

# Patterns in the abundance, phenology, and hatching of the resting egg stage of the invasive zooplankter *Bythotrephes longimanus*: implications for establishment

Meghan E. Brown · Donn K. Branstrator

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**Abstract** To examine how dormancy contributes to the establishment and persistence of *Bythotrephes longimanus*, we investigated resting egg production and hatching in relation to the demography of the planktonic stage and environmental conditions in Island Lake Reservoir (USA). During a 3-year study, the largest contribution to the egg bank occurred in autumn and most eggs hatched in spring, but we also detected some resting egg production and hatching in summer. The difference between summer and late autumn densities of eggs in sediments averaged 47% (range 0–98%) for 18 sites throughout the reservoir, which was similar to experimental estimates of in situ hatching fraction of 67% for eggs in the spring and summer following their production. Based on emergence traps, neonates hatch in the field during May and June. We estimated mortality rates of 64% for resting eggs and embryos, and 59% for newly emerged neonates. Although hatching fraction saturated at the same level, eggs incubated offshore hatched later than those nearshore where water temperature was warmer and light was detectable at the sediment surface. Low dissolved oxygen

concentration did not significantly reduce hatching fraction but resulted in some eggs that initiated development but failed to hatch. Collectively, our results demonstrate substantial annual turnover in the resting egg bank of *B. longimanus* and high mortality of resting eggs during recruitment from the egg to the first molt of the planktonic stage. These patterns suggest that propagule pressure in the form of resting eggs requires large numbers for establishment, and that considerable post-establishment resting egg production is necessary for inter-annual persistence.

**Keywords** *Bythotrephes* · Dormancy · Resting eggs · Hatching experiments

## Introduction

*Bythotrephes longimanus* is a predatory zooplankter, native to Eurasia, which expanded its range to North America and became invasive during the last three decades (Lehman and Cáceres 1993; Yan et al. 2001; Pangle et al. 2007; Strecker and Arnott 2008). As with many cladocerans that inhabit seasonal environments, *B. longimanus* reproduce parthenogenetically during spring and summer when conditions are favorable, but switch to gametogenesis in the autumn. In *B. longimanus*, the product of gametogenesis is a dormant resting egg (about 0.4–0.5 mm diameter)

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M. E. Brown (✉)  
Department of Biology, Hobart and William  
Smith Colleges, Geneva, NY 14456, USA  
e-mail: mbrown@hws.edu

D. K. Branstrator  
Department of Biology, University of Minnesota Duluth,  
Duluth, MN 55812, USA

that has a firm outer shell but no ephippium (Yurista 1992). Resting eggs of *B. longimanus* are broadcast from the mother and sink rapidly (about  $1 \text{ cm s}^{-1}$ ) to the lake floor (Jarnagin et al. 2000). They can tolerate extreme chemical conditions (Brown 2008) and survive gut passage in waterfowl and fish (Jarnagin et al. 2000; Charalambidou et al. 2003), which may facilitate overwintering and dispersal.

Annual resting egg production in the autumn appears to be ubiquitous in North America with no planktonic individuals reported to overwinter (Yurista 1992; Jarnagin et al. 2000; Yan et al. 2001). Thus, in the absence of inoculation from an external source, in situ resting egg production and hatching appear to be essential to the species inter-annual persistence and establishment. Previous studies suggest that resting egg banks of *B. longimanus* are dynamic and turnover more rapidly than those of other cladocerans (Herzig 1985). In Lake Michigan, egg banks of *B. longimanus* fluctuate with seasonal inputs from maternal production and vary spatially (Yurista 1997). Temperature and prey abundance are hypothesized to be the primary cues that initiate egg hatching and production, respectively (Yurista 1997; Yan et al. 2001; Jarnagin et al. 2004), but low dissolved oxygen concentrations (DO) and extreme pH also influence egg hatching success in laboratory studies (Brown 2008).

Despite the critical role of resting eggs in inter-annual persistence, and their likely role in dispersal within and among lakes, we still know appreciably little about the egg's demographic linkages with the planktonic stage and turnover rates of egg banks. To our knowledge, only two prior studies have estimated *B. longimanus* resting egg densities in lake sediments (Herzig 1985; Yurista 1997) and only one estimated the timing of neonate emergence from resting eggs in situ (Herzig 1985). Considering that *B. longimanus* has colonized over 100 lakes in North America, and tens of thousands more lakes offer suitable habitat (MacIsaac et al. 2000), a better understanding of its full life cycle, that includes passage through the resting egg stage, is needed.

Here, we used a variety of approaches to quantify *B. longimanus* resting egg production and hatching patterns in the wild to determine the role of resting eggs in the establishment and persistence of this invader. Specifically, we addressed the following questions: (1) What is the seasonal timing of resting

egg production and hatching and the demographic linkages between the resting egg and planktonic stages? (2) What are the annual inputs (production) and outputs (hatching, mortality) from the resting egg bank? (3) How do environmental conditions influence hatching fraction and emergence rates? Our research was conducted mainly in Island Lake Reservoir (hereafter called Island Lake; St. Louis County, Minnesota, USA), but to address our third question we also conducted an experiment in the laboratory and a transplant experiment in a nearby system (Fish Lake Reservoir, hereafter called Fish Lake). Fish Lake experienced a failed introduction in the early 1990s that may have resulted from its inability to support resting eggs through dormancy, including under-saturated DO in the hypolimnion (Brown 2008). Some of the results from Island Lake in 2003 were previously reported by Brown and Branstrator (2005) and are reprinted here with permission.

## Methods

We used three approaches to investigate the ecology of resting eggs of *B. longimanus*: (1) sediment and water column surveys in Island Lake, (2) field and laboratory hatching experiments that used newly produced eggs harvested from field-caught mothers, and (3) emergence traps that quantified the timing and density of neonates emerging directly from Island Lake sediments.

### Sediment and water column surveys

The sediment egg bank of *B. longimanus* in Island Lake (surface area =  $32.4 \text{ km}^2$ , Secchi  $z = 1\text{--}2 \text{ m}$ ) was examined to characterize its temporal and spatial variability. To describe weekly changes in the egg bank, areal density was estimated from duplicate Ekman box cores ( $0.02 \text{ m}^2$ ), collected at a single location ( $z = 13 \text{ m}$ ,  $47.0178^\circ\text{N}$ ,  $92.2006^\circ\text{W}$ ; hereafter IL deep). Sediment samples were collected every 6–8 days from May 2003 to July 2005, and examined 1–4 days after collection. Collections were not conducted during ice cover, except once in January 2004. Sediments that passed through a  $500\text{-}\mu\text{m}$  sieve, but were retained by a  $250\text{-}\mu\text{m}$  sieve, were searched in

entirety for intact resting eggs and the empty shell hemispheres of hatched eggs.

Concurrent with the sediment surveys at this location, water column collections of the planktonic life stage were conducted weekly. Triplicate vertical net tows were taken after sunset with a Puget-sound style net (0.25-m diameter opening, 500- $\mu$ m pore mesh) during the ice-free period, and with a Wisconsin net (0.15-m diameter opening, 53- $\mu$ m pore mesh) before sunset during ice cover. All tows spanned from 1 m off the bottom to the surface and were preserved in 70% Ethanol by volume. Triplicate tows were pooled and half of each pooled sample (split with a Folsom-style splitter) was searched for *B. longimanus*, which were scored for spine morphology, presence of resting egg broods, and gender. A straight caudal spine, as opposed to a kinked spine, is diagnostic of individuals hatched from resting eggs (Yurista 1992).

To characterize spatial variation and estimate annual inputs (production) to the resting egg bank, additional Ekman box core samples were collected in Island Lake. At eighteen sites that ranged from 6 to 22 m depth and occurred inside and outside the former river channel, duplicate samples were collected on 29–30 July 2004 and again on 11 November 2004, which captured the period of peak resting egg production (Brown and Branstrator 2005). The overlying water depth of sampling sites fluctuated between summer and late autumn by as much as 2 m due to water drawdowns by the hydroelectric utility. With the exception of sample frequency, collection and processing methods were the same as those described for IL deep.

The mean sediment egg densities between the summer and late-autumn surveys were compared with a two-sample *t* test assuming equal variance. Within each collection period (summer or autumn) a linear regression model (REML) was used to determine if sediment egg density (dependent) was predicted by the depth of the sampling location (PASW Statistics 17). The models met assumptions of normality and equal variance.

#### Egg hatching experiments

Experiments were conducted in the field and laboratory to estimate the annual outputs (hatching) from the resting egg bank. Resting eggs of *B. longimanus*

that settle to different locations in a stratified lake will experience different environmental conditions (e.g., light levels, temperature, DO) that could influence hatching as shown in *Daphnia* (Cáceres and Tessier 2003; Allen 2010). To test the effect of temperature and light, we deployed eggs in Island Lake at deep (IL deep, described above) and shallow (IL shallow,  $z = 6$  m) locations, separated by about 500 m. To test the effect of low DO, we deployed eggs in nearby Fish Lake (surface area = 12.4 km<sup>2</sup>, Secchi  $z = 1$ –2 m) where the hypolimnion can become hypoxic during summer. We also conducted a laboratory experiment to test whether a factor other than temperature, but unknown to us, may have influenced hatching rate in the field.

Experiments were deployed in the autumn and were recovered the following spring or summer. Table 1 provides location and dates of deployment and recovery for each experiment. To initiate an experiment, fecund females were collected in the autumn from Island Lake and reared in the laboratory for several days. Naturally released eggs were harvested and randomized to treatments as part of a larger pool of eggs used for tolerance experiments conducted in the laboratory (Brown 2008). Egg diameter was measured by optical reticle (Leica MZ-125 dissecting microscope). Maternal dry weight was recorded on a Cahn C-33 microbalance (accuracy of  $\pm 2$   $\mu$ g) after mothers had been preserved in 70% Ethanol. To account for weight loss from preservation in Ethanol, maternal weights were increased by 1.74 $\times$  (Giguère et al. 1989). Within 12 h of their release, eggs were placed into wells of tissue culture plates (1 egg per well, 8 or 12 wells per plate) and maintained at 4°C. The lids of the plates were modified with mesh windows (1.3 cm diameter, 210- $\mu$ m pore mesh) to permit water exchange once deployed.

For field deployments, the plates were affixed to moorings that suspended them within 0.5 m of the lake bottom. In 2002–2003 and 2003–2004, the moorings had surface expression and were recovered by SCUBA. In 2004–2005, sub-surface moorings were equipped with acoustic releases that enabled remote recovery of batches of eggs at staggered dates the following spring and summer to allow us to test the effect of time and depth (temperature, light) on hatching in Island Lake. One mooring with three batches of eggs was used at IL deep and another

**Table 1** Location and deployment information for in situ and laboratory hatching experiments

Location	Station (Lat, Lon)	Depth (m)	Deployment	Recovery	<i>n</i>
Island lake	IL deep (47.0178°N, 92.2006°W)	13	3 November 2002	11 August 2003	60
			15 October 2003	3 August 2004	60
			5 November 2004	13 May 2005	24
			5 November 2004	3 June 2005	24
			5 November 2004	29 July 2005	24
	IL shallow (47.0197°N, 92.2017°W)	6	5 November 2004	13 May 2005	24
			5 November 2004	3 June 2005	24
			5 November 2004	29 July 2005	24
			5 November 2004	15 September 2005	24
Fish lake	FL (46.9545°N, 92.2653°W)	9	15 October 2003	3 August 2004	60
Laboratory	Lab	–	5 November 2004	13 May 2005	24
			5 November 2004	3 June 2005	24
			5 November 2004	29 July 2005	24
			5 November 2004	15 September 2005	24

The number of eggs (*n*) used in each experiment is provided in the last column. Results are shown in Fig. 3

mooring with four batches of eggs was used at IL shallow. At each location, batches of eggs were held within 1 m of each other. At all locations, temperature (YSI 85 probe), DO (YSI 85 probe calibrated against Winkler titrations), pH (YSI 85 probe), and light intensity (Protomatic) were recorded near the sediment surface weekly during the ice-free season and monthly during winter as ice conditions permitted.

For the laboratory experiment, eggs were placed in tissue culture plates (as described for the field experiments) and maintained in 4-l glass pickling jars on shelves in an environmental chamber (Percival) as described in Brown (2008). Temperature and DO conditions in the jars were maintained during dormancy and hatching to mirror those that we measured at IL deep in 2004–2005. Temperature changes in the jars occurred gradually since experiments were held in multiple liters of water, which buffered them from the nearly immediate temperature change of the chamber. DO was maintained by bubbling compressed nitrogen or compressed air at 2–5 ft<sup>2</sup> h<sup>-1</sup>, and monitored (YSI 5000 probe). Conditions stabilized within a few hours. Batches of plates were removed from jars on the same dates as batches of plates were removed from the corresponding field experiment, and eggs were scored by the same criteria as the field experiments.

Following recovery from the field or lab, eggs were scored at 50× on the dissecting microscope as hatched or failed to hatch (partially hatched or not hatched) using the criteria of Yurista (1992) and Brown (2008). No attempt was made to describe the condition of hatched neonates because live neonates were uncommon and any remains were typically degraded. Hatching fraction was estimated as the number of eggs hatched over total eggs (i.e., where total eggs = hatched + partially hatched + not hatched) recovered. At the time of recovery, nearly all wells contained eggs.

For all experimental locations and years, we used an ANOVA to compare late season egg hatching fraction (August or July for Island Lake field and laboratory, August for Fish Lake). For the 2004–2005 experiments only, a linear model (REML) was generated to compare hatching fraction (dependent variable) among experimental location and month of harvest (PASW Statistics 17). *F* statistics of each independent variable were examined for significance. The model met assumptions of normality and equal variance. A priori pairwise comparisons were examined to test for differences in the mean hatching fraction among locations on each harvest date. For all experimental years and locations, the median egg diameter and the median maternal dry weight were each compared between eggs scored as hatched

versus those that failed to hatch with separate Mann–Whitney rank sum tests, because a Shapiro–Wilk normality tests failed for both metrics.

### Neonate emergence traps

We used neonate emergence traps to (1) measure the pattern of resting egg hatching in situ (also estimated by the hatching experiments) and (2) estimate mortality rates of gametogenetic neonates pre- and post-hatching. Inverted-funnel emergence traps were secured in the sediments at two locations in Island Lake on 30 April 2004, about 1 week after ice out. One set of traps was secured near IL deep (site A:  $n = 10$  traps,  $z = 10$  m; 47.0189°N, 92.2016°W) and a second set was secured approximately 7 km to the east (site B:  $n = 10$  traps,  $z = 10$  m; 47.0264°N, 92.1108°W). Emergence traps were constructed from transparent, blue, plastic water carboys (18 l, 0.25-m diameter). Mesh windows (153- $\mu$ m pore) and collection ports (at the tapered-end) were installed following the design of Cáceres (1998). The ten traps at each location were bolted to a frame to keep the replicates within 1 m of one another. The frames were piloted by SCUBA to the lake floor and embedded in the sediments. Immediately following deployment, the first set of collection bottles was secured to the traps. The collection bottle at the top of each trap was replaced by SCUBA every 7–8 days from 7 May to 27 June 2004. These dates were selected based on the time period when straight-spined individuals were present in the plankton during field studies in the springs of 2002 and 2003. At the time that bottles were collected each week, temperature and DO (YSI 85 probe) and light intensity (LI-COR, LI-1400) were recorded near the sediment surface.

In the laboratory, the portion of the emergence trap sample greater than 153  $\mu$ m was preserved in Ethanol (70% final volume) and searched in entirety (50 $\times$ ) for *B. longimanus* neonates. Emergence rates were calculated based on the mean number of neonates found in the 10 replicates divided by the period of collection in weeks. The coefficient of variation (CV) was determined for each location and collection period to quantify the variation among replicate traps.

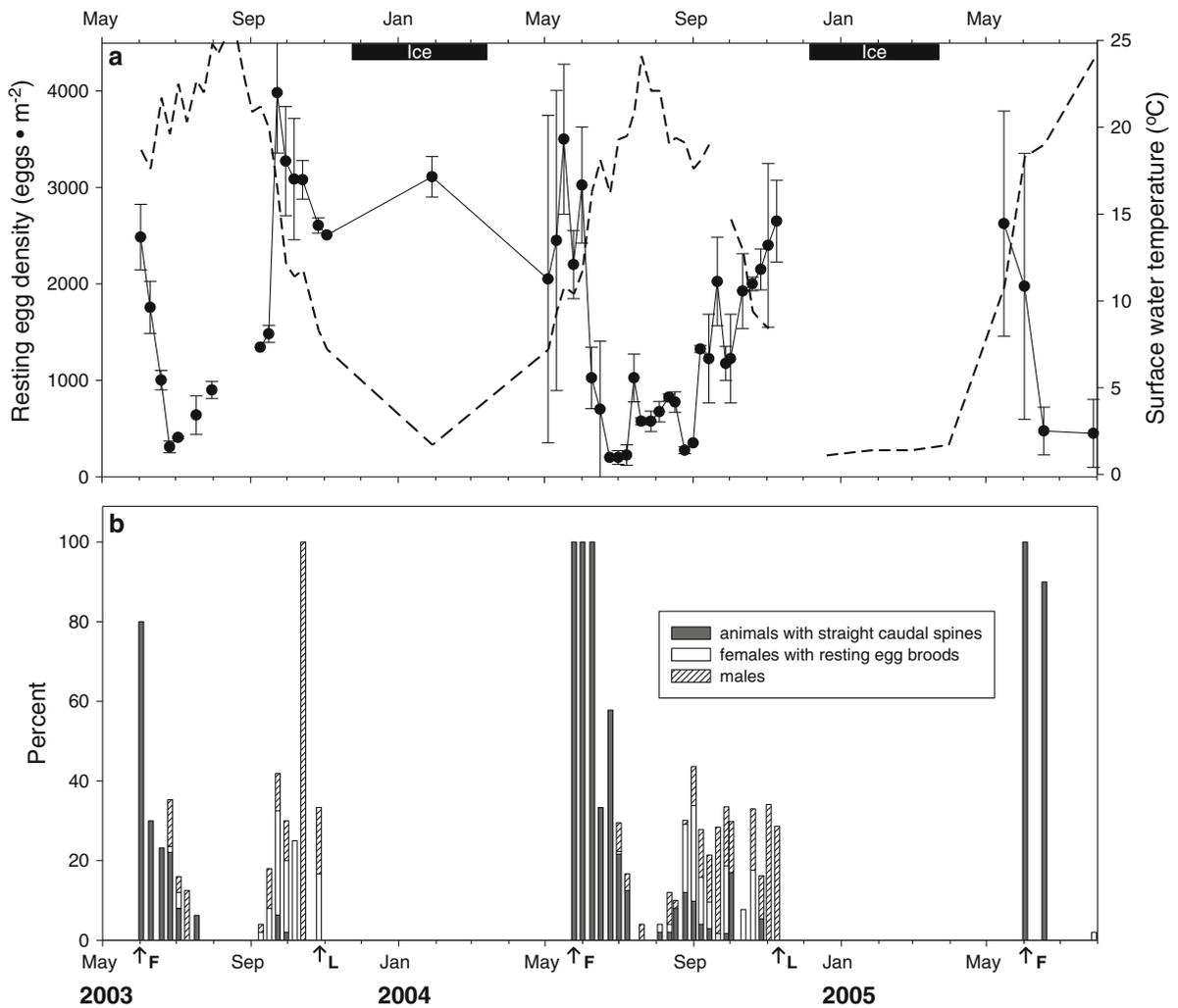
Studies of cladoceran zooplankton rarely track mortality of gametogenetic neonates because once they hatch they cannot be distinguished from parthenogenetic offspring. In *B. longimanus*, however, a

straight caudal spine is diagnostic of a female birthed from a resting egg. We used this anatomical diagnosis to estimate mortality as follows. First, from the sediment survey data from IL deep, we estimated a projected mean emergence rate (eggs  $\text{m}^{-2} \text{d}^{-1}$ ) as the difference in egg density between consecutive collections averaged from 4 May to 24 June 2004 (51 days). We then used the neonate emergence data from the traps at site A, which was near to IL deep, to compute an actual mean emergence rate (ind.  $\text{m}^{-2} \text{d}^{-1}$ ) based on the period of collection from 7 May to 27 Jun (51 days). The difference between the actual mean emergence rate from the traps and that projected from egg depletion in the sediments reflects senescence and predation of eggs and mortality during embryogenesis. Next, we used the water column survey data on the planktonic portion of the population in 2004 to estimate a mean emergence rate of 1-barb, straight-spine planktonic *B. longimanus* (ind.  $\text{m}^{-2} \text{d}^{-1}$ ) into the water column on a weekly basis from 7 May to 27 Jun (51 days) assuming no mortality. At the temperatures observed in Island Lake during this period (12–13°C; Fig. 1), neonates emerging from resting eggs should recruit to stage 2 in about 3.2 days (Yurista 1992). To estimate the rate of emergence on a daily basis, we doubled the density of neonates observed per week and divided by the number of days in the sampling interval (6–8 days). The difference between this emergence rate that assumes no mortality and the actual emergence rate based on the trap data represents short-term, post-hatching neonate mortality.

## Results

### Patterns in the egg bank and planktonic stage

At IL deep, where we monitored sediment resting egg densities and planktonic populations from May 2003 to July 2005, there were strong numerical and temporal relationships between the density of eggs in the egg bank and the demography of the planktonic stage (Fig. 1). In 2003, 2004 and 2005 during late May and June, as water column temperatures increased, egg density in the sediment declined nearly 10-fold. During this period the planktonic stage was detected for the first time following its winter absence, and a large percent of the individuals



**Fig. 1** **a** Areal density (mean  $\pm$  1SD) of *Bythotrephes longimanus* resting eggs in the sediments (solid line and circles) estimated from weekly sediment collections in Island Lake Reservoir at IL deep (see “Methods”). Surface temperature (dashed line) is shown on the secondary axis. Sediment collections were not conducted during ice cover, except once in January 2004. Month labels on the horizontal axis represent the first day of each month and data points are plotted by the date

of collection. **b** The percent of all planktonic *B. longimanus* at IL deep that were animals with straight caudal spines (solid), females carrying resting egg broods (white), or males (stippled). Arrows below the horizontal axis indicate dates when *B. longimanus* was first detected in the plankton (F) and last detected in the plankton (L) each year. Water column surveys for the planktonic stage continued monthly during ice cover

possessed a straight caudal spine, indicating that they had hatched from resting eggs. During autumn each year, some straight spine individuals were also present, indicating a second period of resting egg hatching late in the season. In 2003 and 2004, the largest annual increase in sediment egg density occurred between September and November. During this period, the percent males was on average 16% of the total population in 2003 and in 2004. Each year,

there was some production of males and resting eggs during mid-summer, but at levels much lower than during autumn (Fig. 1).

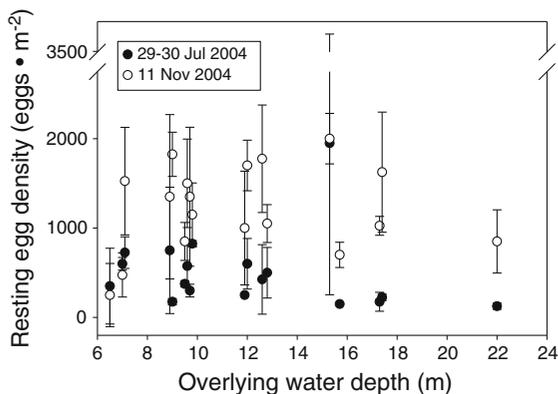
Sediment egg densities at IL deep fluctuated between a few hundred (late spring, summer) and a few thousand (early spring, late autumn) each year (Fig. 1). In 2003, 2004, and 2005 during the month of June the depletion of the egg bank was 88, 93, and 83%, respectively. In our previous report (Brown and

Branstrator 2005) we estimated depletion fractions in 2003 slightly differently and there report 92%. The increases in the egg density at IL deep each year from late summer through late autumn were similar in magnitude to the spring decreases, but occurred much more gradually.

Across the 18 sites that we surveyed between July (summer) and November (autumn) 2004, there was a mean increase of 47% (SD = 38, range 9–98% for 16 values that were positive) or 718 eggs m<sup>-2</sup> (SD = 495) between summer and autumn (Fig. 2), and the difference was highly significant ( $t_{34} = -4.719$ ,  $P < 0.001$ ). At the two shallowest sites, summer density slightly exceeded autumn density. The seasonal replenishment of the egg bank at IL deep (Fig. 1) was among the highest increase measured across the 18 sites (Fig. 2). Although sediment egg density was not predicted by overlying water depth of the site during summer ( $F_{1,16} = 0.309$ ,  $P = 0.586$ ) or autumn ( $F_{1,16} = 0.189$ ,  $P = 0.669$ ), egg density during the summer at the four deepest sites (>16 m) was the lowest and least variable of all collection sites. The two shallowest sites (<7 m) had a low density of resting eggs during both the summer and autumn collection periods.

Egg hatching patterns

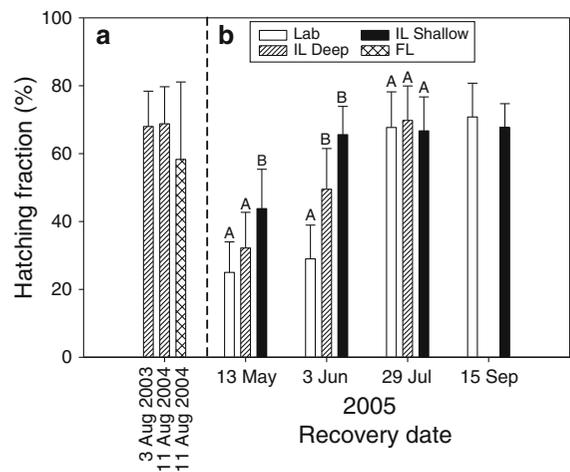
The fraction of newly produced eggs that hatched by July or August was remarkably consistent across years and locations, including comparisons between



**Fig. 2** Areal density (mean ± 1SD) of *Bythotrephes longimanus* resting eggs in the sediments at 18 lake-wide sampling sites (including IL deep) reported by overlying water depth. Collections were made at each site in July 2004 (solid circles) and November 2004 (open circles). Note break in the y-axis

the field and laboratory (Fig. 3). In all cases, at least 58% of the eggs hatched by July or August (mean = 67%), and there was no overall statistical difference among locations (lake, lab, depth within lake) or years ( $F_{5,87} = 0.808$ ,  $P = 0.493$ ). Although not statistically significant, Fish Lake had the lowest hatching fraction of all experiments, as scored in July or August, and was the only location where partially hatched eggs were observed. The aborted eggs were recorded in plates that also had the lowest hatching fraction, and accounted for 17–25% of the eggs in those plates.

In the 2004–2005 experiment, there was a marked chronology to hatching in Island Lake and in the laboratory (Fig. 3). Overall, there was a significant effect of month ( $F_{3,87} = 8.445$ ,  $P < 0.001$ ) on hatching fraction. At IL deep, hatching fraction increased from May to June to July. At IL shallow, hatching fraction increased from May to June and then remained steady. In the laboratory, hatching fraction increased from June to July and then remained steady. A statistically larger fraction of eggs hatched



**Fig. 3** Percent (mean ± 1SD) of *Bythotrephes longimanus* resting eggs that hatched by the time of recovery during **a** in situ experiments in 2002–2003 and 2003–2004 and **b** laboratory and in situ experiments in 2004–2005. Experiments were deployed in autumn and recovered the following spring or summer on the dates indicated on the x-axis (see Table 1 for additional experimental details). Means are based on the percent of eggs that hatched in replicate tissue culture plates ( $n = 5$  plates for 2002–2003 and 2003–2004;  $n = 4$  plates for 2004–2005; see Table 1 for total numbers of eggs). Different letters above error bars (**b**; 2005 recovery) indicate pairwise differences in means within May, June and July (see Table 2)

at the shallow compared to the deep station in May, but by June and July these two locations had statistically indistinguishable hatching fractions. During May and June, statistically fewer eggs hatched in the laboratory than the field (shallow site only in May), but by July the hatching fractions in the field locations were indistinguishable from the laboratory treatments (Table 2; Fig. 3).

There were notable differences in the abiotic environments at IL deep and IL shallow in 2004–2005 (Fig. 4). Although both locations had temperatures above 4°C by mid-May 2005, spring and early summer temperatures were warmer at IL shallow than IL deep (Fig. 4a). At IL shallow, but not IL deep, measurable light reached the sediment surface (Fig. 4b). The pH varied little during the 10-month deployment (pH range = 7.4–7.6). Limnological conditions during laboratory experiments in 2004–2005 were comparable to conditions at IL deep in 2004–2005 (Fig. 4). Although thermal conditions were similar between Island Lake and Fish Lake, DO concentrations were generally 1–2 mg l<sup>-1</sup> lower in Fish Lake during the winter, and the hypolimnion became severely oxygen depleted during summer (Fig. 4c).

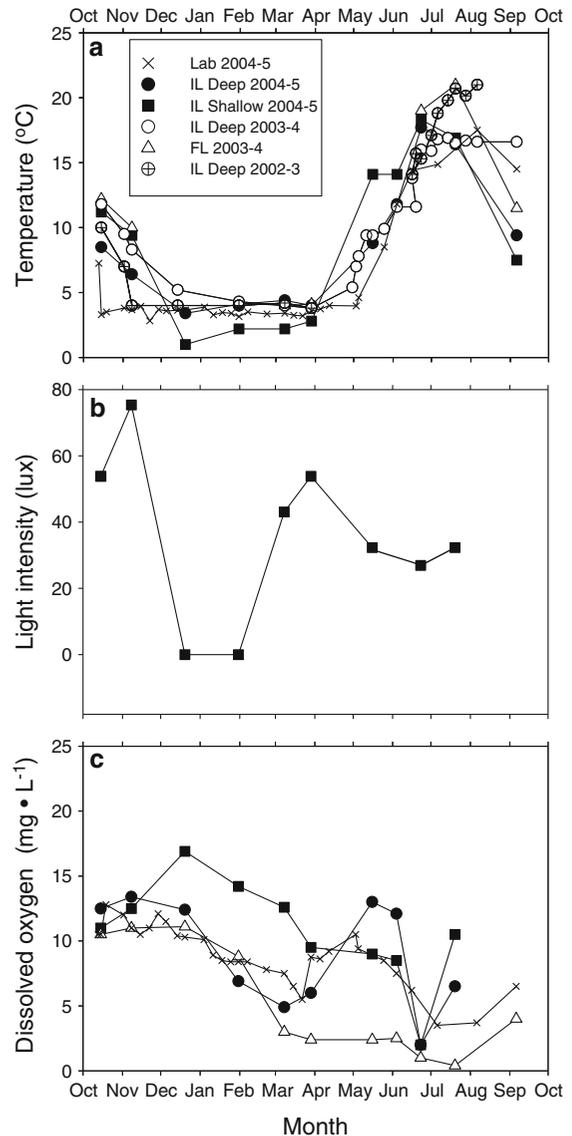
For all of the field and laboratory hatching experiments combined, the median diameter of eggs scored as hatched was statistically indistinguishable from those that failed to hatch (median of all eggs = 430 μm,  $U = 1.57454 \times 10^5$ ,  $n_{\text{hatched}} = 713$ ,  $n_{\text{not hatched}} = 475$ ,

**Table 2** Statistical results from selected a priori pairwise comparisons for in situ and laboratory hatching experiments that were conducted during 2004–2005 (Fig. 3)

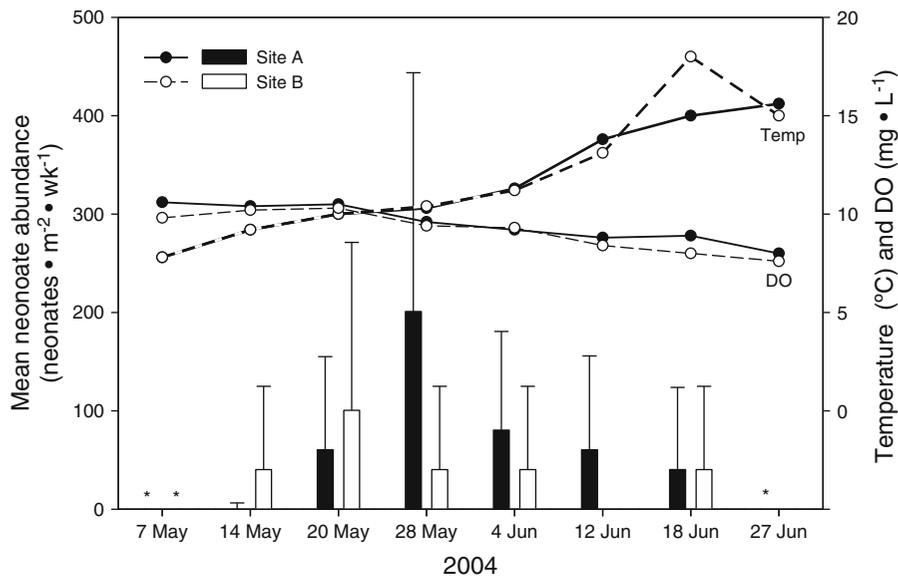
Month	Comparison	Mean difference	SE	<i>P</i>
May	Deep × shallow	17.98	5.74	<b>0.002</b>
	Deep × lab	5.19	5.99	0.387
	Shallow × lab	12.79	6.17	<b>0.038</b>
June	Deep × shallow	0.57	5.39	0.916
	Deep × lab	26.63	5.84	<b>&lt;0.001</b>
	Shallow × lab	26.06	5.99	<b>&lt;0.001</b>
July	Deep × shallow	6.70	5.74	0.243
	Deep × lab	5.20	5.99	0.385
	Shallow × lab	11.90	6.17	0.064

Experimental locations are given in Table 1, and abbreviated in column 2 as IL Deep = Deep and IL Shallow = Shallow. In column 3 the mean difference is the absolute value of the estimated marginal mean difference generated by the linear model. Statistically significant differences are shown in bold

$P = 0.755$ ). However, eggs scored as hatched were produced by heavier mothers than were eggs that failed to hatch (median<sub>hatched</sub> = 187 μg, median<sub>not hatched</sub> = 178 μg,  $U = 1.5656 \times 10^5$ ,  $P = 0.017$ ).



**Fig. 4** Field conditions measured between 0.5 and 1 m above the sediment surface including **a** temperature (°C), **b** light intensity (lux), and **c** dissolved oxygen (mg l<sup>-1</sup>) during the in situ and laboratory hatching experiments. See Table 1 for experimental locations. Month labels on the horizontal axis represent the first day of each month for the deployment and recovery years (2002–2003, 2003–2004, and 2004–2005), and data points are plotted by the date of collection. No light was detected at the sediment surface at IL deep or FL, and the laboratory experiments were conducted in the dark



**Fig. 5** Emergence rate (mean  $\pm$  1SD) of *Bythotrephes longimanus* neonates during May and June 2004. Estimates are based on collections from emergence traps embedded in lake sediments at two sites (see “Methods”). Stars denote dates when no neonates were detected in the traps. Temperature and dissolved oxygen (DO) concentrations taken just above the

sediment surface at the time of each collection are shown for site A (solid line and black circles) and site B (dashed line and open circles). Temperature trends are bolded to differentiate them from DO trends, but data points for the temperatures at the two sites overlap for the first several collections in May

Neonate emergence patterns

At both sites A and B, the first neonates were detected on 14 May 2004 and no neonates were detected at either site on 27 Jun 2004 (Fig. 5). On each of the intervening collection dates, the rate of emergence among replicates at each site was highly variable (CV ranged between 1.25 and 2.11). Although not statistically different from other dates, the maximum neonate abundance was observed near the end of May at both sites. There were no notable differences between the two sites in egg emergence rates or timing, or in temperature or DO near the sediment surface (Fig. 5). When neonates were initially detected, temperature near the sediment surface was approximately 9°C at both sites (Fig. 4). Light conditions were also comparable; On 7 May and 14 May light was  $\leq 0.015 \mu\text{E m}^{-2} \text{s}^{-1}$  at both locations, and was below detection thereafter. The hatching period based on the emergence traps (Fig. 5) is consistent with changes in the density of the egg bank and the window of presence of straight-spined individuals in the water column (Fig. 1).

**Table 3** The potential daily contribution ( $\pm$ SD) of the sediment egg bank to the planktonic phase during spring and early summer based on estimates of (1) the depletion of the sediment egg bank (Sediment surveys), (2) the presence of neonates in emergence trap samples (Emergence traps), and (3) the density of one-barb, straight-spined plankton (Plankton surveys)

	Estimated mean emergence rate (ind. $\text{m}^{-2} \text{d}^{-1}$ )
Sediment surveys	24.5 ( $\pm 152.0$ )
Emergence traps	8.7 ( $\pm 8.5$ )
Plankton surveys	3.6 ( $\pm 4.3$ )

All three estimates are based on field studies from early May until late June 2004

Estimates of neonate emergence rate from the sediment egg bank during May and June in 2004 differed greatly depending on the method (Table 3). This was anticipated given that the three methods differentially accounted for mortality (see “Methods”). Using the data, we estimated a 64% mortality rate of eggs and embryos prior to neonate emergence, followed by a 59% mortality rate for newly emerged neonates during their first few days of life in the plankton. Combined, data suggest that 85% of eggs

depleted from the egg bank in spring 2004 at IL deep failed to recruit to stage 2 in the plankton. This high death rate is corroborated by the observation that straight-spined individuals dominated the population (100%) during the first 3 weeks of 2004 (Fig. 1), indicating that no individuals had yet recruited to maturity and birthed parthenogenetic offspring.

## Discussion

In Island Lake, *B. longimanus* exists solely in the dormant resting egg phase during winter (Fig. 1). This is consistent with other invasive *B. longimanus* populations (Yurista 1992; Jarnagin et al. 2000; Yan et al. 2001) and indicates that resting eggs are critical to the invader's inter-annual persistence. High annual turnover in the resting egg bank in Island Lake indicates that contributions to the dormant phase may need to be large and frequent in order to sustain established populations. Even among long-established populations, *B. longimanus* egg banks appear to turn over frequently. In Mondsee, Austria, where *B. longimanus* is native, Herzig (1985) detected a 98% decline in the resting egg bank of *B. longimanus* in the top 2 cm of sediment between winter and summer. Recent  $^{210}\text{Pb}$  dates for Island Lake sediments (unpublished) suggest that our Ekman core collections penetrated deep enough to retrieve most sediments deposited since the arrival of *B. longimanus*. Thus, our reported temporal and spatial differences in egg densities (Figs. 1, 2), probably reflect changes to the entire lacustrine repository, not just eggs produced the previous year.

High mortality—64% for resting eggs and embryos and 59% for newly emerged neonates (Table 3)—may be one explanation for fast turnover, and may be coupled to food resources and predation. *B. longimanus* emerging from resting eggs rely on the vernal development of cladoceran populations whose own growth rates can be variable (Wetzel 2001). This could lead to temporal and spatial mismatch in resource availability. Compared to other zooplankton, *B. longimanus* eggs and gametogenetic neonates are also relatively large and visible (Branstrator 2005), which should increase their vulnerability to macroinvertebrate and fish predators that are size-selective in their feeding habits. That there was variable duration in population dominance by gametogenetic neonates in the springs of

2003, 2004, and 2005 at IL deep suggests that vernal population growth varied among years (Fig. 1).

Among founding populations of *B. longimanus*, the requirement to produce a resting egg bank could be a bottleneck to establishment. This is particularly true if founding propagules arrive too late in a season to mount sufficient densities of females and males necessary to produce resting eggs. The proportion of males manufactured does not appear to be trivial. In Island Lake, 16% of the total population was males during autumn and similar proportions have been noted in other lakes (Garton et al. 1990; Straile and Hälbig 2000; Yan et al. 2001). Acknowledging the need for male and resting egg production to establish, Drake et al. (2006) proposed that arrival of planktonic propagules during middle to late summer maximized invasion success.

The high mortality rate among resting eggs and emergent neonates that we measured, collectively 85%, suggests that colonization based on resting eggs only will require relatively large numbers of propagules. Moreover, the autumn peak in resting egg production does not overlap with anticipated windows of the highest vector activity (recreational boating) in regions of North America where *B. longimanus* is expanding its range (MacIsaac et al. 2004). Thus, propagule pressure from resting eggs may be smaller than from planktonic-stage individuals and subject to more Allee effects (Drake 2004).

In some invasive populations of *B. longimanus*, including Island Lake, mid-summer resting egg production has been noted (Garton et al. 1993; Burkhardt 1994; Yan and Pawson 1997). This may reflect deterioration in local conditions (e.g., food abundance), but alternatively it may be an adaptive strategy to hedge against environmental uncertainty in the new habitat. Initiation of resting egg production in mid-summer could facilitate establishment (overwintering) as well as dispersal (e.g., by fish, Jarnagin et al. 2000) and be under selection in genotypes that are expanding their ranges.

We found clear evidence for environmental control of hatching. Hatching was not only concentrated during May and June, but was accelerated at near-shore locations which received more light and warmed faster than offshore locations. This is consistent with previously measured hatching cues near 4°C, and with warmer temperatures and more light accelerating the hatching rate (Yurista 1992, 1997).

The fact that hatching patterns in the laboratory closely resembled those in the field at IL deep is further evidence that temperature, and not some unknown factor in the lake, was the primary hatching cue. Our survey results revealed that there were fewer eggs at the very shallowest sites during summer but also during autumn. This spatial pattern probably reflects variation in overlying densities of *B. longimanus*, which is a pelagic-based species. Similar horizontal gradients in egg bank density have been found in Lake Michigan (Yurista 1997) and Mondsee (Herzig 1985). Collectively, our results suggest that shallow, well-mixed environments may facilitate faster spring hatching. This could potentially improve establishment success in ecosystems such as reservoirs that tend to be well-mixed (Havel et al. 2005; Johnson et al. 2008), compared to deep, cold lakes where a spring thermal cue  $>4^{\circ}$  is less likely in the profundal zone. The only basin for comparison is Mondsee, Austria, which is a deep (average depth = 36 m), cold alpine lake (Dokulil and Skolaut 1986). There, resting egg hatching is delayed about 1 month (beginning mid-June) compared to Island Lake (Herzig 1985).

In addition to temperature and light, our results suggest that DO also impacts egg hatching. Only in the Fish Lake experiments did we observe partial hatched eggs (failed development). This has been observed previously in low DO environments in the laboratory (Brown 2008). A potential reduction in hatching fraction in Fish Lake could, in part, explain why *B. longimanus* failed to establish in this reservoir in the early 1990s, especially when combined with an estimated 85% background mortality rate. Further, eggs incubated in undersaturated DO conditions give rise to smaller neonates than eggs experiencing saturation (Brown 2008), which could accentuate mortality in the hypolimnia of productive lakes.

Variation in hatching fraction of cladoceran zooplankton has been widely considered but rarely in the context of dispersal and invading species (Allen 2010). Theoretical models that combine dispersal and dormancy, however, predict that as more habitats becomes available to colonize, it is advantageous for a population to invest more in dispersal than dormancy (McPeck and Kalisz 1998). For a species expanding its range, such as *B. longimanus*, dispersal (short dormancy) could be adaptive and selected on. The hatching fractions that we measured for

*B. longimanus* here (67% average) are similar to other studies on *B. longimanus* (Yurista 1997; Jarnagin et al. 2000) but are on the upper range of hatching fractions reported in *Daphnia* (Cáceres and Tessier 2003; Allen 2010). High hatching fractions in *B. longimanus* are, at minimum, consistent with invasion, but there are alternative explanations. For example, it is feasible that high hatching fractions reflect common exposure to a hatching cue ( $4^{\circ}\text{C}$ ), which was exceeded here and in the laboratory trials of Yurista (1997) and Jarnagin et al. (2000). It is also possible that high hatching fraction is a life-history trait in *B. longimanus* that has been selected to balance low, potentially stochastic, survivorship in the resting egg and neonate. Until more populations are studied, and clonal variation and founder effects are considered, the relative contributions of genetic and environmental factors on hatching will remain unclear.

Finally, we note that there was a measurable maternal effect on hatching in that heavier mothers produced resting eggs that were more likely to hatch the following spring. Maternal effects on resting egg hatching have been demonstrated in *Daphnia* (De Meester and De Jager 1993; Boersma et al. 2000). The maternal contribution may be genetic and/or environmental (related to lipid provision). In either case, the pattern reflects inherent feedbacks between the planktonic stage and the resting egg stage that could have consequences for the establishment success of invading populations.

In conclusion, although most studies of *B. longimanus* to date have focused on the ecology of the planktonic stage, the resting egg clearly plays a pivotal role in inter-annual persistence and establishment. Neglecting the full life cycle of *B. longimanus* in models of range expansion could mislead our understanding of factors controlling the process. As a group, cladocerans have had an exceptionally high invasion rate and present a variety of future invasion risks (Grigorovich et al. 2003). Understanding the full range of mechanisms that facilitate their establishment is imperative.

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