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REACTIONS OF TWO SPECIES OF AQUATIC INSECTS TO THE CHANGES OF TEMPERATURE AND OXYGEN CONCENTRATION

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ABSTRACT

The reactions of larvae of the eurytherm, *Cloeon dipterum*, and of the stenotherm, *Perlodes intricata*, to the changes of temperature and of oxygen concentration were determined. The lethal O_2 concentrations for *C. dipterum* were lower, and their survival in similar conditions was higher than for *P. intricata*. The Q_{10} coefficient for *P. intricata* was markedly lower than the values expected from the Krogh's "normal curve". The working of the ventilatory systems of the two species was also analysed.

CONTENTS

1. INTRODUCTION

The investigation was carried out on larvae of two species with different environmental requirements, namely *Cloeon dipterum* (Linné), Ephemeroptera and *Perlodes intricata* (Pictet), Plecoptera. The study concerned the reactions of both species to the changes of temperature and of oxygen concentration. Special care was taken to obtain a systemic image of the reactions, and thus both factors were manipulated parallelly and various reactions were tested, like survival, O_2 lethal values, respiration and respiratory movements. The task of the study was also to establish, to what degree the physiological requirements of the animals were related to their habitats, and to try to explain the cause of their different resistance to high temperatures and low O_2 concentrations.

2. MATERIALS

The larvae of *C. dipterum* were collected from among the plants in a clay pit in a Warsaw suburb, and the larvae of *P. intricata* from stones of the Olczyski stream in Tatra Mountains. *C. dipterum* lives among the plants in pools, ponds, in the littoral of lakes, and in slow-running waters. Water temperature in such environments can assume high values (e.g., Gieysztor 1934 reported 30.6°C in a pool) and it is

apt to be changeable (e.g., Pattee 1965 observed a daily amplitude of 10.5°C in a reservoir inhabited by *C. dipterum*). Oxygen content is also highly changeable (e.g., air saturation of water can range from 10 to 170% in a pond — Lewkowicz and Wróbel 1971). On the other hand, *P. intricata* lives in stony mountain streams, with low water temperatures and high oxygen contents (Fig. 1). Figure 2 shows the present author's measurements of water temperature in the Olczyski stream (Tatra Mountains). It can be seen that both the annual and daily amplitudes are fairly small. The geographical distribution of both species is different, too. *C. dipterum* is a widespread species in lower parts throughout Europe, while *P. intricata* appears only in the mountains (Fig. 3). Both species are numerous in their habitats; e.g., *C. dipterum* accounted for 25.8% of all the Ephemeroptera larvae caught among the plants in pools near the Bug and Narew rivers (Kamler unpublished), and *P. intricata* constituted 5.8% of all the Plecoptera collected in Tatra Mountains (Kamler 1964).

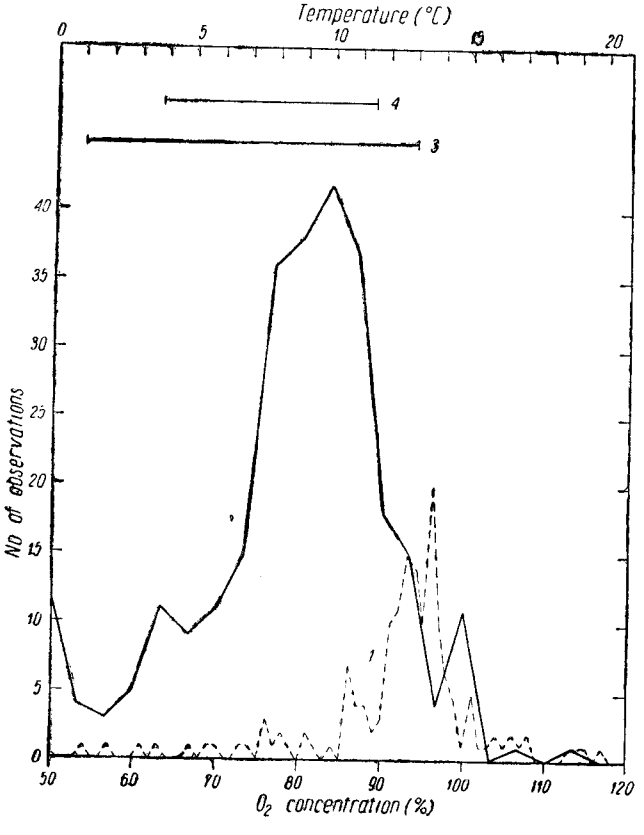

Fig. 1. Oxygen and temperature conditions of *P. intricata* larvae in their natural habitat. 1 — distribution of oxygen content, 2 — distribution of temperature in Tatra streams (compiled from Oleksynowa and Komornicki 1956, 1957a, b, 1960, 1965), 3 — temperature range of *P. intricata* after Wojtas (1964), 4 — temperature range of *P. intricata* after Pleskot (1951)

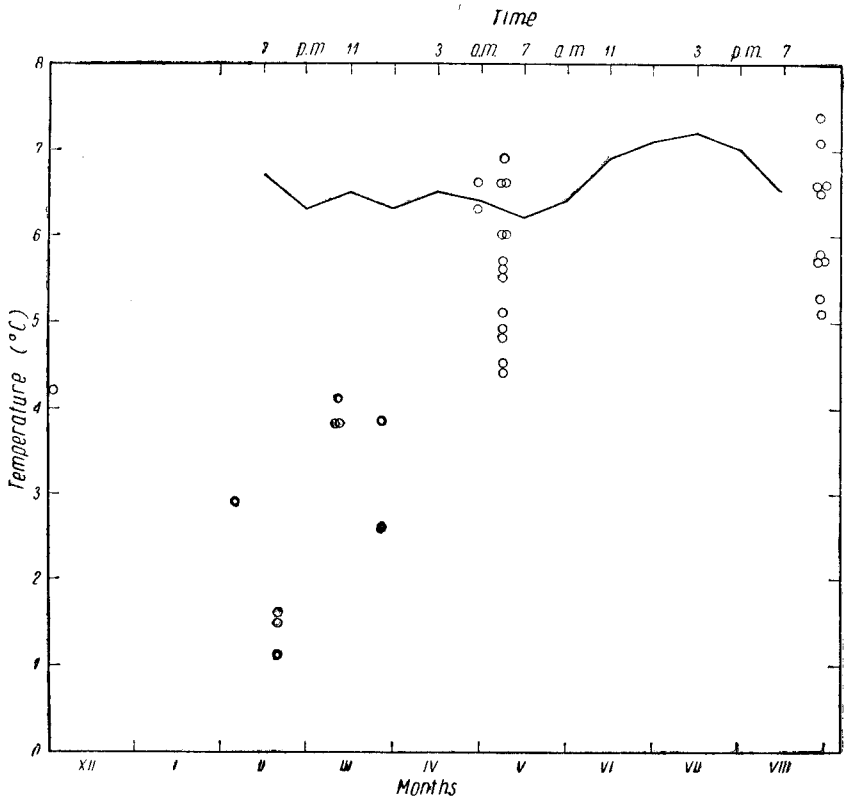

Fig. 2. Water temperatures in Olczyski stream. Points — seasonal temperature variation (winter–summer 1963, 1966, 1967, 1968, 1970), line — temperature changes over 24 hr, Aug. 27–28, 1963

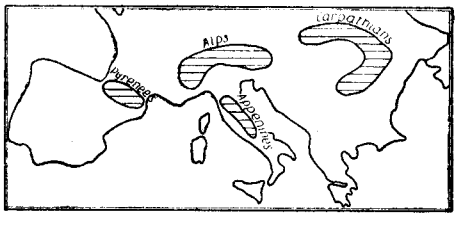

Fig. 3. Distribution of *P. intricata* in Europe

3. METHODS

Before the experiment the larvae had been placed for 24 hr in glass basins, water had been ventilated and the basins kept in a thermostat maintained at the required temperature with an accuracy of ±0.015°C.

The required oxygen contents in water were obtained by nitrogen, air or oxygen bubbling. Oxygen concentration was determined by Winkler's method, the standard deviation of measurements was 0.02 mg/l. In this paper, the oxygen content is expressed in per cent of air saturation of water, 100% means water saturated with air at a given temperature (°C) and atmospheric pressure (mm Hg). When the atmospheric pressure is not known (data from literature), it is assumed to have been 760 mm Hg.

Table I presents the scheme of the experiment. 15 combinations of temperatures and oxygen concentrations were tested. The temperatures were selected according with the environmental requirements of the studied animals, while the oxygen concentrations were similar for both species. The examined reactions were survival, lethal O_2 concentration and respiration; ten animals were tested separately in each temperature/oxygen combination (Table I, experiment 1a and 2). Besides, respiratory movements were investigated. For *P. intricata* they were analysed together with the other indices (Table I, experiment 2).

Table I. Scheme of the experiments

Experiment 1a. Measurements of survival, lethal O_2 concentration and respiration in *C. dipterum* (each O_2 concentration/temperature combination repeated 10 times)

Temp. (°C)	O_2 concentration (%) and number of readings									
	%	No.	%	No.	%	No.	%	No.	%	No.
5.5	15.4	341	29.3	638	56.5	893	104.3	726	142.2	896
15.5	12.0	86	32.3	196	64.4	348	101.2	342	137.6	368
25.5	25.2	61	47.3	111	68.9	157	99.6	104	156.3	138

Experiment 1b. Measurements of respiratory movements in *C. dipterum* (each O_2 concentration/temperature combination repeated 5 times)

Temp. (°C)	O_2 concentration (%)				
5.5	15.5	33.6	57.3	101.4	142.0
15.5	16.3	29.9	47.4	101.9	130.2
25.5	27.0	35.9	43.7	105.2	149.4

Experiment 2. Measurements of survival, lethal O_2 concentration, respiration and respiratory movements in *P. intricata* (each O_2 concentration/temperature combination repeated 10 times)

Temp. (°C)	O_2 concentration (%) and number of readings									
	%	No.	%	No.	%	No.	%	No.	%	No.
5.5	26.0	10*	47.1	159	69.1	257	113.2	278	194.0	576
10.5	33.6	23	42.5	39	76.1	105	108.3	190	133.5	201
15.5	25.8	10*	57.2	32	79.9	55	106.5	92	123.1	128

* Means the death of all animals before the first reading.

The procedure in experiments 1a and 2 was as follows:

1. The initial control bottle I was filled with water having the required temperature and O_2 concentration. Oxygen was fixed and titrated immediately.

2. Ten experimental bottles containing single animals were filled one by one and placed in a thermostat together with the respective final control bottles. The volume of the bottles used for *C. dipterum* was about 60 ml, and of those for *P. intricata* about 170 ml.

3. The initial control bottle II was filled and the oxygen content in it was measured. The oxygen content at the start of the experiment was calculated as a mean from the initial controls I and II.

4. The observations of survival and the readings of respiratory movements were continued during 1 min at regular time intervals until the death of the last animal; total numbers of the readings are quoted in Table I, experiment 1a and 2.

5. The oxygen contents in the experimental and final control bottles, lengths and weights of the animals were determined. Dry weights were determined after drying at 50°C. The latter data allowed to establish the lethal O_2 concentrations and O_2 consumption.

The part of the experiment concerning the role of O_2 concentration has a cross--reference pattern, as the results can be read either by comparing the animals' behavior in bottles with the different initial O_2 concentrations, or by examining the animals in bottles with the same initial O_2 concentration, since the oxygen content is gradually lowered by the animals themselves.

Respiratory movements of *C. dipterum* must have been measured separately (Table I, experiment 1b) because of the complex character of the movements, small size of the larvae, and their great mobility. 75 measurements were taken separately (3 temperatures and 5 oxygen concentrations, 5 repetitions). It was found that the best conditions of observation were obtained by placing single larvae in glass photometric cells of 10 ml capacity and 1 cm of the cell length. A cell was flushed twice with water supplied by means of a thin glass tube attached to the bottom, which was subsequently taken away. The cell was then closed with a glass plate covered with dense vaseline. Former tests by means of dyes had proved such a closure to be sufficiently tight. The vessels with animals were placed in constant temperature baths, and about ten minutes later the measurements started. The animals were watched under 8× lens during about 7 min. The records of ventilation periods (Fig. 4 A) and of rest periods (Fig. 4 B) were taken by means of a tapping key on a kymograph. Clock records every 10 sec (Fig. 4 C) were taken automatically. The ventila-

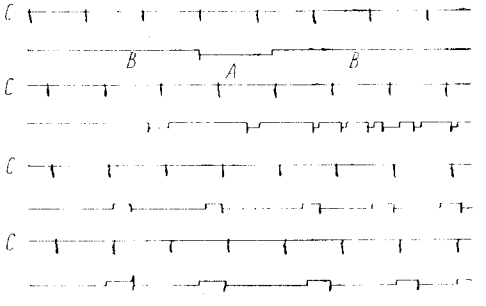

Fig. 4. Fragment of registration of gill movements of *Cloeon dipterum* (A), gills ventilating (B), gills motionless (C); clock records every 10 sec

tion and rest periods were then read from the graph with 0.1 mm accuracy, 0.1 mm being equal to $868 \cdot 10^{-6}$ min. Next, the percentage of ventilation time was calculated. Separate measurements of oxygen consumption allowed to calculate that during a 7 min period of measurement of the respiratory movements of *C. dipterum*, the oxygen content in the cells fell down by not more than 3% of its initial value. Besides, a statistical analysis of the gills' movements record was performed. The whole measuring period was divided into two halves. The percentages of ventilation times in the first and second halves were compared using the t-test, method of paired comparisons (B a i l e y 1959). In the first halves the mean was 43.07, and in the second ones it was 40.12; $t = 1.578$. This result is not significant at the 5% level which cor-

responds to $t = 2.042$. Thus it can be supposed that the measurement of the respiratory movements of *C. dipterum* has been carried out in more or less constant conditions.

Single gill movements of *C. dipterum* are so rapid and complex that they cannot be subject to visual observation, so that resort must have been made to cinematography. A 16 mm film, 32 shots per second, was realized in the Biological Institute for Inland Water Research of the Academy of Sciences, Borok, USSR. The full-grown larvae were placed in a perspex "artificial stream" with some gravel on the bottom. Two temperatures, 13 and 23°C, were tested in stagnant water and in water flow of 0.06 m/sec. The number of movements performed by the larvae in each series of measurements was counted simultaneously by two persons five to seven times during a slow projection of the film on a screen. From the 10 to 14 readings thus obtained the mean with its standard deviation was calculated, and coefficient of variation ranged in various series of measurements from 6.01 to 23.58%. Subsequently, the number of frames corresponding to the established number of gill motions was counted, and the frequency of single gill movements per 1 min was computed. Because of the great mobility of *C. dipterum* larvae, only a few sections of the film could be used for the readings of frequency of single gill movements, and thus the results must be considered as preliminary.

4. RESULTS

Survival

The results of measurements of survival of *C. dipterum* larvae are presented in Fig. 5. It can be seen that in higher temperatures the death

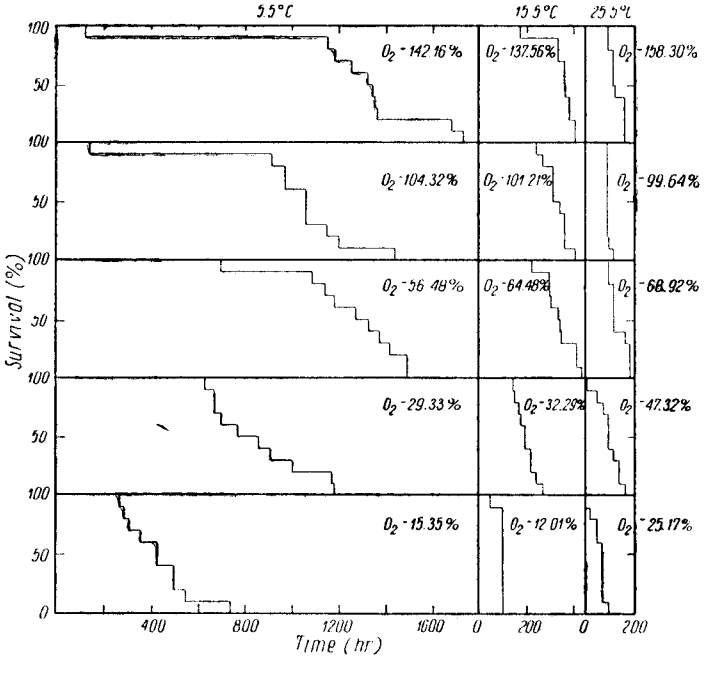

Fig. 5. Survival of *C. dipterum* larvae in different temperatures (°C) and initial O_2 concentrations (%)

of the larvae occurs earlier than in lower ones. It was also found that the larvae died earlier in those sets of experiments in which the initial O_2 concentration was low than in those in which there was much oxygen at the start of the experiment (by a set of experiments is meant here 5 or 10 repetitions of individual measurements taken at the same temperature and O_2 conditions; see Table I). Analogous results were obtained for *P. intricata* (Fig. 6).

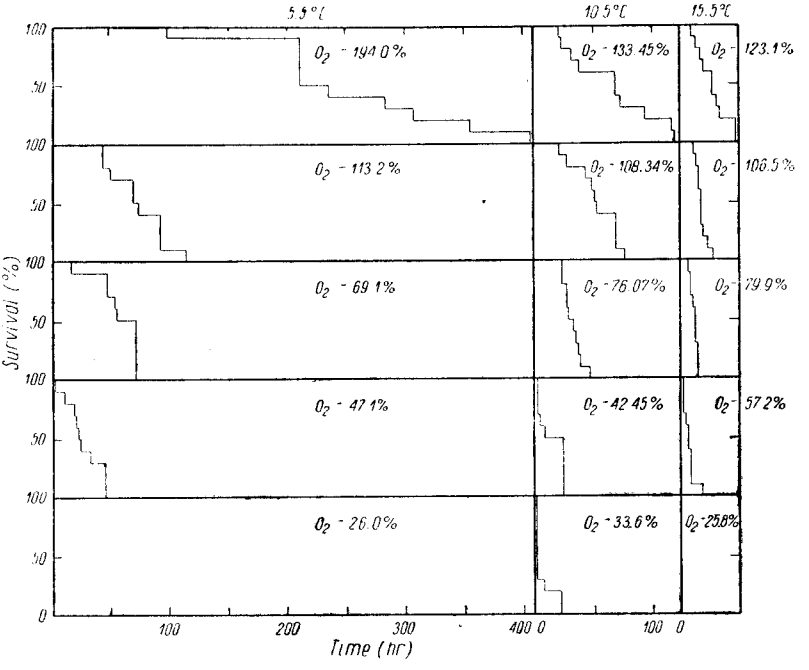

Fig. 6. Survival of *P. intricata* larvae in different temperatures (°C) and initial O_2 concentrations (%)

It can be seen from these illustrations that the survival times of separate individuals in one set were not identical. For a better comparison of the several sets of experiments, in each set the mean survival time for all the 10 individuals was computed together with the 95% confidence interval. The results for *C. dipterum* are presented in Fig. 7 A. Undoubtedly, there were significant differences in survival time of *C. dipterum* larvae, depending on temperature (5.5, 15.5 and 25.5°C). Such differences have been found at all the tested initial O_2 concentrations. In high initial O_2 concentrations (about 60% or more) the survival time did not change much with the change of O_2 concentration, but below this O_2 value the survival time falled down markedly with the decrease of the

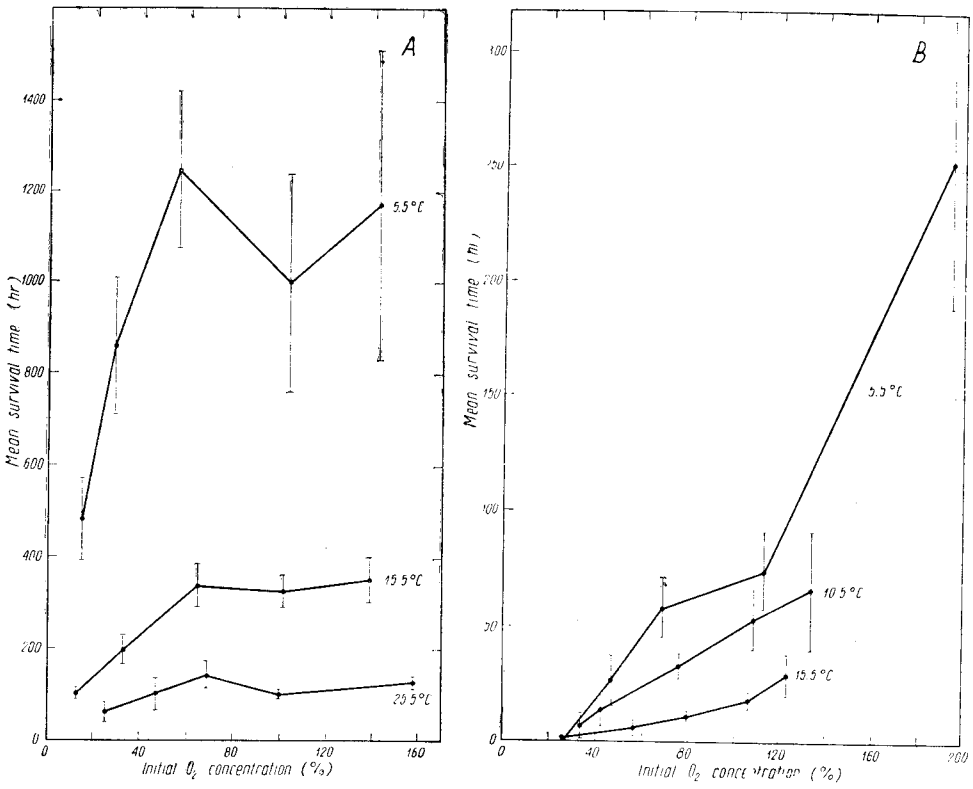

Fig. 7. Mean survival time (hr) in different temperatures (°C) and initial O_2 concentrations (%). Vertical lines — 95% confidence intervals. A — *C. dipterum* larvae, B — *P. intricata* larvae

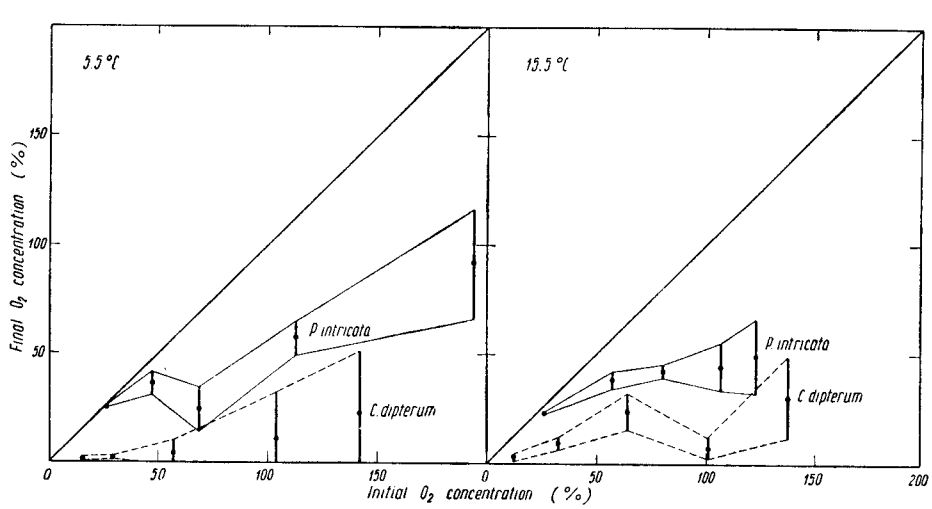

Fig. 8. Lethal O_2 concentrations of *C. dipterum* and *P. intricata* larvae. Points — means for 10 measurements, vertical lines — 95% confidence intervals

inital O_2 concentration; this effect was particularly remarkable in higher temperatures. A significant difference of the mean survival time of *P. intricata* larvae was also found in the tested temperatures 5.5°C, 10.5°C and 15.5°C (Fig. 7 B), but only at the initial O_2 concentrations higher than about 60%.

It is of course impossible to compare directly the survival times of *C. dipterum* and *P. intricata* because of the differences of the animals' weights, of the experimental vessels' capacities and of the oxygen consumption rates of both species of larvae. However, it is remarkable that at O_2 concentration 26.0% (temp. 5.5°C) and O_2 concentration 25.8% (temp. 15.5°C) all the larvae of *P. intricata* died very soon after they had been put in the vessel (Fig. 6, and 7 B), while the larvae of *C. dipterum* have been living much longer at a much lower initial O_2 concentration (12.0% at 15.5°C), as can be seen in Fig. 5 and 7 A.

Lethal O_2 concentrations

As it was stated above, the larvae of *P. intricata* transported from water rich in oxygen into water containing about 26% of oxygen at temperatures 5.5 and 15.5°C died almost immediately. At the medium tem-

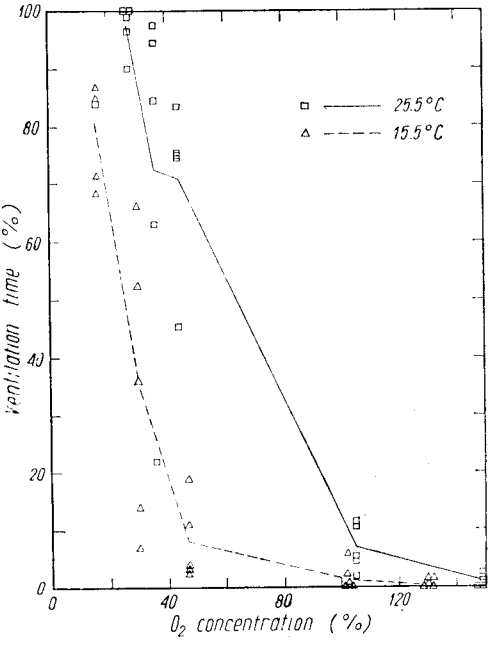

Fig. 9. Per cent of ventilation time in *C. dipterum* larvae in different O_2 concentrations at 25.5 and 15.5°C. Marks stand for the results of individual experiments, lines represent their averages

Table II. Lethal O_2

O_2 concentration at the start of experiment (%)	*Cloeon dipterum* (25.5°C)				
	25.2	47.3	68.9	99.6	158.3
Mean O_2 concentration at the end of experiment (%) ± 95% confidence intervals	6.95 ±4.895	16.43 ±9.390	9.21 ±4.225	2.25 ±1.000	13.14 ±14.859

perature (10.5°C) the lowest O_2 content was somewhat higher, i.e. 33.6% (Table I). The mean survival time was 5.8 hr. Thus, it can be supposed that the lethal O_2 concentration for the *P. intricata* larvae, not adopted formerly to low oxygen concentrations, should be placed between 26 and 34%.

The lethal O_2 concentrations after exposure to an environment with decreasing oxygen content are presented in Fig. 8. For more ready comparison, we confronted only the results of experiments conducted at 5.5°C and at 15.5°C, in which both species were tested. It can be seen that in general larvae of *C. dipterum* died in lower oxygen concentrations than *P. intricata*. The areas defined by the 95% confidence intervals almost do not overlap. It is remarkable that the *C. dipterum* larvae are able to exhaust oxygen down to very low contents values, e.g. 0.02 ml O_2/l at 5.5°C, which is equivalent to 0.23% of air saturation. The results not presented in Fig. 8 are shown in Table II. The lethal O_2 concentrations seem to be fairly stable for each species; no clear relations with temperature or initial O_2 concentrations were observed.

Respiration

To compare oxygen consumption at various temperatures, we selected the results obtained for larvae of similar weight, exposed in water equilibred with air (Table I, experiment 1a and 2, one column before the last). For *C. dipterum* Q_{10} was 2.70 in temperatures ranging from 5.5 to 15.5°C, and 2.62 for the temperature range from 15.5 to 25.5°C. For *P. intricata* Q_{10} was 1.43 at temperatures ranging from 5.5 to 10.5°C.

Respiratory movements

In *C. dipterum* the frequency of single gill movements is high, 350 to 750 movements per 1 min (Table III). We did not observe a change of this frequency resulting from the change of the current speed from 0 to 0.06 m/sec, or from the change of temperature from 13 to 23°C.

concentrations

Perlodes intricata (10.5°C)				
33.6	42.5	76.1	108.3	133.5
29.50 ±3.910	27.72 ±7.210	29.30 ±8.290	46.61 ±13.760	64.83 ±8.150

The ventilatory activity of *C. dipterum* is intermittent and very irregular. E.g., successive ventilation periods and rest periods of an individual larva at 15.5°C and 47.4% air saturation of water were (min. 10^{-3}): ventilation 795, rest 13; v. 48, r. 16; v. 71, r. 8; v. 136, r. 98, and so on. This intermittency and irregularity can be observed in Fig. 4. In different environmental conditions, an animal changes the proportion of the ventilation and rest periods. In Figure 9, percentage of ventilation time in different O_2 contents at 25.5°C and at 15.5°C are presented. They increase with the increase of temperature and with the decrease of oxygen content. The increase of the proportion of ventilation time as the O_2 content decreases is not rectilinear: at higher O_2 contents, i.e. above 100% for 25.5°C, and above 50% for 15.5°C, it is less intensive than at lower concentrations. It can be supposed by extrapolating the data from Fig. 9 that at oxygen concentrations below 20% the gill movements are continuous.

Table III. Frequency of the single movements of gills per 1 min in different temperature/water current speed combinations

Temperature (°C)	Current speed (m/sec)				
	0.00			0.06	
23	478.2			724.6	
13	734.9 357.0	565.9 632.7	596.3 716.0	672.5	714.6

In *P. intricata*, the maximum observed frequency of the respiratory movements was 91 per minute. The respiratory movements frequencies at the tested temperatures and O_2 concentrations are shown in Fig. 10. The experiments conducted at 5.5 and 15.5°C had only 4 sets each, because of an almost immediate death of all the animals in the lowest O_2

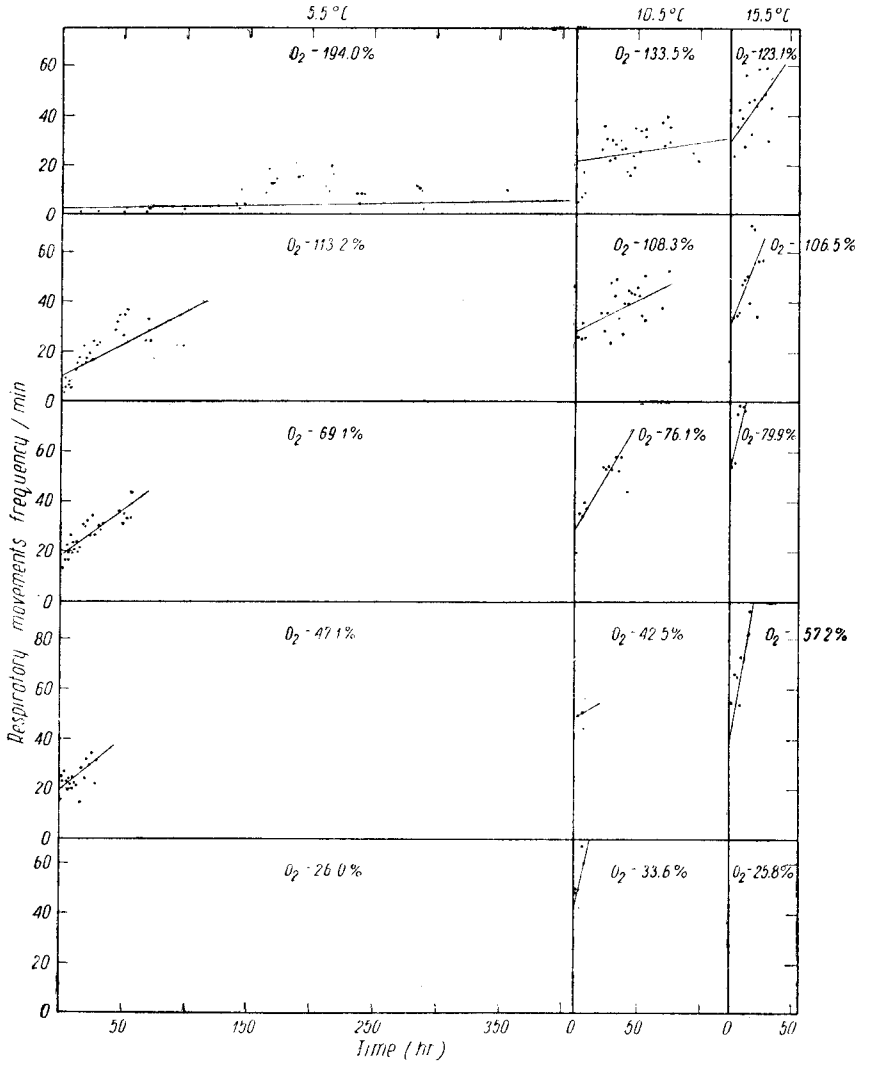

Fig. 10. Respiratory movement frequencies of *P. intricata* larvae in different temperatures (°C) and initial O_2 concentrations (%). Points — mean frequencies of 10 individuals in given time, lines — respective regression lines

concentrations, which was mentioned above. The respiratory movements frequency was proportional with temperature and reversely proportional with the oxygen content at the start of an experiment. A decrease of O_2 concentration brought about by its exhaustion from the experimental bottles by the animals themselves also caused an increase of frequency of the respiratory movements in all the sets of experiments. This increase can be described by regresions of the respiratory movements fre-

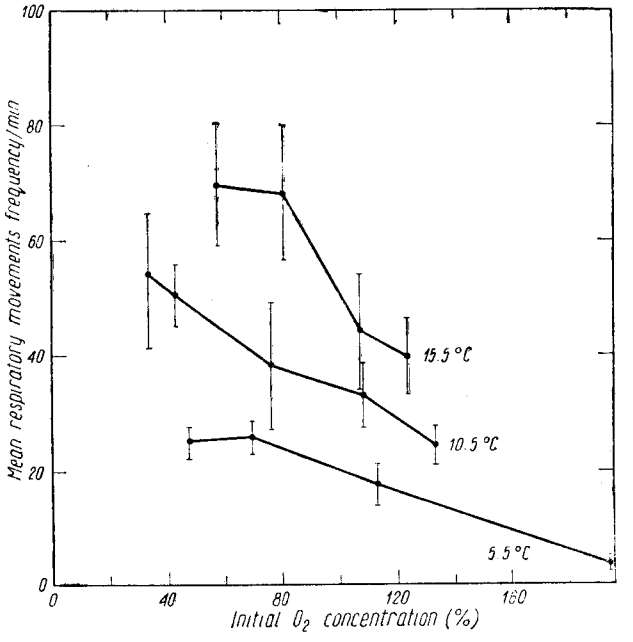

Fig. 11. Mean of all respiratory movement frequencies observed in all ten *P. intricata* larvae in each temperature (°C) and initial O_2 concentration (%). Vertical lines — 95% confidence intervals

quency/min on exposition time. The regression line at 5.5°C and 194% O_2 runs lower than might be inferred from the positions of the visible points, as 46 points in which the mean frequency was 0 could not be presented graphically on the drawing. The regressions were calculated

Table IV. Parameters of the regressions of the *P. intricata* larvae movements/min on time at different temperatures and initial O_2 concentrations

Temperature 5.5°C					
O_2 concentration (%)	26.0	47.1	69.1	113.2	194.0
a_0	—	19.5	17.45	9.87	2.63
a_1	—	+0.416	+0.372	+0.253	+0.006
Temperature 10.5°C					
O_2 concentration (%)	33.6	42.5	76.1	108.3	133.5
a_0	43.15	48.68	28.46	27.25	20.69
a_1	+2.247	+0.283	+0.863	+0.261	+0.077
Temperature 15.5°C					
O_2 concentration (%)	25.8	57.2	79.9	106.5	123.1
a_0	—	49.90	53.10	25.73	28.52
a_1	—	+2.225	+2.217	1.444	+0.680

by means of the formula: $y = a_0 + a_1 x$, where y stands for the respiratory movements frequency per 1 min and x designates the exposition time in hours. The regression equation parameters are presented in Table IV. It can be seen that the regression coefficients a_1 are always positive and usually their value increases with the increase of temperature and decreases with the increase of the initial oxygen content. The only exception is the set of experiments conducted at 10.5°C and initial O_2 concentration 42.5%, where a_1 equal to 0.283 is unexpectedly low.

For more ready comparison of the results of the particular sets of experiments, the mean respiratory movements frequencies performed by all the 10 individuals in each set during the whole exposition time were computed. Those means, together with their 95% confidence intervals, are presented in Fig. 11. It can be seen that the respiratory movements frequencies at the three tested temperatures differ significantly, and the difference is maintained for all the tested initial O_2 concentrations. The decrease of the respiratory movements frequency as the initial O_2 concentration increases can be clearly seen.

5. DISCUSSION

Fox and Simmonds (1933) used a method similar to the one employed in the present work to determine the survival time of *C. dipterum* and of *Baetis rhodani* living in cold streams. They exposed larvae in closed bottles at 16°C in higher and lower oxygen concentrations (6.4 to 7.4 ml O_2/l and 2.6 to 3.1 ml O_2/l respectively). The larvae of *B. rhodani* died after a few minutes in the lower oxygen concentration, while

Table V. Comparison

Species	Ref. No.	Temperature range (°C)				
		1-6.4	5-10	5.5-10.5	6.4-12.3	5-15
Perlodes intricata	1			1.43		
Perla abdominalis	2					
Perla marginata	3					
Pteronarcys californica	4		2.10; 2.19			1.3; 3.2
Acroneuria pacifica	4		1.19; 2.24			1.8; 1.9
Claassenia sabulosa	4					
Arcynopteryx signata	4					
Hexagenia recurvata	5	3.32			2.65	
Krogh's "normal curve"		5.59	3.65	3.47	3.31	3.22

1 — present paper,
2 — Pattee and Rougier 1969,
3 — Istenič 1963,
4 — Knight and Gaufin 1966,
5 — Morgan and Wilder 1936 (winter measurements).

the *C. dipterum* larvae survived for several hours. The authors found that the death of the larvae was not caused by CO_2 accumulation.

The lethal O_2 value for *Brachyptera risi* (Plecoptera), a species with similar ecological requirements as *P. intricata*, were determined by Madsen (1968) in standing water, at 10°C; his method was the same as the present one. The recalculated lethal O_2 value for *B. risi* is approximately 46% of air saturation of water, with a standard deviation equal to 7. The analogous value for *P. intricata*, determined at 10.5°C and 108.3% of air saturation at the start of the experiment was 46.6 (Table II), with a standard deviation 19.23, and thus it was almost identical.

Determinations of oxygen consumption performed in this work are not quite precise. The sources of the lack of precision in determining oxygen consumption by means of the closed-bottle technique were discussed in an earlier work (Kamler 1969). Hence, the present procedure was limited to a comparison of oxygen consumption at different temperatures.

Table V presents a comparison of Q_{10} values describing approximately the Krogh's "normal curve", Q_{10} values for *P. intricata* taken from the research data, and Q_{10} values for larvae of other Plecoptera (ref. No. 2–4) and Ephemeroptera (ref. No. 5), living in low and constant temperatures. The values describing approximately the Krogh's "normal curve" were read directly from the graph published in Ege and Krogh (1914) and computed, as the lists offered in the last mentioned paper and in Winberg and Pechen (1968) do not contain Q_{10} values for all the relevant temperature intervals, and besides, the Q_{10} value 2.63 for the 5°C to 15°C temperature range, quoted in Winberg and Pechen on page 66 as close to the "normal curve", seems to be

of Q_{10} values

5.5-15.5	11-15	10-20	11-20	15-20	15-25	20-25	15.52.5	30-20
	1.52-3.65	1.40 1.2-2.2 1.2-1.4 1.4-1.5 1.7	0.96-1.91	1.13-2.33	0.93-2.11 1.3-2.0 1.3; 1.3	0.65-2.22		0.55; 1.8 0.91-1.5
3.14	2.81	2.63	2.61	2.43	2.40	2.37	2.35	2.19

too low. There are many data in literature concerning the relationship between the metabolism of various non cold-adapted animals and temperature e.g., Winberg (1956) gave an extensive comparison for fish; he found these data to be highly consistent with the Krogh's "normal curve". Pattee (1965) quoted the Q_{10} value for *C. dipterum* measured by means of the closed-bottle (chemical) method as equal to 2.6, and obtained by means of the manometric method as equal to 3.4. Oxygen consumption was measured just before and 24 hr after the raising of temperature by 5°C, and the obtained values were adjusted to those obtained from the measurements of control animals at a constant temperature. The temperature intervals for these measurements were not stated in Pattee (1965), but according to his personal information, in the case of the closed-bottle method lower temperatures were 7 to 12°C and higher 9 to 14°C, while for the manometric method lower temperatures ranged from 3 to 8°C and higher from 9 to 14°C, but most measurements by the latter method were performed at 4 to 9°C. The available data do not allow for a full analysis of Pattee's results, but it can be supposed that his Q_{10} values for *C. dipterum* are not very remote from the Krogh's "normal curve". Also the Q_{10} values for *C. dipterum* obtained in the present work (2.70 at 5.5 to 15.5°C and 2.62 at 15.5 to 25.5°C) are close to the values expected from the Krogh's "normal curve" for the same temperature intervals. However, the Q_{10} value for *P. intricata* is markedly lower. In literature only a few data can be found for the cold-adapted Plecoptera and Ephemeroptera larvae, and it can be seen from the comparison in Table V that they are also much lower from those expected from the Krogh's "normal curve". There are, however, two exceptions: one out of the 31 Q_{10} values stated in Istenič (1963), 3.65 for the temperature interval 11 to 15°C, which is higher from the expected one (2.81), and one out of the 30 Q_{10} values stated in Knight and Gaufin (1966), 3.2 for the 5 to 15°C temperature interval, which is only slightly lower from the expected value (3.22). Scholander et al. (1953) proved the Q_{10} values to be in general consistent with those expected from the Krogh's "normal curve" for animals inhabiting steady warm environments (tropical seas, tropical rain forests), and for those living in cold but fluctuating environments (arctic terrestrial animals). The Q_{10} values quoted by these authors for temperate climate animals were also close to the "normal curve" values. However, the Q_{10} values for arctic fish and Crustacea living in steady cold environments were lower than the expected ones for the same temperatures. It is remarkable that also in the temperate climate there are some relatively steady cold environments, and the animals inhabiting them, as e.g. the Plecoptera and Ephemeroptera larvae, reveal much less vulnerability to

temperature changes than might be expected from the Krogh's "normal curve" within the range of low temperatures natural for them. Such discrepancy, however, is by no means surprising, if are reminded that the "normal curve" is an empirical device, and that it has not been based on measurements of cold-stenothermes. Prosser (1961), presents schematic graphs of log stabilized rates over a wide temperature range for cold-adapted and warm-adapted animals. He remarks that the most frequently encountered is such pattern of acclimatization (rotation and translation) in which the curve at low temperatures runs higher for the cold-adapted animals than for the warm adapted ones, and its slope is less steep, which is an evidence of lower Q_{10} values. However, we should not think that the Q_{10} is low for all the animals living in low temperatures. Prosser (1961) gives other examples besides the discussed one. Recently, Klekowski et al. (1970) found extremely high Q_{10} values at temperatures close to 0°C for an antarctic sea Amphipoda, *Paramoera walkeri*.

The anatomy of the respiratory apparatus and the mechanism of its work in *C. dipterum* has been fully described by Eastham (1958). *C. dipterum* is provided with 7 pairs of gills, in the first six pairs each gill consists of two lamellae. The gills are situated laterally along the abdomen. They move in metachronal rhythm, which causes water to flow between the gills and along the abdomen. Data on the role of gills in *C. dipterum* are contained in Wingfield (1939). He measured O_2 consumption at various oxygen concentrations by normal *C. dipterum* larvae and by those with amputated gills. The respiration of both groups was of the "independent" type; the critical oxygen concentration was 19% of air saturation of water for the larvae with their gills intact, but for those with amputated gills it was much higher, i.e. about 44% of air saturation. By comparing these results with analogous data obtained from observations of operated and unoperated larvae of *Baetis sp.* whose gills are motionless, Wingfield (1939) arrived at a conclusion that the main role of the gills does not consist in immediate withdrawal of oxygen from the environment but rather in causing water movements near the respiratory surfaces. Similarly, Eriksen (1963) proved that oxygen diffusion through the gills' surface was by no means more intensive than through any other respiratory surfaces. Thus the main role of the mobile gills consists in decreasing the oxygen gradients in the boundary layers around the respiratory surfaces.

An application of the cinematographic method allowed for the first time to estimate the rate of single gill movements in *C. dipterum*. This method was employed by Cukerzis (1966) for registration of the respiratory movements frequency of two species of Astacus. The present

results did not prove that *C. dipterum* modified the amount of oxygen near its body by slowing down or accelerating its gills movements. Hence, the mechanisms of adaptation to the changing environmental conditions had to be sought for in other behaviours. It turned out that the changes in the proportion of ventilation and rest periods could constitute such an adaptation. A pattern of respiratory behavior similar to *C. dipterum* can be observed in larvae of Chironomidae. Walshe (1950) took kymograph records of periods of irrigation of a tube, and of pauses in *Ch. plumosum*, at 15 to 19°C. Similarly as in *C. dipterum*, they were rather irregular; in normal larvae, the percentage of the time of irrigation of the tube increased, as oxygen contents in water fell down: this increase was more intensive at low oxygen contents than at higher ones. Such a "turning point" for *Ch. plumosus* is situated at about 20% of air saturation of water, i.e., at lower O_2 contents than that found for *C. dipterum* (about 50% at 15.5°C and about 100% at 25.5°C). This finding seems to be obvious, as *Ch. plumosus* is much more exposed to low oxygen concentrations than *C. dipterum*. In the latter species, a continuous motion of the gills can be expected below about 20% of air saturation. It was remarkable that Fox et al. (1937), who measured oxygen consumption of *C. dipterum* larvae at 10°C, found the critical O_2 content at about 18% of air saturation, and Wingfield (1939) at about 19% at the same temperature.

Knight and Gaufin (1963) describe the behavioral pattern of *Acroneuria pacifica* (Plecoptera) related with a decrease of oxygen content in their environment. Its part are respiratory movements consisting in "bendings" of the whole body. They found that the frequency of the respiratory movements increased as the oxygen content decreased, and the current speed fell down. The respiratory behavior of *A. pacifica* is similar to that of *P. intricata*. Knight and Gaufin (1963) observed in *A. pacifica* the maximum frequency of 92 undulations per minute; the same authors (1964) found it to be 145 per minute in *Pteronarcys californica*, and Pattee and Rougier (1969) who observed *Perla abdominalis* counted 117 movements per minute. All these figures are close to the maximum frequency of the respiratory movements noted in *P. intricata*, which is 91 per minute.

The results of the present work allow to suppose that one of the causes of the presented differences between the reactions of *C. dipterum* and *P. intricata* to temperature and oxygen contents can be the difference in the efficiency of their ventilatory systems. Very quick motions of the gills of *C. dipterum* can probably restore the oxygen supply in the boundary layers near the respiratory surfaces more efficiently and with lesser energy expense than the relatively slow move-

ments of the whole body performed by *P. intricata*. It is not thereby excluded, however, that regulation can occur parallelly at the tissue level in the circulatory system, or by blood system.

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6. SUMMARY

Laboratory experiments were carried out on an eurytherm, *Cloeon dipterum* (Ephemeroptera) and a stenotherm, *Perlodes intricata* (Plecoptera). 15 combinations of oxygen and temperatures were tested (3 values of temperature and 5 concentrations of oxygen); each combination was repeated 10 times. The investigated reactions were: survival, lethal concentrations of oxygen, oxygen consumption and respiratory movements frequency.

The larvae of *C. dipterum* live longer in low oxygen concentrations than those of *P. intricata*. The *C. dipterum* larvae can exhaust oxygen down to very low values, e.g. to as little as 0.02 ml O_2/l at 5.5°C. They exhaust oxygen to lower values than *P. intricata* at the same initial temperatures and oxygen concentrations. Q_{10} for the *C. dipterum* larvae is consistent with the Krogh's "normal curve" expectations, for *P. intricata* it is markedly lower. The gills of *C. dipterum* perform complicated respiratory movements at the rate ranging from about 350 to 750 per minute. No relationship between these movements and environment conditions was found out. Regulation consists in the change of the percentage of time occupied by ventilation. It increases with temperature and as the oxygen concentration decreases. Respiratory movements of *P. intricata* consist in the "bendings" of the whole body; their frequency increases as temperature rises up and oxygen concentration falls down. Their maximum observed frequency was 91 movements per minute.

It is suggested that the difference in the resistance of both species to high temperatures and low O_2 concentrations is connected, among other things, with the different efficiencies of their ventilatory systems.

7. STRESZCZENIE

Przeprowadzono eksperymenty laboratoryjne na eurytermicznym gatunku *Cloeon dipterum* (Ephemeroptera) i stenotermicznym gatunku *Perlodes intricata* (Plecoptera). Stosowano po 15 kombinacji tlen/temperatura (3 temperatury i 5 stężeń tlenu), każdą kombinację powtarzano 10-krotnie. Badano: letalne stężenia tlenu, przeżywalność, zużycie tlenu i częstotliwość ruchów oddechowych.

Larwy *C. dipterum* w niskich stężeniach tlenu żyją dłużej niż larwy *P. intricata*. Larwy *C. dipterum* mogą wyczerpywać tlen do wartości bardzo niskich, np. aż do 0.02 ml O_2/l w 5.5°C. Wyczerpują one tlen do wartości niższych niż *P. intricata* w tych samych temperaturach i wyjściowych stężeniach tlenu. Q_{10} larw *C. dipterum* jest zgodne z oczekiwanym z „krzywej normalnej" Krogha, zaś larw *P. intricata* — wyraźnie niższe. Skrzelotchawki *C. dipterum* wykonują skomplikowane ruchy oddechowe o częstotliwości rzędu 350—750 ruchów/min. Nie stwierdzono zależności tych ruchów od warunków środowiskowych. Regulacja polega na zmianie procentu czasu zajętego na wentylację. Jest on tym wyższy, im wyższa jest temperatura i im mniej tlenu zawiera woda. Ruchy oddechowe *P. intricata* polegają na „przysiadach" całego ciała; ze wzrostem temperatury i ze spadkiem zawartości tlenu w środowisku częstotliwość ruchów oddechowych wzrasta. Maksymalna zaobserwowana częstotliwość wynosi 91 ruchów/min.

Przypuszcza się, że różnica w odporności na wysokie temperatury i niskie zawartości O_2 związana jest u tych gatunków m.in. z różnicą efektywności ich systemów wentylacyjnych.

8. REFERENCES

Bailey, N. T. J. 1959. *Statistical methods in biology.* London, English Universities Press.

[Cukerzis, J. M.] Цукерзис, Я. М. 1966. Исследования некоторых эколого-физиологических особенностей широкопалого и длиннопалого раков в связи с их межвидовыми отношениями. [Investigations of some ecological and physiological requirements of *Astacus astacus* and *A. leptodactylus* in relation to their intraspecific relations]. *Liet. TSR Mokslu. Akad. Darb., Ser. C,* 2 (40), 279–286.

Eastham, L. E. S. 1958. The abdominal musculature of nymphal *Cloeon dipterum* L. (Insecta: Ephemeroptera) in relation to gill movement and swimming. *Proc. zool. Soc. Lond.,* 131, 279–291.

Ege, E., Krogh, A. 1914. On the relation between the temperature and the respiratory exchange in fishes. *Int. Rev. ges. Hydrobiol. und Hydrogr.,* 7, 48–55.

Eriksen, C. H., 1963. Respiratory regulation in *Ephemera simulans* Walker and *Hexagenia limbata* (Serville) (Ephemeroptera). *J. exp. Biol.,* 40, 455–467.

Fox, H. M., Simmonds, B. G. 1933. Metabolic rates of aquatic arthropods from different habitats. *J. exp. Biol.,* 10, 67–74.

Fox, H. M., Wingfield, C. A., Simmonds, B. G. 1937. The oxygen consumption of Ephemerid nymphs from flowing and from still waters in relation to the concentration of oxygen in the water. *J. exp. Biol.,* 14, 210–218.

Gieysztor, M. 1934. Limnologische Untersuchungen an einigen Kleingewässern. *Arch. Hydrobiol. Ryb.,* 8, 75–148.

Istenič, L. 1963. Poraba kisika pri ličinkach *Perla marginata* Pz. v odvisnosti od velikosti telesa in temperature [Rate of oxygen consumption of larvae *Perla marginata* Pz. in relation to body size and temperature]. *Razpr. Slov. Akad. Ljubljana,* Cl. IV, 7, 201–236 [Engl. summ.].

Kamler, E. 1964. Badania nad Plecoptera Tatr [Recherches sur les Plécoptères des Tatras]. *Pol. Arch. Hydrobiol.,* 12, 145–184 [French summ.].

Kamler, E. 1969. A comparison of the closed-bottle and flowing-water methods for measurement of respiration in aquatic animals. *Pol. Arch. Hydrobiol.,* 16, 31–49.

Klekowski, R. Z., Opaliński, K. W., Rakusa-Suszczewski, S. 1970. Oddychanie *Paramoera walkeri* (Stebbing) (Amphipoda) z przybrzeżnych wód Antarktydy (Zatoka Alasheyeva, Ziemia Enderby). [Respiration of *Paramoera walkeri* (Stebbing) (Amphipoda) living in antarctic coast-side waters (Alasheyev Bight, Enderby Land)]. *8 Zjazd Hydrobiol. Pol., Białystok 16–19.IX.1970, Streszcz. ref.,* 66–67, Warszawa 1970, Pol. Tow. Hydrobiol.

Knight, A. W., Gaufin, A. R. 1963. The effect of water flow, temperature, and oxygen concentration on the Plecoptera nymph, *Acroneuria pacifica* Banks. *Proc. Utah Acad. Sci.,* 40, 175–184.

Knight, A. W., Gaufin, A. R. 1964. Relative importance of varying oxygen concentration, temperature and water flow on the mechanical activity and survival of the Plecoptera nymph, *Pteronarcys californica* Newport. *Proc. Utah Acad. Sci.* 41, 14–28.

Knight, A. W., Gaufin, A. R. 1966. Oxygen consumption of several species of stoneflies (Plecoptera). *J. Insect Physiol.,* 12, 347–355.

Lewkowicz, S., Wróbel, S. 1971. Some chemical factors and primary production of phytoplankton in fingerling ponds. *Pol. Arch. Hydrobiol.,* 18, 189–201.

Madsen, B. L. 1968. The distribution of nymphs of *Brachyptera risi.* Mort. and *Nemoura flexuosa* Aub. (Plecoptera) in relation to oxygen. *Oikos,* 19, 304–310.

Morgan, A. H., Wilder, J. F. 1936. The oxygen consumption of *Hexagenia recurvata* during the winter and early spring. *Physiol. Zool.,* 9, 153–169.

Oleksynowa, K., Komornicki, T. 1956. Materiały do znajomości wód w Tatrach. I — Dolina Strążyska [Some new data on the composition of the waters in the Tatra Mountains. I — Strążyska Valley]. *Zesz. nauk. WSR Krak., Ser. Rolnictwo,* 1, 33–51 [Engl. summ.].

Oleksynowa, K., Komornicki, T. 1957a. Materiały do znajomości wód w Tatrach. II — Dolina Białego [Some new data on the composition of the waters in the Tatra Mountains. II — Białego Valley]. *Zesz. nauk. WSR Krak., Ser. Rolnictwo,* 3, 113–126 [Engl. summ.].

Oleksynowa, K., Komornicki, T. 1957b. Materiały do znajomości wód w Tatrach. III — Dolina Małej Łąki [Some new data on the composition of the waters in the Tatra Mountains. III — Mała Łąka Valley]. *Zesz. nauk WSR Krak., Ser. Rolnictwo*, 3, 127–146 [Engl. summ.].

Oleksynowa, K., Komornicki, T. 1960. Materiały do znajomości wód w Tatrach. V — Dolina Chochołowska [Some new data on the composition of the waters in the Tatra Mountains. V — Chochołowska Valley]. *Zesz. nauk. WSR Krak., Ser. Rolnictwo*, 7, 17–48 [Engl. summ.].

Oleksynowa, K., Komornicki, T. 1965. The chemical composition of water in the Polish Tatra Mountains and the problem of its variation in time. In: *Limnological investigations in the Tatra Mountains and Dunajec River Basin.* 91–111. Warszawa, Wydz. Nauk Roln. i Leśn., Komitet Zagospodarowania Ziem Górskich PAN, zesz. 11.

Pattee, E. 1965. Sténothermie et eurythermie. Les Invértébres d'eau douce et la variation journalière de température. *Annls Limnol.*, 1, 281–434.

Pattee, E., Rougier, C. 1969. Étude de la respiration chez deux larves aquatiques d'insectes. Travaux pratiques d'ecologie. *Bull. mens. Soc. linn. Lyon*, 38, 7–18.

Pleskot, G. 1951. Wassertemperatur und Leben in Bach. *Wett. u. Leben*, 3, 129–143.

Prosser, C. L. 1961. Temperature. In: C. L. Prosser, F. A. Brown. *Comparative animal physiology.* 2nd ed., 238–284, Philadelphia, W. B. Saunders.

Scholander, P. F., Flagg, W., Walters, V., Irving, L. 1953. Climatic adaptation in arctic and tropical poikilotherms. *Physiol. Zool.*, 26, 67–92.

Walshe, B. 1950. The function of haemoglobin in *Chironomus plumosus* under natural conditions. *J. exp. Biol.*, 27, 73–95.

[Winberg, G. G.] Винберг, Г. Г. 1956. Интенсивность обмена и пищевые потребности рыб [Rate of metabolism and food requirements of fish]. Minsk, Izdat. Belgos-universitet.

[Winberg, G. G., Pechen, G. A.] Винберг, Г. Г., Печень, Г. А. 1968. Рост, скорость развития и плодовитость в зависимости от условей среды [Growth, rate of development and fecundity depending on the environmental conditions]. In: G. G. Winberg [Ed.] *Metody opredelenija produkcii vodnych životnych.* 45–77. Minsk. Izdat. „Vyšejšaja Škola".

Wingfield, C. A. 1939. The function of mayfly nymphs from different habitats. *J. exp. Biol.*, 16, 363–374.

Wojtas, F. 1964. Widelnice (Plecoptera) Tatr i Podhala. The stoneflies (Plecoptera) of the Tatra Mountains and Podhale region. Łódź, Uniw. Łódzki [German summ.].