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SUITABILITY OF MORPHOLOGICAL PARAMETERS FOR INSTAR DETERMINATION OF PESTIFEROUS MIDGES *CHIRONOMUS CRASSICAUDATUS* AND *GLYPTOTENDIPES PARIPES* (DIPTERA: CHIRONOMIDAE) UNDER LABORATORY CONDITIONS

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ABSTRACT. The midges *Chironomus crassicaudatus* and *Glyptotendipes paripes* were reared in the laboratory on artificial food under constant temperature (30°C) and a 14:10 h light:dark photoperiod for 31 days, from eggs laid by field-collected females. Sequential samples of developing immature stages were taken and measured. Eggs were on average 254 μm long and 102 μm wide at the widest point for *C. crassicaudatus* and 286 μm long and 113 μm wide at the widest point for *G. paripes.* Mean larval lengths ranged from 0.9 mm after hatching to 16.3 mm before pupation in *C. crassicaudatus* and from 0.8 mm after hatching to 9.7 mm before pupation in *G. paripes.* Mean length of pupae was 8.7 mm and 8.3 mm in *C. crassicaudatus* and *G. paripes,* respectively. Four morphometric head parameters (length, width, mentum width, and cephalolabial length) were tested for differentiation of larval instars. All parameters revealed 4 larval instars in both species, with head capsule width apparently the most sensitive indicator for instar differentiation. The cephalolabial length was the most sensitive indicator of sex differentiation in last instar. All investigated morphological parameters of head capsule of both species followed Dyar's law.

KEY WORDS Development, Dyar's law, immature stages, head width, Florida

INTRODUCTION

In many situations around the globe, chironomid midges emerge from urban and suburban lakes and other aquatic habitats in very large numbers and can pose severe nuisance, economic, and health problems for humans (Ali 1991, 1995). This is particularly true for central Florida, where adult chironomid swarms frequently emanating during summer months from some natural lakes may inflict losses to businesses and tourism-related industry amounting to several million dollars per year (Anonymous 1977). In central Florida, *Chironomus crassicaudatus* Malloch and *Glyptotendipes paripes* Edwards are the major pestiferous species of chironomids (Ali 1996). These species have been extensively investigated in several laboratory and field studies for management purposes (Ali et al. 1983, 1996; Xue and Ali 1994a, 1994b). The present study was conducted to expand information on growth, duration, and morphometry of individual larval instars of these species.

In insects, head capsule width or length often provides a reliable criterion for differentiation between larval instars (Daly 1985). In chironomids, in addition to head capsule width and length, various other head capsule parameters, such as antenna length, mandible size, mentum size, or cephalolabial length, have been used for instar determination (Ford 1959, McCauley 1974, Roback 1989, Maier et al. 1990, Stevens 1993). The present study evaluates application and sensitivity of 4 morphological characters: head length and width, mentum width, and cephalolabial length for determining larval instars of *C. crassicaudatus* and *G. paripes* and also describes instar duration of these species under laboratory conditions.

MATERIALS AND METHODS

Resting adult female *C. crassicaudatus* were collected on May 2, 2001, with an aspirator from a building by Lake Apopka, Orange County, Florida, and adult female *G. paripes* were collected in the same manner on June 14, 2001, from a lakefront area of Lake Eustis, Lake County, Florida. In the laboratory, females were released in a 30 × 30 × 30-cm screen cage provided with a 15-cm-diameter dish containing 400 ml of preaerated 1× Martin's rearing solution (Martin et al. 1980), supplemented with thiamine hydrochloride (1.2 mg/liter) (Stevens 1998), and allowed to lay eggs overnight. The next morning, 1 egg mass of a species was transferred to a plastic rearing dish (15 cm diameter, 4 cm high) filled with 400 ml of preaerated rearing solution. The dish was kept in a growth chamber (Model E-30B, Percival Mfg. Co., Boone, IA) at 30°C constant temperature under a cool white fluorescent lamp at a 14:10 h light:dark photoperiod. To examine morphological cues that may enable instars to be distinguished in summer generations, 30°C was selected to replicate the summer water temperatures of Florida lakes. Subsequently, the rearing dish for each species was checked every morning. About 350 ml of rearing solution was removed by vacuum and replaced with the same quantity of fresh preaerated rearing solution; 4 ml of food suspension was added daily into the dish. The food suspension was prepared from 35 mg of K9® fish food (Friskies, Victoria, Australia) (Ste-

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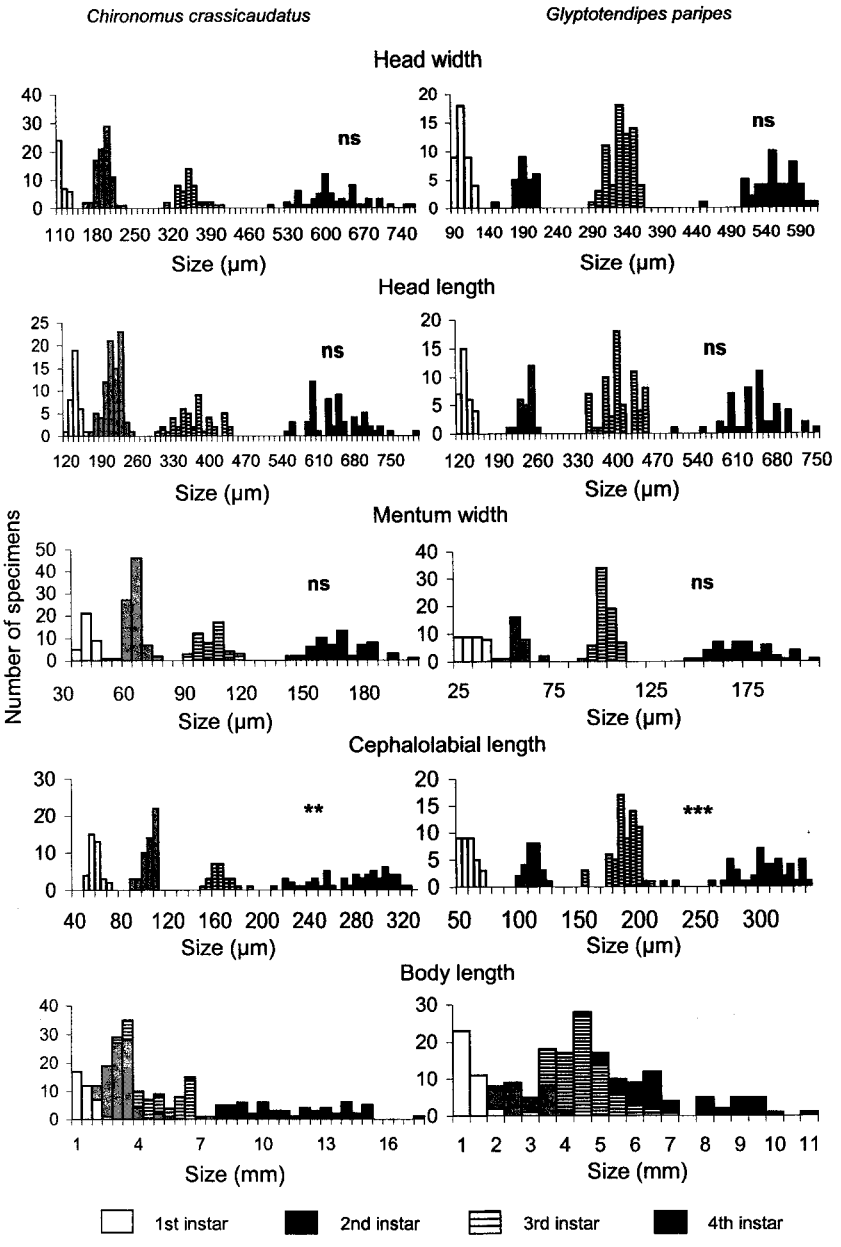

Fig. 1. Frequency distributions of head width, head length, mentum width, cephalolabial length, and body length of laboratory-reared larvae of *Chironomus crassicaudatus* and *Glyptotendipes paripes* (30°C and 14:10 h light:dark photoperiod). Distribution of 4th-instar head structures marked ns were not significantly different from a normal distribution; those marked ** or *** were significantly different ($P < 0.01$ or 0.001, respectively; χ^2 test).

vens 1993, 1998), supplemented with 8 mg of baker's yeast, and was macerated by a tissue grinder in 4 ml of rearing solution (Lobinske 2001).

For both species, starting immediately after egg hatch, 15 specimens of a particular immature stage were taken randomly from rearing dishes every 24 h during the 1st 5 days and thereafter every 48 h for the next 26 days. For *C. crassicaudatus,* only 7 specimens (4 pupae and 3 larvae) remained on the last sampling day. All collected material was preserved in 70% ethanol for 2–3 wk. Body lengths of larvae and pupae were measured (± 0.05 mm) by using a dissection microscope. Eggs dissected from excess egg masses were mounted on slides to measure (± 0.5 μm) length and width with an Olympus BHA microscope equipped with a calibrated eyepiece micrometer. The larvae, after body length measurement, were individually mounted

Table 1. Mean (± SD) head capsule morphometric parameters for individual instars of *Chironomus crassicaudatus* and *Glyptotendipes paripes*; minimum and maximum observed values are given in parentheses.

	Instar			
	1st	2nd	3rd	4th
	Chironomus crassicaudatus			
Head width (μm)	113 ± 6 (105–125)	191 ± 13 (160–225)	350 ± 21 (310–410)	611 ± 51 (500–750)
Head length (μm)	137 ± 11 (105–165)	213 ± 22 (175–250)	370 ± 36 (300–435)	643 ± 51 (550–800)
Mentum width (μm)	36 ± 4 (30–50)	59 ± 4 (55–70)	97 ± 6 (85–110)	159 ± 13 (135–195)
Cephalolabial length (μm)	48 ± 5 (40–60)	95 ± 6 (80–105)	162 ± 16 (95–190)	280 ± 32 (200–330)
Body length (mm)	1.2 ± 0.4 (0.6–2.1)	3.0 ± 0.6 (1.8–5.3)	4.9 ± 1.2 (3.0–7.5)	11.1 ± 2.6 (5.0–17.5)
	Glyptotendipes paripes			
Head width (μm)	102 ± 7 (90–120)	191 ± 14 (145–210)	329 ± 16 (290–360)	549 ± 34 (450–660)
Head length (μm)	130 ± 9 (115–150)	239 ± 13 (210–260)	402 ± 30 (350–450)	652 ± 55 (500–800)
Mentum width (μm)	36 ± 6 (25–55)	57 ± 5 (50–70)	101 ± 4 (90–110)	175 ± 14 (145–220)
Cephalolabial length (μm)	60 ± 12 (50–115)	109 ± 12 (60–125)	188 ± 11 (155–210)	301 ± 28 (190–340)
Body length (mm)	1.1 ± 0.3 (0.7–1.6)	2.7 ± 0.6 (1.7–3.8)	4.4 ± 0.8 (2.5–7.0)	8.3 ± 1.7 (4.5–12.0)

dorsoventrally on temporary slides by using a small drop of cold agar on each slide. This drop created a small chamber under the coverslip and prevented crushing of the head capsule by the cover glass, as described by Frouz (1994). Length and width of head, width of mentum, and cephalolabial length were measured (± 0.5 μm) with the Olympus BHA microscope. Head length was measured as the distance between the most distant anterior and posterior points of head capsule and width was measured as the distance between most distant lateral sides of head capsule margins. Cephalolabial length was measured as distance between the ventroposterior margin of the head capsule and the apex of the medial mentum tooth. The mentum width was measured as distance between most distant lateral margins of the mentum (Ford 1959, Stevens 1993).

Linear regression of $\log_{10}$-transformed mean values of larval head capsule parameters to instar number (Daly 1985, Stevens 1993) was used to test Dyar's hypothesis (Dyar 1890).

RESULTS AND DISCUSSION

Eggs of both species were elliptical, in common with most chironomid species investigated thus far (Pinder 1995). Eggs of *C. crassicaudatus* were 254 ± 23 μm long and 102 ± 7 μm wide at the widest point. The freshly laid eggs were greenish silver in color. Eggs of *G. paripes* were brown in color, 286 ± 15 μm long, and 113 ± 6 μm wide at the widest point.

The size distribution of all investigated morphological structures of head capsules in both species revealed the presence of 4 larval instars (Fig. 1), as in other Chironomidae studied so far in detail (Oliver 1971, McCauley 1974, Stevens 1993). The 1st-instar head capsule widths of both species (Fig. 1) agreed well with corresponding egg widths. The size frequency distribution of all measured parameters became more flat (platykurtic) for 4th instars, especially cephalolabial length, where bimodality can be observed in both species (Fig. 1). This ap-

Table 2. Linear regression values for $\log_{10}$-transformed mean data of individual head capsule parameters with instar number for *Chironomus crassicaudatus* and *Glyptotendipes paripes*. All regression coefficients are significant ($P < 0.01$).

	C. crassicaudatus			*G. paripes*		
	Slope	Intercept	r^2	Slope	Intercept	r^2
Head width	0.246	1.800	0.999	0.243	1.779	0.998
Head length	0.225	1.896	0.998	0.232	1.896	0.998
Mentum width	0.215	1.341	0.999	0.231	1.313	0.998
Cephalolabial length	0.251	1.446	0.998	0.233	1.558	0.998

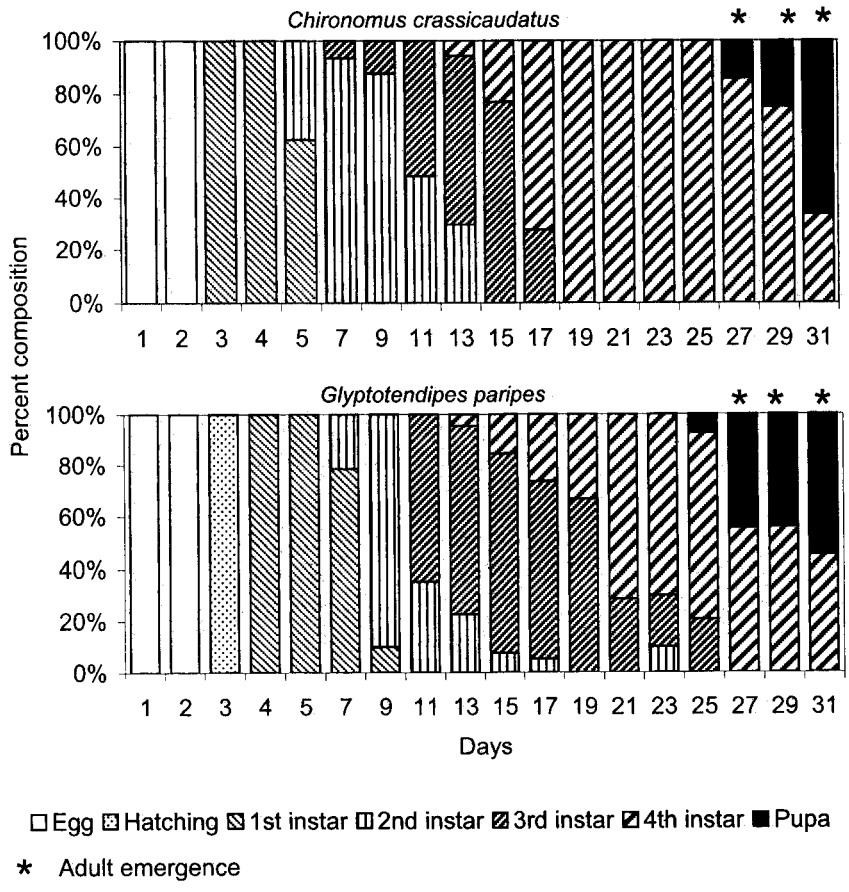

Fig. 2. Percent composition of immature stages in laboratory cultures of *Chironomus crassicaudatus* and *Glyptotendipes paripes* (30°C, 14:10 h light:dark photoperiod), determined from sequential samples.

parently corresponds with sexual dimorphism of 4th instars also observed in other chironomid species (Ford 1959, Atchley and Martin 1971, Stevens 1993). Measurement of 4th-instar exuviae of *C. crassicaudatus* revealed that females had significantly wider heads than males, although an overlap exists between both sexes (J. Frouz, unpublished data). Thus, despite differences between sexes for other investigated morphological parameters, cephalolabial length most sensitively reflects sexual dimorphism in the 4th instar.

Head capsule length and width did not overlap between instars, whereas mentum width and cephalolabial length did overlap. Overlap of mentum width occurred between 1st and 2nd instars in both species and cephalolabial length overlap occurred between 3rd and 4th instars in *G. paripes* (Fig. 1). By using the range that includes 99.9% of all the-

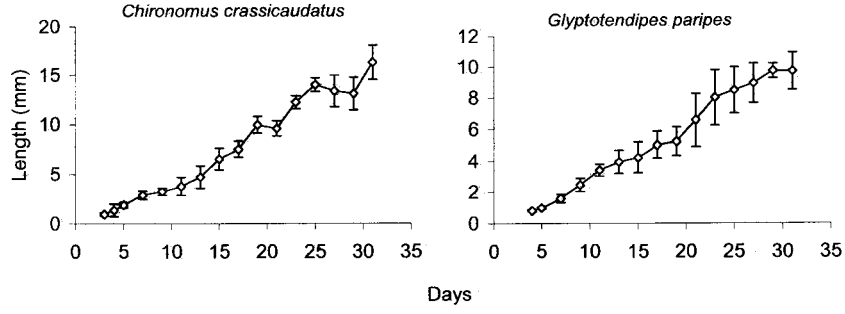

Fig. 3. Mean (± SD) larval body length of laboratory-reared (30°C, 14:10 h light:dark photoperiod) *Chironomus crassicaudatus* and *Glyptotendipes paripes* at various times after oviposition.

oretically possible values (mean ± 3 SD) in Table 1, some overlap may occur in head length (between all instars of *C. crassicaudatus* and between 3rd and 4th instars of *G. paripes*) and in cephalolabial length (between 3rd and 4th instars of *C. crassicaudatus* and in all instars of *G. paripes*). Considering all morphological parameters used in this study, head capsule width allowed the best and easiest instar differentiation. This agrees with the study of McCauley (1974), but some other authors do not recommend the use of head capsule width for larval instar differentiation because of the risk of crushing the head capsule when mounting the larva on a slide (Ford 1959). In this study, no damage to head capsules was observed when using the temporary slide technique of Frouz (1994). However, accurate microscopic measurements of head capsule width or length are more dependent on precise dorsoventral head orientation than is cephalolabial length. Additionally, size of a measured structure should be considered, particularly in younger instars, because the absolute increase in a structure's size between instars was greater for proportionately larger structures. However, the sensitivity differences between investigated morphological structures were minor because theoretically possible overlaps disappeared when a range covering 99% of theoretically possible values was used. Thus, both head length and cephalolabial length allow instar determination with a high level of certainty.

Stevens (1993) observed that head capsule size depends on rearing temperature, but temperature-induced variation did not hide differences between instars. Observation of larval exuviae of 4th instars of *C. crassicaudatus* reared at a wide range of temparatures (15–32.5°C) revealed a similar range of head capsule widths (J. Frouz, unpublished data) as in this study. This indicates that use of the range that covers 99.9% of the theoretically possible values of head capsule width (mean ± 3 SD of data in Table 1) may be recommended for instar differentiation in field studies.

The relationship between $\log_{10}$ of all investigated parameters and instar number indicated consistent geometric progression of these structures during development in agreement with Dyar's rule (Dyar 1890), and as also observed in *Chironomus tepperi* (Skuse) by Stevens (1993). The slopes of these relationships were similar in both species for all studied morphological parameters (Table 2).

Larval body length overlapped between all instars in both species (Table 1 and Fig. 1). In *C. crassicaudatus* some 2nd and 4th instars of similar body length were found (Fig. 1). Thus, body length can be used only for very rough estimation of larval instar. However, as emphasized by Maier et al. (1990), body length is a simple, nonlethal measurement that can provide estimates of larval development. The accuracy of instar assessment when lengths overlap can be increased by comparison of thorax and head width. Observations in both species in the present study indicated that the head width of larvae immediately before molting typically was smaller than the width of the thorax, whereas head width of larvae just after molting was usually as wide as the 1st thoracic segment. Larvae used in this study were stored in ethanol, and therefore reasonable caution should be applied for use of observed body length thresholds for live larvae. Also, the body size may be affected more by artificial food as well as by rearing temperature than by head capsule characteristics.

In both species, the 1st adults emerged after 27 days of development (Fig. 2). Based on data in Fig. 2, mean duration of *C. crassicaudatus* egg development was estimated at 2 days and duration of individual instars (1st to 4th) at 2–3, 6, 5, and 16 days, respectively. Mean egg duration of *G. paripes* was estimated at 2 days, followed by a 1-day hatching period where the 1st instars remained inside the gelatinous egg sheath. Mean individual instar (1st to 4th) durations were 3, 3, 11, and 11 days, respectively.

Larvae of both species roughly doubled their body length during each progressive instar (Table 1). Body length growth in both species showed several steplike waves, the most obvious between 17 and 21 developmental days in both species (Fig. 3). Body length of pupae, 8.7 ± 0.3 mm and 8.3 ± 0.7 mm in *C. crassicaudatus* and *G. paripes,* respectively, was shorter than body length of larvae.

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