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Effects of chemical and physical conditions on hatching success of *Bythotrephes longimanus* resting eggs

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Abstract

Dormant eggs of zooplankton are considered to be tolerant of harsh environmental conditions, yet lethal thresholds of exposure have rarely been determined. We describe 3 yr of dose–response experiments that evaluate hatching success of the resting egg of *Bythotrephes longimanus* (Cladocera: Cercopagidae) in response to salinity, chlorination, heat, desiccation, and freezing. There was no decline in hatching success in response to salinity (up to 4 weeks at 35 g L^{-1} salt) or chlorination (including up to 5 min at 3400 mg L^{-1} chlorine). In response to heat there was an interaction between time and temperature; hatching occurred after exposure to 40°C for 1 min, 5 min, or 10 min but declined with the two longer exposures. Hatching also occurred after exposure to 50°C for 1 min, but no hatching followed exposure to 50°C for 5 min or to higher temperatures for 1 min. In response to desiccation at 17°C, exposure for ≤ 4 h had no effect on hatching but ≥ 6 h resulted in no hatching. When frozen, hatching success depended on whether eggs were frozen in water or without water. Collectively, the results are congruent with an evolutionary origin of *B. longimanus* in permanent, euryhaline basins. The results advance our understanding of dispersal limits of *B. longimanus*, including vector potential, and may inform management practices for reducing range expansion of *B. longimanus* by humans.

Crustacean zooplankton that inhabit temporally variable environments commonly have life cycles that alternate between active and dormant stages (Hairston 1996; Hairston and Cáceres 1996; Gyllström and Hansson 2004). The dormant stage is more durable and able to withstand conditions that are unfavorable or lethal to the active stage (Panov and Cáceres 2007). For example, experiments and direct observations demonstrate that dormant stages can tolerate exposures to a variety of adverse environmental conditions, including desiccation (Brewer 1964; Moghraby 1977; Arnott and Yan 2002), freezing (Schwartz and Hebert 1987; Meijering 2003), heat (Carlisle 1968; Schwartz and Hebert 1987; Raikow et al. 2007*b*), salinity (Bailey et al. 2004; Gray et al. 2005), hypoxia (Marcus et al. 1994; Brown 2008), non-neutral pH (Arnott and Yan 2002; Brown 2008), and vertebrate digestion (Mellors 1975). Reports dating to Darwin (1859) that implicate wind and animals as dispersal vectors of aquatic invertebrates also imply that dormant stages can tolerate desiccation, heat, and gut passage (Cáceres and Soluk 2002; Havel and Shurin 2004; Louette and De Meester 2005).

In addition to environmental durability, dormant stages of crustacean zooplankton can survive for years to decades longer than their active-stage counterparts, and accumulate as viable, persistent egg banks in lacustrine sediments (Hairston 1996). Some species can remain viable in dormant stages for more than a century (Hairston et al. 1995; Cáceres 1998). Together, durability and longevity of the dormant stage permit species to extend individual survival across spatial and temporal scales (e.g., overland dispersal, pond drying, and overwintering) and this can have far-reaching ecological and evolutionary implications (Hairston and De Stasio 1988; Cáceres 1997; Panov and Cáceres 2007).

Despite a long history of research on dormancy in crustacean zooplankton, gaps remain in our understanding of lethal tolerance thresholds. For most taxa there are either limited data or no data on the ecological and physiological survival thresholds of the dormant stage. Likewise, few studies have employed a systematic, dose–response approach, and precise survival thresholds remain poorly resolved (for exceptions *see* Raikow et al. 2007*a*,*b*) even in groups that have been well examined (e.g., daphnids and calanoid copepods; Schwartz and Hebert 1987; Hairston 1996). Discussion on the role of dispersal in controlling zooplankton community structure (Bohonak and Jenkins 2003; Louette and De Meester 2005) has highlighted the need for better resolution of survival thresholds and range capacities of dispersing life stages. This lack of information has also become a deficit in the prevention of aquatic invasive species transport by ship ballast and other anthropogenic vectors (Bailey et al. 2003).

Here we assessed the tolerance limits of the dormant life stage of *Bythotrephes longimanus* to a variety of environmental conditions. *B. longimanus* is a large (1 cm total length), pelagic, predaceous, freshwater zooplankton. Its distribution is primarily in meso-oligotrophic lakes and reservoirs in Asia, Europe, and North America (MacIsaac et al. 2000; Therriault et al. 2002; Brown et al. 2012). It prefers well-oxygenated (≥ 2.4 mg L^{-1}), cool-temperature (10–24°C), low-salinity (0.04–0.06 g L^{-1}) conditions but has been reported outside these ranges including salinities up to 8.0 g L^{-1} (Grigorovich et al. 1998). Reproductively, *B. longimanus* produce the dormant stage (hereafter

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referred to as resting egg) following gametogenesis, and fertilization by a male. Resting eggs are easily distinguished from parthenogenetic eggs. The latter are minute when produced and develop immediately in the brood chamber into first-instar juveniles with large, black compound eyes (Yurista 1992; Branstrator 2005). By contrast, resting eggs of *B. longimanus* measure 0.4–0.5 mm diameter and are golden brown in color (Brown 2008). They have a rigid shell but no ephippium. They are typically brooded in clutches of 2–7 and sink rapidly (up to 9–12 mm s^{-1}) after release from the mother (Jarnagin et al. 2000; Brown 2008). They can withstand gut passage in fish and waterfowl (Jarnagin et al. 2000; Charalambidou et al. 2003) and prolonged exposure to extreme hypoxia and pH (Brown 2008), but are intolerant of dry conditions according to Makrushin (1985) as cited in Fryer (1996).

Because *B. longimanus* is invasive in North America (Lehman and Cáceres 1993; Yan et al. 2001; Pangle et al. 2007), there has been considerable interest in understanding its life cycle and the role of the resting egg during range expansion (Brown and Branstrator 2011; Wittmann et al. 2011). Human recreational equipment (e.g., bait bucket, boat hull, or fishing line) is believed to be the primary overland dispersal vector of *B. longimanus* (MacIsaac et al. 2004; Weisz and Yan 2010). Outreach protocols for decontamination of equipment against aquatic invasive species often recommend drying or rinsing with hot water or a light bleach solution (e.g., the Minnesota Department of Natural Resources Fishing Regulations [www.dnr.state.mn.us] and the Michigan Department of Natural Resources Fishing Guide [www.michigan.gov/dnr]). While these treatments may be lethal to the active stage of *B. longimanus* (Grigorovich et al. 1998), their efficacy at killing resting eggs has not been evaluated. A lack of data on precise desiccation thresholds of the resting egg has also hampered efforts to model dispersal distances that could be achieved by human and nonhuman vectors.

To address this dearth of information, we performed a series of dose–response experiments to determine the lethal thresholds of *B. longimanus* resting eggs to salinity, chlorination, heat, desiccation, and freezing. The results demonstrate that resting eggs can endure a variety of chemical extremes when kept hydrated, but are intolerant of even short-term desiccation, which is atypical of many crustacean zooplankton (Brewer 1964; Moghraby 1977; Arnott and Yan 2002). The lethal thresholds of heat and desiccation, in particular, can be applied to dispersal models and management protocols for *B. longimanus*.

Methods

Resting egg harvest—Resting eggs were harvested from Island Lake Reservoir (Minnesota), a tannin-stained, mesotrophic impoundment (surface area = 32.4 km^2, Z_{max} = 28.7 m, Z_{Secchi} = 1–2 m, chlorophyll *a* concentration = 8.5 $\mu g\ L^{-1}$) which has supported *B. longimanus* since at least 1990 (Branstrator et al. 2006). We collected gravid females with conical nets (0.5 m diameter opening, 500 μm pore width synthetic mesh) towed through the pelagia during September and October nights each year that experiments were initiated (2003, 2004, and 2006). In the laboratory, gravid females were isolated in individual wells of 12-well tissue culture plates with 6 mL capacity per well (hereafter referred to as a tissue culture plate) with Island Lake Reservoir water that was passed through a glass microfiber filter, C gauge (Whatman; hereafter referred to as filtered lake water). Plates were stored at 18°C in environmental chambers in the dark.

We conducted experiments on eggs prior to any overwintering storage because newly produced eggs settle out of the water column rapidly (Jarnagin et al. 2000) and are arguably most vulnerable to an overland dispersal vector (e.g., bird feather, bait bucket, or bilge water) shortly after their release or while still in the brood chamber. Using both recently released and currently brooded eggs, as opposed to eggs retrieved from lake sediments, was also beneficial because it allowed us to constrain egg age to within a few months.

Most experiments were conducted on eggs that were released from mothers in the laboratory, hereafter referred to as free eggs (Table 1). Every 12 h, mothers were supplied with fresh filtered lake water and fed a diet of either live *Daphnia* or immature *B. longimanus*. Resting eggs released from a mother's brood chamber were transferred on a daily basis by pipette into individual wells of tissue culture plates in filtered lake water and stored in the dark at 4°C before experiments began (typically 2–7 weeks). All eggs released within 5 d of the mother's collection were harvested in this fashion. Free eggs with obvious cracks, dents, or fungal growth were discarded. This procedure led to the establishment of banks of ~ 2000 free eggs in 2003, 2000 free eggs in 2004, and 1000 free eggs in 2006.

In addition to free eggs, we studied whole clutches of resting eggs that had not yet been released from the mother's brood chamber, hereafter referred to as in-clutch eggs (Table 1). Gravid females used for the in-clutch egg experiments were collected from Island Lake Reservoir on the same day that experiments were initiated. Only mothers carrying resting eggs in advanced stages of development (golden brown in color; Jarnagin et al. 2000) were used.

General experimental protocol—Resting eggs were exposed to one of several putative stressors (salinity, chlorination, heat, desiccation, or freezing, each described below) designed to simulate a possible overland transport event (natural or anthropogenic vectors) or a treatment employed to decontaminate recreational or commercial equipment. Experiments in 2003–2004 (year 1) examined salinity, heat, desiccation, and freezing. The experiments in year 1 on heat, desiccation, and freezing were designed to estimate upper thresholds of tolerance and thus used broad ranges in the levels of conditions applied. Experiments in 2004–2005 (year 2) examined narrower ranges for heat, desiccation, and freezing, as well as chlorination. Experiments in 2006–2007 (year 3) examined desiccation only. In total, 4771 resting eggs were tested. Of these, 33 eggs (< 1%) were omitted from statistical analysis due to damage recognized during egg scoring.

Each experimental year, free eggs from the egg bank were randomly assigned to treatments and controls. A

Table 1. Summary of conditions administered in controls. Shown are letter codes used in Figs. 1–5, year of study, egg type (free or in-clutch), exposure temperature (T, °C), duration in filtered lake water, and corresponding treatment against which the control was compared. All other experimental details followed the methods for corresponding treatments. Years of study are 1 (2003–2004), 2 (2004–2005), and 3 (2006–2007). Controls were often exposed for durations that bracketed, but may not have duplicated, the treatment conditions as given in Figs. 1–5. Free eggs were released from mothers before exposure. In-clutch eggs were in the mother's brood chamber during exposure.

Code	Experimental year	Egg type	Exposure T (°C)	Exposure duration	Corresponding treatment
a	1	free	4	2 weeks	salinity
b	1	free	4	4 weeks	salinity
c	2	free	4	5 min	chlorination
d	2	free	4	1 d	chlorination
e	1	free	4	1 min	heat, freezing
f	2	free	4	1 min	heat, freezing
g	1	free	4	5 d	desiccation
h	2	free	17	1 d	desiccation
i	2	free	17	2 d	desiccation
j	2	in-clutch	17	1 d	desiccation, freezing
k	2	in-clutch	17	2 d	desiccation
l	3	free	17	2 h	desiccation
m	3	free	17	10 h	desiccation

batch of 12 free eggs served as an experimental replicate. Replicate eggs were treated simultaneously during the exposure phase of an experiment and subsequently housed in individual wells of a single tissue culture plate during the dormancy and thermal cue phases of an experiment. A total of 48 free eggs (four replicates) comprised a treatment or control unless noted.

For the in-clutch egg experiments, which occurred in year 2 only, batches of 12 gravid females served as a replicate and were manipulated together as described above for the free-egg experiments. A total of 48 gravid mothers (four replicates) comprised a treatment or control.

Postexposure, eggs were incubated in the laboratory for several months to simulate the physiochemical setting and the temporal schedule that they would experience in a lake's hypolimnion during winter, spring, and summer (Table 2). We imposed a dormancy phase of only one winter and spring period because our demographic evidence had indicated that a major fraction of *B. longimanus* resting eggs lie dormant for < 1 yr in Island Lake Reservoir (Brown and Branstrator 2005). At the end of the dormancy phase, eggs were scored for hatch success. Each year of experimentation, from egg collection to final scoring, required about 11 months. All experimental phases, aside from final egg scoring, were conducted in a darkened room with occasional use of red, incandescent light (< 5 μE m^{-2} s^{-1}).

Experimental exposure—Salinity: Previous studies have evaluated saltwater exposure as a method to reduce freshwater zooplankton resting egg survival in transoceanic ship ballast tanks (Gray et al. 2005; Bailey et al. 2006; Gray and MacIsaac 2010). To mimic the salinity conditions in ballast tanks, we exposed free eggs in year 1 to 15, 25, or 35 g L^{-1} salt solutions for 2 weeks or 4 weeks. The salt solutions were developed from Scientific Grade Marine Salt (Coralife) and deionized water. The chemical elements and their proportions in the salt solutions were similar to natural seawater. Before exposure, salt solutions were aerated and cooled to 4°C. To begin a replicate, eggs were transferred by pipette from the egg bank into a 1 liter glass jar with 750 mL of the prescribed salt solution. A separate jar was used for each treatment or control. Eggs were held in the dark at 4°C in an environmental chamber for the duration of the exposure. Control eggs were exposed for 2 weeks (control a) or 4 weeks (control b) to deionized water at 4°C (Table 1). Only 24 eggs (two replicates) were used for each control.

Chlorination: Outreach protocols for decontamination of equipment for inland waters often prescribe rinsing with a light bleach solution (e.g., the Michigan Department of Natural Resources Fishing Guide [www.michigan.gov/dnr]). To evaluate this protocol, we exposed free eggs in year 2 to dilute solutions of bleach (Clorox) which contains sodium hypochlorite (NaOCl). Total chlorine concentrations were determined by a wet chemistry method (Hach) with a resolution limit of 0.2 mg L^{-1} chlorine. We exposed eggs to 1 mg L^{-1} chlorine for 1 h or 1 d; to 500 mg L^{-1} chlorine for 1 min, 5 min, 1 h, or 1 d; or to 3400 mg L^{-1} chlorine for 1 min or 5 min. The highest chlorine concentration that we tested (3400 mg L^{-1}) was obtained by following the Clorox product instructions for household cleaning (47 mL to 1 liter water), which produced a 4.7% solution of the Clorox product. Deionized water was used to establish stocks and make dilutions. To begin a replicate, eggs were transferred by pipette from the egg bank into a 1 liter glass jar with a residual film of filtered lake water that kept eggs hydrated. At exposure start, 100 mL of the prescribed solution (chilled to 4°C) were added. Eggs were held in the dark at 4°C in an environmental chamber for the duration of the exposure. To end an exposure, $\sim$ 70 mL of the solution were removed by aspiration and 700 mL of deionized water (chilled to 4°C) were added. After 2–3 min, the solution was aspirated to

Table 2. Mean ± 1 standard deviation of the duration (days), temperature (T, °C), dissolved oxygen concentration (DO), and pH of the filtered lake water in the aquaria in which resting eggs passed the experimental dormancy and thermal cue phases. Years of study are 1 (2003–2004), 2 (2004–2005), and 3 (2006–2007). In year 1, the thermal cue was delivered in two stages. The initial temperature was administered for 129 d and then increased to a higher temperature for the last 31 d. In year 2, the chlorine-exposed eggs were not subject to a dormancy phase but received a longer thermal cue phase (193 d) compared to other eggs that year (*see* Methods for explanation).

	Dormancy				Thermal cue			
Year	Days	T (°C)	DO (mg L^{-1})	pH	Days	T (°C)	DO (mg L^{-1})	pH
1	132±1	3.9±0.7	12.1±1.3	7.4±0.4	129	6.3±0.6	11.5±1.5	7.6±0.4
	—	—	—	—	31	10.0±0.1	no data	no data
2	134±1	3.7±0.3	11.9±1.7	7.5±0.4	160	10.1±0.3	10.4±0.6	7.8±0.3
3	135±1	3.4±0.1	11.7±1.1	no data	160	10.0±0.1	10.5±0.6	no data

100 mL and an additional 700 mL of deionized water were added. In total, eggs were flushed three times with 700 mL of deionized water in an attempt to remove all residual chlorine. Post-dilution testing indicated that the 3400 mg L^{-1} chlorine test solution had been diluted to ~ 3.4 mg L^{-1} chlorine. Control eggs were exposed for 5 min (control c) or 1 d (control d) to deionized water at 4°C (Table 1). Chlorine concentrations were below detection in the deionized water and in the filtered lake water in which eggs were housed postexposure during the experimental dormancy and thermal cue phases (*see* below).

Heat: Outreach protocols for decontamination of equipment for inland waters often prescribe rinsing surfaces with hot tap water (e.g., the Minnesota Department of Natural Resources Fishing Regulations [www.dnr.state.mn.us]). We used a wide range of water temperatures that could be used in decontamination attempts. Our range of test temperatures also bracketed bird core body temperature (about 40°C) and this permitted extrapolation of the results to thermal conditions during gut passage. We exposed free eggs in year 1 to 30°C, 40°C, 60°C, or 100°C for 1 min or 10 min; and in year 2 to 40°C or 50°C for 1 min, 5 min, or 10 min. To begin a replicate, eggs were transferred by pipette from the egg bank into a 125 mL Erlenmeyer flask. Eggs were flushed with 100 mL of filtered lake water at the target temperature and held there in an insulated bath for the exposure. At the end of the exposure, eggs were diluted with 750 mL of filtered lake water at 4°C. Control eggs were exposed for 1 min in year 1 (control e) and 1 min in year 2 (control f) to filtered lake water at 4°C (Table 1).

Desiccation: Zooplankton resting eggs have been shown to tolerate drying for different lengths of time depending on taxa (Brewer 1964; Moghraby 1977; Makrushin 1978). Here, we exposed free eggs to desiccation (as gently circulating air) in year 1 for 1 h, 5 d, or 1 month (30 d); in year 2 for 1 h, 12 h, 1 d, or 2 d; and in year 3 for 30 min, 1 h, 2 h, 3 h, 4 h, 6 h, 8 h, 10 h, 12 h, or 1 d. To begin a replicate with free eggs, eggs were transferred by pipette from the egg bank into individual wells of a tissue culture plate with ~ 0.25 mL of residual filtered lake water per egg. Uncovered plates were placed in dark environmental chambers. Exposure time began when the residual film of water surrounding an egg exterior had evaporated completely. This was carefully verified for each egg, which required a difference in start time between replicate eggs of up to 30 min in some cases. Temperature and relative humidity were measured inside the environmental chambers with wireless monitors (Onset or Oregon Scientific) during the desiccation phase. In year 1, during this phase the treatment eggs were held at a mean temperature of 18°C and a mean relative humidity of 33% (1 h and 5 d trials) or 29% (1 month trial). In year 2, treatment eggs were held at a mean temperature of 17°C and a mean relative humidity of 53%. In year 3, treatment eggs were held at a mean temperature of 17°C and a mean relative humidity of 45%. Control eggs were maintained submerged in filtered lake water at 4°C (year 1) or 17°C (year 2 and year 3) for periods that reflected the median or range of treatment exposures in year 1 (control g), year 2 (control h and control i), and year 3 (control l and control m; Table 1).

In year 2, we also exposed in-clutch eggs to desiccation for 1 h, 12 h, 1 d, 2 d, or 5 d. To begin a treatment replicate, live, gravid females were placed into dry, individual wells of a tissue culture plate which was immediately placed into a dark environmental chamber. Treatment mothers were exposed to a mean temperature of 17°C and a mean humidity of 46% during the exposure phase. Control mothers were sacrificed by pinching the head with forceps while not disturbing the brood chamber. Bodies were transferred to individual wells of tissue culture plates in filtered lake water at 17°C and incubated for 1 d (control j) or 2 d (control k; Table 1).

Freezing: While the resting eggs of some zooplankton species can tolerate freezing for up to 18 yr (Meijering 2003), we examined freeze tolerance in *B. longimanus* resting eggs for shorter time periods that could be relevant for range expansion. To begin a replicate with free eggs, eggs were transferred by pipette from the egg bank into individual wells of a tissue culture plate. In year 1, prior to freezing, each well was filled with 3 mL of filtered lake water at 4°C. In year 2, no water was added to the well prior to freezing. Individual plates were immediately wrapped in aluminum foil and placed in a freezer set to −10°C to −20°C. In year 1, the freeze period was 1 d, 1 week, or 1 month (31 d). In year 2, the freeze period was 1 d. Control e and control f, initiated with the heat experiments in year 1 and year 2, respectively (*see* above), were used for statistical comparison.

In year 2, we also exposed in-clutch eggs to freezing. To begin a treatment replicate, live, gravid females were placed into individual wells of a tissue culture plate without water.

Plates were wrapped in aluminum foil and placed in the freezer. The freeze period was 1 d. Control j, initiated with the desiccation experiment on in-clutch eggs in year 2 (*see* above), was used for statistical comparison.

Experimental dormancy and thermal cue—Egg transition from the exposure phase to the dormancy phase differed depending on the experiment. For resting eggs that were exposed in glass containers (salinity, chlorination, and heat), eggs were transferred by pipette with a small amount of water into individual wells of tissue culture plates. For resting eggs that were exposed in tissue culture plates (desiccation and freezing), eggs remained there. All tissue culture plates were submerged in filtered lake water in 80 L aquaria housed in environmental chambers. The lid of each plate had been predrilled to produce a 2 cm diameter hole over which a sheet of 210 μm pore width synthetic mesh was secured to provide a window for water and dissolved gas exchange. Plates were secured $\sim$ 2 cm apart onto racks prior to submersion. Aquaria were aerated continuously. A dormancy phase was maintained for 132–135 d (Table 2). During this period, temperature, dissolved oxygen, and pH of the aquaria water were monitored biweekly with electronic probes.

To cue resting eggs to develop, the environmental chambers were warmed progressively over several days (Table 2) to a target temperature (Yurista 1997). In year 1, chambers were warmed to 6.3°C where they were maintained for 129 d, followed by warming to 10.0°C where they were maintained for an additional 31 d. In year 2 and year 3, chambers were warmed to 10.1°C and 10.0°C, respectively, where they were maintained for 160 d. Because neither exposure to light nor variation in photoperiod appear to cue development of *B. longimanus* resting eggs (Herzig 1985; Yurista 1997; Brown 2008), eggs were held in the dark during the entire hatching phase. In-clutch eggs remained with their mothers for the duration of the dormancy and thermal cue phases.

In most cases with free eggs, the exposure phase was initiated within 2–7 weeks after the eggs were harvested from mothers in the laboratory. The only exception was with chlorination for which eggs were held 13 weeks prior to exposure. During this time, some eggs had begun to develop as evidenced by a darkening of the embryonic tissue. This is not surprising because other studies have shown that *B. longimanus* resting eggs can hatch within 2–3 months of their release from the mother (Herzig 1985; Brown and Branstrator 2005). However, as a result of their development, after exposure to chlorination the eggs were not subject to a dormancy phase but entered directly into the thermal cue phase that was slightly longer than for other eggs (Table 2). The same procedure was carried out for the chlorination controls.

Egg scoring and statistical analysis—At the end of the thermal cue phase, eggs were scored (Leica MZ125, 50×) as hatched, partially hatched, or non-hatched. Hatched was assigned if the outer eggshell had separated into hemispheres along its natural suture line (Yurista 1992) and there was no remaining soft tissue in the shell. Hatched eggs were often accompanied by a dead neonate in the well. Partially hatched was assigned if the two shell hemispheres had begun to separate but the embryonic tissue was partially decomposed or covered with fungal hyphae. Non-hatched was assigned if there was no evidence of embryonic development or eggshell hemisphere separation (Yurista 1992). We regard the percentages of eggs that hatched as measures of egg survival, and we regard the exposure levels at which no eggs hatched as lethal conditions. The possibility that some non-hatched eggs may have been alive but bet hedging (Cáceres and Tessier 2003; De Stasio 2004) is considered in the Discussion.

For each replicate, the number of eggs scored as hatched or partially hatched was computed as a percentage. Because the hatched, partially hatched, and non-hatched categories always summed to 100%, results of the latter category were not shown in Figs. 1–5. For free-egg experiments, egg number per replicate was typically 12. For in-clutch egg experiments, egg number per replicate ranged from 47 to 82. Small sample sizes precluded estimation of underlying distributions of the data and therefore we used nonparametric statistics (Kruskal–Wallis test or Mann–Whitney test) with a critical level of $p = 0.05$ to compare the percentages hatched or partially hatched among replicate groups (SYSTAT 10.0, Systat Software). The test statistics were compared against a table of the chi-square distribution. With salinity, we also summed the hatched and partially hatched categories in each replicate to estimate the total percentage of eggs expressing some degree of hatch attempt, and compared the values between 2 week and 4 week trials. With controls, we also compared hatch success among years but because there were only two replicates for some control groups, we compared the means of the replicate groups as opposed to individual replicates. Owing to our general use of four replicates (12 eggs per replicate for free-egg experiments), sample sizes were small which reduced statistical power of the nonparametric tests. Failure to reject null hypotheses (no differences between exposures) should be interpreted with this in mind.

Results

Salinity—Eggs hatched in all salinity trials (Fig. 1). No difference was detected in the percentage of hatched eggs among the control and treatments in the 2 week trials (Kruskal–Wallis, $H = 3.50$, $p = 0.32$, degrees of freedom [df] = 3) or among the control and treatments in the 4 week trials (Kruskal–Wallis, $H = 2.42$, $p = 0.49$, df = 3). Also, no difference was detected in the percentage of partially hatched eggs among the control and treatments in the 2 week trials (Kruskal–Wallis, $H = 7.16$, $p = 0.07$, df = 3) or among the control and treatments in the 4 week trials (Kruskal–Wallis, $H = 3.57$, $p = 0.31$, df = 3). No difference was detected in the percentage of hatched eggs between the 2 week and 4 week trials (Mann–Whitney, $U = 111.00$, χ^2 approximation = 0.96, $p = 0.33$, df = 1), but there was a larger percentage of partially hatched eggs in the 4 week compared to the 2 week trials (Mann–Whitney, $U = 51.00$, χ^2 approximation = 3.89, $p = 0.05$, df = 1). No difference was detected in the sum of hatched and partially hatched eggs between the 2 week and 4 week trials (Mann–Whitney, $U = 75.50$, χ^2 approximation = 0.57, $p = 0.45$, df = 1).

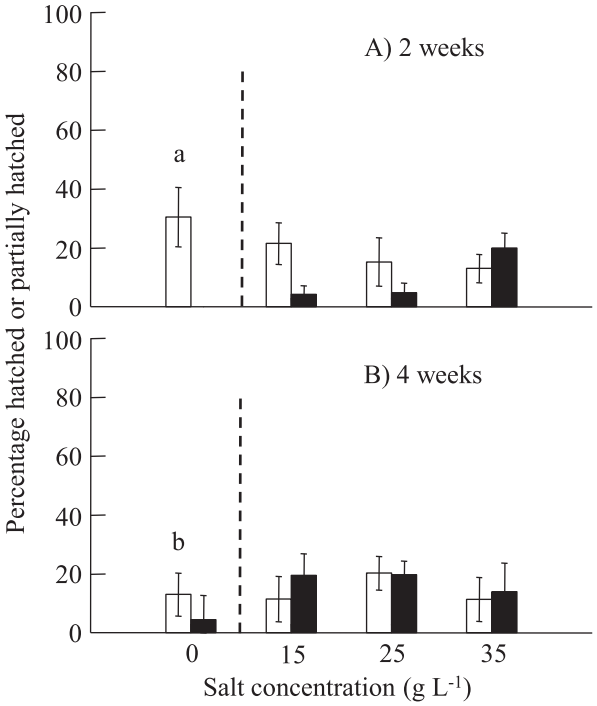

Fig. 1. Percentage of free resting eggs scored as hatched (open) or partially hatched (darkened) after exposure to saltwater for (A) 2 weeks or (B) 4 weeks in year 1. Each value is the mean ± 1 standard error of four replicates (12 eggs per replicate) except for controls which had two replicates each. Dashed vertical lines separate controls (*see* Table 1) from treatments.

Chlorination—Eggs hatched in all chlorination trials (Fig. 2). Across all trials, no difference was detected in the percentage of hatched eggs among the controls and treatments (Kruskal–Wallis, $H = 8.27$, $p = 0.51$, df = 9).

Heat—Across both experimental years, exposure to 50°C for 5 min was the lowest temperature and shortest duration that prevented hatching, but there was considerable variation among trials within and between years (Fig. 3). In year 1, no eggs hatched after exposure to 60°C or 100°C for 1 min, or after exposure to 40°C, 60°C, or 100°C for 10 min. In year 1, among the control and treatments where hatching occurred, there was no difference detected in the percentage of eggs that hatched (Kruskal–Wallis, $H = 0.67$, $p = 0.88$, df = 3). In year 2, unlike year 1, some eggs hatched after exposure to 40°C for 10 min (Fig. 3). In year 2, among the control and treatments where hatching occurred, there was a difference in the percentage of eggs that hatched (Kruskal–Wallis, $H = 12.17$, $p = 0.02$, df = 4) and the general trend was a decline in hatching success with longer exposure and higher temperature. There were no partially hatched eggs in year 1 but they were common in year 2 and represented a larger fraction than hatched eggs in the 50°C treatments but not in the 40°C treatments.

Desiccation—Hatching was prevented by many of the desiccation exposures (Fig. 4). In year 1 and year 2, free eggs and in-clutch eggs hatched only in the controls and 1 h treatments. Within years, no difference was detected in the

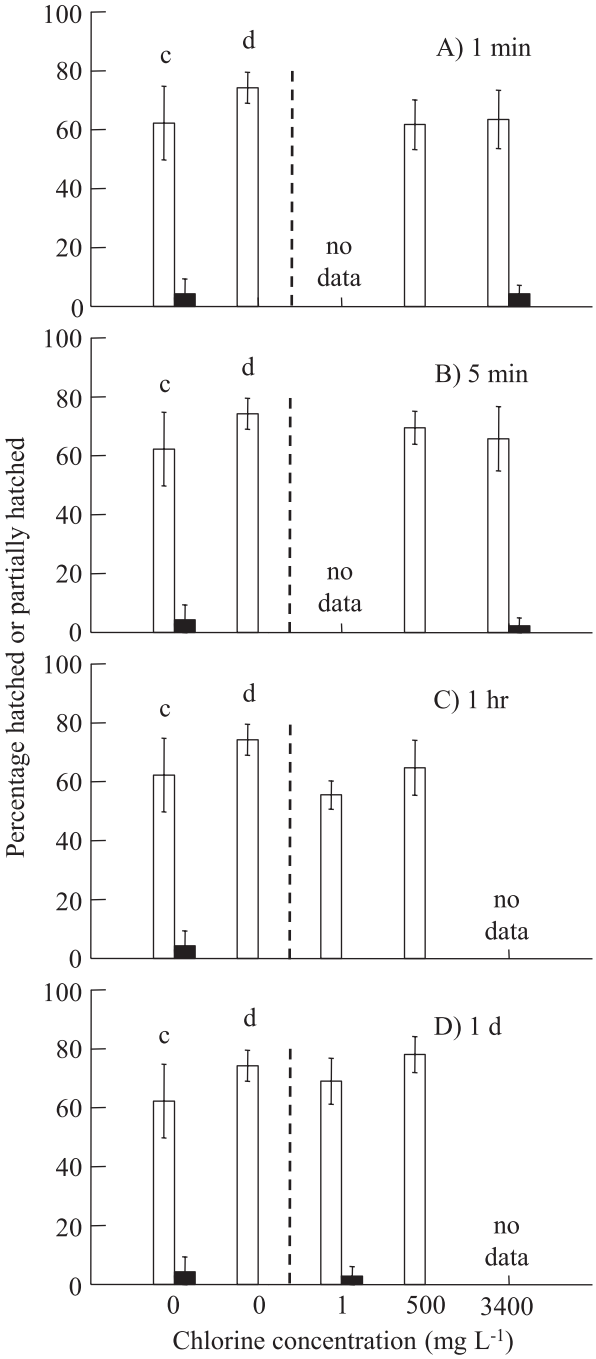

Fig. 2. Percentage of free resting eggs scored as hatched (open) or partially hatched (darkened) after exposure to chlorinated water for (A) 1 min, (B) 5 min, (C) 1 h, or (D) 1 d in year 2. Each value is the mean ± 1 standard error of four replicates (12 eggs per replicate). Dashed vertical lines separate controls (*see* Table 1) from treatments.

percentage of free eggs that hatched among the control(s) and 1 h treatments (year 1: Mann–Whitney, $U = 13.00$, χ^2 approximation = 2.22, $p = 0.14$, df = 1; year 2: Kruskal–Wallis, $H = 0.93$, $p = 0.63$, df = 2). Also, no difference was detected in the percentage of in-clutch eggs that hatched among the controls and 1 h treatments (year 2 only: Kruskal–Wallis, $H = 0.59$, $p = 0.75$, df = 2). In year 3,

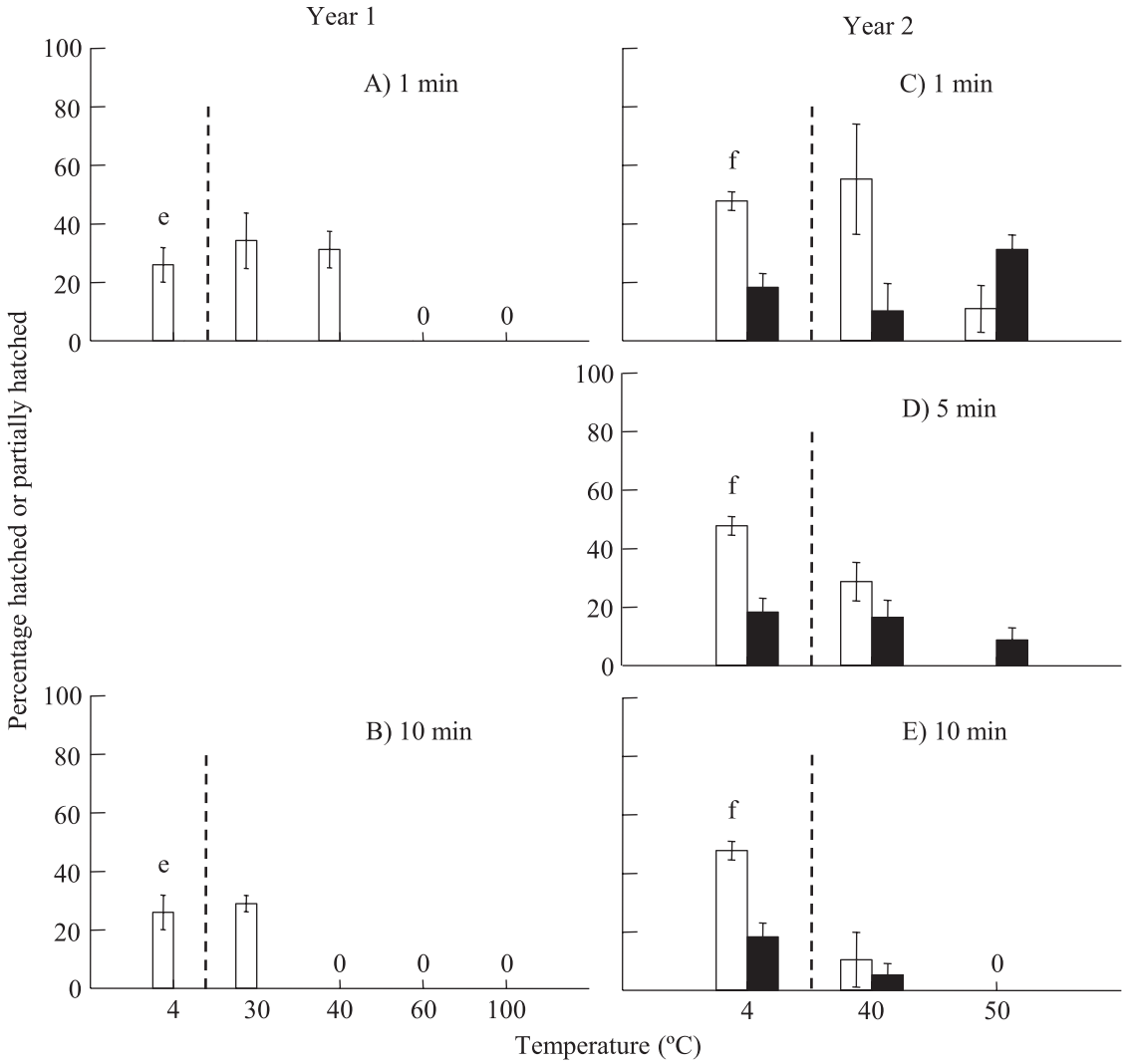

Fig. 3. Percentage of free resting eggs scored as hatched (open) or partially hatched (darkened) after exposure to heated water for (A) 1 min or (B) 10 min in year 1; and for (C) 1 min, (D) 5 min, or (E) 10 min in year 2. Each value is the mean ± 1 standard error of four replicates (12 eggs per replicate). Treatments with a 0 indicate values of 0 in both the hatched and partially hatched categories. Dashed vertical lines separate controls (*see* Table 1) from treatments.

when free eggs were desiccated for finer increments (30 min to 1 d), eggs hatched in the controls and in the 30 min, 1 h, 2 h, 3 h, and 4 h treatments but no eggs hatched or partially hatched when exposure was ≥ 6 h. No difference was detected in the percentage of free eggs that hatched among "control l" and the treatments where hatching occurred (Kruskal–Wallis, $H = 9.30$, $p = 0.10$, df = 5); comparisons were made with "control l" and not "control m" because the time period of exposure of "control l" was most similar to the treatments where hatching occurred (Table 1).

Freezing—In year 1, when free eggs were frozen in water, no eggs hatched following the 1 d, 1 week, or 1 month exposures (Fig. 5). In year 2, by comparison, when free eggs were frozen without water, some eggs hatched following the 1 d exposure but fewer hatched than in the control (Mann–Whitney, $U = 16.00$, χ^2 approximation = 5.40, $p = 0.02$, df = 1). In year 2, when in-clutch eggs were frozen without water, they also hatched following the 1 d exposure and the percentage that hatched was indistinguishable from the control (Mann–Whitney, $U = 10.50$, χ^2 approximation = 0.54, $p = 0.46$, df = 1).

Controls—The percentage (mean ± 1 standard deviation) of eggs that hatched in the controls for the free-egg experiments ranged within and among years (year 1: 26.2% ± 9.6%; year 2: 59.8% ± 9.7%; year 3: 49.0% ± 16.7%). The mean percentages hatched were not different between year 2 and year 3 (Mann–Whitney, $U = 7.00$, χ^2 approximation = 0.60, $p = 0.44$, df = 1). When year 2 and year 3 were combined, the mean percentages hatched were larger than in year 1 (Mann–Whitney, $U = 0.00$, χ^2 approximation = 7.00, $p = 0.01$, df = 1).

Discussion

The hatching success of *B. longimanus* resting eggs varied widely in response to chemical and physical conditions.

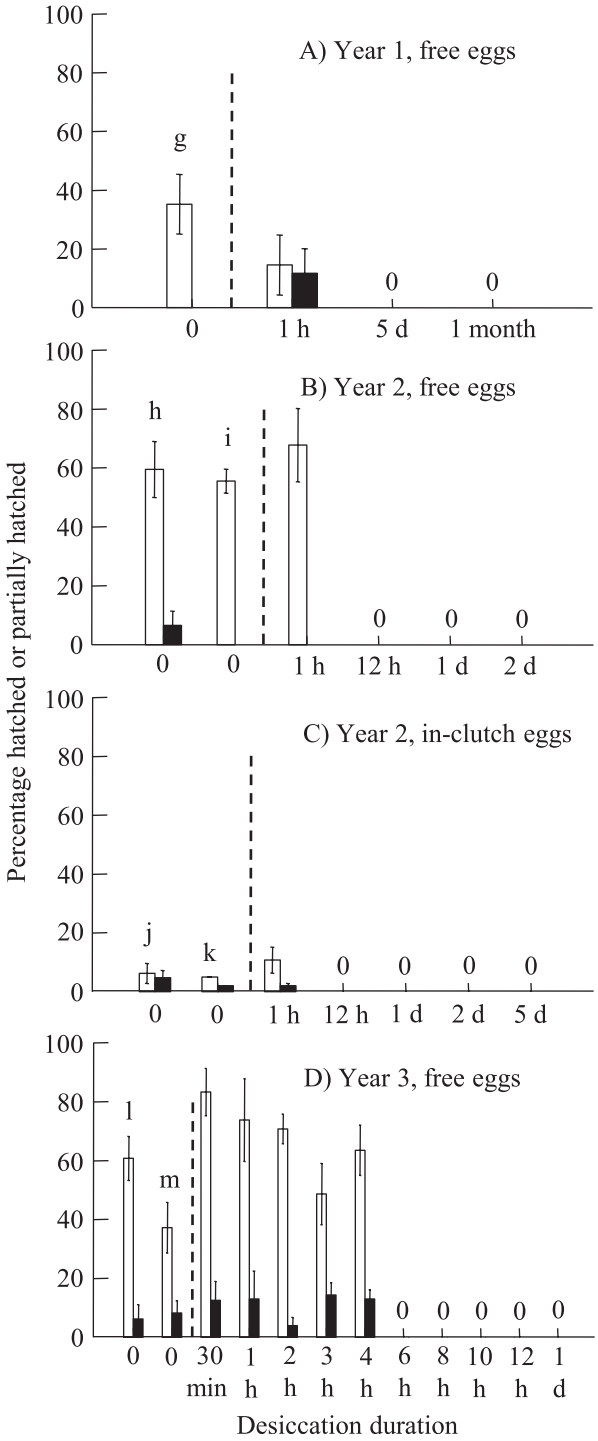

Fig. 4. Percentage of resting eggs scored as hatched (open) or partially hatched (darkened) after exposure to desiccation for (A) free eggs in year 1; (B) free eggs or (C) in-clutch eggs in year 2; and (D) free eggs in year 3. Each value is the mean ± 1 standard error of four replicates (12 eggs per replicate for free eggs, 12 females per replicate for in-clutch eggs). Treatments with a 0 indicate values of 0 in both the hatched and partially hatched categories. Dashed vertical lines separate controls (*see* Table 1) from treatments.

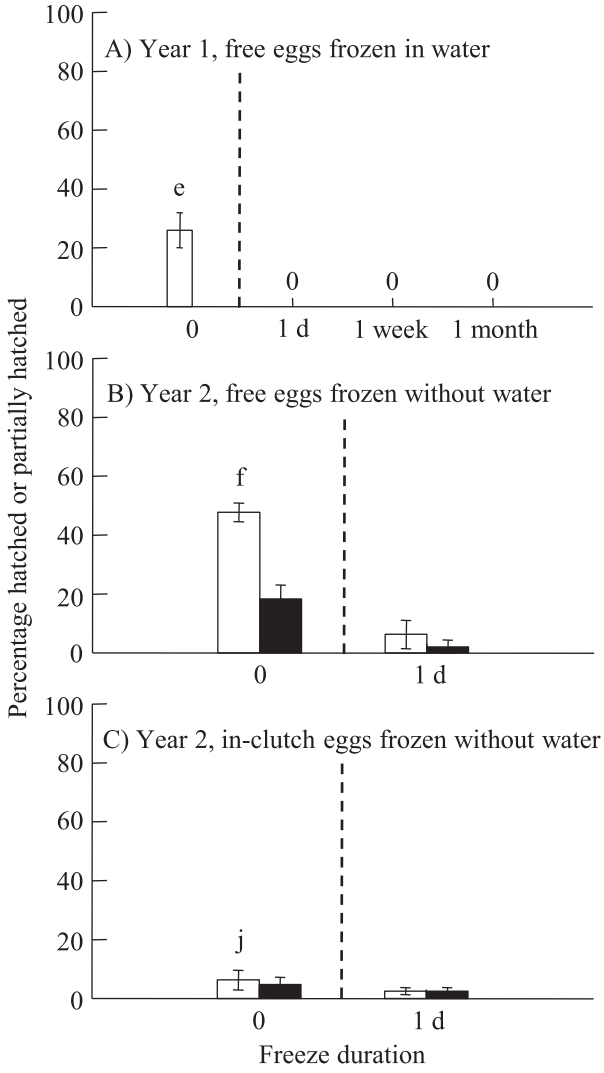

Fig. 5. Percentage of resting eggs scored as hatched (open) or partially hatched (darkened) after exposure to freezing for (A) free eggs frozen in water in year 1, and (B) free eggs frozen without water or (C) in-clutch eggs frozen without water in year 2. Each value is the mean ± 1 standard error of four replicates (12 eggs per replicate for free eggs, 12 females per replicate for in-clutch eggs). Treatments with a 0 indicate values of 0 in both the hatched and partially hatched categories. Dashed vertical lines separate controls (*see* Table 1) from treatments.

When free eggs were hydrated, they survived exposures to high salinity and chlorination, and to moderate heat. The robust nature of the resting egg is also supported by previous research that documented resting egg hatching after extended exposures to hypoxia and extreme pH (Brown 2008), and after gut passage through fish and waterfowl (Jarnagin et al. 2000; Charalambidou et al. 2003). By contrast, our results indicate that resting eggs of *B. longimanus* are highly sensitive to desiccation as free eggs failed to hatch when desiccated for ≥ 6 h at room temperature (17°C).

Before discussing the biological underpinnings and ecological and management implications of the results, it is important to note that control eggs in our experiments provided a target expectation for hatching success under

non-stressed conditions. Hatching fractions in the controls were comparable to, or slightly lower than, previous reports on *B. longimanus* resting egg hatching in the laboratory and field (49% to 67%: Herzig 1985; Yurista 1997; Brown and Branstrator 2011). Some variation in hatching fraction is to be expected as a result of environmental and genetic factors (De Meester and De Jager 1993; Cáceres and Schwalbach 2001; Allen 2010). The lower mean hatching percentage in year 1 compared to the later two years may reflect conditions during the thermal cue phase (Table 2) or clonal variation between years (Schwartz and Hebert 1987; De Meester and De Jager 1993). Within years, the range of hatching percentages among controls was relatively constrained, which suggests that slight variations in experimental procedure that were inherently necessary among controls did not strongly influence baseline hatch success.

Salinity—The salinity concentrations that we administered (15–35 g L^{-1}) were nonlethal to the resting egg stage of *B. longimanus* despite being well above the typical salinity range (0.04–0.06 g L^{-1}) of the planktonic stage (Grigorovich et al. 1998; Ellis and MacIsaac 2009). Aladin and Potts (1995) suggest that the closed brood chamber of *B. longimanus* is an adaptation that improves osmoregulatory control of the embryonic environment. Thus, despite the species' current preference for freshwater, salinity exposure may have been relevant in the evolution of *B. longimanus* and a condition that is not stressful to its resting egg.

Like *B. longimanus*, a wide variety of predominantly freshwater zooplankton taxa produce resting eggs that can tolerate salinity exposure (Bailey et al. 2003, 2006; Gray et al. 2005). This may be adaptive in sediments of lakes where resting eggs generally accumulate and where decomposition and groundwater seepage can cause rapid spatial and temporal shifts in ion concentration (Wetzel 2001). Salinity tolerance may also be adaptive in temporary ponds where evaporation can produce stressful ion concentrations and during gut passage in birds.

There were no differences in egg hatching success across the salinity concentrations and time periods of exposure. Because the exposures were done at a single temperature of 4°C, however, caution should be applied when extrapolating this result to warmer conditions. We chose 4°C in order to prevent temperature from inducing development (Yurista 1997). Like our study, Gray et al. (2005) also found no negative effect of high salinity exposure (10 d to 32 g L^{-1} salinity) on hatching of cladoceran resting eggs when exposures were done at 4°C. By contrast, Bailey et al. (2006), who used a range of temperatures and salinity exposures, found a strong temperature by salinity interaction; for example, their egg exposures to 8 g L^{-1} salinity at 10°C or 30°C had greater hatching success than those at 20°C. Complex interactions may be possible among temperature, egg metabolic rate, membrane permeability, dormancy cues, and protection of eggs by overlying sediments during exposure (Aladin and Potts 1995; Gray et al. 2005; Bailey et al. 2006). The only evidence of a negative salinity effect in our study was an increasing frequency of partially hatched eggs in the 4 week trials compared to the 2 week trials, but there was no difference in the total number of eggs expressing some degree of activity (hatched plus partially hatched) between the two time periods.

There are important management implications for the tolerance of *B. longimanus* resting eggs to salinity. (1) Saline exposure under cold conditions is not lethal to resting eggs and should not be prescribed for disinfecting equipment. (2) *B. longimanus* resting eggs may be able to survive in residual films of water (e.g., live wells or bait buckets) even after evaporative loss, and these areas should be specifically noted in decontamination protocols. (3) *B. longimanus* resting eggs could have seeded the original invasion of North America following survival in saline ballast water, which was first hypothesized by Lehman (1987) and Sprules et al. (1990), as eggs hatched following our longest experimental incubation (4 weeks) that extends beyond the typical voyage length of transoceanic ships entering the Laurentian Great Lakes (Bailey et al. 2003). That said, when the Laurentian Great Lakes were initially invaded by *B. longimanus* in the early 1980s, ballast water exchange was not mandated (Bailey et al. 2003), thus the planktonic life stage could feasibly have founded the initial North American population as well. (4) If ballast water exchange remains the only method of decontamination in the shipping industry, given the tolerance observed in this and previous studies (Gray et al. 2005; Bailey et al. 2006; Gray and MacIsaac 2010), we should expect transcontinental invasion of viable zooplankton resting eggs, including those of new species, to continue albeit at reduced levels. For *B. longimanus*, this is consistent with ongoing genetic homogenization of its North American and Eurasian gene pools (Berg et al. 2002; Colautti et al. 2005).

Chlorination—Chlorine is widely prescribed as a microbial disinfectant. Chlorination causes protein unfolding and irreversible cell damage in bacteria (Winter et al. 2008). Previous research also supports its use as a biocide against metazoans including the cladoceran *Daphnia magna* in its active life stage (Sano et al. 2004). By contrast, our results demonstrate that *B. longimanus* resting eggs are remarkably tolerant of short-term (up to 1 d) chlorination. There was also no reduction in hatching among eggs exposed to the highest concentration (3400 mg L^{-1} for 5 min) where the solution was nominally pH 10.7. Brown (2008) reported that *B. longimanus* resting eggs can survive up to 4 months in 4°C water of pH 11 (adjusted with sodium hydroxide), which is substantially longer than our experiments. Given this level of tolerance to alkaline pH, our study in association with Brown (2008) predict that commercial biocides whose mode of action is oxidation will not be effective barriers against survival of resting eggs at cold temperatures.

We cannot say whether or not chlorination coupled with higher temperatures might alter chlorine toxicity. A study on *Daphnia mendotae* resting eggs conducted at 20°C found 90% mortality after 24 h exposure to 78.3 mg L^{-1} sodium hypochlorite (Raikow et al. 2007*a*), which is strikingly lower than our highest 1 d exposure concentration (500 mg L^{-1}). While interpretation of the chlorination results may also be biased by the fact that a fraction of experimental eggs had initiated development prior to

exposure, the expected effect of this is unclear. Hatching was still detected in these trials, and given that the planktonic stage is more restricted than the resting egg in its range of pH tolerance, embryonic development probably had the effect of decreasing egg tolerance to chlorination (Grigorovich et al. 1998; Brown 2008).

Heat—The structure and function of organic molecules, particularly enzymes, is strongly heat sensitive (Somero 1995). Examples of extreme thermal tolerance (near 100°C) have been reported for dormant stages of some aquatic crustaceans (Carlisle 1968), but the thermal maximum for most metazoans is between 30°C and 45°C (Willmer et al. 2005). Hatching has been demonstrated for daphnid and other cladoceran resting eggs exposed to 30°C or 35°C for various amounts of time (Moghraby 1977; Schwartz and Hebert 1987). Moghraby (1977) found no hatching for eggs exposed to 36°C or 40°C.

Our results indicate that the lethal heat threshold for *B. longimanus* resting eggs is between 40°C and 50°C, and is time sensitive. Time sensitivity is suggested by the results in year 2 at 40°C where the hatching success of resting eggs declined progressively under longer exposures. Most likely, longer exposures enabled greater molecular damage and contributed to the reduction in hatching success between 1 min and 10 min. Our results are consistent with heat-stress experiments on *Daphnia mendotae* resting eggs that indicate a rapid, time-sensitive increase in mortality at exposure temperatures of 40°C to 50°C (Raikow et al. 2007*b*). Our results are also consistent with a study by Charalambidou et al. (2003), who found that *B. longimanus* resting eggs retrieved from waterfowl guts (bird core body temperature is near 40°C) within 1 h of ingestion hatched but those retrieved after longer periods did not. The thermal tolerance for *B. longimanus* resting eggs thus appears to be consistent with short-term, but not long-term, gut residence and dispersal by birds (Charalambidou et al. 2003).

If 40°C is a time-sensitive lethal threshold, we may expect minor experimental fluctuations in temperature around 40°C to result in large differences in survival, which may explain why exposure to 40°C for 10 min produced no hatching in year 1 but some hatching in year 2. In year 2 we also saw more partial hatching, which may reflect the fact that temperatures were near lethal thresholds. However, in the controls there was also more partial hatching in year 2. An alternative explanation for the difference in hatching success between year 1 and year 2 for exposures to 40°C for 10 min may be reduced egg ''health'' or reduced responsiveness to the thermal cue in year 1 as suggested by the lower mean hatching percentage in the controls.

Because *B. longimanus* is a northern-latitude species that prefers cold water (Yurista 1999; Kim and Yan 2010), it is noteworthy that resting eggs tolerate 40°C even for short periods. This may be an artifact of biochemical conservation, or a derived trait under selection if heat tolerance does in fact enhance fitness, for example, by increasing endozooic or epizooic dispersal by homeotherms. Regardless of its origin, high heat tolerance of the resting egg has implications for decontamination. Current outreach recommendations in Minnesota include the use of very hot tap water to decontaminate surfaces of aquatic invasive species. While hot-water rinses may be effective at killing the active stage of *B. longimanus*, our study indicates that sustained exposure to 50°C for 5 min, or shorter durations at higher temperatures, are required to eliminate hatching of the resting egg. This suggests that equipment that can be submerged or flooded (e.g., live wells or bait buckets) may be amenable to heat treatment, but decontaminating boat hulls or other large surfaces with heat is probably impractical.

Desiccation—*B. longimanus* resting eggs are intolerant to short-term desiccation; at 17°C the maximum tolerance to desiccation was 4 h. This is consistent with Makrushin (1978, 1981), who reported that *B. longimanus*, *Leptodora kindtii*, *Sida crystallina*, and other species—all whose resting eggs have large-grained yolks and vacuoles present—are less tolerant of desiccation than resting eggs with small-grained yolks and no vacuoles. It is also consistent with the observation that *B. longimanus* resting eggs could not be hatched from dried mud (Makrushin 1985) as cited in Fryer (1996). In addition to the structure of the egg cytoplasm, the structure of the eggshell may affect desiccation intolerance. Yurista (1997) described a suture around the eggshell where separation occurs during embryogenesis, and this could be a possible zone of weakness or porosity. Melanin in the eggshell may also play a strengthening role as it does in the keratin of bird feathers (Burtt 1979); *B. longimanus* resting eggs appear to be only moderately discolored by melanin. Desiccation intolerance in *B. longimanus* resting eggs may also result in part from the lack of an ephippium, but this is probably not the only factor as Schwartz and Hebert (1987) demonstrated desiccation resistance up to 1 week in daphnid resting eggs that had been experimentally excised from the ephippium.

A limited temporal window of desiccation tolerance in *B. longimanus* resting eggs has several implications for dispersal capacity. It supports the hypothesis that dispersal to North America was by ship ballast water (Sprules et al. 1990), and argues against vectors involving air exposure such as bird feathers. Intolerance to all but short-term desiccation is also consistent with recent observations in North America that motorized watercraft appear to be far more likely than nonmotorized watercraft at spreading *B. longimanus* between inland lakes (MacIsaac et al. 2004; Weisz and Yan 2010). Motorized watercraft support more enclosed wet spaces (e.g., engine coolant water and live well water). They are also generally transported upright (unlike canoes), increasing the chance that standing water will remain intact and serve as a medium for propagule hitchhiking. These temporal constraints on desiccation tolerance provide for the first time a basis to predict range capacities of overland vectors. For example, with a survival threshold of 4 h out of water, recreational traffic moving 90 km h^{-1} could transport dry resting eggs 360 km. Models could be developed to apply this to various geographic regions, but as a cursory illustration most invaded lakes in

Minnesota are within a 4 h drive of Lake Superior (the presumed invasion hub). A short period of desiccation tolerance may also explain why *B. longimanus* range expansion in North America has been slower than *Daphnia lumholtzi*, another recent invader. Studies documenting early range expansion of each species indicate that during a 12 yr period (1990–2001) *D. lumholtzi* expanded its range to about 180 lakes (Havel and Shurin 2004), whereas during a 28 yr period (1982–2009) *B. longimanus* expanded to only about 130 lakes (Weisz and Yan 2010). One major challenge for management of nonnative species will be application of multiple decontamination methods simultaneously as short-term (< 1 d) desiccation, for example, may be effective against some species but not others (Ricciardi et al. 1995). A short period of desiccation tolerance is also consistent with the observation that range expansion of *B. longimanus* in North America thus far has been exclusively by diffusion with no recorded episodes of cross-continental jumps, which has been noted already for *D. lumholtzi* (Havel and Shurin 2004). Finally, because resting eggs are so vulnerable to desiccation, it is tempting to speculate that eggs brooded by females (in-clutch) might survive longer out of water due to additional moisture associated with the brood membrane. While our experiments could not resolve this, we did show that survival of in-clutch eggs out of water was still < 12 h.

Of the conditions we tested, desiccation may translate into the most effective management solution for disinfecting equipment of *B. longimanus*. Compared to manual removal (e.g., rinsing) or chemical exposure (e.g., chlorination or salinity), drying is probably more cost-effective and potentially easiest to apply. Current outreach recommendations in Minnesota include that equipment be dried for at least 5 d to eradicate aquatic invasive species. This duration appears to be more than sufficient, when done at 17°C, to prevent *B. longimanus* resting egg hatching. Finally, we note that a short desiccation window of the resting egg offers at least the possibility of successful eradication of *B. longimanus* from an entire ecosystem if temporary drying could be managed as, for example, in reservoirs which are ecosystems that can accelerate the spread of *B. longimanus* (Johnson et al. 2008; Brown et al. 2012).

Freezing—The hatching success of *B. longimanus* resting eggs in response to freezing depended on whether eggs were frozen in water or not. The lack of hatching among resting eggs frozen in water was likely the result of lethal impaction by water expansion both inside and outside the egg. When eggs were frozen without water, even though the surface was in direct contact with circulating air, freezing may have slowed water loss and extended egg tolerance to 1 d of exposure. Tolerance to freezing by resting eggs has been demonstrated by others. For example, Meijering (2003) hatched several cladoceran species from wet mud that had been frozen at −18°C for 18 yr, and Schwartz and Hebert (1987) reported short-term (1 week) freezing tolerance in several daphnid species. Moreover, cladocerans and other crustaceans regularly survive extended freezing in temporary ponds. For decontamination, the distinction between desiccation coupled with freezing as opposed to desiccation at warmer temperatures is important. Specifically, during late-fall periods, when resting eggs are commonly produced by *B. longimanus*, desiccation of equipment outdoors under cold conditions could increase survival frequency. An interaction between temperature and desiccation on hatching deserves further study.

Synthesis—The suite of chemical and physical tolerance limits of *B. longimanus* resting eggs reported here and elsewhere (Jarnagin et al. 2000; Charalambidou et al. 2003; Brown 2008) suggest that there has been strong historical selection for survival and dispersal of the resting egg within lakes but not between lakes as even short-term air exposure is lethal. Dehydration is not a seasonal or even occasional event in the preferred habitat of *B. longimanus* in deep, permanent basins (Grigorovich et al. 1998). Desiccation intolerance coupled with strong salinity tolerance is also consistent with a marine or brackish origin of *B. longimanus* (Cristescu and Hebert 2002), although few populations inhabit saline environments today (Grigorovich et al. 1998). Dispersal by wind or other vectors that entail desiccation has apparently not been an avenue of selection for *B. longimanus* and may now fortuitously be a key to its modern containment.

High lethal chlorination thresholds endured by *B. longimanus* resting eggs may be an artifact of resistance to other factors such as microbial or fungal invasion, or natural conditions such as salinity or pH extremes (Brown 2008) encountered in sediment environments. How the egg structurally or functionally remains ostensibly impervious to chemical extremes, yet unable to withstand modest air-drying, remains an open question for investigation. Nutritional limits, size constraints, and design limitations dictate that we may expect investment tradeoffs in the evolution of resting eggs between traits that enhance survival and dispersal in the dormant state (e.g., eggshell integrity) vs. those that promote post-birth survival and recruitment (e.g., yolk storage).

One shortcoming of our study was that we did not assess the survival status of non-hatched eggs. Because dormancy length can be under genetic and maternal control (De Meester and De Jager 1993; De Meester et al. 1998; Allen 2010), it is possible that bet hedging accounts for a portion of non-hatched eggs across our experiments (Cáceres and Tessier 2003; Gyllström and Hansson 2004). However, given the short lifespan of the resting egg of *B. longimanus* (Andrew and Herzig 1984; Herzig 1985; Brown and Branstrator 2011), it seems more likely that death, as opposed to bet hedging, explains the status of most non-hatched eggs that experienced conditions that we heretofore identify as lethal. Under a bet-hedging scenario for a short-lived egg, we would expect that at least a portion of them would commit to hatch the first season, which is not the case for the treatments that we assign as lethal thresholds in our study. It could also be argued that small sample sizes reduced our ability to detect hatching, but two factors suggest this was unlikely and give us confidence that failure to hatch in our study was due to impaired survival. (1) There was general consistency between years in the treatments that resulted in no hatching. (2) Brown (2008)

found reduced hatching, but not zero hatching, for resting eggs exposed to extreme pH in her study of *B. longimanus* from Island Lake Reservoir that used similar sample sizes to ours.

A second potential limitation of our study is that the single-lake focus may highlight regional specificity and not the species' general tolerance range. In other cladocerans, regional responses to hatching cues have been observed (Schwartz and Hebert 1987; Allen 2010). While this deserves investigation, and has implications for management, hatching cues established by other researchers in other systems (Herzig 1985; Yurista 1997) were effective in our study. Further, the work of Makrushin (1978, 1985) on Eurasian *B. longimanus* anticipated our results on desiccation intolerance and thereby implies that sensitivity to drying is a broad-scale trait of the *B. longimanus* resting egg and not a local artifact. Moreover, generations that preceded the resting eggs that we used from Island Lake Reservoir, where *B. longimanus* was first detected in 1990, had presumably survived overland transport, and therefore may have already been self-selected for desiccation tolerance, which would suggest that if biased, our study probably overestimated the duration of desiccation tolerance.

In developing management applications from this work, it is important to consider that we used bare eggs (or eggs in brood chambers) as opposed to eggs buried in sediments. Sediments may provide protective barriers against potential toxins, including salt (Gray et al. 2005; Gray and MacIsaac 2010) and sodium hypochlorite (Raikow et al. 2007*a*) added to overlying waters. In our experiments with salinity and chlorination there were no detectable concentration or time effects on hatching; thus, adding sediments as a variable probably would not have modified the results. In the case of heat exposure, however, where a graded reduction in hatching success was detected with longer exposures and higher temperatures, protection by sediments might have influenced the outcome, including the upper tolerance threshold. One can imagine that sediment may also differentially influence the effect of freezing on egg survival and this should be noted if freezing is prescribed for decontamination.

In closing, our ability to identify exposure thresholds where growth or survival of a species is zero in relation to an environmental characteristic is foundational to the concept of an ecological niche. The emerging discipline of invasion biology has brought renewed interest and application to this body of research because of its relevance to models of range expansion and the development of alternative management solutions for prevention and eradication of nonnative taxa. In this regard, freshwater zooplankton are particularly interesting because the insular nature of lakes and ponds requires zooplankton to rely on vectors (some anthropogenic) to overcome terrestrial and marine barriers (Havel and Shurin 2004). Our results with *B. longimanus* should be of considerable interest in this capacity as they underscore the efficacy of desiccation as a method to destroy resting eggs, and highlight some of the shortcomings of alternative approaches. Specifically, thorough drying for $\geq$ 6 h was 100% effective at preventing hatching of resting eggs, whereas salinity, chlorine, heat, and freezing were less effective or not effective at all as agents to prevent hatching when applied at concentrations or levels that would be practical in a policy context. Thus, a management message that encourages humans to limit their usage of lakes to one lake a day, and to dry their equipment thoroughly during intervening periods, is reasonable stewardship that should help limit range expansion of *B. longimanus*.

Acknowledgments

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