

Effects of chronic hypoxia and reduced temperature on survival and growth of burrowing mayflies, *Hexagenia limbata* (Ephemeroptera: Ephemeridae)

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Abstract: *Hexagenia* nymphs are sensitive to hypoxia, and their abundance has been proposed as an ecosystem indicator for assessing the recovery from eutrophication of shallow, mesotrophic lakes. Acute oxygen tolerance limits are known for *Hexagenia* spp., but effects of prolonged exposure to sublethal levels of hypoxia are not. A series of 21-day laboratory experiments was conducted to determine the influence of hypoxic stress (range 2–12.6 mg/L dissolved oxygen) and temperature (range 4–20°C) on survival and growth of *Hexagenia limbata* nymphs. Oxygen and temperature together explained 89% of the variability in survival among *H. limbata*, and 71% of the variability in average growth. Survival increased with increasing oxygen concentration, reaching an asymptote at 7–8 mg/L. Survival increased with temperature to an asymptote at approximately 9.5°C. Higher temperatures magnified the effects of hypoxia on survival. Growth increased with both oxygen and temperature, and did not reach an asymptote at levels up to 12.6 mg/L oxygen and 20°C. Persistent, sublethal oxygen stress (concentrations <7 mg/L) and reduced temperature (<20°C) can influence both survival and size of *H. limbata*.

Résumé : Les nymphes d'*Hexagenia* sont sensibles à l'hypoxie, et on a proposé d'utiliser leur abondance comme indicateur écosystémique pour évaluer le rétablissement après eutrophication des lacs mésotrophes peu profonds. On connaît les limites de la tolérance à une concentration aiguë d'oxygène chez *Hexagenia*, mais pas les effets d'une exposition prolongée à des niveaux sublétaux d'hypoxie. Nous avons mené une série d'expériences de 21 jours en laboratoire pour déterminer l'influence du stress hypoxique (fourchette 2–12,6 mg/L d'oxygène dissous) et de la température (fourchette 4–20°C) sur la survie et la croissance des nymphes d'*Hexagenia limbata*. Combinés, l'oxygène et la température expliquaient 89% de la variabilité de la survie parmi les *H. limbata* et 71% de la variabilité de la croissance moyenne. La survie augmentait avec la concentration d'oxygène, pour atteindre une asymptote à 7–8 mg/L. La survie augmentait en fonction de la température pour atteindre une asymptote à environ 9,5°C. La hausse des températures amplifiait les effets de l'hypoxie sur la survie. La croissance augmentait en fonction à la fois de l'oxygène et de la température, et n'avait pas atteint une asymptote à des niveaux de 12,6 mg/L d'oxygène et 20°C. Un stress persistant et sublétaux dû à la concentration de l'oxygène (<7 mg/L) et une réduction de la température (<20°C) peuvent avoir un effet sur la survie et sur la taille d'*H. limbata*.

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Introduction

Hexagenia mayflies were formerly abundant benthic macroinvertebrates in many shallow, mesotrophic water bodies, including extensive regions of the Great Lakes. Organic pollution eradicated *Hexagenia* spp. in western Lake Erie (Carr and Hiltunen 1965) and other basins (Schuytema and Powers 1966; Schneider et al. 1969; Howmiller and Beeton 1971) but the Lake Erie population is presently showing signs of recovery (J.J.H. Ciborowski, personal observation; L.D.

Corkum, University of Windsor, personal communication; Krieger et al. 1996).

For those Great Lakes areas in which mayflies were once abundant, Reynoldson et al. (1989) have recommended the recovery of *Hexagenia* populations as an indicator of the ecosystem's rehabilitation from eutrophication. Nymphs are acutely sensitive to anoxia in that they generally die when subjected to dissolved oxygen concentrations less than ~1 mg/L for more than 24 h (Hunt 1953; Eriksen 1963). In western Lake Erie, the absence of *Hexagenia* spp. since the 1960s has been attributed to low dissolved oxygen levels (Britt 1955a; Reynoldson et al. 1989). This population crash occurred when unusually warm, calm conditions caused exceptionally stable thermal stratification of the water column. This prevented near-bottom waters from becoming reoxygenated, and resulted in *Hexagenia* mortality rates of up to 100% in open waters of the western basin (Britt 1955a). In addition to such episodes of sudden extinction, oxygen depletion will potentially have long-term effects on a *Hexagenia* population. The increased efforts required by nymphs for respiration may cause reduced growth and thus smaller adult size, which would result in lower fecundity (Clifford and Boerger 1974).

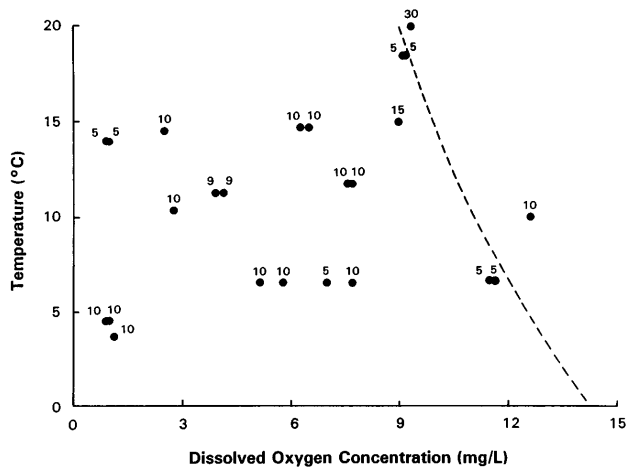
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Fig. 1. Plot of combinations of oxygen and temperature conditions employed. Numerals above each point indicate number of replicate rearing tubs simultaneously exposed to the oxygen–temperature combination. Broken line indicates isopleth of 100% saturation.



Western Lake Erie stratifies sporadically during summer months. Depending on their frequency and duration, these episodes can strongly influence oxygen availability in profundal zones. At the same time, temperature itself determines the solubility of oxygen (Carpenter 1966), the rate of bacterial respiration in hypolimnetic water and sediment (Blumberg and DiToro 1990), and the metabolic rates of nymphs themselves (Morgan and Wilder 1936, cited in Craven and Brown 1969).

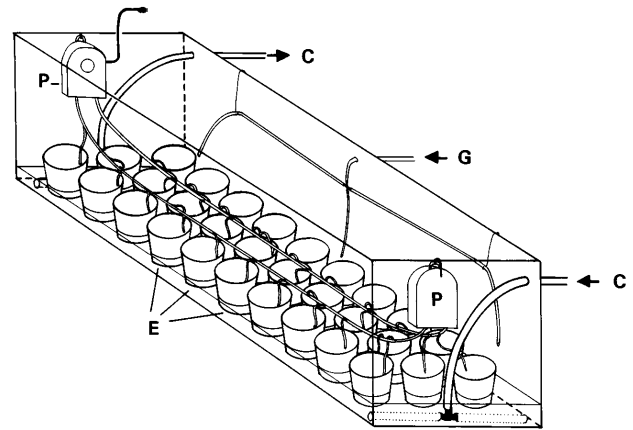
Assessing the recovery of *Hexagenia* spp. in Lake Erie and other mesotrophic lakes therefore requires an understanding of the mayflies' physiological responses to the effects of chronic, low dissolved oxygen concentrations and temperature. Here, we report results of a laboratory study evaluating the survival and growth of *Hexagenia* nymphs over a range of dissolved oxygen concentrations and temperature levels comparable with conditions that can be found in western Lake Erie. Our study provides information needed to model the recovery of mayfly communities in systems recovering from cultural eutrophication.

Materials and methods

Experimental organism

Nymphs of *Hexagenia limbata* were obtained from eggs collected from emergent females on the north shore of Lake Erie near Colchester, Ontario. The eggs were cooled and stored at 8°C following the procedures of Friesen (1981). Eggs were removed from cold storage as required, and hatched within 7 days of being returned to room temperature. Newly hatched nymphs were reared at 22–25°C in aerated aquaria containing dechlorinated tap water and Lake Erie sediment for at least 60 days. Hanes and Ciborowski (1992) found that exogenous factors did not become significant to the growth and development of *Hexagenia rigida* until they were more than 3 months old; therefore, we avoided using very young animals. Nymphs used in experiments were selected by eye for being vigorous (i.e., swimming actively in the water dish in which they were screened) and spanning a range of sizes (body length ~4–16 mm). Because both natural and laboratory-reared *Hexagenia* populations commonly exhibit great size variation (Corkum and Hanes 1992; Hanes and Ciborowski 1992), a broad size range was employed to provide a more realistic representation of nymphal population structure.

Fig. 2. Diagram of an experimental chamber containing rearing tubs (not to scale). Fewer tubs than illustrated were used in experimental trials. E, rearing tubs; P, internal atmosphere recirculating pumps; C, cooling water supply and return hoses; G, gas supply hose (from compressed nitrogen tank or line air supply). Arrows indicate direction of flow. The chamber was sealed with polyethylene sheeting.



Experimental design

Experiments consisted of 21-day trials in which nymphs were cultured at 1 of 24 combinations of fixed levels of oxygen and temperature (Fig. 1). Oxygen and temperature levels were selected to encompass the prevailing range of conditions characteristic of bottom waters of Lake Erie (Schertzer et al. 1987; Lam and Schertzer 1987; Schertzer and Sawchuk 1990). Each trial comprised a minimum of five replicate containers, which were cultured simultaneously in a common experimental chamber. Each replicate container held eight nymphs (equivalent to 1143 individuals/m² of substrate). This density is typical of historical values for western Lake Erie (Reynoldson et al. 1989), and nymphs grow and survive well in containers of this size (Hanes and Ciborowski 1992).

Experimental chambers and conditions

We conducted experiments in three black, 125 × 40 × 40 cm Plexiglas water baths (Corkum and Hanes 1992; hereafter called chambers), covered in transparent, utility weight (1-mil = 0.025 mm) polyethylene sheeting, sealed with masking tape to isolate the atmosphere inside (Fig. 2). Within these chambers, nymphs were placed in each of 10 or more 1-L, heavy polyethylene containers (bottom area 70 cm² × 15 cm tall; hereafter called tubs), containing a 4 cm depth of Lake Erie sediment and 11 cm depth of dechlorinated City of Windsor water. Sediment had been collected by box corer from two locations in western Lake Erie (stations 357 and 358 of Reynoldson and Hamilton 1993). Sediments were stored at 4°C for several months until required and then autoclaved. Sediments were predominantly silt (60–70% of particles <40 μm, determined by hydrometry; loss on ignition 6–7% (Winter 1994)).

Water temperature was controlled by circulating water through the chambers, each cooled (when appropriate) by Aquachiller water refrigeration units (Jewel Industries Inc., Chicago, Ill). Temperature conditions were also stabilized prior to the start of each trial.

Rearing tubs were individually aerated by a system of capillary tubing and hypodermic needles (Corkum and Hanes 1989) fed from one of two manifolds attached to electrically powered, twin-output pumps (Model Elite 802, Rolf C. Hagen Inc., Montréal, Que.). The pumps were mounted inside the chambers on opposite walls, one attached to each end of both manifolds, thus constantly recirculating the atmosphere inside the chamber.

The atmospheric oxygen concentration inside the experimental

chambers was regulated by venting in nitrogen gas, pressurized atmospheric air, or both, through Nalgene plastic tubes supplied from either a gas cylinder (nitrogen) or the laboratory line air system (atmospheric air). In some trials, air and nitrogen were separately injected directly into opposite manifolds connecting the two electric air pumps. Although the gases subsequently mixed within the chamber, this separate delivery system ensured that one row of five or more rearing tubs, fed from the nitrogen-injected manifold, would always receive a higher incipient level of nitrogen, and the other row of five or more tubs would receive a higher level of air. Depending on the incoming pressure, this arrangement sustained differences of as much as 0.7 mg/L O₂ between separate rows of rearing tubs within the same chamber, for the 21-day duration of an experiment. These two oxygen treatments, each consisting of a row of five or more tubs, were then analysed as separate trials. Thus, a single chamber generated either one or two of the 24 oxygen–temperature combinations (Fig. 1), depending on whether gas was fed to the chamber (as illustrated in Fig. 2; one trial) or directly to the manifolds (two trials). Oxygen concentrations were stabilized for at least 12 h prior to the start of every trial by adjusting the flow of nitrogen and air.

One millilitre of a mixture of baker's yeast (15 g), Tetramin fish food flakes (20 g), alfalfa powder (15 g), and water (500 mL) (Hanes and Ciborowski 1992) was added to each rearing tub prior to the start of the trial. The food mixture was pipetted just beneath the water surface, allowing it to settle evenly over the sediment. Nymphs were not fed again during a trial, to minimize exposure to outside air.

Experimental procedure

The nymphs destined for each rearing tub were individually marked by clipping a combination of caudal filaments (Ciborowski et al. 1992). With three caudal filaments per nymph, 2³ = 8 clipping patterns are available (Ciborowski et al. 1992). The particular clipping pattern was randomly assigned to each of the eight individuals allocated to a rearing tub. Such clipping does not influence growth or survival of the nymphs (J.J.H. Ciborowski, E.C. Hanes, A. Winter, and L.D. Corkum, unpublished data; Winter 1994). Marked nymphs were then photographed against a 1-cm² grid. Head width (to the outside of the eyes) of each individual was determined by measuring the size of its photographic image to the nearest 0.04 mm at 25× magnification with an ocular micrometer in a dissecting microscope (Hanes 1992).

Immediately before the nymphs were introduced, the oxygen concentration in each rearing tub was measured using either a YSI model 57 oxygen meter (Yellow Springs Instrument Company, Yellow Springs, Ohio) or the Winkler titration method (Wetzel and Likens 1991). Oxygen concentrations were measured to the nearest 0.2 mg/L. Temperature was also measured in each rearing tub, at the sediment–water interface, using thermometers graduated to 0.1°C. The chamber was then resealed and maintained at the required inflow of air–nitrogen and cooling water for the 21-day duration of the trial. At the conclusion of the trial, oxygen and temperature measurements were repeated in each rearing tub as soon as the chamber was opened. All remaining live nymphs were then retrieved and photographed, and images of their head width were remeasured for determination of growth (see below).

Determination of growth and survival

Survival was expressed as the proportion of the eight nymphs per rearing tub recovered alive at the end of each 21-day trial. Survival data from the five or more replicate rearing tubs within the chamber were then averaged for a given trial. Growth was calculated as the percentage change in head width (100 × ln(final head width/initial head width)) of a nymph during trial. Negative growth (value less than zero) occurred when the final head width of a nymph was less than the initial head width. The values for all surviving nymphs in a rearing tub were averaged. Growth in a trial was expressed as the mean of the means of each rearing tub in which at least one nymph survived.

Table 1. Nonlinear and multiple regression models predicting survival and growth of *Hexagenia limbata*.

Equation no.	Constant	[O ₂]	Temp. ²	[O ₂]×Temp.	IHW	R ²
Survival						
1	0.825	-0.482	-0.043	—	—	0.89
2	0.755	-0.212	—	—	—	0.84
3	0.700	—	-0.039	—	—	0.38
Growth						
4	-1.739	—	0.012	0.036	—	0.71
5	6.581	—	0.008	0.048	-4.351	0.77
6	-0.446	0.501	—	—	—	0.25
7	-1.035	—	0.025	—	—	0.57

Note: Nonlinear models (survival) are in the form $y = a(1 - e^{-b \cdot X_1})$. (1 - e^{-c·X₂}). Polynomial models (growth) are expressed as $y = a + b \cdot X_1 + c \cdot X_2 + \dots$.

Survival and growth data were each analysed by forward stepwise multiple regression analysis (Draper and Smith 1981). Independent variables included in the analyses were oxygen concentration (mg/L), temperature (°C), (oxygen concentration)², (temperature)², and oxygen × temperature. Quadratic terms were included to detect possible nonlinearity of the relationships, which would have indicated maximal growth or survival at some intermediate level of one or more of the independent variables.

For survival, stepwise multiple regression was initially used to select significant variables, but the polynomial function was subsequently changed to a nonlinear regression function as this produced a saturation curve, which was biologically more realistic. For growth, we additionally tested initial head width (mm) as an independent factor in the regression analysis.

Because field surveys and records of physical data are often incomplete, oxygen and temperature measurements may not both be available for a given location or period of interest. Therefore, we also calculated regressions using oxygen and temperature variables separately.

Results

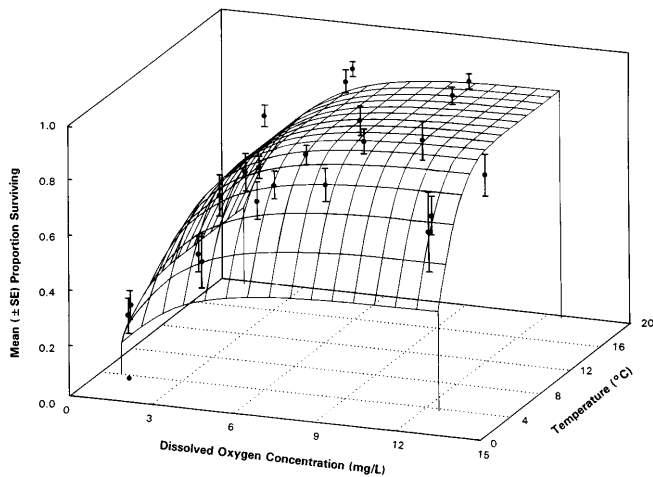
Survival

Survival increased asymptotically with increasing oxygen concentration up to the level of approximately 7–8 mg/L (eq. 1 in Table 1; Fig. 3), well above the 24-h tolerance thresholds of 0.8–1.0 mg/L reported by Hunt (1953) and Eriksen (1963). The influence of temperature on survival was positive as well, and reached an asymptote at approximately 9.5°C (Fig. 3). The mortality curve with respect to either oxygen or temperature became increasingly steep as the complementary independent variable increased in magnitude (compare opposite edges of the response surface in Fig. 3). As a single independent variable, oxygen concentration (eq. 2 in Table 1) provided a better fit by nonlinear regression than did temperature (eq. 3 in Table 1; Figs. 4a, 4b).

Growth

Results of a nested analysis of variance (ANOVA) showed variation in nymphal growth among treatments ($F_{[23,1141]} = 31.90$, $p < 0.0001$) but not among replicate tubs within treatments ($F_{[185,1141]} = 1.18$, $p > 0.05$). Therefore, a regression model for growth as a function of oxygen and temperature was calculated with all replicate tubs per treatment pooled (eq. 4 in Table 1; Fig. 5).

Fig. 3. Response surface plot of eq. 1 (Table 1) showing influence of oxygen concentration and temperature on survival of *Hexagenia* nymphs. Actual experimental data (means of replicate rearing tubs ± 1 SE) are superimposed.



The interaction of oxygen \times temperature removed approximately 44% of the total variation in growth among treatments, and (temperature)² accounted for a further 27%. The inclusion of initial head width in the regression analysis produced an additional significant increase in the coefficient of determination (eq. 5 in Table 1). However, because growth was defined as the log ratio of a nymph's final head width to its initial head width, this improvement came mostly as a result of self-correlation. Growth, in fact, was a significant function of initial size alone (Winter 1994). The inclusion of initial head width in the model thus contributes little explanatory power to the growth response of *Hexagenia* nymphs, but improves the ability to predict growth for individuals whose size can be determined beforehand.

The calculation of regressions using oxygen and temperature separately is represented in eqs. 6 and 7 in Table 1 (Figs. 6a, 6b). Temperature was the most important predictor variable for growth, but the influences of both temperature and oxygen were statistically significant.

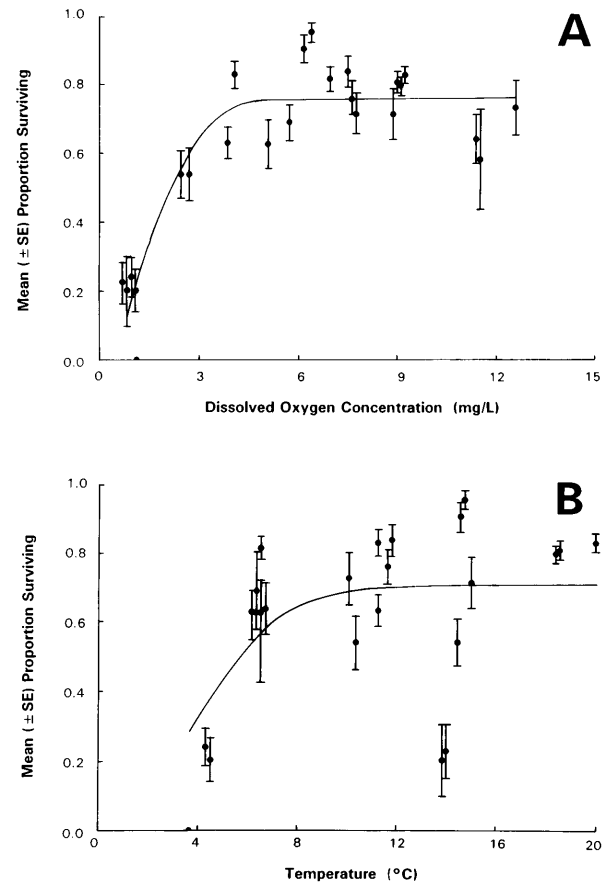
Discussion

Survival

Survival of *Hexagenia* nymphs over 21 days was positively related to the concentration of dissolved oxygen, up to an asymptote at the level of approximately 8 mg/L. This result implies that even light to moderate oxygen stress can increase the mortality rate of the nymphs, if the hypoxic conditions persist.

It has not been previously reported that prolonged sublethal hypoxia influences survival rates among *Hexagenia* spp. Experimental investigations with aquatic invertebrates have usually been restricted to evaluating acute tolerance limits. However, the history of the mayfly population in western Lake Erie suggests that the response relationship to moderate oxygen stress may be as important as the tolerance threshold. The numbers of *Hexagenia* spp. in the western basin of Lake Erie fluctuated considerably between 1928 and 1960 (Fig. 1 of Reynoldson et al. 1989), dropping almost to zero in 1953. However, young nymphs were reported in 1954 (Britt 1955b),

Fig. 4. Mean (± 1 SE) survival of *Hexagenia* nymphs as a function of (a) oxygen concentration and (b) temperature.



and this recovery suggests that a single, catastrophic event is not enough to completely eradicate a population (perhaps because eggs are resistant to anoxia (Hunt 1953)).

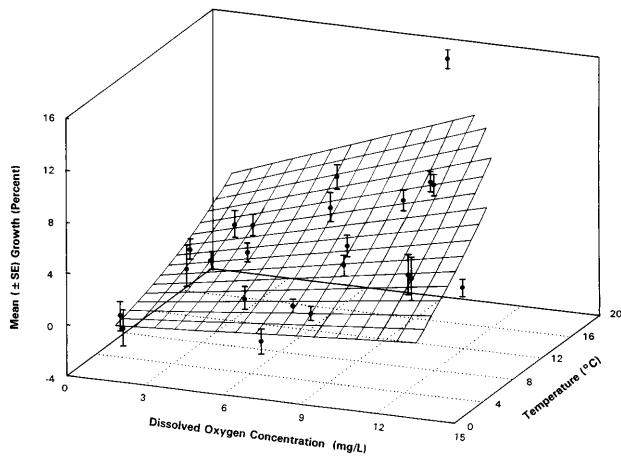
Our data are consistent with the events of 1953–1954 (Britt 1955a, 1955b). Even the harshest hypoxic conditions in our experiments (0.82–0.84 mg O₂/L) killed fewer than 80% of nymphs over 3 weeks. Given the ephemeral and intermittent nature of thermal stratification in large, shallow waters like western Lake Erie (Bartish 1984), it is unlikely that a single episode of total anoxia could cover the whole western basin for long enough to have eradicated the entire mayfly population there at one time. High levels of mortality are thus more likely due to prolonged exposure to oxygen stress.

In our study, dead nymphs were recovered after 21 days in various states of decomposition (or not at all), indicating that individuals died over a range of time intervals. Oxygen stress thus increased the probability of mortality rather than inflicting outright mass mortality. This implies that the duration as well as the severity of an episode of hypoxia can affect the survival of *Hexagenia* populations. Natural recovery of populations may therefore be anticipated even after brief periods of anoxia.

Nymphs that were initially larger were significantly more likely to survive than small nymphs (as determined by analyses of maximum-likelihood estimates for logistic regressions of survivorship versus initial body length for larvae in individual rearing tubs (Winter 1994)).

Mean survival increased with increasing temperature as

Fig. 5. Response surface plot of eq. 4 (Table 1) showing the influence of oxygen concentration and temperature on growth of *Hexagenia* nymphs. Actual experimental data (means of replicate rearing tubs ± 1 SE) are superimposed.



well, up to an asymptote at approximately 9.5°C. This is close to the temperature generally regarded as the developmental threshold for *Hexagenia* spp. (10°C; Heise et al. 1987 and references therein; Giberson and Rosenberg 1992). As temperatures decreased below this point, the increasing likelihood of developmental arrest thus correlates with increasing chances of mortality. Nevertheless, temperature by itself explained only 38% of the variability in survival among treatments, as opposed to 84% for oxygen. This suggests that cold stress also increases the probability of mortality indirectly, rather than directly. Developmental stasis perhaps inhibits recovery from tissue damage or other injury, leaving nymphs more vulnerable to death by other causes.

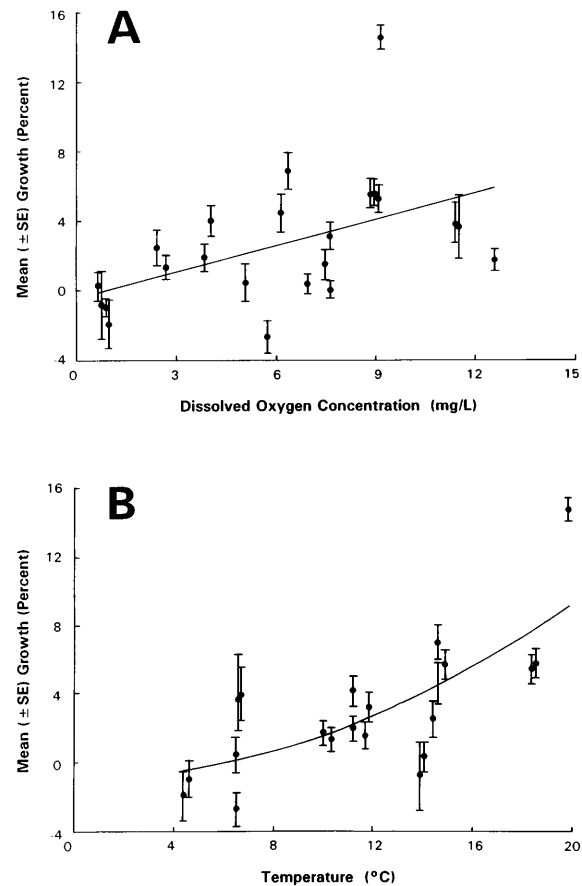
In our study, high temperatures produced a steeper mortality response to oxygen concentration. A reduction in dissolved oxygen concentration at a high temperature would thus be more harmful to nymphs than the same degree of hypoxia at a lower temperature. In a water body such as western Lake Erie, this has important implications for the survival of a mayfly population. Stratification leading to hypoxia in these waters occurs in the summertime, when temperature is high (Bartish 1984). A temperature a few degrees celcius higher or lower during hypoxia might make the difference between complete mortality or sustainable survival. Notably, the severe basin-wide extinction in 1953 followed stratification estimated at less than 14 days duration, but at the elevated bottom temperature of 24°C (Britt 1955a).

Our results suggest that a summer regime of ≥ 8 mg/L oxygen and $\geq 10^\circ\text{C}$ is adequate to sustain *Hexagenia* nymphs without limiting their survival. Persistent, sublethal oxygen stress (concentrations < 7 mg/L) and reduced temperature ($< 20^\circ\text{C}$) measurably reduce *Hexagenia* survival. We believe we can now begin to predict the recovery and survival potential of mayfly populations, given daily estimates of temperature and oxygen at the level of the substrate.

Growth

The interaction of oxygen and temperature was the single most important determinant of nymphal growth over 21 days: as temperature rose, metabolic rates increased, resulting in faster

Fig. 6. Mean (± 1 SE) growth of *Hexagenia* nymphs as a function of (a) oxygen concentration and (b) temperature.



growth, presumably creating higher oxygen demand for *Hexagenia* nymphs. Oxygen and temperature also influenced growth singly, but their predictive power increased when they were considered together. Unlike survival, growth did not approach an asymptote with respect to either oxygen or temperature, over the range of conditions used. For temperature, this result was expected. Previous work has shown that the maximum growth temperatures for *Hexagenia* spp. are just above 20°C (Wright and Mattice 1981; Wright et al. 1982; Corkum and Hanes 1992); temperatures in our study thus represent a range below the optimal zone.

The observed relationship between growth and oxygen concentration has not been previously reported. Average survival peaked at around 7–8 mg/L dissolved oxygen. It is noteworthy that the capacity for growth continued to increase above this point. In warm, shallow water bodies such as western Lake Erie, the water column mixes easily (Bartish 1984) and a storm or high winds can quickly entrain oxygen-rich surface waters to the sediment interface. Our results suggest that *Hexagenia* nymphs can exploit such oxygen conditions to produce faster growth than would occur under calm conditions.

The mechanism that stimulated nymphal growth at high, saturated oxygen levels in our experiments probably involves a reduction in the need to actively ventilate (Eriksen 1963), or an increase in the efficiency of feeding. The energy requirements of processing and assimilating food demand oxygen, and positive correlations between oxygen availability and food

consumption rates have been determined in various fish species (Warren 1971; Muir and Niimi 1972; Beamish 1974; Ross et al. 1992). The same principle may be valid in mayflies.

Many of the animals reared under low oxygen or temperature conditions were smaller when recovered after 21 days than when introduced into their rearing tubs. Most of the animals displaying negative growth were dead, but a number of living nymphs became smaller as well, indicating that use of stored reserves prior to moulting to the next instar is one end of the continuum of growth responses, and not a qualitative symptom specifically associated with moribund or severely stressed animals.

It would be instructive to determine, in future experiments, the full range over which the positive relationship between growth, oxygen, and temperature extends. In our experiments, the predictive power of oxygen and temperature was substantially lower for growth than for survival.

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