
23 **Habitat variation in female size and egg characteristics of *Hexagenia limbata* Serville (Ephemeroptera: Ephemeridae)**

Lynda D. Corkum and Jan J.H. Ciborowski
Department of Biological Sciences, University of Windsor,
Windsor, ON Canada N9B 3P4

*Our objective was to determine whether or not some of the broad variation in life history features of *Hexagenia limbata* could be attributed to habitat type. We collected female imagos of *H. limbata* from three lakes (Erie, St. Clair, Simcoe) and three rivers (Detroit, Grand, St. Clair) in southern Ontario, Canada, during July 1990. The range of female imago body length was equivalent between lakes and rivers (18.12 - 21.5 mm). There were no significant differences in body length of female imagos from Detroit River samples over the emergence span, yet there were significant differences in body length of females among sampling dates at Lake St. Clair.*

There were significant differences in egg size ($p < 0.001$) among replicate waterbodies within each habitat, yet there was no difference in egg size between habitats. Although data were variable for day of first hatch, hatching tended to be earlier in lakes (three or five days) than in rivers (three, six, eight days). Number of days required for 50 per cent and 95 per cent of eggs to hatch was shorter in river than in lake populations, indicating that egg hatching was more synchronous in rivers than lakes. Also, hatching success was higher in rivers (72-80 per cent) than lakes (62-73 per cent).

*Our findings reveal that life history traits of *H. limbata* located in different habitats are significant and may be greater than differences in life history traits along a latitudinal gradient.*

Introduction

The burrowing mayfly, *Hexagenia limbata* Serville is abundant throughout North America in lakes and large rivers, where the substrate is composed of soft mud and clay (Edmunds et al. 1976). Life history interpretation of this ubiquitous

species is difficult because of the protracted emergence period, presence of multiple cohorts (Heise et al. 1987), delayed hatching of eggs, differential growth of males and females and a wide variability in growth rates of individuals from the same egg mass (Hunt 1953). *Hexagenia limbata* co-occurs with *Hexagenia rigida* McDunnough, contributing further to the complexity of life history analysis.

Variation in life history development within and among sampling locations has been attributed to several exogenous factors. Factors known to influence size variation in larvae of *H. limbata* include temperature, photoperiod, food and density (Zimmerman et al. 1975; Corkum and Hanes 1992; Giberson and Rosenberg 1992a, 1992b; Hanes and Ciborowski 1992).

The influence of latitude (and correspondingly, temperature) often has been used to account for variation in life cycles of aquatic insects. Life cycle length in *H. limbata* may vary from less than one year in the southern part of its range to over three years in the northern region (Giberson and Rosenberg 1992a). Degree days have frequently been used to predict both egg hatch (Giberson and Rosenberg 1992b) and length of larval development prior to emergence (McCafferty and Pereira 1984; Heise et al. 1987) across a range of latitudes.

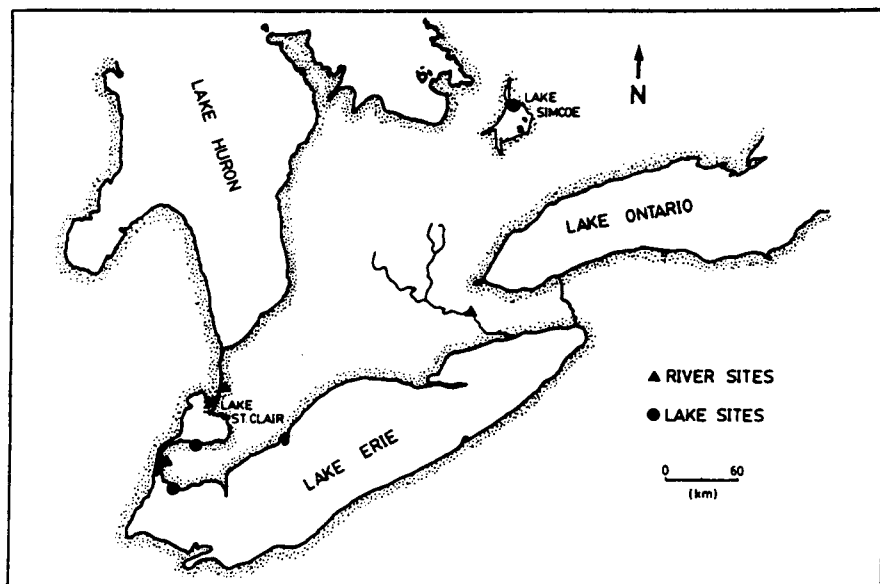
Surprisingly, little research has focussed on the effect of habitat (river versus lake) differences on size variation or emergence patterns in aquatic insects. Significant differences in hatching time of eggs at identical temperatures have been reported for a stonefly, *Diura bicaudata* (L.) (Khoo 1968) and a mayfly, *Ecdyonurus dispar* (Curt.) (Humpesch 1980) when lake and river populations of these species were compared. Corkum (1985) also illustrated that life cycle patterns in the mayfly, *Caenis simulans* McDunnough, differed between habitats. Riverine populations of *C. simulans* were characterized as bivoltine and exhibited an expanded emergence period, whereas lake populations were univoltine with an emergence span of less than three weeks (Corkum 1985). Interestingly, the shortest life cycle period reported for natural populations of *H. limbata* was in irrigation canals, located in Utah, U.S.A., about midway within the distributional range of the species (Edmunds et al. 1976).

In this study, we present results for female size and egg characteristics of *H. limbata* collected from three lakes and three rivers within a similar latitudinal range in southern Ontario, Canada. Our objective was to determine whether some of the broad variation in life history features of the species could be attributed to habitat type.

Materials and Methods

We collected imagoes of *H. limbata* from three lakes (Erie, 4 July; St. Clair, 21 July; Simcoe, 9 July) and three rivers (Detroit, 4 July; Grand, 8 July; St. Clair, 8

Figure 1. Map of southern Ontario illustrating sampling locations for *Hexagenia limbata* female imagos.



July) in southern Ontario in 1990 (Fig. 1). Sample dates were based on results of a questionnaire sent to provincial parks in 1989 and 1990 in which we requested the date of peak emergence dates for *Hexagenia*. Imagos also were collected weekly throughout the emergence period from one lake (Lake St. Clair) and one river (Detroit River) to determine size variation in females. A battery-powered ultraviolet lamp, placed on a white sheet, was used to attract imagos of *Hexagenia* at each of the six locations (Ciborowski and Corkum 1988).

Eggs were retrieved from females at the time of collection (Corkum and Hanes 1992). Females were grasped by their wings and placed in plastic bags (up to 50 females per bag) with ca. 1.5 L of aerated distilled water. Female imagos immediately released eggs into the water. Females were subsequently preserved in 70 per cent ethanol. In the laboratory, eggs that were stored in collection bags were cooled by 4°C steps to 8°C for incubation (Friesen 1981). Cold storage induced dormancy.

Body length (excluding cerci) of each female imago was measured with calipers to the nearest 0.1 mm. Since female imagos cannot be identified (McCafferty 1975), we relied on the identity of simultaneously collected male imagos for

verification. Species identity also was confirmed by examining eggs of female imagos (Koss 1968).

Body length size class analysis of female imagos was used to compare populations from the three sampling locations within each habitat [analysis of variance (ANOVA) test] (Sokal and Rohlf 1981). Also, one-way ANOVA was used to test for temporal variation in body size at the Detroit River and Lake St. Clair sampling sites. If significant differences were detected, a Student-Newman-Keuls (SNK) multiple comparison test was performed to determine which samples did not differ from one another.

On 10 March 1991, six replicate samples of eggs were removed from cold storage, counted (100 eggs per sample x six samples) and transferred to aerated, distilled water in covered petri plates (5.5 cm diameter) for each of the six waterbodies. Eggs were maintained at room temperature (19° to 23°C) and exposed to a 12 h L: 12 h D light regime. Water in each petri dish was aerated continuously using capillary tubing. Eggs were monitored each day (1300 h) for hatching using a dissecting microscope. After hatching began, newly hatched larvae were removed and counted daily. Once hatching had apparently ceased in all plates, eggs were monitored for an additional week.

Samples of eggs (n=50) also were obtained from each site to be stained (CMC mounting media) and measured (egg length and width) to the nearest 0.01 mm using a compound microscope with an ocular micrometer. In all cases, eggs were measured after eight months of cold storage and it is likely that egg size changed with storage time (E.C. Hanes, University of Windsor, personal communication). However, since all samples were exposed to the same treatment, results are comparable.

Egg size was estimated using length and width measurements to calculate the area of an ellipse, the approximate shape of *Hexagenia* eggs. An ANOVA was used to determine the effect (if any) of habitat and waterbodies within habitat on egg size.

Finally, we examined if any relationship existed between body length of female imagos and mean egg size using regression analysis.

Results

Female Size

In the lower Great Lakes region, emergence of *Hexagenia* usually ranges from late June to early August (personal observation). Temporal variation in total body length of female imagos was examined at the Lake St. Clair and Detroit River sampling locations. There was no significant difference in mean body length of

females from the Detroit River samples obtained from 30 June to 25 July 1990 (Table 1).

In contrast to the findings at the Detroit River, there were significant differences in body length of female imagos among sampling dates at Lake St. Clair. Largest mean body length was recorded during the sampling period two, 7 July 1990. Results of the SNK multiple comparison test indicated that there was size overlap among two groups of sampling periods at the Lake St. Clair sampling site. There was no significant difference in mean size among sampling periods 1, 3 and 4 or between periods 4 and 2. Typically, 4 - 10 July has been the time of peak emergence for *H. limbata* at Lake St. Clair during four years in which we have monitored the population. At both lake and river locations, no imagos were found the week before or after the four to five week emergence span.

Variation in the mean body length of female imagos among the six sampling locations also was examined (Fig. 2). There were significant differences ($p < 0.001$) in female body size among waterbodies within each habitat, but there was no significant difference in mean body length of female imagos between habitats (ANOVA). Site variation greatly exceeded the amount of variation in body size found over the emergence season at Lake St. Clair and the Detroit River. The smallest mean body length was recorded for the Detroit River (18.12 ± 0.569 mm, $n=13$); overlap in mean body length was observed between the St. Clair (21.15 ± 0.338 mm, $n=40$) and Grand (20.43 ± 0.421 mm, $n=18$) rivers. In lake populations, mean body length of female imagos increased from Lake Erie (18.23 ± 0.391 mm, $n=38$) to Lake St. Clair (19.19 ± 0.281 mm, $n=52$) to Lake Simcoe (21.46 ± 0.353 mm, $n=36$) with no overlap.

Egg Size and Development

Egg size of *H. limbata* populations was compared among the six locations (Table 2). In each case, a sample of 50 eggs was examined from a combined collection of female imagos at each site. Results of an ANOVA indicated that there were significant differences in egg size ($F=24.071$, $p < 0.001$) among replicate waterbodies within each habitat. However, there was no significant difference in egg size (surface area) between lakes and rivers (ANOVA, $F=1.041$, $p > 0.05$). No significant relationship between mean body size of female imagos and egg size ($R^2=0.25$; t -value=1.158) was detected when data from the six sampling locations were examined.

A summary of egg development in *H. limbata* revealed marked differences in hatching of eggs between habitats (Table 3). An incubation period of three to eight days at room temperature was required before the first eggs hatched. Although data were variable for day of first hatch, hatch tended to occur earlier in lakes (three and

Table 1. Temporal variation in mean total length (mm, excluding cerci) and standard error (SE) of female imagos collected throughout the emergence period at Lake St. Clair and the Detroit River, 1990. Summary results are provided for the ANOVA. V = coefficient of variation (per cent). NS = not significant.

Sample Period	Date	n	Mean±S.E.	V	ANOVA comparison		
					df	F	P
Lake St. Clair							
1	29 June	36	18.69±0.411	13.2	3,359	4.339	<0.005
2	7 July	104	19.87±0.199	10.2			
3	21 July	161	19.09±0.146	9.7			
4	28 July	62	19.26±0.277	11.3			
Detroit River							
1	30 June	6	20.43±1.366	16.4	4,174	1.102	NS
2	4 July	13	18.12±0.569	11.3			
3	15 July	5	19.08±1.703	20.0			
4	19 July	107	19.17±0.214	11.5			
5	25 July	48	19.10±0.338	12.3			

five d) than in rivers (three, six, eight d). However, number of days required for 50 per cent and 95 per cent of egg hatch of the viable eggs that hatched was longer in lakes than in rivers. Hatching success was higher in rivers (72-80 per cent) than in lakes (62-73 per cent). Results for 50 per cent and 95 per cent hatch time show that more eggs hatch within a shorter time period in rivers than in lakes (Table 3), indicating that egg hatch is more synchronous for river than lake habitats. However, median hatch date was quite uniform among all sample locations, varying from 8-12 d.

Figure 2. Size frequency distributions of body lengths of female imagos collected from lakes (A, B, C) and rivers (D, E, F). Arrows represent medians. A. Lake Erie; B. Lake St. Clair; C. Lake Simcoe; D. Detroit River; E. St. Clair River; F. Grand River.

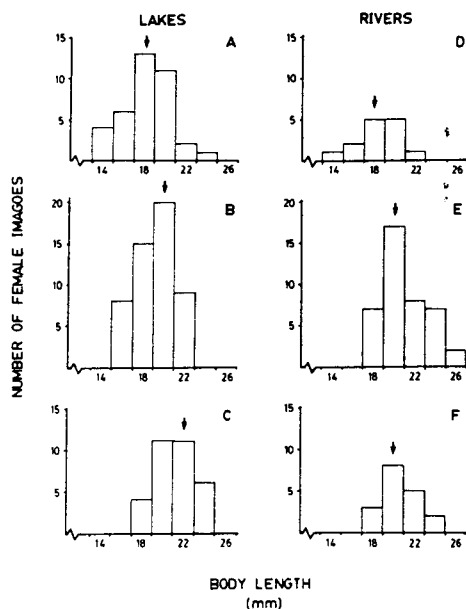


Table 2. Mean and standard error (S.E.) of size (μm^2) of eggs ($n=50$) collected at the lake and river habitats.

Site	Mean ($\times 10^4$)	S.E. ($\times 10^3$)
Lake Erie	4.75	0.892
Lake St. Clair	5.55	1.192
Lake Simcoe	5.82	0.928
Detroit River	5.90	1.329
Grand River	5.25	1.160
St. Clair River	6.38	1.295

Table 3. Egg development in *H. limbata* from three lakes and three rivers reared at room temperature (19-23°C), 12 h L:12 h D light regime after an eight month incubation at 8°C. Replicate samples were based on samples of 100 eggs per petri dish.

Habitat	Total No.	First hatch (d)	Days for % hatch (after 1st hatch)		Hatching success (%)
			50%	95%	
Lake Erie	500	3	8	10	68
Lake St. Clair	500	5	7	9	62
Lake Simcoe	600	5	4	7	73
Detroit River	600	8	2	5	80
Grand River	600	6	3	5	78
St. Clair River	600	3	5	7	72

Discussion

There is tremendous size variation in adults of aquatic insects, particularly mayflies. Temperature is a primary factor that has been used to explain size variation in mayflies, including populations of *Hexagenia limbata* (Corkum and Hanes 1992). Sweeney (1984) emphasized that intraspecific variation in life history characteristics of aquatic insect populations along a thermal gradient may be as great as the individual variation within a single population. Few studies have been conducted comparing life history traits in rivers and lakes in any aquatic insects. Although several life histories studies have been conducted on the burrowing mayfly, *H. limbata*, at different geographical (latitudinal) locations, this is the first study that compares life history traits of this species between habitats and at one geographically similar location.

In this study, we compared female size and egg characteristics of *H. limbata* in three populations of both lake and river habitats of southern Ontario. There were significant differences in female body size among waterbodies within habitats, but there were no significant differences in female size between habitats. Our findings revealed that egg hatching in *H. limbata* was more synchronous in rivers than in

lakes. Specifically, time from first hatch to 50 per cent and 95 per cent hatch is faster in rivers than lakes (Table 3).

Physical characteristics of lakes and rivers may contribute to the difference in life history traits of *H. limbata* between habitats. Typically, female imagos oviposit on the water surface near shore. The oviposited eggs may fall through the water column or may be carried by currents. In lakes, wave action may transport eggs shortly after oviposition from warm water near shore to colder profundal regions, resulting in a delay of egg hatch (Corkum and Hanes 1992). Hanes and Ciborowski (1992) speculated that relatively minor differences in egg hatching time could translate into significant growth and survival differences, owing to competitive effects that may occur later in the life cycle.

There were no significant differences in body length of female imagos from Detroit River samples over time, yet there were significant differences in body length of females among sampling dates at Lake St. Clair. An apparent advantage of synchronous emergence in rivers would be the increased likelihood of finding mates. If riverine emergence was asynchronous the likelihood of successful mating would be reduced owing to the downstream transport of currents scattering the subimagos as they attempted to leave the aquatic environment. In lakes, it is less likely that large imagos such as *Hexagenia* disperse before mating (Corkum 1987).

Intraspecific differences in time required for egg hatching have been observed in various animals including zooplankton, insects and fish (Humpesch 1980). Researchers have suggested that differences in egg development within a species may be attributed to differences in egg size (Bottrell 1975; Munroe and White 1975), suggesting that larger eggs take longer to develop than small eggs at a given temperature. In our study, there was no difference in mean egg size between habitats, but significant differences were noted among waterbodies within each habitat (Table 2).

In other studies (Khoo 1968; Humpesch 1980), significant differences in hatching at identical temperatures have been observed when lake and river populations were compared. Khoo (1968) reported that adults of the stonefly, *Diura bicaudata* (L.), from the stream population laid only diapause eggs, whereas adults from the lake population laid non-diapause and diapause eggs with a higher percentage of non-diapause eggs. It is noteworthy that egg development of *H. limbata* in southern Ontario represents a period of dormancy, not diapause (Corkum and Hanes 1992); however, Giberson and Rosenberg (1992b) have demonstrated diapause in *H. limbata* in northern populations.

In contrast to our findings, Humpesch (1980) noted that the time required for 50 per cent of eggs to hatch was longer in a single river population than in lakes for the mayfly species, *Ecdyonurus dispar* (Curt.). Also, hatching was prolonged for

the riverine population compared to lakes. Humpesch (1980) speculated that eggs of *E. dispar* from the river population exhibited a delayed hatching or dormancy, while eggs from lakes did not.

In summary, we showed that differences in time of hatch, synchrony of egg hatch and size variation among female imagos of *H. limbata* occur between river and lake habitats. The direction in which these differences occur, however, is not consistent among species (cf. Humpesch 1980). Results of our study suggest that differences in life history traits of a species located in different habitats are significant and may be more prevalent than differences in life history traits in populations along a latitudinal gradient.

Acknowledgements

We thank E.C. Hanes for constructive comments on the manuscript and R.J. Thibert for technical assistance. The research was funded by a grant from the Natural Sciences and Engineering Research Council of Canada.

References

- Bottrell, H.H. 1975. The relationship between temperature and duration of egg development in some epiphytic Cladocera and Copepoda from the River Thames, Reading, with a discussion of temperature functions. *Oecologia* 18:63-84.
- Ciborowski, J.J.H. and L.D. Corkum. 1988. Organic contaminants in adult aquatic insects of the St. Clair and Detroit rivers, Ontario, Canada. *J. Great Lakes Res.* 14:148-156.
- Corkum, L.D. 1985. Life cycle patterns of *Caenis simulans* McDunnough (Caenidae: Ephemeroptera) in an Alberta, Canada, marsh. *Aquatic Insects* 7:87-95.
- Corkum, L.D. 1987. Patterns in mayfly (Ephemeroptera) wing length: adaptation to dispersal? *Can. Entomol.* 119:783-790.
- Corkum, L.D. and E.C. Hanes. 1992. Effects of temperature and photoperiod on larval size and survivorship of a burrowing mayfly (Ephemeroptera, Ephemeridae). *Can. J. Zool.* 70:256-263.
- Edmunds, F.G., Jr., L. Berner and S.L. Jensen. 1976. *The Mayflies of North and Central America*. Minneapolis: University of Minnesota Press.
- Friesen, M.K. 1981. *Hexagenia rigida* McDunnough. P. 127-142 in S.G. Lawrence (Ed.), Manual for the Culture of Freshwater Invertebrates. *Can Spec. Publ. Fish. Aquat. Sci.* No. 54.

- Giberson, D.G. and D.M. Rosenberg. 1992a. Effects of temperature, food quantity, and nymphal rearing density on life-history traits of a northern population of *Hexagenia* (Ephemeroptera: Ephemeridae). *J. N. Am. Benthol. Soc.* 11:181-193.
- Giberson, D.G. and D.M. Rosenberg. 1992b. Egg development in *Hexagenia limbata* (Ephemeroptera: Ephemeridae) from Southern Indian Lake, Manitoba: temperature effects and diapause. *J. N. Am. Benthol. Soc.* 11:194-203.
- Hanes, E.C. and J.J.H. Ciborowski. 1992. Effects of density and food limitation on size variation and mortality of larval *Hexagenia rigida* (Ephemeroptera: Ephemeridae). *Can. J. Zool.* 70:1824-1832.
- Heise, B.A., J.F. Flannagan and T.D. Galloway. 1987. Life histories of *Hexagenia limbata* and *Ephemera simulans* (Ephemeroptera) in Dauphin Lake, Manitoba. *J. N. Am. Benthol. Soc.* 6:230-240.
- Humpesch, U.H. 1980. Effect of temperature on the hatching time of eggs of five *Ecdyonurus* spp. (Ephemeroptera) from Austrian streams and English streams, rivers and lakes. *J. Anim. Ecol.* 49:317-333.
- Hunt, B.P. 1953. The life history and economic importance of a burrowing mayfly, *Hexagenia limbata*, in southern Michigan lakes. *Mich. Dep. Conserv. Bull. Inst. Fish. Res.* No. 4.
- Koss, R.W. 1968. Morphology and taxonomic use of Ephemeroptera eggs. *Ann. Entomol. Soc. Am.* 61:696-721.
- Khoo, S.G. 1968. Experimental studies on diapause in stoneflies II. Eggs of *Diura bicaudata* (L.). *Proc. Royal Entomol. Soc. Lond. (A)* 43:49-56.
- McCafferty, W.P. 1975. The burrowing mayflies of the United States (Ephemeroptera: Ephemeroidea). *Trans. Am. Entomol. Soc.* 101:447-504.
- McCafferty, W.P. and C. Pereira. 1984. Effects of developmental thermal regimes on two mayfly species and their taxonomic interpretation. *Ann. Entomol. Soc. Am.* 77:69-87.
- Munroe, I.G. and R.W.G. White. 1975. Comparison of the influence of temperature on the egg development and growth of *Daphnia longispina* O.F. Miller (Crustacea: Cladocera) from two habitats in southern England. *Oecologia* 20:157-165.
- Sokal, R.R. and F.J. Rohlf. 1981. *Biometry*. Second Edition. San Francisco: W.H. Freeman and Co.
- Sweeney, B.W. 1984. Factors influencing life-history patterns of aquatic insects. P. 56-100 in V.H. Resh and D.M. Rosenberg (Eds.), *The Ecology of Aquatic Insects*. New York: Praeger.
- Zimmerman, M.C., T.E. Wissing and R.T. Rutter. 1975. Bioenergetics of the burrowing mayfly, *Hexagenia limbata*, in a pond ecosystem. *Verh. Int. Ver. Theor. Angew. Limnol.* 19:3039-3049.