Influence of salinity and temperature on the growth and production of a freshwater mayfly in the Lower Mobile River, Alabama

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Abstract

Secondary production of the burrowing mayfly, Hexagenia limbata, was quantified from four sites differing in seasonal salinity within the Lower Mobile River, Alabama, from October 1995 to September 1996. This population was univoltine, with emergence occurring from late May through early August. Comparisons with other populations of this species showed latitudinal trends suggesting that summer temperatures may exceed an upper thermal threshold for growth. Longitudinal differences in riverine salinity (i.e., upriver sites, 0%e; downriver sites, 5.5%e maximum salinity) explained most of the differences among sites, both for average density (upriver sites, 75.6 mayflies m⁻²; downriver sites, 2.54 mayflies m⁻²) and annual production (upriver, 1,669 mg m⁻² yr⁻¹; downriver, 46.6 g m⁻² yr⁻¹). Laboratory bioassays indicated that H. limbata nymphs could survive elevated salinity (LC₅₀ of 6.3%e at 18°C, 2.4%e at 28°C), although growth experiments showed similar growth at 0, 2, 4, and 8%e salinity treatments. Results from field observations and laboratory experiments demonstrated that these mayflies are tolerant of increases in salinity and showed that individuals surviving the stress of elevated salinity can grow at similar rates as mayflies in freshwater.

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Study site

The Mobile River Basin encompasses 113,960 km² in Alabama, northwestern Georgia, and eastern Mississippi (Pat-
Hexagenia in a salinity-influenced river

Fig. 1. Map showing the four study sites (A–D) sampled in the Lower Mobile River, October 1995 to September 1996. Sites A and B experience seasonal increases in salinity, whereas sites C and D typically remain fresh throughout the year.

This is a river-dominated delta, similar in structure to the Mississippi Delta, which begins at the confluence of the Alabama and Tombigbee Rivers and flows southward into the Northeast Gulf of Mexico (Fig. 1). The Mobile River and its major distributary, the Tensaw River, discharge into Mobile Bay; average discharge at the mouth is 1,728 m³ s⁻¹ (Patrick 1994). The river corridor is mostly forested, with cypress (Taxodium sp.) and tupelo (Nyssa sp.) being the dominant woody vegetation. Emergent macrophytes (e.g., Zizania sp., Phragmites sp.) line the river banks in depositional areas. River-water specific conductivity in freshwater reaches ranges from ~100 to 260 µS cm⁻¹; dissolved oxygen in all habitats sampled (i.e., <3 m depth) is typically high (>5.9 mg L⁻¹); and water clarity is low (Secchi depth usually <0.5 m; M. Chadwick, unpubl. data).

Salinity of the main channel of the Mobile River varies inversely with freshwater discharge. During low flow, saline water from the Gulf of Mexico flows up Mobile Bay to the mouth of the river, which typically increases salinity of lower reaches. However, benthic salinities fluctuate widely depending on storms and associated freshwater inflows, ranging at the river’s mouth from zero at peak discharge (winter) to ~25% during summer base flow (Schroeder and Lysinger 1979).

As a result of saltwater intrusion, benthos of the Lower Mobile River (i.e., from Mobile Bay to approximately 20 km upstream) is dominated by euryhaline species, including shrimp, crabs, and polychaetes (Alabama Coastal Area Board, unpubl. data). Sites farther upstream (i.e., approximately 20–45 km upstream) become saline only at extremely low discharge, and the most common invertebrates are polychaetes, oligochaetes, mussels, and a few aquatic insects (mostly mayflies; Alabama Coastal Area Board, unpubl. data). In preliminary benthic samples collected October 1994, we found the burrowing mayfly Hexagenia limbata along with nereid polychaetes, the brackish-water mussel (Rangia cuneata), and hogchokers (Trinectes maculatus).

Biology of study organism

Hexagenia limbata (Serville) (Ephemeroptera: Ephemeridae) is a hemimetabolous benthic insect common in large rivers and lakes. The genus Hexagenia occurs throughout the Western Hemisphere from Manitoba, Canada to Argentina (Berner and Pescador 1988). Nymphs construct U-shaped burrows in soft sediments and consume large quantities of sediment containing algae and bacteria (Hunt 1953; Charbonneau and Hare 1998). Substrate size is particularly important in controlling spatial distribution, in that nymphs preferentially burrow in areas containing mostly fine sediments (Wright and Mattice 1981).

The life history of H. limbata is well known (Hunt 1953; Berner and Pescador 1988). Growth and development occur only in the nymphal stage and show strong temperature dependence (Sweeney and Vannote 1978; Corkum and Hanes 1992; Newbold et al. 1994). Mass emergences of H. limbata have been linked to water temperatures of ~19°C, the suggested threshold for nymphs to grow past the final instar (Fremling 1964, 1973). In northern populations where annual temperatures range from 0 to 18°C, at least 3 yr are required to complete the life cycle (Riklik and Momot 1982; Giberson and Rosenberg 1994). In contrast, laboratory-reared nymphs at 24 to 27°C can emerge in only 79 d (Fremling 1973). However, elevated temperatures may suppress nymphal growth (Sweeney and Vannote 1978; Wright and Mattice 1981; Newbold et al. 1994), a situation we hypothesized would occur for H. limbata during summer in the Mobile River.

Methods

Field study—We sampled four equally spaced, 10-km river reaches (sites A–D, Fig. 1) beginning 20 km below the confluence of the Alabama and Tombigbee Rivers and ending at the branch of the Spanish River. This 40-km section provided a natural gradient of increasing salinity at periods of low discharge, with sites closest to Mobile Bay experiencing the highest salinities (i.e., sites A and B). Specific habitats in which mayflies were quantified were selected based on the presence of emergent macrophytes and fine substrates, which ensured appropriate habitat and maximum
mayfly densities (Wright and Mattice 1981; M. Chadwick pers. comm.).

For each study site, on each sampling date single benthic water samples were collected <0.5 m off the bottom with an alpha bottle, and water temperature (YSI Model 55), salinity (YSI Model 33), and dissolved oxygen (YSI Model 55) were measured. Water temperature was recorded continuously over the study using two HoboTemp® data loggers <0.5 m from the bottom at either location immediately downstream (at site A) and upriver (at site D) of the 40-km study reach. Sediment samples were collected quarterly with a Petite Ponar grab sampler (0.024 m²), frozen, and subsequently analyzed for percent total organic carbon and percent grain size.

Mayflies were collected every 4–6 weeks from each reach using a Petite Ponar sampler from October 1995 through January 1996 (i.e., 10 grabs site⁻¹ date⁻¹) and from March 1996 through September 1996 (i.e., 15 grabs site⁻¹ date⁻¹). Samples were field sieved (500-µm mesh) and sorted for mayfly nymphs, which were then transported live to the laboratory in chilled water.

All nymphs were measured for total length under a dissecting microscope, from the base of the caudal filaments to the anterior portion of the frontal process. Dry weight of nymphs was calculated using the length-weight regression from Benke et al. (1999).

Annual secondary production of *H. limbata* was estimated using the size-frequency method (Benke 1984, 1993) for salt-exposed (sites A and B) and fresh water (sites C and D) reaches, and from these data we calculated annual production to mean biomass ratios (P/B). Of the methods available for estimating secondary production (i.e., Allen Curve, instantaneous growth, removal summation, and size frequency), the size-frequency method was considered most appropriate because it does not require synchronous development (Benke 1984), the situation for *H. limbata* in the Mobile River. We used the cohort product interval (CPI), a measure of development time used to correct annual secondary production estimates for cohorts that develop in < or >1 yr (see Benke 1984). This was done by graphing monthly changes of the relative proportions of 10 size classes (3-mm groups), chosen on the basis of morphological and developmental condition (i.e., nymphal size, appearance of wing pads).

Differences in physicochemical variables among the four sites were determined using a Kruskal-Wallis test (α = .05); if significant differences were found, sites were then compared using Tukey’s multiple-range test on ranks (Zar 1984). Density of *H. limbata* from all sites throughout the year was regressed against all abiotic variables (i.e., sediment and water parameters) that were significantly different among the sites using simple linear regression to assess the main factors potentially influencing abundance of *H. limbata*.

**Laboratory experiments**—Salinity bioassay: A 96-h salinity bioassay was conducted to assess acute mortality of *H. limbata* to salinity and to establish the sublethal salinity levels used in growth experiments (see below). We used a 2 × 5 factorial design with two temperature treatments (18°C and 28°C) and five salinity treatments (0, 2, 4, 8, and 16‰), with five replicates per treatment. Salinity treatments were made by adding aquarium salt to aerated Mobile River water (800 mL) contained within 1-liter mason jars and placed in growth chambers. Salinity was measured with a salinity meter (YSI Model SCT 33). One or two nymphs (length 15–20 mm, collected from the Mobile River) were placed in each treatment and checked for mortality at 6, 12, 24, 48, and 96 h. Salinity and temperature were monitored during each sampling interval, and dissolved oxygen was measured at the beginning and end of the experiment. Nymphs were assumed dead if they did not move when touched with a blunt probe; dead nymphs were removed immediately.

**Growth experiments**: To examine the effects of salinity on growth of *H. limbata*, we conducted experiments on two different life stages (first and late instars used in experiments 1 and 2, respectively). Nymphs of both stages are potentially exposed to seasonal increases in salinity in the Lower Mobile River during summer base flow.

In experiment 1, we used three salinity treatments (0, 2, 4‰) at one temperature (28°C), with five replicates per treatment. Salinity levels were determined by the salinity bioassay (see above), and the temperature represented the typical level at the time of increased salinities in the Lower Mobile River. Experimental containers were 1-liter mason jars filled with 8 cm of prepared sediment (potter’s clay and potting soil enriched with fish food, see Corkum and Hanes 1992). Mayflies were given no additional food other than organic matter present in the sediment. Water was prepared as described in the salinity bioassay. We used five nymphs per container, representative of a maximum density that occurs in the study area (i.e., ~200 nymphs m⁻², pers. obs.). Nymphs (first instars) were hatched from eggs from gravid females collected from the upper Mobile River (~15 km upriver from site D) and were apportioned randomly to containers. After 90 d, nymphs were measured for total length under a dissecting microscope and these values were converted to dry weight using the length-weight regression. First instars were assumed to have started at ~4.6 × 10⁻³ mg initial weight (based on a starting length of 1.25 mm and the length-weight regression). An instantaneous growth rate (*W*) was then calculated as

\[ g = \ln(W_{\text{final}}/W_{\text{initial}})/\Delta t. \]

In experiment 2, we used a 5 × 2 factorial design, with five salinity treatments (0, 1, 2, 4, 8‰) at two temperatures (18°C and 28°C), with three replicates per treatment. Containers (1-liter mason jars) were prepared with natural sediment collected from Dead Lake, a tributary of the Mobile River near the study sites. Water was prepared as described in the salinity bioassay. We used late-instar nymphs (one per container; length 17–28 mm), which also were collected from Dead Lake. Each nymph was measured initially for total length. After 21 d, all surviving nymphs were remeasured, lengths were converted into dry weights, and instantaneous growth was calculated.

Data analyses: For the salinity bioassay, a two-factor ANOVA was used to test whether both temperature and salinity
Table 1. Summary of differences in salinity, temperature, and dissolved oxygen recorded from bottom water at the four sites (A–D) in the Mobile River. Samples were collected every 4–6 weeks, October 1995 to September 1996. For A, sites are ordered by decreasing distance from the mouth of the river (i.e., A, closest to mouth; D, farthest from mouth).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>MSE</th>
<th>F</th>
<th>P</th>
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<tbody>
<tr>
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<td>Site</td>
<td>3</td>
<td>263.4</td>
<td>5.53</td>
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<td>Error</td>
<td>38</td>
<td>47.6</td>
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<td>Temperature</td>
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<td>0.97</td>
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<td>Error</td>
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<td></td>
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<tr>
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<td></td>
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<td>0.23</td>
<td>0.87</td>
</tr>
<tr>
<td>Error</td>
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<td>159.3</td>
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<tr>
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<tr>
<td>x salinity (‰)</td>
<td>A</td>
<td>(1.52)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>B</td>
<td>(0.23)</td>
<td></td>
<td></td>
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<tr>
<td></td>
<td>C</td>
<td>(0)</td>
<td></td>
<td></td>
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<td></td>
<td>D</td>
<td>(0)</td>
<td></td>
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</table>

Fig. 2. (A) Mean monthly discharge from October 1975 to September 1993 for the Mobile River. Discharge was calculated by combining daily discharge from below the last lock and dam for the Alabama (Claiborne Lock and Dam) and Tombigbee (Coffeeville Lock and Dam) Rivers (~50 km upriver from the study reach); (B) Maximum salinity recorded from all sites in the Mobile River, October 1995 to September 1996. Salinity was measured from single samples collected every 4–6 weeks from each sampling site (A–D). Decreased salinity during July was the result of increased river discharge.

Results

Field study—Physicochemical variables: Mean annual salinity (0–1.52‰) differed significantly among the four sites (Table 1), whereas mean water temperature (22.1 ± 1.23°C) and dissolved oxygen (7.9 ± 0.27 mg L⁻¹) did not differ. Examination of mean monthly discharge (N = 18 yr) and maximum salinity (present study) showed an inverse relationship between salinity and discharge (Fig. 2). During periods of low flow in Fall 1995 and Summer 1996, the downriver sites experienced saltwater intrusion (with salinities of up to 5.5‰ for site A and 2.0‰ for site B), whereas the upriver sites (C and D) remained fresh throughout the study. Sediment analysis also revealed that site B had a higher percentage of sand ($F_{1,12} = 55.1; p = 0.038$) and smaller proportion of total carbon ($F_{3,12} = 59.8; p = 0.028$) than the other three sites.

Continuous temperature measurements were compromised by the loss of some data from one of the two temperature loggers. The logger at site A recorded temperature only from 14 September 1995 to 9 May 1996, whereas the logger at site D recorded from 12 October 1995 to 6 September 1996. Over the entire river, a minimum of 4.6°C was recorded on 7 February 1996 (site D) and a maximum of 31.3°C was recorded on 22 July 1996 (site A, monthly samples). Unlike the temperature measurements made during sampling, temperatures recorded from 12 October 1995 to 9 May 1996 (i.e., when continuous data were available at both reaches) did not differ significantly between the two reaches (t-test, $p < 0.05$; Fig. 3).

Density, size, and secondary production of *Hexagenia limbata*: Nymphs of *H. limbata* were collected from at least one of the four sites in all months except May 1996 (Fig. 4), when low water and substrates covered by a thick algal mat
prohibited grab sampling. Sites C and D had similar mean mayfly densities (≈75 m⁻²), which were ~25 times higher than sites A and B (≈3 m⁻²; Fig. 4). Despite high density variation in sites C and D, there was a significant negative relationship between maximum observed salinity and mayfly density across the four sites ($r^2 = 0.42, n = 64, p < 0.001$).

Examination of length-frequency distributions for *H. limbata* nymphs suggested a univoltine life cycle and a cohort production interval close to 1 yr (Fig. 5). A large increase in the percent of preemergent mayflies (i.e., >22 mm, with enlarged wing pads) occurred from late April through August, close to the period of adult emergence. The greatest proportion of small nymphs (i.e., <12 mm) occurred in October 1995 and September 1996, but nymphs <10 mm were rarely collected because they were too small to be retained by the grab sampler and sieve.

Secondary production estimates using the size-frequency method and a CPI of one differed greatly between sites in terms of salinity exposure. Production was much lower in sites within salt-exposed reaches (A and B: 46.6 g m⁻² yr⁻¹) compared with sites within freshwater reaches (C and D: 1,669 mg m⁻² yr⁻¹). In contrast, annual $P/B$ values for both reaches were similar (~4, Table 2).

**Laboratory experiments—Mortality:** Salinity and temperature both significantly affected mortality of *H. limbata* (Table 3). At 18°C, only the 16%e treatment showed higher mortality than the 0%e control (after 96 h), whereas at 28°C mortality was higher than the control at both 8 and 16%e (Dunnett’s test). Mortality tended to increase with time at
Table 2. Annual secondary production estimates of *H. limbata* as dry weight (using the size-frequency method), for salt-exposed and freshwater reaches of the Lower Mobile River, October 1995 to September 1996. Negative production values are not included. Negative values for the first two size classes are assumed to have resulted from inaccurate sampling of smaller nymphs.

<table>
<thead>
<tr>
<th>Size class (mm)</th>
<th>Density (No. m(^{-2}))</th>
<th>Mass (mg)</th>
<th>No. lost</th>
<th>Biomass (mg m(^{-2}))</th>
<th>Loss</th>
<th>Biomass loss (mg)</th>
<th>Times No. of size classes (8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt-exposed reaches (sites A and B)*</td>
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<tr>
<td>7–9</td>
<td>0.16</td>
<td>0.712</td>
<td>-0.641</td>
<td>0.112</td>
<td>1.201</td>
<td>-0.77</td>
<td>(-6.16)</td>
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<td>10–12</td>
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<td>1.69</td>
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<td>2.475</td>
<td>0.792</td>
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<td>13–15</td>
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<td>3.26</td>
<td>0</td>
<td>1.568</td>
<td>4.395</td>
<td>0</td>
<td>0</td>
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<tr>
<td>16–18</td>
<td>0.481</td>
<td>5.53</td>
<td>0</td>
<td>2.66</td>
<td>7.06</td>
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<td>0</td>
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<tr>
<td>19–21</td>
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<td>4.137</td>
<td>10.575</td>
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<td>21.44</td>
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<td>Freshwater reaches (sites C and D)†</td>
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<td>1.603</td>
<td>6.872</td>
<td>21.44</td>
<td>12.006</td>
<td>96.051</td>
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</table>

* Density, 2.564 m\(^{-2}\); biomass, 11.84 mg; production, 46.592 mg m\(^{-2}\) yr\(^{-1}\); production to biomass ratio (*P/B*), 3.94.

† Density, 75.641 m\(^{-2}\); biomass, 437.462 mg; production, 1669.705 mg m\(^{-2}\) yr\(^{-1}\); production to biomass ratio (*P/B*), 3.82.
both temperatures (Fig. 6). However, contrary to predictions, we observed no salinity–temperature interaction (Table 3). The LC₅₀ for salinity at 28°C was estimated as 2.4‰, whereas at 18°C the LC₅₀ was 6.3‰ (Fig. 7).

Growth and development: For experiment 1 (first instars) we observed high mortality (~80%) across all salinity treatments, and there were significant differences in instantaneous growth rates between the 0 and 4‰ treatments ($F_{2, 26} = 5.79; p = 0.009$; Fig. 8A). Further, when data were expressed as total accumulated mayfly biomass, we observed substantially higher biomass in the 0‰ salinity treatment than in the 2 or 4‰ treatment (Fig. 8B).

For experiment 2 (late instars), mortality was low (6.7%) and there was no significant difference in growth among salinity treatments (Table 4). However, we did observe a temperature effect (Table 4), with growth rates being significantly higher at 28°C (0.018 ± 0.010 mg mg d⁻¹; mean ± SE) than at 18°C (0.011 ± 0.008 mg mg d⁻¹). As a consequence of differential growth between temperature regimes, we observed a large difference in development and emergence of subimagos (i.e., first adult stage) during the experiment: at 28°C, ~20% of all mayflies emerged from containers (many of which successfully molted within 24 h to the imago stage) compared with only one individual (<1%) emerging at 18°C.

Discussion

Temperature and life history—Compared with that of other more northern Hexagenia limbata populations, the univoltine life cycle observed for the Mobile River population is not surprising because of the relatively high temperature regimes at this latitude. H. limbata required 2,588 degree
Fig. 8. Results of the 90-d growth trial (experiment 1) in which *H. limbata* nymphs (first instars) were exposed to one of three salinity levels (0, 2, and 4 ppt), showing (A) mean growth (+SE), and (B) total biomass accrued. Letters in panel A indicate the Tukey's multiple comparison groupings.

days (dd) to develop to emergence based on laboratory rearing with temperature regimes from 6 to 26°C, and based on a minimum threshold of 10°C (McCafferty and Pereira 1984). In the Lower Mobile River, nymphs accumulated ~3,250 dd for emergence (based on daily temperatures from 23 September 1995 to 6 September 1996) in a thermal regime from ~5 to 32°C. This dd value is considerably higher than the McCafferty and Pereira (1984) laboratory value, and those from other northern and southern populations (Ta-
ble 5). The high accumulated dd observed in our study can be explained by three possible mechanisms. First, dd for southern populations would decrease if an upper temperature threshold was reached, beyond which development slowed or ceased altogether. Inclusion of an upper thermal threshold is consistent with operation of the thermal equilibrium hypothesis, which states that there is an optimal thermal range for growth and development (Sweeney and Vannote 1978). An upper thermal limit of ∼25°C could be assumed for H. limbata based on reduced ingestion rates and gut-clearance times observed for laboratory populations (see Zimmerman and Wissing 1978). Inclusion of both upper (25°C) and lower (10°C) thresholds for H. limbata development in the Lower Mobile River would decrease accumulated dd to ∼1,050, a value that is more similar to that of northern populations (Table 5). Second, because metabolic activity increases with increasing temperature, high summer water temperatures in the Lower Mobile River could reduce rates of biomass accumulation under elevated activity. In this context, H. limbata’s life cycle may be constrained to at least 1 yr throughout most of its geographic range (i.e., by reduced growth rates in low and high temperate latitudes and maximized growth in midlatitudes). This mechanism would be supported if maximal sizes of preemergent nymphs occurred at midlatitudes and smaller final sizes occurred both north and south of this ‘thermally optimal range’ (Sweeney and Vannote 1978; Welch and Vodopich 1989; Giberson and Rosenberg 1994). However, the lack of a clear latitudinal pattern on Table 5 suggests that this is not case. Third, suppression of the lower threshold for growth (i.e., <10°C) may occur in cold-adapted populations (see Giberson and Rosenberg 1994). In such a case dd would be underestimated for northern populations. Decreasing the lower threshold values for cold-adapted populations would increase dd to values similar for less cold-adapted populations developing at lower latitudes. Development in northern latitudes is limited by low temperatures and accumulation of enough dd for emergence can require >1 yr. In contrast, mayfly development in southern latitudes may be limited by high temperatures and its effect on metabolic rate such that it may greatly extend development time.

**Salinity–mayfly associations**—Annual secondary production estimates clearly showed lower production in the harsher salt-exposed (vs. freshwater) reaches of the river. Substantially lower production in salt-exposed reaches is related to the decreased biomass due to individual mortality associated with increased salinity (i.e., observed as lower densities, Fig. 4). In contrast, the laboratory experiments show that there are similar individual growth rates for H. limbata between these two habitat types, in spite of the putative physiological constraints of salinity for individual mayflies in salt-exposed reaches.

Because H. limbata was present in the lower sites (A and B) exposed to seasonal increases in salinity, and all sites (A–D) had similar annual P:B, this mayfly apparently is tolerant to salinity exposure at levels found in the Lower Mobile River. However, because nymphal densities in salt-exposed sites (A and B) were significantly lower than the freshwater sites (C and D), seasonal salinity appears to reduce resident mayfly populations through increased individual mortality. The present study did not assess whether mayflies are continuously drifting from freshwater reaches of the river or whether resident individuals occur in salt-exposed sites throughout development. However, H. limbata is not known to drift (Hunt 1953), although it is possible that flighted adults from freshwater reaches could repopulate salt-exposed reaches by oviposition to some extent.

Comparisons of mayfly density between our study and that during 1982–1983 (Alabama Coastal Area Board, unpubl. data) revealed that densities in the Lower Mobile River can vary greatly from year to year. For example, in November 1981 density approached 400 mayflies m⁻², whereas densities the following September 1982 decreased to ∼10 m⁻². Because 1980 was considered a wet year (i.e., the third wettest year from 1975–1993, U.S. Geological Survey hydrological records), it is likely that abundant freshwater inputs could have provided a salt-free condition in lower reaches for most of the year. This could have increased survivorship and thus maintained the high densities observed in 1981. In contrast, because 1981 was a dry year (i.e., the third driest year from 1975–1993, U.S. Geological Survey hydrologic records), increased salinities in the lower river could have caused a coincident decrease in mayfly density.

Like that observed in the field, results of the salinity bioassay and growth experiments clearly demonstrated some salinity tolerance by H. limbata. However, tolerance varied among individuals, which suggests that salinity can influence individual fitness and, hence, mayfly population dynamics (see Sweeney 1984). At 96 h, 16‰ salinity was lethal at both temperature regimes; however, some nymphs were able to survive exposures of up to 12‰. Mayfly exposure to our bioassay trials probably represents a response to conditions that were potentially harsher than those in nature. Maximum exposures for nymphs were >2 times higher than salinities measured in the Lower Mobile River. Moreover, the length of salinity exposure in the Lower Mobile River is much less than 96-h duration of the bioassay because of the diurnal tidal cycle, which may enable nymphs to survive in these variable-salinity habitats. It is also possible that the presence of interstitial freshwater found in sediments may provide burrowing mayflies a refuge from increasing salinity.

Mayfly growth can be regulated by both endogenous (e.g., hatching date) and exogenous factors (e.g., temperature, nymphal density and food, McCafferty and Pereira 1984; Corkum and Hanes 1992; Hanes and Ciborowski 1992). In our growth experiments, H. limbata grew at similar rates in salinity conditions ranging from zero to about 8‰. In the experiment involving first instars, the lowest growth rates and total mayfly biomass (i.e., lowest total growth) was associated with the highest salinity treatment, indicating low tolerance to salinity in this life stage. In contrast, mortality during the second growth experiment involving late instars was negligible (possibly due to the shorter exposure time, 90 vs. 21 d for experiments 1 and 2, respectively). Results of the bioassay and the second growth experiments are consistent with our field observations of decreased mayfly density. Because the appearance of first instars (from eggs) coincides with seasonal increases in salinity (i.e., June–September), and this life stage was particularly vulnerable
to mortality from salinity (i.e., high mortality at high salinities), it appears that differential mortality of these nymphs was the main factor producing lower densities in salt-exposed reaches of the river. However, most mayflies that survive initial increases in salinity appear to be capable of completing development and can emerge and reproduce, thereby potentially providing eggs for the next generation.

Little is known about the specific modes of action causing mortality from increased salinity, or the physiological pathways (e.g., loss of intracellular water balance) involved in the tolerance of salinity by aquatic insects (Attrill et al. 1996). Irrespective of the mechanisms, the combination of field observations of mayflies in seasonally salt-exposed reaches of the Mobile River and the results of the salinity bioassay and growth experiments show that \textit{H. limbata} is not a halophobic organism (sensu Gallardo-Mayenco 1994) and can survive, grow, and emerge in seasonally saline environments.

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