and survival of mosquitofish

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Abstract

The perchlorate anion—an oxidizer found in rockets, missiles, some ammunition, flares, airbags, and fireworks—occurs as a contaminant in ground and surface water in many parts of the United States. Its toxic effects include inhibition of thyroid hormone synthesis. To investigate its chronic toxicity, mosquitofish (*Gambusia holbrooki*) adults and fry were exposed to aqueous sodium perchlorate at 1, 10, and 100 mg/L, and growth and reproductive performance (fecundity, eggs/embryos mass, and gonadosomatic index [GSI]) were determined. Five-day acute toxicity tests were also performed. Perchlorate had a stimulatory effect on fecundity, GSI, and egg/embryo mass, at least for some treatments. The LC_{50} of sodium perchlorate was 404 mg/L. Growth was enhanced at 1 mg/L but inhibited at 10 mg/L. These results suggest that, at environmentally relevant concentrations, perchlorate does not induce acutely toxic effects but may have mild stimulatory or hormetic effects on fitness parameters in this species.

Keywords: Perchlorate; Reproduction; Growth; LC_{50}; Fish; Mosquitofish; *Gambusia*; Acute toxicity; Chronic toxicity; Thyroid

1. Introduction

Ammonium perchlorate (AP) is used as an oxidizer in the propulsion systems of solid fuel rockets and missiles and is used in the manufacture of flares, fireworks, and automobile airbags in the US (Greer et al., 2002). Because of its limited shelf life, periodic replacement of perchlorate in rocket and missile propulsion systems is required. Therefore, large quantities have been removed and disposed of at solid fuel rocket and missile processing facilities. Recently, improved detection methods have revealed widespread perchlorate contamination of groundwater at such facilities located in several states in the US, especially Utah, California, Nevada, Arizona, and Texas (Motzer, 2001; Smith et al., 2001).

Because perchlorate salts have relatively high solubility in water, once released into aqueous systems, they can readily dissociate, producing their corresponding cations and the perchlorate anion. The resultant perchlorate ion has been characterized by rapid mobility, stability, and nonreactivity in the environment. Thus, perchlorate in aquatic systems can persist for years to decades (Volkoff et al., 1999; Clark, 2000). Consequently, fish in contaminated surface waters may be affected by perchlorate contamination.

Environmental perchlorate (ClO_4^-) contamination is of concern because it is known to cause adverse effects on the thyroid gland in vertebrates. Due to its use as a treatment for Grave's disease (Wolff, 1998) and research into effects of exposures to humans and other

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vertebrates (Manzon et al., 2001; Brechner et al., 2000; Lawrence et al., 2000; Goleman et al., 2002; York et al., 2001; Kao et al., 1999; Patiño et al., 2003; Zoeller, 2003), much is known about the effects of perchlorate on thyroid function. The mechanism of thyrotoxicity is inhibition of iodide uptake via competitive binding with the sodium/iodide symporter in thyroid follicles. This results in reduced uptake of iodide and diminished thyroid hormone production, which may lead to reduced serum concentrations of thyroxin (T_4) and triiodothyronine (T_3) (Saito et al., 1983; Wolff, 1998; Clark, 2000; Yu et al., 2002). Thyroid hormones have important roles in regulating growth, cell differentiation, embryonic and neurological development, reproduction, and the metabolism of lipids, proteins, and carbohydrates in vertebrates (Clark, 2000). In fish, they may also affect larval metamorphosis and osmoregulation (Kime, 1998). Therefore, perturbation of thyroid homeostasis by environmental contaminants may affect fitness components (e.g., growth, development, and reproduction) in fish (Power et al., 2001; Volkoff et al., 1999), which could ultimately lead to effects at the population, community, or ecosystem levels. However, there are relatively few studies on toxicological effects of perchlorate in fish (Patiño et al., 2003; Dean et al., 2004; Crane et al., 2005).

The purpose of this study was to investigate the growth rate, reproductive effects, and 5-day median lethal concentration (LC_{50}) in eastern mosquitofish (*Gambusia holbrooki*) exposed to a range of sublethal or lethal concentrations of sodium perchlorate in water. The hypotheses were that exposure to perchlorate would decrease growth and reproductive performance in this species and that the LC_{50} would be comparable to that found in other fish species (Patiño et al., 2003; Dean et al., 2004).

2. Materials and methods

2.1. Test chemical

Sodium perchlorate (EM Science, Gibbstown, NJ) was used to make a stock solution (pH 7.4) at several different perchlorate concentrations. Sodium perchlorate was chosen over the ammonium salt to examine the effects of perchlorate ion alone, without the confounding effects of ammonium toxicity. Reconstituted water (60 mg/L; Instant Ocean sea salts in reverse osmosis water) was used for acclimation and testing.

2.2. Test animal and rearing conditions

Eastern mosquitofish were used because they are common in the eastern US and are of ecological importance and *Gambusia* are commonly found in abundance in perchlorate-contaminated areas (Smith et al., 2001; Theodorakis et al., 2005).

Adult fish were purchased from hatcheries and were acclimated to laboratory conditions for 1 week before the start of the experiments. An 18/6-h light/dark photoperiod was maintained. During the acclimation period, fish were fed commercial flake goldfish food twice daily. Debris and uneaten food were removed from the bottom of the tank each morning prior to feeding. Dissolved oxygen, conductivity, temperature, pH, and salinity were measured with a multiparameter water quality meter (YSI Model 85 meter; Yellow Springs, OH), and ammonia in water was measured spectrophotometrically (Hach spectrophotometer Model DR/2000; Loveland, CO). Measurements were recorded every other day.

To obtain fry, gravid females were placed in 80-L aquaria and fry were collected for 1 week. Fry that were less than 1 week old were used for the survival and growth experiments.

2.3. Reproduction

Adult mosquitofish (two males and four females) were randomly assigned to aquaria and exposed to various concentrations (0, 1, 10, and 100 mg/L; nominal concentrations) of sodium perchlorate for 8 weeks. There were five replicates at each dosing concentration of sodium perchlorate. During the exposure, photoperiod and feeding frequency were the same as the acclimation period. Water in the aquaria was maintained at 25 °C. Breeding activity was induced by maintaining fish in 180 mg/L Instant Ocean sea salts in water and adding artificial plastic plants that occupied at least 50% of the volume of the aquaria. The water in each aquarium was aerated and continuously filtered with a glass wool filter.

A 0.5-cm-thick layer of activated charcoal was added to the filtration system to remove any contaminants that may have been leached from the artificial plastic plants. Phthalate esters are routinely used as plasticizers and typically leach from plastic products that are in contact with aqueous solutions, and di-(2-ethylhexyl)-phthalate is one of the phthalate esters most commonly detected in aqueous samples (Suzuki et al., 2001). Thus, 50 mL of water was collected once each week from each aquarium. This water was extracted with a C-18 solid phase extraction cartridge, eluted with 50:50 methanol:chloroform, and analyzed by gas chromatography–mass spectrometry as described in Suzuki et al. (2001) and Fatoki and Noma (2002).

One-third of the water in the aquaria was changed every other day. An appropriate amount of sodium perchlorate was added to adjust the concentration to the original nominal value. Water quality was checked and recorded every other day during the exposure period.

Water samples in each aquarium were collected twice per week and analyzed to monitor perchlorate concentration using ion chromatography. At the end of exposure, female mosquitofish were euthanized with MS-222 (1.0 g/L in bicarbonate-buffered, reconstituted water, pH 7.0) and dissected to obtain their broods.

2.4. Survival

Ten fry were randomly assigned to 500-mL beakers and continuously exposed to 100, 300, 500, 800, 1000, and 2000 mg/L (nominal concentrations) of sodium perchlorate in ambient water for 5 days. These test concentrations were chosen based upon results of preliminary range-finding tests (data not shown). Tests were run at 25 °C in controlled-environment beakers. There were five replicates of each concentration plus six control (no perchlorate) beakers. During the exposure, fry were fed commercial frozen brine shrimp once per day. Eighty percent of the water in each beaker was changed every other day and an appropriate amount of sodium perchlorate was added to adjust the concentration to the original nominal value. Water quality was checked and recorded every other day. To monitor concentration of perchlorate in the beaker, water samples were collected every other day and analyzed using ion chromatography. Mortality and abnormal behavior were monitored daily and dead fry were removed immediately.

2.5. Growth

One hundred and seventy fry (<1 week old) were weighed and randomly assigned to 18 500-mL beakers (10 fish per beaker). Fry were continuously exposed to 1, 10, and 100 mg/L sodium perchlorate for 4 weeks. There were five replicates at each dose plus three control beakers. During the exposure, beakers were kept in an incubator at 25 °C and 18/6-h light/dark photoperiod. Feeding, frequency of water changes, and monitoring of water quality were similar to those used in the survival experiment. Fry mortality and abnormal behavior were also recorded. To monitor the concentration of perchlorate in the beakers, water samples were collected every other day and analyzed using ion chromatography.

2.6. Effects of NaCl on growth, reproduction, and survival

To determine possible confounding effects of osmolarity on growth, reproduction, and survival, a duplicate experiment was conducted using the methods described above by adding NaCl to reconstituted water. The concentrations of NaCl were equal to the concentrations of sodium perchlorate described above.

2.7. Analysis of perchlorate in water samples

Analytical methods for assessing perchlorate in water followed those described in Smith et al. (2001). Water samples were filtered through a 0.45-μm Acrodisc filter into a 5-mL vial (Dionex Corp., Sunnyvale, CA) and were analyzed using a DX-500 Ion Chromatography System equipped with a GP50 pump, a CD 20 conductivity detector, and an AS40 automated sampler. Ion separation was accomplished with a Dionex Ion Pac AS16 analytical column. Eight-point standard curves consisting of 2.5, 5, 10, 20, 50, 100, 200, and 500 μg/L perchlorate (for reproductive/growth effects) and 10, 50, 100, 250, 500, 1000, 2000, and 3000 μg/L perchlorate (for acute toxicity) were constructed using a 100-mg/L certified sodium perchlorate standard (AccuStandard, Inc., New Haven, CT). Sodium hydroxide (100 mM) was used as eluent at a flow rate of 1 mL/min. Resulting chromatograms were optimized and the perchlorate peak (when present) was identified based on the retention time of the standard.

2.8. Endpoint analysis

Fecundity, average mass per egg/embryo, and gonadosomatic index (GSI; the ratio of gonad weight to body weight) were used as the reproductive endpoints. Because the number of eggs produced in *Gambusia* is linearly related to standard length (Theodorakis et al., 1997), fecundity was recorded as number of eggs/standard length. For fry growth, percentage mass gain was calculated as final average mass/initial average mass, on a per-fish basis (i.e., the average mass per fry was calculated as total mass of all fry/number of fry); specific growth rate was calculated as $((\ln(\text{final mass}) - \ln(\text{initial mass})) \times 100)/\text{days of exposure}$ (Crossland, 1985). For fry survival, the endpoints were the number of survivors and the LC_{50}.

2.9. Water quality

All water quality parameters were measured on the day that the water was collected. Dissolved oxygen, conductivity, temperature, pH, and salinity were measured with a YSI Model 85 multiparameter meter, and unionized ammonia was measured with a HACH spectrophotometer Model DR/2000.

2.10. Histopathology

Histological preparations followed the methods of Carr et al. (2003) and Bradford et al. (2005). Histological effects were determined in the adult females used for reproductive studies at the end of the 6-week (42-day) reproductive studies. Heads of fish were cut off and fixed for 24 h in Bouin's solution (75% saturated picric

acid solution, 20% formalin, and 5% glacial acetic acid) for thyroid follicle examination. Prior to processing the samples, head were decalcified in Bouin's fixative. The tissues were then dehydrated in a series of ethyl alcohol and xylene baths and embedded in paraffin. Thin sections (5 μm) were cut using a rotary microtome cut 4055 (Olympus American Inc., Melville, NY), mounted on slides, stained with hematoxylin and eosin, and examined by light microscopy. As the thyroid tissues are usually found in fish around the ventral aorta and brachial arteries near the gills and tongue on the lower-jaw region, serial sections were made in this region. Five sections per fish were randomly chosen and percentages of follicles affected by hyperplasia (multiple layers of follicular cells), hypertrophy (simple columnar rather than cuboidal epithelium), and/or colloid depletion (as evidenced by reduction or absence in colloid, follicular epithelium infolding, or a pale, lacy, or vacuolated appearance of the colloid) were determined. The severity of thyroid damage was also assessed based upon the guidelines established by the EPA Pathology Working Group (Mann, 2000). According to these guidelines, the degrees of hypertrophy, hyperplasia, and colloid depletion were given scores of 0, 1, or 2 in order of increasing severity. The individual score was then calculated as the mean scores for each of the three metrics of colloid depletion, and the overall score was determined by summing each of these means (Hooth et al., 2001). Histological effects were also determined for adult female fish exposed to 0, 1, 10, and 100 mg/L (nominal concentration) NaCl for 6 weeks.

2.11. Statistical analyses

All statistical analyses were performed using SAS (version 8.0). For survival data, mean values and standard deviation (SD) were calculated for each group test. Percentage data (growth, survival) were arcsine square root transformed prior to statistical analysis (Sokal and Rholf, 1981). One-way ANOVA followed by Duncan's multiple range test was used to determine differences between mean values. Statistical differences were considered significant at overall α of 0.05. The LC_{50} for mosquitofish fry was obtained using probit analysis. For reproduction and histology endpoints, data were analyzed using the nonparametric Kruskall–Wallis test and were reported as medians with first/third quartiles. This was done because of the skewed distribution of the data, the fact that the shape of the distributions differed between treatments, and the fact that no transformation could satisfactorily normalize the data and homogenize the variances.

3. Results

Water quality parameters are reported in Table 1. For the growth and survival experiments, conductivity and salinity varied with perchlorate exposure concentration (Table 1). The relationships between actual and nominal perchlorate concentrations are reported in Table 2. In all samples DHEP was below detection limits. Detection limits calculated using water spiked with DHEP in

Table 1
Water quality parameters in the aquaria or beakers used for determination of acute toxicity and effects of sodium perchlorate on reproduction and growth in eastern mosquitofish (*Gambusia holbrooki*)

Experiment	Treatment	n[a]	pH	Temperature	Dissolved O_2 (mg/L)	Conductivity (mS/cm)[b]	Salinity (ppt)[c]	Total ammonia (mg/L)
Reproduction	Control	155	6.5±0.3	25.2±1.6	81.8±10.4	372.6±29.3[1]	0.2±0.0[1]	0.3±0.2
	1 mg/L	155	6.5±0.3	25.6±1.6	80.0±12.2	382.1±28.1[1]	0.2±0.0[1]	0.3±0.2
	10 mg/L	155	6.4±0.3	25.5±1.4	78.4±12.1	383.8±24.8[1]	0.2±0.0[1]	0.3±0.2
	100 mg/L	155	6.5±0.3	25.4±1.4	79.9±12.7	474.9±33.2[1]	0.2±0.0[1]	0.4±0.2
Growth	Control	30	6.7±0.2	24.4±0.7	50.0±14.9	140.0±9.9[2]	0.1±0.0[2]	0.7±0.3
	1 mg/L	75	6.7±0.2	24.9±0.5	48.3±15.8	133.3±17.5[2]	0.1±0.0[2]	0.7±0.2
	10 mg/L	75	6.6±0.2	24.2±0.7	48.1±11.2	148.0±11.0[2]	0.1±0.0[2]	0.7±0.2
	100 mg/L	75	6.6±0.1	23.9±0.7	48.7±13.1	228.6±16.0[3]	0.1±0.0[2]	0.6±0.2
Acute toxicity	Control	14	6.7±0.5	22.6±0.7	89.8±222.5	128.5±21.8[4]	0.1±0.0[1]	0.2±0.2
	100	8	6.7±0.5	23.3±0.6	64.1±19.4	190.4±13.9[5]	0.1±0.0[1]	0.3±0.2
	300	10	6.9±0.5	22.5±0.5	62.8±16.0	295.8±131.8[6]	0.2±0.1[1]	0.2±0.1
	500	10	6.9±0.5	22.8±0.3	58.7±12.6	513.1±177.3[7]	0.3±0.1[1,2]	0.3±0.1
	800	10	6.4±0.5	22.7±0.3	56.4±8.5	866.1±32.3[8]	0.4±0.0[2]	0.3±0.1
	1000	8	6.6±0.5	22.2±1.0	79.9±5.9	950.1±30.9[9]	0.5±0.0[3]	0.2±0.2
	2000	4	6.7±0.5	22.8±0.1	75.5±2.8	1840±24.0[10]	1.0±0.0[4]	0.3±0.0

Mean±SD.

[a] n, total number of water samples collected.

[b] Within each experiment, conductivity values that are labeled with a different number are statistically significantly different ($P<0.05$, ANOVA).

[c] Within each experiment, salinity values that are labeled with a different number are statistically significantly different ($P<0.05$, ANOVA).

Table 2
Comparison of nominal and measured concentrations[a] (mg/L) of perchlorate used for determination of acute toxicity and effects of sodium perchlorate on reproduction and growth in eastern mosquitofish (*Gambusia holbrooki*)

Experiment	Nominal concentration	Measured concentration	
		Range	Mean (±SD)
Reproduction	1	0.93–1.23	1.15 (±0.1)
	10	6.57–8.28	7.31 (±3.32)
	100	80.96–110.29	99.25 (±9.96)
Survival	100	75.30–90.24	81.57 (±3.84)
	300	253.64–293.06	270.27 (±15.44)
	500	403.63–511.76	427.24 (±32.49)
	800	435.50–602.47	565.25 (±37.12)
	1000	919.00–1044.17	926.76 (±72.25)
	2000	1596.5–1986.5	1803.15 (±126.64)
Growth	1	0.74–1.45	0.8 (±0.06)
	10	8.04–15.73	15.17 (±11.08)
	100	63.52–104.63	86.62 (±30.46)

[a]Perchlorate was not detected in controls (detection limit = 1 ng/mL).

methanol were 0.05 μg/L, which was at least an order of magnitude lower than concentrations found to elicit reproductive and developmental effects in fish (Kim et al., 2002; Chikae et al., 2004a, b). Percentage recoveries of DHEP ranged 75–83%.

3.1. Reproduction

Because there were no statistically significant differences among aquaria ($P>0.05$, Kruskall–Wallis test), all individuals for each treatment were pooled for reproductive analyses. Standard length ($P>0.05$) and weight ($P>0.05$) were not significantly different among treatments (Table 3; ANOVA). Gonadal somatic indices were greater in the 1- and 100-mg/L treatments than in the controls (Fig. 1A; $P<0.05$, Kruskall–Wallis test). Egg/embryo mass was greater in treated than in control samples only at 100 mg/L (Fig. 1B). Median fecundity was greater in all treatments compared to control (Fig. 1C; $P<0.05$, Kruskall–Wallis test). Concentration of NaCl (0, 1, 10, 100 mg/L) had no effect on GSI, egg mass, or fecundity ($P>0.30$, Kruskall–Wallis test; data not shown).

3.2. Survival

The results of the one-way ANOVA indicated that survival rates of mosquitofish fry were significantly different among treatments ($P<0.001$) and decreased with increasing doses of sodium perchlorate (Fig. 2). The estimated LC_{50} of mosquitofish fry was 404.4 mg/L, calculated using actual (Table 2) rather than nominal perchlorate concentrations. Abnormal behaviors and physical changes were observed during the exposure, including inverted (upside down) swimming, curvature of the spine, and cephalic lesions. Mortality of fry exposed to NaCl was not different from that of control for concentrations of NaCl in reconstituted water ranging 1–2000 mg/L ($P>0.30$, Kruskall–Wallis test; data not shown).

Table 3
Standard length and body mass of adult female mosquitofish used for the reproduction experiments and number surviving after 8 weeks of sodium perchlorate exposure

Nominal concentration (mg/L)	n[a]	Length (cm)[b]	Mass (g)
0	11	3.6±0.3	0.82±0.23
1	14	3.4±0.4	0.74±0.20
10	11	3.6±0.3	0.88±0.24
100	12	3.6±0.3	0.88±0.29

[a]Number of females surviving after 8 weeks.

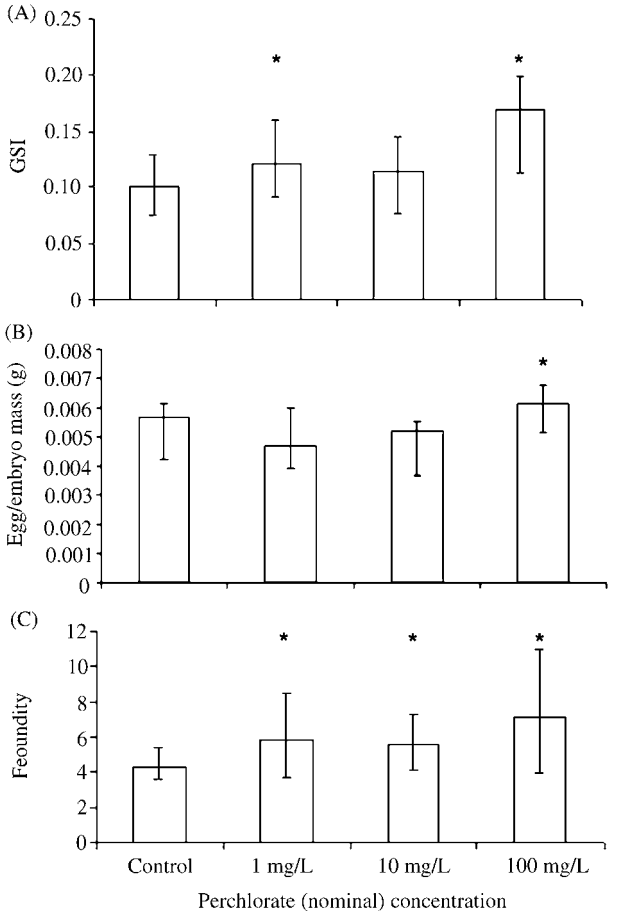

Fig. 1. (A) Gondal somatic index (GSI), (B) average mass per egg or embryo, and (C) fecundity (brood size/body length) of mosquitofish exposed to sodium perchlorate for 8 weeks. Bars represent medians and error bars are first and third quartiles. Bars labeled with an asterisk are statistically significantly different from controls ($P<0.05$, Kruskall–Wallis test).

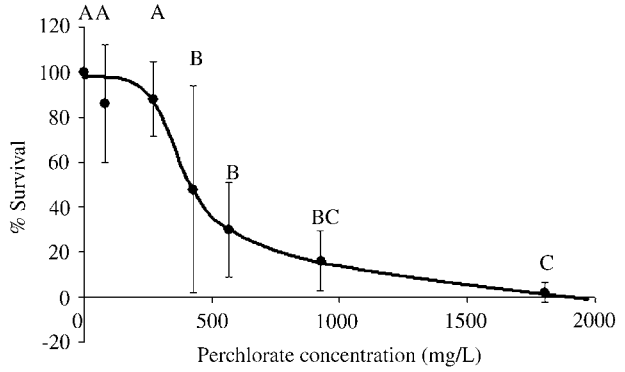

Fig. 2. Mean survival rates of mosquitofish fry exposed to different concentrations of sodium perchlorate for 5 days. Error bars represent standard deviation. Data points labeled with different letters are statistically significantly different from each other ($P<0.05$, ANOVA) ($n=7$ replicate beakers for control; $n=5$ for treated groups).

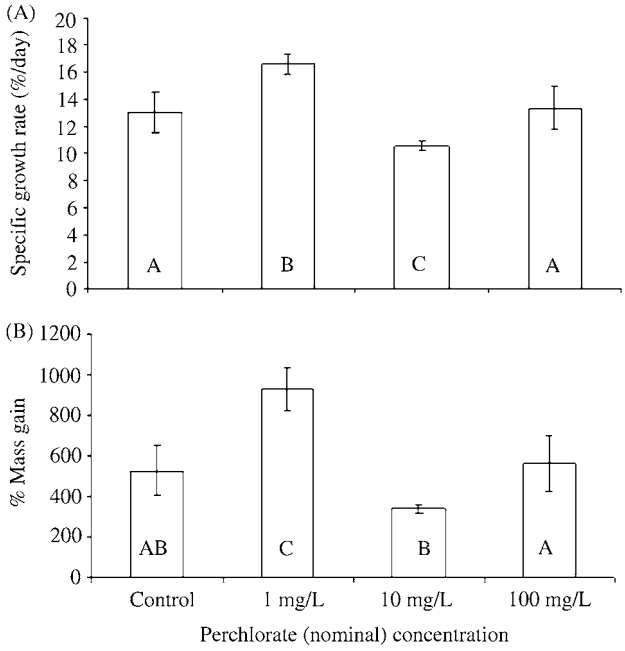

Fig. 3. Specific growth rate (SGR) (A) and mass gain (B) of mosquitofish fry exposed to perchlorate for 4 weeks. Mass gain = [(final weight−initial weight)/initial weight] × 100; SGR = [ln(final weight)–ln(initial weight)] × 100/period of exposure. Bars and error bars represent means and standard deviations. Bars labeled with different letters are statistically significantly different from each other ($P<0.05$, ANOVA).

3.3. Growth

The final masses among treatment groups were 0.058 ± 0.006, 0.09 ± 0.009, 0.056 ± 0.003, and 0.048 ± 0.01 g/fish for control, 1, 10, and 100 mg/L of sodium perchlorate, respectively. Final mass per fish in the 1-mg/L sodium-perchlorate-treated group was significantly greater than those of other groups ($P<0.01$; ANOVA), and there were no other significant differences among treatments. For specific growth rate, the 1- and 10-mg/L treatments were significantly higher and lower, respectively, than controls ($P<0.05$, ANOVA; Fig. 3A). For percentage mass gain, the 1-mg/L treatment group was significantly different from all groups. There were also differences among the 10- and 100-mg/L groups (Fig. 3B). Concentration of NaCl (0, 1, 10, 100 mg/L) had no effect on growth for either percentage mass gain or specific growth rate ($P>0.30$, Kruskall–Wallis test; data not shown).

3.4. Histopathology

For both the individual scores and their sum, the effects generally increased with increasing exposure concentration (Fig. 4A). In all cases, effects were seen at all exposure concentrations. With regard to percentage follicles affected, there was also an increase in response for increasing concentrations of perchlorate, however, at 10 mg/L and above (nominal concentrations), 100% of the follicles observed exhibited hyperplasia and depleted colloids (Fig. 4B). No histopathological effects were seen in fish exposed to NaCl.

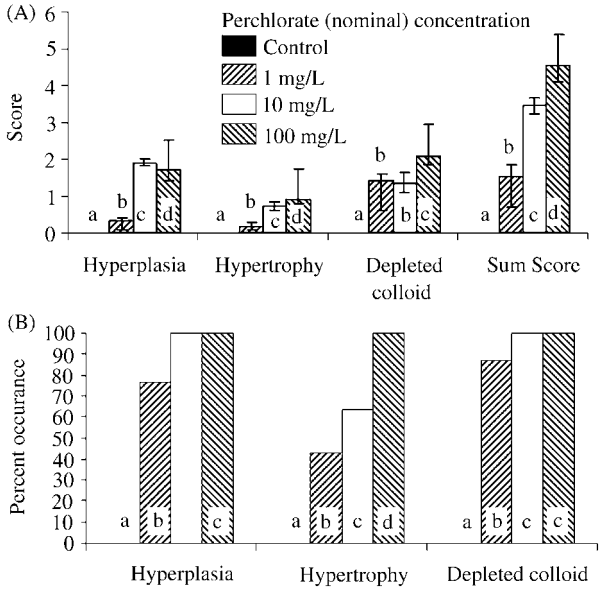

Fig. 4. Histopathological effects in adult female mosquitofish (*Gambusia holbrooki*) exposed to various concentrations of sodium perchlorate for 42 days. (A) Median (first and third quartile) scores for severity of histopathological effects with regard to thyroid follicle hyperplasia, hypertrophy, colloid depletion, and their sum. Letters denote statistical differences among treatments ($P<0.05$, Kruskall–Wallis test). (B) Percentage thyroid follicles that exhibit hyperplasia, hypertrophy, and colloid depletion. Letters denote statistical differences among treatments ($P<0.05$, χ^2 test).

4. Discussion

4.1. Reproductive effects

The results of the present study indicate that perchlorate does not reduce fecundity of mosquitofish at the dosage associated with effects on the thyroid. On the contrary, there seemed to be a mild stimulatory effect on GSI, egg mass, and fecundity. With regard to GSI, an effect was seen at 1 mg/L (nominal concentration). The average mass of the females at this concentration was not statistically different from that in other treatments but was about 10–15% lower than that in the other treatments. This may have influenced the patterns of GSI, but further research would need to be done to determine whether this was the case.

In contrast to growth and reproduction effects, perchlorate has been shown to affect thyroid-level responses. Bradford et al. (2005) found that a 30-day exposure of eastern mosquitofish to perchlorate at the same concentrations as those used in the present study induced perturbations in thyroid histopathology, as indicated by an increase of the epithelial cell height and hyperplasia. Thus, it appears that these concentrations of perchlorate are capable of disrupting the thyroid hormone axis in this species, but this does not result in reduction of reproductive performance.

Other studies have found that perchlorate did not produce any effects, either stimulatory or inhibitory, on reproductive parameters. For example, environmentally relevant concentrations of AP 18 mg/L did not affect the packed egg volume (index of spawning success) and the rate of natural egg fertilization (index of egg quality) of zebrafish (*Danio rerio*) but affected histology of thyroid follicles (Patiño et al., 2003). Higher concentrations of AP (677 mg/L) suppressed spawning activity of zebrafish, but these authors suggested that effects at the higher concentrations might be due to the toxic effects of AP other than thyrotoxicity. These results are also consistent with reproductive effects of AP in rats (York et al., 2001), for which AP exposure did not affect reproductive performance up to 30 mg/kg-day, exposed over two generations. However, low dosages (below 30 mg/kg-day) did cause histopathologic changes in thyroid tissue, such as hypertrophy and hyperplasia (York et al., 2001). Thus, it appears that perchlorate exposure results in thyroid disruption in many species, but subsequent effects on reproduction may be species specific.

There may be several possible explanations for the mild stimulatory effect of perchlorate on fecundity in mosquitofish. First, an increased number of eggs may be a compensatory response. In some teleosts, the balance between large numbers of smaller eggs and small numbers of larger eggs may be affected by environmental stresses such as pollutants and toxicants (Kime, 1998). In fact, stimulatory effects of toxicants on fitness parameters may be an adaptive response to a variable environment (Forbes, 2000). Second, there is some indication that perchlorate induces oxidative stress (as reflected by DNA damage and glutathione levels) in zebrafish (Liu and Theodorakis, 2003), and oxidative stress may stimulate cell proliferation (Esposito et al., 2004; Klaunig and Kamendulis, 2004). This could affect oogenesis in perchlorate-exposed mosquitofish. Third, there may be some unmeasured covariate of fecundity, such as spawning frequency, which is affected by perchlorate. Finally, there may be pleiotropic effects of thyroid disruption, oxidative stress, or other mechanisms of perchlorate toxicity that may be indirectly responsible for the effects of perchlorate on fecundity.

Although thyroid disruption may play a role in the effects found in the current study, the effects of thyroid hormones on reproductive capacity in fish are still not well established. Some negative correlations between increased thyroid hormone concentrations and reproductive performance in fish have been reported (McBride and Overbeeke, 1975; Olivereau, 1977; Rangneker and Latey, 1977; Cyr and Eales, 1988; Tyler and Sumpter, 1996). In other fish, thyroid hormones may affect the secretion of reproductive hormones (Cyr and Eales, 1996; Parhar et al., 2000). Because 30-day exposures of perchlorate affected mosquitofish thyroid hormone levels and histopathology, there may be a mechanistic linkage between the thyroid disruption and the effects seen in the current study, but the precise mechanism is unknown at this point.

4.2. Survival

In the current study, elevated levels of sodium perchlorate significantly affected survival rate of mosquitofish fry. The estimated LC_{50} of perchlorate was lower than that (529 mg/L of AP) reported in zebrafish larvae (R. Patiño, Texas Tech University, Lubbock, TX, unpublished data). This difference could be due to the species specificity or to differences in ionic form of the perchlorate salts (sodium perchlorate vs. AP). The estimated 5-day LC_{50} of AP for *Xenopus laevis* larvae (510 mg/L) was similar to that found for mosquitofish exposed to sodium perchlorate in the current study (Goleman et al., 2002). Other investigators have determined the LC_{50} of perchlorate in rainbow trout (*Salmo gairdneri*), fathead minnows (*Pimephales promelas*), and bluegill sunfish (*Lepomis macrochirus*) juveniles or larvae to be 2100, 1665, and 1500 mg/L, respectively (EAEST, 1998; Dean et al., 2004). Thus it appears that mosquitofish are more sensitive to the acute toxic effects of perchlorate than other species of fish.

4.3. Growth

The results of the current study indicate that there is a stimulatory effect of perchlorate on growth at 1 mg/L and an inhibition at 10 mg/L. One mechanism for these results may be due to the effects of thyroid hormones on growth of fish, excreted either directly or via thyroid modulation of other hormones such as growth hormone. Although the regulation of growth hormone by thyroid hormones is well established in mammals (Muller et al., 1999), there are conflicting results on the role of thyroid hormones on growth hormone production in fish. Different studies have found that thyroid hormones may either negatively (Nishioka et al., 1985; Rousseau et al., 2002) or positively (Luo and McKeown, 1991; Melamed et al., 1995; Gomez et al., 1997) affect growth hormone production in fish. Alternative hypotheses for the effects of perchlorate on mosquitofish growth are that the effects seen are due to oxidative stress or some other mechanism of toxicity, pleiotropic effects, or compensation to toxicant stress.

The higher growth rate in the fish exposed to 1 mg/L of sodium perchlorate might be also explained by growth hormesis. Growth hormesis is the acquisition of tolerance in organisms by adaptation of growth control mechanisms when they were exposed to small amounts of toxicants and is the by-product of normal responses of biological systems that counteract the effects of the inhibitors (Stebbing, 1997). Such occurrences of hormesis are very common in toxicological studies (Calabrese and Baldwin, 2000, 2001, 2003). For example, in the mummichog (*Fundulus heteroclitus*), fin regeneration of 0.05–1.0 mg/L cadmium-exposed fish was more rapid than that of control fish. The authors suggested that this phenomenon was due to the hormetic overcompensation by homeostatic regulatory mechanisms (Weis and Weis, 1986). Similar mechanisms may be involved in the patterns seen in the present study.

4.4. Histopathology

The results of the histopathological analysis of female mosquitofish exposed for 6 weeks to perchlorate were in accordance with other studies with mosquitofish (Bradford et al., 2005) and other fish (Crouch, 2003; Patiño et al., 2003; Crane et al., 2005; Theodorakis et al., 2005): environmentally relevant concentrations of perchlorate anion affect thyroid histopathological parameters in fish. In general, the differences between control and treated fish were greater in fish treated for 6 weeks in this study and were greater than those seen in mosquitofish exposed for 30 days or less (Bradford et al., 2005). This could be due (at least in part) to the fact that the fish in the present study were exposed to perchlorate for a longer period of time than were the fish in the Bradford et al. (2005) study. Also, the mosquitofish examined in the present study were undergoing reproduction, whereas the fish examined by Bradford et al. (2005) were in nonreproductive condition. Although this may have affected the relative degree of difference among the fish in the two studies—because reproductive condition in fish has been shown to affect thyroidal responses (MacKenzie et al., 1989)—other studies have found that the relative degree of thyroid histopathological response increases with increasing exposure durations (Crouch, 2003; Patiño et al., 2003; Bradford et al., 2005). This suggests that the longer exposure duration contributed to the differences in the degree of effects between this study and that of Bradford et al. (2005).

5. Conclusions

This study found unexpected effects of perchlorate on reproductive performance and growth in mosquitofish, including mild stimulation and possible hormesis. These effects are probably not due to differences in salinity or Na^+ concentrations, because NaCl treatments at similar concentrations to sodium perchlorate had no effects on reproduction, growth, and survival. Thus, the concentrations of Na^+ used in this study were probably not a significant stressor to *G. holbrooki*, which is in accordance with previous studies that have found that this species has a relatively high salinity tolerance. Adverse effects of salinity on growth, reproduction, survival, or metabolic rate of *G. holbrooki* have not been found at salinities less than 7 ppt (Chervinski, 1983; Newman and McCloskey, 2000; Akin and Neill, 2003). Although the perchlorate concentrations used in this study are within the range of concentrations found in the field in some studies (Smith et al., 2001), they are lower than those found in others (Motzer, 2001). In addition, the LC_{50} determined in the current study is much higher than water concentrations found in the field (Smith et al., 2001), indicating that acutely toxic effects of perchlorate are unlikely for this species. However, this is not meant to imply that perchlorate contamination is beneficial to natural populations of fish or that there may be no detrimental ecological effects of environmental perchlorate contamination. Also, because of bioenergetic tradeoffs in life history traits, hormesis or other toxicological stimulation of fitness parameters may not necessarily increase overall fitness, primarily owing to the fact that different fitness parameters may respond in different directions (Forbes, 2000). In addition, potential ecological effects of alterations of fitness parameters, either inhibitory or stimulatory, are not fully understood. In particular, the effects seen here were relatively small, so the significance of these effects to higher-level processes (population, community) have yet to be established. Hence, future studies using population simulations and modeling would have to be carried out to determine

these issues. These issues need to be further addressed to fully reveal the ecological risk of low levels of perchlorate contamination.

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