**Bythotrephes cederstroemi** Diapausing Egg Distribution and Abundance in Lake Michigan and the Environmental Cues for Breaking Diapause

**Peder M. Yurista**  
Department of Biology  
University of Michigan  
Ann Arbor, Michigan 48109-1048

**ABSTRACT.** Bythotrephes diapausing eggs found in the sediments of Lake Michigan ranged in density from less than 100 m⁻² in shallow waters (20 m) to densities over 5,000 m⁻² in offshore waters (> 60 m). The density of diapausing eggs was observed to increase with depth of the water column and is probably the result of higher input from greater adult abundance in off-shore waters. Temperature and photoperiod are environmental cues that are known to affect the time and success in hatching of diapausing eggs in many species. An increase in temperature above the storage temperature (4°C) was found to increase the success of hatching Bythotrephes diapausing eggs and shorten the incipient time to hatching, however, all temperature treatments had an equivalent effect on success implying that the cue is discrete and occurs close to 4°C. The onset of maximum hatching rate following the cue to break diapause was dependent on temperature, which is the probable result of development rate being a function of temperature. Length of photoperiod did not have a significant effect on the hatching success or development time of Bythotrephes diapausing eggs, unlike past observations for the cladoceran Daphnia.

**INDEX WORDS:** Bythotrephes, eggs, diapause, Lake Michigan.

**INTRODUCTION**

The production of resting or diapausing eggs is a means by which organisms survive stressful environmental conditions. These conditions may be abiotic (e.g., temperature), or biotic (e.g., resources, predator avoidance tactic: Hairston 1987). Diapausing eggs do not hatch immediately but remain in a state of suspended development until the stressful condition has passed or an associated environmental cue has been detected. Environmental cues such as the length of photoperiod or temperature (Stross 1966) can signal the return to favorable conditions and break diapause, which allows normal development to resume. Successful recruitment of a zooplankton population each year often depends on the diapausing egg stock and the emergence of young following stressful conditions.

*Bythotrephes cederstroemi* is a predatory cladoceran that has invaded the Laurentian Great Lakes and has potential implications for energy flow in the Great Lakes food web (Lehman 1991, Yurista and Schulz 1995). *Bythotrephes* is now an important member of the plankton and reproduces by cyclical parthenogenesis. In the fall they produce gametogenic diapausing eggs that overwinter and hatch in the spring. The adult *Bythotrephes* does not overwinter, consequently, the development each year of a *Bythotrephes* population is a result of the successful hatching of diapausing eggs and the subsequent parthenogenic reproduction by emergent animals.

The goals of this research were to answer the questions: (1) How extensive is the distribution of *Bythotrephes* diapausing eggs and what are their abundances in Lake Michigan? (2) What environmental conditions affect the success of egg hatching and seasonal population dynamics of *Bythotrephes*?

**METHODS**

The benthic area of Lake Michigan was surveyed to assess *Bythotrephes* diapausing egg abundance and distribution by collecting gravity cores. Initial Ponar grab samples indicated the feasibility of collecting *Bythotrephes* eggs from bottom sediments while gravity core samples were employed for the ease of quantifying their contents. Sediment cores were collected at inshore (20 m) and offshore (100
Bythotrephes Diapausing Eggs

m) reference stations monthly during the summers of 1990–1991 (May through September), monthly along the transect between these stations (at depths of 35, 52, and 75 m) (1991), and at other stations on synoptic cruises of Lake Michigan in August of each year (Fig. 1). Cores of 6.7 cm diameter were obtained by allowing a gravity corer to free fall the last 10 m of water depth. Four replicate samples were obtained when possible. Lake bottom substrate varied with location, and sandy locations were difficult to sample.

Diapausing egg density was quantified from the hydraulically extruded sediment cores. Early samples were examined centimeter by centimeter to a depth of 3 cm with eggs not found below a depth of 2 cm. Sedimentation rates in Lake Michigan are a few tenths of a millimeter per year (Robbins and Edgington 1975) and the top 2 cm completely encompass the time span during which Bythotrephes have been present in the Laurentian Great Lakes. The top 2 cm of each sediment sample was sieved through 500 µm nylon mesh to presort and remove large items (such as Monoporia, Mysis, oligochaetes, molluscs, and debris), and then passed through 300 µm nylon mesh to retain particles in the size range of Bythotrephes diapausing eggs. Previous measurements of egg diameters (434 ± 24 µm, with a range of 340 to 490 µm, n = 156) indicated that these were appropriate mesh sizes. Samples were preserved in 10% sugar-Formalin solution and retained for microscopic observation and quantification. Each sample was searched completely for Bythotrephes diapausing eggs using a dissecting microscope. Intact eggs that appeared to have been attacked by fungi or bacteria were tallied separately and not used in the analyses. Replicates taken on the same date for each station were averaged and the results reported on an areal basis. A total of 280 sediment core samples were analyzed during this investigation.

The effects of temperature and photoperiod as potential cues for the hatching of diapausing eggs were investigated with a fully factorial design. Temperature treatments were 6, 8, and 10°C maintained in a darkened walk-in culture room and photoperiod treatments were 8 hours Light:16 hours Dark (8L), 12 hours Light:12 hours Dark (12L), and 16 hours Light:8 hours Dark (16L). Photoperiod was controlled with timers on 4-watt incandescent lights and attenuated with neutral density screening to an intensity of less than 0.1 µE-m⁻²·sec⁻¹, the approximate light intensity at 20 m in Lake Michigan.

FIG. 1. Locations at which benthic samples were collected to assess Bythotrephes diapausing egg densities in Lake Michigan during the summers of 1990 and 1991. Samples were collected at several sites near Grand Haven, Michigan, from May until September each year whereas other sites were sampled on a synoptic cruise once each year. The 20 m and 100 m reference stations are indicated by stars.
Diapausing eggs were obtained as they were released by gametogenic *Bythotrephes* that had been collected from Lake Michigan and maintained in the laboratory (Yurista 1992). The eggs were placed in GF/F filtered Lake Michigan water and stored at 4°C in the dark for a necessary refractory period (Herzig 1985). After 4 months of storage approximately 430 eggs were sorted into nine culture trays, one for each treatment. The remaining diapausing eggs (193) were stored in the dark at 4°C as controls and observed at the conclusion of the experiment. Each tray contained approximately 48 eggs with 2 eggs per 2-mL well. The eggs were observed daily and the dates of hatching events were recorded. The development stage of eggs (Yurista 1992) was observed in order to anticipate hatching. Observations were made using either a low-light video camera or a dissecting scope under low intensity red light during the light cycle of the photoperiod. The experiment was allowed to run for 110 days at which time the hatching rate decreased to near zero. Statistical analyses were performed using SYSTAT (Wilkinson 1990). A nonlinear equation was fit to the accumulated number of hatched eggs as a function of elapsed time for each temperature and photoperiod treatment (Y = a/(1 + e^(bX+c))). The second derivative was evaluated numerically and the point of maximum hatching rate (inflection point) for each treatment was determined. Two-way ANOVA was used to test for treatment effects on time to maximum hatching rate and the percent success of hatching. Tukey's test for additivity (F*) was used to test for additivity of the factors temperature and photoperiod because of one replicate per treatment (Neter et al. 1990). Nonparametric statistics (Mann-Whitney rank test, MWU) were used in other statistical comparisons because comparisons involved variables with sample sizes of less than 10. The level of significance used for hypothesis testing was α = 0.05.

RESULTS

*Bythotrephes* diapausing eggs are relatively abundant and widely distributed throughout Lake Michigan waters. Densities ranged from less than 100 to over 5,000 m⁻². The average density for all sample dates and locations was 2,410 m⁻² (± 1,600 SD, n = 73). Higher densities of *Bythotrephes* eggs were observed at offshore stations (> 60 m) than inshore stations (20 m) (Fig. 2). Average density for all stations less than 60 m in depth was 1,327 m⁻² (± 1,308 SD, n = 36) and for stations greater the 60 m, 3,464 m⁻² (± 1,070 SD, n = 37). Sample locations in Green Bay were relatively shallow (20 m) and had low densities (0–140 m⁻²) of *Bythotrephes* diapausing eggs. Egg density increased rapidly beyond a depth of 35 meters and appeared to plateau at depths of 75 m and greater (Fig. 2), although the data were variable. Egg density at the 75 m (n = 7) and 100 m (n = 7) transect stations (1991) did not differ (MWU, p = 0.65) (Fig. 2). A logistic function suitably described the entire data set (R² = 0.88) (Fig. 2).

Abundance appeared to increase slightly with the progressing season, however the regression slope was significantly different from zero (p = 0.04) for only the 20 m depth in 1990 (p ≥ 0.2 for the other regressions) (Fig. 3). Mean abundance at the 100 m station increased from 3,613 m⁻² (± 733 SD, n = 6) to 6,000 m⁻² (Fig. 2).

**FIG. 2.** *Bythotrephes* diapausing egg density in Lake Michigan sediments as a function of water column depth for all stations sampled in 1990 and 1991. The solid symbols are *Bythotrephes* resting egg abundance collected in 1991 along a transect off Grand Haven, Michigan (43.00° N). The open symbols are for all other locations. The 1991 densities at the transect 75 m (n = 7) and 100 m (n = 7) stations do not differ significantly (MWU, p = 0.65). The regression line for all data points is Y = 3,490/(1 + exp(3.59 - 0.0904X)) (R² = 0.88, n = 73).
Temporal trends in Bythotrephes diapausing egg abundance at 20 m and 100 m reference stations off Grand Haven, Michigan (43.00° N) for 1990 and 1991. The solid symbols are for the 20 m station and the open symbols are for the 100 m station. The regression slope was significant only for the 20 m station in 1990 (p = 0.04), all other regression slopes were not significantly different from zero (p > 0.2).

in 1990 to 4,385 m⁻² (± 591 SD, n = 7) in 1991 indicating a slight build up over time but again it was not statistically significant (MWU, p = 0.074).

The hatching rate of diapausing eggs in all experimental treatments for environmental cues was slow at first, rose to a maximum, and then slowed down and stopped near the end of the observations. Hatching occurred over a moderate time period dependent on temperature (range of 25 days at 10°C to 62 days at 6°C). Empirical nonlinear curves fit to accumulated egg hatching as a function of time (Fig. 4) described this observation well (R² ≥ 0.98 for all treatments).

Time to maximum hatching rate and the final percent success of hatching did not exhibit any interaction effects between the factors temperature and photoperiod. Tukey's one degree of freedom analysis indicated no departure from additivity for both maximum hatching rate (F* = 2.0, p = 0.25) and percent hatching success (F* = 0.01, p = 0.93). The analysis of time to peak hatching rate, using a two-way ANOVA without an interaction term, indicated that the factor temperature had an effect (F_Temp = 12.78, p = 0.037) but the factor photoperiod did not have an effect (F_Phot = 0.44, p = 0.85). For final percent hatching success, the two way ANOVA with no interaction term did not indicate an effect for either factor (F_Temp and F_Phot, p > 0.8). The average hatching success was 49% (14.3 SD, n = 9) for all treatments, while the diapausing eggs stored at 4°C had a hatching success of only 8% (16 out of 193). Because no photoperiod effects were observed and there was a greater than five fold increase for all treatments, it was interpreted that the treatment temperatures all exceeded a thermal cue. Although Herzig (1985) did not find a difference in hatching of Bythotrephes longimanus diapausing eggs in continuous light or continuous dark, the lack of a control for presence or absence of light in this experiment does not rule out light as a cue.
DISCUSSION

*Bythotrephes cederstroemi* diapausing eggs are widely distributed and found at significant densities in Lake Michigan. The diapausing egg distribution and densities are comparable to those observed for *Bythotrephes longimanus* in Mondsee by Herzig (1985). Offshore densities in Mondsee ranged up to 7,000 m⁻² with inshore locations having much lower densities. An explanation for the observed pattern in Mondsee was that water basin flow patterns created by inflowing streams transported the eggs to deeper basins (Herzig 1985). The offshore egg abundance in both Mondsee and Lake Michigan are oligochaetes, amphipods, bivalves, and chironomids (Alley and Mozley 1975). Of these the most likely to redistribute sediments in Lake Michigan offshore waters are the oligochaete *Stylodrilus heringianus* and amphipod *Monoporia affinis* (Krezoski et al. 1980). Redistribution of radionuclides by *Stylodrilus heringianus* to a maximum depth of 4.4 cm was observed by Krezoski et al. (1984) in the laboratory. These experiments were performed with animal densities 2–10 times those in offshore Lake Michigan and at elevated temperatures of 10.5–11.5°C (normal benthic temperature is approximately 4°C). Sieved and homogenized sediments were allowed to settle in their experimental procedure. These experiments demonstrated bioturbation, however, the animal densities, thermal regime, and consolidation and compaction of sediments within microcosm chambers do not appear to translate directly to my field observations. Maximum mixing depths of in situ ¹³⁷Cs profiles at locations within kilometers of our sampling stations show the mixed sediment layer to be 2 cm (Robbins and Edgington 1975). Additionally, transport and redistribution of large items such as *Daphnia* ephippia where not studied in the laboratory and it is unknown whether ephippia or *Bythotrephes* diapausing eggs would be redistributed in a manner similar to the sediments. Bioturbation by *Monoporia* occurs in the less dense top centimeter (Krezoski et al. 1984) and would not be a factor in extensive egg redistribution. The top 2 cm at Lake Michigan stations sampled encompass the time span during which *Bythotrephes* have been present in the Laurentian Great Lakes and do not appear to be confounded by bioturbation.

The spatial distribution of diapausing eggs in Lake Michigan sediments is the result of *Bythotrephes* abundance in the water column as a source term rather than processes that redistribute eggs. First, focusing of lake sediments (Likens and Davis 1975) is unlikely to be a major factor involved in the transport of *Bythotrephes* diapausing eggs from inshore to offshore locations. Focusing in Lake Michigan has been found to occur in studies using atmospherically deposited radionuclides (Edgingon and Robbins 1990). *Bythotrephes* diapausing eggs are lighter than most sediments (specific density is approximately 1.13, personal observation) and could be easily resuspended and transported offshore during unstratified periods (Edgington and Robbins 1990, Eadie et al. 1984), storm events (Mortimer 1988), or internal seiches. A qualitative test to determine the magnitude of a focusing effect on the distribution of *Bythotrephes* diapausing eggs is to compare between inshore and offshore stations the ratio of accumulated egg abundance in the benthic region below the water column with females found in the water column for previous years (Lehman 1991). A significant difference in ratios would indicate that differential input or loss processes are acting at each location. Transport of eggs from shallow water to deep waters would result in a lower ratio at inshore regions and a higher ratio at offshore regions. The ratio of eggs to animals was similar at both stations (Table 1) and does not support focusing as a significant transport mechanism from shallow to deep waters. Secondly, predation by fish on *Bythotrephes* with diapausing eggs followed by migration to deep water habitats and defecation is not a likely mechanism for significant transport offshore of diapausing eggs. The predominant planktivorous fish species of inshore shallow water habitats of Lake Michigan are rarely found in deep offshore waters (Baker et al. 1992, Crowder et al. 1987, Jude and Tesar 1985, Christie 1974). Additionally the ratio of *Bythotrephes* densities in shallow waters to those offshore is low (Lehman 1991, Lehman and Caceres 1993) and complete transport of the measured egg density would not substantially alter the offshore density. In addition to transport, differential loss through hatching of eggs may also contribute to density differences between inshore and offshore waters (DeStasio 1989). The absolute magnitude in differential hatching loss between inshore and offshore
TABLE 1. A comparison between an inshore (20 m) and offshore (100 m) station in Lake Michigan of the seasonal average of adult Bythotrephes in the water column (from Lehman 1991, Lehman and Caceres 1993, Branstrator 1995) to the number of diapausing eggs found in the sediments (1990). The ratio is an estimate of potential input from the average abundance of adults. A large difference in ratios between stations would indicate that differential input or loss occurs at either location.

<table>
<thead>
<tr>
<th>Station</th>
<th>Diapausing eggs in sediments (m⁻²) (SD)</th>
<th>Adults in water column (m⁻²) (SD)</th>
<th>Ratio (eggs/adults)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 m</td>
<td>284 (± 250, n = 4) 1990</td>
<td>36 (± 33, n = 9) 1987,89</td>
<td>7.9</td>
</tr>
<tr>
<td>100 m</td>
<td>3,613 (± 733, n = 7) 1990</td>
<td>321 (± 210, n = 13) 1988,89</td>
<td>11.3</td>
</tr>
</tbody>
</table>

stations is limited by the large difference in the egg bank size and in the potential input from adult Bythotrephes (Table 1). Although differential hatching may be important (see below), the most likely explanation for the higher abundance of diapausing eggs in deep waters appears to be the larger source term of adults found in the water column at offshore stations (Lehman 1991, Lehman and Caceres 1993).

Photoperiod length was not found to be a cue for breaking diapause in the diapausing eggs of Bythotrephes. Diapausing eggs have been observed to hatch in continuous dark for *B. longimanus* at 2.5 and 4.6°C (Herzig 1985) and *B. cederstroemi* at 5.6 and 10.6°C (Yurista 1992). During the current study, eggs stored in the dark at 4°C and checked at the completion of the experiment showed some hatching (8%). The present study did not find an increase in hatching with increasing photoperiod length (but did not investigate presence/absence of light), while Herzig (1985) did not observe an increase in hatch success with continuous light compared to continuous dark in his treatments. These two results indicate that photoperiod is probably not a cue for breaking diapause in *Bythotrephes* as has been observed for *Daphnia* (Stross 1966) and some species of rotifers (Pourriot and Snell 1983). Cues due to photoperiod are unlikely to have evolved as a result of low light penetration to the deep waters where *Bythotrephes* diapausing eggs are predominantly distributed.

Temperature appears to be a positive cue for hatching in *Bythotrephes* diapausing eggs. As temperature was increased in this experiment above storage temperature (4°C), there was a greater than five fold increase in percent hatching. However, within the range of temperature treatments used there was no significant difference in total success. Herzig (1985) also found an increase of hatching success greater than four fold in going from 2.5 to 4.6°C but did not find a difference between continuous light and continuous dark at 2.5°C, which reduces the implication for presence or absence of light as the cue. The results from these two studies indicate that an increase in temperature increases the success of hatching, and the temperature threshold at which this occurs is near 4°C. The cue appears to be discrete because further increases in temperature (8 and 10°C) did not stimulate greater success. The higher temperatures also increased the onset of maximum hatching rate, probably through increased development rate. Profundal waters of most deep temperate lakes are often near 4°C throughout the year, sometimes cooling to temperatures near 2°C if mixing conditions continue before ice cover. A small increase in temperature to or near 4°C after a long storage would be a reliable environmental signal that spring conditions have arrived. A much higher temperature in the benthos is less likely to be reached once large lakes stratify and accordingly a cue greatly above 4°C is not evolutionarily favored.

Diapausing eggs in shallower waters (< 35 m), even though at low densities, may be a significant factor in the yearly population development of *Bythotrephes*. Eggs in shallow waters are more likely to (1) experience a thermal cue with a five fold increase in hatching success rate, (2) experience a thermal cue earlier in the season lengthening the time for population development, and (3) have a faster development rate during the early season when water temperatures are higher inshore compared with offshore. Early season temperatures in Lake Michigan are below 4°C. Following winter, the lake begins to warm and a thermal bar develops in Lake Michigan separating warmer shallow waters from cold offshore waters. As the lake continues to warm, the bar moves outward toward deeper
water and is replaced by water warmer than 4°C. The mixed water column offshore continues to warm to 4°C and the thermal bar eventually disappears. The shallower benthic regions inshore, which are subject to the earlier warming, are likely to realize the five fold increase in hatching success following receipt of a thermal cue and the first to contribute inocula to each years population. As the thermal bar progresses to deeper waters, the thermal conditions (Garton et al. 1992, Yurista in prep) and cladoceran prey populations (Lehman 1991, Mor- dukhau-Boltovskaya 1958) permit emerging Bythotrephes to increase their population through parthenogenic reproduction. The development of the seasonal population may be fast because time to primaparity (first clutch release) is 14 days at 12.7°C (Yurista 1992). Eggs in deeper waters will hatch later if or when the thermal cue extends to deep water. The occasional hatch without any apparent cues may be a bet hedging strategy if the thermal environment does not exceed the cue to signal a break in diapause.

Early seasonal development of a Bythotrephes population is probably dependent on the diapausing eggs produced the previous season. Respiration rates of Bythotrephes diapausing eggs are such that they are capable of surviving for approximately 280 days on their energy stores (Andrew and Herzig 1984). As oxygen tensions decrease, the respiration rate of Bythotrephes diapausing eggs also decreases (Andrew 1995) and diapausing eggs may remain viable for a longer time period. In the laboratory, Herzig (1985) observed a diapausing egg hatch after 17 months, but most hatched earlier. Although Bythotrephes diapausing eggs may have energy stores for at least a couple of years under appropriate conditions, this raises a question as to how long Bythotrephes diapausing eggs remain viable. A long-term egg bank (De Stasio 1989), as found for many Daphnia species and copepods, may retain viable eggs for many decades (Hairston 1996). Based on lab results (Andrew and Herzig 1984, Andrew 1995), Bythotrephes eggs which have not hatched within a couple of years following their production may be unlikely to contribute to subsequent population rejuvenation.

ACKNOWLEDGMENTS

I would like to thank George Kling, Earl Werner, Hank Vanderploeg, David Allan, and Gary Fowler for their constructive comments on the manuscript. I also would like to thank Nelson G. Hairston, Jr. and an anonymous reviewer for their helpful comments. This work was supported in part by grants from the Rackham School for Graduate Studies and the Department of Biology, University of Michigan, and grant NSF OCE 89-10999.

REFERENCES


Andrew, T.E., and Herzig, A. 1984. The respiration rate of the resting eggs of Leptodora kindtii and Bythotrephes longimanus (Crustacea, Cladocera) at environmentally encountered temperatures. Oecologia 64:241-244.


Submitted: 7 November 1996

Accepted: 11 April 1997