ANALYSIS OF THE POPULATION STRUCTURES OF BITHYNIIDAE IN OPISTHORCHIASIS NIDI IN SVERDLOVSK REGION

D. N. Ponomarev and I. M. Khokhutkin

UDC 594.32:591.67

During 1987-1988 populations of Codiella troscheli (252 specimens) and Bithynia tentaculata (123 specimens) were sampled from waters of River Tobol basin, eastern Sverdlovsk Region, in nidi of opisthorchiasis. Factor and multivariate dispersion analysis methods were used to evaluate the size/age structure of the populations. It was concluded that differences in the population structure of the same species from different waterbodies are strongly correlated with chronographic fluctuations in the hydroregime. It was suggested that the reproductive group of the intermediate host populations is a very important element in the functioning of the "helminth—intermediate—additional—definitive host" system.

Opisthorchiasis, a severe human parasitic disease, remains a significant socio-economic problem on the territory of Western Siberia, which includes the Ob'-Irtysh hyperendemic region. Most of the Ob'-Irtysh nidus affects man and is of natural origin (Sidorov, 1983). It is rightly assumed that independent disease nidi also occur in eastern Sverdlovsk Region (Ponomarev, 1974). The complex opisthorchiasis transmission cycle and the regional specifics of its nidal functioning require much further study.

It is now known that Codiella troscheli (Paasch) and C. inflata (Hans.) are the intermediate hosts of Opistorchis felineus (Riv.) in Western Siberia and Kazakhstan. A possible additional host may be Bithynia tentaculata (L.) (Fedorov, 1979), though this has yet to be confirmed. The aim of the present study is to analyze population variation in two species of Bithyniidae, B. tentaculata and C. troscheli, from various waters of eastern Sverdlovsk Region. A brief literature review on the biology and ecology of this group of mollusks has also been attempted, for they play an important role in the functioning of the opisthorchiasis nidus.

Table 1 presents data on material collection and their localities. All waterbodies surveyed belong to the River Tobol basin, a tributary of the Irtysh River. Specimens were collected using hand nets and occasionally by hand from the shores and also from a boat, especially during the fall floods, with a view to studying the channel and permanent waterbody populations to a depth of 2.5 m. The populations were evaluated both by counting the numbers on measured sites (1 m^2) on a clear bed and with good visibility, and also via a hand-net survey. Mollusks were found in the substrate and on vegetation, as well as in the ooze and detritus layers. Table 1 shows mean density at several sites. Sample 4 gives the combined density of both species; C. troscheli comprises one third of this figure. In addition to the bithyniid mollusks, four species of bivalves and 18 species of gastropods were also found; certain biotopes were dominated by orb shells, Viviparus, and pond snails. Most prevalent are the widely-distributed species characteristic of the Irtysh province of the European/Siberian subregion of the Palaearctic. Shell measurements were taken using standard vernier calipers with a 0.05 mm scale; the number of axial ribs and shell whorls were estimated using a hand-held magnifying glass (7×). All calculations were fed into a SM-3 computer and processed by factor and multivariate dispersion analysis programs (FACA, MANOVA) at the Computer Center of the Institute of Plant and Animal Ecology, Urals Branch, Russian Academy of Sciences.*

*The authors are grateful to O. A. Zhigal'skii and L. M. Rozhdestvenskaya for their assistance with the mathematical programs and computer calculations.

Institute of Plant and Animal Ecology, Urals Branch, Russian Academy of Sciences.
Translated from Ékologiya, No. 5, pp. 62-69, September-October, 1991. Original article submitted November 21, 1989.

 0096-7807/91/2205-0328$12.50

TABLE 1. Material and Habitats of Codiella troscheli and Bithynia tentaculata Populations

Sample No.	District and Collection Point	Number of animals in sample, ind.	Density, ind./m^2	Biotope
1	Gari District, Ust' Loz'va settlement, 28.07.88	60	15*	Former bed of River Tura. Argillaceous ooze. Vegetation: Carex, Stratiotes.
2	Tavda District, Beloyarka village, 15.07.88	41	0.5*	Former bed of River Tavda. Silted ooze. Elodea, Potamogeton.
3	Turinsk District, Dubrovino village, 20.08.88	49	8.5*	Former bed of River Tura. Sandy substrate with thick detritus layer. "Carpet" of vegetation on bottom: Elodea, Sagittaria; littoral overgrowth of Carex and Typha. Also inhabited by B. tentaculata (58 ind., 7.2 ind./m^2
4	Artemovskii District, Sosnovyi bor settlement, 20.08.87	102	52.8**	Lotic pond on River Irbit, tributary of River Nitsa. Sandy and gravelly substrate, warp, and thick detritus layer. Areas overgrown with Carex, Typha, Elodea, Myriophyllum, Lemna. Also inhabited by B. tentaculata, 66 ind.

*C. troscheli.
**Both species.

Brief Ecology of Bithyniid Mollusks. Fairly detailed studies have been made of the habitat conditions of bithyniid mollusks both in the European and Asiatic waterbodies of the USSR. However, generally speaking, the data refer to Bithyniidae from a specific waterbody, which makes it difficult to assess the individual species' requirements with regard to certain environmental conditions. By and large the bithyniid mollusks are euryhaline, though B. tentaculata is more often found in lotic freshwater. With a more or less even water chemistry most bithyniid mollusks are found in the shallows in muddy substrates or substrates rich in organic matter. A number of basic factors affecting their distribution have already been identified: vegetation, depth, oxygen, hydrogen sulfide, nitrite and iron content, and soil acidity (Goryachev, 1952; Miroshnichenko, 1954; Myasoedov and Nikonov, 1964; Sosipatrov, 1967, Zabolotskii and Pirogov, 1968; Beer, 1968, 1985; Shustov, 1975; Beer et al., 1976; Sidorov, 1983). Bithyniid mollusks can tolerate large variations in abiotic conditions (Vinogradov, 1952; Lisitskaya, 1957; Sidorov, 1983). This tolerance is shown both in the individual organism and in the response of the population structure. Thus the life cycle of B. tentaculata may vary considerably. It may take from one to four years to complete even in close populations and is strongly dependent on water temperature. The size/age structure of the population is primarily linked to the bottom sediment temperature regime and the speed of the current. Water chemistry plays a significant role in adult shell height variation, though it does not affect the age of sexual maturation. Disruptions in the age structure may be explained by fluctuations in the replacement value of the younger generation related to periodic anomalies in the thermal and hydrological regimes (Young, 1975; Dussart, 1979).

Animal groups from different waterbodies must be directly comparable in order to properly analyze their data. In animals with a long life cycle (many years) this means that care must be taken that identical age groups are being compared. However, great difficulties are involved in mollusk age determination. A specific indicator has been proposed for bithyniids: "the number of annual rings on the shell" or, more accurately the number of costae or axial ribs (Beer et al., 1969). In our opinion this is a sufficiently reliable means of determining the age of bithyniid mollusks. Discounting the rib that is formed after emergence from the egg, marking the line between embryonic and definitive shell whorls, we may view all subsequent rings as annual growth markers. The drawback is that usually the rings are not very clear, as shown by the photographs of certain individuals in

TABLE 2. Correlation Coefficients (r) between Character Pairs in Two Species

Characters		*Bithynia tentaculata*					*Codiella troscheli*				
		SW	MH	MW	R	W	SW	MH	MW	R	W
SH	1	0,87	0,90	0,76	0,77	0,63	0,75	0,65	0,69	0,55	0,69
	2	0,90	0,93	0,90	0,71	0,77	0,90	0,79	0,82	0,73	0,81
SW	1	—	0,87	0,81	0,77	0,45	—	0,70	0,66	0,61	0,68
	2	—	0.93	0,88	0.62	0,69	—	0,72	0,80	0,68	0,86
MH	1	—	—	0,72	0,71	0.60	—	—	0,68	0,54	0,51
	2	—	—	0,90	0,79	0,77	—	—	0,80	0.69	0,58
MW	1	—	—	—	—	—	—	—	—	0,52	0,47
	2	—	—	—	0,73	0,74	—	—	—	0,61	0,58
R	1	—	—	—	—	0,65	—	—	—	—	0,51
	2	—	—	—	—	0,65	—	—	—	—	0,67

Notes. 1) All size/age groups; 2) reproductive group; SH, SW, MH, MW, R, W denotes, respectively, shell height, shell width, mouth height, mouth width, axial rib number and shell whorl number.

the above-cited reference; in many cases they have almost completely disappeared from view. Study of the annual rings on the operculum is of little assistance either. Starobogatov (1977) was quite right to express caution that "the annual rings" merely indicate interruptions in the shell's growth, which may have arisen for any number of reasons other than that of overwintering. If the rings are barely visible it might be impossible to distinguish between rings formed for a variety of reasons. A review of the literature confirms that age can only be reliably determined by using special methods, which cannot be easily replicated outside of the laboratory. In most cases an analysis of the size/age groupings is sufficient for solving most ecological problems.

Analysis of Bithyniid Population Structures. The concept of "breeding size" (reproductive group numbers) comes from the field of population genetics. It refers to the number of actual forerunners of the next generation,i.e., individuals making an active contribution to the genetic composition of the next generation (Li, 1978). In certain cases their actual number has been fairly accurately determined in the laboratory using specimens from the wild (Khokhutkin, 1984). Other studies have tended to feature a wider group, i.e., individuals which have laid eggs. It has been reported that some of these, however, fail to hatch, though this occurs in no more than 11% of the total; this is half the standard error by which the breeding size is usually determined. Thus the group of animals which have laid eggs may be substituted in cases where the breeding size has not been accurately determined. The problem is made easier by the fact that animals with sterile eggs are generally smaller in size. This can be demonstrated in a graph.

Mean breeding size (in shell height) in a population of B. tentaculata from a lake in New York State (USA), according to long-term studies (Mattice, 1972) is 9.5 mm. We will use this figure also in our studies for want of alternative data. Preliminary analysis of a graph based on our sample data from Tavda District (40 indiv.) shows a distinct compact grouping of individuals measuring from 8 to 11 mm in shell height and a model size of 10 mm. Shell width analysis gives similar results. The mean age of the reproductive group in the American population is 17-18 months; it has been calculated that the oldest individuals may reach five or six years of age. Sexual dimorphism has been noted in the shell measurements of a number of bithyniid mollusk species: the female shell is relatively shorter and wider than the male's. There are no sex differences in the morphological characters of B. tentaculata, although protandrous hermaphrodites have been observed in C. troscheli (Logachev and Serebrennikova, 1969). Thus it was decided to perform size analysis on a homogeneous group. Minimum shell height in the reproductive group of the latter species, according to the graph, is 7 mm.

Initial measurements and calculations were made of the entire size/age structure of the sample population; this was then repeated for the reproductive group. The number of characters examined varied from sample to sample — from two to six. The raw data were then processed as follows using the FACA program. To begin with, we have a series of coordinates for initial characters, simultaneous analysis of which proves difficult past three or more variables. By applying a factor analysis, we can reduce the characters' range down to one

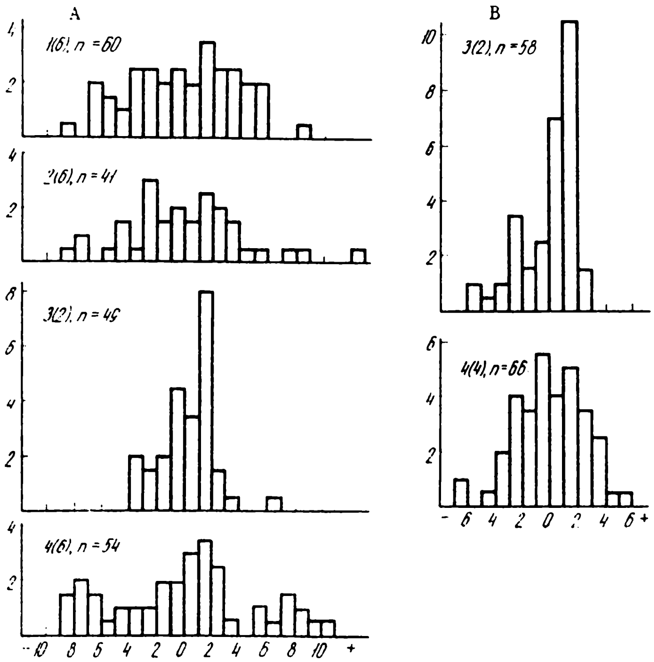

Fig. 1. Size/age structure of Codiella troscheli (A) and Bithynia tentaculata (B): 1, 2, 3, 4) sample Nos. corresponding to Table 1 (No. of characters analyzed — in parentheses), n) number of specimens. abscissa: coordinate deviation (individual characters) from norm; ordinate: object frequency for each specific class.

or two variables, and in so doing lose a mean 15.5% of the initial data, which we deem satisfactory for our particular analysis.

Statistically significant correlation coefficients (r) (in deviation from zero) for selected morphometric characters are presented in Table 2. Since there were no significant variations in the coefficients of the various samples, they have been combined. Correlation between characters was found to be close to $r \geq 0.70$. All age groups of B. tentaculata exhibited a correlation coefficient of $r \geq 0.70$ in their character pairs, with the exception of "shell width—rib and whorl number" and "rib—whorl number". In the reproductive group a coefficient of $r \geq 0.70$ was only recorded in the intercorrelations between all characters and shell whorl number. C. troscheli exhibits a coefficient of $r \geq 0.70$ in the correlation between shell height and all other characters; the same applies to shell width, except in relation to rib number and the "mouth height—width" character pair; in all other cases the value for r is much less dependent.

In the reproductive group coefficients of $r \geq 0.70$ were observed in the pairs "shell height—width" and "shell width—mouth height"; in all other character pairs the mean correlation was $r \geq 0.50$, with one exception, where it was even less. In B. tentaculata the highest values ($r \geq 0.80$) are noted in the intercorrelations of the majority of plastic characters, especially for the sample as a whole. Correlation coefficients for plastic and meristic characters are somewhat lower: $r \geq 0.70$-0.79. Both intra- and intergroup character pairs in C. troscheli generate high r values. These values were not recorded in the reproductive group.

The FACA program enables linear combinations of initial characters to be analyzed graphically with their correlation coefficients plotted against each other on one or two factor (canonical) axes; the groups of characters are arranged according to the principle of imminent proximity, thus enabling the biological correlations between them to be successfully analyzed. As shown in preliminary analysis, this means that closely-correlated characters are arranged in relatively compact groups. This program also enables the role of each of the initial characters on either axis to be analyzed with regard to population variation. Shell height and width are the most significant characters on the first axis; they account for 72.5% of total variation. The characters on the second axis are much less informative;

they include rib and whorl number, and account for only 21.5% variation. The first two characters are responsible for a factor matrix value of 0.90, whilst the other two characters and those of mouth height and width generate values of 0.62 and 0.83 respectively. The first axis represents true values. The first two characters taken separately generate the highest values. Both the entire sample and the reproductive group were analyzed simultaneously with more or less the same results.

The program also displays the object coordinates openly within the factor space. In this case the "object" is understood to mean the characters of a specific sample of animals. Further analysis of the size/age structure can be easily carried out, once a histogram is made of the individual's distribution within this factor space, derived from the multivariate space of the initial characters (see Fig. 1). Plot the object's coordinates in the factor space on the horizontal (abscissa), and the frequency of encounter of specific classes on the vertical (ordinate) axis. One further stipulation is that for each sample the position of the object coordinates must be considered in relation to their own (factor) axis. Since the histogram is plotted either on a single axis, or, if there are two, along the first axis, and it should include the main variation assessment component for the first two plastic characters, the resultant distribution may therefore be seen to represent the intercorrelated variation in size characters, primarily shell height and width. Furthermore, since the shell grows in size with age, the resultant distribution structure of the individuals may be seen to represent the size/age structure of the population. There is no essential difference in coordinate distribution in the factor space from that of the initial characters, though it provides much more significant information about the intercorrelation between the characters from different size/age classes. It has been noted already that all these tests generate animal groups with r values very close to each other, thus enabling an accurate assessment of the sample's morphological composition.

The samples with the highest number of specimens are presented in Fig. 1. In the sample from Gari District, where larger-sized individuals predominate, individual distribution for the six characters is fairly even, with a slight peak in the more senior age groups. The sample from Tavda District exhibits a peak at either end of the age scale; most members of the Turinsk sample belong to the reproductive group, forming a rather compact group with a high peak. Lastly, the sample from Artemovskii District, characterized by a wide diversity of different age groups, exhibits several peaks. The Turinsk sample of the second species is similarly compact; it exhibits a significant peak in the older classes and a much lower one in the young. Distribution in the Artemovskii sample is more even, though even here at least two groups — young and adult — can be distinguished.

The second part of our study involves the use of the single-factor dispersion analysis method (MANOVA) to analyze the population variation of the two species. The Mahalonobis distance (D) of the reproductive group of the C. troscheli population from Gari District is very different from those of the populations from Tavda and Artemovskii Districts; the difference between the last two is slight (D — from 2.64 to 4.32). All the samples of this species generated very different values (from 2.85 to 3.49) from the B. tentaculata sample from Artemovskii District. Maximum shell heights for C. troscheli were as follows: Artemovskii District — 9.30 mm, Tavda — from 8.15 to 9.30 mm, Gari — 11.8 mm; and in B. tentaculata 13.4 mm from Artemovskii District and 11.4 mm in Turinsk.

The limnological features of the studied waterbodies in eastern Sverdlovsk Region are fairly similar. Study of the variation of C. troscheli, the transmitter of opisthorchiasis, and a closely-related species has shown that these two species are euryoecious, i.e., capable of enduring wide variations in environmental conditions. We have observed, however, in a number of our samples a certain degree of limitation or preference for one or other environmental feature; this is borne out by the literature. We have analyzed the size/age structure of B. tentaculata and C. troscheli populations. Particular attention was given to the reproductive group, for we believe that it is a major stabilizing element in the entire population system.

By taking the B. tentaculata sample as a whole we can establish a close correlation between all pairs of plastic characters; in C. troscheli a close correlation has been found between pairs of different characters and shell height—width. All character pairs in the reproductive group of B. tentaculata are closely correlated with the exception of "whorl number" and other characters. In C. troscheli only the "shell height—width" and "shell width—mouth width" character pair exhibits a close correlation. Shell height and width (on the first axis) account for a major part of the variation (more than 70%). The size/age structure of both species samples differ greatly according to the waterbody; the differences are largely related to chronographic, sometimes seasonal, variations, rather than the biotope itself.

A factor analysis of the reproductive groups reveals their more or less "compact distribution"; this undoubtedly reflects the differences in age and the associated differences in size. A multivariate dispersion analysis enables us to conclude that the reproductive groups of B. tentaculata differ from those of the other species. In other words, they are larger in size. This concurs with the findings of other authors (Beer and Makeeva, 1973) and suggests that individual B. tentaculata are larger than the other species of Bithyniidae from the eastern regions of the range. Further study is needed on the differences in the C. troscheli reproductive groups from various waterbodies; this may similarly be the result of chronographic variation.

From a practical viewpoint if we had to recommend a single parameter for measurement in the field when collecting and processing material, it would be shell height. It is by far the simplest and quickest parameter to measure and is an adequate reflection of the true characteristics of population size/age structure. Future research should perhaps be aimed at proving the hypothesis that the reproductive group of the intermediate host population is a very important element in the functioning of the "helminth—intermediate—additional—definitive host system".

LITERATURE CITED

Beer, S. A., "Genesis and structure of opisthorchiasis areal. Communication 2. The structure of areal of Bithynia leachi (Shepp.) and factors conditioning it," Med. Parazitol. Parazit. Bolezni, 37, 677-686 (1968).

Beer, S. A., "Opisthorchiasis," in: Helminthoses of Man (Epidemiology and Treatment) [in Russian], Moscow (1985), pp. 102-119.

Beer, S. A., Koroleva, V. M., and Livshits, A. V., "Age determination of Bithynia leachi (Mollusca, Gastropoda)," Zool. Zh., 48, No. 9, 1401-1404 (1969).

Beer, S. A., Livshits, A. V., Maslova, L. K., and Zavoikin, V. D., "Local prevalence and ecology of Bithynia inflata in the north of Tomsk Region. Communication 1. The influence of abiotic factors on Bithynia prevalence," Med. Parazitol. Parazit. Bolezni, 45, No. 1, 69-81 (1976).

Beer, S. A., and Makeeva, V. M., "Systematic status and variability of genus Bithynia (Gastropoda) in West Siberia," Zool. Zh., 52, No. 5, 668-675 (1973).

Dussart, G. B. J., "Life cycles and distribution of the aquatic gastropod molluscs Bithynia tentaculata (L.), Gyraulus albus (Müller), Planorbis planorbis (L.) and Lymnaea peregra (Müller) in relation to water chemistry," Hydrobiologia, 67, No. 3, 223-239 (1979).

Fedorov, K. P., "Ecology of Opisthorchidae in Novosibirsk Region," in: Helminth Ecology and Morphology in West Siberia [in Russian], No. 38, Novosibirsk (1979), pp. 5-55.

Goryachev, P. P., "Some questions on the biology of Bithynia leachi, intermediate host of Opistorchis felineus," Tr. Omsk. Med. Inst., No. 18, 147-157 (1952).

Khokhutkin, I. M., "Organization and variation in the polymorphic structure of terrestrial mollusc species," Zh. Obshch. Biol., No. 5, 615-623 (1984).

Li, Y., Introduction to Population Genetics [Russian translation], Mir, Moscow (1978).

Lisitskaya, L. S., "Some questions on the biology of Bithynia leachi, intermediate host of Opistorchis felineus," in: Proceedings of Scientific Conference, Rostov-on-Don Med. Inst. (1956), pp. 833-835.

Logachev, E. D., and Serebrennikova, N. G., "On the development of sex cells in Bithyniidae," in: Malacological Questions in Siberia. Material from Scientific Conference on the Study of Freshwater Molluscs of Siberia [in Russian], Tomsk (1969), pp. 24-26.

Mattice, J. S., "Production of a natural population of Bithynia tentaculata L. (Gastropoda, Mollusca)," Ékol. Pol., 20, No. 39, 525-539 (1972).

Miroshnichenko, M. P., "Bithyniidae of Western Siberia (study of mollusc ecology in relation to its role in the prevalence of opisthorchiasis)," Dissertation for Candidacy of Biol. Sci., Tomsk (1954).

Myasoedov, V. S., and Nikonov, S. P., "Biocenoses of the mollusc Bithynia leachi in the waters of Tyumen' Region," in: Material from Scientific Conference on Medical Parasitology [in Russian], Tyumen' (1964), pp. 99-103.

Ponomarev, D. N., Nosogeography of Infectional and Parasitological Pathology in the Central Urals [in Russian], Sredne-Uralsk Publ. House, Sverdlovsk (1974).

Shustov, A. I., "Features of the biology of Bithynia leachi (Shep.) in the waters of Central Kazakhstan," in: Helminths of Birds and Fish of Kazakhstan and their Intermediate Hosts [in Russian], Alma-Ata (1975), pp. 131-141.

Sidorov, E. G., Natural Foci of Opisthorchiasis [in Russian], Nauka, Alma-Ata (1983).

Sosipatrov, G. V., "Some observations on the biology and ecology of freshwater mollusc – intermediate hosts of Echinochasmus perfoliatus (B. leachi)," in: Collected Works on the Helminthology of Agricultural Animals [in Russian], Vol. 13, Moscow (1967).

Starobogatov, Ya. I., "Class Gastropoda," in: Key to Freshwater Invertebrates of European USSR [in Russian], Leningrad (1977), pp. 151-174.

Vinogradov, L. I., "On the biology of the mollusc Bithynia leachi (Sheppard, 1823)," Zool. Zh., 41, No. 3, 464-465 (1962).

Young, V. R., "The life cycles of six species of freshwater molluscs in Worcester-Birmingham Canal," Proc. Malac. Soc. London, 41, No. 6, 533-548 (1975).

Zabolotskii, V. I., and Pirogov, V. V., "Biology of Bithynia leachi Shep. and the structure of the opisthorchiasis focus in Astrakhan Region," Parazitologiya, 2, No. 6, 509-513 (1968).