SOME STUDY ON THE BIOLOGY AND BIOENERGETICS OF *CLOEON DIPTERUM* (L.), *EPHEMEROPTERA* (PRELIMINARY DATA)

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1. Introduction

As it is well known, the larvae of *Ephemeroptera* are rather abundant in fresh waters; for instance, their density in Morgan's Creek, Meade County and Kentucky, was about 65 individuals/m², which accounted for 5% of the total number of invertebrates and for about 40% of primary consumers (MINSHALL, 1967). An average biomass of mayflies in large rivers (Amur and its tributaries) in summer varied from 0.4 to 11.8 g/m² with a maximum of 17.8 g/m² in July (LEVANIDOVA, 1968).

The larvae of *C. dipterum* are of a swimming type. They live among the plants or at the bottom mud (BERTRANDT, 1954, BROWN, 1961a) in pools, ponds, lake littoral zones and slow-running waters. The temperature of water can assume high values in such habitats (GIEYSZTOR, 1934 recorded 30.6°C in a pool) and varies considerably (PATTEE, 1965 observed a daily amplitude of 10.5°C in a water body inhabited by *C. dipterum*). Oxygen content is also highly variable (air saturation water can range from 10 to 170% in a pond — LEWKOWICZ and WRÓBEL, 1971). Larvae of *Cloeon dipterum* are very tolerant of the changes in environmental conditions. Thermal index (temperature at which 50% of animals die) for young and old forms of this species is 30.2°C and 28.5°C, respectively (WHITNEY, 1939). A change in oxygen concentration from 1.5 to 9.0 ml O_2/l (about 19 and 110% air saturation) has no effect on *C. dipterum* respiratory rate (WINGFIELD, 1939 and CHAURIN, 1956). They exhaust oxygen to such low concentrations as 0.02 ml O_2/l at 5.5°C or 0.80 ml O_2/l at 25.5°C (0.24 and 9.60% air saturation, respectively), as reported by KAMLER, 1971. Fox and SIMMONDS (1933) determined that *C. dipterum* is not sensitive to the changes in pH from 6.0 to 9.0. BRITTAIN (1974) observed pH of the water bodies inhabited by this species ranged between 6.6 and 8,8. Water flow up to a rate of 0.6 m/sec. is tolerated by the larvae (DORIER and VAILLANT, 1954). High resistance of *C. dipterum* to the environmental changes accounts for its wide geographical distribution. This is a Paleoartic species common in lentic waters of the whole Europe (ILLIES, 1967). It occurs mainly in·lowlands and only occasionally in waters located 200 m above the sae level (BRITTAIN, 1974). In favourable habitats it is generally more abundant than other *Ephemeroptera* species (HARNISCH, 1957 and IKONOMOV, 1960). In Poland it is the main mayfly species (KEFFERMÜLLER, 1972) and it occurs in large quantities particularly in ponds (SOWA, 1959). KAMLER (1971) reports that *C. dipterum* accounted for 25.8% of all *Ephemeroptera* larvae caught among plants in the overflow of the Bug and Narew rivers in 1961. Their average biomass (KAMLER, unpubl.) in summer was about 2.2 g/m² (in June up to

7,0 g/m²). Mass occurrence, particularly in spring and summer, is an indication of the great importance of larval *C. dipterum* to the community. They form an important link in the trophic chain and can be the main component in the diet of young predatory fish.

In ecological and bioenergetical studies developmental stages of animals should be recognized. In the holometabola, which develop by steps, it is not difficult. The developement of the hemimetabola is uniform. The larva moults many times becoming more and more like the adult insect. Moults are numerous and it is impossible to count them, particularly in the field. Morphological differences after successive moults are slight, so it is not possible to estimate the number of them, which is possible for instance for the *Chironomidae*. But some attempts to distinguish stages in the development of the *Ephemeroptera* were made. SCHMIDT (1951) distinguished the stage of larvulla during which gills are being developed, and the stages of larvae or nymphs when the wings develop, till the time of subimago emergence. BERTRAND (1954) recognized seven to ten instars during the development of gills, and then the nymphs. PLESCOT (1958) proposed to accept that the number of age groups is equal to the number of moults, and divided the whole mayfly development into four stages according to the development of gills and wings. BRETSCHKO (1965), LEVANIDOVA (1968), and LEHMKUHL (1970) distinguished larvullae, new-hatched, without respiratory organs, and four — six larval stages according to the degree of wing development.

If the stages really exist in the development of the *Ephemeroptera*, it must be indicated by their biometrical characters, and by physiological processes in these insects. BORUCKIJ (1958), RUBANIENKOVA (1964), and LEVANIDOVA (1968) suggested that the measurements of the body length and the width of head capsule can be used to determine the body weight of the larvae and the develop-
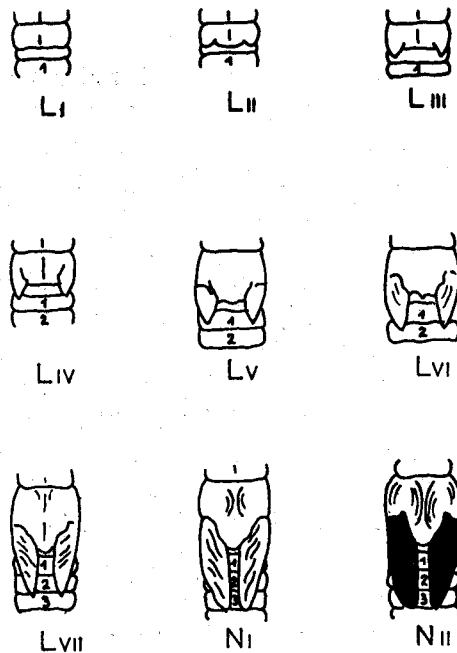

Fig. 1. Degree of wing development in aquatic forms: larvae (L_I—L_{VIII}) and nymphs (N_I and N_{II}) of *Cloeon dipterum* as a criterion of distinguishing developmental stages. 1, 2, 3 — abdominal segments

mental cycles of the *Ephemeroptera*. Only a few authors take into account the developmental stages of the *Ephemeroptera* when studying their metabolism. TRAMA (1957) determined energy budget of larval *Stenonema pulchellum* of different body length. IVANOVA (1958) and HILMY (1962) analysed the changes in the respiratory rate during mayfly development.

The objective of the present study is to ascertain whether a tendency to the occurrence of distinct stages in the development of *Cloeon dipterum* really exists, and whether the accepted criteria of the degree of larval development are confirmed by the biometry and bioenergetics of this species.

2. Results and discussion

2.1. Developmental stages of *Cloeon dipterum*

The following aquatic stages were identified in the development of *C. dipterum* on the basis of readily distinguished morphological characters (Fig. 1.):

larvullae L_0 — new-hatched without or with poorly developed gills, absence of wing-pads, two caudal filaments; they do not resemble much a typical;

larvae: L_I — gills well developed, three caudal filaments, absence of wing-pads;

L_{II} — the mesothoracic wing-pads slightly marked;

L_{III} — the mesothoracic wing-pads readily visible, they cover a half of metathorax;

L_{IV} — the mesothoracic wing-pads cover the metathorax;

L_V — wing-pads reach a half of the first abdominal segment;

L_{VI} — wing-pads exceed the first abdominal segment;

L_{VII} — veins are readily visible on the wing-pads reaching the second abdominal segment;

nymphs: N_I — the wing-pads long, with well developed venation, prothorax large and protuberant;

N_{II} — wings and prothorax dark; these forms are ready to undergo metamorphosis into subimago.

Stage L_0 is not analysed in the present paper because very small larvullae were rarely found in the field samples. The body size and respiratory metabolism were measured for particular stages, as well as the relationships between the duration of development and number of moults for particular stages were analysed. Some measurements were taken for the subimaginal (S) and adult (A) stages, which are terrestial and can fly.

2.2. Duration of the development in relation to the diet

The development of *C. dipterum* from stage L_I to the emergence of subimago was studied in laboratory cultures maintained at 20°C ($\pm$ 1.0°C). There were 10 individual cultures in two variants:

D — larvae fed on organic detritus;

S — larvae fed on algae (*Spirogyra*).

Food was supplied in excess. Increases in the body length and width of head capsule were measured, stages were indentified and moults were counted for each larva.

It was found that the larvae fed on detritus grew more rapidly and they emerged after 146.5 days and 27.6 moults. The larvae fed on algae needed 211.3 days to complete their development and they moulted 38.4 times (Fig. 2A and 2B). To compare statistically the results obtained in both variants the confidence limits were calculated for the difference between the means of two normal distributions of independent variables (OKTABA, 1974). The difference between the means $\bar{x}_1$ and $\bar{x}_2$ lies within the limits:

$$\bar{x}_1 - \bar{x}_2 - L_{0.05} < \bar{x}_1 - \bar{x}_2 < \bar{x}_1 - \bar{x}_2 + L_{0.05}$$

where $L_{0.05}$ is confidence interval;

$$L_{0.05} = t_{0.05} \cdot \sqrt{\frac{n_1 \cdot SD_1^2 \mp n_2 \cdot SD_2^2}{n_1 + n_2 - 2} \cdot \left(\frac{1}{n_1} + \frac{1}{n_2}\right)};$$

The value of $t_{0.05}$ is given in Student's t-distribution tables for $n_1 + n_2 - 2$ degrees of freedom and selected significance level (95%).

The difference in the duration of development and in the number of moults between variants D and S are highly significant ($p < 0.001$). The 95% confidence interval for the difference between the means in 64.8 $\pm$ 20.1 days of development and 10.8 $\pm$ 2.5 moults. The average frequency of moults

(Fig. 2C) was similar and reached 5.31 (variant D) or 5.50 days (variant S). There were not significant differences in mean maximum body dimension (length, width of head) between the larvae from both the cultures (Tab. I).

In both variants females developed less rapidly than males and they passed through a greater number of moults. Their sizes were larger but only the differences between males and females in the body length from stages L_V to N were statistically sifgnificant ($p < 0.01$).
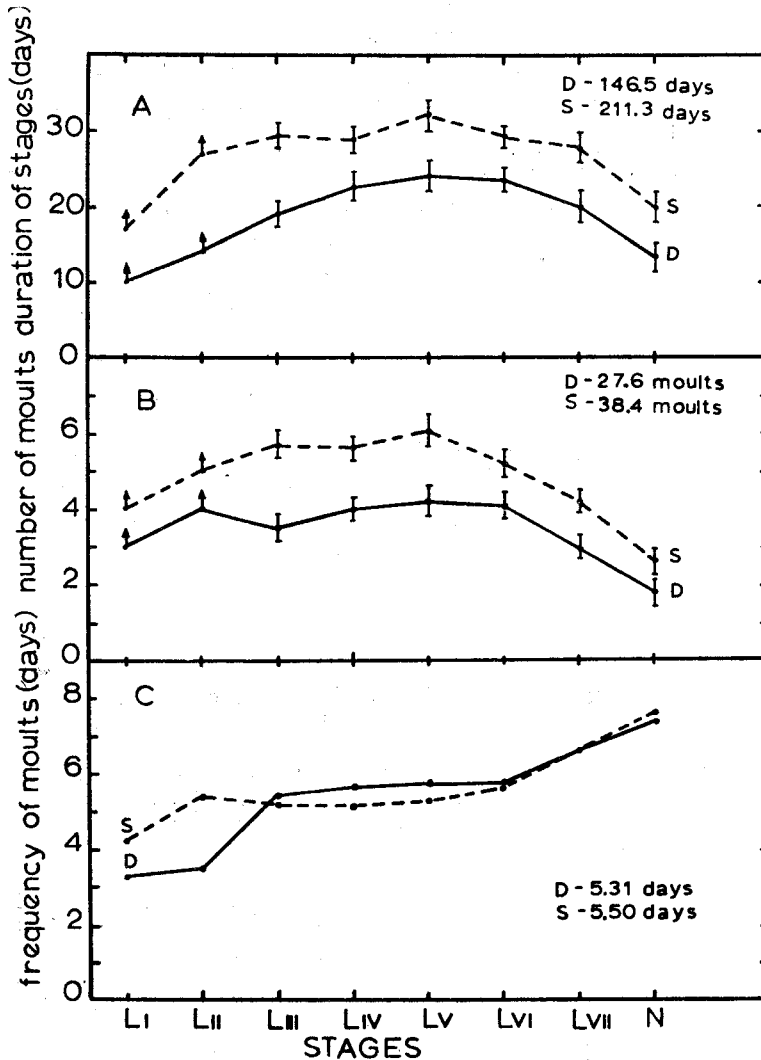

Fig. 2. Development of *Cloeon dipterum* cultures on detritus (D) and *Spirogyra* (S). Vertical lines indicate 95% confidence limits. A — duration of stages, B — number of moults in each stage, C — frequency of moults

The final larval size was the same on each food, but the duration of development and the number of moults differed. The number of moults in the development of *Cloeon dipterum* can vary and depend on trophic conditions for larvae, while the frequency of moults is rather stable. The number of moults can not correspond to the number of size groups as it was suggested by PLESKOT (1958), because the size of a larva is not proportional to the number of moults (KHOO, 1964). In addition to trophic conditions also other environmental factors can influence the number of moults, such as light (KHOO, 1964) and temperature (SCHMIDT, 1951). Schmidt also studied the effect of wounds and subsequent regeneration on the number and frequency of moults in mayfly larvae. A rise in the frequency of moults during regeneration results from an increase in the rate of metabolic processes at that time, and it does not speed up the development. Moults not only enable growth but also represent a form of the excretion of the products of metabolism which are stored in the skin.

Two kinds of it were selected on the basis of food preference study. That study indicates that detritus is the most preferred food, while *Spirogyra* with not destroyed cells is of an average quality (2.01 and 1.55, respectively, as compared to the control taken as 1.00). Calorific values of both kinds of food are similar: 4.100 cal/mg dry wt for *Spirogyra* (IVANOVA, 1958) and 3.950 cal/mg dry wt for detritus (COFFMAN et al., 1971). A delay in the development of the larvae fed on algae probably resulted from

Table I

Comparison of the larval development of Cloeon dipterum cultured on two kinds of food at 20°C. The figures denote t (BAILEY, *1959, comparison of two means) and* $L_{0.05}$ (OKTABA, *1974, 95% confidence interval of differences between two means).* NS — *no significance,* HS — *high significance* (p — *more than* 1%)

Compared features	Food / Sex	Detritus		average animal (♀ and ♂)	average animal (♀ and ♂)	Spirogyra	
		♀♀	♂♂			♂♂	♀♀
Duration of development (days)	mean±SD	152.6±14.6	140.4±21.9	146.5±18.7	211.3±12.8	198.8±16.9	223.8±19.7
	t	1.04—NS		7.13—HS		2.15—NS	
	$(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$	—		64.8±20.1		—	
Number of moults	mean±SD	28.4±1.8	26.8±3.3	27.6±2.6	38.4±2.5	37.2±2.3	39.6±2.3
	t	0.96—NS		9.47—HS		1.66—NS	
	$(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$	—		10.8±2.5		—	
Frequency of moults	mean±SD	5.37±0.18	5.24±0.21	5.31±0.20	5.50±0.37	5.34±0.34	5.65±0.38
	t	1.05—NS		1.43—NS		1.36—NS	
	$(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$	—		—		—	
Maximum body length (mm)	mean+SD	9.16±0.24	8.15±0.43	8.66±0.63	8.80±0.61	8.31±0.43	9.28±0.31
	t	4.59—HS		0.54—NS		4.09—HS	
	$(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$	1.01±0.58		—		0.97±0.62	
Maximum width of head (mm)	mean+SD	1.43±0.07	1.35±0.05	1.39±0.07	1.43±0.08	1.41±0.09	1.45±0.07
	t	1.57—NS		1.19—NS		0.79—NS	
	$(\bar{x}_1-\bar{x}_2)+L_{0.05}$	—		—		—	

$$t = \sqrt{\frac{n_1-1\,(SD_1^2+)\,n_2-1/SD_2^2}{n_1+n_2-2}}\;\frac{\bar{x}_1-\bar{x}_2}{\sqrt{\tfrac{1}{n_1}+\tfrac{1}{n_2}}}\;; \qquad L_{0.05} = t_{0.05}\sqrt{\frac{n_1 SD_1^2+n_2 SD_2^2}{n_1+n_2-2}\cdot\frac{1}{n_1}+\frac{1}{n_2}}.$$

lower assimilation of *Spirogyra*. Because of the absence of cellulase in the *Ephemeroptera*, only injured plant cells can be digested efficiently. Algae are consumend in large quantities but living cells are frequently met in faeces (WISSMAYER, 1926, BROWN, 1960). In the already mentioned experiment on food preference, homogenized algae (*Spirogyra*), thus with destroyed cells, were more preferred (2.14 in relation to the control) to not destroyed algae. This supports the suggestion that the walls of plant cells can not be efficiently digested by larval *C. dipterum*. Particulate detritus, however, consisted of amorphous organic matter and numerous microbes is a readily assimilated food item.

2.3. Biometry

Body length (from the apex of the head to the end of the abdomen), width of head capsule, as well as wet and dry body weights were measured for the successive developmental stages of *C. dipterum*. A total of 1,149 measurements were taken. Statistically significant differences were found in the average values of these parameters between successive stages. Body length (Fig 3A) and width of head capsule (Fig. 3B) increased uniformly from stage to stage, while the increase in the wet and dry body weights (Fig. 4) followed a sigmoid curve. In the nymphs (N_I and N_{II}), subimago and imago, sex can be distinguished. There were generally 113 females and 116 males in a samples, thus each sex contributed to 50% of the total. The females were longer and heavier than the males; the differences between the mean values are highly significant ($p < 0.01$). There were no significant differences (with 95% confidence

interval) in the average width of head capsule between the both sex (Fig. 3B) and in the adult body length (Fig. 3A), probably because of the insufficient number of individuals: $t = 2.16$, $n = 9$ for females and $n = 7$ for males, winged forms not being an essential subject of the present paper.

The percentage content of the dry weight in the body of the larvae was calculated (Fig 5). It slightly decreased in L_I to L_V (from 23.66% to 21.83%) and then increased to 33.72% in the body of adult females and to 41.38% in males.
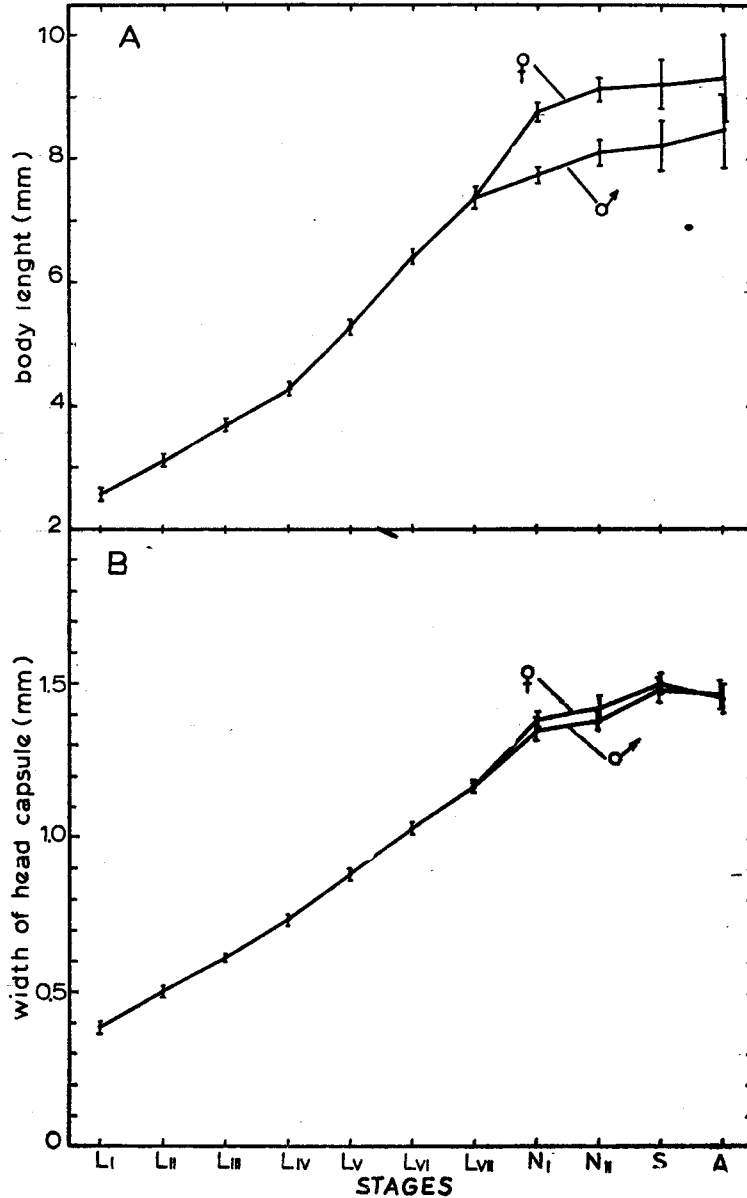

Fig. 3. Average dimension of *Cloeon dipterum* in each stage. Vertical lines indicate 95% confidence limits. A — body length B — width of head capsule. L_I—L_{VII} — larvae, N_I, N_{II} — nymphs, S — subimago, A — adults

The relationships between the wet and dry body weights, on one hand, and body length and head capsule width, on the other, were calculated using the equations of logarithmic regression. To test the significance of the difference in the coefficients a and b between the regressions for two successive stages t-test was used (BAILEY, 1959, SIMPSON et al., 1960). Common regressions were calculated for the stages between which there were not significant differences. The relationship between the dry weight (in mg) and body length (in mm) has a constant value in aquatic larvae of this species and follows
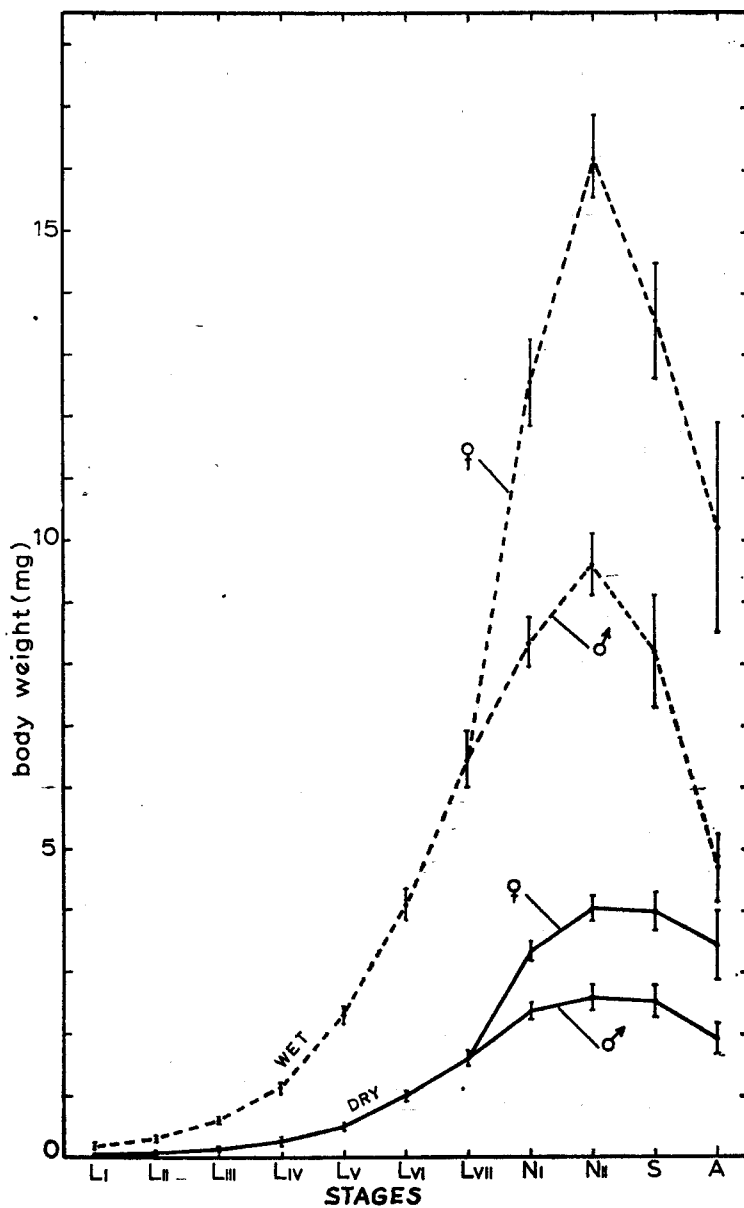
Fig. 4. Average wet and dry body weights of *Cloeon dipterum* in each stage. Vertical lines indicate 95% confidence limits.
L_I—L_{VII} — larvae, N_I, N_{II} — nymphs, S — subimago, A — adults
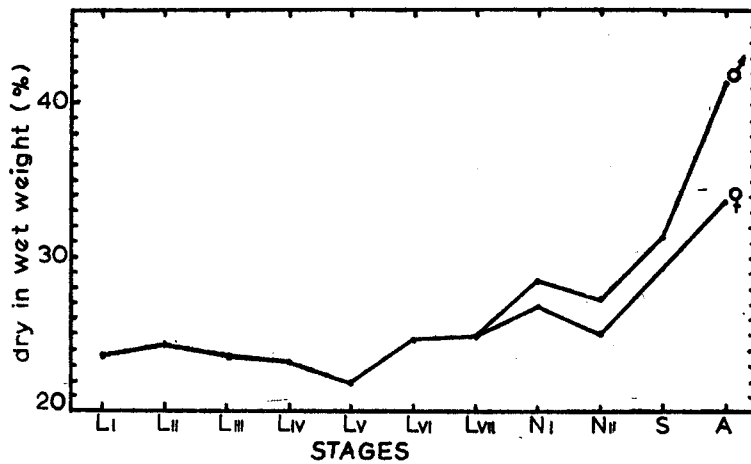

Fig. 5. Percentage of dry weight content in the wet weight throughout the development of *Cloeon dipterum*

the formula: $W_{dry} = 0.001 \cdot L^{3.68}$. Logarithmic form of this equation with 95% confidence interval for a and b is: $\log W_{dry} = (\bar{3}.0155 \pm 0.0342) + (3.6802 \pm 0.0489) \cdot \log L$;

The correlation coefficient $r = 0.98$ indicates for a highly significant relationship between the body length and dry weight of the larvae (Fig. 6). When the regression of the body weight on linear dimensions

Table II

The relation of wet and dry weights (W — in mg) to body length (L — in mm) and width of head-capsule (H — in mm) for Cloeon dipterum. The figures denote t_a and t_b (BAILEY 1959, SIMPSON et al., 1960, comparison of two regression coefficients). NS — no significance, HS — high significance (p — more than 1%)

| Compared regression	Stages	n	Formula regression (with 95% confidence limits of a and b)	r	Differences between coefficient a and b regression in two groups of stages
Wet weight (mg) on body length (mm)	$L_I - L_V$	712	$W = 0.0041 - 0.0054 \cdot L^{3.5342 - 3.7438}$	0.9312	$t_a = 4.37 - HS$
	$L_{VI} - N$	384	$W = 0.0071 - 0.0135 \cdot L^{3.1066 - 3.4212}$	0.9019	$t_b = 3.90 - HS$
Dry weight (mg) on body length (mm)	$L_I - N$	1096	$W = 0.0009 - 0.0011 \cdot L^{3.6313 - 3.7291}$	0.9756	$t_a = 162 - NS$ $t_b = 1.04 - NS$
Wet weight (mg) on width of head (mm)	$L_I - L_V$	712	$W = 2.4155 - 2.7227 \cdot H^{2.8249 - 3.0231}$	0.9089	$t_a = 11.99 - HS$
	$L_{VI} - N$	384	$W = 3.9120 - 4.3152 \cdot H^{2.6909 - 3.0985}$	0.8191	$t_b = 0.25 - NS$
Dry weight (mg) on width of head (mm)	$L_I - L_V$	712	$W = 0.5580 - 0.6229 \cdot H^{2.8394 - 3.0220}$	0.9214	$t_a = 14.18 - HS$
	$L_{VI} - N$	384	$W = 0.9101 - 1.0025 \cdot H^{3.0782 - 3.4806}$	0.8537	$t_b = 3.10 - HS$
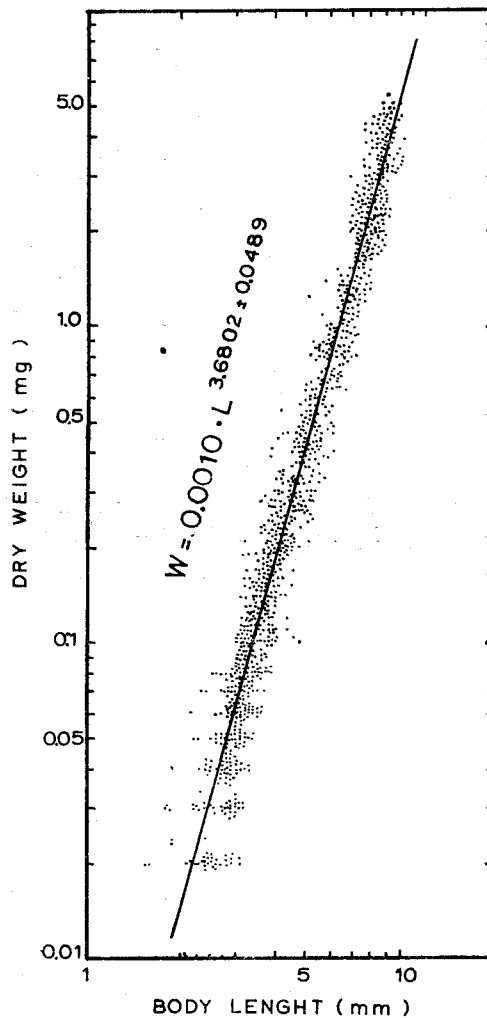
Fig. 6. Dry weight (W — in mg) as a function of body length (L — in mm) for *Cloeon dipterum*

is calculated (Winberg 1968), the coefficient of regression $b = 3$ if growth is not accompanied by the changes in the body shape, $b > 3$ if the ratio of the body dimension to body weight decreases, and $b < 3$ if it increases. The obtained value of $b = 3.68$ indicates for "getting fat" of aquatic forms of this species.

In the remaining cases (Tab. II) the values of a and b for the stages $I_I - L_V$ differ significantly $(Pp < 0.01)$ from those for later stages. The obtained results are in accordance with the statement by TEISSER (1931) that the increase in the wet weight of *C. dipterum* is discontinuous, while the increase in the dry weight is continuous.

The biometrical data suggest that there is a clear tendency towards the occurrence of distinct stages in the development of *C. dipterum*, and they confirm the validity of the accepted criterion for determination of the degree of development. Highly significant relationship between dry weight and body length makes it possible to calculate with great accuracy the weights of the larvae of known sizes.

2.4. Energy value

Energy value of the body and moults of larval *C. dipterum* was found by the method of combustion in a calorimeter. Calorific value increased from 5.0233 cal/mg dry wt in L_I to 6.5011 cal/mg in subimaginal females or 6.1843 cal/mg in subimaginal males (Tab. III). Assuming that the calorific value of the moults has a constant value of 5.4125 cal/mg dry wt throughout the whole period of development, calorific equivalents of the moults in successive stages were calculated (Tab. III).

Table III

Calorific values and calorific equivalents for each developmental stage of Cloeon dipterum

	Stages	Calorific value cal/dry mg (mean+SD)	Calorific equivalent cal/indiv.	Weight of moult in the dry body weight	Calorific equivalent cal/1 moult
	L_I	5.0233±0.0743	0.2009		0.0108
	L_{II}	5.2578±0.1480	0.3943		0.0203
	L_{III}	5.5698±0.5302	0.7798		0.0379
	L_{IV}	5.7092±0.3574	1.4958	5%	0.0709
	L_V	5.7168±0.3044	2.8012		0.1326
	L_{VI}	6.0088±0.1914	6.0869		0.2741
	L_{VII}	6.0716±0.4248	9.7692		0.4354
females	N_I	6.2808±0.3884	21.1475	5%	0.9112
	N_{II}	6.4172±0.4008	25.9640	7.5%	1.6424
	S	6.5011±0.3075	25.9004	13.0%	2.8032
	A	6.4553±0.3185	22.1869	—	—
males	N_I	6.0698±0.3678	14.4461	7.0%	0.9017
	N_{II}	6.1239±0.3204	16.0262	11.5%	1.6289
	S	6.1843±0.3200	15.8998	16.0%	2.2265
	A	6.0045±0.4408	11.7328	—	—
average moults		5.4125±0.0960	—	—	—

The obtained calorific value of *C. dipterum* corresponds to the average calorific value of aquatic invertebrates, which ranges from 4.419 to 6.637 kcal/g dry wt (PRUS, 1970).

Similar values are quoted by TRAMA (1957) for *Stenonema pulchellum*, another species of the order *Ephemeroptera*. Trama also observed an increase in the calorific value of stages; it was 5.290 cal/mg for the 4 mm larvae and 5.710 for 7 mm larvae.

2.5. Respiration

A total of 232 measurements of oxygen consumption by *Cloeon dipterum* at $20\pm0.015°C$ were taken by volumetric respirometer (GRODZIŃSKI and KLEKOWSKI, 1968). The changes were observed in the respiratory rate throughout the developmental period (Fig. 7). A highest respiratory rate of $5.067 \pm 0.911 \,\mu l \, O_2/hr \cdot mg$ dry wt (mean and 95% confidence interval) was found in the young larvae. It dropped considerably to $1.302 \pm 0.111 \,\mu l \, O_2$ in L_{VI} and increased to $2.590 \pm 0.484 \,\mu l \, O_2$ in N_{II} females or to $2.645 \pm 0.367 \,\mu l \, O_2$ in N_{II} males. Then, it decreased in winged forms. There were no differences in the respiratory rate between the males and females in the nymphal stage ($t = 0.36$). The respiratory rate of the males after metamorphosis was higher; the 95% confidence interval for the difference between means is $0.721 \pm 0.029 \,\mu l \, O_2/hr \cdot mg$ dry wt (subimago) and $0.742 \pm 0.299 \,\mu l \, O_2$ (imago).
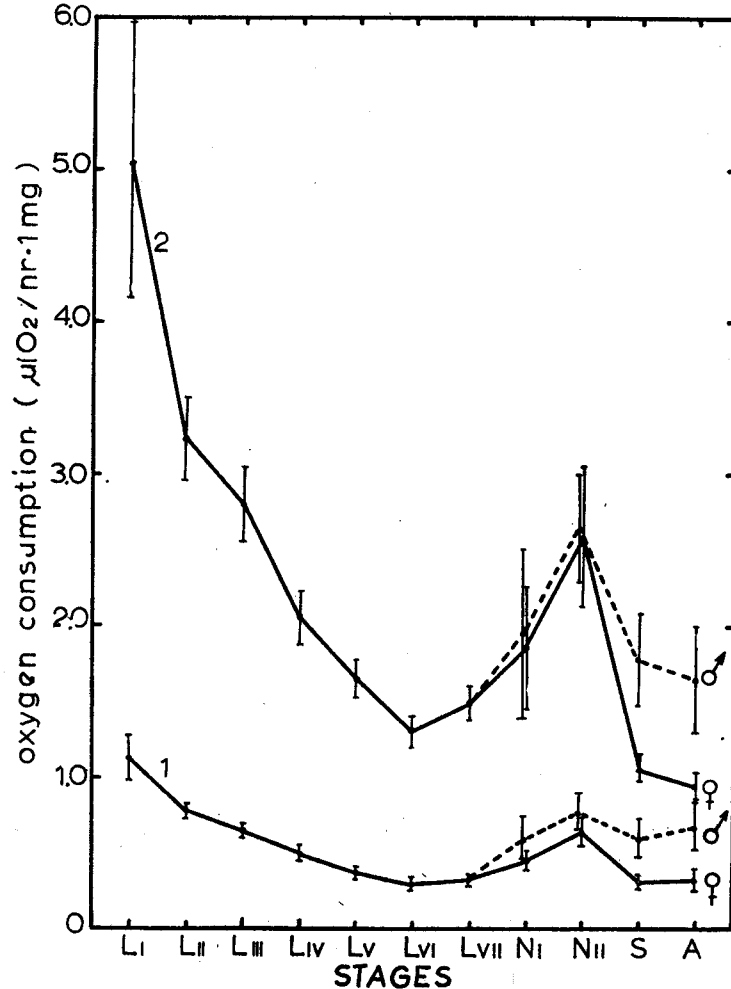

Fig. 7. Oxygen consumption rate by each stage of *Cloeon dipterum*. Vertical lines indicate 95% confidence limits. 1 — per weight, 2 — per dry weight

The amount of oxygen used by an average individual of each stage was calculated (Fig. 8). With at 95% confidence interval the differences in oxygen consumption between successive stages were significant ($p < 0.01$); they indicate, like biometrical measurements, for a distinct character of the stages analysed.

Only the females in the stage ready to emerge (N_{II}) use more oxygen than the males (95% confidence interval for the differences between means is $3.585 \pm 2.510 \,\mu l \, O_2/hr \cdot indiv.$). For the other stages the differences between the males and the females were not significant.

Daily losses of energy respiration (Fig. 8) were calculated multiplying the oxygen consumption (μl O_2/24 hr · indiv.) by 0.005 cal, a calorific equivalent of 1 μl O_2 (TRAMA, 1957). A decrease in the value of calorific equivalent for non-feeding forms (subimago and adult) was 3.7771 cal (females) and 4.2934 cal (males), including 2.8032 cal and 2.2265 cal for moults of subimago, respectively. Daily losses for respiration of the adults were 0.3769 cal and 0.3883 cal per individual. Therefore, a decrease in the calorific equivalent for the winged forms would correspond to 2.6 days of life of the females to 5.3 days of life of the males on the average. INAGAKI (1970) reports that the male of *Cloeon* sp. live about 4 days at 50% air humidity and up to 3 weeks at 100,% humidity. LEHMKUHL and ANDERSON (1970) studied the surviving of adult mayflies of the species *Cinygmula reticulata* and also found that the males could survive more than one day.

The analysis of the relationship between the oxygen consumption and body weight of successive stages shows that some period can be distinguished in the development of *C. dipterum*. During the period including the stages L_I-L_V (115 measurements of the respiration of 0.14—2.82 mg larvae), the respiratory metabolic rate followed the formula $R = 0.48\ W^{0.56}$, where R is respiratory rate in μl O_2/hr · indiv., and W is wet weight in mg, the coefficient of correlation $r = 0.91$. (Fig. 9). Logarithmic form of this equation with 95% confidence interval is: $\log R = (\bar{1}.6810 \pm 0.0191) + (0.5624 \pm 0.0488) \cdot$ · $\log W$. In the next period, involving the stages $L_{VI}-N_{II}$ (76 measurements of the respiration of 1.93—21.24 mg individuals) the regression equation is $R = 0.17\ W^{1.47}$, with $r = 0.96$ (Fig. 9). Logarithmic form of this equation with 95% confidence interval is: $\log R = (\bar{1}.2409 \pm 0.0794) + (1.4658 \pm 0.0955) \cdot \log W$.
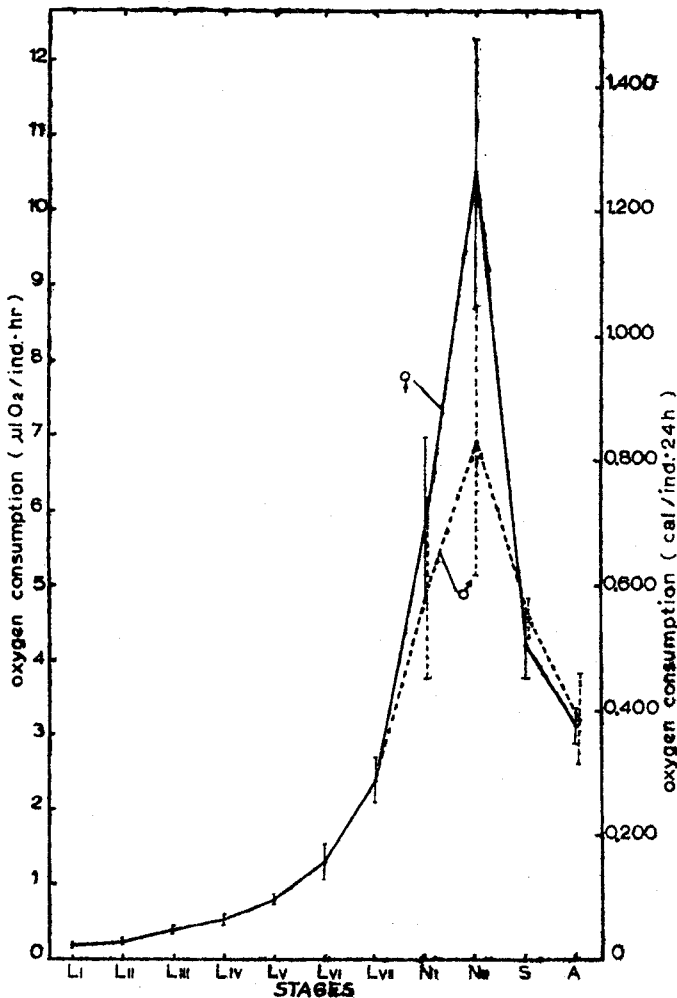

Fig. 8. Oxygen consumption by each stage of *Cloeon dipterum*, at 20°C (mean values with 95% confidence limits)

WINBERG (1956) found that the fol'owing equation for two species of larval *Cloeon* ranging from 0.19 to 10.25 mg in weight: $R = 0.872\ W^{0.88}$. IVANOVA and ALIMOV (1973) calculated a general formula for the relationship between the respiratory rate (in μl O_2/hr · indiv.) and body weight (in mg) in larval *Ephemeroptera* of 0.6—151.0 mg: $R = 1.435\ W^{0.803}$.

The regression calculated in the present study for all measurements of all aquatic stages ($n = 191$) is: $R = 0.55\ W^{(0.835 \pm 0.040)}$. The value of b is similar to that quoted by the above cited authors. Comparing the value of b from this regression with that for the first stages (by t-test, BAILEY, 1959) $t_b = 8.62$ is obtained. When it is compared with b for older stages, $t_b = 12.14$. Consequently, the relationship between the respiration and body weight of *C. dipterum* can not be described by one common regression equation, the difference between such equation and the regressions for particular periods being highly significant ($p < 0.001$).
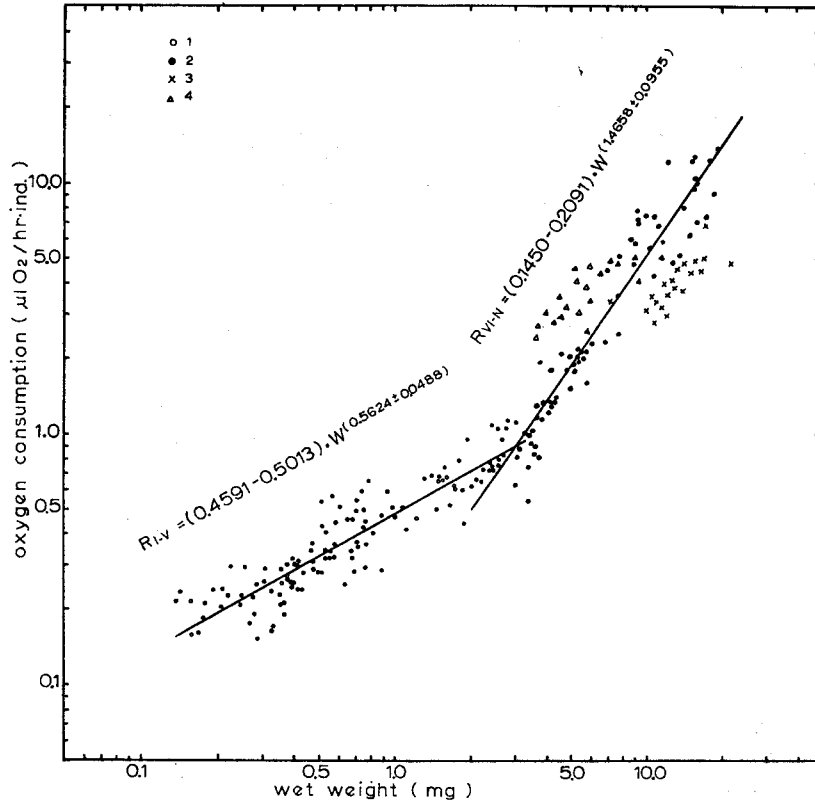

Fig. 9. Oxygen consumption (R — in ul/hr · indiv.) at 20°C as a function of wet weight (W — in mg) for *Cloeon dipterum*. 1 — young larvae, (L_I—L_V), 2 — older forms (L_{VI}—N_{II}), 3 — females (subimago and imago), 4 — males

The above results were obtained using the predictive regression. RICKER (1972) proposed to use the functional regression to describe natural variability of this kind. Because the major part of the variability of R and W is an inherent quality of the material rather than of the measurement technique — geometric mean regression (GM regression) should be used, which minimizes the sum of squared deviations of each point from the line both vertically and horizontally. The comparison of both methods (Tab. IV) indicate that the slope of the GM regression line, v, is higher than that of the predictive regression line (b) but the differences between them are not considerable; for younger stages they are significant with 95% confidence interval and have a value of 0.0579 ± 0.0485, for older stages they are not significant. The value of the difference between coefficient b and v depends on the number of measurements and on the coefficient of correlation (r).

The changes in the metabolic rate during the development of this species and a high value of $b = 1.47$ before metamorphosis are probably related to a rapid development of gonads at that time.

2.6. Changes in the coefficient of net production efficiency

$$K_2 = \frac{P}{P+R} \; ;$$

Energy budget is calculated in energy units (cal) in relation to a definite time period (usually 24 hours). The formula of energy budget shows the rates of food taken by an individual or a population, as a sum of parameters representing different ways of energy utilization. Using the symbols proposed by PETRUSEWICZ (1967) and KLEKOWSKI (1970) the equation of energy budget $C = P + R + FU = A + FU$, where C is consumption (food uptake), P is production, R is respiration (costs of maintenance), A is assimilation (sum of production and respiration, assimilated food less excreta), and FU are rejecta (not utilized part of the food energy). The parameters of the energy budget (C, P, R) can be used for the calculation of the efficiency of energy conversion (IVLEV, 1939, WINBERG, 1956). The following basic indices of efficiency are used in ecology:

$$U^{-1} = \frac{P+R}{C} = \frac{A}{C} \; ; \text{ the efficiency of assimilation,}$$

$$K_1 = \frac{P}{C} \; ; \text{ the gross production efficiency,}$$

$$K_2 = \frac{P}{P+R} = \frac{P}{A} \; ; \text{ the net production efficiency.}$$

From the data presented in preceding chapters, K_2 was calculated for particular stages of *C. dipterum* fed on detritus and *Spirogyra*. It was calculated separately for the males and the females only in the nymphs because when the biometry, calorific values and respiration were analysed, sex differences for L_I to L_{VII} were not taken into account, and any significant differences were found between the males and the females in the duration of these stages or in the number of moults. The duration of
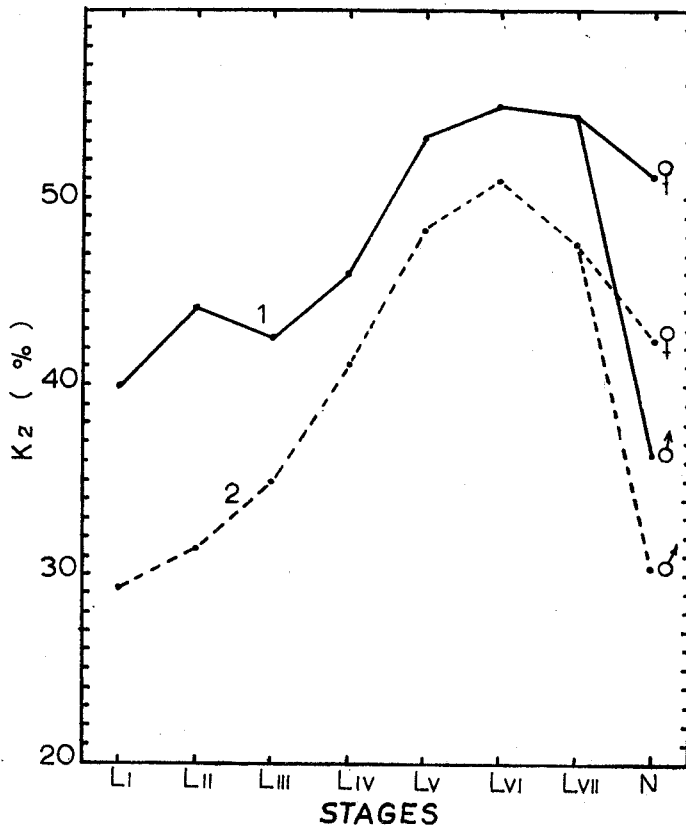

Fig. 10. Changes of instantaneous efficiency K_2 for *Cloeon dipterum* cultured on detritus (1) and *Spirogyra* (2)

Table IV

Comparison of predictive GW functional regression of oxygen consumption on wet body weight

Stages	n	r	equation of regression		confidence limits b	coefficient v
			predictive	GM functional		
L_I—L_V	115	0.91	$R = 0.48 \cdot W^{0.56}$	$R = 0.49 \cdot W^{0.62}$	0.5136—0.6112	0.6203
L_{VI}—N_{II}	76	0.96	$R = 0.17 \cdot W^{1.47}$	$R = 0.16 \cdot W^{1.52}$	1.3703—1.5613	1.5221
♀♀	21	0.88	$R = 0.67 \cdot W^{0.70}$	$R = 0.53 \cdot W^{0.79}$	0.4510—0.9468	0.7906
♂♂	20	0.86	$R = 1.10 \cdot W^{0.69}$	$R = 0.89 \cdot W^{0.80}$	0.4649—0.9159	0.8041

development and number of moults per stage were taken from the cultures maintained at 20°C. Using calorific equivalents, production of the body was calculated, or production due to the body growth (P_g) and production of the moults (P_m) in reach stage. Daily losses of energy for respiration are R values.

It was found that the individuals fed on detritus used for production more of assimilated energy than those fed on *Spirogyra* (Fig. 10). An average production efficiency is 50.5 and 43.6%, respectively. In both the cases the changes in the value of K_2 were observed. For L_I the respective values are about 40 and 29%, then they increase and reach a maximum of about 55 and 51%, respectively, in the final larval stages (L_V, L_{VI}, L_{VII}), a decrease is observed in the nymphs, the respective values being 51 and 42% for the females and 36 or 30% for the males.
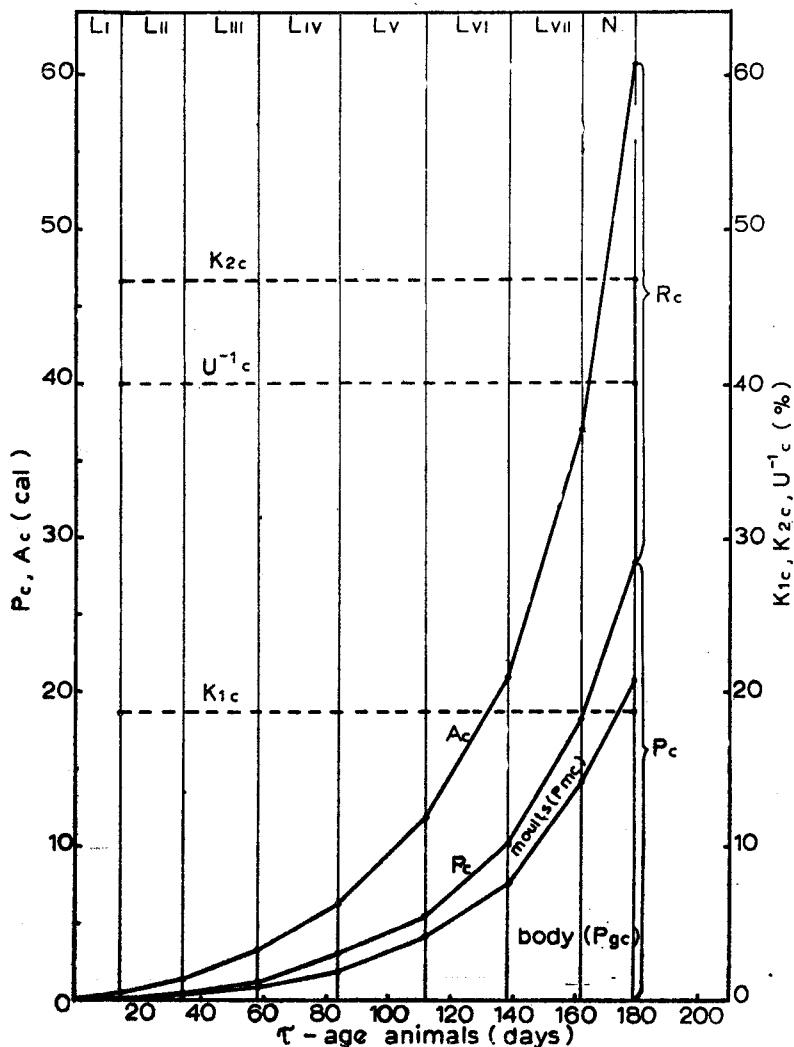
Fig. 11. Cumulative energy budget for an average individual (o and 0) of *Cloeon dipterum*

Similar changes in the efficiency of net production during the development of a terrestrial phyto-phage, *Tribolium castaneum*, were reported by KLEKOWSKI et al. (1967). During the larval development of this species K_2 increased from 13 to 30%, and also decreased before metamorphosis.

Assuming that under natural conditions *Cloeon dipterum* live on mixed food, the cumulative budget for an average individual was calculated (Fig. 11). Summing up average values of the parameters of energy budget, cumulative values of P_c, R_c, and A_c were obtained, and a cumulative coefficient of net

production $K_{2c} = \dfrac{P_c}{P_c + R_c} = \dfrac{P_c}{A_c}$ was calculated; an average value of K_{2c} is 46.7% for the whole

developmental period of *C. dipterum*.

Assuming that assimilation efficiency $U^{-1} = 40\%$ (COFFMAN et al. 1971), gross production efficiency $K_{1c} = 18.7\%$ was calculated. This value lies within a range of 6–37% reported by PHILLIPSON (1966), and slightly exceeds a value of 14.5% obtained by TRAMA (1957) for *Stenonema pulchellum* and corresponds to a value of 18.4% found by LINDEMAN (1941) for progressive efficiency of primary consumers.

2.7. Life cycle of *Cloeon dipterum*

Field samples were taken in a small pond near Warsaw during March, 1972 — December, 1973. A total of 65 samples included 12,024 individuals of *Cloeon dipterum*. The developmental stage of each larva was determined according to the accepted criterion of the degree of development. Percentage of particular stages in the samples was calculated. Two generations of this species per year were observed (Fig. 12). The first generation emerged in May—June, 1972, and in April—May, 1973. The second generation emerged on the turn of July and August in both years. During the autumn and spring young stages (L_{II} and L_{III}) predominated, and they accounted for 50–70% of the total number of individuals caught. This is a period of the slow growth, which is followed by a period of the rapid growth in the summer.
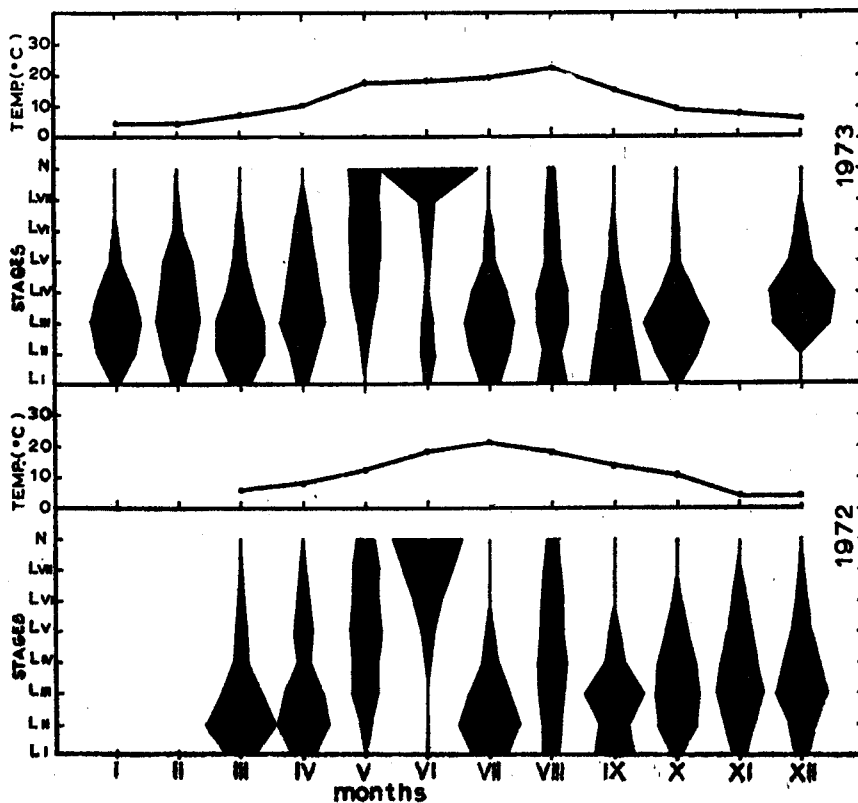

Fig. 12. Life cycle of *Cloeon dipterum* in a pond near Warsaw

LANDA (1968) stated that the development of *Cloeon dipterum* represented type B_1, which included *Ephemeroptera* species overwintering in the form of small, slowly growing larvae, and having two generations per year: a long-living winter generation and a short-living summer generation. Also BRETSCHKO (1965) gives similar characteristics of the life cycle of *Cloeon dipterum*, but MACAN (1961) observed two generations only during very hot summers. BRITTAIN (1974) observed only one generation in Norway. An increase in the growth rate of the lárvae in autumn is not only the effect of a drop in water temperature (MACAN and MAUDSLEY, 1966) but also the effect of the shortening of the length of the day (PLESKOT, 1961). The absence of real diapause, which is replaced by a period of the slow growth in winter, plasticity of the life cycle and possibility of rapid regeneration show that *C. dipterum* is well adapted for the life in small, temporary water bodies (BROWN, 1961a).

SUMMARY

Some study on the biology and bioenergetics of Cloeon dipterum (L.), Ephemeroptera (preliminary data)

In studies on the biology and bioenergetics of cultured *Cloeon dipterum*, morphometric features were applied as criteria of stages in larval development. Eight stages were distinguished on the basis of wing development. It was found that the duration of each stage was dependent on food type. Larvae cultured on two kinds of foods reached approximately the same size, however, the duration of development and number of moults differed. The number of moults was variable and dependent on environmental conditions. It cannot be taken as a basis for estimating the degree of the larval development of this species. However, the frequency of moults is relatively constant. The body length, width of head, wet and dry weights of each larval stage were measured. Statistically significant differences were found in the mean values of these parameters as well as in the mean rate of oxygen consumption between the stages. Therefore, it seems that the chosen criteria can be reasonably used to estimate larval development. The coefficient of net production efficiency K_2 was calculated for all the stages. The value of K_2 showed a small increase (from about 40% to 55%) during the L_I to L_{VII} stages and decreased during the final period of larval development. An analysis of the relationship between metabolic rate and larval body weight revealed the existence of two larval periods in which the relationships differed significantly; these were stages $L_I - L_V$: $R = 0.48 \cdot W^{0.56 \pm 0.05}$ and stages L_{VI} to nymphal stage: $R = 0.17 \cdot W^{1.47 \pm 0.09}$. In the field two generations of *Cloeon dipterum* were found throughout the year — the winter generation (emerging in June) and the summer generation (emerging in August).

DISCUSSION

U. HUMPESCH: How was the photoperiod in your culture?

St. CIANCIARA: "The day" (electric light) was 7 hours long in my experiment.

U. HUMPESCH: Did you find a difference moulting frequency throughout the year like LUBBOCK (1863), LICHTENBERG (1972) found in *Cloeon dipterum?*

St. CIANCIARA: I led my cultures in laboratory at approximately stable conditions (temperature, lighting, food), the differences were smaller than ones observed by these authors in the field.

U. HUMPESCH: Is there a moult between N_I and N_{II}?

St. CIANCIARA: Between N_I and N_{II} I found at least one moult.

U. HUMPESCH: I want to remember to the work of HILMY, she also found differences in respiration peaks in different stages!

St. CIANCIARA: Yes, HILMY (1962) and also IVANOVA (1958) found the similar changes in the respiratory rate during mayfly development.

B. NAGELL: The change in the relation metabolic rate/larval weight between the L_V and L_{VI} stage is very significant. Do you have any opinion about the physiological reason for this?

St. CIANCIARA: I have no good explanation of that today, but I suppose that at this stage of the larval development the gonads start to grow probably.

J. E. BRITTAIN: The term "instar" refers to the stages separated by moulting. Therefore I would suggest that you use term "stage" or "developmental stage" in your studies where several instars are considered together.

St. CIANCIARA: Yes, your suggest is right.

T. SOLDÁN: The term "instar" is used incorrectly I think. According to LUBBOCK, GRANDI and LANDA *Cloeon dipterum* has about 20 instars. It is no right instars. It is "morphological degrees" according to Rawlinson.

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