Morphometrics of two Californian populations of *Gumaga nigricula* (McLachlan 1871) (Trichoptera: Sericostomatidae)[1,2]

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ABSTRACT

Eighty *Gumaga nigricula* larvae from Hopland Spring and Big Sulphur Creek, northern California, USA, were studied to quantify possible dissimilarities among them. The populations are statistically different in two meristic characters: number of setae on the anterior margin of the pronotum and number of pleural sclerites on abdominal segment VIII. They also appear to be different in a continuous character, head width/length ratio. In all cases, specimens from Big Sulphur Creek have statistically significant higher values. These data are congruent with the hypothesis that there is biologically significant genetic isolation between these populations.

Key words: Morphometrics, *Gumaga*, Trichoptera, morphology, systematics, ecological indicator

RESUMEN

Morfometría en dos poblaciones californianas de *Gumaga nigricula* (McLachlan 1871) (Trichoptera: Sericostomatidae)

Ochenta larvas de *Gumaga nigricula* provenientes del Hopland Spring y del Big Sulphur Creek en el norte de California, Estados Unidos, se estudiaron para cuantificar las posibles diferencias entre ellas. Estas poblaciones son estadísticamente diferentes en dos características merísticas: el número de cerdas en el margen anterior del pronoto y el número de escleritos pleurales en el segmento abdominal VIII. Estas poblaciones también parecen ser diferentes en la característica continua, cociente ancho/largo de la

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cabeza. En todos los casos, los ejemplares del Big Sulphur Creek tienen valores significativamente mayores. Estos datos son congruentes con la hipótesis de que existe aislamiento reproductivo significativo entre estas dos poblaciones.

INTRODUCTION

Gumaga nigricula is a widespread sericostomatid caddisfly whose larvae inhabit streams in the western United States, from Arizona to Oregon (Resh et al., 1981). Larvae lie exposed over gravel, hidden beneath rocks (Wiggings, 1977), or on sandy bottoms (Wood to Santiago-Blay, personal communication 1984), and always occur in lotic environments, where they may reach densities of 10,000 individuals per square meter (Resh, 1983; Resh et al., 1981). Larvae of these species are also found in streams perturbed by heat, sedimentation, or high concentration of sulfates, ammonia, and heavy metals. In these disturbed situations, *G. nigricula* larvae may occur in numbers greater than all other aquatic macroinvertebrates considered together (Resh et al., 1981). The membrane-attached maxilla and labium, the maxillolabium, probably facilitates feeding in shovel-like fashion. The asymmetrical mandibles (Lepneva, 1971) suggest their function in a mortar-pestle fashion. Indeed, a preliminary study of the gut contents of four *G. nigricula* disclosed over 95% particles < 0.1 mm diameter (Wiggins, 1977). As many trichopterans do, G. nigricula construct pupal cases and aggregate in dense clumps (Resh et al., 1984). Adult *G. nigricula* are common in the terrestrial-lotic ecotone, with highest densities in the immediate five-meter vicinity of streams and at relatively high levels (about 8 m) above ground (Jackson and Resh, 1989). Pheromone-mediated sexual attraction has been demonstrated for adult *G. nigricula* (Resh et al., 1987; Resh and Wood, 1985).

Several authors have suggested that some trichopteran genera, including *Gumaga*, can be used as water quality indicators (Nielsen, 1974; Resh et al. 1981; Schuster and Etnier, 1978). According to these authors, to be considered as a potential indicator, an organism must meet these conditions: first, have a widespread distribution for comparative and predictive purposes; second, be abundant at the site of investigation before possible pollutants are dumped for quantitative evaluations; and third, have a well-defined taxonomic status in order to help establish sound cause-effect relationships. Solid species-level taxonomic knowledge is of paramount importance in the selection of reliable water quality indicators (Resh and Unzicker, 1975).

Although *G. nigricula* satisfies conditions 1 and 2 for indicator species, there are conflicting data regarding its precise taxonomic status. For example, two larval morphs can be distinguished on the basis of dorsal cranial coloration patterns: a darkheaded form occurs in Hopland Spring, whereas a striped-headed form inhabits Big Sulphur Creek. In addition, offspring from transplanted eggs retain the larval characteristics of their site of origin, at least during the first stages of larval development (Wood, 1988). Data on the oviposition patterns of each morph suggest that the populations are separate taxa (Wood, 1988). However, the adults are morphologically indistinguishable. In addition, males respond to pheromone extracts from females of the other population, but production of viable laboratory hybrid progeny is limited (Wood, 1988).

This paper further quantifies differences between Hopland Spring and Big Sulphur Creek populations of *G. nigricula*.

METHODS

Forty preserved *G. nigricula* larvae from Hopland Spring and from Big Sulfur Creek were studied. Both localities are 100-150 km NNE of Berkeley, CA. Hopland is a first order spring located at the University of California Hopland Field Station, Mendocino Co., CA, USA. Big Sulphur creek is a second order stream located at The Geysers, Sonoma Co., CA. The collection data for Hopland Spring specimens: site 4, 15 March 1981, V. H. Resh, 21 specimens; under walnut trees, midway between trough and tire tracks, 20 March 1982, 19 specimens. The collection data for Big Sulphur Creek specimens: exclu. Pool 1, Surber samples, 17 Dec. 1979, 40 specimens, J. R. Wood collector. Specimens are maintained in Resh's and Wood's research holdings.

Resh (1982) found that it is very difficult to determine larval instar of *G. nigricula* on the basis of head capsule width alone. Population head width also changes with environmental conditions (Resh 1992). Thus, to minimize the effects of possible differential ages and time of collection, as well as to facilitate comparisons, I selected only the largest specimens (i.e. those whose head capsule maximum width was, 1.0-1.5 mm) from each population. The mean and variation of head capsule widths was similar in both populations.

I counted three meristic characters: the number of setae on the anterior margin of the pronotum, the number of setae on the metanotal area 1, and the number of pleural sclerites on abdominal segment VIII (Figures 1-3). The counts were done with a dissecting microscope and the drawings with a calibrated reticle eyepiece (100 squares/cm^2).

Hotelling's t^2-test, the multivariate counterpart of the Student's t-test (Afifi and Allen, 1972; Hull and Nie, 1981), was used to analyze the data with a pre-set alpha, $\alpha = 0.05$. Bonfevroni's technique (t value/total number of pairwise comparisons) was employed to determine the

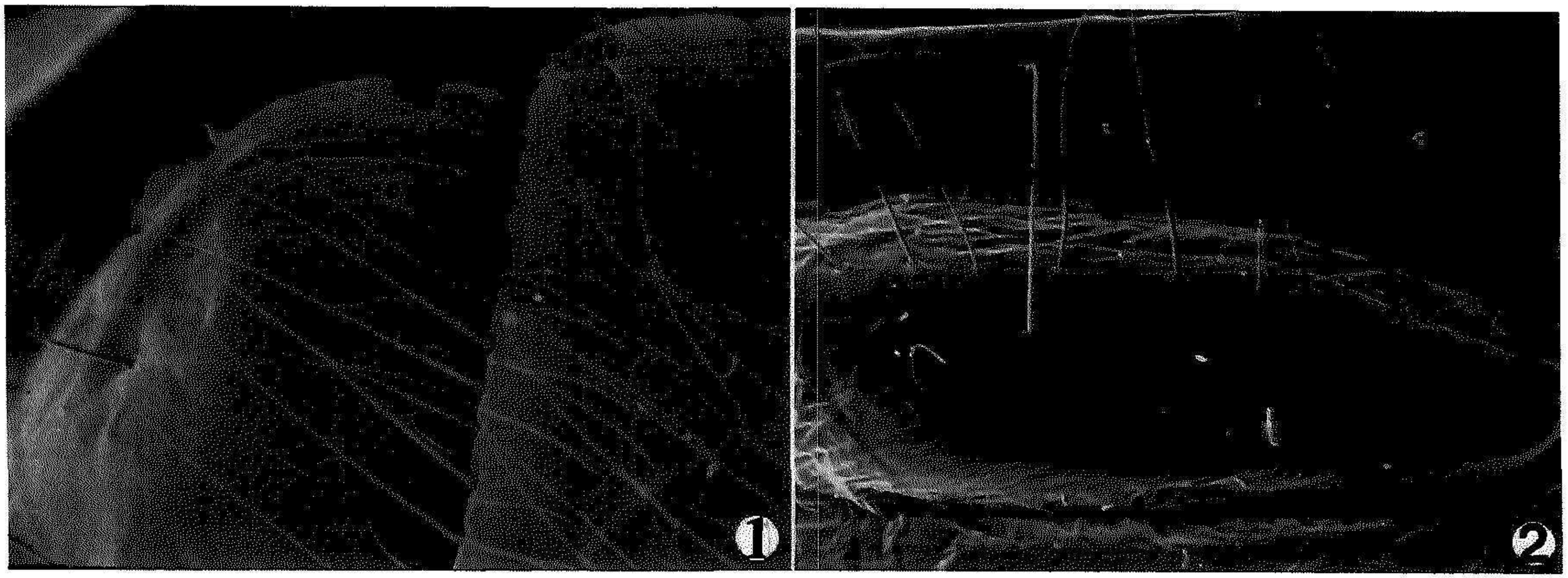
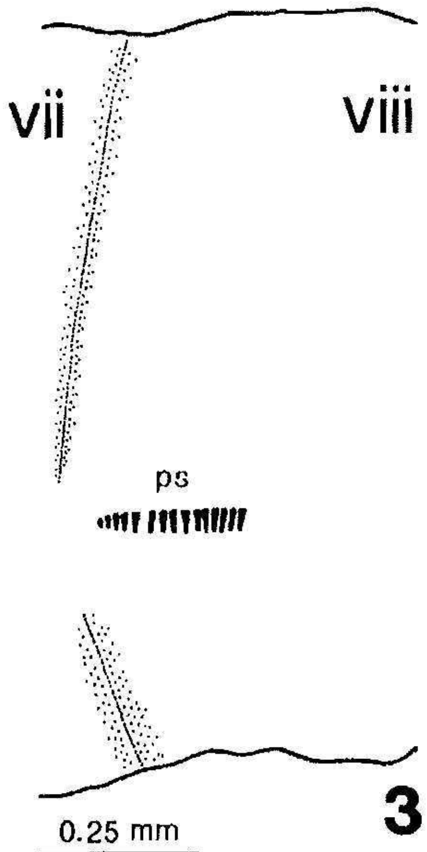


FIGURE 1. Head and pronotum of *G. nigricula* larvae, lateral, showing setae on anterior pronotal margin.
FIGURE 2. Right metanotal setal area one of *G. nigricula*.



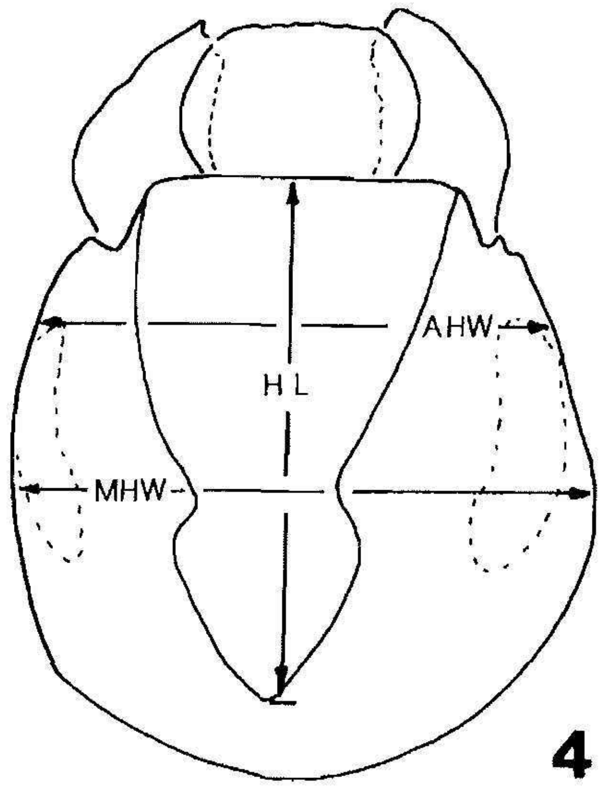


FIGURE 3. Pleural sclerites (ps) on abdominal segment VIII of *G. nigricula*. (See also Figure 5).

FIGURE 4. Head capsule of *G. nigricula* larvae showing operational definitions used in this study. AHW = anterior head width, HL = head length, MHW = median head width.

characters responsible for the interpopulational differences. In practice, Bonfevroni's technique is analogous to a reduction in the α value established for rejecting the H_0 of no difference in the Hotelling t^2-test. The data input and calculations, except Bonfevroni's, were done with "S" (Becker and Chambers, 1981).

With a calibrated eyepiece (100 units/cm), two other measurements were taken on 20 other dissected 10% KOH-cleared heads per population and t-tested: head width/length ratio, and the anteroposterior divergence [= (maximum head width - anterior head width, at eye level)/ head length] (Figure 4). Untransformed as well as transformed [logarithmic (ln x + 1) and arcsin $\sqrt{X}$, see Sokal and Rohlf 1995, Zar 1996] ratio data was t-tested (both tails, unpaired, $\alpha = 0.05$) by using StatView (Haycock et al., 1994).

Electron micrographs were taken with an ISI-DS 130 scanning electron micrograph after specimens were sputter-coated with a 60-nm layer of gold.

RESULTS

The Hotelling t^2-test value is $t_{23,78} = 102.47$ ($P < 0.001$); hence, the two samples are statistically different. Bonfevroni's test showed that two of the three characters studied, mean number of sclerites on abdominal pleuron VIII and mean number of setae on the anterior margin of the pronotum, account for these results. In both cases, the specimens from Big Sulphur (BS) Creek have more meristic units than those from Hopland (H): $\overline{X}_H = 34.00$ sclerites, sd = 3.81; $\overline{X}_{BS} = 50.45$, sd = 5.56, $t_{78} = 5.12$, $P < 0.001$) (Figure 7); $\overline{X}_H = 45.55$ setae, sd = 5.33, $\overline{X}_{BS} = 55.23$, sd = 5.89, $t_{78} = 11.48$, $P < 0.01$; The mean number of setae on setal area 1 is not statistically different in the two populations: ($\overline{X}_H = 19.48$ setae, sd = 3.25; $\overline{X}_{BS} = 18.70$, sd = 2.08, $t_{78} = 0.43$; $P > 0.50$).

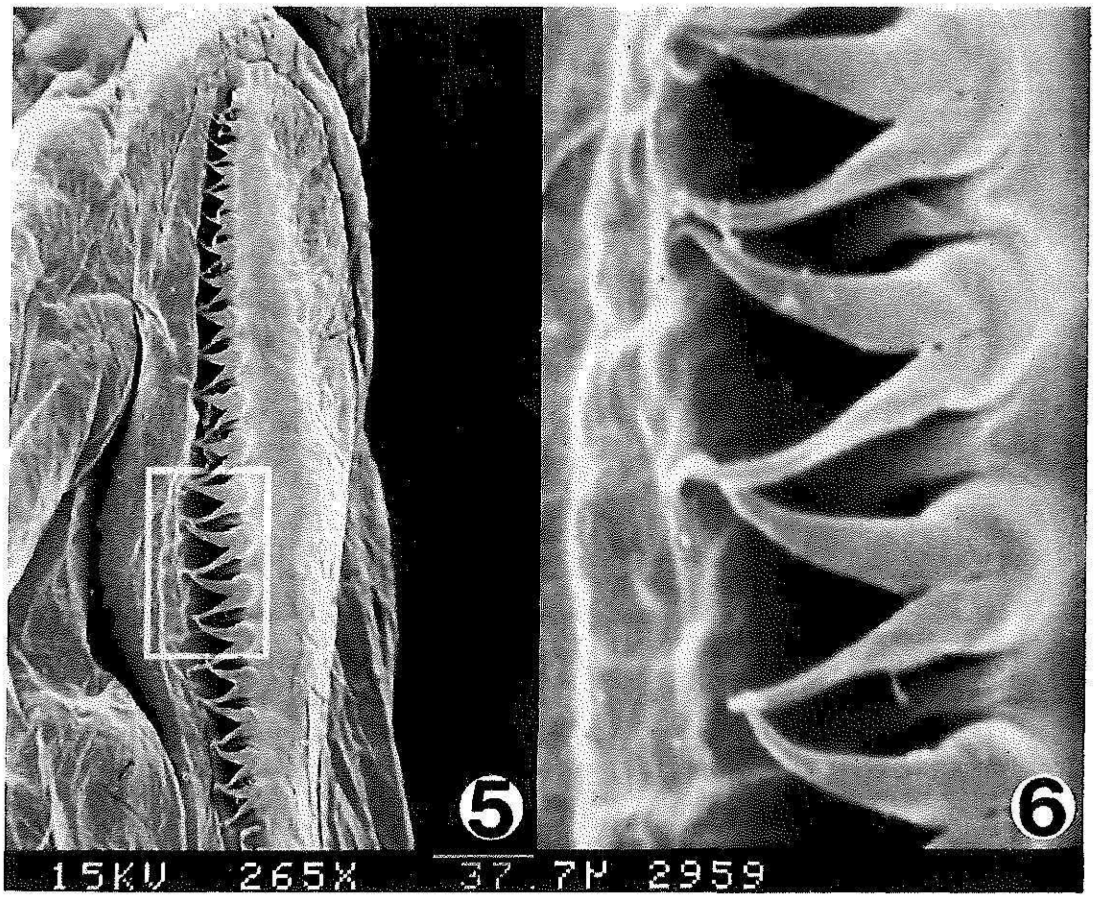
Furthermore, *G. nigricula* populations at Hopland Spring and at Big Sulphur Creek are also statistically different in head capsule width/length ratio ($\overline{X}_{BS} = 0.89$, sd = 0.04, $\overline{X}_H = 0.96$, sd = 0.06; t = 2.69 $P < 0.05$) (Figure 8) but similar in anteroposterior divergence ($\overline{X}_{BS} = 0.09$, sd = 0.04, $\overline{X}_H = 0.12$, sd = 0.06; t = 0.58, $P > 0.50$). The differences in head width/length ratio remained statistically significant ($P < 0.05$) when data were ln X + 1 or arcsin $\sqrt{X}$ transformed (results not shown).

DISCUSSION

The total number of sclerites on abdominal pleuron VIII appears to be a reliable character. There is a large and significant difference in the counts (Figure 7, x values). This character lacks the drawbacks noted for setae (see below) except for finding the two or three anteriormost sclerites because the integument of pleuron VII may be folded over them. It must be noted that *G. nigricula* populations from other localities in California examined by Wood (1988) show no difference for the characters studied here nor for other traits. This finding strongly suggests that genetic flow between Big Sulphur Creek and Hopland Spring populations is much less than that between other studied combinations of Californian populations.

Lepneva (1970, 1971) provides no data on the functional significance of those sclerites; Wiggins (1977) does not mention them although the sclerites are illustrated. These sclerites are bifid (Figures 5, 6), about 20 µm long, 15 µm wide (maximum dimensions), and they are possibly functional in mechanoreception by holding the larvae secured within the case. The larval case has a reduced diameter distally; abdominal growth, particularly posteriorly, must be accompanied by ease construction apically.

Setal counts, the other meristic character used, are unreliable for they may be difficult to see. Moreover, setae may break off and, if large



FIGURES 5-6. Pleural sclerites on abdominal segment VIII of *G. nigricula*. Scale bar, Figure 6 = 7.1 um.

setal pits are absent, their actual location of setae may be difficult to discern (Figures 1, 2). However, if group differences in setal counts are large and/or if the pits are conspicuous, such as in trichobothria of arachnids (Foelix, 1982) and of some insects (Wygodzinsky and Lodhi, 1989), this character is useful. The possible setal count difference between Big Sulphur and Hopland Spring specimens had been previously noted but not quantified (Resh et al., 1981). Although there is considerable overlap in the setal counts of the two populations, (Figure 7, y values), the differences are still statistically significant. Accurate counts were obtained with some difficulty, yet interpopulation differences were clearly large enough to be detectable.

Morphometric data are difficult to interpret (Barlow, 1961; Bookstein, 1991; Brown, 1959; Daly, 1985). In *G. nigricula*, a hypothesis of variation with temperature does not explain the observed differences because both streams are temperature stressed: Hopland because of its shallowness; Big Sulphur because of human-made developments (Resh et al., 1981). Although Hopland Spring is less than 40 km north of Big

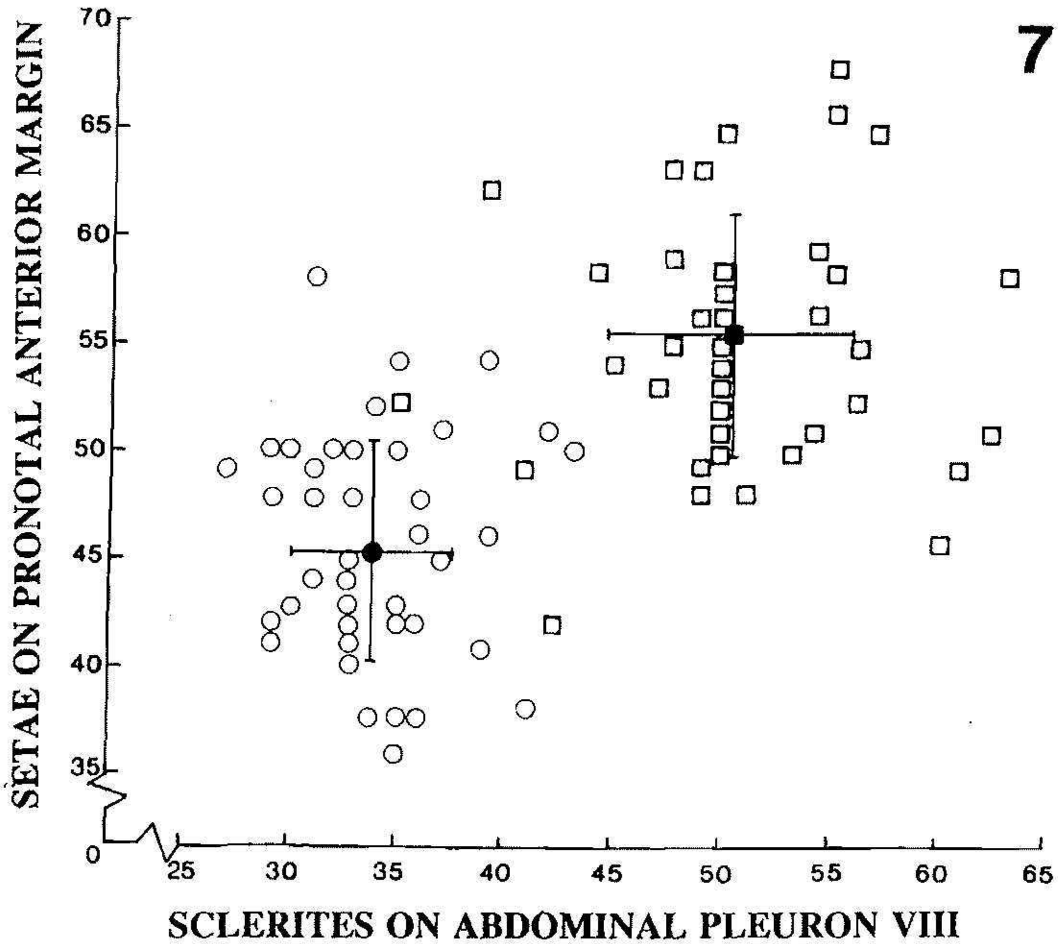


FIGURE 7. Number of setae on pronotal anterior margin in relation to the number of sclerites on abdominal pleuron VIII of *G. nigricula* larvae. ○, □ = actual data for Hopland Spring and Big Sulphur Creek specimens, respectively; darkened symbols and lines crossing them represent the mean and one standard deviation, respectively.

Sulphur Creek, the possible slight temperature decrease with latitude is probably compensated by the higher elevation (about 300 m) of Big Sulphur Creek (U.S. Geological Survey, 1969). Seasonal morphometric variations in *Tinodes waeneri* L. (Trichoptera: Psychomyiidae) have been associated with corresponding temperature variations (Jones, 1974). If the environment is related to morphometric differences found in this study, its mode of action is more complex.

Another possibility is that between these populations there is biologically significant isolation, forming biotypes (Diehl and Bush, 1981) or "ecological species" (conspecific populations that are morphologically similar in many, but not in every character, but whose biochemistry or ecological preferences are different (Ferguson, 1980; Wood, 1988), or cryptic species (White, 1973). Several head capsule characters have been successfully used to separate populations of adult caddisflies

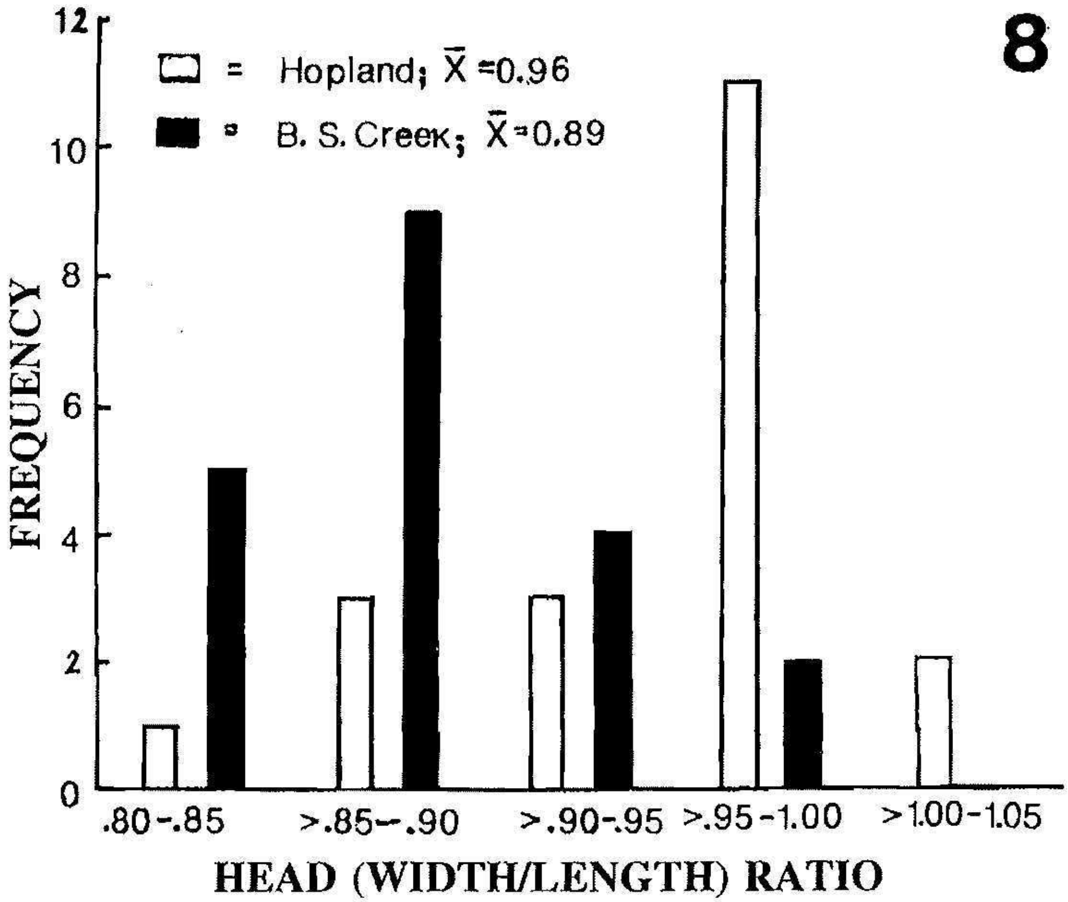


FIGURE 8. Frequency of head capsule (width/length) ratios in two samples of *G. nigricula* larvae.

(Malicky, 1977). Evidently, *G. nigricula* still needs a more precise taxonomic characterization in order to be confidently used as a long-term biological indicator.

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