STAGES AND PHYSIOLOGICAL PERIODS IN THE DEVELOPMENT OF *CLOEON DIPTERUM* (L.) (BAETIDAE)

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ABSTRACT

The postlarvullar period in the development of *C. dipterum* covers eight aquatic stages, which differ in morphology, biometry and respiration value. Body size, number of moults, and the duration of development may vary, and may depend on environmental conditions. With the constant increase of dry weight in relation to body length, and the constant increase of body caloric value there occur two periods which vary in physiological processes: period I (L_I - L_V stages), is characterized by the accelerated increase of wet weight and body hydration and period II, (L_{VI} - N stages), is characterized by increased oxygen consumption and a decrease in body hydration.

INTRODUCTION

Cloeon dipterum, an extremely common species, frequently exhibiting mass occurrence, may have an important ecological role. Unfortunately, in the literature, discrepancies both in terminology and methods used to evaluate degree of development, result in physiological and bioenergetical data which are often not comparable. The same problem also occurs in other species of Ephemeroptera.

The criteria for evaluating the degree of development of an insect should be based on easily distinguishable morphological features and should divide the development of the species into equal periods. There are two important periods of morphogenesis in the

Table 1. Stages in development of Ephemeroptera (terminology and methods of dividing).

<table>
<tr><th colspan="16">Periods in morphogenesis with forming of:</th><th rowspan="2">Authors</th></tr>
<tr><th colspan="4">Gills</th><th colspan="12">Wing-pads</th></tr>
<tr><td colspan="16">LARVAE</td><td>Weber 1933</td></tr>
<tr><td colspan="16">NYMPHS</td><td>Macan and Worthington 1972</td></tr>
<tr><td colspan="6">N_{I}</td><td colspan="7">N_{II}</td><td colspan="3">N_{III}</td><td>Macan 1961</td></tr>
<tr><td colspan="3">N_{I}</td><td colspan="8">N_{II}</td><td colspan="4">N_{III}</td><td>N_{IV}</td><td>Clifford 1970</td></tr>
<tr><td>N_{I}</td><td colspan="2">N_{II}</td><td colspan="5">N_{III}</td><td colspan="7">N_{IV}</td><td>N_{V}</td><td>Lehmkuhl 1970, Hall et al. 1975</td></tr>
<tr><td colspan="3">LARVAE</td><td colspan="13">NYMPHS</td><td>Bertrand 1954</td></tr>
<tr><td colspan="3">LARVULLAE</td><td colspan="6">LARVAE</td><td colspan="7">NYMPHS</td><td>Mikulski 1936, Schmidt 1951</td></tr>
<tr><td colspan="4">(L_{o}) LARVAE</td><td colspan="3">N_{I}</td><td colspan="2">N_{II}</td><td colspan="6">N_{III}</td><td>N_{IV}</td><td>Pleskot 1958, Hilmy 1962</td></tr>
<tr><td colspan="2">L_{o}</td><td colspan="2">L</td><td colspan="3">N_{I}</td><td colspan="2">N_{II}</td><td colspan="6">N_{III}</td><td>N_{IV}</td><td>Bretschko 1965, Levanidova 1968,
Levanidova and Rubanienkova 1965</td></tr>
<tr><td colspan="2">L_{o}</td><td colspan="2">L_{I}</td><td>L_{II}</td><td colspan="3">L_{III}</td><td>L_{IV}</td><td>L_{V}</td><td colspan="2">L_{VI}</td><td colspan="2">L_{VII}</td><td colspan="2">N</td><td>Cianciara 1979a</td></tr>
</table>

⟵ time of development ⟶

development of the Ephemeroptera from an egg to a subimago: the period of formation of the respiratory abdominal organs (gills), and the period of formation of the wing-pads. Changes in these morphological features constitute the basis for distinguishable stages.

DEVELOPMENTAL STAGES

The criterion proposed for evaluating the degree of development and the basis for differentiating stages is the size of the mesothoracic wing-pads in relation to the segments of the thorax and the abdomen (Cianciara 1979a). Larvullae (L_O), larvae (L) and nymphs (N) are distinguished in a similar way to Mikulski (1936), Schmidt (1951), Pleskot (1958) and others (see Table 1). Larvullae cover all the developmental forms up to the moment when gills were fully formed, the term having wider connotations than in the classification of Pleskot (1958). The larvae (L_I - L_{VII}) covered the whole period of formation and growth of the wing-pads, and the nymphs (N) have wing-pads of maximum length. The term "larvae" is broadened, while the term "nymphs" is restricted in relation to the other authors except Weber (1933)(see Table 1). Many authors use the term "nymphs" for all the aquatic forms in the development of Ephemeroptera, starting with hatching from an egg (Macan 1961, Clifford 1970, Lehmkuhl 1970, Hall *et al.* 1975), or when the gills are fully formed (Bertrand 1954), or for the entire period of growth of the wing-pads (Pleskot 1958, Hilmy 1962), or when their length exceeds that of the metathorax (Mikulski 1936, Schmidt 1951). Most authors distinguish subsequent stages on the basis of distinct morphological features, but the younger stages are too "frequent" compared with the older ones which comprise longer physiological periods of development. Presuming that the assumed time axis, divided into stages on the basis of laboratory culture (Cianciara 1979b), represents, at least approximately, the physiological possibilities of the species studied in the natural environment, the division of time for particular stages gives the most equal sections (Table 1).

A number of experiments were carried out (Cianciara 1979a, b, c, d) in order to provide evidence supporting these divisions of developmental stages.

NUMBER OF DEVELOPMENTAL STAGES

In holometabolous insects the number of stages is determined by a constant and small number of moults. Contradictory data on the number of moults in hemimetabola, including the Ephemeroptera, exist in the literature. Some authors (e.g. Pleskot 1958, Pescador and Peters 1974) give a constant and definite number of moults for a given species, in others (e.g. Mikulski 1936, Schmidt 1951, Clifford

1970) the number changes and may even reach 50 moults. The latter was confirmed by culture (Cianciara 1979b) where, 27 to 40 moults were observed, depending on the type of food. The number of moults may also be influenced by other environmental factors, e.g. temperature (Schmidt 1951), and light (Khoo 1964). In the same species, the number of moults is greater in the winter generation than in the summer one (Schmidt 1951, Bretschko 1965). In addition, injuries and resulting regeneration may increase the number of moults with out influencing the rate of development (Schmidt 1951). It is impossible to define the moult number on the basis of any metrical parameters, since, under environmental pressure the larger nymphs may be physiologically younger than the smaller ones (Clifford 1970). The size of the larva need not bear any relationship to the number of moults (Schmidt 1951, Khoo 1964), these being dependent not just on growth but being, primarily, the method of excretion of the waste products of metabolism, collected in the exuvium.

DURATION OF DEVELOPMENT

The duration of total development and of development of each stage may also change, and may be influenced by environmental factors. *C. dipterum* usually has two generation per year (Landa 1968, Schmidt 1951, Bertrand 1954, Bretschko 1965, Cianciara 1979c), but only one in unfavourable climatic conditions (Macan 1962, Brittain 1972). Thus, the total development may last from two months (for the summer generation) to ten months (for the winter one) or even to 12 months. In natural conditions, environmental changes (i.e., temperature, day length) influence changes in the rate of the development, and subsequently the duration of particular stages. In the winter generation the stages L_{II} - L_{IV} last for about 150 days in the period of cessation of growth (Bretschko 1965, Cianciara 1979c). Under relatively stable laboratory conditions, (Cianciara 1979b) cultures showed a steady rate of growth, but type of food greatly influenced the duration of development (from about 5 to 7 months).

LIFE CYCLE ANALYSIS

When analysing the life cycles or the physiological processes of Ephemeroptera, many authors identify age groups on basis of body size (Trama 1957, Brown 1961, Lehmkuhl and Anderson 1970, Brittain 1972, 1974; Zimmerman *et al.* 1975) or body weight (Winberg 1956, Ivanova 1958, Pattee 1968, Kamler 1970, Alimov 1971 and others). However, the above parameters may be modified due to geographical location, food supplies or other variables. Independent of the type of food, the cultures (Cianciara 1979b) showed a uniform growth and approximately the same body sizes (both in early stages and in the final ones), though at different times and with a different number of moults. In natural conditions, however, it was observed (Cianciara

1979c) that the summer generation was represented by individuals smaller than those in the winter one. Seasonal changes in body size of *C. dipterum* was also observed by Bertrand (1954), Macan (1961) and Bretschko (1965). The typical growth pattern may also be influenced by diapause or periodical cessation of growth (Britt 1962), or by delayed egg hatching (Clifford 1970). The same body size (and weight) may then correspond to different age and different degrees of advancement in development of the insects (Clifford 1970).

Thus, the number of moults, duration of development, body size, and weight of mayflies are changeable and cannot be the starting point for defining the degree of their development.

BIOMETRIC AND BIOENERGETIC RELATIONSHIPS

If, however, stages in the development of Ephemeroptera exist, the fact should be confirmed by biometrical features and physiological properties of the insects. To test this, a number of biometrical and bioenergetical measurements were made for all the identified stages, and the course of changes in these parameters during the development of *C. dipterum* was studied (Cianciara 1979d). It was found that changes of the linear body dimensions (body length, head capsule width) were relatively uniform and in proportion to the growth of a given stage (Fig. 1). A uniform increase in the caloric value of the body was also observed. The increase in the wet and dry body weights (Fig. 2) followed a typical S-shaped curve (with flying forms also considered). The pattern of change of the parameters is not dissimilar to the patterns observed in other animals (Winberg 1968). No stepwise changes were observed, which would confirm the occurrence of stages in the development of *C. dipterum*. The only distinct differences between the parameters concerned the sexual differences in nymphs. It was found that in *C. dipterum* there were constant positive relationships between dry body weight and body length and between caloric equivalent and dry body weight (Fig. 3). The increase in the wet body weight in relation to the linear dimensions, however, was more rapid in the young larval stages (L_I - L_V) than in the older forms (L_{VI} - N). Between the stages L_V and L_{VI} the change is associated with an initial increase and then with a decrease in body hydration (Fig. 4A). Significant differences were also observed in intensity of oxygen consumption (Fig. 4B): initially it decreased (from maximum in stage L_I to minimum in stage L_{VI}) whereas in older stages it increased. These changes were observed in *C. dipterum* by Ivanova (1958) and in three other species of mayflies by Hilmy (1962). Significant changes between a group of younger stages (L_I - L_V) and a group of older ones (L_{VI} - N) were noted, oxygen consumption being dependent on body weight (Fig. 4C). The data confirms the occurrence of two periods with different intensity of physiological processes in the development of *C. dipterum*. The initial period of larval development (stages L_I - L_V) is characterized

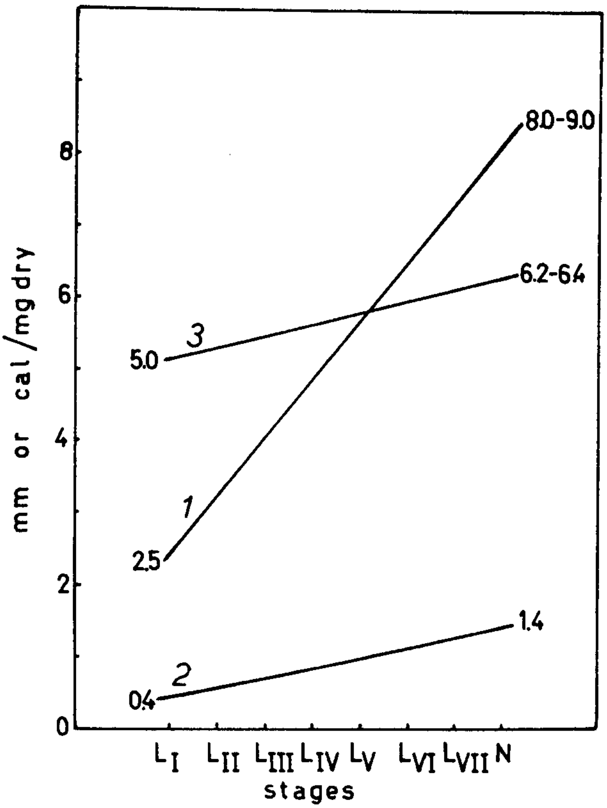

Figure 1. Parameters in the development of *C. dipterum* showing linear growth: 1 - body length (mm), 2 - width of head capsule (mm), 3 - caloric value (cal/mg dry wt); L - larvae, N - nymphs; I - VII - number of stages.

by a rapid rate of increase in body weight (and increase in body hydration). In the second period (stages L_{VI} - N) the increase in wet body weight is slower and body hydration decreases, but the processes of metabolism occur with greater intensity which is reflected by higher oxygen consumption. Most probably it is connected with a rapid development of gonads, first in females, then in males (Soldan, personal communication).

CONCLUSION

Thus the biometrical and bioenergetical data revealed two periods in the development of aquatic forms of *C. dipterum* (i.e. two

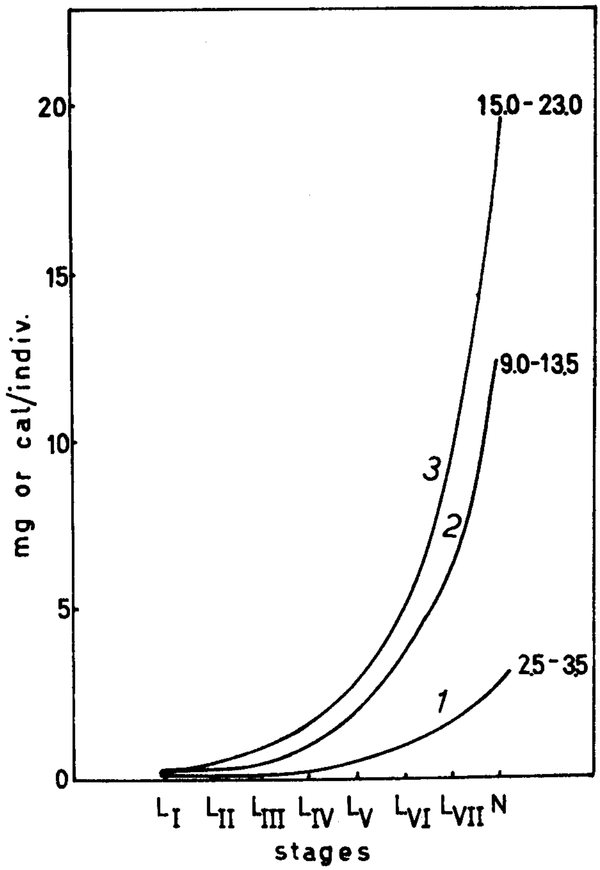

Figure 2. Parameters in the development of *C. dipterum* showing parabolic growth: 1 - dry weight (mg), 2 - wet weight (mg), 3 - caloric equivalent (cal/indiv.). Symbols as in Fig. 1.

physiological stages are confirmed). Since the two periods cover very long stages in the development, it seems more useful, for experimental reasons, to use the earlier accepted division (Table 1). This appears to be substantiated by statistical analysis of measured parameters (Cianciara 1979d). Highly significant statistical differences were confirmed between stages from L_I to N. However, there were no such differences (or not significant ones) between primarily (Cianciara 1979a) distinguished nymphs N_I and N_{II}. In the literature (Pleskot 1958, Hilmy 1962, Bretschko 1965 and others - Table 1), the final nymphal forms (N_{II}) are usually identified in mayfly development as certain, evidently different, stages (ripe nymphs). There is

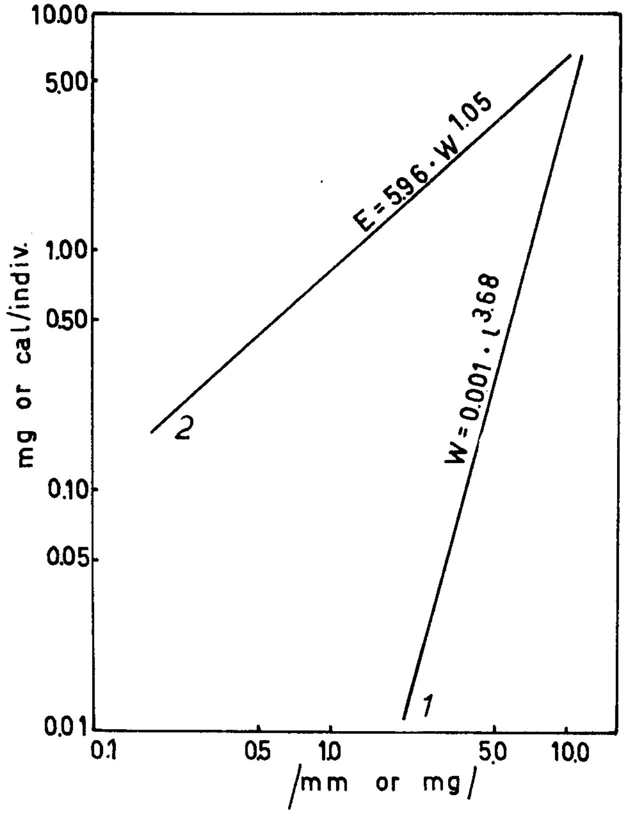

Figure 3. Constant dependence in stages L_I - N in development of *C. dipterum:* 1 - the relationship between dry body weight (mg) and body length (mm), 2 - the relationship between caloric equivalent (cal/indiv.) and dry weight (mg). Symbols as in Fig. 1.

no evidence whatsoever in the analysis of results of biometry and respiration for this division. Thus, in spite of distinct morphological differences, N_{II} nymphs cannot constitute a separate stage from N_I nymphs, since they are only the final instar before metamorphosis and not the stage. In summary, from the above data it can be concluded that in the postlarvullar period of the development of *C. dipterum* eight stages can be distinguished (Table 1): seven larval ones (L_I - L_{VII}) and one nymphal stage (N).

Since the development of *C. dipterum* shows a gradual metamorphosis, as do all hemimetabolous insects, the identified stages cover

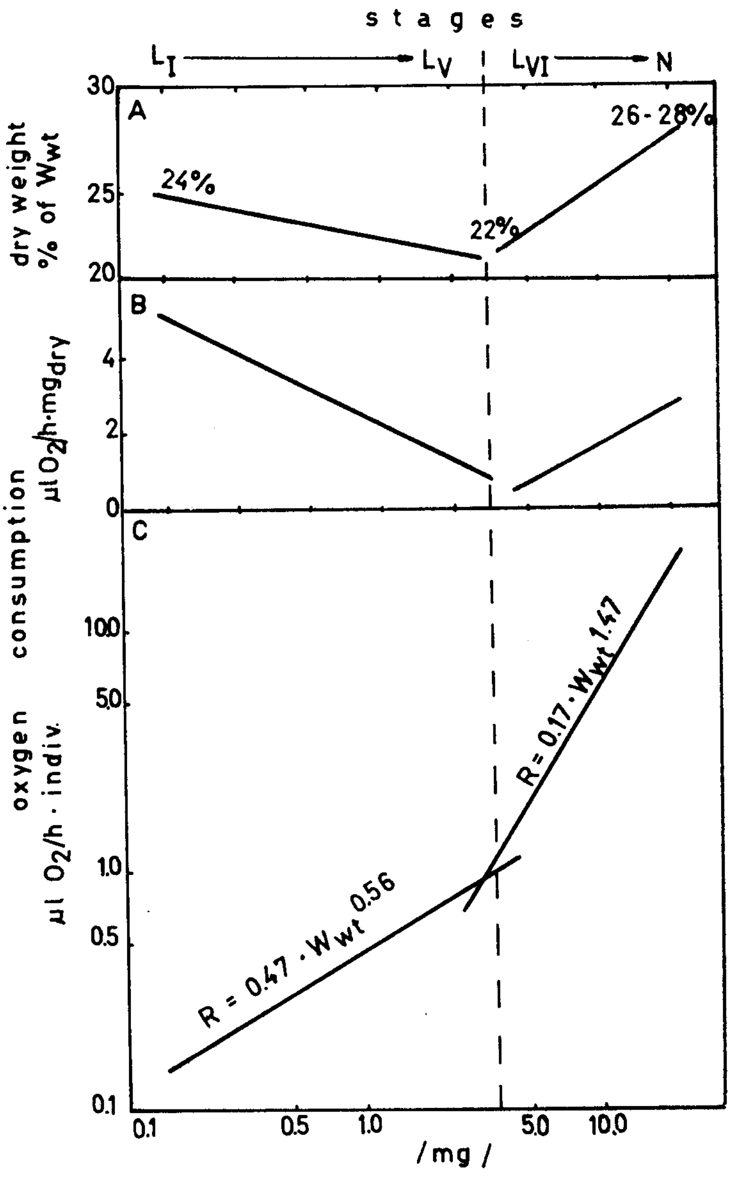

Figure 4. Parameters and their relationships in the development of *C. dipterum*, showing the existence of two periods: I - stages L_I - L_V, II - stages L_{VI} - N; A - dry body weight (percent of wet body weight), B - oxygen consumption (µL O_2/h/mg dry wt), 3 - the relationship between oxygen consumption (µL O_2/h/indiv.) and wet body weight (mg). Symbols as in Fig. 1.

the developmental phase determined by morphological criteria where particular larvae or nymphs may be ascribed definite biometrical and bioenergetical features. Though the division into the stages is arbitrary and not univocal as in holometabola, such separation may be helpful in experimental work.

RESUME

Le *Cloeon dipterum* passe par huit étapes aquatiques de développement, différant les unes des autres par leur morphologie, leur biométrie et leur capacité de respiration. La taille du corps, la quantité de mues et le temps que l'espèce met à se développer varient en fonction des conditions du milieu. En maintenant constant le taux d'augmentation du poids à l'état sec par rapport à la taille du corps et à l'augmentation constante de son pouvoir calorifique, il se produit deux périodes aux processus physiologiques distincts. La période I, comportant les étapes L_I à L_V, se caractérise par une augmentation accélerée du poids à l'état mouillé (concomitante à l'hydratation de l'organisme) et la période II, comportant les étapes L_{VI} à N, se caractérise par une augmentation de la consommation d'oxygène (concomitante à la déshydratation de l'organisme).

ZUSSAMENFASSUNG

Die Entwicklung von *Cloeon dipterum* erstreckt sich über acht aquatische Stadien, die sich unterschieden in Bezug auf Morphologie, Biometrie und Respirationswerte. Körpergröße, Zahl der Häutungen und Entwicklungsdauer der Arten können variieren und von Bedingungen der Umgebung abhängen. Innerhalb der konstanten Vermehrungsrate des Trockengewichts im Verhältnis zur Körperlänge und der konstanten Zunahme der vom Körper erzeugten Wärmewerte treten zwei Perioden auf, die in physiolgoischen Prozessen variieren. Periode I enthält die Stadien L_I bis L_V und ist charakterisiert durch beschleunigte Zunahme sowohl an Naßgewicht, als auch an Körperhydration. Periode II erstreckt sich über die Stadien L_{VI} bis N und zeichnet sich sowohl durch vermehrten Oxygenverbrauch, als auch verminderte Körperhydration aus.

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