Emergence Patterns of *Hexagenia bilineata*: Integration of Laboratory and Field Data

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Abstract. Laboratory findings regarding water and air temperature effects on the growth rate and emergence success of *Hexagenia bilineata* and field observations of emergence patterns of *H. bilineata* under known thermal regimes are compared. Several hypotheses based on laboratory results are evaluated. The hypothesis that degree-day accumulation requirements could be used to predict the intervals between onset of emergence each year was rejected. The laboratory derived regression showing that approximately 2337 degree-Celsius days (days having temperature above 10°C) were required for development from oviposition to emergence correlated very well with the degree-days actually occurring (2322 and 2192 degree-Celsius days) in two succeeding years in a cove representing a normal thermal condition. However, in a thermally enriched cove, the predicted time required for development was exceeded by 1500 to 1600 degree-Celsius days in two years of field observations. The hypothesis that a critical thermal threshold was required for complete development into the adult stage was neither strongly rejected nor supported by our field observations. Laboratory tests suggested a critical temperature threshold (between 15 and 20°C) might be involved in determining the onset of emergence. These results lead to a prediction of a four-week to six-week difference in the onset of emergence between the two field sites; however, only a two-week difference was observed. Air temperature is shown to affect survival after emergence but is irrelevant to the onset of emergence. A series of laboratory tests may be required to arrive at a good predictive model for thermal effects on any target species.

Very few studies can be found in the literature that evaluate the effects of thermal discharges on emergence patterns of freshwater insects even though early predictions identified off-season emergences as a potential problem (Bregman 1969; Coutant 1962; Hawkes 1969). These early predictions were based on the belief, supported by numerous

studies of Ephemeroptera and Trichoptera in natural temperature regimes, that degree-day accumulation or thermal thresholds were predominant factors determining the growth rate and onset of emergence in these freshwater insects (Clifford, Hamilton & Killins 1979; Coutant 1968; Flannagan & Lawler 1972; Hunt 1953; Macan & Maudsley 1966; Makaravian 1980; Neave 1932; Nebeker 1971b; Wise 1980). Laboratory studies also have provided strong support for the concept that temperature is a controlling factor in determining onset of emergence. For instance, several studies have demonstrated that when immature mayfly nymphs (or eggs) are collected from the field, then reared at higher temperatures, emergence will begin earlier than in the field population (Brittian 1976a; Fremling 1964, 1973; Nebeker 1971a, 1971b; Wright & Mattice 1981a). However, investigators who have studied the effects of thermal discharges on insects in the field have found either no change in emergence pattern (Langford 1975; Langford & Daffern 1975) or relatively minor changes (Mattice & Dye 1978; Tennessen & Miller 1978). Thus, it appears that laboratory studies have provided insufficient information for predicting the effects of thermal discharges.

Studies that have evaluated the effects of temperature on Ephemeroptera through a combination of controlled laboratory experiments and extensive field observations are rare. Brittian (1976b) and Humpesch (1979, 1981) are notable exceptions; however, they were not specifically studying emergence patterns and thus reported incomplete information for predicting effects on emergence. We conducted an integrated laboratory and field study of the mayfly, *Hexagenia bilineata*, over a two-year period. Our data include (1) information on emergence patterns in an intake channel of a power plant, a cove receiving thermal discharge, and in a reference cove; (2) extensive daily temperature records from the intake channel and discharge cove; (3) monthly thermal profiles from the intake channel, the discharge cove, and a nearby reference cove; (4) two sets of laboratory experiments on growth and emergence under four constant temperature regimes; and (5) laboratory observations on emergence success under four air temperature conditions. Using data previously published (Mattice & Dye 1978; Wright & Mattice 1981a, 1981b; Wright, Mattice & Beauchamp 1982) as well as data reported here for the first time, we examined the hypotheses that (1) degree-day accumulation and/or thermal thresholds in the aquatic environment are correlated with onset of emergence, (2) that air temperatures can affect onset of emergence, and (3) laboratory observations can be relied upon to predict the effects of thermal alterations in a natural system.

STUDY SITE

The populations of *H. bilineata* discussed in this paper were found in three coves of Watts Bar Lake in association with the Tennessee Valley Authority's (TVA) Kingston Power Plant (Fig. 1). This coal-fired generating plant is located near the junction of the Emory and Clinch Rivers (84° 30'W, 35° 52'N) near Kingston, Tennessee. The intake channel (hereafter called intake cove) is separated from the Emory River by a skimmer wall which has a 5-m opening 8 m below the surface. The water source is not always the Emory River, however, since the lower volume of flow in the Emory often results in Clinch River water flowing back through the mouth of the Emory into the intake cove. Information on the size and operational specifications of the power plant can be found in Mattice and Dye (1978).

The discharge cove's primary source of water circulation is the discharge from the power plant. There is a second small source of flow into the discharge cove entering from the western-most finger.

The reference cove receives water from either the Emory or Clinch rivers, depending on relative volumes of flow. There is also a small intermittent source of flow entering the eastern-most tip of the cove.

ORNL-DWG 85-1343

INTAKE COVE

REFERENCE COVE

KINGSTON POWER PLANT

DISCHARGE COVE
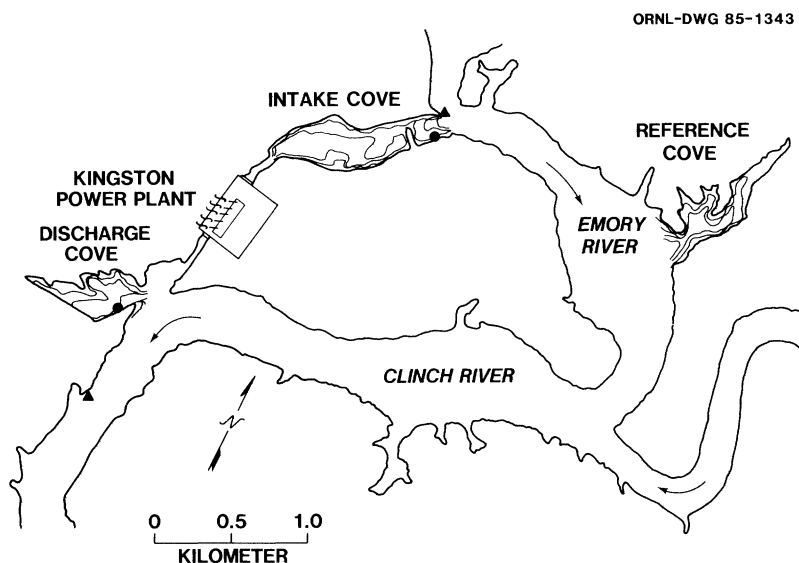
EMORY RIVER

CLINCH RIVER

0   0.5   1.0
KILOMETER

Fig. 1. Schematic diagram of the study site indicating the location of Ryan thermographs (●) that collected temperatures at a single depth in each cove and the Tennessee Valley Authority temperature recording stations (▼) that collected temperatures at a series of depths

The three coves are similar in many respects except for temperature. For instance, all three have substrate conditions that are conducive to *H. bilineata* habitation (see Wright & Mattice 1981b). The mean depth of the coves is approximately 4 m; the maximum depth in the discharge and reference coves is about 8 m at the mouth. The maximum depth in the intake cove is 10 m, but depths greater than 8 m are confined to a very small area next to the skimmer wall. All three coves have trees around about one-half of the shoreline that provide good locations for mating swarms of mayflies. The coves differ with respect to thermal stratification patterns and mean temperature of the water column. Preliminary data indicated that the discharge cove stratified during the summer, the intake cove was nearly homothermal year-round, and the reference cove showed decreasing temperatures with depth in the summer but did not clearly stratify. Preliminary measurements also indicated that discharge cove water temperatures were on average 4 to 5° C warmer than temperatures in the intake cove. The reference cove temperatures were usually intermediate. The detailed temperture differences occurring between the intake cove and the discharge cove are important for understanding temperature effects on *H. bilineata* emergence and thus are amplified later in this paper.

METHODS

Laboratory Experiments

Experiments to determine temperature effects on *H. bilineata* emergence were conducted in the laboratory. In one experiment, previously reported in Wright and Mattice (1981a), nymphs collected from the field at a temperature of 15° C were divided into groups by sex and size class (body length) then reared to the adult stage at 15, 22.5, and 30° C. The emphasis was on determining the effect of temperature, sex, and initial body length (8-10 mm, 13-14 mm, and 16-18 mm) on adult body length and emergence success. In a second experiment, previously reported in Wright, Mattice and Beauchamp (1982), eggs collected from postmating flight females were introduced into laboratory aquaria at temperatures of 10, 15, 20, 25, 30, and 35° C to determine temperature effects on growth rate, growth patterns, and survival. The total number of degree-days required for development from the egg to the adult stage was estimated based on the relationship

between temperature and development rate. This relationship was well described by the equation $1/D = a + bT$, where $1/D$ = development rate, T = time, and a and b are constants. The minimum threshold temperature for development was also determined from this relationship by extrapolation.

A third experiment (not previously reported) addressed the question of survival of adults emerging into low air temperatures. One aquarium was placed in each of four environmental chambers with air temperatures of 10.0, 15.5, 20.0, and 26.5°C. Water temperatures were heated to 22.0°C in the 10.0, 15.5, and 20.0°C chambers. The water temperature in the fourth chamber was the same as ambient air temperatures (26.5°C). Relative humidity was maintained at 30%. Mature nymphs were collected from the field and 25 were placed in each aquarium. Ovservations were made daily to determine dates of emergence and length of survival as subimagoes and imagoes.

Temperature Measurement in Field
Temperature information was gathered from several sources. The primary temperature data were collected using Ryan thermographs located on the bottoms of the intake and discharge coves, at a depth of between 2.0 and 3.5 m (depending on water level) (Fig. 1). The strip charts in the thermographs continuously recorded data for up to four weeks and thus were changed approximately monthly. Thermograph data were collected from December 1975 through November 1976 in the intake cove and from December 1975 through June 1977 in the discharge cove. A secondary source of temperature information was from hourly temperature recording devices operated by the Tennessee Valley Authority (TVA). One TVA recording station was located outside the skimmer wall of the intake cove, the second was located in the Clinch River approximately 1200 m downstream from the discharge outlet (Fig. 1). Computer printouts of data from both stations showing temperatures at several depths were obtained from TVA for the total period of time over which the field research was conducted. A third source of temperature information was obtained by measuring bottom substrate temperatures with a YSI telethermometer. We took measurements once per month during 1976 at 20 to 30 locations along three transects in the intake and discharge coves and in a nearby reference cove. Two to four times a month, temperature measurements were also taken with the YSI telethermometer near the Ryan thermographs and the TVA temperature recording stations, to provide an independent check of those measurements.

Because the Ryan thermographs at both field sites were lost during the period of observation, the TVA readings had to be relied upon to supply missing data. Comparisons were made between the TVA and thermograph data collected over the same period to develop correction factors where needed. Comparable data were available for an eight month period in the intake area and for 18 months in the discharge area. The comparison was between the daily mean temperature estimated (visually) from the continuously recorded thermograph strip charts and the daily mean temperature calculated from hourly measurements by TVA.

In the intake cove, the thermograph temperatures collected at 2.0 to 3.5 m depth and TVA's temperatures recorded at the level of the skimmer wall opening differed at most by 2.8°C and the average difference was only 0.4°C. Since this difference was within the accuracy limits of both recorders and since there was no consistent pattern of difference, it was decided that no correction factor was needed. The thermograph data were used preferentially until the thermograph was lost in November 1976, then TVA data were used.

In the discharge cove, substitution of TVA temperature records for the missing thermograph data was less straightforward. TVA temperature records collected in the Clinch River approximately 1200 m downstream from the power plant were carefully

evaluated to determine if they could be used to estimate temperatures in the discharge cove at a location only about 650 m from the power plant. The shallow-depth (0.8 m) TVA temperature recorder was the only recording depth clearly and consistently affected by the thermal plume at 1200 m from the power plant discharge. Fortunately a clear relationship was found between temperatures measured at the 0.8 m depth at TVA's Clinch River station and those recorded by a Ryan thermograph at 2- to 3.5-m depth in the discharge cove, although the relationship changed with the season. During the period of stratification (April to October 1976 and May to October 1977) mean daily water temperatures estimated from the discharge cove thermograph records were never more than 2.4° C above or 0.9° C below the daily means estimated from TVA's Clinch River temperature records. About 70% of the time, the differences were less than ± 1.0° C. Spot checks with a thermistor at both sites usually indicated even less difference. Thus, TVA's Clinch River temperature data were used with no correction during periods of stratification to supply missing temperature information. After destratification, however, daily mean water temperatures in the discharge cove were consistently higher than those in the Clinch River with differences ranging from 0.4 to 5.5° C and averaging 2.2° C in 1976 and 2.6° C in 1977. Independent comparisons between the two sites based on telethermometer readings averaged 2.1° C in 1976 and 1.9° C in 1977. Given the accuracy limitations of each temperature measurement method, a conservative value of 2.0° C was selected as a correction factor to avoid the possibility of exaggerating the intake and discharge cove thermal differences. TVA's Clinch River temperature data were used to supply missing information during the destratified (winter to spring) period by adding a 2.0° C correction factor. Starting and ending dates for the stratification period were easily determined by using TVA's hourly temperature records from several depths to identify the 1- to 3-day period of rapid temperature change during fall overturn and spring stratification development.

Field Observations of Emergence Patterns

Data on *H. bilineata* emergence dates and relative numbers emerging in each cove were collected primarily using sticky traps and direct observation. Sticky traps sampled the relative abundance of the adult population in trees along the shorelines of the coves. These traps consisted of 12-oz beverage cans suspended from tree limbs, wrapped in rectangular pieces of heavy plastic, and sprayed with Tree Tanglefoot (Tanglefoot Co., Grand Rapids, MI). Twelve traps were spaced more or less equally around each cove between June 9 and October 1 in 1976 and between May 1 and September 10 in 1977. The heavy plastic sheet on each can was removed, replaced, and sprayed at least once per week and more frequently during periods of heavy emergence to avoid saturation of the collecting surface. Sampling was more frequent in 1977 than in 1976. In 1978, only two sticky traps were placed in each cove, but they were placed at sites where emergents were most consistently collected the previous two years. The traps were placed in the discharge cove on June 6, 1978, and in the intake cove on June 13, 1978. Traps were checked frequently only until the first emergence in each cove was determined.

Direct observation produced qualitative information that aided us in estimating dates of emergence in each cove. For instance, if only fresh subimagoes were found on the sticky traps, it indicated that an emergence had occurred the night before collection. If the traps collected subimagoes plus imagoes, it indicated that an emergence had occurred since the last sample collection, and the freshness of the material assisted in estimating the dates. The presence or absence of flying imagoes and subimagoes and exuvia on the water surface was noted during our sampling trips and helped to define dates and duration of emergences.

RESULTS

Laboratory Experiments

Results reported in Wright and Mattice (1981a) demonstrated that adult tissue maturation was temperature and size dependent. The majority of nymphs in the initial body length size groups of 13-14 mm and 16-18 mm emerged simultaneously when reared at temperatures of 22.5° C and 30° C; however, nymphs in the 9-mm size group emerged synchronously two to four weeks later. Adult tissue naturation occurred in all size groups at temperatures of 15° C over a five-month period but was not synchronized. Emergence was attempted only by mayflies in the largest size group in the 15° C tests but death occurred during metamorphosis in nearly all cases. Size of nymphs at the large-wing-pad stage was a function of interactions between temperature and initial size and between temperature and sex.

The laboratory temperature study reported in Wright, Mattice and Beauchamp (1982) showed that the degree-days required for development of *Hexagenia bilineata* from oviposition to first emergence of subimagoes were 2337° C days above a threshold temperature of 10.1° C. The 95% confidence interval around the degree-day estimate was 2045-2727° C days and the 95% confidence interval around the threshold temperature was 7.0-13.2° C,

Air temperature clearly had an effect on the survival time of individuals after emergence and the time required to shed the subimaginal skin after emergence (Table 1). Not all nymphs introduced into the lab aquaria attempted to emerge; however, there was no correlation between emergence attempts and air temperature. Eighty-four percent of all emergence attempts occurred on the same three dates in all test conditions. Of the 20 to 25 mayflies in each temperature condition that attempted emergence, 68 to 95% successfully completed the subimaginal molt. Although successful completion of the imaginal molt also did not appear to be a function of air temperature, the time to completion of the molt was temperature-related. It is significant that the imaginal molt did occur successfully at air temperatures as low as 10-12° C. A meaningful estimate of mean number of days adults survived after emergence was not obtainable under the laboratory conditions since the limited flying space above the water probably contributed to the drowning of several adults. The maximum observed survival time, however, provides an indication of the biological limits of survival at given air temperatures: maximum survival time at 26.5° C was two days and at 10-12° C was eight days.

Mean Daily Temperature and Thermal Stratification Differences Between Coves

Mean daily temperatures representative of bottom temperatures throughout the homothermal intake cove and representative of a portion of the thermally complex

TABLE I

Testing effect of emergence from warm water temperatures into cooler air temperatures on survival and molting.

Water temp (°C)	Air temp (°C)	No. nymphs	No. emerging	% of emergents reaching adult stage	Mean days to adult molt[1]	Range of days of survival after emerg.[2]
22	10-12	25	21	86	2.6	1-8
22	15.5	25	25	68	2.5	1-6
22	20	25	21	95	1.3	1-3
26.5	26.5	25	20	85	0.6	1-2

[1] The mean is only approximate since some of the values from which it was calculated were estimates of events which occurred during a weekend.
[2] Many of the deaths were a result of drowning; thus the maximal number of days surviving is most representative of the potential for survivorship.

discharge cove are shown in Figure 2. Discharge cove temperatures were estimated for two weeks in February 1977, mid-June to mid-August in 1977, and from October 1977 through June 1978 using seasonally corrected TVA data as previously explained. Temperatures in the intake cove ranged from a seasonal low of $1°C$ to a seasonal high of $25°C$. The seasonal range was from 5 to $30°C$ in the discharge cove. Daily temperature information was not collected in the reference cove, but monthly measurements indicated that it represented thermal conditions fluctuating between that of the discharge and intake.

The effect of depth and thermocline formation on thermal patterns in the intake, discharge and reference coves is demonstrated in Figure 3. The relationship of temperature to depth is based not on measurements from top to bottom of the water column but rather on bottom temperatures from shallow to deep areas along three different transects in the discharge and intake coves. Temperatures on the bottom are very uniform throughout the intake cove, regardless of depth throughout the year. Substrate temperatures in the discharge and reference coves vary with depth on a seasonal basis. The change in bottom temperatures with depth is most pronounced in the discharge cove, and is primarily explained by the development of a thermocline between May and October of each year. However, there is additional thermal variation which results from the presence of a small stream flowing into the western tip of the discharge cove. The dark circles in Figure 3 represent temperature measurements that were made in the area (roughly one-third of the cove) affected by the stream inflow. Temperatures in this area were not measured every month. Available measurements indicate that temperatures were always 1 to $5°C$ cooler near this inflow than in other areas of the discharge cove at similar depths. The Ryan thermograph was always located above the thermocline and out of the influence of the small stream. Thus, the temperature values shown for the discharge in Figure 2 were representative of a seasonally variable portion of the discharge cove bottom. When the cove was not stratified (approximately October to May) the temperatures in Figure 2 represented about two-thirds of the cove, but only about one-third during the summer (May to September).

Emergence Patterns
    The pattern of mayfly emergence was similar in 1977 to the observations previously reported for 1976 (Mattice & Dye 1978) (Fig. 4a,b and Fig. 5a,b). Figures 4a and 5a show the dates of trap sampling and the mean number of mayflies per trap collected on those dates in three coves. Figures 4b and 5b show the dates of emergence based on observation
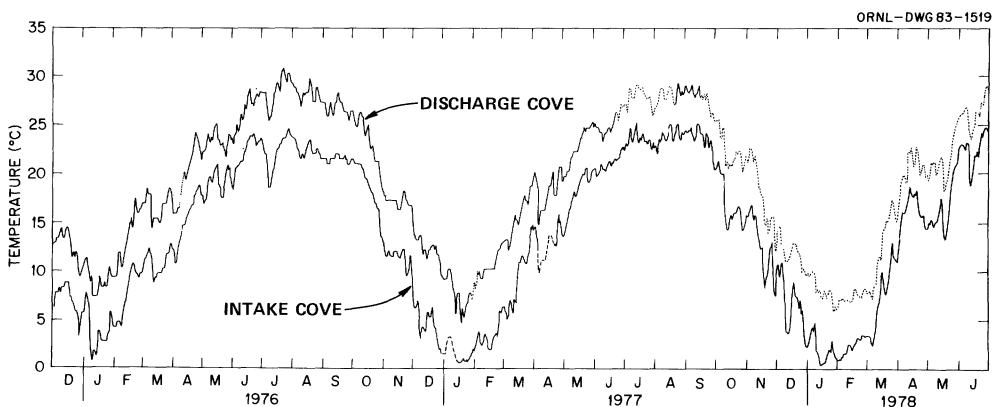

Fig. 2. Mean daily temperatures measured at a single location in each cove. The intake cove data are representative of the whole cove, since it is homothermal year round. Since the discharge cove stratifies in the summer, the data shown are representative only of the bottom area above the thermocline. Dotted lines represent periods when temperatures were estimated based on TVA temperature data incorporating seasonal correction factors. Dashed lines represent educated guesses.
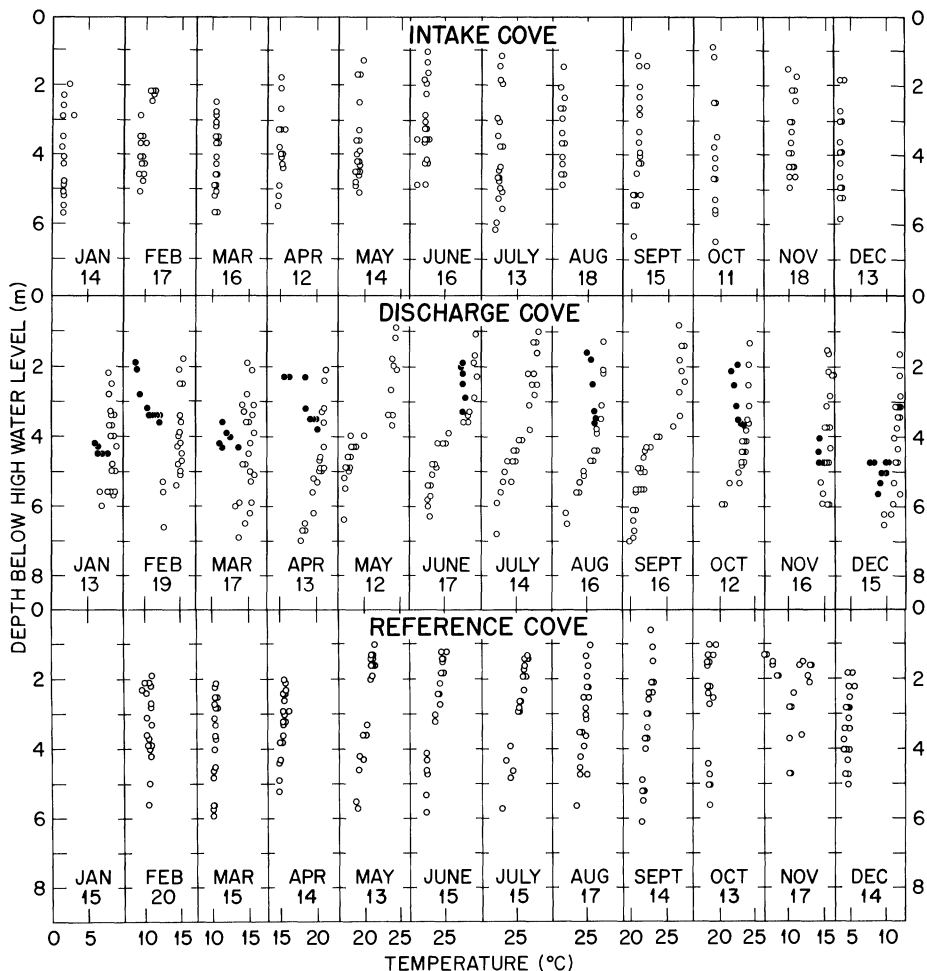
Wright & Mattice

Fig. 3. Bottom temperatures are shown as a function of depth over a 12-month period in 1976. In the discharge cove, the dark circles (●) represent the temperature measurements taken in the transect closest to a small stream flowing into the western tip of the cove. All other temperature measurement location in the discharge cove (○) are assumed to be affected by the thermal discharge from the power plant.

and extrapolation from the material collected on the traps. The similarities in emergence patterns between the coves are more evident than the differences. For instance, the first major emergence (one consisting of more than a few scattered individuals) occurred in late June in 1976, 1977 and 1978 in all coves. The emergences throughout the season were also highly synchronized between coves in both years with a few exceptions. Emergences were considered to be synchronous if there was any overlap in the presence of adults. Since *Hexagenia* males frequently survive two to three days after emergence, a difference in emergence of up to two days was considered synchronous.

Differences in emergence patterns between the coves can be demonstrated. In 1976, the early emergence of *Hexagenia* on May 27, 1976 in the discharge cove, was reported by a co-worker who had been in the area on that date. He did not observe flying mayflies at any other location in the vicinity of the Kingston Steam Plant; however, the intake and reference coves were not specifically checked. A survey of local residents produced no evidence for any emergence in the intake and reference coves on that date. In 1977 sticky
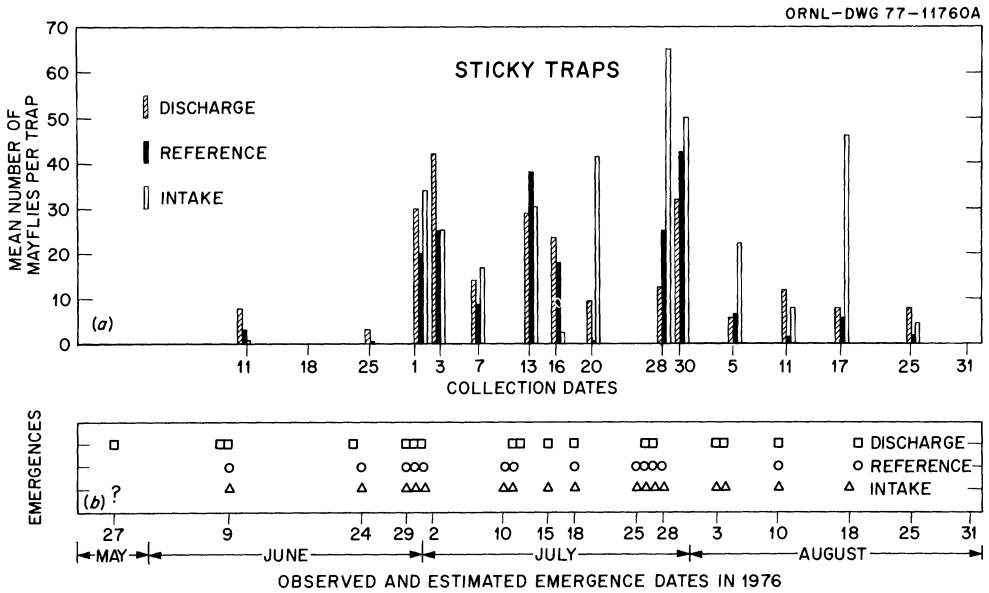
Fig. 4. Mean numbers of mayflies collected per sticky trap in three coves on Watts Bar Lake in 1976 (a) and pattern of emergence events in 1976 (b). Sticky traps were placed on June 9, 1976. A small emergence observed on May 27 was believed to occur only in the discharge cove. (Modified from Mattice & Dye 1978.)
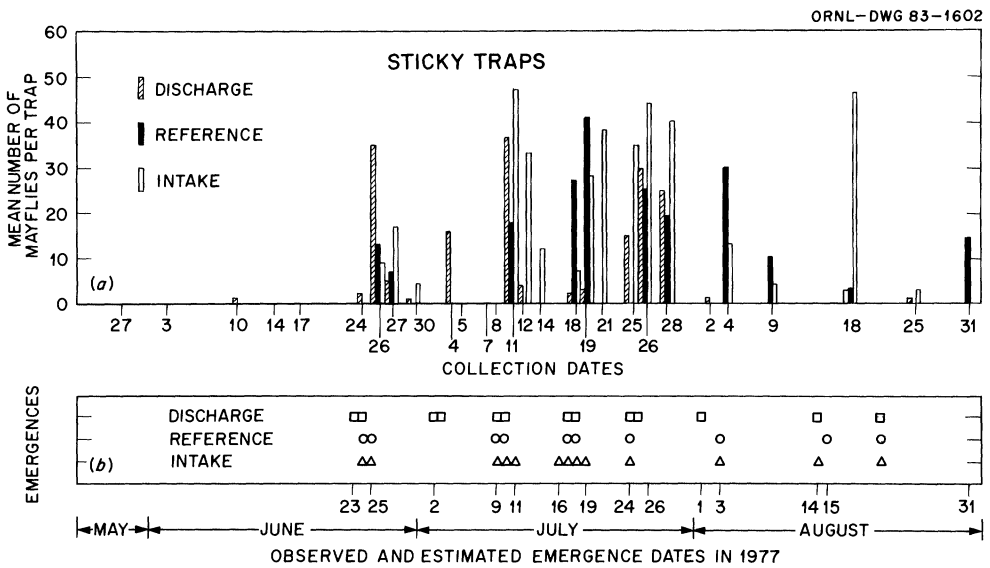

Fig. 5. Mean numbers of mayflies collected per sticky trap in three coves on Watts Bar Lake in 1977 (a) and pattern of emergence events in 1977 based on direct observation and sticky trap collections (b). Sticky traps were placed on May 1, 1977, but no emergence was detected until June 10 in the discharge cove.

traps were set on May 1 to specifically test for early emergence in the discharge cove. *Hexagenia* were first found in small numbers on traps in the discharge cove on June 10. No *Hexagenia* were collected in the intake and reference coves prior to the first synchronous emergence (June 23-25). In 1978, there was no indication of an early emergence in the discharge cove, however, fewer sticky traps had been placed.

Although emergences were generally synchronized in all three coves, there were differences in the relative proportion of the population in each cove that emerged during each emergence. This is best shown by comparing the cumulative percent of the total number of mayflies collected on sticky traps over time (Figs. 6a and b). Statistical analyses of differences in the distributions of the cumulative percent emergence between coves are summarized in Table 2. The 1977 data confirmed the 1976 data which indicated earlier emergence in the discharge cove than in the intake or reference coves. However, the 1977 data contradicted the 1976 indication that the reference cove represented an in-between emergence pattern corresponding to the in-between thermal regime.
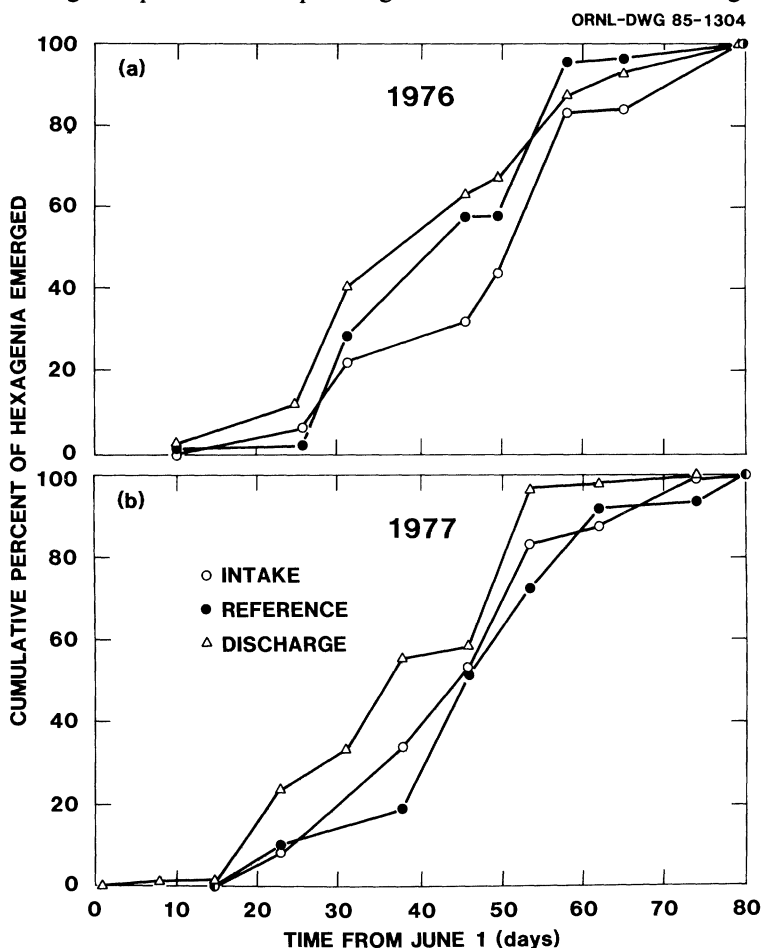
ORNL-DWG 85-1304

Fig. 6. (a) and (b). Cumulative percent emergence in each of the coves as a function of time from June 1 in 1976 and 1977. (The 1976 graph (6a) is modified from Mattice & Dye, 1978.) In 1976 the tendency toward early emergence paralled the relationship of mean monthly temperatures in the three coves (discharge > reference > intake). In 1977 the tendency toward earlier emergence in the warmer (discharge) cove was continued, however, the relative emergence patterns in the intake and reference coves differed between the two years. Since no temperatures were measured in the reference cove in 1977, it is not known whether the pattern change is temperature related.

TABLE II

Comparison of cumulative percent emergence using the Kolmogorov-Smirnov two-sample nonparametric test.

Comparison	1976		1977	
	Relationship	Significance level	Relationship	Significance level
D vs I	D >I[1]	p <0.001	D >I	p <0.001
D vs R	D >R	0.05 < p <0.10	D >R	p <0.001
I vs R	R >I	p <0.001	I >R	0.001 < p <0.01

[1]The symbol '>' means 'earlier than'.

Degree-Day Differences

Based on the daily temperature information and the dates of first emergence in each cove, monthly estimates of degree-days available for mayfly growth were made for the intake cove and the shallow bottom area above the thermocline in the discharge cove (Table 3). Only temperatures above 10° C were used to calculate degree-days based on the threshold temperature for growth estimated from the laboratory experiments (Wright, Mattice & Beauchamp 1982). The total degree-days occurring in the intervals between onset of emergence in 1976-1977, and 1977-1978 were 2322 and 2192 in the intake cove and 3881 and 3771 in the discharge cove. Thus, in either year there were approximately 1500 to 1600 more degree-days available for growth in portions of the discharge cove than for the intake cove. The calculated number of degree-days occurring between the onset of emergence in the 1976-77 period and the 1977-78 period differed by 130 in the intake cove and 110 between years in the discharge cove.

DISCUSSION

Emergence patterns differed between the discharge and intake coves in 1976 and 1977. One difference observed was that onset of emergence began two weeks earlier in the discharge cove than in the intake cove. A second difference was that a greater proportion of *Hexagenia* emerged during the early part of the summer in the discharge cove. However, these differences were small compared to the maximum degree-day differences that existed between the two coves.

Estimated degree-day differences represent only the differences in the vicinity of the single Ryan temperature recorder in each cove rather than the average cove-wide differences. Although the intake cove was very homogeneous with respect to bottom temperatures, the discharge cove was found to be very heterogeneous. This may be characteristic of water bodies receiving thermal discharge from a power plant. In a paper summarizing the biological effects of thermal discharges on benthos, Voigtlander (1980) noted that the extent of effects is often limited by the behavior of the thermal plume; modern discharge designs tend to force the plume towards the surface, thus minimizing contact of the warmest effluent with the substrate. Therefore, a single temperature recorder in a field location is insufficient to characterize thermal regime even within a small cove.

There is an additional complicating factor to be considered. Even if the temperature measurements collected are representative of one-third of the discharge cove's bottom area, it is not possible to know what proportion of the mayfly population in the cove may have experienced those tempertures. We cannot assume that the nymphs reside in one location at all times. There is some information to suggest that *Hexagenia* nymphs do, at

TABLE III

Total number of degree-days above 10° C found per month in the intake and discharge coves.

Year	Month	Intake		Discharge[1]	
		Monthly degree-days	Between emergences degree-days[2]	Monthly degree-days	Between emergences degree-days[2]
1976	JAN	0		5.5	
	FEB	2.5		105.4	
	MAR	27.8		209.7	
	APR	177.8		312.1	
	MAY	298.8		412.3	
	JUN	371.5		500.0	
	JUL	368.7		577.3	
	AUG	372.6		560.3	
	SEP	347.5		500.5	
	OCT	258.8		407.0	
	NOV	43.8		214.0	
	DEC	0	2322	68.1	3881
1977	JAN	0		0.9	
	FEB	0		16.5	
	MAR	35.8		182.8	
	APR	114.8		251.0	
	MAY	262.3		405.3	
	JUN	317.6		457.0	
	JUL	421.3		565.3	
	AUG	377.1		565.0	
	SEP	404.4		532.6	
	OCT	212.5		384.0	
	NOV	80.4		241.9	
	DEC	4.0	2192	63.4	3771
1978	JAN	0		0.3	
	FEB	0		0	
	MAR	14.3		98.6	
	APR	193.4		321.8	
	MAY	226.9		369.5	
	JUN	386.0		502.4	

[1] This information applies only to that portion of the discharge cove which is above the 3-5 m depth contour and outside of the area affected by the cool stream.
[2] The first observed emergence of each year in the intake cove began June 9 in 1976, June 24 in 1977, and June 19 in 1978.
[3] The first observed emergence of each year in the discharge cove began May 27 in 1976, June 10 in 1977, and June 20, in 1978.

least during some portions of the year, migrate and swim throughout the water column (Hudson & Swanson 1972; Mundie 1959; Swanson 1967; Hunt 1953). Regardless of these limitations and uncertainties, it still seems evident that at least some of the mayflies in the discharge cove should have experienced considerably more degree-days than any mayflies in the intake cove. Thus, some alternative hypotheses concerning effects of temperature on onset of emergence in the laboratory can be evaluated against the field observations.

The first hypothesis we are evaluating is that degree-day accumulation is a primary factor determining onset of emergence. The linear relationship observed in the laboratory between development rate and temperature would tend to suggest that degree-day accumulation is a viable hypothesis. We would strongly support this hypothesis if our field observations had been made only in the intake cove. The degree-days accumulated in the intake cove during the 12 month intervals between onset of emergence in 1976-1977, and 1977-1978 of 2322 and 2192 were both well within the 95% confidence limits of the

minimum degree-day requirement (2045-2727) estimated by our laboratory experiments. However, predictions based on the minimum degree-days required for development would indicate that eggs laid in the discharge cove in June would emerge as adults approximately four months later (in October).

Sticky traps were not sampled beyond October 1, 1976, or September 10, 1977, to test for the possibility of fall emergence; however, nymphal sampling was continued throughout the year. While large nymphs were present during all months, nymphs with the characteristic pre-emergent dark wing pads were collected only in the months of April, May, June, July and August (Wright & Mattice, unpubl. data). Neither our relatively frequent fall and winter visits to the discharge cove during sampling of nymphs over 2-1/2 years nor that of other Oak Ridge National Laboratory staff investigating fish populations produced any evidence of fall or winter emergences. Thus the pattern of emergence observed in the discharge cove demonstrates that degree-day accumulation is not the primary factor determining onset of emergence.

The second hypothesis we are evaluating is that a specific water temperature is the key to onset of emergence. This hypothesis assumes that a critical temperature threshold is reached during a period of rising temperatures that triggers the development of adult tissue in both small and large nymphs at the same time and results in a synchronous onset of emergence. A good example and discussion of the effect of a critical temperature threshold on emergence and adult size in a mayfly population is provided in Vannote and Sweeny (1980). Our observations on *H. bilineata* nymphs reared in laboratory tests led us to conclude that the existence of a temperature threshold for maturation and metamorphosis between 15 and 20°C was very probable (Wright & Mattice 1981a). Fremling's (1973) report that emergence of *H. bilineata* would not occur in the laboratory at temperatures <19°C further suggests that a critical thermal threshold may be controlling onset of emergence. Under field conditions with temperatures rapidly rising at a similar rate in both coves, temperatures in the expected critical threshold range (15 to 20°C) were reached four to eight weeks earlier in the discharge cove than in the intake cove. Clear support for the hypothesis would have been demonstrated by similar water temperatures in both coves at onset of emergence or by a four- to eight-week advances in onset of emergence in the discharge. This was not observed. Water temperatures at onset of emergence were within 0.1 of 24.1°C in the discharge cove in both years and within 0.2 of 21.5°C in the intake cove in both years. Onset of emergence in 1976 and 1977 was separated in the two coves by about two weeks. Thus, the hypothesis of a critical temperature threshold as a key determinant of onset of emergence cannot be strongly supported by our field data; however, it is a better predictor than degree-days.

A third hypothesis being tested by our data is that air temperature may have some effect on onset of emergence. Langford (1975) speculated that laboratory experiments may provide optimum conditions for early onsets of emergence such as 'correct' air conditions, abundance of food and stable flows, which do not represent natural conditions in river systems in early spring. Our tests of air temperature effects performed in the laboratory on late instar nymphs suggest that air temperature *per se* is irrelevant to onset of emergence. The primary effect of air temperature was to vary the amount of time the subimagoes and imagoes survived after emergence. Similar observations on the relationship of air temperature to length of survival in the subimago stage have been made by Lyman (1944) and Humpesch (1971). Our observation regarding the effect of laboratory air temperatures on emergence has two implications, the first being that the speculation that mayfly nymphs may be able to detect and reject unfavorable air temperatures seems unfounded. The second is that emergence from Watts Bar Lake could occur earlier in the spring and later in the fall than is normal without having a detrimental effect on subimago or imago survival.

While none of the hypotheses evaluated provide a good prediction of onset of emergence under the thermal regimes observed in each cove, the laboratory observations were sufficiently replicated to demonstrate clearly that thermal factors do affect development rate and emergence success. Based on circumstantial evidence, it seems reasonable to predict that degree-days may control development up to a certain point, but development is stopped until a particular temperature key is reached. The fact that nymphs in the discharge cove did not develop to the adult stage during early fall suggests that rising temperatures may be necessary. Once development is restarted, there may be factors other than just time or degree-days that determine onset of emergence, e.g., nutrition or photoperiod. Since emergence is not size-dependent above a minimum size of about 15 mm for females (Wright & Mattice 1981a), it would appear that nutritional factors are not of major importance in determining onset of emergence. Photoperiod appears to be relatively unimportant to mayflies based on a review of 30 to 40 papers relating to mayfly or aquatic insect larval development and emergence. However, photoperiod may deserve further consideration since the interactive effect of photoperiod and temperature on larval development of Odonates has been well documented (Ingram 1976; Lutz 1968; Procter 1973; Trottier 1971). It is clear that further experimentation will be necessary to fully explain the factors that determine onset of emergence. It would be desirable to conduct laboratory experiments under a varying temperature regime and incorporate photoperiod as an experimental variable.

The findings of this paper are summarized as follows. The thermal conditions in our field sites were homogeneous in the intake cove and spatially heterogeneous in the discharge cove with a portion of the discharge cove providing an environment with 1500 to 1600 additional degree-days for development. Our laboratory tests on *Hexagenia bilineata* indicated strongly that a minimum number of degree-days are required to complete development. While this was not sufficient information for predicting the timing of onset of emergence under altered thermal conditions in the field, the laboratory-derived information did predict the approximate interval between onset of emergence under normal thermal conditions (i.e., the intake cove). Laboratory studies also indicated that a critical temperature threshold might be involved. Predictability of onset of emergence under the altered thermal conditions in the field was improved but not fully explained by assuming that a critical temperature threshold was required to complete development. Our series of laboratory tests (Wright & Mattice 1981a; Wright, Mattice & Beauchamp 1982; and this paper) were not exhaustive but with each test more information relevant to understanding the factors affecting growth and emergence of *H. bilineata* became apparent. It is our conclusion that prediction of thermal effects is achievable with carefully integrated field and laboratory studies, but the level of detail of the studies must be much greater than is normally undertaken.

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