Observations on the Bionomics of the Freshwater Nematode Chromadorina bioculata

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Abstract: The biology and morphology of *Chromadorina bioculata* is presented. The nematode was abundant on the alga *Cladophora* of one lily pond, but absent from similar habitats in nearby ponds. The setae, caudal glands, high locomotory rates and positive photo response have been interpreted in relation to maintenance on, amongst and between algal filaments, suspended over large volumes of water.

When placed in tap or distilled water, *C. bioculata* became inactive and died. The influence of pH buffers, tonicity, temperature and starvation on activity were investigated. *C. bioculata* survived longer in artificial sea water diluted 10 or 100 times with distilled water, or in NaCl isotonic with sea water diluted 100 or 1000 times, than in tap or distilled water. No evidence of wide osmotic toleration or osmoregulation was observed. Activity was influenced by temperature, with peak activities occurring at the seasonal normal temperature. These findings are discussed in terms of general nematology and habitat selection. *Key Words:* osmotic tolerance, activity, behavior, distribution, morphology, setae, caudal glands.

The Chromadoridae consist mostly of marine species, but a few are adapted to soil and fresh water habitats. *Chromadorina bioculata* (Schultze) occurs in fresh water and is one of the few nonmarine nematodes with photoreceptors (4, 5). The species has been reported from North, East and Central Europe, including the Balkans. Almost nothing is known of the biology of freshwater nematodes, and some observations made between June and September 1971 on the morphology, biology and physiology of *C. bioculata* are presented below.

MORPHOLOGY: *Chromadorina bioculata* (Schultze *in* Carus, 1857), Wieser, 1954 used in these investigations had the following measurements:

Females: L = 0.56-0.63 mm; a = 20-31; b = 5.2-6.4; c = 5.4-6.2; V = 46-60%.

Males: L = 0.49-0.57 mm; a = 21-28; b = 5.5-6.4; c = 5.0-6.4.

The population corresponded well with the description of the species made by Andrássy (1). In addition, we found that the female had a total of 130-132 setae, and the male 124-128 setae. These included four cephalic setae, two subdorsal and two subventral, all about 7 μ long. In the cervical and oesophageal regions there were four rows of somatic setae also about 7 μ long. The setae in these rows became shorter towards the middle of the body. The distribution of setae had the following pattern (based on the examination of 5 females and 4 males).

$$♀: 4\ \frac{6}{5}\ \frac{10}{8\text{-}9}\ \frac{12}{10}\ \frac{7\text{-}8}{3\text{-}4} \qquad ♂: 4\ \frac{6}{5}\ \frac{20\text{-}22}{19\text{-}22}\ \frac{6\text{-}7}{3\text{-}4}$$

The first number represents cephalic setae,

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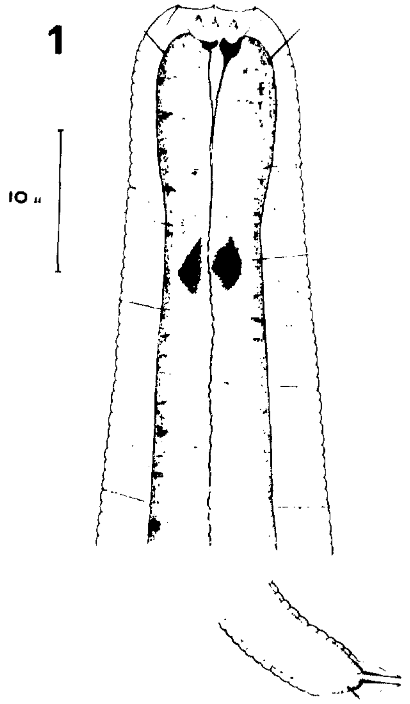

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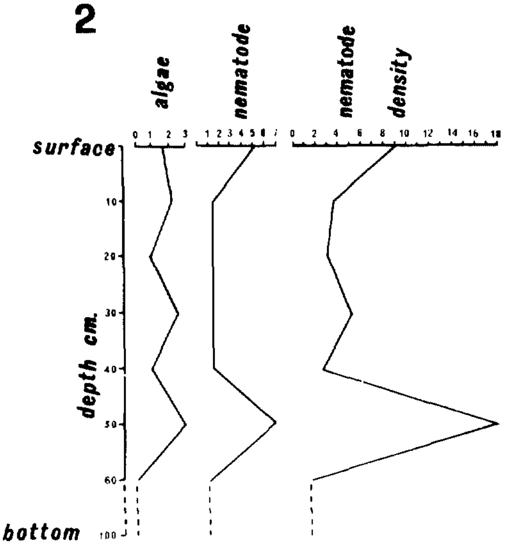

FIG. 1-2. **1. A.** Anterior part of *Chromadorina bioculata* (Schultze) and **B.** tail end showing caudal gland secretions. **2.** Algae and *C. bioculata* showing depth distribution, and their coincident occurrence. Algae are assessed in relative quantities; *C. bioculata* data is expressed as the number of specimens of *C. bioculata*/mg dry weight of alga.

the following numbers above the line are subdorsal somatic and below the line subventral somatic setae. In the female, each pair of figures represents the oesophageal, pre-vulval, post-vulval and tail regions, respectively. In the male they represent the oesophageal, genito-intestinal and tail regions. Unlike Andrássy (1) illustrated in his Fig. 6B, we observed two setae (one on each side) at the base of the caudal gland duct (Fig. 1).

DISTRIBUTION OF *CHROMADORINA BIOCULATA* AND OTHER NEMATODES: We failed to recover *C. bioculata* from any of the ponds and lakes around the Imperial College Field Station except for one lily pond in which it abounds. The pond is concrete-lined and measures 7 X 15 m, having a depth of about 1 m. Tap water is added monthly to replace water lost by evaporation. Other than the planktonic habitat, from which no *C. bioculata* were recovered, the pond presents two distinct habitats. For the summer months the vertical walls are covered in algae, chiefly of the genus *Cladophora*, growing outward in dense mats of up to 2-3 cm. This periphyton also includes Ciliata, *Hydra* and Rotifera.

The pond bottom detritus is largely derived from decaying beech tree litter, and shelters Chironomida larvae, Ciliata, Cladocera, Diatomeae, Gastrotricha, Ologochaeta, Rotifera and many Thecamoebae, together with species of the algal genera *Closterium* and *Pediastrum* as well as nematodes.

Of the 748 nematode specimens identified in the pond, nine species of nematodes were found, eight in the algae and four in the bottom mud (Table 1). Seventy-seven percent of all nematodes on the algae were *C. bioculata*, and on the deeper algae it was virtually the only species present. In contrast, only 4% of the nematodes in the bottom detritus were *C. bioculata*. The mean density of *C. bioculata* was three individuals/mg dry weight of alga, although up to 9/mg have been found. Males and females were present in equal proportions. *C. bioculata* occurred in highest mean densities (7/mg dry wt. alga) 40-60 cm below the

TABLE 1. Nematodes collected from the algae on the sides of the pond and from the mud bottom.

Habitat	Nematode	Number
Algae on the pond sides	*Chromadorina bioculata* (Schultze)	523
	Aphelenchoides sp.	108
	Rhabdolaimus terrestris de Man	39
	Plectus parvus Bastian	2
	Camallanus sp.[a]	2
	Monhystera vulgaris de Man	2
	M. paludicola de Man	1
	Eudiplogaster sp.	1
Mud on the pond bottom	*Monhystera vulgaris*	34
	M. paludicola	28
	Tobrilus sp.[b]	5
	C. bioculata	3

[a] L_1 larva of fish parasite, probably in pond fish.

[b] *Tobrilus* close to *T. consimilis* and *T. aberrans* in the Andrássy key (1964). (2)

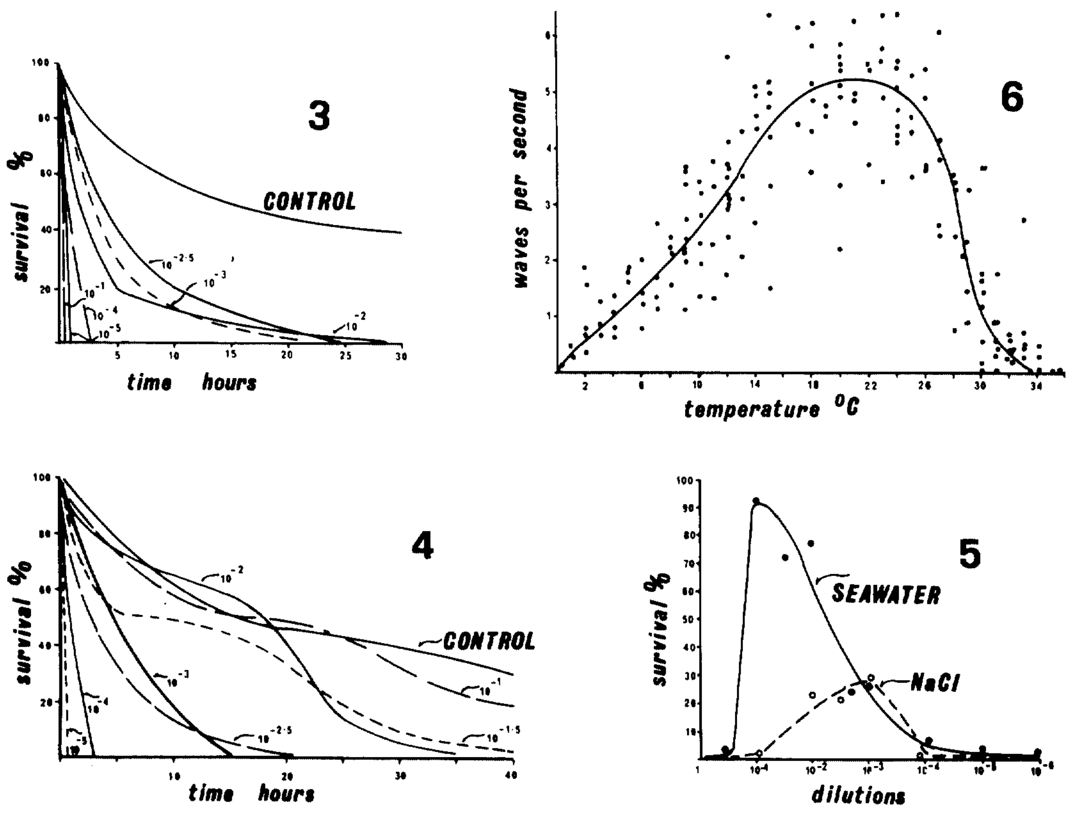

FIG. 3-6. 3. Mean survival of *Chromadorina bioculata* at various aqueous dilutions of NaCl at 20 C. The control was in filtered pond water. Each line is the mean for 50 individuals. 4. Mean survival of *C. bioculata* in a range of aqueous dilutions of artificial seawater at 20 C. The control was in filtered pond water; each line is the mean for 50 individuals. 5. Comparison of *C. bioculata* survival in NaCl and seawater of equal osmotic values (derived from Fig. 3 and 4). Ordinate axis is percent survival × time, abscissal axis is aqueous dilution. 6. Locomotory rates of *C. bioculata* over a range of temperatures. Each point represents a single observation; line fitted by eye.

surface, and showed a second peak density at the surface (Fig. 2).

LOCOMOTORY HABITS OF *C. BIOCULATA*: Adult *C. bioculata* are capable of very rapid movements, reaching a maximum of 6.5 undulations/sec at 20-22 C (Fig. 6), and swim freely by their own efforts. However, no individuals were found in samples of pond water any distance from the algae; instead they moved in and around the algal filaments. Typically they moved over the surface of the filaments in rapid, but highly coordinated bursts of movements following rectilinear or helical paths. On the filaments the body "flowed" so that there was minimum lateral displacement and each part of the body followed the previous position (8). As it flowed over the alga the body appeared to be attached, and did not fall off when on the under side (as one might have anticipated). The setae may be secretory (7), but also provide considerable sensory input regarding posture and nature of the substratum.

When not actively changing location, movement is dominated by the caudal gland and the slightly hooked tail, the body pivoting from a subterminal position. Individuals spend considerable periods attached to the substratum by caudal gland secretions. This is a viscous, mucoid elastic, often copious secretion which we were unable to stain using Alcian blue for acid mucopolysaccharide and oil red O and Sudan Black A for lipids (10). Occasionally adults were seen attached to the substrate or to algae by means of the four cephalic setae. In this position they may have been feeding, since their intestinal lumens frequently contained green particles, but none was ever seen engulfing food.

ACTIVITY AND SURVIVAL LIMITS OF *C. BIOCULATA*: When removed from the pond and kept in the laboratory on algae in pond water or in filtered pond water (pH 8.0-8.2), *C. bioculata* survived for several days. If placed in tap water (pH 7.6-8.1) or distilled water (pH 4.8-6.0), they quickly became inactive and died. Because of the survival in filtered pond water, it seemed unlikely that they were starving in the tap and distilled water, but rather were highly susceptible to some physicochemical conditions. Preliminary to any physiological laboratory studies, some factors likely to influence survival were examined.

Osmotic and ionic conditions. Five adults in each experiment were placed in 0.1 ml of NaCl in microtiter plates using a range of concentrations calculated in osmotic pressures equivalent to sea water, and each concentration was tested with 50 individuals. At aqueous dilutions of 10^{-1}, 10^{-4} and 10^{-5} NaCl of the solution isosmotic to sea water, with which we started, all specimens were irreversible when inactivated within 2 hr, but they survived 20-30 hr at dilutions between 10^{-2} and 10^{-3} NaCl at 20 C (± 2). The controls in filtered pond water survived over 50 hr (Fig. 3). When repeated using artificial sea water (9), survival was longer than in NaCl of equal osmotic pressures (Fig. 4).

The area subtended by each hyperbola in Fig. 4 corresponding to the different dilutions was calculated at each dilution. This value was plotted as the ordinate (percent survival × time) against the dilution values expressed as a logarithmic scale of the abscissa (Fig. 5). This shows a maximum survival in seawater between 10^{-1} and 10^{-3} in NaCl dilutions.

In undiluted seawater, *C. bioculata* did not move after 38 sec (± 10), and in NaCl solutions of equal osmosity, they died after 36 sec (± 10), these differences being statistically insignificant. Analysis of filtered pond water established a mineral salt equivalent, equal to 0.16%, corresponding to a sea water dilution of $10^{-2.5}$.

pH buffers. C. bioculata were placed in a range of buffers, but the results were irregular, suggesting that the phosphate and biphosphate ions were having an influence additional to that of the pH.

Temperature. The water temperature of the pond between June and September fluctuated from 15 to 29 C. Preliminary tests in constant temperature rooms showed marked differences in locomotory rates at 12 and 20 C, with very small variance compared to the means.

Single specimens were placed in 2 ml of filtered pond water in solid watch glasses, and, using ice and hot water in the medium around the watch glasses, they were taken from 0 to 35 C. We measured locomotory rates at each temperature for 30-40 sec by counting the anterior undulations per second. Highest speeds were reached between 16 and 26 C, the rate dropping sharply above and below this region (Fig. 6). By measuring displacements over a distance of 1 cm it may be estimated that an individual, under ideal conditions and with continuous activity, could travel 1.2 m/hr.

DISCUSSION

Tonicity of the fluids of nematodes from various habitats has been shown to be isotonic with the surrounding medium (3, 6). The ability to regulate water balance has been measured in some (3, 6). The marine species *Deontostoma californicum* has an osmotic pressure equivalent to 0.6 M NaCl (6). The osmotic pressure in many nematode-parasites of vertebrates is equivalent to 0.2 M NaCl, and free-living soil and plant parasites about 0.15 M NaCl (3). Survival of *C. bioculata* was longest between 5.20 mM and 0.52 mM NaCl; this may be the lowest reported isotonicity for a nematode so far. This emphasizes the isotonic range for nematodes of about 1:50. *C. bioculata* appeared to be unable to regulate sufficiently to survive in conditions of osmotic or ionic stress. Greater survival in artificial seawater dilutions rather than in equivalent osmotic values of NaCl may have resulted from NaCl toxicity, through an improved physiological condition being maintained in the more 'balanced' artificial seawater.

Temperature data is liable to acclimatization and seasonal and regional factors, but for the period observed, this population's greatest activity occurred at the mean daily temperatures of the season. Winter activities are to be investigated in a comparable manner. These activities are extremely high when compared with the data of Wallace and Doncaster (11).

Distribution of *C. bioculata* was discontinuous, as it dominated one pond but was not found in similar nearby ponds containing adequate algae. Within the pond it was found on the sides in areas of greatest algal density. This species is photopositive in visible light (4) with paired anterior photoreceptors (5). It may be deduced that such a response leads the nematodes to areas of algal growth. Their absence in the bottom mud may be due to an absence of food, lower oxygen tensions or the presence of species potentially predatory to nematodes.

The caudal gland and somatic setae have been observed in the behavior of *C. bioculata*; they are probably associated with attachment and with the considerable agility and sensory coordination required by a species living suspended or precariously attached to algal filaments over a deep water habitat. The high activity rates may also be related to the need to maintain or regain their station in an aqueous habitat. Such high activity rates, while doubtless permitting entry to an area of algal growth, could not be maintained, for it would interfere with feeding and mating and would consume large quantities of energy.

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