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The morphology of the larval instars of *Chaoborus flavicans* (Meigen, 1818) (Diptera, Chaoboridae)

S. PARMA

ABSTRACT

This paper contains a description of the four larval instars of *Chaoborus flavicans* (Meigen, 1818). Several morphometric data, especially of head parts, are given. Except the length of the head capsule other quantitative and qualitative characters prove to be instar specific. A key for macroscopic identification is included.

1. INTRODUCTION

Chaoborus flavicans (Meigen, 1818) has a holarctic distribution (Cook, 1956; Peus, 1967). Larvae and pupae can be observed in a large variety of aquatic habitats which illustrates their great ecological plasticity (Parma, 1969). High densities are found especially in stratified eutrophic and dystrophic lakes, where the fourth instar larvae and the pupae usually burrow in the bottom during daytime.

For ecological research an ability to identify all larval instars is important. Head capsule length proved to be a good criterion (Parma, 1969), but can only be applied to preserved specimens and to living specimens of the more robust fourth instar. In studying the ecology of this species (Parma, in press) we needed macroscopical identification characters, so that the instar of free swimming larvae could be determined. Initially we only looked for such criteria, but in this way many other qualitative and quantitative morphological data came available.

Studies on larval morphology have mainly been done on the fourth instar (Peus, 1934; Hirvenoja, 1961; Saether, 1967; Sikorowa, 1967a; Parma, 1969). Published data on younger instars are scanty. Some information was given by Prokešová (1959), Saether (1967) and Sikorowa (1967a, b; 1970). Weismann (1866), von Frankenberg (1915), Smith (1960) and Sikorowa (1970) gave some details of the younger instars of *C. crystallinus*. Sikorowa

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(1970) and Deonier (1943) reported on younger instars of *C. pallidus* (Fabricius, 1792) and *C. astictopus* Dyar & Shannon, 1924 respectively.

The scarcity of published data justifies the presentation of our morphological data on the different larval instars of *C. flavicans*. All measurements were carried out on larvae from lake „Vechten", a sandpit in the municipality Bunnik, Netherlands (surface area 4.7 ha, maximum depth 12 m). The larvae were preserved in 4% formalin.

We compared the morphological data of *C. flavicans* with those of other species, e.g. *Co borealis* and *C. alpinus*. *Chaoborus borealis* Cook, 1956, is a junior synonym of *C. crystallinus* (De Geer, 1776) (see Saether, 1970). *Chaoborus alpinus* Peus, 1938, is a junior synonym of *C. flavicans* (see Saether, 1967).

2. Morphology

2.1. Total length, length of the head capsule and setae on dorsal side of the head capsule.

Total length is exclusive of the anal papillae. Measurements were carried out on fully stretched animals. These were obtained by slowly adding 4% formalin to a sample of living larvae. First and second instar larvae were measured with an ocular micrometer, third and fourth with mm graph paper. The results, summarized in table I, show the overlap between the successive instars. Total length is not a valid criterion for instar distinction (see also Malueg, 1966: 77; Teraguchi & Northcote, 1966: fig. 2).

The length of the head capsule (measured according to Prokešová, 1959: fig. 7) seems to be a good character to distinguish the four larval instars (table I; see also Parma, 1969: fig. 2).

At the dorsal side of the head of the first instar three pairs of setae are present. The middle pair can have two or three branches. The others are always unbranched (fig. 1a). In the second, third and fourth instars the setae are all of a plumose type. Here also the middle ones are most strongly branched (fig. 2E). In these stages a short seta is present just above the base of the antenna. This hair is simple in the second and third instar, but plumose in the fourth. Saether (1967: 578) mentioned this as the only qualitative difference between third and fourth instar (see also Weismann, 1866: pl. IV).

Table I. Total length and length of the head capsule of the four larval instars.

instar	length of the larvae		length of the head capsule		
	number of animals	range in mm	number of animals	mean in µm	range in µm
1	180	1.7— 3.0	143	227	200—250
2	161	2.8— 5.0	160	419	325—525
3	184	4.0— 8.0	400	744	575—875
4	> 3000	7.0—15.0	1808	1152	925—1325

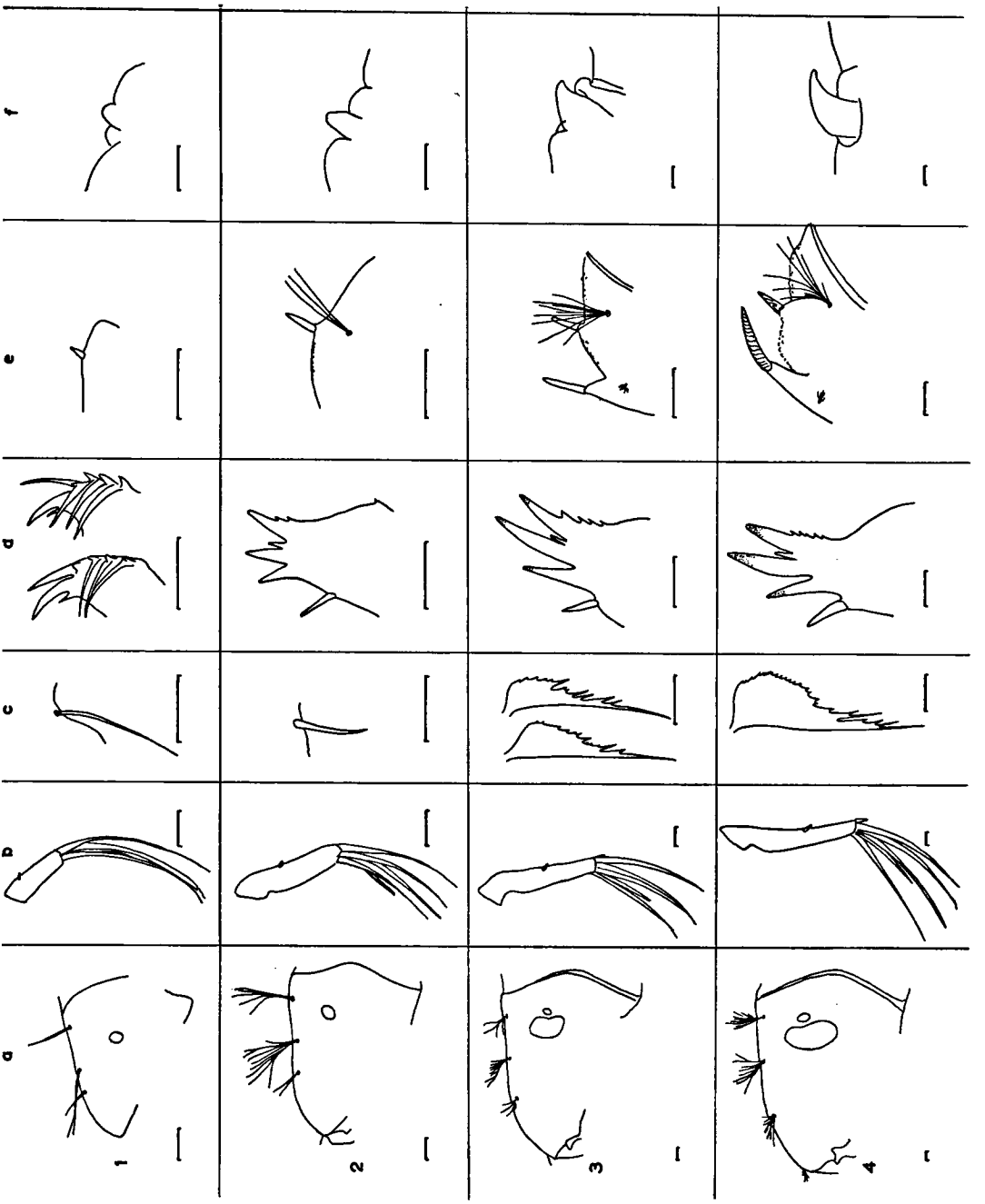

FIG. 1. Some morphological features of the first (1), second (2), third (3) and fourth (4) instar of *Chaoborus flavicans*. a, head capsule with dorsal setae; b, antenna; c, prelabral appendage; d, mandible; e, maxilla; f, dorsal process. All horizontal bars represent 50 μm.

2.2. Antennae and postantennal filaments.

The antenna of the first instar does not have the incision at the proximal end, which is characteristic for the following instars (fig. 1b). A short seta is present on the anterior face of the antenna. The distance of this seta from the proximal end as a percentage of the total length is characteristic for the instar (table III).

The first instar has four terminal setae. These are rather long with respect to the antenna (table III). One of them is a little shorter than the others. The ratio long/short terminal seta is 1.1—1.2. The other instars have 5 terminal setae of which one is clearly shorter. The ratio long/short seta decreases in the course of the development (table III). Finally in the third and fourth instar a very short terminal spine is present (fig. 1b).

The second, third and fourth instars always have 10 postantennal filaments, but these are absent in the first instar (Weismann, 1866: 71; Prokešová, 1959: 148). This difference is visible with a pocket lens and can be used for macroscopic identification. The length in the three instars is represented in table II.

Saether (1967: table 1) gave some corresponding measurements of antennae and postantennal filaments of the third instar larvae from other localities, which are included in tables II and III. Differences in the relevant measurements seem to be present. The possibility cannot be excluded that a closer investigation of younger instar larvae from more localities might lead to splitting up the species.

Table II Length in μm of antenna, longest terminal seta of the antenna and postantennal filaments from the four larval instars. n — number of animals; s.d. — standard deviation. Saether (1967) — figures from third instar larvae, calculated from his table I.

	antenna			long. terminal seta			postantennal filaments		
instar	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.
1	46	63	5	45	152	9	—	—	—
2	49	176	8	42	215	10	53	172	6
3	50	330	11	49	321	12	43	319	11
4	50	510	22	50	499	27	50	561	27
Saether (1967)	42	388	—	42	375	—	42	387	—

2.3. Prelabral appendages.

Prokešová (1959: 148) mentioned the absence of prelabral appendages in the first instar. However, we found that in the first and second instar larvae these head appendages are present as firm awl-shaped setae (fig. 1c) (see also Weismann, 1866: pl. V; von Frankenberg, 1915: 551; Deonier, 1943: 385; Smith, 1960).

Table III. The ratio of long/short terminal setae of tne antenna, the ratio of long terminal seta/antenna and the place of the small seta on the anterior face of the antenna of the four larval instars. s.d. — standard deviation. Saether (1967) — figures for third instars calculated from his table I.

	ratio long/short terminal seta			distance seta on ant. face of antenna from prox. end as perc. of total length			ratio longest terminal seta/antenna		
instar	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.
1	—	—	—	37	30.0	2.5	43	2.41	0.13
2	23	2.11	0.14	49	43.3	3.2	40	1.22	0.07
3	47	1.79	0.08	50	54.9	4.1	48	0.94	0.04
4	50	1.62	0.08	50	66.6	3.8	50	0.98	0.04
Saether (1967)	42	1.8	—	—	—	—	42	0.96	—

In third and fourth instar larvae the typical knife shaped blades, important for the species distinction within the genus, are developed. In the third instar the blades are more slender than in the fourth. This finds expression in the length/width ratio (table IV). Third instar larvae measured by Saether (1967: table 1) showed a range in the corresponding length/width ratio of 4.0—6.0, which is lower than in our lake "Vechten" specimens.

The anterior side of the appendages of third and fourth instar larvae is distinctly S-curved (fig. 1c).

Table IV. Length, width and the ratio length/width of the prelabral appendages in the four larval instars. n — number of animals; s.d. — standard deviation.

	length in μm			width in μm			ratio length/width		
instar	n	mean	s.d.	n	mean	s.d.	n	mean	s.d.
1	39	61	4	—	—	—	—	—	—
2	42	52	4	—	—	—	—	—	—
3	46	165	16	46	29	2	46	5.6	0.6
4	50	297	22	50	61	7	50	4.9	0.5

2.4. Labrum.

In all four instars three pair of setae occur on the anterior side of the labrum.

The terminal armature of the labrum is diverse and may include labral and sublabral setae, bifurcate setae and bristled scales (Eckstein, 1936; Parma, 1969). Smith (1960: fig. 2,5) gave a picture of labral and sublabral setae from first and second instar larvae of *C. borealis*. This is contrary to our results in *C. flavicans*, where first instar larvae have only bristled scales (fig. 2C). In the second instar bristled scales, bifurcate setae (fig. 2D) and 3 labral setae are present. In the third also sublabral setae appear. According to Saether (1967: table 1) 8—10 labral and 6—8 sublabral setae occur in

this stage. The mean number of labral and sublabral setae in the fourth instar is 14,7 and 12,0 respectively (Parma, 1969).

2.5. Mandibles.

In the first instar the mandible is provided with three teeth, two large and one small. The number of setae in the mandibular fan is 3 (fig. 1d). Smith (1960: fig. 3) gave a similar picture of *C. borealis*. Sikorowa (1967b) mentioned 4 setae in the mandibular fan of first instar larvae of *C. alpinus*, but the possibility that one of the big teeth has been taken for a seta cannot be excluded. The mandibular fan is absent in the first instar of *C. astictopus* (Deonier, 1943: 385). The mandibles of our first instar larvae are colourless.

In the second instar the mandible has three big teeth and a small one situated between the second and the third tooth. In the third instar the tips of the big teeth are brown, whereas this colour extends further towards the base in the last larval instar (fig. 1d).

The number of setae in the fan increases during development. Variation in number is represented in table V. Sikorowa (1967b) mentioned for her Polish specimens of *C. alpinus* 5, 7—10 and 9—16 for the second, third and fourth instar respectively. Saether (1967: table 1) found 8—12 setae in the third instar of *C. flavicans*.

Table V. Number of setae in the mandibular fan of second, third and fourth instar.

number of setae / instar	5	6	7	8	9	10	11	12	13	14
2	15	16	1							
3			2	11	4					
4						2	10	21	6	2

2.6. Maxillae.

Changes in the shape of the maxille in the successive instars are represented in figure 1e. The maxillary palpus appears in the third instar. The "four but relatively broad bristles" at the base of the maxillary palpus (Saether, 1967: 574) are present in both third and fourth instar larvae. Smith (1960: fig. 2,5) found a maxillary palpus already in the first and second instar. But Weismann (1866: pl. V) in his picture of *C. crystallinus*, drew in the first instar only a peg on the stipes.

2.7. Eyes.

In the first instar simple eyes are present. According to Weismann (1866: 61), von Frankenberg (1915: 551) and Smith (1960: fig. 5) the development of a compound eye starts in the second instar. Deonier (1943: 386) on the contrary found only simple eyes in the second instar of *C. astictopus*.

Examination of at least 75 second instar larvae showed us that in *C. flavicans* the development of the compound eye indeed starts during this stage, but the pigmentation is restricted to only a few ocelli. Macroscopically this is hardly visible. The "absence" of a compound eye in the second instar and the presence in the third is a reliable feature to identify the two instars macroscopically.

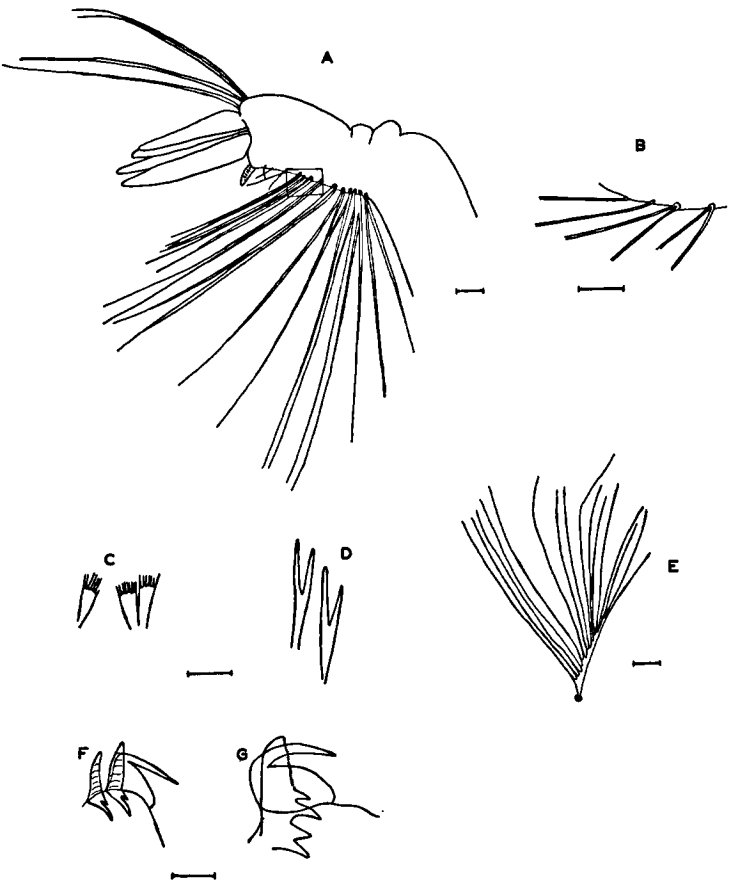

FIG. 2. Some morphological details of larval instars of *Chaoborus flavicans*. A, first instar. Last abdominal segments with anal fan; B, detail of A; C, first instar. Bristled scales of the labrum; D, second instar. Bifurcate setae of the labrum; E, fourth instar. Middle seta of dorsal side of head capsule; F, second instar. Detail of anal apparatus; G, third instar. Detail of anal apparatus. All horizontal bars represent 25 μm.

2.8. Tracheal bladders.

In the fourth instar larvae of *C. flavicans, crystallinus, obscuripes* and *pallidus* the anterior pair of air-sacs is always larger than the posterior pair (Parma, 1969). This applies also to the younger instars of *flavicans*. The ratio can vary greatly owing to transformation during preservation, but is mostly 1.2—1.3.

In the living state we found the air bladders of all instars to be always kidney-shaped.

2.9. Abdomen.

The protuberance on the ninth abdominal segment, known as the "dorsal

process" (Eckstein, 1936; Cook, 1956) is present in all instars. Initially the process is knob-shaped, but in the third and fourth instars the typical conical hook has developed (fig. 1f). The four anal papillae are pointed at the top. The lower pair is always 1.1—1.2 as long as the upper two. In all instars a cluster of two pairs of setae occurs above the papillae. In the first instar the four setae are smooth (fig. 2A), in the others they are pectinate. Smith (1960: 398) found pectinate setae in the first instar of *C. borealis*.

The mean lengths of the upper pair and the lower pair are represented in table VI. The ratio between lengths of upper and lower setae is 1.1—1.4.

Table VI. Mean length in μm of the upper and lower anal setae. n — number of animals. s.d. — standard deviation.

	upper pair			lower pair		
instar	n	mean	s.d.	n	mean	s.d.
1	40	160	15	40	223	23
2	44	177	18	44	236	17
3	44	278	16	43	355	22
4	50	461	38	50	583	48

The anal apparatus described by von Frankenberg (1915: 513), Peus (1934: 652) and Parma (1969) is present in the first instar, but in an underdeveloped form. Figures 2F and 2G show this apparatus in the second and third instar.

In the anal fan of the first instar the setae arise in pairs (figs. 2A, B; see also Sikorowa, 1970). Only towards the posterior extremity a few setae do arise singly. These are usually shorter than the rest. The setae are all smooth. Smith (1960: 398) found 15—22 pectinate setae in the first instar and 18—22 in the second instar of *C. borealis*. This points also to paired setae in the first instar.

The setae in the fan of the following instars are pectinate. The number of setae in successive instars is indicated in table VII. For the first instar these figures refer to the number of paired setae. Saether (1967: table I) mentioned 11 setae for a second and 18—23 for 49 third instar larvae. Sikorowa (1970) found 10—13, 9—11 and 4 pairs of setae in anal fans of first instar larvae of *C. crystallinus*, *C. flavicans* and *C. pallidus* respectively.

The fan of second, third and fourth instar larvae is stiff and its setae lie in one plane. In the first instar they protrude to all sides and the fan has an entangled aspect. This is macroscopically visible and makes it possible to distinguish the first from the second instar.

Table VII. Number of rays in the anal fan of the four instars.

instar	number of animals	mean	standard deviation	range actually observed
1	33	9.9	0.6	9—11
2	80	15.2	0.6	14—16
3	88	19.6	0.8	16—22
4	50	23.6	0.8	22—26

3. Discussion

From our study it can be concluded that besides the length of the head capsules several other characters are reliable for instar distinction. For instance the length of the antenna, longest terminal setae of the antenna, postantennal filaments and prelabral appendages proved to be instar specific. Moreover, the length of the median tooth is a reliable character to separate the instars (Stahl, 1959: 83).

For macroscopic identification of first, second and third instar larvae the absence or presence of the postantennal filaments, the visibility of the compound eye, the aspect of the anal fan and the ratio between length of antenna and its longest terminal setae are of importance. No reliable characters for macroscopic separation of free swimming third and fourth instar larvae were found. It is too difficult to count the number of setae in the anal fan of this rather lively animal. Generally, however, these instars are more robust so that microscopic identification is possible without injuring the larvae.

4. Key to the larval instars: macroscopic identification

1a. Simple and complex eye visible ..... *3rd or 4th instar*
b. Only simple eye visible 2
2a. Longest terminal setae of the antennae more than twice as long a the antennae. No postantennal filaments. Anal fan has an entangled aspect ..... *1st instar*
b. Longest terminal setae of the antennae about as long as the antennae. Postantennal filaments present. Setae of anal fan stiff and lying in one plane ..... *2nd instar*

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Drs. S. PARMA
Limnologisch Instituut
„Vijverhof"
Rijksstraatweg 6
Nieuwersluis (U) — The Netherlands

present address:
Delta Instituut voor Hydrobiologisch Onderzoek
Vierstraat 28
Yerseke — The Netherlands

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