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# FOOD PREFERENCE OF *CLOËON DIPTERUM* (L) LARVAE AND DEPENDENCE OF THEIR DEVELOPMENT AND GROWTH ON THE TYPE OF FOOD

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## ABSTRACT

Studies on food preference showed that *C. dipterum* larvae feed preferably on organic detritus and homogenized algae (*Spirogyra*). Under regular conditions of laboratory cultures the type of food bears on daily increments in body length, duration of the whole development and the number of moulting, on the other hand, it does not bear on the final size of the body of nymphs of neither sex nor on the frequency of moulting in the course of development.

## 1. INTRODUCTION

The larvae of Ephemeroptera have mouth structure of a biting type (Brown 1961b, Mikulski 1936). They feed mainly on algae and vascular plant tissues and are rarely carnivorous. Organic detritus (from the bottom sediment) may be of great importance in the feeding of Ephemeroptera; Kendeigh (1961) found in the food consumed by ephemerids about 66% of detritus, Minshall (1967) — 50 to 100%, and Coffman et al. (1971) up to 55%. The remaining part of food composition constists of plants (algae — mostly periphyte — and vascular plant tissues); animal food is consumed rarely, occasionally — it makes up about 4% of the diet of Ephemeroptera (Kendeigh 1961).

It is a well known fact that the type of food may have a substantial effect on the development and physiological functions of an organism. The aim of this study was to investigate what kind of food the larvae of *C. dipterum* are taking by preference and which parameters of the development of this species are affected by changes different diets.

## 2. MATERIAL AND METHODS

### Experimental material

*Cloëon dipterum* for laboratory cultures was taken from a small (surface area — 40 $m^2$, depth — about 2 m deep), isolated water body situated near Wawer (southeastward from Warsaw). The examined species — *Cloëon dipterum*

(Linné) of genus *Cloëon* (Leach 1815) (Ephemeroptera : Baetidae) was identified using the keys by: Mikulski (1936), Bogoescu (1958) and Sowa (1975).

Two kinds of food used in the cultures: algae (*Spirogyra*) and organic detritus were taken from the same water body.

Methods of the investigations of food preference of the larvae of *C. dipterum*

Studies on food preferences of *C. dipterum* larvae were carried out using the method of the so-called "enclosures" (or "dvoriks" — after Gaevskaya 1939) — it is a variant of the "cafeteria test". Petri dishes (20 cm in diameter) were divided into 8 equal parts by means of glueing the longer edge of eight glass microscope-slides (2.5 cm × 7.5 cm) to the bottom of the Petri dish. In this way "external" enclosures (Fig. 1A) and "internal" enclosures (Fig. 1B) were

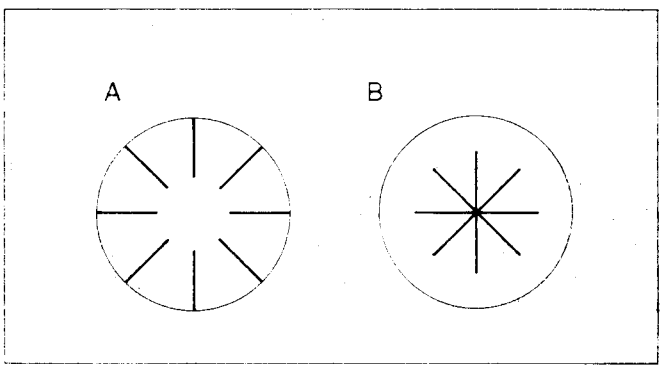


Fig. 1. Schematic draft of "dvoriks" used in the studies on food preference in *C. dipterum* larvae. A — internal enclosures, B — external enclosures

made. Dishes were filled up with water. Food was placed in the set up compartments, leaving one of them without food — as control. In the experiments the following types of food were used:

$A_1$ — algae (*Spirogyra*) — homogenized,
$A_2$ — organic detritus,
$A_3$ — leaves of lettuce — slightly decayed — homogenized,
$A_4$ — algae (*Spirogyra*) — whole, live threads,
$A_5$ — living yeast,
$A_6$ — animal remains (*Cloëon dipterum*, *Tubifex tubifex*) — homogenized,
$A_7$ — animal remains — nonhomogenized,
K — control (without food).

Food preferences of medium-sized (larval stages: $L_{III}$ and $L_{IV}$) and large (stages: $L_V$ and $L_{VI}$) larvae of *C. dipterum* were observed. (Classification of larval stages in groups after Cianciara 1979 a). Two replications of every variant of the experiment (in external and internal enclosures) were carried out in both groups of the larvae; each time 100—150 individuals were introduced into each dish. During 8 to 10 days of the experiment the number of larvae was counted in each compartment twice a day. Altogether 35 observations of medium-sized larvae and 32 observations of large larvae were conducted. Frequency of food selection ($A/K$) was evaluated in relation to the control (in the control $A/K = 1.00$). Food

preference of *C. dipterum* larvae was evaluated as well using the Ivlev's coefficient of food selection (*W*) calculated from equation:

$$W = \frac{x_2 - x_1}{x_2 + x_1}$$

where: $x_1$ — ratio of a given food in the environment ($x_1 = 12.5\%$ — invariably),
$x_2$ — proportional frequency of larvae recorded in the compartment with a given food.

The food tested in the experiment was regarded as "selected", when the Ivlev's coefficient of food selection (1955) was positive ($W \geqslant 0$) and as "avoided", when the obtained values were negative ($W < 0$). The additional estimation of food selection in relation to the control (*K*) has enlarged the range of evaluation of various types of food. Consequently, within the group of "selected food" a group of "good food" has been differentiated (in the case when not only the coefficient, *W* was positive, but when, moreover, the frequency of larvae in the given food was twice higher than in the control, i.e. $A/K \geqslant 2.0$) and a group of "adequate food" (the remaining types of food in the group of the selected food but with a lower frequency of larvae occurrence, i.e. $A/K < 2.0$). In the group of the "avoided food" two sub-groups were distinguished, likewise — "indifferent food" (in the case when the coefficient *W*, was negative, indeed, yet not as low as in the control, i.e. $0 > W > -0.2$) and completely "bad food" (when the obtained values were lower than in the control, i.e. $A/K < 1.0$, while at the same time the coefficient of food selection was lower than the mean value in the control, i.e. $W < -0.2$).

## Methods of the investigations of the effect of two different types of food on duration of development of *C. dipterum*

The development of *C. dipterum* larvae from the stage $L_I$ to the emergence of sub-imago was observed in the laboratory cultures from September 1972 until May 1973. Animals were cultured individually in porcelaine evaporating dishes, 5 cm in diameter, filled with about 20 $cm^3$ of water (aerated). Water temperature (20°C±1.5°C) was measured regularly, artificial light (7 hours a day) was applied.

There were 10 cultures for each of the two food variants:

D — larvae fed on organic detritus,

S — larvae fed on algae (*Spirogyra* — living, whole threads). Food was supplied in excess. Water in the culturing dishes was changed and fresh food provided every 24 hours. Measurements of body lenght (*l*, mm) and width of head discus (*h*, mm) of each individual in the cultures were carried out every 3—4 days. At the same time, stage of development (Cianciara 1979a) and the number of moults were also determined.

Since the larvae taken for experiment were in stage $L_I$ or $L_{II}$ the maximum number of days of these stages duration in the cultures was accepted as the time equal to or shorter than the period of the real duration of these stages. The number of moults in stages $L_I$ and $L_{II}$ were also estimated in the same way. Mean body length and mean width of head discus were calculated on the basis of the results from all the measurements of these parameters in various larval stages. The minimum and maximum values of the body length in various stages were calculated assuming a regular linear increment within each stage. The

increments of body length per stage (mm/stage), per moult (mm/moult) and per day (mm/24 h) were also calculated. Data on the duration of each stage, number and frequency of moults, and the measurements of females and males from both variants of cultures were worked out statistically (after Bailey 1959, Oktaba 1974, Croxton 1975).

Using probability tests ($t$ — for comparison of two mean values of one variable — after Bailey (1959) and $L_{0.05}$ — 95% limit of confidence level of differences between the means — after Oktaba (1974) the measurements of the parameters of the development from the variants of the experiment were compared and the magnitude of the differences was evaluated. It is assumed that at the probability: $p \geqslant 0.05$ — there is no statistical significance (NS), when $0.05 > p \geqslant 0.01$ — low statistical significance (S), when $p < 0.01$ — high statistical significance (HS).

## 3. RESULTS

### A. FOOD PREFERENCES OF *C. DIPTERUM* LARVAE FROM MIDDLE AND OLDER AGE-GROUPS

Results from the experiments did not show any significant differences between the data obtained from the "external" and "internal" enclosures (Figs. 1A and 1B), despites the observed inclination of *C. dipterum* larvae to swim along the walls of the culturing dishes, therefore, the findings from both versions of the experiment are presented together (Fig. 2, Tab. I).

Data from Fig. 2 show clearly that *C. dipterum* larvae of both age-groups selected most frequently homogenized cells of *Spirogyra* algae ($A_1$) and organic detritus ($A_2$) out of various types of food supplied in the experiment. Homogenized, slightly rotten lettuce leaves ($A_3$), algae in the form of living threads ($A_4$) and yeast ($A_5$) were, as regards *C. dipterum* larvae, in the category of indifferent food (the coefficient of food selection — $W = \pm 0$; Tab. I). Homogenized remains of *C. dipterum* and *T. tubifex* ($A_6$), i.e. animal food, were avoided (especially by younger larvae) or decidedly "bad", when nonhomobenized ($A_7$).

Some differences depending on the age-group of the larvae were found as regards the preference of some types of food (Fig. 2, Tab. I). Larvae of the medium age-group (stages $L_{III}$ and $L_{IV}$) distinctly preferred homogenized cells of algae ($A_1$), whereas older larvae (stages $L_V$ and $L_{VI}$) selected more often detritus ($A_2$). Yeast ($A_5$), selected readily by younger larvae, was the avoided (or bad) type of food for older individuals.

### B. DEVELOPMENT OF *C. DIPTERUM* FED IN THE CULTURES ON DETRITUS (D) OR ON LIVING ALGAE — *SPIROGYRA* (S)

Results of *C. dipterum* cultures are shown in Table IIA (larvae fed on detritus — variant D) and in Table IIB (larvae fed on *Spirogyra* — variant S). According to the assumed proposition (see: methods), duration of the larval stages $L_I$ and $L_{II}$ was the same for both sexes, i.e. at least 10 and 14 days, respectively (in the variant D cultures) and 17 and 27 days, respectively (in the variant S cultures). The number

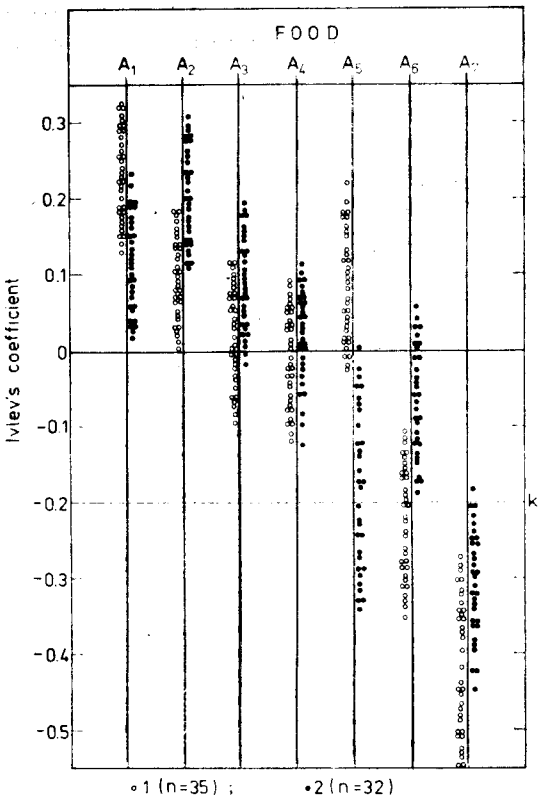


Fig. 2. Food preference in medium (1 — stages $L_{III}$ and $L_{IV}$) and large (2 — stages $L_V$ and $L_{VI}$) larvae of C. *dipterum*, (expressed by Ivlev's coefficient of food selection). $A_1$—$A_7$ — types of food (as in Tab. I). K — control (without food)

of moults in these two stages was equal — at least 3 and 4 (variant D) and 4 and 5 (variant S).

In the investigated cases (Tabs. IIA and IIB, Fig. 3), the larval stage $L_V$ was of longest duration and showed the greatest number of moults; the frequency of moulting decreases with the progressing development of the larvae.

The development of females in the cultures was slightly slower than that of males. The various stages of development lasted longer in females than the respective stages in males: by 0.6—5.0 days (Tab. IIA — variant D) and by 1.0—7.6 days (Tab. IIB — variant S), but only in nymphal stage (N) this difference was statistically significant. The number and the frequency of moults in consecutive stages was similar in individuals of either sex. Females surpassed males in size (Tab. III, Figs. 4A and 4B); already in the stage $L_V$ the differences between the mean body length of males and females were statistically significant. On the other hand, no significant differences were observed in the width of head discus (Tab. III). Absence of statistical differences (Tab. IV) between females and males fed on the same type of food, in respect to the duration of the whole period of their development and

Table I. Food preference in medium ($L_{III}$–$L_{IV}$) and large ($L_V$–$L_{VI}$) larvae of *Cloëon dipterum* (expressed by $A/K$ and by the Ivlev's coefficient of food selection ($W$))

| Food | $L_{III}$–$L_{IV}$ | | $L_V$–$L_{VI}$ | | $L_{III}$–$L_{VI}$ | | | |
|---|---|---|---|---|---|---|---|---|
| | $A/K$ | $W$ | $A/K$ | $W$ | $A/K$ | $W$ | evaluation | |
| $A_1$ Algae (*Spirogyra*) — homogenized | 2.41 | +0.233 | 1.87 | +0.110 | 2.14 | +0.174 | good | selected |
| $A_2$ Organic detritus | 1.81 | +0.093 | 2.33 | +0.216 | 2.07 | +0.152 | good | selected |
| $A_3$ Lettuce leaves — homogenized | 1.56 | +0.019 | 1.80 | +0.091 | 1.68 | +0.052 | adequate | selected |
| $A_4$ Algae (*Spirogyra*) — whole, living threads | 1.49 | −0.004 | 1.65 | +0.047 | 1.57 | +0.020 | adequate | selected |
| $A_5$ Yeast — living | 1.80 | +0.091 | 1.04 | −0.181 | 1.42 | −0.039 | indifferent | avoided |
| $A_6$ Animal remains — homogenized | 0.96 | −0.220 | 1.31 | −0.068 | 1.14 | −0.147 | indifferent | avoided |
| $A_7$ Animal remains — nonhomogenized | 0.62 | −0.416 | 0.79 | −0.310 | 0.70 | −0.365 | bad | avoided |
| K Control (without food) | 1.00 | −0.200 | 1.00 | −0.200 | 1.00 | −0.200 | — | avoided |

total number and frequency of moults, makes possible the comparison of these parameters of the development of an average individual from the experimental variant D and variant S.

It was found (Tab. IV) that in the animals fed on detritus the swarming of sub-imago occurred much earlier and that the total number of moults was less frequent than in the individuals fed on algae; also in various stages of development (Tab. V, Figs. 3A and 3B) the differences between variants D and S were statistically significant. On the other hand, the effect of different types of food on the frequency of moults was not observed (Tab. IV, Fig. 3C).

Females and males fed on detritus attained similar values in body measurements to those of females and males fed on *Spirogyra* (Fig. 4), as well in respective stages of development (Tab. III), as in the maximum values — in nymphs just before the swarming of subimago (Tab. IV).

In both variants of the cultures the increments in the body length of females were slightly higher (NS) than the increments in the body length of males. Regardless of the type of feeding the increment in the body length of an average individual was relatively regular (in the period between stage $L_{III}$ and stage N) amounting to about 1 mm in every consecutive stage (Tabs. IIA and IIB). On the other hand, the elongation of the body per one moult in the larvae fed on detritus was significantly (S) greater than in the larvae fed on *Spirogyra*, whereas statistically highly significant (HS) differences were noted in the daily (mm/24 h) increments in body length. The elongation of the

Table II. Development of *C. dipterum* in laboratory cultures at 20°C

A. Food — detritus (D)

| Sex | Stage | Duration of stage (days) | Number of moults in stage | Frequency of moults (days) | Body length (mm) | | | Increment in body length | | | Head width (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\bar{x}\pm SD$ | $\bar{x}\pm SD$ | $\bar{x}$ | $x\pm SD$ | min. | max. | mm/stage | mm/moult | mm/24h | $x\pm SD$ |
| ♀♀ | $L_I$ | ⩾10 | ⩾3 | (3.33) | 2.12 | — | — | — | — | — | 0.35 |
| | $L_{II}$ | ⩾14 | ⩾4 | (3.50) | 2.68±0.29 | — | — | — | — | — | 0.47±0.10 |
| | $L_{III}$ | 20.0±3.0 | 3.4±0.6 | 5.88 | 3.09±0.53 | 2.85 | 3.64 | 0.79 | 0.232 | 0.040 | 0.57±0.11 |
| | $L_{IV}$ | 24.0±4.4 | 4.2±0.8 | 5.71 | 4.32±0.65 | 3.64 | 4.94 | 1.30 | 0.310 | 0.054 | 0.69±0.12 |
| | $L_V$ | 24.8±2.5 | 4.4±0.6 | 5.64 | 5.58±0.78 | 4.94 | 6.03 | 1.09 | 0.248 | 0.044 | 0.89±0.14 |
| | $L_{VI}$ | 23.8±2.8 | 4.2±0.4 | 5.67 | 6.46±1.03 | 6.03 | 7.15 | 1.12 | 0.267 | 0.047 | 1.10±0.13 |
| | $L_{VII}$ | 20.2±3.6 | 3.0±0.7 | 6.73 | 7.73±0.93 | 7.15 | 8.33 | 1.18 | 0.393 | 0.058 | 1.23±0.16 |
| | N♀♀ | 15.8±1.8 | 2.2±0.4 | 7.18 | 8.80±0.62 | 8.33 | 9.16 | 0.83 | 0.377 | 0.052 | 1.36±0.14 |
| ♂♂ | $L_I$ | ⩾10 | ⩾3 | (3.33) | 2.16 | — | — | — | — | — | 0.37 |
| | $L_{II}$ | ⩾14 | ⩾4 | (3.50) | 2.74±0.52 | — | — | — | — | — | 0.48±0.09 |
| | $L_{III}$ | 18.2±1.6 | 3.6±0.9 | 5.60 | 3.16±0.54 | 2.92 | 3.59 | 0.67 | 0.186 | 0.037 | 0.57±0.10 |
| | $L_{IV}$ | 21.2±2.5 | 3.8±0.4 | 5.58 | 4.09±0.74 | 3.59 | 4.58 | 0.99 | 0.260 | 0.047 | 0.71±0.12 |
| | $L_V$ | 23.4±4.9 | 4.0±1.0 | 5.85 | 5.13±0.62 | 4.58 | 5.47 | 0.89 | 0.222 | 0.038 | 0.86±0.14 |
| | $L_{VI}$ | 23.2±4.8 | 4.0±0.7 | 5.80 | 5.81±0.64 | 5.47 | 6.42 | 0.95 | 0.238 | 0.041 | 1.07±0.15 |
| | $L_{VII}$ | 19.6±7.6 | 3.0±0.7 | 6.53 | 6.94±0.69 | 6.42 | 7.48 | 1.06 | 0.353 | 0.054 | 1.19±0.15 |
| | $N^{00}$ | 10.8±3.3 | 1.4±0.6 | 7.71 | 7.77±0.54 | 7.48 | 8.15 | 0.67 | 0.479 | 0.062 | 1.29±0.13 |

B. Food — *Spirogyra* (S)

| Sex | Stage | Duration of stages (days) | Number of moults in stage | Frequency of moults (days) | Body length (mm) | | | Increment in body length | | | Head width (mm) |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | $\bar{x}\pm SD$ | $\bar{x}\pm SD$ | $\bar{x}$ | $\bar{x}\pm SD$ | min. | max. | mm/stage | mm/moult | mm/24h | $\bar{x}\pm SD$ |
| ♀♀ | $L_I$ | ⩾17 | ⩾4 | (4.25) | 1.98 | — | — | — | — | — | 0.41 |
| | $L_{II}$ | ⩾27 | ⩾5 | (5.40) | 2.47±0.40 | — | — | — | — | — | 0.52±0.11 |
| | $L_{III}$ | 31.8±4.1 | 5.8±0.8 | 5.48 | 3.11±0.50 | 2.76 | 3.67 | 0.91 | 0.157 | 0.029 | 0.61±0.12 |
| | $L_{IV}$ | 30.4±4.2 | 5.8±0.4 | 5.24 | 4.21±0.59 | 3.67 | 4.99 | 1.32 | 0.228 | 0.043 | 0.71±0.12 |
| | $L_V$ | 34.8±4.0 | 6.2±1.1 | 5.61 | 5.88±0.82 | 4.99 | 6.50 | 1.51 | 0.244 | 0.043 | 0.92±0.16 |
| | $L_{VI}$ | 29.8±3.3 | 5.2±0.8 | 5.73 | 7.03±0.84 | 6.50 | 7.51 | 1.01 | 0.194 | 0.034 | 1.14±0.18 |
| | $L_{VII}$ | 29.4±3.2 | 4.4±0.6 | 6.68 | 7.99±1.04 | 7.51 | 8.50 | 0.99 | 0.225 | 0.034 | 1.26±0.18 |
| | N♀♀ | 23.6±2.6 | 3.2±0.4 | 7.38 | 8.91±0.62 | 8.50 | 9.28 | 0.78 | 0.244 | 0.033 | 1.40±0.15 |
| ♂♂ | $L_I$ | ⩾17 | ⩾4 | (4.25) | 2.03 | — | — | — | — | — | 0.33 |
| | $L_{II}$ | ⩾27 | ⩾5 | (5.40) | 2.54±0.48 | — | — | — | — | — | 0.46±0.10 |
| | $L_{III}$ | 27.2±2.6 | 5.6±0.6 | 4.86 | 3.25±0.55 | 2.89 | 3.82 | 0.93 | 0.166 | 0.034 | 0.55±0.12 |
| | $L_{IV}$ | 27.2±2.2 | 5.4±0.6 | 5.04 | 4.38±0.66 | 3.82 | 4.84 | 1.02 | 0.188 | 0.038 | 0.67±0.13 |
| | $L_V$ | 29.4±3.4 | 6.0±0.7 | 4.90 | 5.33±0.75 | 4.84 | 5.67 | 0.83 | 0.138 | 0.028 | 0.88±0.17 |
| | $L_{VI}$ | 28.8±2.0 | 5.2±0.8 | 5.54 | 6.00±0.72 | 5.67 | 6.48 | 0.81 | 0.156 | 0.028 | 1.08±0.17 |
| | $L_{VII}$ | 26.2±2.6 | 4.0±0.7 | 6.55 | 6.92±0.97 | 6.48 | 7.52 | 1.04 | 0.260 | 0.040 | 1.20±0.19 |
| | N♂♂ | 16.0±2.1 | 2.0±0.0 | 8.00 | 7.88±0.55 | 7.52 | 8.31 | 0.79 | 0.395 | 0.049 | 1.36±0.16 |

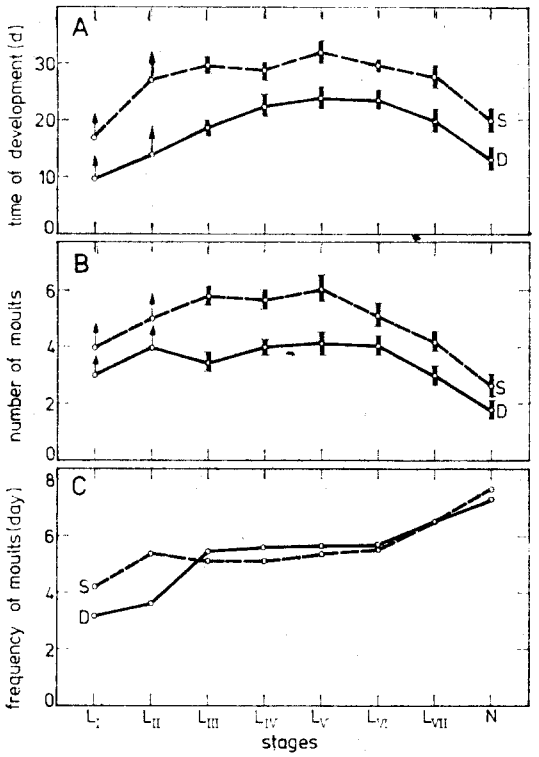


Fig. 3. Development in laboratory cultures of *C. dipterum* fed on detritus (D) and *Spirogyra* (S), at 20°C. Mean values for males and females: A — duration of larval stages (days); B — number of moults in each stage; C — frequency of moults in each stage (days). (A and B: arrows in stages $L_I$ and $L_{II}$ — $x \geqslant$ value; in other stages: $x \pm ci$)

body length by 1 mm lasted about 21 days in the cultures fed on detritus (variant D) and about 28 days when fed on algae (variant S).

## 4. DISCUSSION

*C. dipterum* is regarded as an algaphagous species (Wissmeyer 1926, Bertrand 1954, Ivanova 1958, Brown 1961a), yet under natural conditions, certainly, some detritus is taken, as addition to the diet. From among other algae *Spirogyra* is selected most willingly and may constitute up to about 70% of plant food of *C. dipterum* (Ivanova 1958). Algae may be taken in large, often excessive, quantities (Ivanova 1958) but living cells are often found in faeces of ephemerids (Wissmeyer 1926, Brown 1960). Due to the lack of cellulose in Ephemeroptera only the impaired plant cells are thoroughly digested (Brown 1960). That is undoubtedly the reason why homogenized algae (*Spirogyra*), i.e. with destroyed cells, were selected more willingly in the carried out experiments than complete algae supplied

Table III. Comparison of linear measurements (body length and width of the head discus) of *Cloëon dipterum* fed on detritus (D) and on *Spirogyra* (S)

| Stage | Size | Body length (mm) | | | | Head width (mm) | |
|---|---|---|---|---|---|---|---|
| | Variant: | D♀♀ | D♂♂ | S♂♂ | S♀♀ | D | S |
| $L_I$ | $\bar{x}$ | 2.12 | 2.16 | 2.03 | 1.98 | 0.36 | 0.37 |
| $L_{II}$ | $\bar{x}\pm SD$ | 2.68±0.29 | 2.74±0.52 | 2.54±0.48 | 2.47±0.40 | 0.48±0.09 | 0.49±0.11 |
| | $t$ | 0.39—NS | | 0.55—NS | | 0.41—NS | |
| $L_{III}$ | $\bar{x}\pm SD$ | 3.09±0.53 | 3.16±0.54 | 3.25±0.55 | 3.11±0.50 | 0.57±0.10 | 0.58±0.12 |
| | $t$ | 0.48—NS | | 1.23—NS | | 0.57—NS | |
| $L_{IV}$ | $\bar{x}\pm SD$ | 4.32±0.65 | 4.09±0.74 | 4.38±0.66 | 4.21±0.59 | 0.70±0.12 | 0.69±0.12 |
| | $t$ | 1.32—NS | | 1.25—NS | | 0.50—NS | |
| $L_V$ | $\bar{x}\pm SD$ | 5.58±0.78 | 5.13±0.62 | 5.33±0.75 | 5.88±0.82 | 0.88±0.14 | 0.90±0.16 |
| | $t$ | 3.11—HS | | 3.33—HS | | 0.87—NS | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 0.45±0.29 | | 0.55±0.33 | | — | |
| $L_{VI}$ | $\bar{x}\pm SD$ | 6.46±1.03 | 5.81±0.64 | 6.00±0.72 | 7.03±0.84 | 1.08±0.14 | 1.11±0.18 |
| | $t$ | 3.09—HS | | 5.96—HS | | 1.10—NS | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 0.65±0.43 | | 1.03±0.35 | | — | |
| $L_{VII}$ | $\bar{x}\pm SD$ | 7.73±0.93 | -6.94±0.69 | 6.92±0.97 | 7.99±1.04 | 1.21±0.16 | 1.23±0.19 |
| | $t$ | 3.63—HS | | 4.71—HS | | 0.65—NS | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 0.79±0.44 | | 1.07±0.46 | | — | |
| Nymphs | $\bar{x}\pm SD$ | 8.80±0.62 | 7.77±0.54 | 7.88±0.55 | 8.91±0.62 | 1.33±0.14 | 1.38±0.16 |
| | $t$ | 6.11—HS | | 6.43—HS | | 1.64—NS | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 1.03±0.39 | | 1.03±0.33 | | — | |

Table IV. Comparison of the parameters development of *C. dipterum* fed in the cultures on detritus (D) and *Spirogyra* (S) (at 20°C)

| Index parameters | Food variant: | Detritus | | | *Spirogyra* | | |
|---|---|---|---|---|---|---|---|
| | | D♀♀ | D♂♂ | $D_{mean}$ | $S_{mean}$ | S♂♂ | S♀♀ |
| Duration of development (days) | $\bar{x}\pm SD$ | 152.6±14.6 | 140.4±21.9 | 146.5±18.7 | 211.3±21.8 | 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 | $\bar{x}\pm SD$ | 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 (days) | $\bar{x}\pm SD$ | 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 | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | — | | — | | — | |
| Max. body length (mm) | $\bar{x}\pm SD$ | 9.16±0.24 | 8.15±0.43 | 8.66±0.63 | 8.80±0.63 | 8.31±0.43 | 9.28±0.31 |
| | $t$ | 4.59—HS | | 0.54—NS | | 4.09—HS | |
| | $(\bar{x}_1-\bar{x}_2)+L_{0.05}$ | 1.01±0.58 | | — | | 0.97±0.62 | |
| Max. head width (mm) | $\bar{x}\pm SD$ | 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 | |
| | $(\bar{x}_1-\bar{x}_2)+L_{0.05}$ | — | | — | | — | |

Table V. Comparison of the parameters development of *C. dipterum* fed in the cultures on detritus (D) and *Spirogyra* (S)

| Stage | Parameter of development | Duration of stage (days) | | Number of moults in stage | | Frequency of moults | |
|---|---|---|---|---|---|---|---|
| | variant: | D | S | D | S | D | S |
| $L_I$ | $\bar{x}$ | ⩾10 | ⩾17 | ⩾3 | ⩾4 | 3.33 | 4.25 |
| $L_{II}$ | $\bar{x}$ | ⩾14 | ⩾27 | ⩾4 | ⩾5 | 3.50 | 5.40 |
| $L_{III}$ | $\bar{x}\pm SD$ | 19.1±2.5 | 29.5±4.0 | 3.5±0.7 | 5.7±0.7 | 5.46 | 5.18 |
| | $t$ | 6.97—HS | | 9.80—HS | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 10.4±3.3 | | 2.2±0.7 | | — | |
| $L_{IV}$ | $\bar{x}\pm SD$ | 22.6±3.7 | 28.8±3.6 | 4.0±0.7 | 5.6±0.5 | 5.65 | 5.14 |
| | $t$ | 3.80—HS | | 5.97—HS | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 6.2±3.6 | | 1.6±0.6 | | — | |
| $L_V$ | $\bar{x}\pm SD$ | 24.1±3.8 | 32.1±4.5 | 4.2±0.8 | 6.1±0.9 | 5.74 | 5.26 |
| | $t$ | 4.30—HS | | 5.08—HS | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 8.0±4.1 | | 1.9±0.8 | | — | |
| $L_{VI}$ | $\bar{x}\pm SD$ | 23.5±3.7 | 29.3±2.7 | 4.1±0.6 | 5.2±0.8 | 5.73 | 5.63 |
| | $t$ | 4.00—HS | | 3.57—HS | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 5.8±3.2 | | 1.1±0.7 | | — | |
| $L_{VII}$ | $\bar{x}\pm SD$ | 19.9±5.6 | 27.8±2.9 | 3.0±0.7 | 4.2±0.6 | 6.63 | 6.62 |
| | $t$ | 3.96—HS | | 4.13—HS | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 7.9±4.4 | | 1.2±0.6 | | — | |
| N | $\bar{x}\pm SD$ | 13.3±3.65 | 19.8±4.6 | 1.8±0.6 | 2.6±0.7 | 7.39 | 7.62 |
| | $t$ | 3.50—HS | | 2.69—S | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 6.5±4.1 | | 0.8±0.7 | | — | |
| N♀♀ | $\bar{x}\pm SD$ | 15.8±1.8 | 23.6±2.6 | 2.2±0.4 | 3.2±0.4 | 7.18 | 7.38 |
| | $t$ | 5.52—HS | | 3.51—HS | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 7.8±3.6 | | 1.0±0.7 | | — | |
| N | $\bar{x}\pm SD$ | 10.8±3.3 | 16.0±2.1 | 1.4±0.6 | 2.0±00. | 7.71 | 8.00 |
| | $t$ | 2.97—HS | | 3.01—S | | — | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | 5.2±4.5 | | 0.8±0.8 | | — | |

intact. As regards other types of food used in the experiments testing food preferences in *C. dipterum*, such as: homogenized lettuce leaves and yeast, they were, certainly, easily accessible (subject to the action of digestive enzymes) but nonspecific for the examined species. Brown (1961a) emphasizes the fact that individuals of the species *C. dipterum* under laboratory conditions pick out from among the supplied food most often that kind of food that they find commonly in their natural environment. On the other hand, they avoid noticeably the algae

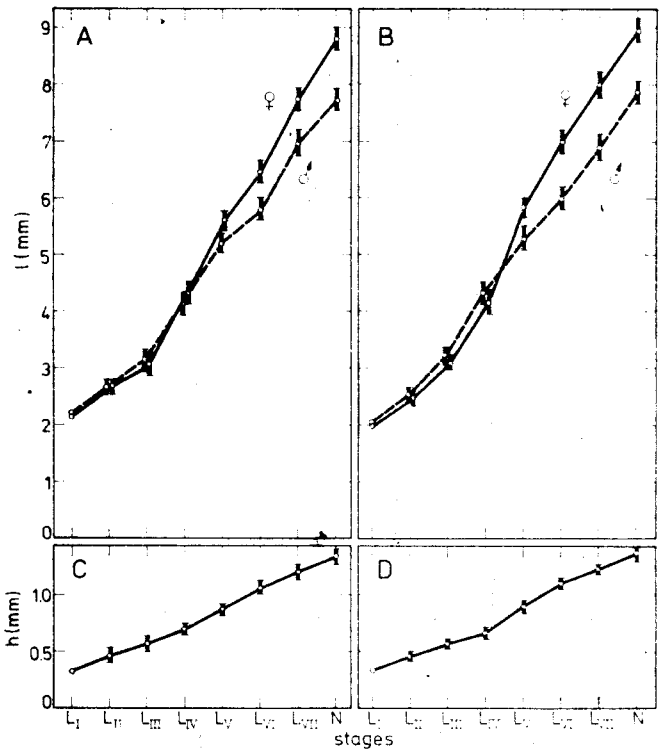


Fig. 4. Changes in body length ($x \pm ci$) of *C. dipterum* fed on detritus (A, C) and on algae — *Spirogyra* (B, D) in laboratory cultures (at 20°C). A, B — body length ($l$, mm) — males and females, C, D — width of head discus ($h$, mm) — males and females

Cyanophyceae and living animals (B r o w n 1961a). The phenomenon of cannibalism, observed sometimes in the laboratory cultures of the larvae of *C. dipterum* occurs sporadically and only under the conditions of starvation.

The observations of the development of *C. dipterum* in the laboratory cultures show that the number of moults in this species of Ephemeroptera is variable (the minimum — 27, the maximum — 40) and may be dependent on the feeding conditions. C l i f f o r d (1970) also reports — by example of *Leptophlebia cupida* (Say) — that nymphs in the course of their development may pass through a varying number of moults and that larger nymphs may be physiologically younger than individuals larger in size. The size of the larvae does not have to be proportional to the number of moults (K h o o 1964, S c h m i d t 1951).

Besides feeding conditions other environmental agents, such as, e.g. temperature (S c h m i d t 1951), light (K h o o 1964) may also offect the number of moults. Hence, the differences in the data given in the literature in respect of the number of moults in the course of the development of ephemerids — M i k u l s k i (1936) gives for Ephemeroptera

Table VI. Comparison of the increments in body length of *Cloëon dipterum* fed on detritus (D) and *Spirogyra* (S)

| Increment in body length | Food | detritus | | | *Spirogyra* | | |
|---|---|---|---|---|---|---|---|
| | variant: | D♀♀ | D♂♂ | $D_{mean}$ | $S_{mean}$ | S♂♂ | S♀♀ |
| mm/stage | $\bar{x}\pm SD$ | 1.05±0.20 | 0.87±0.17 | 0.96±0.21 | 1.00±0.17 | 0.90±0.11 | 1.09±0.27 |
| | $t$ | 1.68—NS | | 0.45—NS | | 1.60—NS | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | — | | — | | — | |
| mm/moult | $\bar{x}\pm SD$ | 0.304±0.067 | 0.290±0.108 | 0.294±0.079 | 0.216±0.042 | 0.217±0.097 | 0.215±0.034 |
| | $t$ | 0.27—NS | | 2.16—S | | 0.05—NS | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | — | | 0.078±0.072 | | — | |
| mm/24 h | $\bar{x}\pm SD$ | 0.049±0.007 | 0.046±0.010 | 0.048±0.008 | 0.036±0.003 | 0.036±0.008 | 0.036±0.006 |
| | $t$ | 0.60—NS | | 3.44—HS | | 0.00—NS | |
| | $(\bar{x}_1-\bar{x}_2)\pm L_{0.05}$ | — | | 0.012±0.008 | | — | |

generally: 19—30 moults in the course of their development, Schmidt (1951) reports that the number of moults varies and may come up to as much as 50, whereas, Bretschko (1965) noted in the development of *C. dipterum* 20—21 moults. Individuals of the same species may show a different number of moults in various generations; Schmidt (1951) gives: 27—32 moults in summer generation and 40—45 moults in winter generation of *C. dipterum*. Moreover, Schmidt (1951) investigated the effect of injuries and consequent regeneration of the larvae of ephemerids on the number and frequency of the moults. The increased frequency of moults comes as a result of the increased intensity of metabolic processes during the time of regeneration. Moults are connected not only with the growth of larvae but are also a form of excretion of the products of metabolic processes, accumulated in the exuviae.

In the laboratory cultures, regardless of the type of the supplied food, a decrease in the frequency of moults was observed together with the progressing development of the cultured individuals (from every 3—4 days to every 8 days). When the relative stability of the conditions of development is maintained, which may occur only in the laboratory culture, this regularity has physiological grounds since younger individuals with a higher degree of metabolism must moult more often than the older ones. Mikulski (1936) reports likewise that young larvae of Ephemeroptera moult more often, i.e. every 4—5 days whereas the older one — every 10—12 days. Schmidt (1951) observed, also, in the development of *C. dipterum* a decrease in the frequency of moulting with increasing age: very young larvae moulted every 1—3 days, older individuals every 5—7 days, whereas, nymphs every 14 days. Under natural conditions besides physiological factors environmental agents (changes of the temperature, light, fluctuations of food resources, etc.) exert also an influence upon the frequency of moulting. According to Bretschko (1965) — moulting in *C. dipterum* occurs in autumn (September) every 3 days, in winter (December—February) not oftener than every 14 days, whereas in spring (March—April) again more frequently i.e. every 10 days. Humpesch (1975) noted in the development of three species of mountain ephemerids (*Baetis alpinus, B. lutheri* and *B. rhodani*) moultings in winter — every 14 days in young larvae and every 20 days in older individuals, whereas in summer — every 7 days.

Furthermore, it has been found in the present investigations that the type of the supplied food has a substantial effect not only on the number of moults but also on the rate of development of *C. dipterum* (the duration of the development was either about 5 months or more than 7 months). The duration of consecutive stages was nearly equal, except stage $L_V$ lasting a slightly longer time (in this stage the number of moultings was greater than in any other stage).

Under natural conditions (Cianciara 1979b) the duration of the whole development of *C. dipterum* may range from 2 months (summer generation) up to 10 months (winter generation); young larvae (stages $L_{II}$—$L_{IV}$) predominate during the longest period of about 150 days of retarded growth (September—March).

The increments in the body length of the cultured individuals of *C. dipterum* were fairly equal amounting to about 1 mm in every

stage of development (on the average during the time of 21 or 28 days depending on the type of food; increments per one moult — 0.22 mm or 29 mm, respectively. Trama (1957) noted in laboratory cultures (temperature 20°C, plant feeding), likewise, a relatively constant growth rate in *Stenomena pulchellum* — the increment of the body length by 1 mm lasted on the average 33 days (about 0.28 mm per one moult). Under natural conditions the increments in body length vary in the course of the year (Cianciara 1979b); the lengthening of the body by 1 mm takes less than 20 days in the late spring and summer, about 40 days — in autumn and early spring, whereas, during about five autumn-winter months the growth of the animals is restrained.

It should be emphasized that regardless of the type of food the individuals in *C. dipterum* cultures attained approximately the same body size (as well in various larval stages as in the the final stage before the metamorphosis — in nymphs of either sex), whereas, the observations under natural conditions (Cianciara 1979b) showed that short, summer generation was represented by individuals smaller than those from winter generation.

The two types of food (organic detritus and algae — *Spirogyra*) used in the cultures were selected on account of abundant occurrence in the natural environment and well-known from the literature (Ivanova 1958, Brown 1960, Kendeigh 1961, Minshall 1967, Coffman et al. 1971). The importance of this type of food in the diet of *C. dipterum* was fully confirmed in the present studies on food preference. It has been found that individuals fed on detritus or *Spirogyra* attained approximate final values of body size, but in a different time and the cost of different numher of moults. Since calorific values of both types of food are approximate (*Spirogyra* — 4100 cal/mg, after Ivanova 1958, detritus — 3950 cal/mg, after Coffman et al. 1971), the cause of a considerable retardation in the development of *C. dipterum* individuals is probably connected with a worse "quality" of this food. Algae used in monoculture as food are possibly less useful physiologically and are less rich as regards chemical composition. They contain small quantities of proteins and fats and large quantities of ash carbohydrates in the form of cellulose (Blažka 1966) hardly digested by Ephemeroptera (Wissmeyer 1926, Brown 1960). Thus, algae in the form of living threads constituted a type of food not easily assimilable. On the other hand, crumbled detritus, containing algal cells, rotifers, plant and animal remains, amorphous organic matter and numerous microorganisms (Coffman et al. 1971) was diversified and easily assimilable. So, detritus evaluated in the food preference tests as food selected by *C. dipterum* larvae more frequently than algae proved to be also more useful physiologically. Higher daily increments in body length were obtained and quicker and more economical development was observed, due to a shorter duration and lower number of moults. On the other hand, the type of food did not bear on the final body size of individuals of neither sex nor on the frequency of moults in the course of development of the larvae.

It may be expected that the results obtained from the cultures up to now do not exhaust potential possibilities of the given species. Maybe that another diet, for instance a mixture of detritus and homogenized algae, as well as an appropriate adjustment of the remaining

experimental conditions (e.g. higher temperature, a longer exposure to light, etc.) would shorten the duration of the development and lessen the number of moultings.

## 5. SUMMARY

Food selection by medium and large larvae was investigated in laboratory experiments (using the "dvorkis" method after Gaevskaya 1939). Food preferences were evaluated in relation to the control, Ivlev's tests (1955). Organic detritus and homogenized algae (*Spirogyra*) were selected by *C. dipterum* larvae by preference from among seven types of food used in the experiment.

Development of *C. dipterum* fed on detritus or living algae (*Spirogyra*) was observed in laboratory cultures (20°C) from the larval stage $L_I$ untill the emergence of subimago. It has been found that duration of every consecutive stage and of the whole development, as well as the number of moults in the course of development of the investigated species may be variable and dependent on feeding conditions. The better type of food (in this case: detritus) gave in effect a quicker development, a lower number of moults and higher daily increments in body length. On the other hand, the type of the supplied food had no effect on the final size of the body of the nymphs of either sex and the frequency of moults (likewise, independent of the type of food) decreased with the progressing development of *Cloëon dipterum* larvae.

## 6. STRESZCZENIE

W doświadczeniach laboratoryjnych badano (metodą „podwórek" — wg Gaevskaya 1939) wybiórczość pokarmową średnich i dużych larw *C. dipterum*. Preferencję pokarmową oceniano w stosunku do kontroli i testem Ivlev'a (1959). Spośród zaoferowanych w doświadczeniu 7 rodzajów pokarmów, najchętniej przez larwy *C. dipterum* wybierany był detritus organiczny i glony (*Spirogyra*) — homogenizowane.

W hodowlach laboratoryjnych (w 20°C) śledzono od stadium $L_I$ do wylotu subimago, rozwój *C. dipterum* karmionych detritusem lub żywymi glonami (*Spirogyra*). Stwierdzono, że długość trwania zarówno poszczególnych stadiów, jak i całego rozwoju, oraz ilość wylinek w rozwoju badanego gatunku mogą być zmienne i uzależnione od warunków pokarmowych. Lepszy pokarm (tu: detritus) powodował szybszy rozwój i z mniejszą ilością wylinek oraz wyższe dobowe przyrosty długości ciała. Rodzaj pokarmu nie wpływał natomiast na finalne rozmiary ciała nimf obu płci, a częstotliwość wylinek (również niezależnie od rodzaju pokarmu) malała wraz z rozwojem *Cloëon dipterum*.

## 7. REFERENCES

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

Bertrand, H. 1954. Les insects aquatiques d'Europe. Les Ephemeroptère., *Encyclopédie entomologique*. 30 (1), 215—266, Paris, P. Lechevalier.

Blažka, P. The ration of crude protein, glycogen and fat in the individual steps of the production chain. *Hydrobiol. Stud.*, 1, 395—408.

Bogoescu, C. 1958. *Fauna Republicii Populare Romine. Insecta, Ephemeroptera*, 7 (3), 1—187. Bucuresti, Edit. Acad. Republicii Populare Romine.

Bretschko, G. 1965. Zur Larvenentwicklung von *Cloëon dipterum, Cloëon simile, Centroptilum luteolum* und *Baetis rhodani*. *Z. wiss. Zool.*, 172 (1/2), 17—36.

Brown, D. S. 1960. The ingestion and digestion of algae by *Cloëon dipterum* L. (Ephemeroptera). *Hydrobiologia*, 16, 81—96.

Brown, D. S. 1961a. The food of the larvae *Cloëon dipterum L.* and *Baetis rhodani* (Pictet) (Insecta, Ephemeroptera). *J. anim. Ecol.*, 30, 55—75.

Brown, D. S. 1961b. The morphology and functionning of the mouthparts of *Cloëon dipterum* L. and *Baetis rhodani* (Pictet) (Insecta, Ephemeroptera). *Proc. Zool. Soc. Lond.*, 136, 147—176.

Cianciara, S. 1979b. Life cycle of *Cloëon dipterum* (L.) in natural environment. *Pol. Arch. Hydrobiol.*, 26, 501—513.

Cianciara, S. 1979a. Some study on the biology and bioenergetics of *Cloëon dipterum* (L.) Ephemeroptera (preliminary data). In: Pasternak, K., Sowa, R. [Eds.]. Proc. Second. Int. Conf. Ephem, August 23—26, 1975. Cracow, (4), 501—513.

Clifford, H. F. 1970. Analysis of a northern mayfly (Ephemeroptera) population with special reference to allometry of size. *Can. J. Zool.*, 48, 305—316.

Coffman, W. P., Cummins, K. W., Wuycheck, J. C. 1971. Energy flow in a woodland stream ecosystem. *Arch. Hydrobiol.*, 68, 232—276.

Croxton, F. E. 1975. *Elementary statistics with applications in medicine and the biological sciences.* New York, Dover Publ.

[Gaevskaya, N. S.] Гаевская, Х. С. 1939. Прибор для изучения питания водных животных (дворики) [The aparature for study on the food preference of aquatic animal]. *Zool. Zh.*, 18 (6).

Humpesch, U. 1975. Autökologische Untersuchungen zum Entwicklungszyklus einiger mitteleuropäischer Ephemeropterenarten. Proc. 2nd int. Conf. Ephemeroptera, Cracov, Poland.

[Ivanova, S. S.] Иванова, С. С. 1958. Питание личинок некоторых поденок [Feeding of larvae of some mayflies]. *Tr. Mosk. Tekhnol. Inst. rybn. prom. Khoz.*, 9, 102—120.

[Ivlev, V. S.] Ивлев, В. С. 1955. *Экспериментальная экология питания рыб* [*Experimental ecology of fish feeding*]. Moskva, „Pishchepromizdat".

Kendeigh, S. C. 1961. *Animal ecology.* Englewood Cliffs, New Jersey, Prentice-Hall.

Khoo, S. G. 1964. Studies on the biology of *Capnia bifrons* (Newmann) and notes on the diapause in nymphs of this species. *Gewäss. Abwass.* 34/35, 23—30.

Mikulski, J. S. 1936. Jętki (Ephemeroptera) [Mayflies (Ephemeroptera)]. *Fauna słodkowodna Polski*, 15, 1—168, Warszawa, Kasa im. Mianowskiego.

Minshall, G. W. 1967. Role of allochthónous detritus in the trophic structure of a woodland springbrook community. *Ecology*, 48, 139—149.

Oktaba, W. *Elementy statystyki matematycznej i metodyka doświadczalna* [*Elements of matematical statistics and experimental methods*]. Warszawa, PWN.

Schmidt, H. 1951. Amputation und Regeneration von Schwanzfaden und Abdomidalsegmenten bei Larven der Ephemeridenart *Cloëon dipterum* L. und ihr Einfluss auf die Hautungsintervalle. *Zool. Jb. Abt. Zool. Physiol.*, 62, 395—428.

Sowa, R. 1975. What is *Cloëon dipterum* (Linnaeus, 1761)?. The nomenclatural and morphological analysis of a group of the European species of *Cloëon* Leach (Ephemerida: Baetidae). *Entomol. scand.*, 6, 215—223.

Trama, F. B. 1957. The transformation of energy by an aquatic herbivore *Stenomena pulchellum* (Ephemeroptera). Ph.D. Dissertation, Univ. Michigan.

Wissmeyer, A. 1926. Nahrungsuntersuchungen bei Ephemeridenlarven. *Arch. Hydrobiol.*, 16, 668—698.