

ANALYSIS OF ABDOMINAL MOVEMENTS IN NYMPHAL *Ephemeroptera* BY MEANS OF HIGH-SPEED CINEMAPHOTOGRAPHY*

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Introduction

The purpose of this study is to describe some different types of abdominal movements of ephemerid nymphs, in relation to gill movements and swimming, in order to explain some unusual findings at the electron microscope concerning the ultrastructural pattern of muscular organization [1, 2, 7, 8]. Three different kinds of nymphal mayflies were chosen which present a different type of swimming related to a different habitat: *Cloeon dipterum*, *Ephemera danica*, *Ecdyonurus venosus* (and *E. helveticus*). The general feature of gill movement of these ephemerid nymphs has already been described by means of stroboscopic observations with reference to currents produced by them, analysed by introducing suspensions of lamp black into the water [3, 4, 5]. The normal rate of gill oscillation, however, was not measured because, to make stroboscopic observation possible, the movements of the gills were stimulated under conditions of asphyxiation, so producing sufficiently rapid movements to avoid the phenomenon of "flicker" when observed by the stroboscope. In high-speed films the nymphs may be observed in normal conditions, the swimming behaviour and the oscillation rate of each gill may be analysed by repeated projections of the films and it is possible to observe the changes depending by small time-phase differences which cannot be observed with unaided eye. The movements are studied in this work in relation to their motion mechanism and precautions were therefore taken to maintain identical conditions of the water without chemical-physical changes (salinity, temperature, oxygen tension, etc.). It has been postulated by several workers that gill movement frequency may be related only to oxygen content of the water; it would therefore have been easy to define the maximum and minimum stroke frequency by varying the oxygen tension during our studies. However, recent microscope researches [9, 10, 11] have shown that the tracheal gills contain chloride cells responsible for osmoregulation and electrolytes absorption. Controlling the water oxygen content in our experiment conditions was therefore not sufficient to study the values of the factors related to tracheal gill stroke frequency.

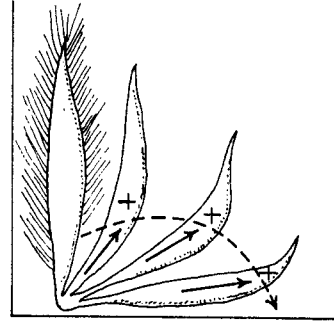
Methods

The quantitative data on the speed and rate of abdominal swimming movements and of gill oscillations were obtained by means of high-speed cinemaphotography and by repeated examinations of the films with the help of a time-lapse projecting apparatus, so measuring and analysing the individual

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photos. The paths covered by each gill was studied by projecting the photographic images onto millimetre-ruled graph paper. The instruments used were: camera Beaulieu 16 mm. for speed cinemaphotography (64 phot./sec.), camera Fastax 16 mm. for high-speed films (800 phot./sec.), objective Wallensak 50 mm. with prolongations of 5 cm. (in *Ephemera* and *Ecdyonurus*) and of 10 cm. (in *Cloeon*); lighting system with normal lamps (30 W) at the microscope or with Hg lamps in direct films; pellicles Plus X Kodak exposed at 64 ASA, Ektachrome Kodak at 125 ASA, and Double X at 160 ASA for high-speed films. For the time-lapse seeing of photos was used a projector-analyzer L-W Photo Kodak. Selecting lengths of film referring to several specimens and showing a constant succession of gill strokes, the data on tracheal gill shifts were set out in tables, divided and put in the same order as the numbers of the photos (see Table 1, for example). We noted that the animals were influenced particularly by

Ephemera danica



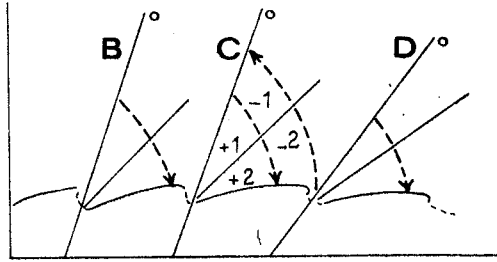
phot.	gills			
	B	C	D	E
1	-	-	-	-
2	+	+	-	-
3	+	+	-	-
4	+	+	-	-
5	+	+	-	+
6	-	+	+	+
7	-	-	+	+
8	-	-	+	+
9	-	-	+	+
10	-	-	-	+
11	+	+	-	-
12	+	+	-	-
13	+	+	-	-
14	+	+	+	-
15	+	+	+	+
16	-	-	+	+
17	-	-	+	+
18	-	-	+	+
19	+	-	-	+
20	+	-	-	-
21	+	+	-	-
22	+	+	-	-
23	-	+	+	-
24	-	-	+	+
25	-	-	+	+
26	-	-	+	+
27	-	-	+	+
28	+	-	+	-
29	+	+	-	-
30	+	+	-	-
31	-	+	+	-
32	-	-	+	+

phot.	gills			
	B	C	D	E
33	-	-	+	+
34	-	-	+	+
35	-	-	+	+
36	+	-	-	-
37	+	+	-	-
38	+	+	-	-
39	+	+	-	-
40	+	+	+	+
41	-	-	+	+
42	-	-	+	+
43	-	-	+	+
44	-	-	+	+
45	+	+	-	-
46	+	+	-	-
47	+	+	-	-
48	+	+	+	-
49	-	+	+	+
50	-	-	+	+
51	-	-	+	+
52	-	-	-	+
53	-	-	-	-
54	+	-	-	-
55	+	+	-	-
56	+	+	-	-
57	+	+	+	-
58	+	+	+	+
59	-	-	+	+
60	-	-	+	+
61	-	-	+	+
62	+	+	-	+
63	+	+	-	-
64	+	+	-	-

Fig. 1. Image of a series of gill movements in *Ephemera danica*, without pauses between a whole movement and another.
(A, B, C, etc. = 1°, 2° 3°, etc. gill)

light and noise: bright light, at least for the first few seconds, inhibited movement, while loud noise excited them. We do not believe that the behavioural differences observed could be due to different degrees of fatigue among the specimens, or at most only relatively, since the same procedure was followed in all cases from catching them to experimenting.

Ecdyonurus venosus



phot.	gills				
	B	C	D	E	F
1	0	0	0	0	0
2	+1	+1	0	0	0
3	-2	+2	+2	+1	+1
4	-1	-1	-2	+2	+2
5	0	0	0	-1	-1
6	0	0	0	0	0
7	0	0	0	0	0
8	0	0	0	0	0
9	+2	+2	+1	0	0
10	-1	+2	+2	+2	+2
11	0	0	-1	-1	-2
12	0	0	0	0	-1
13	0	0	0	0	0
14	0	0	0	0	0
15	+1	0	0	0	0
16	+2	+2	+1	0	0
17	-1	+2	+2	+2	+2
18	0	-1	-1	-2	-2
19	0	0	0	-1	-1
20	0	0	0	0	0
21	0	0	0	0	0
22	0	0	0	0	0
23	+2	+1	+1	0	0
24	-1	+2	+2	+2	+2
25	0	-1	-1	-2	-2
26	0	0	0	-1	-1
27	0	0	0	0	0
28	0	0	0	0	0
29	0	0	0	0	0
30	+1	+1	0	0	0
31	-1	+2	+2	+2	+1
32	0	-1	-2	+2	+2

phot.	gills				
	B	C	D	E	F
33	0	0	0	-1	-1
34	0	0	0	0	0
35	0	0	0	0	0
36	0	0	0	0	0
37	+1	0	0	0	0
38	+2	+2	+1	0	0
39	-1	+2	+2	+2	+2
40	0	0	-2	-2	-2
41	0	0	0	-1	-1
42	0	0	0	0	0
43	0	0	0	0	0
44	0	0	0	0	0
45	+2	+2	+1	0	0
46	-2	+2	+2	+1	+1
47	-1	-1	-2	+2	+2
48	0	0	-1	-1	-2
49	0	0	0	0	0
50	0	0	0	0	0
51	0	0	0	0	0
52	+2	+2	+1	+1	0
53	+2	+2	+2	+2	+2
54	-1	-1	-2	+2	+2
55	0	0	0	-2	-2
56	0	0	0	0	0
57	0	0	0	0	0
58	+1	0	0	0	0
59	+2	+2	+1	+1	0
60	-2	+2	+2	+2	+1
61	-1	-2	-2	-2	-2
62	-1	-1	-1	-1	-2
63	0	0	0	0	0
64	0	0	0	0	0

Fig. 2. An example of a series of gill movements in *Ecdyonurus venosus* with fairly regular pauses between one movement and the next. (+1, +2, is the effective stroke, -1, -2 is the recovery stroke)

Observations

On the basis of the data shown in the Tables and from comparisons with Diagrams, fairly exact observations could be made on the duration and frequency of the individual movements of the gills, the outgoing and return speed, and hence the duration of the effective and recovery strokes and the variations in gill movement in the nymphs observed. For clarity's sake, the results concerning movements of the gills as a whole are reported separately from those referring to movements of individual gills.

I. Movement of the gills as a whole

a) Movement as a whole, pauses and "overlaps"

Each one of the pairs of tracheal gills on the same segment beat together synchronously, so that each gill makes a movement which is the mirror image (symmetrical) of the corresponding gill on the other side; this is true when all the gills are working together and when, exceptionally and only for fractions of a second, only a few gills are moving (for example, the fourth, fifth and sixth in *Cloeon*), the others remaining still. In all the specimens observed the gills show an antero-posterior metachronal rhythm, resulting in each movement of a gill being slightly in advance of the movement of the gill behind it. The intervals between one whole movement of all the gills and the next are also not always regular. In *Ephemera* generally an other movement starts as soon as one finishes, i.e. as soon as the penultimate gill has made its movements (the last one is almost constantly stationary) the first gill again starts moving (Fig. 1). In *Ecdyonurus* and *Cloeon* there may be an interval ranging from 15 to 150 milliseconds between one total movement and the next. Since these intervals between one movement and the next in a series of movements are so brief, we shall call them "pauses", even though they are not easily detected by the naked eye, to distinguish them from the much longer intervals (sometimes lasting several seconds) which we shall term "resting periods" or "standstill". It can thus be seen that in a series of movements, *Ephemera* shows no true pauses (Fig. 1), *Ecdyonurus* shows fairly regular pauses (Fig. 2), while *Cloeon*'s pauses are much more variable (Fig. 3). In view of this extreme variability

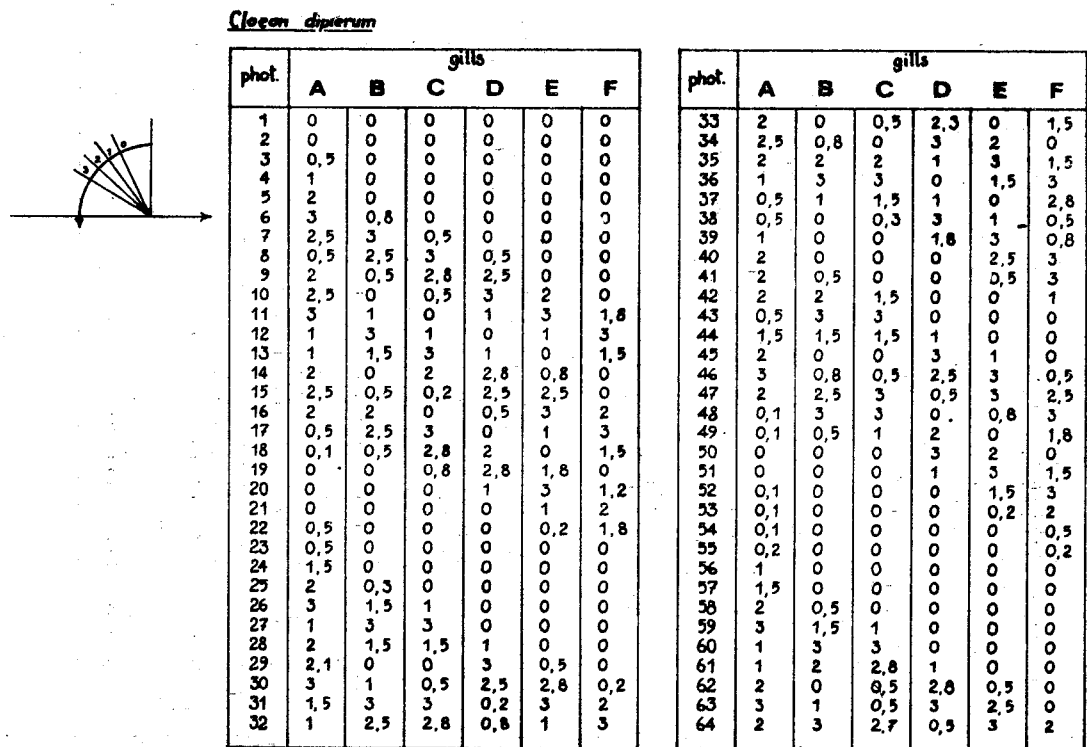


Fig. 3. Image of a series of slow gill movements in *Cloeon dipterum*. One may see the variability of the pauses at the resting position (zero) and the irregular speed of the motion in the effective and recovery stroke

in the latter, the strokes are continuous when the nymphs move the gills at high speed. In some cases in *Cloeon* the movements become so fast and frequent that they "overlap", i.e. the first gill starts moving before the last has finished; and when the movements get faster, the gills start moving again without returning to their original position, each tracheal gill making an "incomplete" stroke (Fig. 4).

b) Duration of the overall movement

From selected lengths of film showing several specimens making a succession of strokes, the number of photograms required to show each complete movement are listed in tables (see T. I, II, III as examples). They show that each complete movement takes up a different number of photos even on the same length of film. This shows that the insect may alter the speed at which all the tracheal gills beat, although only within a limited range. Since our observations were aimed at defining the features of the muscular mechanism which govern these movements, the duration and frequency of the gill opening and closing cycle were analysed. The above mentioned tables gave the duration of each complete movement, and the maximum and minimum cycle times were recorded for each specimen filmed. These are shown below:

Duration of Total Movement

	<i>Ephemera</i>	<i>Ecdyonurus</i>	<i>Cloeon</i>
min.:	106.25 msec.	62.50 msec.	171.87 msec.
max.:	285.90 msec.	109.37 msec.	250.00 msec.

c) Frequency of gill movements

Before analysing the significance of these which may vary so much in duration, or commenting the frequency of gill strokes, several considerations must be remembered on the physico-chemical conditions of the water and the insect's respiratory requirements (above mentioned in introduction and in method's section). The stroke frequency values could, if desired, be obtained from the duration of the total movement, which is very variable, as the stroke frequency per second at the various degrees of activity. This frequency would, however, be merely theoretical and just as varied, and it would therefore appear impossible to establish the rhythm of gill movements and their frequency. *Ecdyonurus* and *Cloeon* showed very irregular pauses or overlappings between one stroke and the next, so this cannot be

Cloeon dipterum:

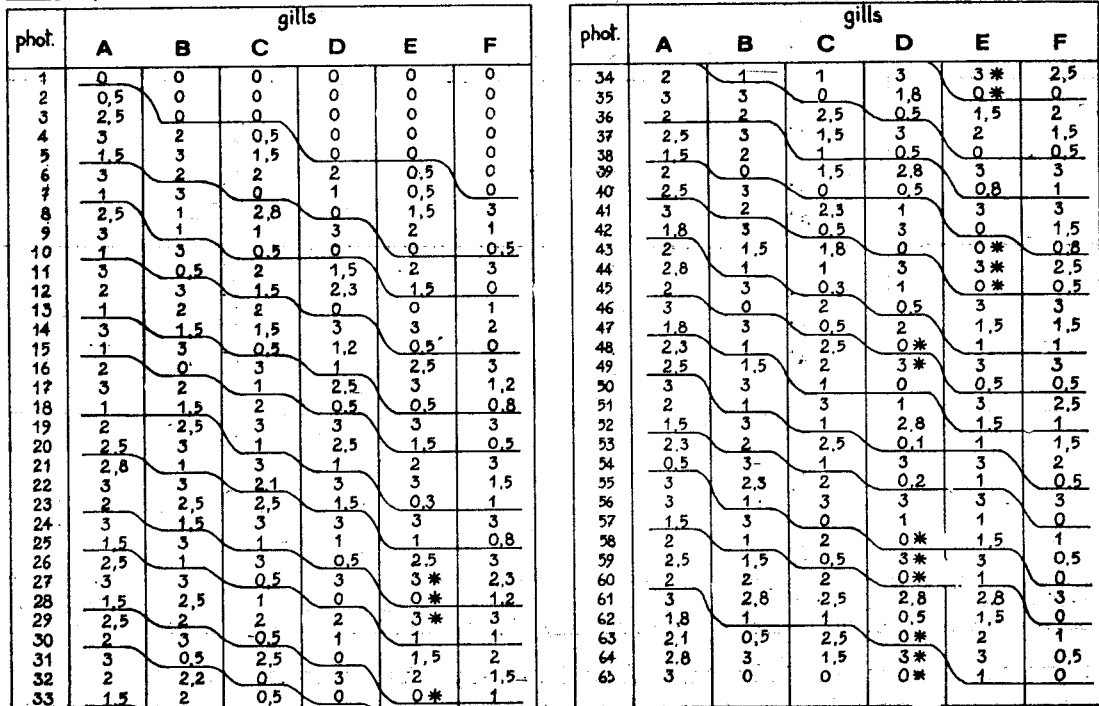


Fig. 4. One example of fast gill movements in *Cloeon dipterum*. The first gill (A) starts moving before the last (F) has finished (gill G is always still). The strokes are often incomplete, the fast complete strokes are marked by asterisks

termed a constant rhythm. *Ephemera* could on the other hand be used to give data on stroke frequency per second, since one movement followed the other without interruption, and in fact we observed that gill movement created constant undulation of the barrier of fringes at the sides of the abdomen. Here again, though, the frequency figures would be minimum or maximum according to whether they were obtained in periods when *Ephemera* was moving the tracheal gills slower or faster; the gill stroke frequency could be considered meaningful only when recorded in periods when several similar movements follow each other for at least a few seconds, i.e. when the rhythm is to a certain extent regular.

d) *Frequency (and variable rhythms) in Ephemera*

Table I gives the average duration of a complete movement in each length of film. There is not much difference between the average times during which each specimen moves the tracheal gills, for most of the film lengths observed. The specimens show movements lasting an average of about 8 to 9

Table I

Several lengths of films where the specimens of Ephemera danica make a succession of gill movements

Films	Movements	N. of photos	Films	Movements	N. of photos
A	1°	19	F	1°	9
	2°	17		2°	9
	3°	12		3°	8
	4°	12		4°	9
	5°	14		5°	8
B	1°	7	G	1°	9
	2°	7		2°	9
	3°	7		3°	9
	4°	9		4°	10
	5°	8		5°	11
	6°	10			
	7°	7			
8°	8				
C	1°	8	H	1°	8
	2°	9		2°	8
	3°	9		3°	8
	4°	9		4°	9
	5°	9		5°	8
D	1°	9	I	1°	12
	2°	6		2°	8
	3°	9		3°	8
	4°	9		4°	8
	5°	9		5°	9
E	1°	8	L	1°	19
	2°	5		2°	15
	3°	7		3°	21
	4°	7			
	5°	7			

Letters indicate the different films, ordinal numbers mark each complete movement, the number of the photographs occupied by one complete movement is in the third column. Transversal dotted lines are the resting periods between one series of movements and the next series.

photograms in seven out of ten lengths of film; the average duration of each movement is therefore approximately 133 msec. It would thus appear that each specimen has a certain standard or rhythm for its movements, which occurs most frequently; the differences between the specimens depend on the general, environmental and physiological conditions of each animal. In Table I, however, it is

also clear that the mean duration of the movement can vary considerably in a few lengths of film, ranging from a minimum of 106.25 msec. to a maximum of 285.90 msec. (in film lengths E and L in Table 1). The normal frequency revealed in this analysis is about 6.1-7.5 strokes per second, although they may be as slow as 4 strokes per second (minimum frequency) or as fast as approximately 12 strokes per second (maximum).

e) *Lack of constant rhythm and maximum frequency in Ecdyonurus and Cloeon*

For the reasons already explained, i.e. the varying lengths of the pauses or the degree of overlapping of movements, any calculation of the stroke frequency in *Ecdyonurus* and *Cloeon* would be purely theoretical based on the data in Tables 6 and 7. For these two specimens, we can, however, assess the maximum stroke frequency on the basis of cases when the whole gill movement follow each other without pauses and without overlappings. In actual fact this situation very probably occurs only extremely rarely because the gills do not have an overall structure, like *Ephemera*, requiring continuity of consecutive movements. In *Ecdyonurus* (Table II) each complete movement of tracheal gill takes

Table II

Several lengths of films where the specimens of *Ecdyonurus venosus* make a succession of gill movements

Films	Movements	N. of photos	Films	Movements	N. of photos	
A	1°	5	D	1°	5	
	2°	5		2°	4	
	3°	6		3°	4	
	4°	5		4°	5	
	5°	5		5°	5	
	6°	6		6°	5	
	7°	5		7°	4	
	8°	5				
	9°	6				
	10°	6				
B	1°	6	E	1°	6	
	2°	6		2°	7	
	3°	6		3°	7	
	4°	6		4°	7	
	5°	6		5°	6	
	6°	6		6°	6	
	7°	6		7°	7	
				8°	6	
				9°	7	
C	1°	7				
	2°	7				
	3°	6				
	4°	6				
	5°	6				
	6°	7				
	7°	6				

Letters indicate the different films, ordinal numbers mark each complete movement, the number of the photograms occupied by one complete movement is in the third column. Transversals dotted lines are the resting periods between one series of movements and the next series.

more or less regularly a minimum of 62.5 msec.; if complete movements were carried out regularly one after the other, this would give a frequency of about 16 strokes per second, or a maximum frequency of 20 strokes per second if one movement starts before the previous one is over (and without reducing the complete distance covered by each gill). In *Cloeon* (Table III) the minimum duration of the complete movement as observed by us was 171.8 msec. (11 photograms); this would give a frequency of about 6 strokes per second assuming movements were carried out without pause, each one starting as soon as the previous one was completed, or a maximum frequency of 10-12 strokes per second assuming complete but overlapping movements.

f) *Faster oscillations in Ecdyonurus and Cloeon*

The frequencies above described are perfectly possible but are not in fact the maximum the two specimens can produce with their gills; they are merely theoretical maximums. Both *Ecdyonurus* and *Cloeon* have two ways of increasing their stroke frequency, firstly by cutting down to the minimum the delay between one gill starting to move and the next (this reduces the duration of the overall movement); at the same time one movement can be overlapped as far as possible on the next, meaning the first gill starts moving again before the others have finished, and the movements of each gill are therefore incomplete. The second method is to oscillate the tracheal gills so fast that incomplete movements are overlapped, and the gills do not even have time to make a complete outgoing or return movement. In *Cloeon* (Fig. 4) a rate of 23 strokes per second was in fact observed, involving not only overlapping movements and reduction of the time, but also incomplete backward and forward gill

Table III

Several lengths of films where the specimens of Cloeon dipterum make a succession of gill movements

SPECIMEN N. 1 Movements with resting intervals			SPECIMEN N. 2 Movements without resting intervals		
Films	Movements	N. of photos	Films	Movements	N. of photos
A	1°	12	Film of 1 sec. (64 phot.)	1°	9
	2°	11		2°	7
	3°	11		3°	8
	4°	13		4°	8
	5°	11		5°	7
	6°	11		6°	8
	7°	13		7°	7
	8°	13		8°	8
	9°	16		9°	7
	10°	12		10°	8
	11°	11		11°	7
B	1°	12		12°	8
	2°	13		13°	7
	3°	12		14°	7
	4°	13		15°	7
	5°	11		16°	8
	6°	11		17°	8
C	1°	14		18°	7
	2°	11		19°	8
	3°	12		20°	8
	4°	12		21°	8
	5°	12		22°	8
	6°	12		23°	8
D	1°	13			
	2°	11			
	3°	13			

Letters indicate the different films, ordinal numbers mark each complete movement, the number of the photograms occupied by one complete movement is in the third column. Transversal dotted lines are the resting periods between one series of movements and the next series.

movements. In conclusion, both *Ecdyonurus* and *Cloeon* can easily exceed the theoretical maximum frequencies, to the point of oscillating the gills incompletely, almost as if they were fibrillating. Since this metrological investigation was concerned with the physiological properties of the muscles which move the tracheal gills, we may compare the data obtained on overall movement with the findings for the movements of the individual gills.

II. Observations of Individual Tracheal Gill Movements

The films clearly show, as already described (3, 4, 5), the position of the individual tracheal gills with respect to the body, and which gills on the first abdominal segment vary in form or have reduced movement (e.g. the first gill in *Ephemera* and *Ecdyonurus*) or on the seventh segment which remain almost stationary and serve to deflect or hold back water currents produced by the other gills. High speed film (800 phot./sec.) gives rise to further observations, some of them made here, particularly as regards *Cloeon*, since the highest speed images were necessary with this specimens.

a) The path of each gill

Generally the duration of movement varies slightly from one gill to another, as also does the distance covered. In *Cloeon*, the duration of the stroke also varies, the last gills moving faster than the first. This is observed without in any way contradicting the metachronal movement described. The paths covered by each gill were studied by projecting the photographic image onto millimetre-ruled graph paper and by calculating the angles of inclination of each gill in start and arrival position (Fig. 5). The data are summarized below (where "Angle X" is the inclination of each gill in the starting position with respect to the perpendicular of the longitudinal axis of the body):

Gills	Angle X, start position	Arrival position	Angle covered
1	38°	57°	19°
2	23°	52°	29°
3	15°	47°	32°
4	10°	44°	34°
5	4°	41°	37°
6	-1°	38°	39°

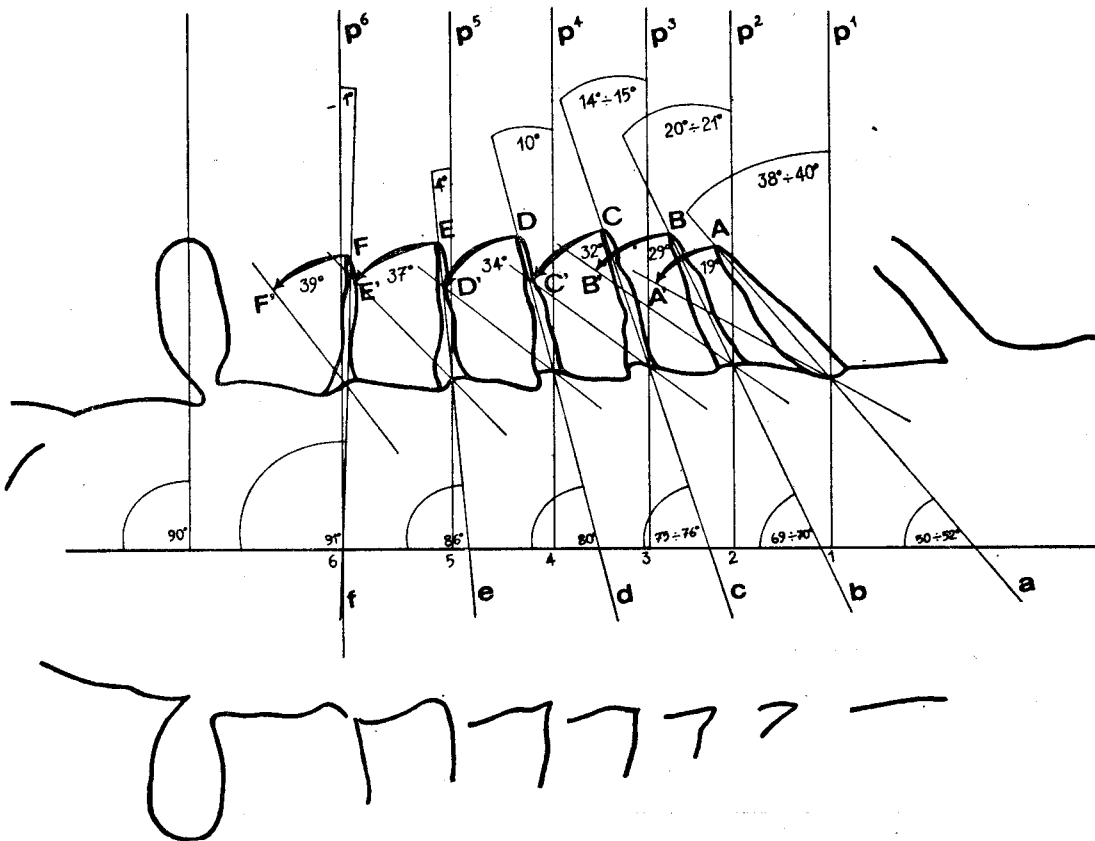


Fig. 5. Projection of the abdomen of *Cloeon dipterum* to show the paths covered by each gill (A-A', B-B', etc.). Other explanations in the text. (a, b, c, etc. = gill axis; p¹, p², etc. = the perpendiculars to the body longitudinal axis)

Taking the angle covered by each gill as an indication of the distance shifted, the path increases from the first to the last moving gill (from 19° to 39°). Bearing in mind that the duration of the movement, as we have already stated, varies in inverse proportion to the angle, each gill therefore covers a shorter path in a greater time than the next one. We have not carried out any particular film studies of the movements of the anterior lamellae of each gill in *Cloeon*, since they adhere completely to the main lamella, at least for part of the distance covered. EASTHAM's observation (5) of the crowding of the anterior gills and lamellae, would appear in actual fact to be explained more by the different positions in which the gills lie with respect to the longitudinal axis of the body, than by the fact that the space is occupied by the very curved anterior lamellae in the movement. For *Ephemera*, this observation was anyway not possible as the fringe of filaments covering the lamellae make it difficult to establish exactly the angle of shift of each individual gill. For *Ecdyonurus*, film observation is possible of the path covered by each tracheal gill, but they move much faster at the end of the effective stroke and beginning of the recovery stroke that the values obtained would vary broadly in relation to the speed of each gill. In all three specimens the tip of the gill follows an elliptical path; this has already been reported by EASTHAM (3, 4) on the basis of stroboscopic observations in *Ecdyonurus* and *Ephemera*, and is detectable also in *Cloeon* by means of high-speed films.

b) *Synchrony and delay between one tracheal gill and the next*

Movement starts with the anterior gill, the rest all moving one after the other up to the sixth (the seventh is stationary). This is therefore a metachronal movement. Although this is immediately evident even to the naked eye, high-speed film enable us to study the values of the delay in gill movement

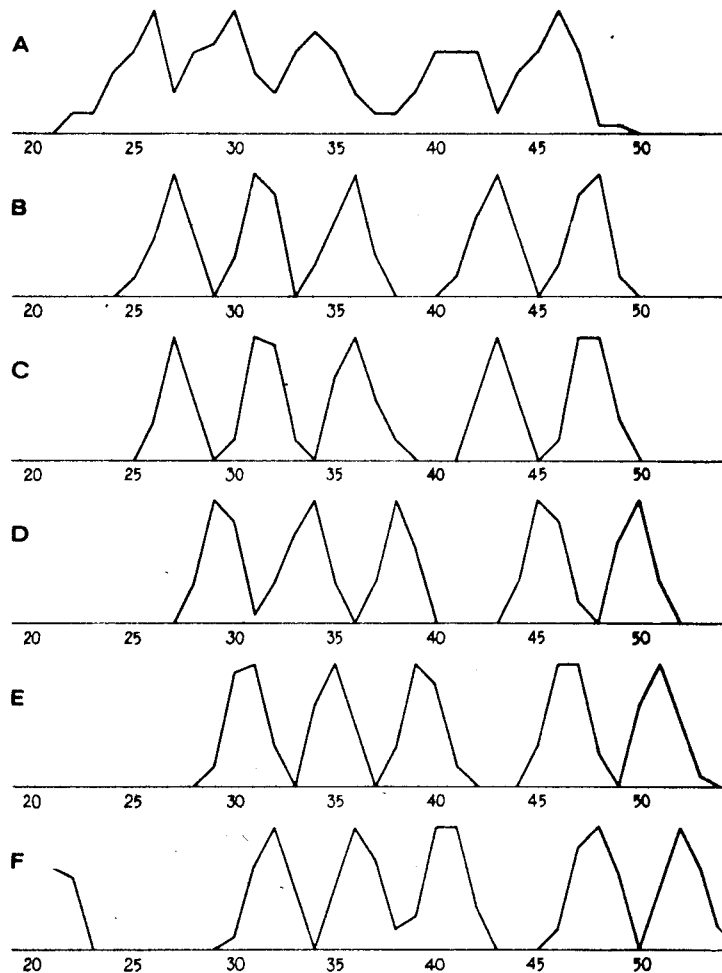


Fig. 6. One example of a diagram of the paths covered by the gill movements, with the delay in start-up and arrival points of each gill in *Cloeon dipterum*. Numbers in the horizontal lines correspond to the photograms

start-up. In *Cloeon*, for example, gill B, the second one, starts moving $3/64$ th of a second ($1/64$ th of a second = 1 photogram) later than the first gill, A. Gill C starts moving $1/64$ th of a sec. after gill B, and so on until gill E, which starts moving $2/64$ th of a sec. after D. These lags appear constantly repeated every time the gills start moving after a standstill (long pause). Gill B starts moving when gill A has reached point 3 (maximum shift) and so on up to the sixth gill, always bearing in mind that only six gills are mobile. The delay in starting, and therefore in arriving, does obviously vary depending on the speed and frequency of movement. Fig. 1, 2, 3 show also the delays in arrival and start-up; for fast movement, the start-up point may be the 0.5 also. This is even clearer if the values given in the tables of analysis are plotted as a diagram representing the movement of each gill with its shift and delay (Fig. 6, for example). These diagrams obtained may be fitted together, marking the movement of different gills with different lines to give an overall view of the movement and of any synchrony or delays (Fig. 7, for example). EASTHAM, in *Cloeon*, stated that the metachronal movement and the lag in starting each movement produces a rhythmical synchrony between the gills in the same row, resulting in the first gill moving in synchrony with the fifth and the second with the sixth (5). Our research failed to confirm this finding. In fact, from Fig. 6, it is clear that there is no constant overlapping of the first gill (A) with the fifth (E), nor of the second (B) with the sixth (F). Probably EASTHAM's findings depend on his observations having been made stroboscopically, which led him to consider as identical some positions which in fact were slightly different. In *Ephemera* the lag between one gill and the next is very constant, although obviously it varies in absolute value according to the speed of the movement, but this can be easily observed because the fringes of one gill should not be separated too much from the fringes of the next. In *Ecdyonurus*, on the other hand, not taking into account the first lamella which is small, there is a fairly constant start-up lag, although this is not evident from here published tables (as Fig. 2), which refers to the sector occupied by the lamellae during the normal path (+1, +2, -1, -2), and not to the departure and arrival points.

c) The stroke of each gill

To calculate with a certain degree of precision the time taken by one tracheal gill in its effective and recovery stroke, one must bear in mind all the previous findings on the overall movement, to interpret any variations observed; high-speed film is essential to observe the actual behaviour and times for each case. Our recordings were therefore made using the projector-analyser on lengths of film

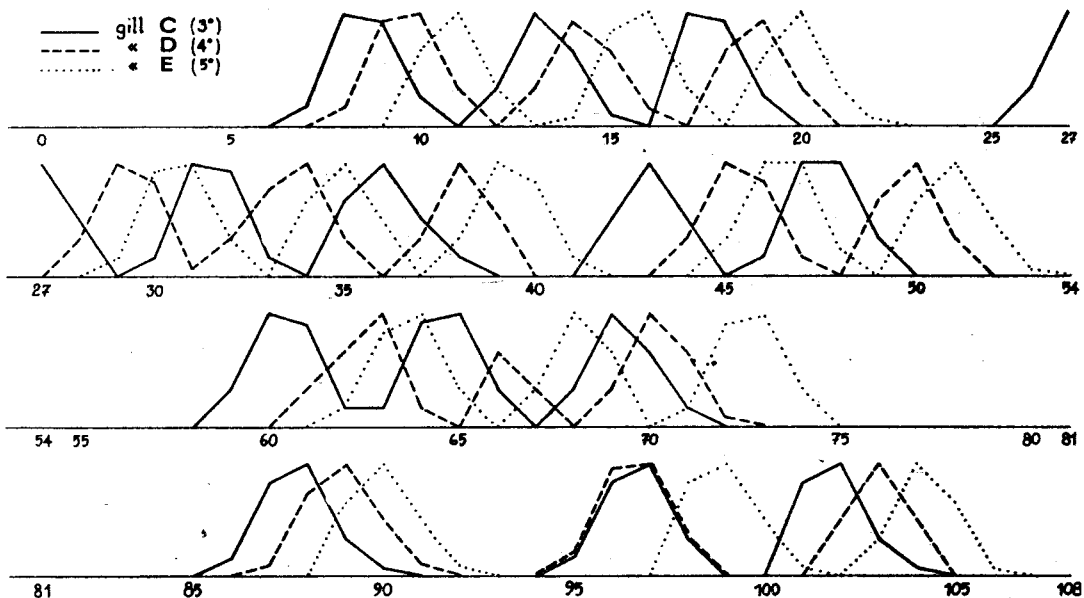


Fig. 7. One example of the diagram obtained by fitting together the movements of three gills, to show any synchrony or delay

taken at a rate of 800 phot/sec. For *Cloeon*, as example, numerous Tables were obtained analysing the shift for each photogram, and a short length is given as an example from a diagram on millimetre-ruled graph paper (Fig. 8) referring to half a second. This nevertheless shows a whole movement in an almost overlapping, continuous pattern (i.e. with no noticeable pauses between one gill movement and the next). High speed film thus shows up even small irregularities in this movement. It can be seen for example from Table II that while gill C never stops, gills D and E both make very short pauses of about 18 msec. The gill paths may be slightly irregular towards the end of the path; this is probably due to resistance factors in the water such as pressure or eddies created by the currents, or else to the fact that at the end of the path the energy driving the gills is produced not only by muscular contraction but also by the energy accumulated in the elastic chitinous components at the point of the insertion of the gills. Curiously, there are some long pauses (for example of 25 msec.) at the end of effective stroke, which were never revealed in the tables obtained from film taken at 64 photo/sec.

d) *The effective and recovery stroke in relation to muscular activity*

In relation to the observations as those referred in (Fig. 6, 7, 8) we can identify the musculature, and in fact the actual muscular activity, which produces the effective and recovery strokes. *Cloeon* and *Ecdyonurus* have two antagonist muscles moving the tracheal gills, one for the effective (backward)

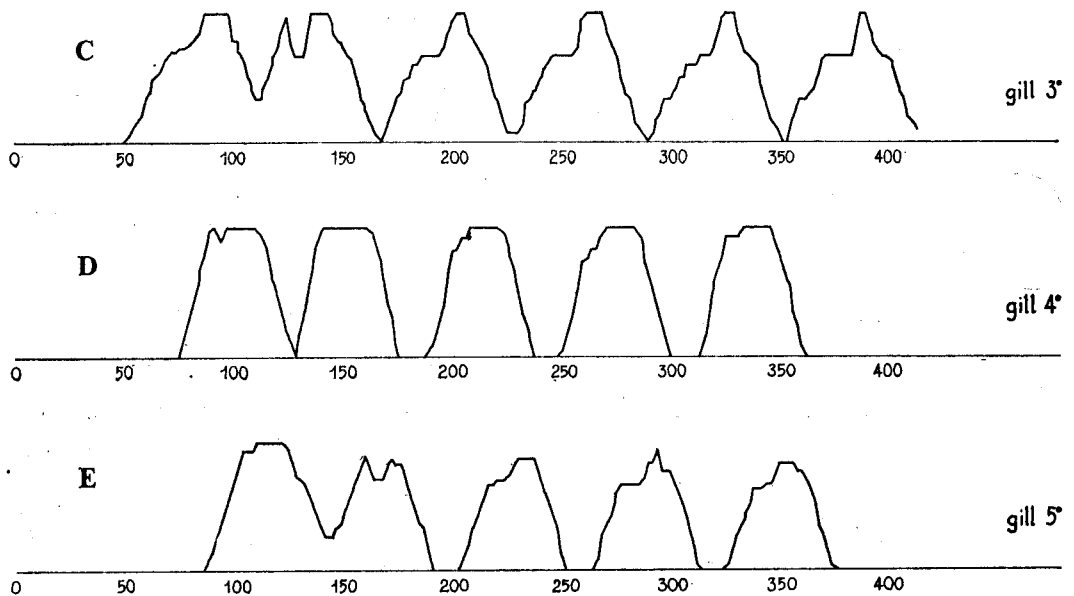


Fig. 8. Diagram of the paths covered by three gills, obtained from a high-speed film projected on millimetre-ruled graph paper, to show the small lags at the end of the effective and recovery strokes. (See the interpretation in the text)

and one for the recovery (forward) stroke [6]. These muscles, in *Cloeon*, make a complete contraction in as little as 15 msec., and in fact the corresponding representations in diagrams have a time interval of this length. Observing the overall movement, we reached the conclusion that the maximum frequency was about 12 strokes per second in *Cloeon*, and 20 strokes per sec. in *Ecdyonurus*, as above stated. However, those data gave us an idea of the frequency of muscular contractions but not of the speed at which the muscle contracts (also the duration of the halt at the end of the gill stroke was not included). Muscle contraction, therefore, in *Ecdyonurus* and *Cloeon* is very fast, independently by the frequency, especially considering that part of the stroke paths is covered by inertia. This is not the case for *Ephemera* where the tracheal gills do not behave like an oar cutting the water, but move more slowly outlining a fairly ample elliptical path; this movement is produced by three muscles inserted at the base of the gill. Considering further that, unlike *Ephemera*, in *Ecdyonurus* and *Cloeon* the gill movement may be incomplete, with faster oscillations, it is even more how extremely fast the muscles involved must contract.

III. Abdominal movements during swimming

The three nymphs under consideration have different abdominal movements during swimming [3, 4, 5], depending on their type of median longitudinal abdominal somatic musculature [6]. The film have succeed in confirming previous observations which it is not necessary to refer in this work. As regards the quantitative data on the movement mechanism, they may be referred briefly. *Ephemera* nymphs, since they have medial muscles mostly intrasegmental, can thus move their abdomen easily in the sandy cavities or tunnels they dig, and when swim they can flex one urite away from the one next to it much more than the other two nymphs do. During swimming they present a rhythmical oscillating movement very slow that may be observed also at the naked eye. *Ecdyonurus* nymphs are lithophilic and rheophilic, and when they are not swimming the abdomen is relatively immobile, moving slowly upwards and downwards. During swimming (even though the nymph is rarely seen to swim) the abdomen helps move the body through the water in very quick darts. *Cloeon* nymphs are typical pelagic swimmers; their abdomen makes a typical circular movement (slow movement) when the nymph is not swimming, but it makes rapid movements both dorso-ventrally and laterally during swimming. From film analysis we may observe that the frequency of abdominal movements is very variable, but in fast swimmers, as *Ecdyonurus* and *Cloeon*, one side to side movement of the abdomen may occupy only 15 msec. or less.

Conclusions

The conclusions, with relation to muscular systems activity, may be summarised as below:

1) In abdominal movements of the nymphs examined there are two kinds of movement mechanism: one rhythmical (gill strokes in *Cloeon* and *Ecdyonurus*, and swimming abdominal movements in all three nymphs), one non really rhythmical (many abdominal movements as the circular one of non swimming *Cloeon*, the slow elliptical stroke of gills in *Ephemera*, and so on).

2) Rhythms of movements are not regular, and frequencies of gill oscillations (or frequencies of muscular contractions) may be distinguished from the high speed movements of individual gills (i. e. from the speed of contraction of the muscle). Thus there are no autooscillatory movements, and all movements may therefore be performed synchronously by nervous system impulses (this finding agrees with the observation of abundant sarcoplasmic reticulum and dyads in all these muscles at the electron microscope).

3) Oscillatory movements of swimming are faster in *Ecdyonurus* and *Cloeon* than in *Ephemera*, and the gill stroke movements, always slow in *Ephemera*, may become fast and faster in *Ecdyonurus* and *Cloeon* by reducing gill path or by overlapping the overall gill motions (this agrees with ultrastructural findings of a myofilament arrangement pattern in these muscles similar to that of flight muscles of Insects [1, 2, 7]. Non oscillating movements observèd are performed in any case slowly (probably by external longitudinal intrasegmental muscles in *Cloeon* or by the direct muscles at the basis of the gills in *Ephemera*, etc., which are ultrastructurally of the toni-slow type).

Further studies in this field could explain which ultrastructural patterns of the muscles are related to oscillatory mechanism of the movement or to the speed of contraction.

SUMMARY

Analysis of abdominal movements in nymphal Ephemeroptera by means of high-speed cinemaphotography

Mayfly nymph muscles show under the electron microscope an unusual character of sarcomere organisation. In order to establish morphofunctional correlations concerning the physiological effects of different kinds of muscular patterns, we report some studies of nymphal mayfly movements by

means of high-speed cinemaphotography, which amplify the observations with light microscopic or stroboscopic methods.

The films record and reproduce the swimming and gill movements of nymphs of *Cloeon dipterum*, *Ephemera danica*, *Ecdyonurus venosus*, *E. helveticus*, etc., that present different types of environmental adaptation and move in all parts of the water body — on the substratum at some depth as well as among water weeds or in fast-flowing streams.

Furthermore, the movements were studied by repeated viewing of the films with the help of a time-lapse projecting apparatus, and by measurement and analysis of individual pictures.

In high-speed films the oscillation rate of each gill and the metachronal rhythmical movement of the gills or the swimming behaviour do not appear so regular as previously described, but it is possible to observe the changes depending on small time-phase differences and on short pauses between movements, which cannot be observed with the naked eye.

The collected data are characterized by the use of exact quantities and rare compared with each other in tables and diagrams.

Some implications of these findings in relation to different ultrastructural patterns of muscle fibres as well as to contraction kinetics are discussed.

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