



## Delayed egg hatching accounts for replacement of burrowing mayflies *Hexagenia rigida* by *Hexagenia limbata* after recolonization in western Lake Erie

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### ABSTRACT

Egg banking may have played a role in the recolonization of two burrowing mayfly species (*Hexagenia limbata* and *Hexagenia rigida*), which recolonized western Lake Erie after over 30 years of near extirpation. *H. rigida* was the first of the two to colonize successfully, but was overtaken by *H. limbata* (the historically dominant species). To understand mechanisms of species replacement, we compared egg hatching between the two species at 20 °C in the lab under typical hatching temperatures (no incubation) and after cold storage (8 °C) for 2, 6 and 12 months. Prolonged storage at cold temperatures simulates temperature conditions to which embryos are exposed when deposited by late-emerging female imagos. Without incubation, *H. rigida* (95.4 ± 2.02) exhibited significantly higher egg hatching survivorship than *H. limbata* (85 ± 3.2) ( $P = 0.01$ ). First hatch date for *H. rigida* was one day earlier than *H. limbata*, but time for 50% to hatch (5 d) and hatching duration (5 d) was the same for both species. After 2 and 6 months of incubation, *H. limbata* exhibited an earlier hatch date, but there was no significant difference in mean percentage of egg survival (ca 63%) between species. After 12 months of cold storage, hatching success for *H. limbata* was 44.6 ± 4.17%, but *H. rigida* eggs did not hatch. Banking of eggs at low temperature increases the chance of successful recolonization, once stressful conditions pass. The viability *H. limbata* eggs after long quiescence may help to explain the shift in dominance from *H. rigida* to *H. limbata*.

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### Introduction

Egg banks, the natural storage of eggs in delayed development, are vital in aquatic habitats to ensure continuity of populations in variable environments, averaging risk over time (Levins, 1979). Darwin (1859) recorded one of the earliest observations of seed banks when he tallied 537 plants that arose from three tablespoons of pond mud he collected and held for six months. Dormant eggs (e.g. epiphya of Cladocera) are particularly beneficial in variable environments because they ensure that organisms survive in temporary aquatic environments (Brendonck and DeMeester, 2003). Egg, seed or germ banks account for variation in the timing of emergence of offspring from a single clutch, leading to bet-hedging against future environmental stochasticity (Evans and Dennehy, 2005). Ephemeroptera (mayflies), as with plants and zooplankton, are able to survive in temporary and variable environments (Williams, 1996).

Eggs of burrowing mayflies, such as *Hexagenia limbata* (Serville) and *Hexagenia rigida* McDunnough (Ephemeroptera: Ephemeridae), drop into the mud bottom of lakes, remaining buried over time. Egg

viability and development in bottom mud is a function of exposure to anoxia (as a result of hypolimnetic stratification or burial in the sediment), and temperature (Britt, 1955; Jacobsen, 1966). At 2 to 2.5 mm below the surface sediment, oxygen levels decline to 0 mg/L, halting egg development (Jorgensen and Revbech, 1985; Ventling-Schwank and Livingstone, 1994). Eggs may ultimately rise to substrate surfaces as a result of sediment mixing by currents and/or burrowing activities by organisms in the upper layers of mud (DeStasio, 1989). *Hexagenia* (*limbata* and *rigida* combined) eggs survive both exposure to anoxia (245 d, Gerlofsma, 1999; 380 d Fremling, 1967) and to cold temperature (Giberson and Rosenberg, 1992a; Hunt, 1953). Once hatched, embryo development is temperature dependent, influencing synchrony in life history patterns (Friesen et al., 1979; Heise et al., 1987; Hunt, 1953).

*H. limbata* and *H. rigida* were historically abundant in the benthic community in the western basin of Lake Erie of the Laurentian Great Lakes, but eutrophication devastated the populations (Britt, 1955; Nebeker, 1972) for over 30 years (Krieger et al., 1996; Winter et al., 1996). Oxygen conditions have improved in the profundal zone of the western basin of Lake Erie due to phosphorus abatement (Makarewicz and Bertram, 1991) and filtering of waters overlying sediments by invasive dreissenids (Makarewicz et al., 1999). Recolonization of *Hexagenia* nymphs began in the early 1990s from successful oviposition from nearby localities (Krieger et al., 1996). The recovery of burrowing mayflies over the past two decades in

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Lake Erie, and their contribution to the diet of forage, sport and commercial fishes, has been remarkable (Ludsin et al., 2001).

Interestingly, the two species of *Hexagenia* exhibited different recolonization patterns. Results of a 12-year monitoring study (1997–2008) of adults showed that *H. rigida* was the initial colonizer in western Lake Erie (Corkum, 2010). Between 2000 and 2002, *H. limbata* became the dominant species and has remained so (Corkum, 2010). Bottom grab sediment samples taken throughout the basin confirmed that *H. rigida* nymphs were replaced over time by *H. limbata* in both density and distribution although both species continue to co-occur (Elderkin et al., 2012). The relative abundance of the two species is reminiscent of species ratios from the 1940s when *H. limbata* was more dominant than *H. rigida* (3:1) (Chandler, 1963 cited in Corkum, 2010).

Reasons for the shift in dominance between the two species are not clear. Populations of *H. limbata* and *H. rigida* in the Laurentian Great Lakes have similar life history traits and morphology. Moreover, there are few, if any, differences between the two species with respect to time of adult emergence, adult dispersal distances or the resulting distance from shore where embryos are found deposited in lake sediments (Corkum, 2010). Both species of *Hexagenia* have an extended emergence period from May to October (Corkum, 2010; Corkum and Hanes, 1992; Corkum et al., 2006), contributing to differential hatching rates of fertilized eggs such that eggs are exposed to different temperature regimes. Adult females oviposit on the water surface and the fertilized eggs sink to the lake bottom, becoming buried within the clay/mud sediment. Corkum et al. (1997) speculated that plasticity in egg development time may account for the ability of burrowing mayflies to recover from brief anoxic episodes in the bottom sediments.

In this study, we examined egg hatching success between burrowing mayfly species to explain the initial dominance of *H. rigida* followed by the replacement and dominance of *H. limbata* in the shallow, well-mixed western basin of Lake Erie. Because *H. rigida* was the early colonizer in western Lake Erie after the massive eutrophication disturbance, we predicted that initial hatching success of this species would be greater than *H. limbata*. However, for embryos that remained in the sediment and were exposed to cold temperatures, we predicted the advantage in hatching success should shift to *H. limbata*, the species that ultimately became widespread and dominant. Friesen et al. (1979) showed that *H. rigida* embryos do not hatch below 8 °C, but remain viable once temperature increases, suggesting that eggs overwinter (Giberson and Rosenberg, 1992b). Cold storage simulates temperature conditions to which embryos are exposed when deposited by late-emerging female imagoes. Our laboratory study was designed to test species differences in hatching rate of embryos exposed to increasing periods of cold storage.

## Methods

*Hexagenia* nymphs are detritivores that burrow as deep as 10 cm in bottom sediments (Charbonneau et al., 1997) and undergo up to 30 molts (Hunt, 1953) before emerging into a winged form. Because nymphal development times vary widely (14 to 22 months) based on temperature, subimago (sub-adult) emergence can occur over an extended period (late May to early October) (Corkum, personal observation). Subimagoes are carried by wind and move onshore to emerge to the final adult (imago) stage, which is short lived (up to 2 days), leaving a narrow window of time for dispersal and oviposition. Adults are attracted to lights so eggs can be collected easily from fertilized females on shore.

### Collection of eggs

After sunset, adult female *H. limbata* and *H. rigida* were individually collected by hand (25 July 2006) from large swarms attracted to

street lights at two locations in southwestern Ontario: Detroit River, Windsor (42° 20' N, 82° 57' W) and Colchester Harbour (41° 59' N, 82° 55' W), western Lake Erie. The collection date corresponded to the peak (maximum) emergence of *Hexagenia* species. Sexually mature adults are identified by clear wings and females are distinguished from males by their lighter (yellow) bodies and small eyes (Corkum, personal observation). Females of each species were distinguished by their abdominal pigmented markings (Elderkin et al., 2012). Sexually mature female imagoes were examined by eye to establish species and placed in separate 2 L polyethylene soil bags containing 1 L dechlorinated, aerated water into which a few mL of sculpture's clay had been added to prevent egg clumping (Corkum and Hanes, 1992). Thirty to 50 gravid females of each species were placed into each of several water-filled bags, where they immediately released their eggs into the water. Females are fertilized when they fly through male mating swarms. On average, each female contains about 4000 eggs (Hunt, 1953). To ensure maximum egg deposition, females were kept in the collection bags until the next morning when the adults were removed and preserved in 70% ethanol. Embryos were retained in the original collection bags and hatched under two different protocols (room temperature and cold storage). We also confirmed species identification by examining the chorionic sculpture of the collected eggs (Koss, 1968).

### Embryos exposed to room temperature

To mimic summer water temperatures (20 °C) near the bottom sediments of the lake, embryos were maintained at room temperature. Embryos were left in their collection bags in the lab at 20 °C for 6 days, then transferred into plastic Petri dishes (2 species × 10 replicates × 100 embryos/replicate). Petri dishes (100 mm dia. × 15 mm depth) were half filled with dechlorinated water that had been aerated for 3 days and maintained at the 20 °C room temperature. Covered Petri dishes were randomly placed on an enamelled tray and all were covered with black cardboard to simulate dark conditions of the lake bottom sediments. Water in the Petri dishes was replenished with aerated dechlorinated water every 2 days to prevent hypoxic conditions. Embryos were monitored daily (1300–1400 h) for hatching using a dissection microscope. Any newly hatched 1st instar nymphs were counted and removed from the Petri dishes. Hatching was monitored until the appearance of new 1st instar nymphs had ceased for at least 3 days.

### Embryos exposed to cold storage

To simulate prolonged quiescence in the laboratory, eggs that were stored in the collection bags at room temperature for 6 days (about 14 days before hatching occurred in control eggs) were then cooled to 14 °C for 6 days before being incubated at 8 °C, following existing protocols (Friesen, 1981; Giberson and Rosenberg, 1992a). Egg hatching does not occur at 8 °C for *H. rigida* (Friesen et al., 1979) or *H. limbata* (Giberson and Rosenberg, 1992b). Embryos of each species were maintained at 8 °C for 2, 6, and 12 months. After each of the 3 incubation periods, embryos of each species were removed from storage bags and placed in Petri dishes (2 species × 10 replicates × 100 embryos/replicate) at room temperature (20 °C) to assess time to hatch and hatching success for each species after exposure to cold storage.

## Results

Without cold storage, mean hatching success was greater in *H. rigida* (95.4 ± 2.02) than in *H. limbata* (84.8 ± 3.20%) (P = 0.01). Hatching patterns between species differed as a function of time that eggs were held in cold storage (Table 1). Most *H. rigida* eggs took similar times to hatch after up to 6 months in cold storage at

**Table 1**

Summary of variables examined during egg hatching with varying exposure to cold storage conditions. All values for each condition were measured as means from 10 replicates (100 eggs per replicate) per species per treatment. Hatching success (survivorship) was measured based on the means from the total number of nymphs that hatched each day per replicate. Day of hatching at 2, 6 and 12 months is the time from when eggs were removed from cold storage and placed at room temperature (20 °C).

Duration of cold storage (months)	Day of 1st instar hatch	Day of 50% of nymph hatch	Duration of egg hatching (days)	Hatching success $\pm$ (SE)%
0 month <i>H. rigida</i>	22	23	5	95.4 (2.02)
0 month <i>H. limbata</i>	23	23	5	84.8 (3.02)
2 months <i>H. rigida</i>	8	14	12	62.2 (3.38)
2 months <i>H. limbata</i>	3	12	17	63.8 (3.59)
6 months <i>H. rigida</i>	5	11	13	65.3 (4.15)
6 months <i>H. limbata</i>	1	4	12	56.1 (4.50)
12 months <i>H. rigida</i>	0	0	0	0.00 (0.00)
12 months <i>H. limbata</i>	2	7	13	44.6 (4.17)

8 °C, but eggs did not hatch after being incubated for a year (Fig. 1). In contrast, *H. limbata* eggs hatched faster when returned to room temperature as the amount of time spent in cold storage increased. Also, *H. limbata* eggs successfully hatched after a 12 month exposure to 8 °C (Fig. 1). When *H. limbata* eggs were returned to room temperature after being incubated for a year, the first eggs hatched on day 2 and duration of egg hatching was 13 days.

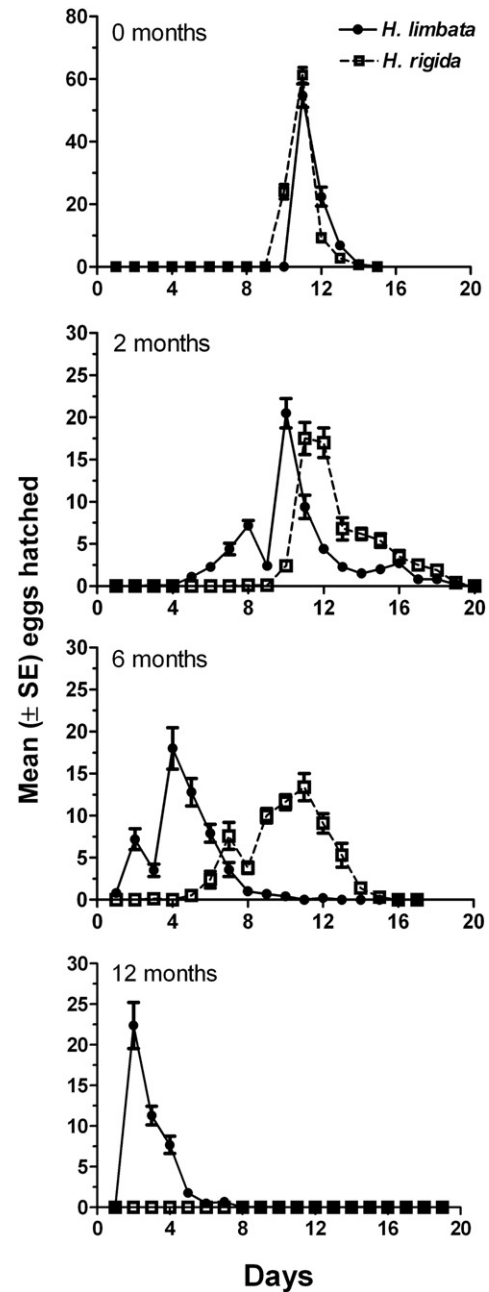
There was no significant difference in mean percentage hatching success between species after 2 months (*H. rigida*,  $62.2 \pm 3.38$ ; *H. limbata*,  $63.8 \pm 3.59$ ;  $t_{18} = 0.324$ ,  $P = 0.749$ ) or 6 months (*H. rigida*,  $65.3 \pm 4.15$ ; *H. limbata*,  $56.1 \pm 4.50$ ;  $t_{18} = 0.324$ ,  $P = 0.152$ ) cold storage. After 12 months of cold storage, hatching success for *H. limbata* was  $44.6 \pm 4.17\%$ , but none of the *H. rigida* eggs hatched.

Other egg hatching traits were monitored (Table 1). For eggs held at room temperature, the first days of hatch for the two species were similar (*H. rigida*, day 22; *H. limbata*, day 23). Time for 50% of embryos to hatch (day 23) and duration of hatch (5 days) were the same for both species. However, as the duration of the cold period increased, differences in timing to 50% hatch began to be seen. After a 2-month exposure to cold storage, about 50% of *H. limbata* eggs hatched (51.9%) after 12 days and *H. rigida* hatched (50.1%) after 14 days. First day of egg hatch for *H. limbata* (day 3) was 5 days earlier than *H. rigida* (day 8). Duration of hatching differed between species: *H. limbata* (17 days) vs. *H. rigida* (12 days). After six months of cold storage, 50.2% of *H. limbata* eggs hatched on day 4 and 49.4% of *H. rigida* eggs on day 11. First day of egg hatch differed between species (*H. limbata*, day 1; *H. rigida*, day 5), but the duration of egg hatching between species was similar: *H. limbata* (12 days) and *H. rigida* (13 days).

## Discussion

Species replacement, demonstrated by the shift from *H. rigida* to *H. limbata*, occurs when a dominant species (measured by density and/or spatial distribution) is supplanted by another. The replacement may result from biotic interactions such as interspecific resource competition, predation and/or from differential responses to physical factors (in the case of burrowing mayflies: anoxia, water temperature, wave action) during oviposition and subsequent nymphal development (Herrmann et al., 2010; Juliano, 1998).

The recolonization of both *H. rigida* and *H. limbata* into western Lake Erie likely initiated from upstream waters (Detroit River-Lake St. Clair) and other tributaries (e.g., Maumee River) surrounding the lake. The Detroit River is the major tributary entering Lake Erie, contributing 90% of inflowing water (Carter and Hites, 1992). Since *H. rigida* is more abundant than *H. limbata* (1.8:1) in the Detroit River (Corkum, 2010), it is not surprising that *H. rigida* was the initial



**Fig. 1.** Mean ( $\pm$  SE) egg hatching over time (days) for *H. limbata* (open squares, dashed line) and *H. rigida* (solid circles, solid line) at 20 °C (i.e., no incubation) and after 2, 6, and 12 months of incubation (8 °C) before return to 20 °C. Note: values on the Y-axis for “no incubation” (0 months) differ from those at other times. The days for hatching at 2, 6, and 12 months are the times after warming back to 20 °C.

colonizer. *Hexagenia* size and fecundity are positively correlated (Giberson and Rosenberg, 1994; Hunt, 1951). Because *H. limbata* is larger in Lake Erie (and therefore more fecund) than *H. rigida* adults, nymphs of *H. limbata* have surpassed *H. rigida* in density and distribution (Elderkin et al., 2012). Nevertheless, both species are highly fecund and have the potential to disperse throughout the basin.

Habitat occupancy has been shown to influence species interaction through priority effects; i.e., interspecific interactions where outcomes depend on the arrival, breeding or activity of interacting species (Lawler and Morin, 1993; Peeke et al., 1995). In this study of mayfly recolonization, we sought to explain the processes that led to the initial dominance of *H. rigida* followed by the replacement and long-term dominance of *H. limbata* in the western basin of Lake Erie. Differences in egg hatching patterns may explain the population



trends of these co-existing species and account for the shift in *Hexagenia* species. *H. rigida* had higher initial egg hatching success than *H. limbata*, and would have been more successful in recolonizing the western basin before *H. limbata*. Since *H. rigida* was the first species to find open habitat, it had the advantage of founder conferred dominance (McAuliffe, 1984). Developmentally, *H. rigida* eggs hatch one day earlier and with greater survivorship than the eggs of *H. limbata*. Hanes and Ciborowski (1992) reported that *H. rigida* nymphs which hatched one day earlier grew slower, but exhibited greater rates of survivorship in comparison to nymphs that hatched one day later. Thus, an earlier hatch date and greater survivorship allowed *H. rigida* to maintain its established initial dominance (Corkum et al., 1997).

The initial population dominance of *H. rigida* in the lake did not last long (Corkum, 2010). Over the next decade, *H. limbata* increased in density and in spatial distribution throughout the western basin of Lake Erie, whereas *H. rigida* declined in both abundance and distribution (Corkum, 2010; Elderkin et al., 2012). This pattern can be explained by examining egg hatching patterns between the two species. After eggs were held at 8 °C for 2 and 6 months, hatching success did not differ between species. However, the eggs of *H. limbata* hatched progressively earlier than those of *H. rigida*, giving nymphs of *H. limbata* growth advantage. After 12 months of egg incubation at 8 °C, *H. limbata* continued to survive, but *H. rigida* did not so eggs that were deposited in cooler water (e.g., deeper water or later oviposition) would gradually favour *H. limbata*. Friesen et al. (1979) also reported low survivorship (1.4%) of *H. rigida* eggs when held at 8 °C for a year. Giberson and Rosenberg (1994) suggested that *H. limbata* are better adapted to cold temperatures than *H. rigida*, which is consistent with the more northerly distribution of *H. limbata* (Harper and Harper, 1981; Oliver, 1960; Rawson, 1953).

Another factor that would favour *H. limbata* over *H. rigida* is the periodic stratification that continues to occur in the relatively shallow (mean depth 7.4 m; maximum depth 18.9 m) waters of the western basin of Lake Erie, resulting in recurring but unpredictable anoxic conditions at the lake bottom (Bridgeman et al., 2006). Variation in the concentration of dissolved oxygen near mayfly nymphal burrows may result in the failure of a *H. rigida* cohort, but in the success of future *H. limbata* cohorts because of the latter's enhanced egg viability in the egg bank. Thus, *H. limbata* can establish a reserve of potentially viable eggs from several generations such as those described in other invertebrate studies (Brendonck and DeMeester, 2003). Delayed hatching (a bet-hedging trait) enables *H. limbata* to contribute to subsequent cohorts after long periods of cold storage or sediment burial, and then successfully hatch once exposed to temperatures that induce egg hatching (Heise et al., 1987).

Environmental stresses often produce bottlenecks or “ecological crunches” where selection is intense (Wiens, 1977). Highly fecund semelparous taxa such as mayflies are particularly sensitive to environmental stresses. Burrowing mayfly nymphs cannot withstand anoxia (Britt, 1955; Winter et al., 1996) and although egg development is arrested by anoxia, eggs resume development once the environment is oxygenated (Gerlofsma, 1999) and waters become warm (Friesen et al., 1979; this study). When eggs of both species are present in the upper few centimetres of clay/mud sediment, both species may develop in warmer waters after exposure to cold temperatures (<8 °C) for less than 6 months. The persistence of *H. rigida*, albeit at low nymphal densities, is typical of a “sink population” supported by continual immigration, rescuing the population from extinction (Pulliam, 1988). Once *H. limbata* and *H. rigida* become established, the populations of each are sustained by nymphal growth and development to emergent adults, dispersal within the basin, and colonization from upstream sources (cf. Sax and Brown, 2000), but *H. limbata* has the advantage of greater fecundity and higher egg viability when eggs are cold for long periods (up to one year) in lake sediment. *H. limbata* can recruit individuals from the current

mating season as well as eggs from previous years that were resting until suitable environmental conditions occur, resulting in the gradual increase and dominance of this species.

Our present findings of delayed egg hatching also account for the extended emergence in both species of *Hexagenia* and this helps to explain why both species persist, even with the observed advantage to *H. limbata*. *H. rigida* remains present in the western basin because eggs continue to hatch within the year of oviposition and because of its continued immigration from upstream areas. Although species evenness can be affected by competition (McAuliffe, 1984), we argue that egg hatch time and prolonged survivorship from the *H. limbata* egg bank favour this species during environmental bottlenecks, explaining the shift in dominance from *H. rigida* to *H. limbata*. As eutrophication symptoms (including anoxic events) return to western Lake Erie from enhanced in-lake total phosphorus and loadings into the lake (Bridgeman et al., 2006; Chaffin et al., 2011; Dolan and McGunagle, 2005; Matisoff and Ciborowski, 2005), we anticipate that densities of burrowing mayflies may decline and species shifts are likely to re-occur as demonstrated in this study.

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