

A method for obtaining in situ growth rates of larval Chironomidae (Diptera) and its application to studies of secondary production¹

Abstract—Methods and growth chambers are described which permit in situ estimates of the growth rates of chironomid larvae inhabiting litter accumulations in lotic habitats. Instantaneous growth rates (IGRs) for larvae of different sizes ranged from 0.01 to 0.24 mg mg⁻¹ ash-free dry mass (AFDM) d⁻¹ at stream temperatures of 2.9°–15.1°C. IGRs were significantly and linearly related to temperature. Regression equations relating IGRs and temperature, combined with field-derived data for chironomid standing stock and stream temperature, enabled calculation of the production of chironomids inhabiting litter accumulations (15-g DM litter bags) in a temperate mountain stream. The annual production per litter bag was about 224 mg AFDM yr⁻¹ and the annual *P:B* was 42, indicating rapid turnover of chironomid biomass. The annual production of chironomids exceeded the mean standing crop biomass of all macroinvertebrates by 4.6×

The recognition of secondary production as a means of quantifying the influence of stream-dwelling invertebrates on ecosystem processes (Benke and Wallace 1980; Parker and Voshell 1983; Fisher and Gray 1983) has resulted in the compilation of a considerable volume of literature (Benke 1984). Most secondary production estimates have been restricted to relatively large organisms with semi-, bi-, or univoltine life cycles which are amenable to the size-frequency and various cohort methods of measurement (Waters 1977; Benke 1984). However, benthic invertebrates with low standing stock biomass, short generation times, and overlapping cohorts (e.g. Chironomidae) may have high annual *P:B* ratios (*P:B* = production : \bar{X} standing stock biomass) and may contribute substantially to total community secondary production (Mackey 1977a; Fisher and Gray 1983; Benke et al. 1984). Such organisms are often overlooked

or ignored in production studies due to difficulties in recognizing specific cohorts or estimating the cohort production interval (CPI of Benke 1979). Shortcut methods, such as the estimation of secondary production as the product of standing stock biomass and some *P:B* ratio (often the empirically derived 3.5 to 5; Waters 1977; Benke 1984) or the uncorrected size-frequency method (Benke 1979; Benke et al. 1984), have been applied to entire benthic invertebrate communities and almost certainly have resulted in significant underestimation (e.g. Fisher and Likens 1973; Fisher 1977; Webster 1983). In these studies, quantification of the influence of macroinvertebrates on the processing of organic matter was attempted. However, the low production estimates utilized may have influenced the final conclusions, thus contributing to our uncertainty of the role of these organisms. Production was probably most underestimated among those groups with short CPIs, low standing stocks, and overlapping cohorts. Among chironomids inhabiting temperate mountain streams, *P:B* values exceeding 30 or 40 may be general (Waters 1979; Benke 1984). Clearly, methodologies that are not extremely labor-intensive yet allow realistic estimates of the production of this group are needed.

We describe a simple field-based method for obtaining community level estimates of chironomid growth rates. These data, combined with temperature and standing stock, will allow a reasonable estimate of secondary production to be calculated by the instantaneous growth method (Waters 1977; Benke 1984). Our method is based on the assumption that changes in weight of groups of chironomid larvae of mixed taxa can be used to estimate an average growth rate reflective of an entire community. The influences of fast growing taxa should generally be balanced by slow growing taxa.

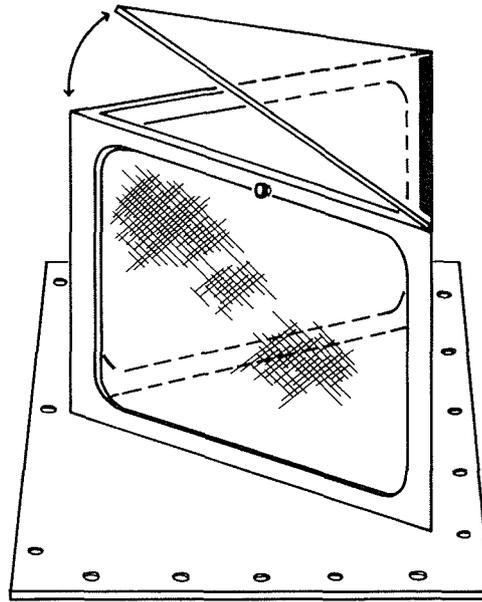
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Wedge-shaped growth chambers, their side panels covered with 63- μm Nitex mesh to exclude the smallest chironomid larvae (Fig. 1), were placed in the stream with the top extending above the water surface and the base anchored to the bottom with four 25-cm galvanized nails. The wedge was oriented directly into the current, reducing drag and clogging of the mesh. Clogging was not a major problem during the study. The chamber cover was secured with several rubber bands. Red maple (*Acer rubrum* L.) leaves for food-substrate were removed from the stream, air-dried, frozen, and 8–10 leaves placed in each chamber. Similar food was used throughout the study to minimize nutritional effects.

In late August, October, and December 1984 and January 1985, samples of litter accumulations (leaf packs and debris dams) were washed through a series of nested sieves with stream water, the sieve contents placed in enamel pans, and chironomid larvae removed with a polyethylene bulb pipette and sorted into three length classes (900–1,299, 1,300–1,999, 2,000–3,000 μm) selected on the basis of the general size distribution of the stream population (pers. obs.). Specimens $>3,000 \mu\text{m}$ usually matured in <10 days and were not used in the growth study. Each specimen was measured to the nearest 0.01 mm with an ocular micrometer under a dissecting microscope. From 32 to 64 specimens of each length class were initially introduced to each chamber. All specimens recovered in each length class were used, regardless of taxon; we assume that this resulted in a representative sample of taxonomic diversity that reflected that of litter accumulations in the stream. After 7–14 days incubation, material in the chamber was removed and the chironomids were measured and counted. During each incubation period a reference chamber with leaves but no larvae was used to determine if immigration occurred. Between incubation periods chambers were removed from the stream, cleaned, and dried.

Stream temperature was measured continuously with a chart thermograph (Kahl



— = 2 cm

Fig. 1. Design of growth chamber constructed of 5-mm Plexiglas with front and rear panels covered with 63- μm Nitex mesh.

Sci. Instr. Co.). The median daily temperatures over each incubation period were averaged to obtain the mean median daily temperature during incubations.

Individual larvae were measured before and after incubation. An average length for the populations of individuals in the chambers was estimated and, from a length-weight regression equation, their average ash-free dry mass (AFDM) was obtained. For the regression equation, 70 larvae (killed and preserved in 3% formaldehyde) of various lengths (range, 879–5,800 μm) and taxa were oven-dried (65°C) for 12 h, placed in a desiccator for 24 h, weighed, and then ashed at 500°C for 1 h. For AFDM, individuals were weighed to the nearest 0.1 μg with an automatic electrobalance (Cahn 25, Ventron Corp.). The resulting data were best described by the following power model:

$$W = 0.452 L^{3.099} \quad (r^2 = 0.81, P < 0.0005) \quad (1)$$

where W is AFDM in μg and L is length in mm. Mackey (1977b) and Ladle et al. (1985) also found that larval length-weight rela-

Table 1. Summary of data used to estimate instantaneous growth rates for three length classes of Chironomidae larvae. I—2,000–3,000 μm ; II—1,300–1,999 μm ; III—900–1,299 μm . T is the mean median daily temperature over the incubation period ($^{\circ}\text{C} \pm 1$ SE), L_i and L_f the mean initial and final lengths over an incubation interval (± 1 SE), n the sample size, t the length of incubation in days, and IGR the instantaneous growth rate coefficient ($\text{mg mg}^{-1} \text{d}^{-1}$).

Class	L_i	n_i	L_f	n_f	t	T	IGR
I	2,251 \pm 47	64	2,993 \pm 286	10	11	15.1 \pm 0.2	0.080
II	1,350 \pm 26	39	2,330 \pm 151	11	11	15.1 \pm 0.2	0.149
III	927 \pm 18	32	2,236 \pm 394	3	11	15.1 \pm 0.2	0.238
I*	2,322 \pm 150	9	3,142 \pm 271	9	14	12.1 \pm 0.5	0.066
II	1,806 \pm 38	41	2,629 \pm 201	7	12	12.2 \pm 0.1	0.097
III	1,127 \pm 18	46	2,322 \pm 150	9	12	12.2 \pm 0.1	0.182
I	2,157 \pm 31	54	2,437 \pm 46	18	7	5.5 \pm 0.4	0.055
II	1,548 \pm 18	51	1,849 \pm 61	18	7	5.5 \pm 0.4	0.073
III	1,153 \pm 21	51	1,477 \pm 50	29	7	5.5 \pm 0.4	0.110
I	2,477 \pm 40	50	2,736 \pm 64	27	10	2.9 \pm 0.2	0.031
II	1,663 \pm 30	59	1,978 \pm 76	10	10	2.9 \pm 0.2	0.055
III	1,130 \pm 26	37	1,213 \pm 64	10	10	2.9 \pm 0.2	0.013
I†	2,736 \pm 64	27	2,596 \pm 96	13	10	2.3 \pm 0.2	-0.016
II†	1,978 \pm 76	10	2,178 \pm 99	10	10	2.3 \pm 0.2	0.029
III†	1,213 \pm 64	10	1,135 \pm 60	6	10	2.3 \pm 0.2	-0.013

* Based on extended incubation of class III population initially incubated at 12.18 $^{\circ}\text{C}$.

† Based on extended incubation of populations initially incubated at 2.87 $^{\circ}\text{C}$.

tionships for various chironomids were best described by power models. We calculated instantaneous growth rate coefficients (IGRs) ($\text{mg mg}^{-1} \text{d}^{-1}$) by the following expression:

$$\text{IGR} = (\ln W_f - \ln W_i)/t \quad (2)$$

where W_i and W_f are the initial and final larval AFDM observed during a period of growth (t) in days (Romanovsky and Polishchuk 1982). The IGRs calculated for each size class at different thermal regimes were regressed (least-squares) against the mean median daily temperature over the incubation period. From these regression equations, temperature-specific IGRs over the annual thermal regime of the study stream could be estimated.

We used the methods described here in a 2nd order headwater tributary of Ball Creek which drains Watershed 27 of the Coweeta Hydrologic Laboratory (Macon Co., North Carolina), an undisturbed 38.8-ha catchment with vegetation characterized as a mixed hardwood-hemlock association. The topography is rugged and of high relief with the elevation of the streambed ranging from 1,035 to 1,188 m. The relatively high elevation, the north-northeastern aspect, the dense forest cover, and the steep ridges forming the catchment's boundaries com-

bine to restrict insolation to the stream during much of the year. The mean median daily stream temperature is 9.0 $^{\circ}\text{C}$ (range, 0.5 $^{\circ}$ –15.8 $^{\circ}\text{C}$) and the stream accumulates only about 3,300 degree days year $^{-1}$.

Above 2.3 $^{\circ}\text{C}$, the average lengths of larvae of all size classes increased (Table 1). The recovery of individuals from the growth chambers ranged from 9 to 60% of the number introduced. The presence of pupae, pupal exuviae, and adult fragments indicated some loss of maturation and emergence. In three of five incubations, a single chironomid larva was recovered from the control chamber to which none had been added.

The size-specific chironomid IGRs for five different temperature regimes are given in Table 1. Above 5.5 $^{\circ}\text{C}$, the smaller larvae grew at higher rates than the larger ones, as expected (Konstantinov 1958; Ladle et al. 1985). However, at 2.3 $^{\circ}$ and 2.9 $^{\circ}\text{C}$ the intermediate-size larvae grew fastest (Table 1, Fig. 2A, B). The IGRs of the chironomid larvae in the three size classes were significantly related to temperature within the range considered (ca. 2 $^{\circ}$ –15 $^{\circ}\text{C}$) (Fig. 2A, B, C); the relationship was best described by a linear model. Growth decreased abruptly when populations were incubated at 2.3 $^{\circ}\text{C}$, probably due to a sudden cessation of feeding. During the incubations at water tem-

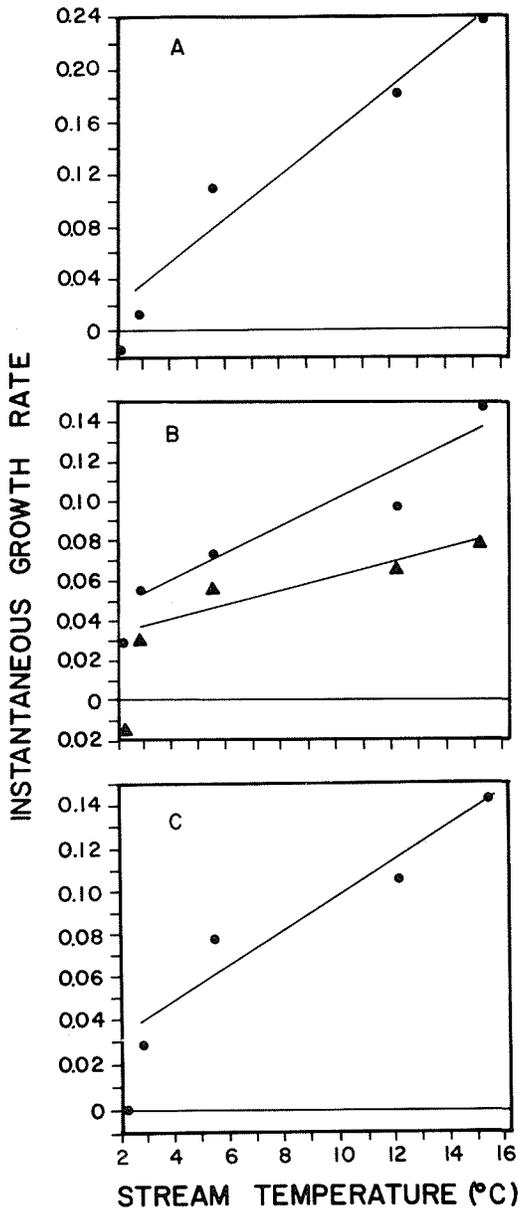


Fig. 2. A. Relationship between instantaneous growth coefficient (IGR) ($\text{mg mg}^{-1} \text{d}^{-1}$) and temperature ($^{\circ}\text{C}$) for chironomid larvae of the smallest length class (900–1,299 μm). Solid line is derived from the following model: $\text{IGR} = -0.012 + ^{\circ}\text{C } 0.017$ ($r^2 = 0.95$, $P < 0.05$). The IGR measured at 2.3°C was not included in the regression analysis (see text). B. Relationships between IGR and temperature for larvae of the intermediate (1,300–1,999 μm : ●) and largest (2,000–3,000 μm : ▲) length classes. IGR vs. $^{\circ}\text{C}$ of the intermediate length class is described by the following model: $\text{IGR} = 0.033 + ^{\circ}\text{C } 0.007$ ($r^2 = 0.89$, $P < 0.10$); and of the largest length class by $\text{IGR} = 0.027 + ^{\circ}\text{C}$

temperatures $< 2.9^{\circ}\text{C}$, the stream was ice-covered and the growth chambers were placed on the substrate through holes cut in the ice. Because of the sudden decrease, the IGRs measured at 2.3°C were not included in the regression analysis. At 2.3°C slight weight losses in the largest and smallest larvae (Table 2, Fig. 2A, B) caused negative IGRs. No weight loss was observed for the intermediate size. The differences in the growth rates among larval size classes at the lowest temperatures may be attributable to the taxonomic composition of the populations of larvae in the chambers, with each taxon characterized by a different temperature at which the IGR is zero or negative. The larvae removed from chambers at the end of each growth study were identified: the number of taxa was diverse, usually with at least four to six major taxa in each chamber. The Orthoclaadiinae were particularly well represented (*Brillia*, *Cheatocladus*, *Eukiefferiella*, *Orthocladus*, *Rheocricotopus*, *Corynoneura*, *Nanocladius*, and various other undetermined orthoclad genera), as were the Tanypodinae (*Ablesomyia* and others).

The geometric means of the IGR values calculated for each length class at each thermal regime were regressed against temperature. Equation 3 relates mean growth rate and temperature (Fig. 2C):

$$\text{IGR} = 0.015 + ^{\circ}\text{C } 0.008 \quad (3)$$

$(r^2 = 0.93, P < 0.05).$

The temperature-specific IGRs derived from Eq. 3 can be combined with standing stock data to calculate secondary production by the instantaneous growth method:

$$P = \text{IGR} \times (B_f + B_i)/2 \times t \quad (4)$$

where B_f and B_i are the final and initial standing stock biomasses observed over a

←
0.004 ($r^2 = 0.90$, $P < 0.05$). For both length classes the IGRs measured at 2.3°C were not included in the regression analysis (see text). C. Relationship between the geometric means of the IGRs of three length classes of chironomids (900–1,299, 1,300–1,999, 2,000–3,000 μm) and temperature ($^{\circ}\text{C}$). The relationship is described by the following model: $\text{IGR} = 0.015 + ^{\circ}\text{C } 0.008$ ($r^2 = 0.93$, $P < 0.05$). The mean IGR measured at 2.3°C was not included in the regression analysis (see text).

Table 2. Calculation of annual production (mg AFDM litter bag⁻¹ yr⁻¹) of Chironomidae larvae inhabiting litter bags placed in a stream (Watershed 54) at the Coweeta Hydrologic Laboratory (unpubl. data). B is the average biomass (mg AFDM \pm 1 SE litter bag⁻¹, $n = 16$) for each date, B_{med} the median biomass over each time interval measured in days (t), B_{mean} the mean annual biomass, T the median temperature over time interval ($^{\circ}$ C), IGR the daily instantaneous growth rate coefficient (mg mg⁻¹ d⁻¹) (specific for the mean median temperature measured over each time interval: see text), P_t the estimated production during each time interval, and P_{annual} the annual production (sum of all P_t).

	B	B_{med}	T	t	IGR	P_t
14 Mar 82	7.34 \pm 0.50	12.5	10.0	34	0.10	42.57
17 Apr 82	17.71 \pm 2.15	11.2	11.4	67	0.11	82.25
23 Jun 82	4.62 \pm 0.53	4.6	14.5	62	0.13	37.08
24 Aug 82	4.59 \pm 0.66	3.1	16.0*	39	0.14*	17.12
2 Oct 82	1.68 \pm 0.28	1.9	13.5	36	0.13	8.75
7 Nov 82	2.06 \pm 0.23	2.6	11.4	63	0.11	18.02
9 Jan 83	3.15 \pm 0.27	2.8	10.2	62	0.10	17.17
12 Mar 83	2.38 \pm 0.26					
	$B_{mean} = 5.34$					$P_{annual} = 223.57$

* 16 $^{\circ}$ C is outside the range of the IGRs used in the regression analysis; therefore, a more conservative IGR calculated for 15.1 $^{\circ}$ C (highest mean median temperature at which IGRs were measured) was used.

time interval (t) measured in days (Romanovsky and Polishchuk 1982).

The ecological significance of the above information can best be demonstrated by a simple example with data from a litter decomposition study in experimental Watershed 54 (unpubl. data) at the Coweeta Hydrologic Laboratory. Due primarily to its lower elevation and to groundwater domination, the stream draining Watershed 54 has a higher average annual temperature (11.5 $^{\circ}$ C; range, 6.1 $^{\circ}$ –17.8 $^{\circ}$ C) than the headwaters of Ball Creek. However, we assume that the chironomid communities in the two streams are generally similar, since we have found taxonomically similar communities in various streams throughout the Coweeta basin. The use of growth rates, standing stock, and temperature data from the two different streams should not affect the overall conclusions below. In practice, however, IGRs derived from a particular stream should be applied only to that stream community.

Fifteen grams of air-dried red maple, dogwood (*Cornus florida* L.), white oak (*Quercus alba* L.), or *Rhododendron maximum* L. leaves in 0.5-cm mesh bags were placed in the stream draining Watershed 54 and four bags of each removed at intervals for a year (Table 2). Invertebrates were removed from the leaf bags and AFDM determined for the various taxa. Temperature was measured with a maximum-minimum

thermometer. The annual chironomid production per litter bag, calculated with the temperature-specific growth rates derived from Eq. 3, was 224 mg AFDM yr⁻¹ (Table 2), the average standing stock was 5.3 \pm 0.5 mg AFDM ($\bar{X} \pm 1$ SE), and the annual $P:B$ was 42. Chironomid production exceeded the mean annual standing stock of all macroinvertebrates (including chironomids) in the litter bags (48.8 \pm 5.8 mg AFDM) by 4.6 \times . Chironomid production alone was similar to the total macroinvertebrate production that would have been calculated if we had assumed that the annual $P:B = 5$. This emphasizes the potential of chironomids for influencing energy flow in temperate mountain streams and their role in particulate organic matter processing and transport (Webster 1983; Fisher and Gray 1983). The potential ecological significance of organisms characterized by low standing stocks and high $P:B$ ratios is not well recognized in most studies of cool mountain streams.

The high chironomid $P:B$ ratio of 42, although not generally suspected for temperate mountain streams, is not surprising. High seasonal turnover rates (>60) have been documented for the temperate Thames River (Mackey 1977a). Short generation times (2–4 weeks), under thermal regimes similar to that above, were reported for *Chaetocladius melaleucus* (Meigen) (Orthocladiinae) by Ladle et al. (1984). As our re-

sults show, the biomass of chironomid communities inhabiting leaf-litter accumulations in temperate mountain streams turns over rapidly, and this profoundly influences their overall production rates.

The method of estimating production outlined here has disadvantages. In the example above the average IGR used was not weighted for the relative biomasses in the various size classes. It would have been more accurate to separate the chironomids from the samples into the same size classes as in the growth study, calculate biomass and production separately, and then sum the values to obtain total annual production. The effects of the rearing chamber must also be considered. The population density in the chambers was probably different from that in the stream and this may affect potential biotic interactions. The red maple leaves offered, although a major species entering the stream, are one of many food resources available. The nature of the litter substrate may influence growth rates (Ward and Cummins 1979). The variation in current flow through the chamber was probably of little importance, as the chironomids were selected from leaf accumulations in debris dam backwater areas and litter bags where flow is similarly limited.

Despite disadvantages, field rearing has some important advantages over laboratory rearing. The water flowing through the chamber is derived directly from the surrounding stream, so that nutrient concentrations, dissolved organic carbon, and other factors potentially influencing leaf conditioning and chironomid growth are minimally affected. Furthermore, the larvae reared in the field chambers are exposed to natural diel thermal regimes which influence growth and development (Sweeney 1984).

Ideally, to estimate chironomid production, each size class of each taxon should be reared at temperatures and under nutritional conditions that are realistic for calculations of the IGRs or CPIs needed for production estimates. The closest approach to this was that of Mackey (1977*a,b*) who obtained growth and developmental rates for many species by rearing individual larvae in the laboratory. Although detailed studies

of the autecologies of specific taxa are most valuable and should be encouraged, this is not possible for most stream studies. More than 70 chironomid taxa occur in some streams of the Coweeta Hydrologic Laboratory. Rearing of each is unrealistic; the present state of their systematics does not allow accurate identification of many late instars, and identification of most early instars is not possible. An autecological approach would also require examination and identification of all specimens at sites where densities may exceed 25,000 m⁻² (unpubl. data). In stream production studies, limited by time and funding, the average community IGR method of production estimation demonstrated here is a practicable alternative for obtaining reasonable estimates of production for the Chironomidae.

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