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Effects of oxygen deficiency on survival and glycogen content of *Chironomus anthracinus* (Diptera, Chironomidae) under laboratory and field conditions

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Abstract

Growth and glycogen content of *Chironomus anthracinus* in Lake Esrom, Denmark was examined during summer stratification in 1992 and 1993. Simultaneously, effects of oxygen deficiency on glycogen utilization and survival were experimentally studied. The population consisted of almost fullgrown 4th instar larvae in 1992 and 2nd and 3rd instar larvae in 1993. Growth rate and glycogen content changed as hypolimnetic oxygen deficiency increased. During a 1st phase of stratification dry weight and glycogen content increased (2nd and 3rd instars) or was almost constant (4th instar) but decreased significantly during the following 2nd phase. This change from growth to degrowth and utilization of endogenous glycogen reserves correlated with a change in the thickness of the microxic layer (<0.2 mg O_2 l^{-1}) above the sediment surface. The layer increased from 2–3 m in phase 1 to 4–5 m in phase 2, and we suggest that this deteriorated the oxygen conditions and resulted in a change in larval energy metabolism from fully aerobic during the 1st phase to partly anaerobic in the 2nd phase. During the 2nd phase larval metabolism was estimated at less than 20% of normoxic rate. Experimental exposure of the larvae to anoxia indicated highly different survival of young larvae (2nd and 3rd instars) and older larvae (large 4th instars). The mortality of young larvae was 50% after three days in anoxia at 10 °C, whereas only 25% of the older larvae had died after 3–4 weeks under similar conditions. Extending the treatment, however, resulted in increased death rate of the 4th instar larvae with only 10% surviving after seven weeks. The anaerobic metabolism of 4th instar larvae as estimated from glycogen degradation at 10 °C was 5% of normoxia in the interval from 0–5 days but 1.5% in the interval from 20–25 days. It is concluded that survival of *C. anthracinus* in anoxia is very limited, but traces of oxygen in the environment allowing for faint aerobic metabolism prolong the survival time of the larvae from a few days (2nd and 3rd instars) or a few weeks (4th instar) to probably 3–4 months.

Introduction

The profundal fauna of Lake Esrom, Denmark is exposed to low oxygen concentrations for two or more months each year during the summer stratification. Four macrofaunal species are permanently present in the bottom sediments, the midge, *Chironomus anthracinus* Zetterstedt, the tubificid, *Potamothrix hammoniensis* Michaelsen and two species of pea mussels, *Pisidium* spp.

The population dynamics of the dominant insect species *C. anthracinus* is well described (e.g. Jónasson, 1972; Lindegaard *et al.*, 1993). Its life cycle is either annual or biennial. During the summer of 1992 the population consisted of almost fullgrown 4th instar larvae in their second year of life. This population emerged during spring 1993 and was replaced by a new generation, which during the summer of 1993 was composed of 2nd and 3rd instar larvae.

The ability of many invertebrates to survive low oxygen concentrations or even anoxia has been correlated with the production of large glycogen stores which may be converted during anaerobic metabolism to ethanol, lactic acid or other organic endproducts (e.g. Augenfeld, 1967; Frank, 1983; Wilps & Zebe, 1976). Previous studies have shown that *C. anthraci-*

nus can regulate its respiratory metabolism and thereby maintain almost full activity down to a concentration of 2–3 mg O_2 l^{-1}. Short term studies have shown that the respiratory metabolism declines sharply below this level but is accompanied by increased anaerobic degradation of glycogen. The compensation for the loss in aerobic metabolism by anaerobic processes, however, is extremely small (Berg *et al.*, 1962; Bairlein, 1989; Hamburger *et al.*, 1994).

This paper presents temporal changes in dry weight and glycogen content of 2nd, 3rd and 4th instar larvae of *C. anthracinus* during summer stratification and compares these results with the effects of experimental anoxia on survival and rate of glycogen utilization.

Materials and methods

Lake Esrom is situated 30 km north of Copenhagen. It has an area of 17.3 km^2 and a maximum depth of 22 m. The profundal zone (>10 m) covers 11 km^2. Detailed descriptions of the lake and its surroundings are given by Berg (1938), Jónasson (1972) and Lindegaard *et al.* (1993). Sampling took place at 21 m depth in the northern part of the lake (station 7 in Jónasson, 1972) in two years during summer stratification, *i.e.* from June 9th to October 8th, 1992, and from July 5th to September 21st, 1993. Temperature and oxygen profiles were measured *in situ* at each sampling date with an YSI oxygen-meter with a resolution of 0.1 °C and 0.1 mg O_2 l^{-1}, respectively.

Larval samples were collected with an Ekman bottom sampler (225 cm^2), sieved with a mesh size of 500 μm and transported back to the laboratory. Within two hours after the sieving, individuals of *C. anthracinus* were sorted out according to instar and size, washed in a proper medium and immediately transferred to experimental flasks or placed in a drying oven (60 °C) for later analyses.

The effect of anoxia on the survival time and glycogen content of *C. anthracinus* was investigated in laboratory experiments. Batches of 200, 100 and 50 individuals, equal to 5, 5 and 100 mg dry weight of 2nd, 3rd and 4th instar larvae, respectively, were placed in 1-litre glass bottles (area of bottom surface = 50 cm^2). The resulting density of larvae are within the range found for populations of *C. anthracinus* in the lake (Jónasson, 1972). The medium in the flasks was 0.45 μm filtered lake water, gassed with nitrogen (99.9% N_2) to reduce the oxygen concentration to initially <0.15 mg O_2 l^{-1}. The bottles were sealed to prevent diffusion of oxygen into the water and placed in thermostat at 5, 9 or 15 °C in darkness. Initial and final oxygen concentrations were measured with an oxygen electrode (Radiometer, Denmark; resolution = 1 mm Hg).

Dry weight (DW) was determined after drying larvae to constant weight at 60 °C for 24 hours. Ash free dry weight (AFDW) was determined after ignition of dried larvae at 550 °C for one hour.

The glycogen content was determined by digestion of dried samples of larvae (3–10 replicates) in 30% KOH for two hours in a boiling water bath. Glycogen was precipitated by the addition of ethanol (96%), concentrated by centrifugation after storage for 24 hours at 5 °C and hydrolysed in one hour in a boiling water bath in 1 N HCl (Augenfeld, 1967). The glucose content was assayed enzymatically (Glucose (HK) Sigma Diagnostics). According to instar and size each sample comprised 10–200 larvae (≈ 5–20 mg DW). The glycogen content was expressed as mg ind^{-1} or as percent of larval dry weight.

Nitrogen concentrations were measured in a CHN-analyzer (E.A. 1108, Carlo Ebra Instr.) on samples of 2–10 mg dried larvae. We assumed 16% N in protein in order to estimate larval content of protein (Gordon, 1972). Lipid content was estimated as total dry weight minus the sum of protein, glycogen and ash.

The following conversion factors were used: 18.83 kJ (4.5 kcal) g^{-1} protein, 16.74 kJ (4.0 kcal) g^{-1} glycogen, 39.75 kJ (9.5 kcal) g^{-1} lipid, and 20.19 kJ (4.825 kcal) l^{-1} O_2 (Gordon, 1972), 226 kJ (54 kcal) mol^{-1} glycosyl-unit (mol wt 162) in anaerobic combustion (Gnaiger, 1991).

Results

Field studies

Stable thermal stratification in 1992 was established in 6 m depth by the end of May and lasted five months until the complete overturn in mid October (Fig. 1A). The profundal temperature increased from 8 to 10 °C during this period. The decline in oxygen content in the profundal zone followed few days after stratification and the microxic period (<0.2 mg O_2 l^{-1}) lasted four months (Fig. 1B). In 1993 a stable thermocline was established in 7 m depth by early May and it lasted almost five months until the overturn by the end of September (Fig. 2A). During this period the profundal temperature increased from 6 to 9 °C. The microxic

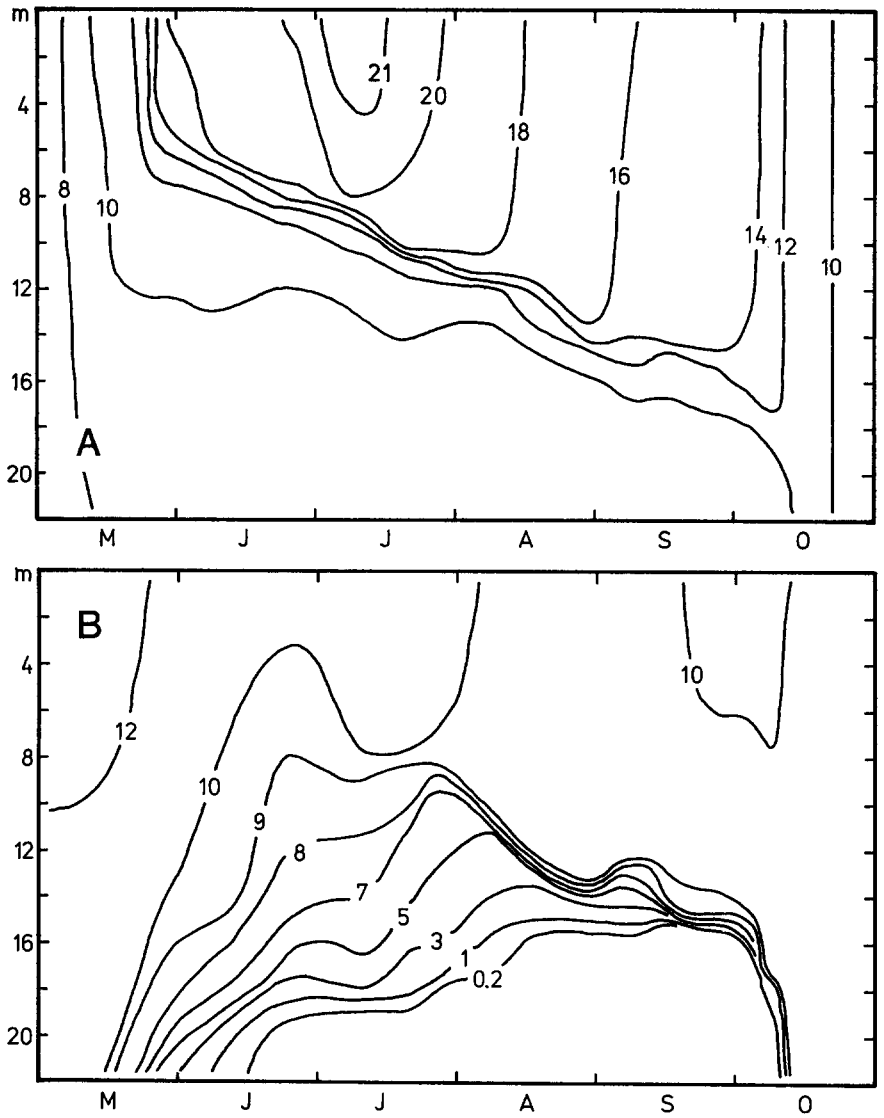

Fig. 1. (A) Temperature (°C) and (B) oxygen (mg O_2 l^{-1}) isopleths for Lake Esrom during the period from May to October 1992.

period lasted from early July to mid September, thus slightly longer than 2 months (Fig. 2B).

During the summer stratification in 1993 the *C. anthracinus* population comprised one cohort which had settled in late April. Young larvae were observed in the samples by the end of June. From July 5th to the overturn in September two thirds of the larval population were in the 2nd instar and one third in the 3rd instar. At July 5th all larvae had full guts, but by August 3rd most of the larvae had empty guts, which suggested that the larvae did not feed from early August until the overturn in late September.

Dry weight of 2nd instar larvae increased from July 5th to August 12th, 1993, but decreased during the following period until the overturn by the end of September when growth was resumed (Fig. 3A). During the first period the glycogen concentration was almost constant but decreased during the second period. The change from growth to degrowth in dry weight and the decline in the concentration of body glycogen reserves coincided with an increase in the thickness of the microxic zone of the hypolimnion. From mid July 1993 until the beginning of August we found less than 0.2 mg O_2 l^{-1} in the water column 2–3 m above the

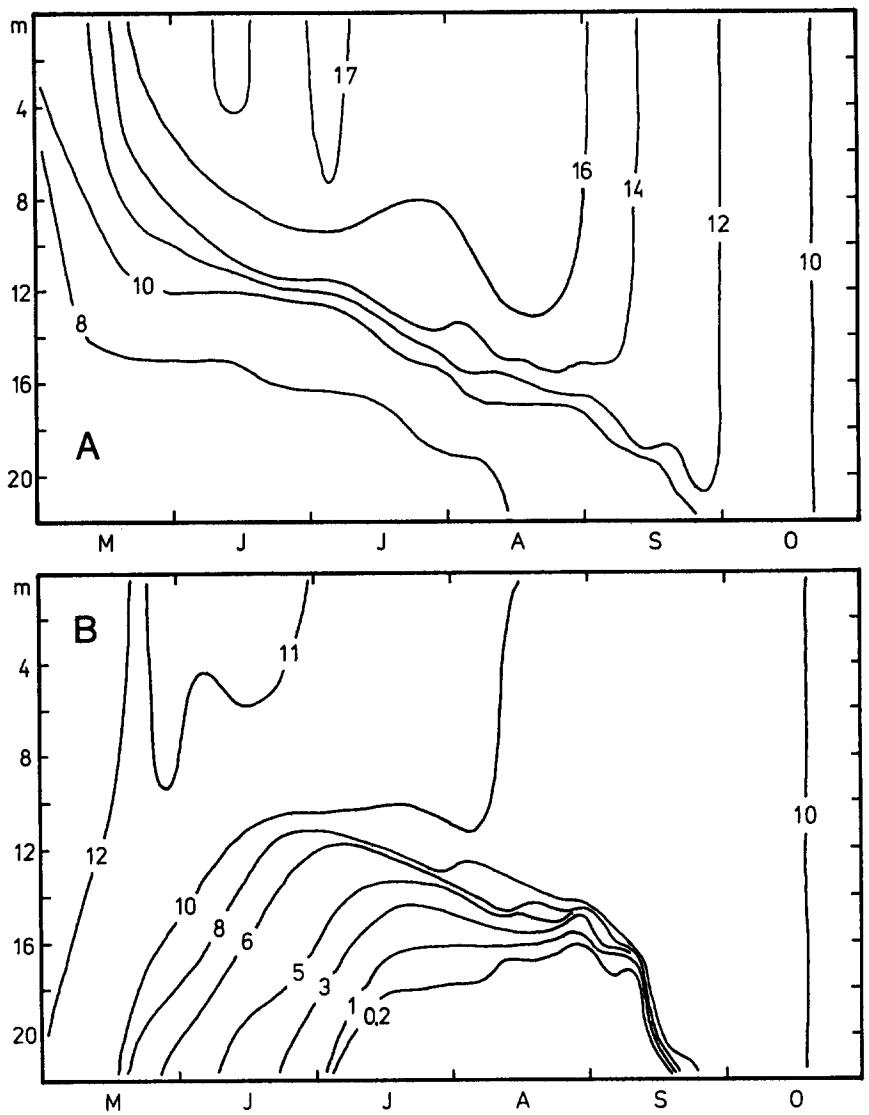

Fig. 2. Isopleths representing temperature and oxygen during the summer of 1993. Legend otherwise as in Fig. 1.

sediment surface. During the following two weeks this zone increased to 4–5 m and remained here until the beginning of September. Although we are unable to demonstrate a decline in the availability of oxygen to the larvae, we suggest that the metabolism of the larvae changed from fully aerobic to partially anaerobic within this interval (hatched area in Fig. 3A). Therefore, we distinguish two phases during the microxic period in 1993: a 1st phase between the sampling dates July 5th and August 12th when the larvae were able to maintain aerobic metabolism, which allowed slight growth, and a 2nd phase from August 12th to the overturn (September 26th) with partially anaerobic metabolism and degrowth of the larvae. The 3rd instar larvae showed almost identical trends in dry weight and glycogen changes (Fig. 3B).

During the summer stratification in 1992 the population of *C. anthracinus* consisted of large 4th instar larvae, which had settled in the spring of 1991 and of which approximately half the population had emerged during April 1992. The remaining larvae were almost fullgrown when collected during June–October 1992. Most of the larvae had some material in their gut at June 9th; all larvae, however, had empty guts by June 15th. Thus the food uptake apparently ceased within this period and was not resumed until after the

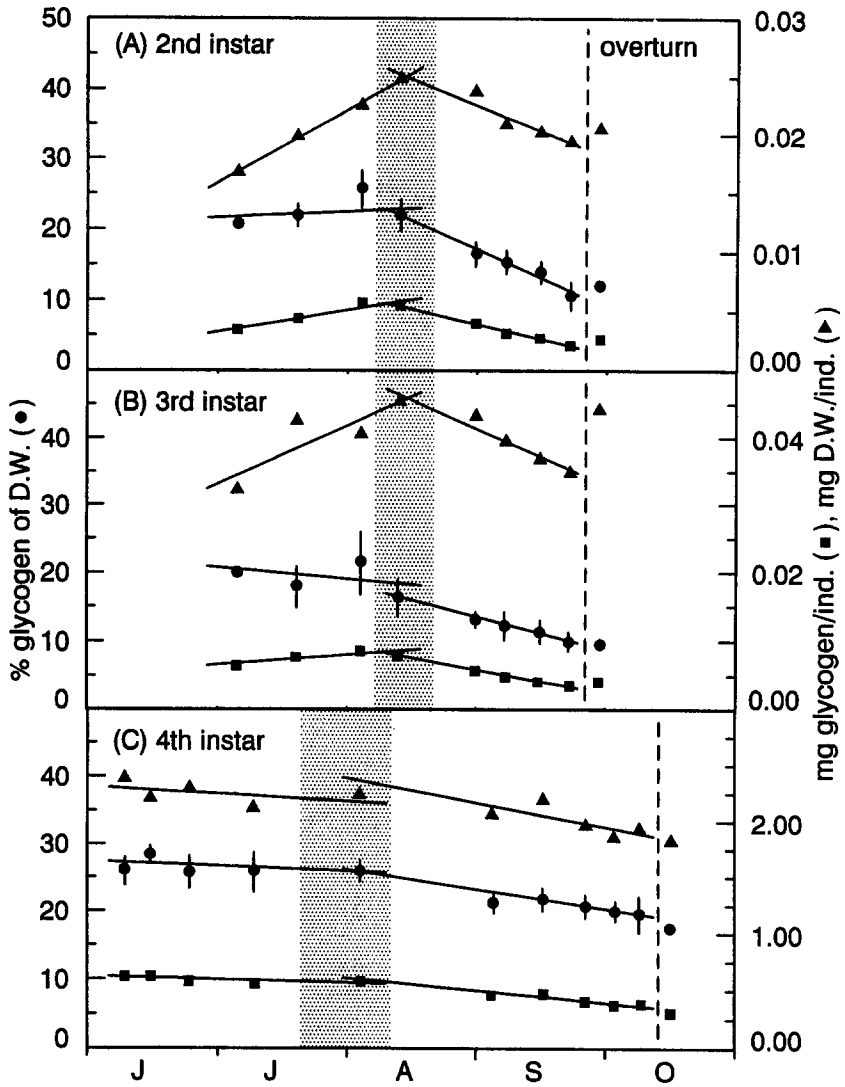

Fig. 3. Glycogen concentration in percent of dry weight (•), dry weight content (▲), and calculated glycogen content (■) of 2nd (A), 3rd (B) and 4th (C) instar larvae of *Chironomus anthracinus* during the periods with hypolimnetic microxia in 1992 (4th instar) and 1993 (2nd & 3rd instar). Hatched areas show periods of increase in thickness of microxic layers. Stippled lines indicate time of autumn overturn. Vertical bars represent standard deviation.

overturn in October. Similar, although less pronounced changes in dry weight and glycogen concentration than found in younger larvae, were observed for 4th instar larvae during the period with hypolimnetic microxia. From June 9th to August 3rd the dry weight content and glycogen concentration of the larvae were almost constant but declined faster thereafter. As observed for the population in 1993, the increase in the utilization of the body energy reserves coincided with an increase of the microxic zone with <0.2 mg O_2 l^{-1} from 2–3 to 4–5 m above the sediment in the period from about July 20th to August 10th. A 1st phase from June 9th to August 3rd with aerobic metabolism of the larvae was therefore distinguished from a 2nd phase with partially anaerobic metabolism lasting from August 3rd to October 9th, 1992 (Fig. 3C).

Linear regressions were calculated for the three parameters, dry weight and glycogen content per individual and percent glycogen of dry weight, in the 1st and 2nd phase (Fig. 3A, B and C), with constants given in Table 1. Except for decline in dry weight of 4th instar larvae, significant differences between 1st and 2nd phase in slopes and/or intercepts in dry weight, glycogen concentration and glycogen content

Table 1. Regressions (Y = A + bX) and number of replicates (n) for individual dry weight (mg DW), individual glycogen content (mg gly.), and glycogen content in % of DW (% gly.) of *Chironomus anthracinus* during the 1st and 2nd phase of the microxic period. Regressions are shown in Fig. 3.

		1st phase			2nd phase		
Instar	Parameter	b (10^{-3})	A	n	b (10^{-3})	A	n
2nd	mg DW	0.2083	0.0168	4	−0.1491	0.0253	5
–	mg gly.	0.0598	0.0036	4	−0.0836	0.0054	5
–	% gly.	27.7699	21.7007	20	−266.4501	21.9137	28
3rd	mg DW	0.2874	0.0344	4	−0.2777	0.0464	5
–	mg gly.	0.0468	0.0068	4	−0.1127	0.0079	5
–	% gly.	55.8659	20.4686	17	−156.9753	16.4021	23
4th	mg DW	−2.0742	2.2894	5	−5.0795	2.2588	6
–	mg gly.	−0.9366	0.6161	5	−3.1304	0.5804	6
–	% gly.	−24.1126	27.2018	41	−95.1640	25.8221	60

were found (Table 2). All slopes, however, differed significantly from zero during the 2nd phase.

Our observations indicate that the feeding activity of the larvae declined during the 1st phase and was fully abolished during the 2nd phase in both years. It is therefore suggested that the energy, necessary to maintain life processes within the 2nd phase was of purely endogenous origin. Consequently, an estimate of the energy metabolism can be made from the loss in larval dry weight and change in glycogen concentration during the 2nd phase.

These calculations, however, require estimates of the mean composition and energy content of the organic compounds (protein, lipid and carbohydrate (= glycogen)) of the larvae. The nitrogen and ash contents of the three larval instars were measured on several dates during the 2nd phase with the results and mean values indicated in Table 3. The mean glycogen concentration and mean dry weight were calculated from the regression equations shown in Table 1. From these measurements and calculations the organic composition and energy content (kJ g^{-1} DW (AFDW)) of each instar was estimated (Table 4).

The ash content of the 2nd and 3rd instar larvae remained relatively constant during the 2nd phase, whereas the three major classes of organic compounds *i.e.* glycogen, protein and lipid were partly decomposed. In the following calculations of the energy metabolism it was assumed that the daily loss in dry weight represented organic dry weight (AFDW). The loss was divided into one part, which was broken down aerobically in proportion to the organic composition of the larvae, and a second part represented by the 'extra' glycogen loss, which was broken down anaerobically.

Table 2. Test of regressions (t-test) representing 1st and 2nd phase, and test of significance of slopes during the 2nd phase of the microxic period. Regressions are shown in Table 1 and Fig. 3.

Instar	Parameter	slopes (b) *P*	intercepts (A)	slope = 0 *P*
2nd	mg DW	<0.001		<0.01
–	mg gly.	<0.001		<0.001
–	% gly.	<0.001		<0.001
3rd	mg DW	<0.01		<0.005
–	mg gly.	<0.001		<0.001
–	% gly.	>0.05*	<0.001	<0.001
4th	mg DW	>0.05*	>0.05*	=0.05
–	mg gly.	<0.05		<0.01
–	% gly.	<0.001		<0.001

* Slopes or intercepts not significantly different.

During the 2nd phase of the microxic period the 2nd instar larvae lost 0.1491 μg DW d^{-1} ($\approx$ AFDW) of which 0.0836 μg was glycogen (Table 4). Aerobic degradation of protein and lipid thus was 0.0655 μg. Together these two components constituted 76.45% of

Table 3. Percentage nitrogen and ash content of dry weight in *Chironomus anthracinus* during stratification in 1992 and 1993.

Instar	Date		%	S.D.	n	Mean
Nitrogen						
2nd	Aug.	16th	8.99	0.13	5	9.39
	Sept.	16th	9.76	0.06	6	
3rd	Aug.	16th	9.34	0.13	4	9.80
	Sept.	16th	10.26	0.14	4	
4th	Aug.	3rd	8.07	0.74	15	8.00
	Sept.	15th	7.89	0.26	7	
Ash						
2nd	Aug.	5th	5.62	0.16	6	
	Sept.	9th	7.16	0.55	9	7.20
	Sept.	28th	9.88	0.66	7	
3rd	Aug.	5th	7.36	0.55	11	
	Sept.	9th	8.17	0.52	7	8.42
	Sept.	28th	10.48	1.25	7	
4th	Sept.	5th	5.45	0.61	16	5.19
	Sept.	19th	4.88	0.87	13	

larval DW. The corresponding aerobic degradation of glycogen (16.35% of DW) was (0.0655/76.45) × 16.35 or 0.0140 μ g glycogen ind^{-1} d^{-1}. Total aerobic combustion therefore was 0.0795 μg DW ($\approx$ AFDW) ind^{-1} d^{-1} (= 0.164 μg AFDW mg^{-1} AFDW h^{-1}) and the energy metabolism equalled 3.6847 10^{-6} kJ mg^{-1} AFDW h^{-1}.

The pathways of the anaerobic metabolism in *C. anthracinus* are unknown. Long-term exposure of *Chironomus plumosus* L. to anoxia lead to release of ethanol as the primary endproduct (Frank, 1983), and in the following calculation we assume that the same pathway is operating in *C. anthracinus*. Total glycogen degradation amounted to 0.0836 μg ind^{-1} d^{-1} of which 0.0140 μg was estimated as aerobic degradation, hence anaerobic energy metabolism constituted 0.0696 μg glycogen ind^{-1} d^{-1} (= 0.821 10^{-9} μmol glycocyl-units mg^{-1} DW h^{-1}) equal to 0.186 10^{-6} kJ mg^{-1} DW h^{-1} (= 0.200 10^{-6} kJ mg^{-1} AFDW h^{-1}). Therefore, total energy metabolism in the 2nd phase of the microxic period was 3.885 10^{-6} kJ mg^{-1} AFDW h^{-1}. Anaerobic energy metabolism from glycogen degradation thus made up 5.4% of total energy metabolism. Similar calculations were carried out for 3rd and 4th instar larvae. As shown in Table 4, total energy metabolism in 3rd instar larvae amounted to 5.079 10^{-6} kJ mg^{-1} AFDW h^{-1} of which 2.7% originated from anaerobic degradation. Corresponding values for the 4th instar larvae were 1.349 10^{-6} kJ mg^{-1} AFDW h^{-1} and 5.7%.

The field studies emphasize that the costly anaerobic metabolic pathway is of very little importance in the overall energy production of the three investigated instars of *C. anthracinus*.

Laboratory studies on the effect of anoxia on survival and glycogen content of C. anthracinus

Two series of experiments with 4th instar larvae each lasting 3–4 weeks were conducted. In the first series larvae were collected in July 1992 and incubated at 9 °C. In the second series, larvae were collected in late September 1992 and incubated at 5, 9 and 15 °C. The 2nd and 3rd instar larvae were collected in late August and late September 1993 and incubated four days at 9 °C. Thus all larvae in these experiments were collected during the period with microxic conditions in hypolimnion and therefore acclimated to low oxygen concentration. Preliminary experiments were carried out with 4th instar larvae to investigate the effect of a bottom substratum (a thin layer of lake sediment) on survival of the larvae. We found no difference in the survival time of the larvae, although the presence of sediment gave rise to an intense smell of presumably hydrogen sulphide. Therefore the sediment layer was omitted in all subsequent experiments.

Survival under anoxic conditions (<0.15 mg O_2 l^{-1}) appeared to be inversely related to temperature. Nearly 75% of the 4th instar larvae incubated at 9 °C survived 25 days (Fig. 4, curves 1 and 3). At 5 °C 80–85% survived 3 weeks (curve 2) whereas at 15 °C only 50% survived the same interval (curve 4). During the experiments the larvae were lying almost quiet on the bottom showing only slight movements. After the treatment the surviving larvae were still bright red and able to curl up immediately after transfer to aerated water. Larvae which appeared rigid and stretched did not regain motility and died within one day after transfer to aerated water.

Second and third instar larvae were much less resistant to anoxia (curve 5). After four days treatment only 20–60% of 2nd and 3rd instar larvae were alive. More than 95% of larvae kept in aerated, 0.45 μm filtered lake water survived the same interval, and after 14 days even 90% of the 2nd instar larvae and 60% of the 3rd instar larvae were still alive. This indicates that the

Table 4. Metabolic characteristics of *Chironomus anthracinus* during the microxic 2nd phase of stratification in 1993 (2nd and 3rd instar) and 1992 (4th instar). A. Mean larval dry weight, mean organic composition and larval energy content. B. Calculated daily loss in dry weight and glycogen, cf. regressions in Fig. 3. C. Estimated microxic energy metabolism, normoxic respiration and microxic energy metabolism as % of energy metabolism at normoxia.

	Instar					
	2nd		3rd		4th	
A.						
mean DW (mg ind^{-1})	0.0218		0.0400		2.0447	
% protein	58.68		61.34		50.00	
% glycogen	16.35		13.06		21.81	
% lipid	17.77		17.18		23.00	
% ash	7.20		8.42		5.19	
kJ g^{-1} DW (AFDW)	20.85	(22.47)	20.57	(22.46)	22.21	(23.43)
B.						
Daily loss						
(μg DW ind^{-1})	0.1491		0.2777		5.0795	
(μg glycogen ind^{-1})	0.0836		0.1127		3.1304	
C.						
Energy production during microxia (10^{-6} kJ mg^{-1} AFDW h^{-1})						
aerobic	3.685	(94.6%)	4.943	(97.3%)	1.273	(94.3%)
anaerobic	0.200	(5.4%)	0.136	(2.7%)	0.076	(5.7%)
total	3.885		5.079		1.349	
Respiration, normoxia (10^{-6} kJ mg^{-1} AFDW h^{-1})	29.18	(8.5 °C)	24.46	(8.5 °C)	7.93	(9.0 °C)
Energy production, microxia as % of normoxia	13.31		20.46		17.01	

mortality was determined by anoxia and that starvation was of minor importance (results not shown).

The 4th instar larvae contained 26 and 20% glycogen in the experiments initiated in July and late September, respectively (cf. Fig. 3C). Glycogen decreased throughout both experimental series, and the rate of decline was highest during the first two weeks (Fig. 5). After two weeks larvae from the first and second series kept at 9 °C (curves 1 and 3) contained 20 and 14% glycogen, respectively, after 3 weeks the concentration was down at 16 and 13%. At 5 °C (curve 2) the rate of glycogen utilization was indistinguishable from that found at 9 °C, but considerably faster at 15 °C. After three weeks at 15 °C the glycogen concentration was down at 7% of DW (curve 4). The glycogen concentration of 2nd instar larvae changed from 12.5 to 8.0% of the dry weight in four days at 9 °C (curve 5).

The curve representing 4th instar larvae collected in July and kept at 9 °C (Fig. 5, curve 1) was used to estimate glycogen contents (μg mg^{-1} larval DW) and rates of glycogen utilization (μg mg^{-1} larval DW d^{-1}) during five successive 5-days intervals (Table 5). Glycogen degradation averaged 6.6 μg mg^{-1} DW d^{-1} during the interval between 0 and 5 days and declined gradually to 2.0 μg between 20 and 25 days. The aver-

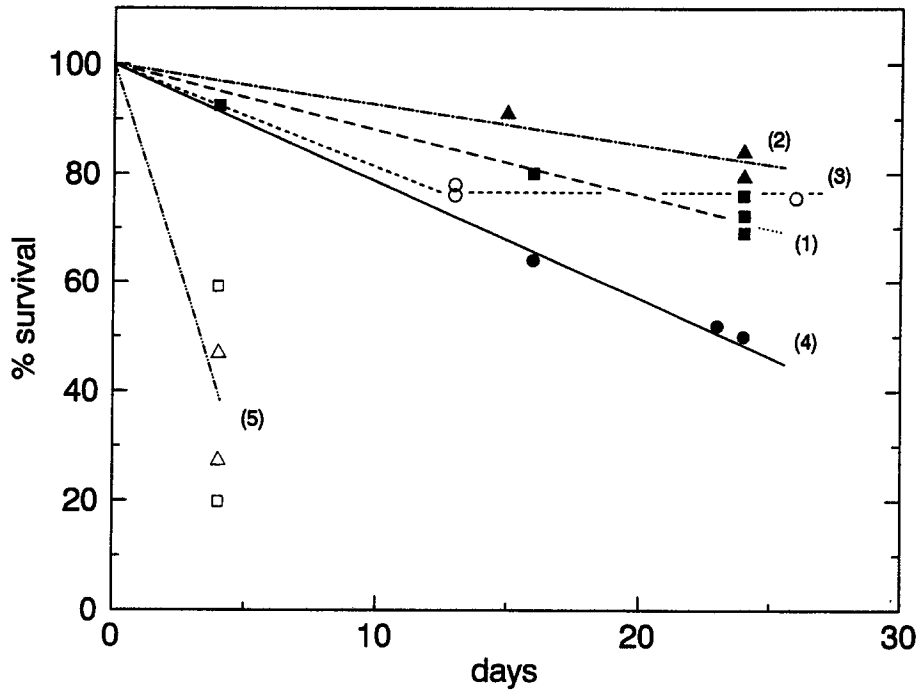

Fig. 4. Survival of *Chironomus anthracinus* under experimental anoxia. 1) to 4) represent experiments with 4th instar larvae in 1992. 1) Larvae from July, 9 °C (■); 2) Larvae from September, 5 °C (▲); 3) Larvae from September, 9 °C (○); 4) Larvae from September, 15 °C (●). 5) 2nd (□) and 3rd (△) instar larvae collected August/September 1993, 9 °C. Curves fitted by eye.

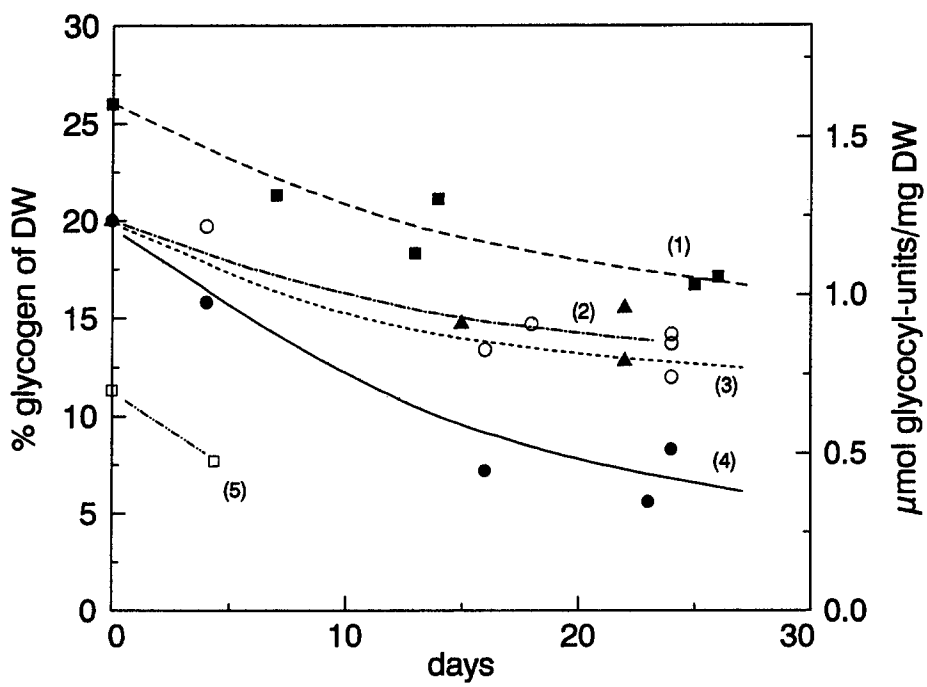

Fig. 5. Changes in the glycogen content in percent of dry weight and in 10^{-6} mol glycosyl units mg^{-1} DW of *Chironomus anthracinus* with time under experimental anoxia. Curves fitted by eye. Curve 5) represents 2nd instar larvae. Symbols otherwise as in Fig. 4.

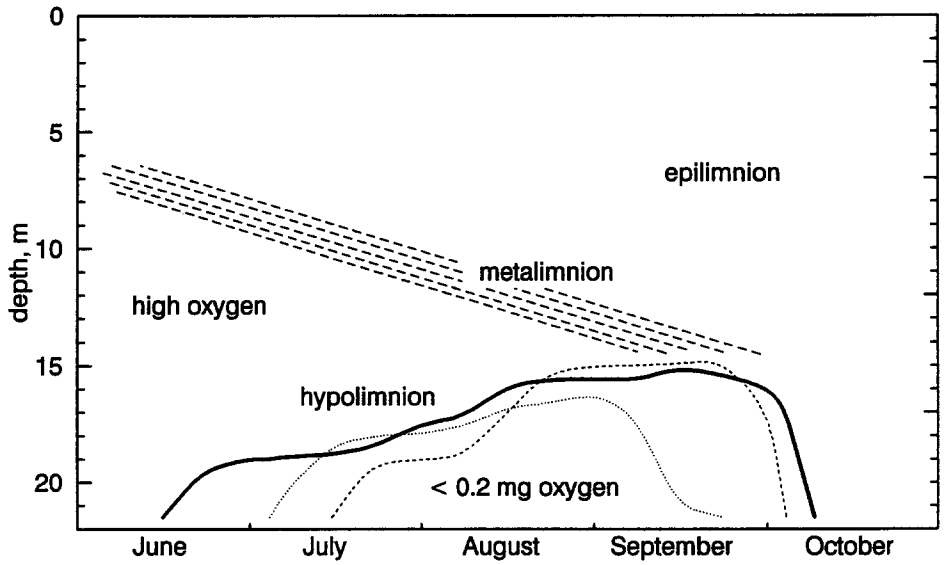

Fig. 6. Schematical presentation of oxygen conditions in the hypolimnion of Lake Esrom during summer stratification. Curves represent oxygen concentrations at 0.2 mg O_2 l^{-1}. Location of metalimnion and full drawn curve shows the situation in 1992 (cf. Fig. 1). Stippled curve and dotted curve show oxygen levels in 1991 and 1993, respectively.

age glycogen degradation in 2nd instar larvae amounted to 11.25 μg mg^{-1} DW d^{-1} during four days of exposure to anoxia (not shown).

Discussion

The results regarding energy metabolism of *Chironomus anthracinus* in the profundal zone of Lake Esrom are based on the observation that the hypolimnetic microxic period can be separated in two phases (Fig. 3). The splitting of the microxic period coincides with an increase in the microxic bottom layer of water. This development is visualised in Fig. 6, which include curves for 0.2 mg O_2 l^{-1} during three successive years together with the approximate location of the metalimnion in 1992 (cf. Fig. 1). The still deeper location of the metalimnion through the summer period gradually reduces the volume of the hypolimnion. Volumetric calculations show that the hypolimnion volume is reduced by 30% from mid June to mid July during which period the upper part of the hypolimnion is relatively oxygen rich. During the following month (mid July to mid August) the remaining volume decreases with about 40%. This increased rate of reduction of the hypolimnion could well explain the observed rather sudden increase in magnitude of the microxic bottom water. The environmental conditions at 21 m depth in the hypolimnion is thus partly related to the specific morphometry of the lake basin, which also governs the patterns of hypolimnetic circulation.

After decline of the oxygen content in the hypolimnion down to 0.5–1.0 mg O_2 l^{-1} feeding of *C. anthracinus* was abolished or markedly reduced in agreement with earlier observations (Jónasson, 1972; Heinis & Crommentuijn, 1992). The estimated energy metabolism during the microxic period is based on the assumptions that the larvae are exclusively dependent upon endogenous reserves to support energy metabolism, and that excretion was insignificant. Many soft-bodied marine and freshwater invertebrates are capable of taking up dissolved organic material (DOM) from the ambient water via epidermal transport systems (review by Gomme, 1982). Only very low levels of DOM uptake are generally found among freshwater organisms, contributing only a few percent to the basal metabolism. This supports the hypothesis that uptake of DOM is incompatible with the processes of ion- and osmoregulation in non-marine organisms. However, under laboratory conditions two freshwater invertebrates can achieve a considerable uptake of short chain, volatile carboxylic acids from the medium; in the pulmonate snail *Biomphalaria glabrata* (Say) acetate contributes about 6% (Thomas *et al.*, 1984) and in *Tubifex tubifex* (Müller) propionate can cover up to 40% of the basal metabolism (Hipp *et al.*, 1986).

Anoxic conditions, however, led to a decrease of the uptake rate in *T. tubifex* to the level of the diffusional component. Nothing is known about integumentary uptake rates in chironomids.

Daily excretion of ammonia nitrogen measured at 17 °C and normoxia was 2.4% of DW in well fed *Chironomus* sp. larvae, thus representing a considerable loss of material (Tátrai, 1982). In the marine copepod, *Acartia tonsa* (Dana), daily ammonia-nitrogen excretion was 2.5% of DW at a food concentration of 200 μg N l^{-1} but 0.5% without food algae (Kiørboe *et al.*, 1985). The effect of prolonged starvation and low oxygen concentration on the excretion in chironomids is unknown.

The energy metabolism, estimated from aerobic and anaerobic degradation of endogenous reserves, during the 2nd phase of the microxic period can be related to the energy metabolism measured at 100% air saturation (normoxia). The energy produced in the respiration of profundal *C. anthracinus* at normoxia is expressed by the equation,

$$\log_{10}(Y) = \log_{10}(19.4984(X)^{-0.2971}) - 2.083e^{-0.0286t}$$

where $(Y) = \mu l\ O_2\ mg^{-1}$ AFDW h^{-1}, (X) = mg AFDW ind^{-1} and t = temperature in °C (Hamburger *et al.*, 1994).

Considering average bottom temperature and mean dry weight of the larvae during the 2nd phase of the microxic period of stratification in 1992 and 1993 the respiration, expressed in kJ, at normoxia was calculated with the values shown in Table 4. The total energy metabolism estimated under microxic conditions in 2nd, 3rd and 4th instar larvae thus constituted 13.2, 20.5 and 17.0% of the energy metabolism at normoxia. We do not consider the difference between instars as significant, and conclude that the rate of the energy metabolism in *C. anthracinus* during the period with hypolimnetic microxia was within the range of 13–21% of the normoxic metabolic rate.

Although these estimates of the energy metabolism in *C. anthracinus* larvae at low oxygen concentrations is encumbered with some uncertainty, it falls within the range reported for other invertebrates. Measurements of invertebrate energy metabolism during dormancy indicate suppression of the metabolic rate to below 20% of normoxic rates and quite often below 5% (Hand, 1991). In *Lumbriculus variegatus* (Müller) the heat flux under microxic conditions (0.5 mg O_2 l^{-1} (20 °C)) amounts to 25% of the normoxic rate. Even at this low oxygen concentration a significant contribution, amounting to 30% of the total heat flux, is aerobic (Gnaiger, 1991). In short term experiments (24 h) with *Tubifex tubifex* the heat production under anoxic conditions amounts to 10–15% of the aerobic rate (Famme & Knudsen, 1984). The present estimate, 13–21% of normoxic rate, for *C. anthracinus* exposed to long-term microxic conditions falls within the range of other invertebrates, capable of surviving micro- or anoxic conditions.

Glycogen concentrations have not been measured earlier in *C. anthracinus*. A field study was carried out with *C. plumosus* from Lake Mendota (Augenfeld, 1967). Lake Mendota is an eutrophic lake in which a definite thermocline usually forms in the beginning of June. A marked oxygen deficit is found in the hypolimnion from about July until late October when the overturn takes place. Within this period (June 22th to October 15th) the glycogen concentration of 4th instar larvae (>30 mg WW, 87% water) dropped from 22% to 10.5% of DW in the first 5 weeks, down to 8% during the following 8 weeks and further down to 4% at the time of the overturn two weeks later. We have calculated that within these three intervals glycogen utilization was 3.3, 0.5 and 2.9 μg glycogen mg^{-1} larval DW d^{-1}, respectively, and the author estimated an average daily loss of 1.7 μg glycogen mg^{-1} DW d^{-1} for the whole period. On average, the *C. anthracinus* population of 4th instar larvae in Lake Esrom degraded 3.13 μg glycogen ind^{-1} d^{-1} (= 1.53 μg mg^{-1} DW d^{-1}) during the 2nd phase of the period with hypolimnetic microxia (Table 4). Thus, in response to severe oxygen limitation glycogen utilization was similar in these two populations of profundal chironomids.

The laboratory studies indicated that large 4th instar larvae of *C. anthracinus* were able to withstand anoxia for a period of 3–4 weeks with less than 30% mortality. Within this interval glycogen loss was 6.6 μg mg^{-1} larval DW d^{-1} during the first 5 days but decreased to 2.0 μg after 20–25 days (Table 5). The anaerobic energy metabolism calculated for the same intervals was 0.384 and 0.116 10^{-6} kJ mg^{-1} DW h^{-1}, approximately corresponding to 5.0 and 1.5% of normoxic level. Longer exposure time to anoxia resulted in further decrease in the rate of glycogen utilization and rapid deterioration of the larvae, with only 10% surviving after 7 weeks (results not shown). Although we can not exclude side effects (e.g. accumulation of ethanol) from the longterm enclosure of larvae in the flasks, the present data suggest that 4th instar larvae of *C. anthracinus* are unable to survive more than a few weeks on fully anaerobic metabolism.

Table 5. Glycogen contents of *Chironomus anthracinus* (in mg glycogen and μmol glycosyl-units mg^{-1} DW read from curve 1, Fig. 4) during exposure to experimental microxia (<0.2 mg O_2 l^{-1}) and the calculated mean daily glycogen degradation (in μg glycogen mg^{-1} DW d^{-1}) and energy production (in 10^{-6} kJ mg^{-1} DW h^{-1} and percent of normoxia).

	0 d	5 d	10 d	15 d	20 d	25 d
mg mg^{-1} DW	0.260	0.227	0.205	0.190	0.178	0.168
μmol mg^{-1} DW	1.605	1.401	1.265	1.173	1.099	1.037
μg mg^{-1} DW d^{-1}		6.6	4.4	3.0	2.4	2.0
10^{-6} kJ mg^{-1} DW h^{-1}		0.384	0.256	0.174	0.141	0.116
% of normoxia [1)]		5.0	3.3	2.3	1.8	1.5

[1)] 100% air saturation, 7.73 10^{-6} kJ mg^{-1} DW h^{-1} (Table 2)

The 2nd and 3rd instar larvae were much less resistant to anoxia than large 4th instar larvae, most likely because of higher weight-specific metabolic rate (cf. Table 4). During four days treatment with anoxia the glycogen concentration of 2nd instar larvae dropped from 12.5% to 8% of DW corresponding to a loss of 11.25 μg glycogen mg^{-1} DW d^{-1} (Fig. 5). The energy metabolism equalled 0.65 kJ 10^{-6} mg^{-1} DW h^{-1} and hence 2.2% of the normoxic metabolic rate (cf. Table 4). The initial concentration of oxygen in the experimental flasks was 0.15 mg O_2 l^{-1} or slightly less, considered as functionally anoxic. A previous study suggests a threshold level at about 0.1 mg O_2 l^{-1} for uptake of oxygen in *C. anthracinus* 4th instar larvae (Hamburger *et al.*, 1994) and a similar threshold level was observed in 2nd and 3rd instar larvae (unpublished results).

Other laboratory studies also indicate limited resistance of *C. anthracinus* to anoxia. Berg and Jónasson (1965) exposed 4th instar larvae to deoxygenated lake water (<0.1 mg O_2 l^{-1} (Winkler titration)) at 6 °C and found 100% survival of 4th instar larvae after 3 weeks and 75% survival after 5 weeks. Nagell and Landahl (1978) observed 90% survival at 4 °C after treatment for 1 month and 50% survival for a period up to 3 months in 4th instar *C. anthracinus* larvae. In these experiments the larvae were enclosed in flasks containing water from an anoxic pond, delivered to the flasks without contact to oxygen. Glucose was added in order to stimulate bacterial consumption of remaining oxygen. In experiments with *C. plumosus* 65% of 4th instar larvae survived 24 days with anoxia at 10 °C. It is interesting to note, however, that 3rd instar larvae were much less resistant, none of them surviving 4 days and 50% mortality was observed after only 2 days exposure (Frank, 1983).

Field and laboratory estimates of glycogen conversion in *C. anthracinus* larvae exposed to severe oxygen deficiency or anoxia emphasized the low capacity of the anaerobic metabolism. Laboratory measurements of the respiratory metabolism in *C. anthracinus* have shown that the oxygen concentration in the environment must be about 0.5 mg O_2 l^{-1} if the larvae shall maintain an aerobic metabolism in the order of 13–20% of normoxic rate (Hamburger *et al.*, 1994). The ability of the field population to survive several months under apparently almost anoxic conditions can only be explained from the hypothesis that oxygen is constantly transferred from the upper to the lower part of the hypolimnion and utilized at the same rate by the bottom sediment and the associated fauna. Wind induced circulation in the hypolimnion of stratified lakes is well documented (e.g. Allanson, 1990), but has not been studied in Lake Esrom. However, in-lake differences in the depth distribution of bivalves is considered a consequence of such phenomena (Jónasson, 1978; Hamburger *et al.*, 1990). Further, the observed increase in bottom temperature with 2–3 °C during the stratification period (Figs 1 & 2) indicates a transfer of warmer and more oxygen rich water to the bottom layer of the hypolimnion.

The period with hypolimnetic microxia in 1992 lasted 119 days and the mean temperature was 8.7 °C. The number of degree days (dd) thereby equalled 1035. Thus, large 4th instar *C. anthracinus* can survive more than 1000 dd of microxic conditions in the hypolimnion at about 17% of the normoxic metabolic level, provided that the access to oxygen allows for mainly aerobic metabolism and hence low rate of glycogen degradation in anaerobic processes. In 1993 the period with hypolimnetic microxia was 80 days at on average 7.5 °C, which equals 600 dd. Both 2nd and 3rd instar

larvae survived this period without significant mortality. In 1991 the period with hypolimnetic microxia was 85 days at 11.9 °C, which equals 1012 dd (Lindegaard *et al.* 1993). Immediately after the overturn this year 3rd instar larvae weighing 0.15 mg DW and fullgrown 4th instar larvae had concentrations of 10 and 20% glycogen, respectively (unpublished observations). Although younger instars are more susceptible to oxygen deficiency, the majority of both 3rd and small and large 4th instar larvae survived the summer stratification in 1991 (Lindegaard *et al.*, 1993).

C. anthracinus has been reported as a common species living in the sublittoral and profundal zone of many natural mesotrophic to eutrophic lakes (e.g. Brundin, 1949; Thienemann, 1954). Maximum density occurs in 15–25 m depth with rapidly decreasing abundance at greater depths (Lundbeck, 1926; Thienemann, 1954). Due to a general eutrophication of the European lowland lakes the abundance of *C. anthracinus* has seriously decreased and the vertical distribution diminished (e.g. Hofmann, 1971). Although Lake Esrom has been eutrophicated during this century resulting in an increase in the microxic period from about zero to several months, the *C. anthracinus* population is still considerable and maintain the highest abundance at maximum lake depth (Jónasson, 1984; Lindegaard *et al.* 1993). We suggest that lake morphometry and the development of metalimnion are important factors in controlling the microxic regime in hypolimnion and thus indirectly influence the survival and vertical distribution of *C. anthracinus*. Though the morphometry of Lake Esrom seems to benefit large populations of *C. anthracinus*, we still consider its existence vulnerable to prolonged duration of the microxic period and threatened by atypical stratifications and temperatures in the hypolimnion.

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