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Proceedings of the 12th International Symposium on Aquatic Oligochaeta

ADRIAN PINDER, NAIME ARSLAN & MARK WETZEL (Eds)



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GÖRAN MILBRINK



12th International Symposium on Aquatic Oligochaeta

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This volume is the proceedings for the 12th International Symposium on Aquatic Oligochaeta (ISAO), held in Fremantle Western Australia from the 10th to the 13th of September 2012. This was the latest in a series of triennial symposia that began in May 1979 when 31 researchers met for four days in Sidney, British Columbia, Canada, hosted by Ralph Brinkhurst, then of the Institute of Ocean Sciences who has played a pivotal role in the field of oligochaete research for many decades. That first meeting set the scene for regular collegiate gatherings of oligochaetologists that have spawned many collaborations and scientific advances over the past 35 years. Continuity in the oligochaete research community is shown by the fact that four of the researchers that attended the 1979 symposium joined us in Fremantle.

The 12th ISAO symposium was the first to be convened in the Southern Hemisphere. While this resulted in a smaller than usual meeting (partly also attributable to world economic issues), it was nonetheless an engaging and invaluable gathering. The symposium was preceded by a workshop on identifying Australian aquatic oligochaetes, presented by Adrian Pinder, Mark Wetzel and Christer Erséus, providing the opportunity for local and regional aquatic ecologists as well as several symposium delegates to improve their expertise in the identification of freshwater, estuarine, and marine oligochaetes. Thirty delegates from 12 countries attended the symposium, presenting 21 oral presentations and 8 posters. In addition to the scientific sessions

convened at the Esplanade Hotel in Fremantle, there was an excursion to Rottnest Island—Wadjemup (in Noongar), just off the coast from Fremantle, and a dinner at Kings Park Botanical Gardens overlooking the city of Perth. A wide range of scientific topics were covered by presenters, including biogeography, systematics, toxicology, methodologies and ecology.

The symposium concluded with a business meeting to discuss issues affecting the aquatic oligochaete community. Discussions centred on 1) production of a symposium planning document (a draft of which assisted organisers of the Fremantle symposium), 2) the role of the General Secretary of the International Symposia on Aquatic Oligochaeta (membership directory maintenance, a mirror website for future symposia, assisting symposia hosts as required, including with proceedings volumes, and to organize and chair the symposium business meetings) and 3) An invitation from Dr Jana Schenková of Masaryk University (Brno, Czech Republic) to oligochaetologists past, present and future to meet in Brno in September 2015. This invitation was accepted by all delegates, and we look forward to joining our colleagues to once again discuss the wonderful world of aquatic oligochaetes. See <http://www.isao2015.cz> for more details.

About half of the presentations were written up as papers in this volume. This proceedings volume also includes a memoriam for Dr. Tamara L. Poddubnaya (1930-2011), who contributed an enormous body of work on the aquatic oligochaetes of Russia and the former Soviet republics and who will be greatly missed by her many colleagues. All papers in this volume were reviewed by two anonymous referees and revised versions were re-examined by one of the three editors.

The symposium organizing committee consisted of Adrian Pinder, David Cale, Anna Leung, Joanne O'Connor, Melita Penniford and Kirsty Quinlan. We are deeply indebted to the sponsors of the symposium that allowed costs to delegates to be greatly reduced: Phoenix Environmental Sciences, Bennelongia Environmental Consultants, Outback Ecology, Biota Environmental Sciences, Subterranean Ecology, Ecologia Environment, Australian Society for Limnology and the Western Australian Department of Parks and Wildlife (then Department of Environment and Conservation).



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In Memoriam Tamara L. Poddubnaya (1930–2011)—Oligochaetologist



Tamara Poddubnaya (right) with Tarmo Timm during a visit to the Võrtsjärv Limnological Station in Rannu, Tartumaa, Estonia in 1974.

Dr. Tamara Leont'evna Poddubnaya (nee Protopopova) was born 18 February 1930 in Rostov-na-Donu, a large city on the Don River in Southern Russia. After graduating the Rostov University in 1953 she joined the Biological Station in Borok, which later became the Institute for Biology of Inland Waters at Rybinsk Reservoir, located on the Upper Volga River in Northern Russia. Here she spent the remaining 57 years of her lifetime, working in the same research institute. In 1962, she received her Cand. Biol. Sci. (= Ph.D.) degree in Moscow; her thesis (in Russian) was entitled, "Studies on the biology of mass species of tubificids (*Limnodrilus newaensis* Mich. and *Limnodrilus hoffmeisteri* Clap.) of the Rybinsk Reservoir".

In 1955 she married Artur G. Poddubnyj, a famous ichthyologist in the same institute. They had a son, Sergej (1956) and a daughter, Elena (1962). Their son, Sergej A. Poddubnyj is working as geographer in Borok while the daughter-in-law Larisa G. Poddubnaya is studying fish parasites.

Throughout her career, most of Dr. Poddubnaya's research involved zoobenthic communities in the Volga reservoirs, especially the aquatic oligochaetes. She published more than 40 papers between 1958 and 1990, mostly in her native Russian language. Although most of her publications focused on the ecology and reproductive biology of Tubificidae and Naididae, several addressed issues in oligochaete taxonomy. Of particular interest were her studies highlighting the taxonomic value of the notch usually observed in the prostomium of *Chaetogaster diaphanus*—correcting misinformation presented by Christina Sperber (1948) who had attributed this character to *C. cristallinus*.

She participated in the first and second International Symposia on Aquatic Oligochaete Biology (ISAOB) convened (respectively) in Sidney, British Columbia, Canada (in 1979, hosted by Ralph O. Brinkhurst at the Institute of Ocean Sciences), and Pallanza, Italy (in 1982, hosted by Giuliano Bonomi at CNR–Istituto Italiano di Idrobiologia). When flying to the first ISAOB in 1979, Dr. Poddubnaya, together with Drs. Antanas Grigelis and Tarmo Timm, inadvertently purchased air tickets between Moscow and Sydney, Nova Scotia (on the east coast of Canada) *instead* of Sidney, British Columbia (on the west coast), where this first international symposium was to convene. After spending a night in the Soviet general consulate in Montréal, and with the extraordinary, long-distance assistance from Ralph Brinkhurst, they reached the correct venue for this important first ISAOB symposium, one day later. When in Pallanza for the second ISAOB, Dr. Poddubnaya and Nonna P. Finogenova (another oligochaetologist from Russia) were hosted for several days by Dr. Bonomi, engaging in collaborative discussions on the life cycles of tubificids.

After retiring from science, Dr. Poddubnaya spent her last years in Borok. She passed away on 30 January 2011 at the age of 80.

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First record of achaetous *Marionina* Michaelsen, 1890 (Annelida: Clitellata: Enchytraeidae) in the southern Atlantic

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Short note

Marionina Michaelsen, 1890 is a polyphyletic enchytraeid genus represented by about 100 nominal species (Rota et al., 2008). When the genus was originally described in 1889, it got a preoccupied name, *Marionina*, which was corrected into *Marionina* one year later by Michaelsen himself. According to the ICZN (1999: Art. 60.3), a new replacement name has its own author and date. Thus, the correct nomenclature of the genus should be *Marionina* Michaelsen, 1890 (Rota et al., 2008).

Marionina has marine and non-marine species (Erséus et al. 2010). The former may be found in tidal debris, on mangrove aerial roots, in clean sandy beaches, in sublittoral sediments, and on rocky shores (Healy & Coates 1999). Some of the marine species are unusual in that they lack chaetae, and are grouped under the name “achaetous *Marionina*” (Matamoros et al. 2012). The first published description of an achaetous *Marionina* occurred under the name *Michaelsena achaeta* Hagen, 1954, a taxon later augmented and transferred to *Marionina* by Lasserre (1964). Two other achaetous taxa are currently named: *Marionina arenaria* Healy, 1979, and the former subspecies *Marionina achaeta nevisensis* Righi & Kanner, 1979, later raised to species status as *Marionina nevisensis* by Coates (1983). A comprehensive review of the diversity of “achaetous *Marionina*” was recently performed by combining morphological and molecular data (Matamoros et al. 2012). According to these authors, specimens of achaetous *Marionina* from a number of worldwide localities (Caribbean, Australia and northern Europe) make up a monophyletic grouping comprised of 11 separately evolving lineages, which could be assigned to seven different morphotypes. Only two of these morphotypes could be identified as nominal taxa, *M. nevisensis* Righi & Kanner, 1979 sensu lato and *Marionina nothachaeta* [= *M. achaeta* sensu Lasserre, 1964]. Based on segment numbers, Matamoros et al. (2012) suggested that *Marionina arenaria* and *M. achaeta* sensu Hagen (1954) may be the same species. *M. arenaria* are only available for study at the Natural Museum of Ireland (Natural History Division) in Dublin (NMI). The only *Marionina* species reported from the southern Atlantic are *Marionina cana* Marcus, 1965 and *Marionina nea* Marcus, 1965, both endowed with chaetae (Prantoni et al. in press).

Individuals of *Marionina* without chaetae were collected in July 2012 in an intertidal pond, in bottoms made up by gravel, shell fragments, and coarse sand, close to the rocky promontory of the Ponta do Baleeiro beach, Municipality of São Sebastião, São Paulo State, southeastern Brazil (23°49.689' S; 45° 25.392' W). The site is exposed to waves, and local salinity is around 35 PSU (Figure 1).

Four individuals were analyzed, two of which were mature and devoid of chaetae. Besides being achaetous, they were identified as *Marionina* based on the following diagnostic characteristics: 31–42 segments; total length between 3.6 and 5.5 mm; cuticle thickness between 2 and 3 µm; prostomium conical, wider than long; clitellum diameter of 168 µm, annular in XII–XIII, with glandular cells arranged in transverse lines; seminal vesicle unilateral; dorsal anterior blood vessel bifurcated in III or IV; coelomocytes dispersed, irregular and egg-shaped, with cytoplasm filled with small grains; sperm funnels (Figure 2).

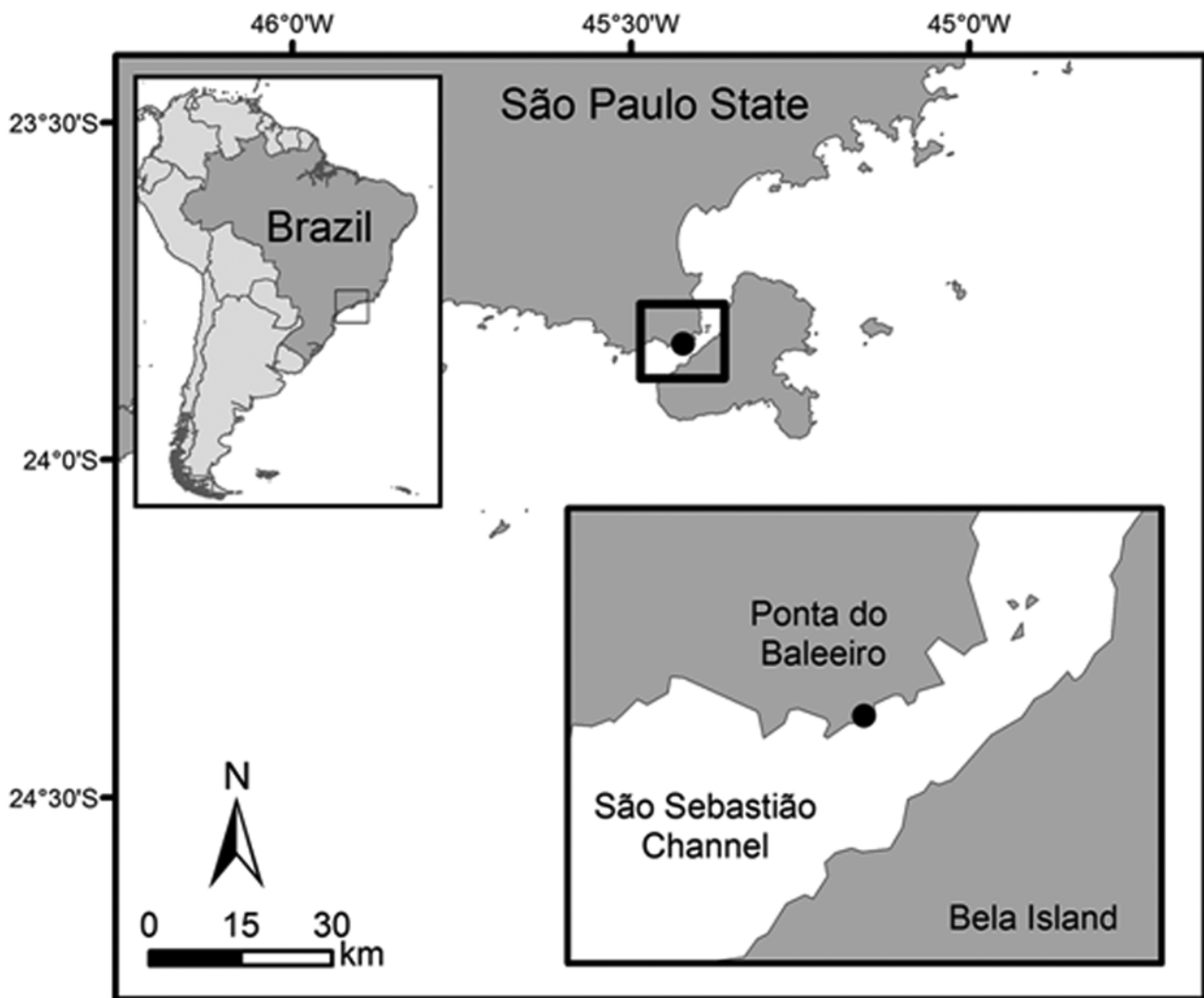


FIGURE 1: Collection site of achaetous *Marionina* in southern Brazil.

This is the first record of an achaetous *Marionina* for the southern Atlantic. The animals collected in São Paulo appear morphologically indistinguishable from *M. nevisensis* Righi & Kanner, 1979 described from Nevis Island in the Caribbean. However, it is possible that *M. nevisensis* as recognized or described subsequently by a number of different authors (Coates, 1983; Erséus, 1990; Erséus et al., 1990; Coates, 1990; Coates & Stacey, 1993; Healy & Coates, 1999) is a complex of globally distributed cryptic species (Matamoros et al. 2012). Further study of the Brazilian specimens is still necessary, including analyses of characters from DNA sequences, and observations with transmission and scanning electron microscopy.

The examination of additional characters will be essential to test whether the shared morphological characteristics are homologous, or whether they represent environmentally convergent adaptations.

Matamoros et al. (2012) suggested that all tropical lineages (e.g., from Central America and Oceania) of achaetous *Marionina* seem to constitute a monophyletic group that originated from ancestors living in temperate climate regions. However, they have also suggested that more extensive sampling, especially in the temperate regions, might reveal a different evolutionary history.

While reporting a new geographical record of achaetous *Marionina*, we emphasize the scarcity of studies on the fauna of brackish-water and marine oligochaetes in the southern Atlantic. Indeed, only 13 of the approximately 600 described species of marine and brackish-water oligochaetes have been reported from Brazil, almost all of them known only from their type localities. A systematic survey of the group in the southern and southeastern Brazil will likely reveal a much higher diversity, considering the variety of regional habitats.

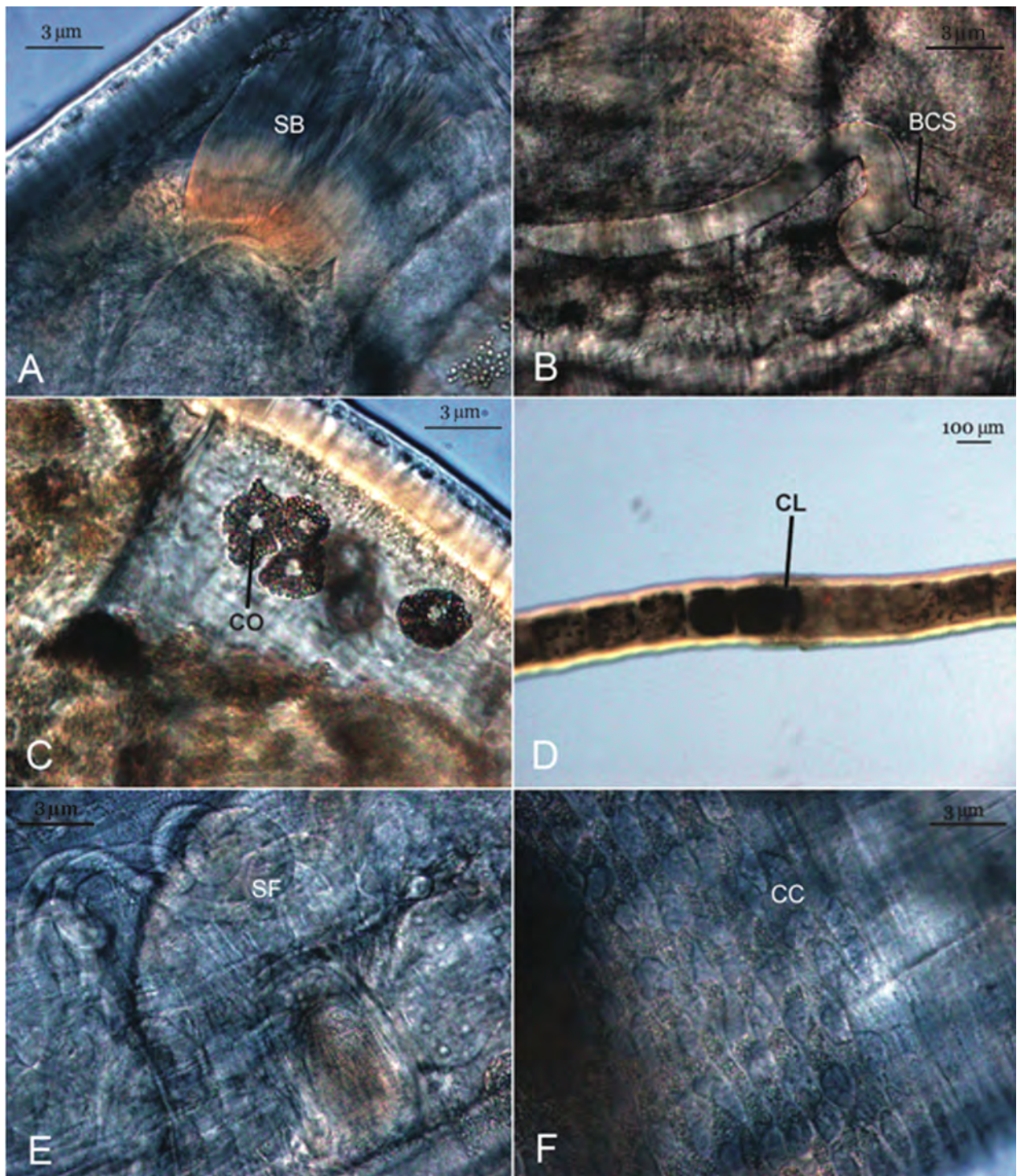


FIGURE 2: Anatomy of the achaetous *Marionina* from Sao Paulo. (A) a bundle of sperm, SB, covering the sperm funnel; (B) bifurcated anterior dorsal blood vessel, BCS; (C) coelomocytes, CO; (D) clitellum, CL; (E) vas deferens; (F) clitellar cells, CC.

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Preliminary survey of aquatic oligochaetes in Eastern Tamil Nadu, India

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Abstract

A random survey of aquatic Oligochaeta was conducted in Chennai, Villupuram, Dindugal, Thiruvallur, and Sivagangai districts of Tamil Nadu from November 2010 to March 2012. Thirteen taxa were identified from a total of 644 aquatic oligochaetes examined from various qualitative samples of aquatic vegetation and sediments. Chennai district had 3 families: Naididae, Pristinidae and Tubificidae along with a polychaete. The naidids were most diverse with 5 species: *Branchiodrilus semperi*, *Aulophorus furcatus*, *Dero digitata*, *Dero indica* and *Dero zeylanica*, the pristinids were represented by *Pristina brevichaeta* and *Pristina jenkinsae* and the tubificids by 2 species, *Branchiura sowerbyi* and *Limnodrilus hoffmeisteri*. The nine aquatic oligochaetes collected from Chennai were already reported from this region. Two naidids, *Branchiodrilus semperi* and *D. indica*, along with the tubificid *Branchiura sowerbyi*, were collected from Villupuram district and they constitute the first reports for that district. *Limnodrilus udekemianus* from Kodaikanal, Dindugal district constitutes the first report for that region of Tamil Nadu. Megadriles belonging to the family Octochaetidae (*Dichogaster bolau?*) and unidentified Megascolecidae collected from Thiruvallur and Sivaganga districts respectively were found to occur in aquatic habitats.

Key words: Aquatic Oligochaeta, Naididae, Pristinidae, Tubificidae, Octochaetidae, Megascolecidae, Polychaeta

Introduction

The monograph by Naidu (2005) was an effort to bring together all the information available in the literature on aquatic oligochaetes known from the Indian subcontinent. It describes a total of 102 taxa belonging to the various families of aquatic Oligochaeta from the Indian subcontinent. A recent review of freshwater oligochaetes of India suggests that a thorough survey of various states is needed to understand the distribution of oligochaetes systematically (Singh et al, 2009). Hence the present work is a preliminary attempt to explore the fauna of aquatic Oligochaeta from a few districts of the state of Tamil Nadu, South India as the studies on this group from Tamil Nadu are scanty, except for some reports by Naidu (2005), Naveed *et al.* (2011) and Naveed (2012).

Sampling sites

A random survey of aquatic Oligochaeta was conducted at Chennai, Villupuram, Dindugal, Thiruvallur and Sivagangai districts of Tamil Nadu (Fig. 1) from November 2010 to March 2012. Qualitative samples of aquatic vegetation and bottom sediments were collected from Adyar River near Saidapet (13°01'00"N, 80°13'03"E), Adyar River near Besant Nagar (13°00'19"N, 80°16'06"E), Adyar River near Kotturpuram (13°00'58"N, 80°14'05"E), Porur Lake (13°02'09"N, 80°09'08"E), by the second and fourth author and from Red Hills Lake (13°11'32"N, 80°10'26"E), Maduravoyal Pond, (13°03'47"N, 80°09'32"E), Nandampakkam River (13°00'59"N, 80°11'15"E), Pallikaranai Pond (12°56'48"N, 80°13'34"E), Tharamani Pond (12°59'36"N, 80°14'22"E), Madhavaram Lake (13°09'53"N, 80°14'57"E), Rettari Lake (13°08'33"N, 80°12'46"E) by the second author and from Chetpet Pond (13°04'30"N, 80°14'31"E) by the second and fifth authors and from Vandalur Lake (12°53'04"N, 80°05'03"E) by the fifth author, all in Chennai. Samples were collected from Alampalam Lake (11°46'26"N, 78°55'30"E) in Villupuram by the fourth author and from

Kodaikanal Boat House Lake ($10^{\circ}14'19''\text{N}$, $77^{\circ}30'12''\text{E}$) by the second author, this Lake is located at an elevation of 2,133m (6,998ft) in Dindugal district (with cool climate throughout the year), and the first four authors had collected the samples from Satharai Lake ($13^{\circ}17'55''\text{N}$, $80^{\circ}03'21''\text{E}$) in Thiruvallur and the second author had collected samples from Sivaganga Pond ($9^{\circ}50'43''\text{N}$, $78^{\circ}29'20''\text{E}$) in Sivaganga district.

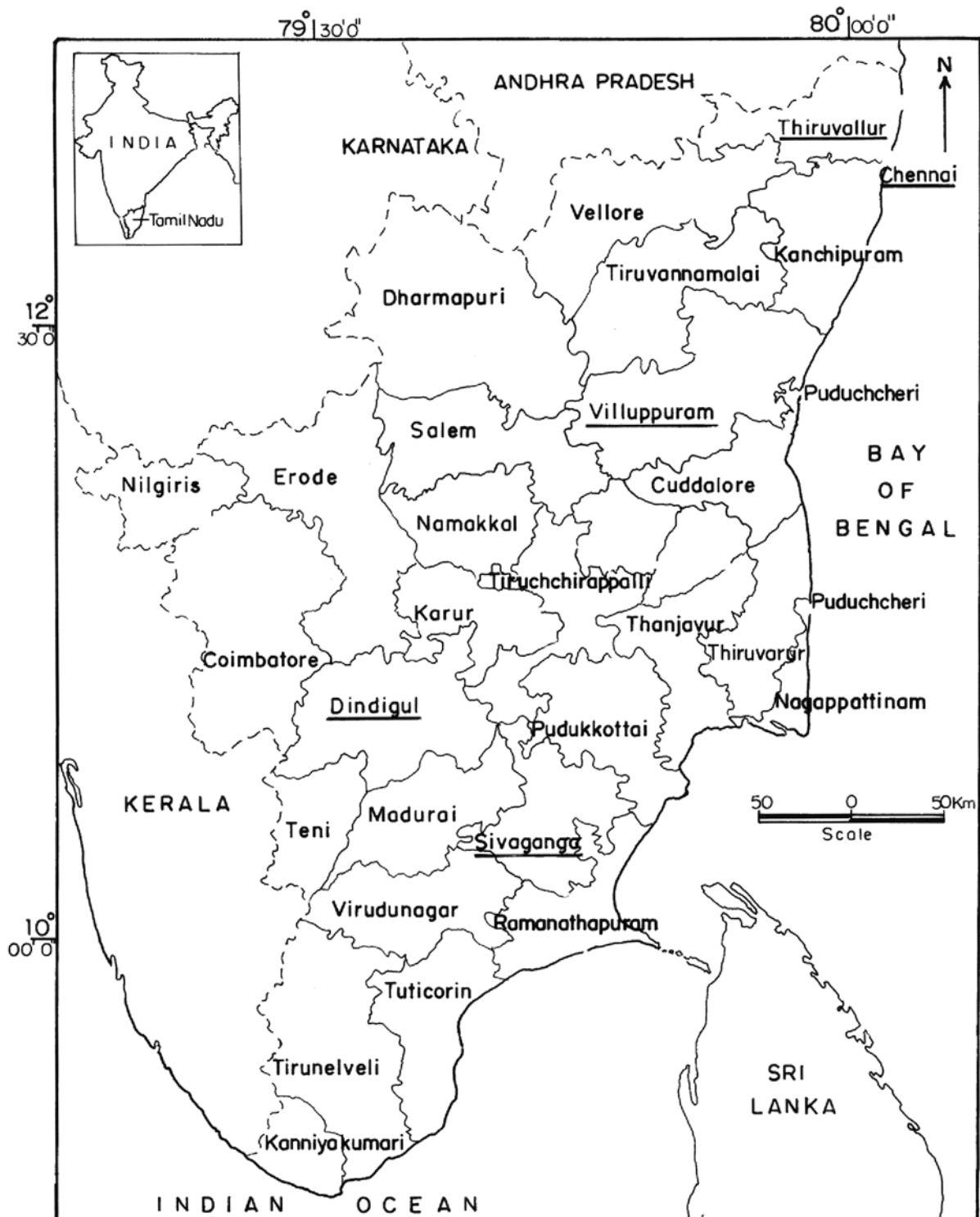


FIGURE 1. Map of Tamil Nadu (the districts surveyed are underlined).

Materials and methods

Hand net and tea strainers were used to collect qualitative random samples of bottom mud while aquatic vegetation was handpicked. Aquatic vegetation was transferred into trays containing water and gently shaken, the Oligochaeta specimens were collected with the help of droppers using magnifying lens and sufficient illumination. The sediment samples were washed through a 200 µm mesh sieve and the worms were sorted manually by diluting a small portion of the sediment residue in a white porcelain dish. Live oligochaetes were also extracted from some sediment samples by wet funnel method (O'Connor, 1995) with minor modifications. The collected worms were transferred into vials containing 10% formaldehyde and were preserved for identification. Live worms were examined under a cover slip in a drop of water. The preserved worms were examined on a slide in a few drops of glycerin under a cover slip. Brinkhurst and Jamieson (1971), Naidu (2005) and Timm (2009) were used for the identification of the worms. A total of 644 individuals were examined and identified by the first author. The drawings were made using a camera lucida. The classification of aquatic Oligochaeta by Timm (2009) is followed in the present work.

Results

Faunistic results

Thirteen taxa were identified (Table 1) from a total of 644 aquatic Oligochaeta examined. Chennai District had by 3 families: Naididae, Pristinidae and Tubificidae along with an unidentified polychaete. The naidids were most diverse with 5 species: *Branchiodrilus semperi*, *Aulophorus furcatus*, *Dero digitata*, *Dero indica* and *Dero zeylanica*, the pristinids were represented by *Pristina brevichaeta* and *Pristina jenkiniae* and the tubificids by 2 species: *Branchiura sowerbyi*, *Limnodrilus hoffmeisteri*. Two naidids, namely *Branchiodrilus semperi* and *D. indica* along with one tubificid, *Branchiura sowerbyi* were collected from Villupuram district and they constitute the first reports for Villupuram district. *Limnodrilus udekemianus* collected from Kodaikanal, Dindugal district constitutes the first report for that region of Tamil Nadu. Megadriles belonging to the family Octochaetidae (*Dichogaster bolau?*) collected from Thiruvallur district and unidentified Megascolecidae collected from Sivagangai districts were found to occur in aquatic habitats.

Systematic account

Family Naididae

Branchiodrilus semperi (Bourne, 1890)

Material examined: Alampalam Lake (Villupuram) on 02.01.2011 (n=1); Porur Lake (Chennai) on 02.05.2011 (n=1).

Morphological observations: Length about 7–20 mm. Worms brownish. Prostomium blunt and conical. A pair of finger-like dorso-lateral gills per segment in the anterior region. Dorsal chaetae from VI consisting of 1–3 hair chaetae and 1–2 needle chaetae entering the lumen of the gills and the hair chaetae protruding out of the lumen of the gills in the anterior region. Hair chaetae smooth. Needle chaetae without a nodulus, simple-pointed in the anterior region and in the posterior region the needle chaetae are peculiarly bayonet-shaped (Fig. 2A). Ventral chaetae 2–3 per bundle with distal tooth longer and thinner than proximal (Fig. 2B) with nodulus in the middle of the chaetae.

Distribution in Tamil Nadu: Chidambaram, Chennai (Naidu, 2005), Tiruvallur (Naveed, 2012).

Remarks: First report from Villupuram. This species is characterized by the presence of dorsolateral gills in the anterior half of the body (Timm, 2009).

It is reported from East and South Asia only (Naidu, 2005).

TABLE 1. Systematic list of aquatic Oligochaeta so far reported from Tamil Nadu. Species recorded during the present study are highlighted in bold.

| Taxa | Records in this paper | Total distribution in Tamil Nadu |
|--|--|---|
| ORDER TUBIFICIDA | | |
| FAMILY NAIDIDAE | | |
| <i>Allonais gwaliorensis</i> (Stephenson, 1920) | Porur Lake, Chennai | Tanjavur. |
| <i>Aulophorus furcatus</i> (Müller, 1773) | | Chennai, Coimbatore, Tandikoudi, Ouralpatti, Thanjavur, Udakamandalam (Ooty). |
| <i>Aulophorus gravelyi</i> Stephenson, 1925 | | Ennur near Chennai. |
| <i>Aulophorus hymanae</i> Naidu, 1962 | | Coimbatore. |
| <i>Aulophorus michaelsoni</i> Stephenson, 1923 | | Chennai, Udakamandalam. |
| <i>Aulophorus tonkinensis</i> (Vejdovský, 1894) | | Coimbatore. |
| <i>Branchiodrilus semperi</i> (Bourne, 1890) | Porur Lake, Chennai | Chidambaram, Chennai. |
| <i>Chaetogaster crystallinus</i> Vejdovský, 1883 | | Udhakamandalam. |
| <i>Dero digitata</i> (Müller, 1773) | Redhills Lake, Chennai | Coimbatore, Chennai. |
| <i>Dero dorsalis</i> Ferronière, 1899 | | Chennai. |
| <i>Dero indica</i> Naidu, 1962 | Tharamani Pond, Chennai, Madavaram Pond, Chennai, Chetpet Pond, Chennai | Chennai. |
| <i>Dero nivea</i> Aiyer, 1929 | | Tandikoudi, Ouralpatti, Palni. |
| <i>Dero zeylanica</i> Stephenson, 1913 | Porur Lake, Chennai | Chidambaram, Chennai. |
| <i>Nais andhrensensis</i> (Naidu and Naidu, 1981) | | Udhakamandalam. |
| <i>Nais communis</i> Piguët, 1906 | | Chennai, Udakamandalam. |
| <i>Nais variabilis</i> Piguët, 1906 | | Yercaud. |
| <i>Slavina appendiculata</i> (d'Udekem, 1855) | | Udhakamandalam. |
| <i>Stylaria fossularis</i> (Leidy, 1852) | | Udhakamandalam. |
| FAMILY PRISTINIDAE | | |
| <i>Pristina aequiseta</i> Bourne, 1891 | | Udhakamandalam. |
| <i>Pristina breviseta</i> Bourne, 1891 | Porur Lake, Chennai | Chennai |
| <i>Pristina jenkiniae</i> (Stephenson, 1931) | Redhills Lake, Chennai | Chennai |
| <i>Pristina longiseta longiseta</i> Ehrenberg, 1828 | | Udhakamandalam, Thanjavur, Tandikoudi, Ouralpatti. |
| <i>Pristina menoni</i> (Aiyer, 1929) | | Yercaud. |
| FAMILY TUBIFICIDAE | | |
| Subfamily Rhyacodrilinae | | |
| <i>Bothrioneurum iris</i> Beddard, 1901 | | Palani, Kodaikanal. |
| <i>Branchiura sowerbyi</i> Beddard, 1892 | Porur Lake, Chennai, Nandampakam Lake, Chennai | Chennai, Annamalainagar, Coimbatore, Coonoor, Udakamandalam. |
| SUBFAMILY TUBIFICINAE | | |
| <i>Aulodrilus pluriseta</i> (Piguët, 1906) | | Annamalainagar. |
| <i>Limnodrilus hoffmeisteri</i> Claparède, 1862 | Porur Lake, Chennai, Nandampakam Lake, Chennai, Pallikaranai Marsh, Chennai | Chennai, Coimbatore, Udakamandalam, Coonoor. |
| <i>Limnodrilus udekemianus</i> Claparède, 1862 | Kodaikanal Boat House Lake, Dindugal | Udhakamandalam. |
| <i>Tubifex tubifex</i> (Müller, 1774) | | Udhakamandalam, Coonoor, Kanyakumari. |
| ORDER ENCHYTRAEIDA | | |
| FAMILY ENCHYTRAEIDAE | | |
| <i>Stephensiella marina</i> (Moore, 1902) | | Ennur near Chennai. |
| ORDER CRASSICLITELLATA | | |
| FAMILY ALMIDAE | | |
| <i>Glyphidrilus annandalei</i> Michaelson, 1910 | | Mettupalyam, Tiruvallur. |
| FAMILY OCTOCHAETIDAE | | |
| <i>Dichogaster bolau</i> (Michaelson, 1891)? | Satharai Lake, Thiruvallur | |
| FAMILY MEGASCOLECIDAE | | |
| Megascolecidae gen. sp. | Sivaganga Pond, Sivaganga | |
| CLASS POLYCHAETA | | |
| Polychaeta gen. sp. | Porur Lake, Chennai | |

Aulophorus furcatus (Müller, 1773)

Material examined: Porur Lake (Chennai) on 02.12.2010 (n=1).

Morphological observations: Length about 6–7 mm. About 50 segments. Prostomium bluntly conical. Dorsal chaetae begin from V with 1 hair and 1 needle chaetae (Fig. 2C). Ventral chaetae 3–4 per bundle with upper tooth slightly longer (Fig. 2D) in the anterior region and reducing to 2 in the posterior segments with almost equal teeth (Fig. 2E). Branchial fossa with a pair of non-contractile palps and 3 pair of gills.

Distribution in Tamil Nadu: Chennai, Coimbatore, Tandikoudi, Ouralpatti, Thanjavur, Udhakamandalam (Naidu, 2005).

Remarks: *Aulophorus furcatus* is cosmopolitan in distribution. It occurs in the detritus of muddy sediments near submerged vegetation and is always accompanied by a diverse benthic fauna (Nesemann *et al.*, 2004). Presence of a pair of palps with 3 pairs of gills in the branchial fossa is a striking character of this species.

Dero digitata (Müller, 1774)

Material examined: Redhills Lake on 16.02.2011 (n=1) and 28.04.2011 (n=2).

Morphological observations: Length 5–7 mm. Prostomium triangular. Dorsal chaetae from VI, each bundle with 1 hair and 1 needle chaeta. Needle chaetae sigmoid, bifid, with distal tooth longer and thinner than proximal (Fig. 2F). Ventral chaetae 4–5 in II–V with distal tooth longer and thinner than proximal (Fig. 2G). Ventral chaetae decreasing to 2–4 in number from the VI segment with almost equal teeth (Fig. 2H). Branchial fossa with 4 pairs of gills. Live worms reddish in colour.

Distribution in Tamil Nadu: Coimbatore (Naidu, 2005), Chennai (Naveed, 2012).

Remarks: Previously reported from Chennai. Cosmopolitan in distribution.

Dero indica Naidu, 1962

Material examined: Alampalam Lake (Villupuram) on 02.01.2011 (n=1); Chetpet Pond on 07.08.2011 (n=40); Tharamani Pond on 08.08.2011 (n=60) and Madavaram Pond on 11.08.2011 (n=35); all in Chennai.

Morphological observations: Worms red in colour. 5–8 mm in length. 30–70 segments with undifferentiated hind regions. Prostomium bluntly triangular. Dorsal bundles of chaetae begin in VI, ventral bundles in segment II. The dorsal bundles consist of 2 hair and 2 needle chaetae in the anterior segments while in the posterior segments the number is reduced to 1 hair and 1 needle. Hair chaetae long and not serrated. Needle chaetae bifid, sickle-shaped with distal nodulus. Distal tooth of the needle chaetae slightly thinner and longer than the proximal (Fig. 2I). Ventral bundles in the foremost segments from II–V consist of 4 long, thin and slightly curved chaetae per bundle with upper tooth longer than the lower (Fig. 2J). In other segments 3–4 ventral chaetae per bundle, shorter than in II–V with upper tooth almost equal to lower (Fig. 2K). Branchial fossa with 4 pairs of gills.

Distribution in Tamil Nadu: Chennai (Naveed, 2012)

Remarks: First report from Villupuram. Dorsal bundles with 2 hair and 2 needles beginning in segment VI, and 4 pairs of gills in the branchial fossa are the conspicuous characters of this species.

Dero zeylanica Stephenson, 1913

Material examined: Porur Lake (Chennai) on 24.04.2011 (n=3).

Morphological observations Length 4–7 mm, segments 40–70. Prostomium triangular. Dorsal chaetae begin in VI, each bundle with 3 hair and 3 needle chaetae. Needle chaetae bifid, sigmoid with distal tooth longer than proximal (Fig. 2L). Ventral chaetae in II–V 4–6 per bundle, longer and thinner than those in more posterior segments and with distal teeth twice as long as proximal (Fig. 2M). In the remaining segments ventral chaetae with almost equal teeth (Fig. 2N).

Distribution in Tamil Nadu: Chidambaram (Naidu, 2005), Chennai (Naveed, 2012).

Remarks: Present only in the samples collected from Chennai. It is reported from Asia and Brazil (Naidu, 2005).

Family Pristinidae

Pristina brevichaeta Bourne, 1891

Material examined: Porur Lake Chennai on 02.01.2011 (n=1).

Morphological observations: Length >2mm and number of segments >17 (posterior missing). Prostomium with short proboscis. Dorsal chaetae begin in II, 1 hair and 1 bifid needle per bundle with weak nodulus and equal teeth (Fig. 2O). Ventral chaetae 3–5 per bundle, with distal tooth equal to the proximal (Fig. 2P).

Distribution in Tamil Nadu: Chennai (Naidu, 2005).

Remarks: Immature worm (spermathecal chaetae not observed). Previously reported from Chennai. Presence of proboscis, the non-serrated hair chaetae and needle chaetae with equal teeth are some of the prominent characters of this species. It is generally distributed in south-east Asia, Europe and North and South America (Naidu, 2005).

Pristina jenkiniae (Stephenson, 1931)

Material examined: Red Hills (Chennai) on 16.02.2011 (n=1).

Morphological observations Length 2.5–3 mm and 30–36 segments. Prostomium conical, proboscis absent. Dorsal and ventral chaetae from segment II. Dorsal bundles consists of 1 hair and 1 needle chaetae per segment. Distal teeth of the needles shorter than the proximal (Fig. 2Q). Ventral chaetae 3–4 per bundle in the anterior segments and 2–3 in the posterior segments. Ventral chaetae all with equally long teeth (Fig. 2R).

Distribution in Tamil Nadu: Chennai (Naveed, 2012).

Remarks: Previously reported from Chennai. This species differs from *P. brevichaeta* in the absence of a proboscis and by the upper tooth of the needle chaeta being much shorter and thinner than the lower. Cosmopolitan (Timm, 2009).

Family Tubificidae

Subfamily Rhyacodrilinae

Branchiura sowerbyi Beddard, 1892

Material examined: Alampalam Lake in Villupuram on 02.01.2011 (n=1); Porur Lake on 09.04.2011 (n=12), 02.05.2011 (n=7); Nandampakam on 04.06.2011 (n=14).

Morphological observations: Length 65–170 mm. Prostomium conical. Posterior third of body with a pair of hollow finger like gills per segment. Dorsal chaetae from II with 1–6 hair and sigmoid bifid chaetae with proximal tooth longer than distal. Hair chaetae bayonet-shaped. Ventral chaetae simple pointed, 5–6 anteriorly, gradually decreasing to 1–2 posteriorly. Sex organs not clearly visible.

Distribution in Tamil Nadu: Chennai, Annamalainagar, Coimbatore, Coonoor, Udhakamanalah.

Remarks: First report for Villupuram district. Presence of gills in the posterior third of the body is a characteristic feature which enables easy identification of this species. It is cosmopolitan in distribution.

Subfamily Tubificidae

Limnodrilus hoffmeisteri Claparède, 1862

Material examined: Porur Lake on 02.01.2011 (n=20), 08.01.2011 (n=25), 08.02.2011 (16 specimens), 12.02.2011 (n=12), 02.03.2011 (n=12), 08.03.2011 (n=13), 23.03.2011 (11 specimens), 03.04.2011 (n=7), 09.04.2011 (n=6), 15.04.2011 (n=32), 24.04.2011 (n=19), 26.04.2011 (n=10), 02.05.2011 (n=3), 25.05.2011 (n=16), 04.06.2011 (n=33), 08.06.2011 (n=25), 12.06.2011 (n=28); Nandampakam on 12.05.2011 (n=7), 04.06.2011 (n=7); Pallikaranai Pond 21.05.2011 (n=14); Vandalur Lake on 03.11.2011 (n=4), 07.11.2011

(n=5), 23.11.2011 (n=3), 02.12.2011 (n=4), 20.12.2011 (n=5), 26.12.2011 (n=3), 07.01.2012 (n=26), 04.02.2012 (n=28), 03.03.2012 (n=24); Chetpet Pond on 13.11.2011 (n=3), 14.12.2011 (n=4), 21.01.2012 (n=2), 18.02.2012 n=(9) and 10.03.2012 n=(8). Many specimens were sexually mature.

Morphological observations: Penis sheaths are conspicuously visible in mature worms, constituting a vital character in the identification of this species. It is much longer than broad (8–10 times longer than its maximum width). Two types of penis sheath, namely the “plate-topped” type (Fig. 2U1) and the “typical” type (Fig. 2U2), were observed. Live worms red in colour. Length 30–60 mm. Segments 40–150. Prostomium bluntly conical. Dorsal and ventral chaetae begin from segment II, all bifid and similar, with distal tooth equal in length to the proximal. Anterior segments bearing 4–8 chaetae per bundle. The number of chaetae per bundle gradually decreases in the succeeding segments, with 1–2 in the most posterior segments.

Distribution in Tamil Nadu: Chennai, Coimbatore, Udhakamandalam, Coonoor.

Remarks: “Plate-topped” penial sheaths along with the “typical” type were observed in the present study. The sheaths of *L. hoffmeisteri* may be straight or curved, the walls are of even thickness and the distal ends are expanded to form a trumpet-shaped head, with a lateral opening. Some of the variation appears to be due to natural causes, but some apparent variation is due to such factors as the degree of pressure exerted upon the sheath when mounting, the position of the sheath and the angle from which it has been viewed. Even taking the variation into account, the shape of the sheath is still quite distinct from that of any other species (Kennedy, 1969). Cosmopolitan in distribution.

***Limnodrilus udekemianus* Claparède, 1862**

Material examined: Kodaikanal Boat House Lake, Dindugal District 14.01.2011 (n=1 immature).

Morphological observations: Length about 48–50 mm. Segments about 90 in number with the posterior-most tail region missing. Prostomium bluntly conical. Both dorsal and ventral chaetae similar and begin from the II segment. Anterior chaetae 3–9 per bundle, with distal tooth thicker and much longer than the proximal tooth and more curved (Fig. 2V). Chaetae in the posterior segments gradually decrease in number. Beginning of the chloragogen tissue from VI.

Distribution in Tamil Nadu: Ooty (Naveed, 2012).

Remarks: First report from Kodaikanal, a cool mountainous region of Dindugal district of Tamil Nadu. *L. udekemianus* differs from *L. hoffmeisteri* by the relative size and shape of the two teeth of the chaetae (Kennedy, 1969). The chaetae in *L. hoffmeisteri* are all similar, with distal tooth thinner and equal in length to the proximal, while in *L. udekemianus* the distal tooth is thicker, much longer and more curved than the proximal tooth in the foremost bundles. It was first reported in India by Naveed (2012) but is cosmopolitan in distribution.

Family Octochaetidae

(*Dichogaster bolau* (Michaelen, 1891)?)

Material examined: Satharai Lake, Thiruvallur district on 10.11.2010 (n=1).

Morphological observations: Length 4 cm (posterior portion missing), breadth 1.0–1.2 mm. Segment number >99. Shape of prostomium conical. Dorsal chaetae start from II, sigmoid, simple-pointed, 2 per bundle. Ventral chaetae almost straight and pointed. Clitellum from XII. Spermathecal pores in 8/9.

Remarks: The identification is provisory. The species is basically terrestrial found in tropical countries.

Family Megascolecidae

Material examined: Sivaganga Pond, Sivaganga District, 13.05.2011 (n=1).

Morphological observations: Simple pointed sigmoid chaetae and clitellum beginning with or in front of segment xv.

Remarks: Live worm crawls out of the petri dish. Its presence in the aquatic habitat may be accidental.

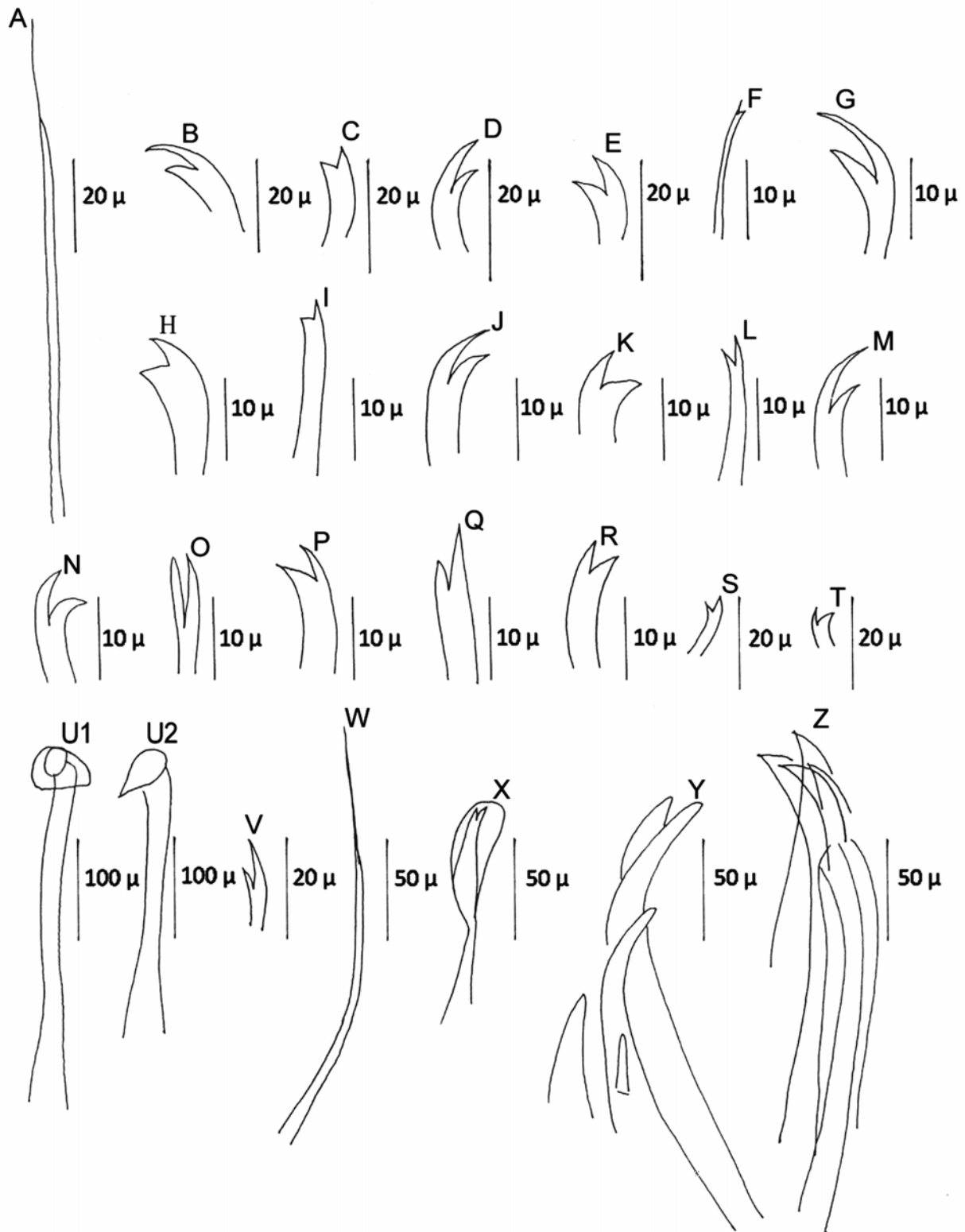


FIGURE 2. *Branchiodrilus semperi*: A) needle chaeta, B) ventral chaeta. *Aulophorus furcatus*: C) needle chaeta, D) ventral chaeta of segment II, E) ventral chaeta of a posterior segment. *Dero digitata*: F) needle chaeta, G) ventral chaeta of segment II, H) ventral chaeta of a posterior segment. *Dero indica*: I) needle chaeta, J) ventral chaeta of segment II, K) ventral chaeta of a posterior segment. *Dero zeylanica*: L) needle chaeta, M) ventral chaeta of segment II, N) ventral chaeta of a posterior segment. *Pristina brevichaeta*: O) needle chaeta, P) ventral chaeta of a posterior segment. *Pristina jenkiniae*: Q) needle chaeta, R) ventral chaeta of a posterior segment. *Limnodrilus hoffmeisteri*: S) dorsal chaeta, T) ventral chaeta, U1) penis sheath “Plate-topped”, U2) penis sheath “typical”. *Limnodrilus udekemianus*: V) dorsal chaeta of the anterior segment. A polychaete: W) hair chaeta, X) dorsal chaetae of segment VIII, Y) ventral chaeta of segment IX, Z) ventral chaeta of segment X.

Polychaeta?

Material examined: Porur Lake in Chennai on 03.03.2011 (n=1) collected from mud and aquatic vegetation.

Morphological observations: Only anterior 10 segments present, with remnants of segment XI and the rest of the worm missing. Length of the first 10 segments about 4–5 mm. Prostomium bluntly conical. Dorsal and ventral bundles starting from segment II, consisting of about 4–6 bayonet-shaped hair chaetae per bundle (Fig. 2W). Eight bifid chaetae beginning from segment VIII, with upper teeth shorter and with thin lateral projections surrounding the distal portion of chaetae, giving them an oar- or spoon-shaped appearance (Fig. 2X). Simple-pointed chaetae about 5 in number ventrally in segment IX (Fig. 2Y) and X (Fig. 2Z). Hair chaetae absent in segments IX and X, but oar-shaped chaetae present dorsally.

Remarks: The worm was immature with the thickest segments VI–VII without any traces of clitellum, genital pores, or internal reproductive organs. The simple-pointed (modified?) ventral chaetae cannot be genital; the respective segments, IX and X, are very thin. At first, the worm was mistakenly thought to be an *Aulodrilus* sp. as the oar-shaped dorsal chaetae resemble some species of that genus, but their structure (with a film surrounding the whole distal end of the chaetae) is not seen among tubificids so far; it may be present in some polychaetes only.

Discussion

A recent proposal of synonymy of the families Naididae and Tubificidae affects the taxonomic classification of most species commonly used in pollution biology. Thus, it has been formally proposed that the former families of Naididae and Tubificidae be merged as synonyms into the family Naididae. The growing evidence from molecular data of an increasing number of oligochaete species suggests that this interpretation may change again in the near future (Rodriguez and Reynoldson, 2011). However, it is reasonable to treat sediment-dwelling tubificids separately from the epibenthic naidids and pristinids that have derived from them, in all other branches of biology except phylogeny (Timm, 2009). Hence the classification of Timm (2009) is followed in the present work.

About 28 aquatic Oligochaeta from Tamil Nadu comprising of 16 species of Naididae, 5 species of Pristinidae, 5 species of Tubificidae, 1 species of Enchytraeidae and 1 species of Almididae were described by Naidu (2005). Recently 1 species of Naididae, 1 species of Pristinidae and 1 species of Tubificidae were reported from Tamil Nadu by Naveed (2012), hence presently 31 aquatic Oligochaeta are known from Tamil Nadu (Table 1). Naididae dominate the aquatic oligochaete fauna in Tamil Nadu and the genus *Dero* dominates over other naidids. Fifty nine species and 2 subspecies of naidids were reported out of the 102 species of aquatic Oligochaeta described from the Indian subcontinent (Naidu, 2005). A similar tendency is also encountered in Indonesian freshwaters so far surveyed, where 77% (27 spp.) of the fauna is occupied by the family Naididae (Ohtaka et al, 2000). The Naididae appear to be more poorly represented in Australia where 38 species of them have been recorded (Pinder, 2001). In the present study the naidids were mostly immature and were collected mostly from aquatic vegetation. Alves et al (2007) has shown that aquatic macrophytes provide an important habitat for naidids. Naidid worms breed mostly in an asexual way; by paratomy (budding) and mature individuals occur seldom. Fortunately, they possess many external characters useful for identification, such as specialized locomotory chaetae, body appendages, and eyes. Living mostly on bottom surface and aquatic plants, many of them are able to swim. The former subfamily Pristininae of the family Naididae which is treated as a separate family Pristinidae includes a single genus *Pristina* (Timm, 2009). The two pristinid species, *P. brevichaeta* and *P. jenkiniae*, identified from Chennai were already reported from this region by Naidu (2005) and Naveed (2012) respectively, but are yet to be reported from other parts of Tamil Nadu and North India. The success of the tubificids *Branchiura sowerbyi* and *Limnodrilus hoffmeisteri* in wide range of aquatic habitats is due to the tolerance to different environmental conditions, as well as their high rates of reproduction (Naveed et al, 2011). The identification of a polychaete found together with oligochaetes is provisory. *Limnodrilus udekemianus*, recently reported from India by Naveed (2012), was collected during this survey from Kodaikanal, a cool mountainous region of Dindugal District of Tamil Nadu. This constitutes the first report of this species for that region and the species is lacking in tropical parts of Tamil Nadu. The occurrence of megadriles belonging to the families Octochaetidae and Megascolecidae in aquatic habitats may be accidental due to flooded rain water.

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Profundal oligochaete faunas (Annelida, Clitellata) in Japanese lakes

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Abstract

Thirty-eight species of oligochaetes (Annelida, Clitellata) belonging to five families were recorded from profundal bottoms of 50 freshwater lakes on Japanese islands. They were mostly widely distributed species, and the composition of fauna is basically explained by the scheme of Timm (2012), with parallel replacement of European species. Oxygen, temperature and surrounding fauna could be main factors determining the profundal fauna in the lakes. The lumbriculids (*Styloscolex japonicus*, *Yamaguchia toyensis* and *Lumbriculus variegatus*), haplotaxids (*Haplotaxis gordioides*) and enchytraeids (*Marionina klaskisharum*) were restricted to several deep and oligotrophic lakes located in Hokkaido and northern Honshu, where *Rhyacodrilus komarovi* often accompanied them. *Limnodrilus hoffmeisteri* was the commonest species, occurring irrespective of the trophic status and bottom temperature of the lake. *Tubifex tubifex* also occurred in wide trophic scale, but it has never been found in shallow eutrophic lakes where the bottom temperature exceeds 15°C, where *L. hoffmeisteri*, *Branchiura sowerbyi* and several *Aulodrilus* species were the main representatives.

Key words: oligochaetes, fauna, lake profundal, Japan

Introduction

In the lake profundal, which is herein defined as the deepest vegetation-free muddy zone in lacustrine benthos as in Thienemann (1925) and Timm (2012), it has been recognized that oligochaetes have played a dominant role in the saprobic system, and their compositions have been discussed in terms of lake typology and other ecological and zoogeographical aspects (e.g., Brinkhurst 1974).

Timm (2012) recently reviewed the profundal oligochaete fauna in Palaearctic lakes and he showed that the fauna is mostly formed of species present also in shallower water, except for those in ancient rift lakes like Lake Baikal where endemic species living in the profundal have arisen through evolutionary radiation. The faunal composition in the “usual” lakes usually depends on geographical region and trophic state of the lake, the latter regulating the amount of oxygen in the bottoms, and parallel replacement species can be recognized between faraway European and Asian lakes (Timm 2012).

Along with Kurile Islands and the Kamchatka Peninsula, Japanese archipelago is part of the northwestern Pacific Rim and has variable kinds of lakes. In contrast to European and North American lakes, which are mostly glacial origin, there are many volcanic and maritime coastal ones in Japanese islands. In addition, the Japanese islands stretch a long way north to south with a corresponding heterogeneous climate, resulting in presence of both dimictic and monomictic lakes (Yoshimura 1936).

The first study on profundal oligochaetes in Japanese lakes was conducted by Stephenson (1917) in the ancient Lake Biwa, based on Annandale's samples collected in 1915 (cf. Annandale 1922). He originally described *Criodrilus bathybates* (syn. *Biwadrilus bathybates* (Stephenson)) and *Kawamura japonica* (syn. *Branchiura sowerbyi* Beddard) along with unidentified tubificids and lumbriculids from the deep profundal of the lake.

Miyadi (1931a,b, 1932a–g) and Kitagawa (1973, 1974a,b, 1975a,b, 1978, 1979) intensively studied the structure of bottom fauna in many Japanese lakes from the viewpoint of lake typology. Miyadi (1933) classified them into eight types on the basis of causal-ecological concept. He classified four deep and

oligotrophic caldera lakes (Lakes Shikotsu, Toya and Kuttara in Hokkaido, and Lake Ideda in Kyushu) into “Oligochaeta lakes” where the profundal bottom fauna was composed of oligochaetes almost exclusively. Miyadi and Kitagawa recorded many “*Tubifex*” in their lists of zoobenthos, however, their “*Tubifex*” could not denote the taxon *Tubifex* but an oligochaete assemblage (Ohtaka and Iwakuma 1993), thus species composition can not be read in their studies.

Faunal records of lake profundal oligochaetes in Japanese lakes have been accumulated since 1980s in accordance with an increase in faunistic and ecological studies of lake zoobenthos (Ito 1978; Ito and Uno 1980; Ito et al. 2002, 2005; Fukuhara et al. 1987; Yasuda and Okino 1987; Ohtaka 1994, 1995, 2001a, b, 2004, 2006, 2009a, b; Ohtaka and Kikuchi 1997; Ohtaka and Ito 2002; Ohtaka and Iwakuma 1993; Ohtaka and Martin 2011; Ohtaka and Nishino 1995, 1999; Ohtaka et al. 1988, 2006, 2010; Nishino et al. 1999; Fend and Ohtaka 2004; Hirabayashi et al. 2007, 2012; Martin and Ohtaka 2008). Comparing these records, species composition of profundal oligochaetes in Japanese lakes can be basically explained by the scheme of Timm (2012), but faunal differences among respective lakes and the factors determining the species composition have not been well understood yet. Therefore, in the present study, I show the present status of profundal oligochaete composition in 50 Japanese freshwater lakes in relation to lake environments and long-term changes.

Study area and methods

Fifty freshwater lakes in Japanese islands were studied for their profundal oligochaete faunas (Table 1; Fig. 1). They include 4 tectonic, 14 volcanic, 23 dammed and 9 other lakes, with variable area, depth and trophic status. The majority of the specimens were collected by the author during 1982–2010 with the aid of an Ekman–Birge bottom sampler (grasp area 225 cm²) from the ecological profundal, i.e., bottoms in aphotic zones or, when the lakes were shallower than the compensation depth, from offshore muddy bottoms without vegetation. The bottom samples were passed through a screen with a mesh pore size of 0.25 mm, and the retained worms were immediately fixed in 10% formalin solution, then dehydrated whole in a graded dilution series of ethanol : water, cleared in methyl salicylate, and mounted in Canada balsam on slides for identification. Selected specimens were dissected directly or cut serially for anatomical and histological observations. In addition, oligochaete specimens collected by colleagues from several lakes (Nos 4, 7, 23, 28, 34, 36), and those in voucher specimens of previous studies were examined and incorporated into the present study.

For environmental factors, bottom temperature was measured in 42 lakes when profundal specimens were collected during summer periods, and a modified Carson’s trophic state index (mTSI; Aizaki et al. 1981) was calculated for every lake on the basis of Secchi disk transparency presented by NCBEA (1993) or author’s observations. Oligotrophic, mesotrophic and eutrophic status of the lake water approximately correspond to lower than 30, from 30 to 50, and higher than 50 for the mTSI values, respectively.

Results

Faunal composition and regional characteristics

Thirty-eight oligochaete species were recorded from profundal bottoms of the 50 Japanese lakes studied (Table 2). Thirty-one species or 82 % of the total fauna belonged to the family Naididae sensu Erséus et al. (2008), which consisted of 11 naidines, 6 rhyacodrilines and 14 tubificines, and they were mostly widely-distributed species. Oligochaetes in other families were composed of three lumbriculid, one haplotaxid, two enchytraeid and one phreodrilid species.

The most frequently recorded species in the list was *Limnodrilus hoffmeisteri*, occurring in 36 lakes. It was followed by *Tubifex tubifex* (31 lakes), *Bothrioneurum vej dovskyanum* (11), *Limnodrilus claparedianus*, *Ilyodrilus templetoni* and *Aulodrilus* sp. (10 each), *Branchiura sowerbyi* and *Teneridrilus mastix* (9 each). The number of oligochaete species occurring in individual lake ranged from one to 14. The sole representatives in the profundal bottom were *Tubifex tubifex* in 5 lakes (Nos 3, 21, 24, 30, 48), *L. hoffmeisteri* in 2 lakes (Nos 31, 50) and *Rhyacodrilus coccineus* in the acidotrophic Lake Inawashiro (No. 20).

Lumbriculids (*Styloscolex japonicus*, *Yamaguchia toyensis* and *Lumbriculus variegatus*), haplotaxids (*Haplotaxis gordioides*) and enchytraeids (*Marionina klaskisharum*) were restricted to five deep and oligotrophic lakes located in Hokkaido and northern Honshu (Nos 5, 9, 10, 11, 15), where *Rhyacodrilus komarovi* was present, except for Lake Shikaribetsu (No. 5). Species composition of Lake Shikotsu (No. 9) and the neighboring L. Toya (No. 10) closely resemble each other, where all six species in L. Shikotsu were common to L. Toya, and five of them (*S. japonicus*, *Y. toyensis*, *H. gordioides*, *M. klaskisharum*, *Rhyacodrilus* sp.) were not found from other Japanese lake profundals. In addition, a minute polychaete, *Aeolosoma hemprichi* Ehrenberg was recorded only from the profundal of L. Shikotsu and Toya.

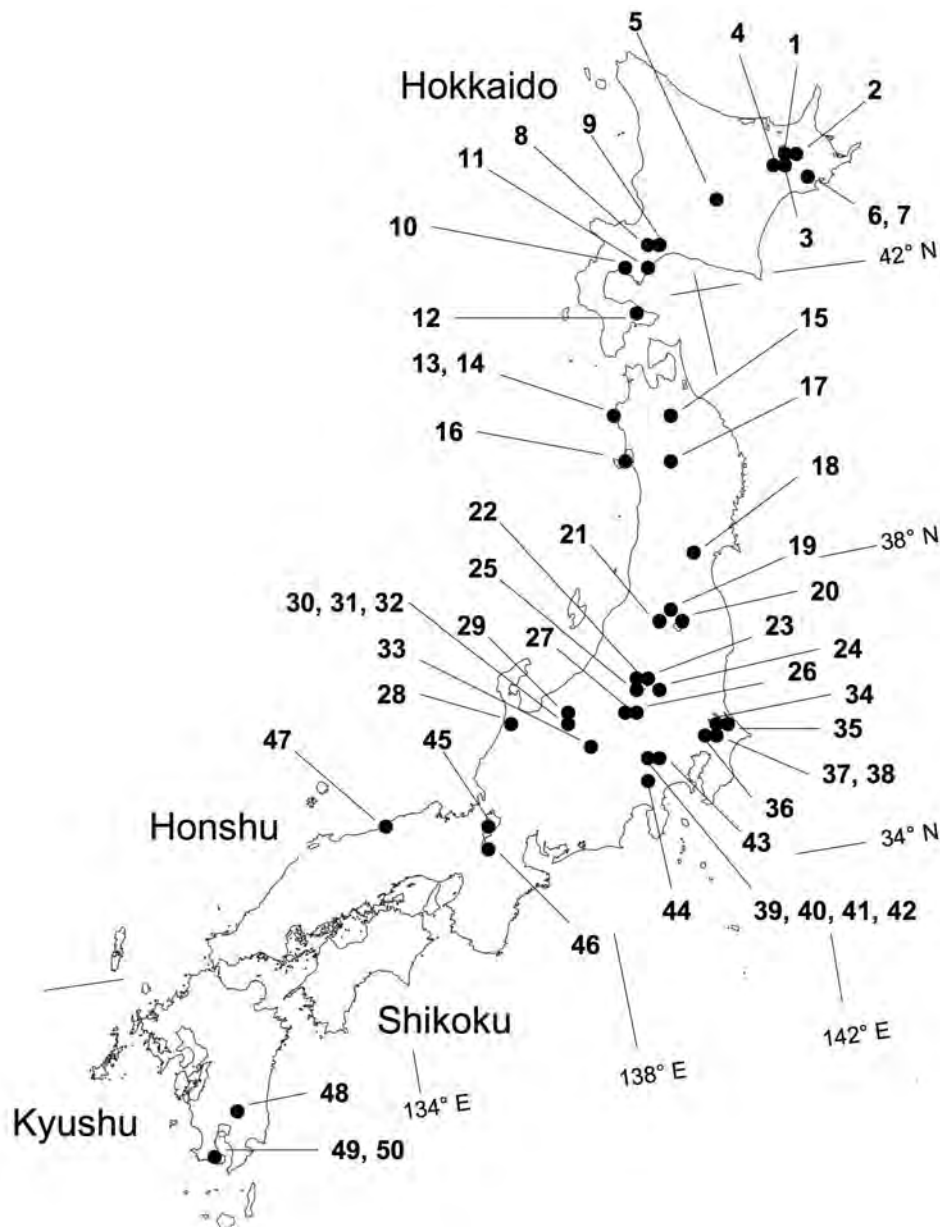


FIGURE 1. Distribution of lakes under study in the Japanese Archipelago. Lake number corresponds to those in Table 1.

Occurrence of oligochaetes in relation to lake environments

Bottom temperature ranged from 3.9 °C to 26.9 °C among 42 lakes in which temperature was measured during the summer season. Although many species occurred at a wide range of bottom temperatures, some were found only in a restricted range of temperatures (Fig. 2). For example, the lumbriculids, haplotaxid, enchytraeids and several naidids (*R. coccineus*, *R. komarovi*, *Krenedrilus towadensis*, *Embolocephalus nikolskyi*) were recorded in the bottoms with temperature lower than 8 °C. On the other hand, occurrences of *Rhyacodrilus hiemalis*, *L. claparedianus*, *Limnodrilus grandisetosus*, *I. templetoni* and *Aulodrilus* sp. were biased toward higher temperatures. They were found in thermally non-stratified shallow lakes where the bottom temperature

exceeded 13 °C. *Branchiura sowerbyi* was usually found at the bottoms whose temperature was higher than 20 °C, but the gill-less variant form in the north basin of Lake Biwa (No. 45) inhabited the ever-cool bottom with 7-8 °C. *Limnodrilus hoffmeisteri* occurred at every temperature, while the second commonest tubificine, *T. tubifex* was never found in shallow lakes with bottom temperature higher than 15 °C.

TABLE 1. A list of Japanese freshwater lakes in which profundal oligochaete fauna were studied.

| No. in Fig. 1 | Lake name | Prefecture | Location ¹ | | Area ² (km ²) | Maximum depth ² (m) | Origin ^{1, 2, 3} | Lake type ^{2, 3} | mTSI ⁴ |
|---------------|--------------------|-----------------|-----------------------|----------|--------------------------------------|--------------------------------|---------------------------|---------------------------|-------------------|
| | | | N | E | | | | | |
| 1 | Kussharo | Hokkaido | 43° 37' | 144° 20' | 79.5 | 117.0 | caldera | acidtrophic | 35 |
| 2 | Mashu | Hokkaido | 43° 35' | 144° 33' | 19.1 | 211.5 | caldera | oligotrophic | 9 |
| 3 | Panke-to (Akan) | Hokkaido | 43° 29' | 144° 11' | 2.8 | 54.0 | dammed | oligotrophic | 21 |
| 4 | Akan | Hokkaido | 43° 27' | 144° 06' | 13.3 | 45.0 | caldera | eutrophic | 38 |
| 5 | Shikaribetsu | Hokkaido | 43° 17' | 143° 07' | 3.4 | 99.0 | dammed | oligotrophic | 27 |
| 6 | Toro | Hokkaido | 43° 09' | 144° 33' | 6.4 | 7.0 | lagoon | eutrophic | 63 |
| 7 | Takkobu | Hokkaido | 43° 06' | 144° 29' | 1.4 | 2.3 | lagoon | oligotrophic | 55 |
| 8 | Okotanpe | Hokkaido | 42° 48' | 141° 16' | 0.4 | 20.5 | dammed | oligotrophic | 30 |
| 9 | Shikotsu | Hokkaido | 42° 45' | 141° 20' | 78.8 | 363.0 | caldera | oligotrophic | 17 |
| 10 | Toya | Hokkaido | 42° 36' | 140° 51' | 70.4 | 180.0 | caldera | oligotrophic | 26 |
| 11 | Kuttara | Hokkaido | 42° 30' | 140° 11' | 4.7 | 148.0 | caldera | oligotrophic | 13 |
| 12 | O-numa (Oshima) | Hokkaido | 42° 00' | 140° 41' | 5.5 | 13.6 | dammed | eutrophic | 50 |
| 13 | O-ike | Aomori | 40° 34' | 139° 58' | 0.05 | 24.0 | dammed | mesotrophic | 36 |
| 14 | Ochikuchi-no-ike | Aomori | 40° 34' | 139° 59' | 0.02 | 20.3 | dammed | mesotrophic | 47 |
| 15 | Towada | Aomori/Akita | 40° 28' | 140° 53' | 61.1 | 327.0 | caldera | oligotrophic | 28 |
| 16 | Hachiro-gata | Akita | 40° 00' | 140° 00' | 27.6 | 12.0 | lagoon | eutrophic | 60 |
| 17 | Tazawa | Akita | 39° 43' | 140° 40' | 25.8 | 423.0 | caldera | acidtrophic | 42 |
| 18 | Izu-numa | Miyagi | 38° 43' | 141° 06' | 2.9 | 1.3 | fluvial | eutrophic | 65 |
| 19 | Hibara | Fukushima | 37° 41' | 140° 03' | 10.8 | 31.0 | dammed | mesotrophic | 40 |
| 20 | Inawashiro | Fukushima | 37° 28' | 140° 06' | 104.8 | 94.6 | tectonic | acidtrophic | 35 |
| 21 | Numazawa | Fukushima | 37° 27' | 139° 35' | 2.9 | 96.0 | caldera | oligotrophic | 28 |
| 22 | Oze-numa | Fukushima/Gunma | 36° 56' | 139° 18' | 1.8 | 9.5 | dammed | mesotrophic | 36 |
| 23 | Yu-no-ko | Tochigi | 36° 48' | 139° 25' | 0.3 | 12.5 | dammed | mesotrophic | 43 |
| 24 | Chuzenji | Tochigi | 36° 44' | 139° 28' | 11.6 | 163.0 | dammed | oligotrophic | 28 |
| 25 | Maru-numa | Gunma | 36° 49' | 139° 21' | 0.5 | 47.0 | dammed | mesotrophic | 28 |
| 26 | O-numa (Akagi) | Gunma | 36° 33' | 139° 11' | 0.9 | 16.5 | caldera | mesotrophic | 41 |
| 27 | Haruna | Gunma | 36° 28' | 138° 52' | 1.2 | 14.0 | caldera | mesotrophic | 36 |
| 28 | Kahoku-gata | Ishikawa | 36° 40' | 136° 41' | 4.1 | 6.5 | lagoon | eutrophic | 73 |
| 29 | Nojiri | Nagano | 36° 49' | 138° 13' | 3.9 | 37.5 | dammed | oligotrophic | 37 |
| 30 | Aoki | Nagano | 36° 37' | 137° 51' | 1.9 | 58.0 | dammed | oligotrophic | 27 |
| 31 | Nakatsuna | Nagano | 36° 36' | 137° 51' | 0.1 | 12.0 | dammed | mesotrophic | 32 |
| 32 | Kizaki | Nagano | 36° 33' | 137° 50' | 1.4 | 29.5 | dammed | mesotrophic | 41 |
| 33 | Suwa | Nagano | 36° 03' | 138° 05' | 13.3 | 6.3 | tectonic | eutrophic | 76 |
| 34 | Kasumi-ga-ura | Ibaraki | 36° 02' | 140° 24' | 168.2 | 7.0 | lagoon | eutrophic | 73 |
| 35 | Kita-ura | Ibaraki | 36° 01' | 140° 34' | 34.4 | 10.0 | lagoon | eutrophic | 73 |
| 36 | Naka-numa | Ibaraki | 35° 53' | 140° 10' | 0.01 | 13.4 | fluvial | eutrophic | 44 |
| 37 | Tega-numa | Chiba | 35° 51' | 140° 05' | 6.5 | 3.8 | dammed | eutrophic | 80 |
| 38 | Inba-numa | Chiba | 35° 46' | 140° 13' | 11.6 | 2.5 | dammed | eutrophic | 69 |
| 39 | Kawaguchi | Yamanashi | 35° 31' | 138° 45' | 5.7 | 14.6 | dammed | eutrophic | 37 |
| 40 | Motosu | Yamanashi | 35° 28' | 138° 35' | 4.7 | 121.6 | dammed | oligotrophic | 25 |
| 41 | Sai-ko | Yamanashi | 35° 30' | 138° 41' | 2.1 | 73.2 | dammed | oligotrophic | 34 |
| 42 | Shoji-ko | Yamanashi | 35° 29' | 138° 37' | 0.5 | 15.2 | dammed | eutrophic | 37 |
| 43 | Yamanaka | Yamanashi | 35° 25' | 138° 52' | 6.8 | 13.3 | dammed | mesotrophic | 36 |
| 44 | Ashi-no-ko | Kanagawa | 35° 13' | 139° 00' | 6.9 | 43.5 | dammed | mesotrophic | 31 |
| 45 | Biwa (north basin) | Shiga | 35° 15' | 136° 05' | 614.7 | 103.6 | tectonic | mesotrophic | 35 |
| 46 | Biwa (south basin) | Shiga | 35° 03' | 135° 54' | 54.5 ⁵⁾ | 6 | tectonic | eutrophic | 60* |
| 47 | Koyama-ike | Tottori | 35° 30' | 134° 09' | 7.0 | 6.3 | lagoon | eutrophic | 65 |
| 48 | Mi-ike | Miyazaki | 31° 53' | 130° 58' | 0.7 | 93.5 | crater | oligotrophic | 46 |
| 49 | Ikeda | Kagoshima | 31° 14' | 130° 34' | 10.9 | 233.0 | caldera | mesotrophic | 34 |
| 50 | Unagi-ike | Kagoshima | 31° 13' | 130° 36' | 1.2 | 56.5 | crater | mesotrophic | 53 |

1) after Horie (1962); 2) after NCBEA (1993); 3) after Japanese Society of Limnology (2006); 4) modified Carlson's Trophic State Index (Aizaki et al., 1981) based on Secchi depth in meter in NCBEA (1993) or author's values (*); 5) after Haga (2006)

The mTSI values calculated from Secchi depth ranged from 9 (No. 2) to 80 (No. 37). Three lumbriculids (*S. japonicus*, *Y. toyensis* and *L. variegatus*), a haplotaxid (*H. gordioides*), an enchytraeid (*M. klaskisharum*) and a tubificine (*E. nikolskyi*) were restricted to oligotrophic lakes with mTSI values lower than 30 (Fig. 3). Occurrence of *R. komarovi* was also biased to oligotrophic lakes. On the other hand, *Aulodrilus* sp., *L. grandisetosus*, *B. sowerbyi* and *A. pluriseta* were invariably found in eutrophic lakes with mTSI values higher than 50. Many other species were found from meso- to eutrophic lakes. As an exception, *L. hoffmeisteri* occurred in lakes of every trophic state. *T. tubifex* was also found from wide trophic range, but it has been never found from the lakes with mTSI values higher than 60, all of which were shallow and with bottom temperature exceeding 15 °C in summer.

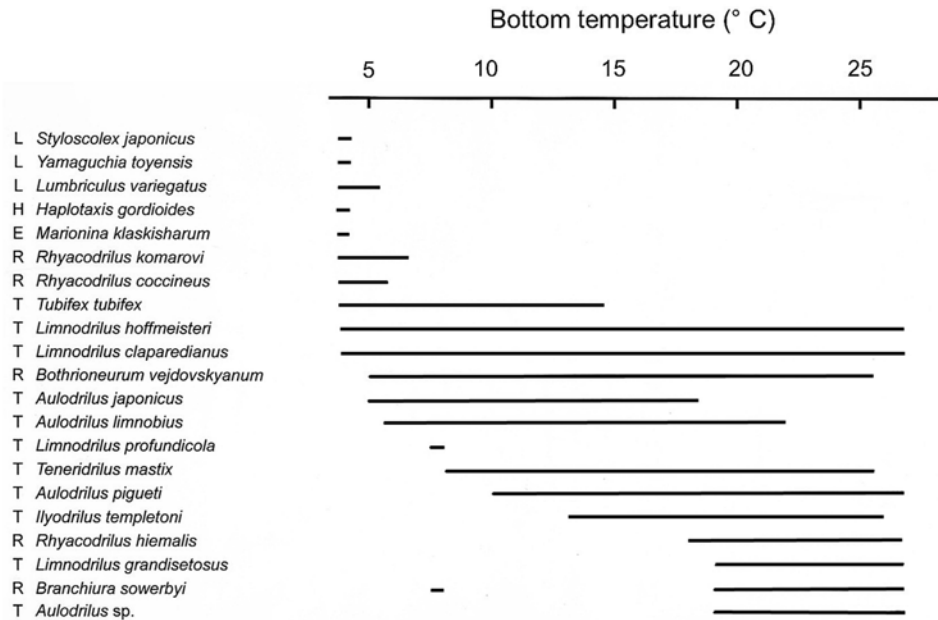


FIGURE 2. Range in summer temperatures in the profundal bottoms where respective oligochaete species were collected. Rare species, including all naidines, were excluded. L, Lumbriculidae; H, Haplotaxidae; E, Enchytraeidae; R, Rhyacodrilinae; T, Tubificinae.

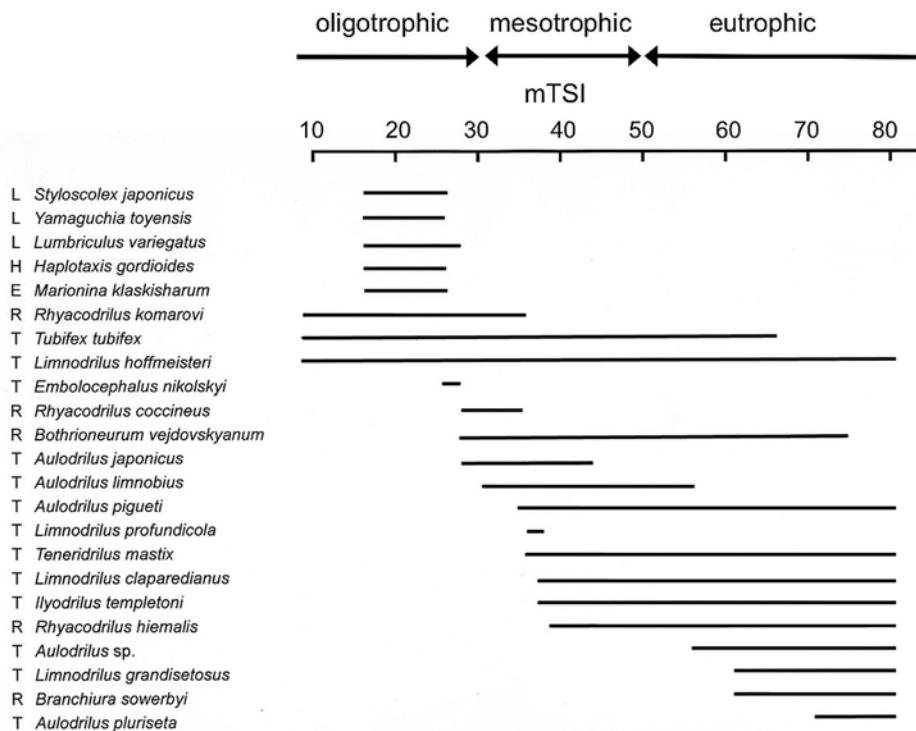


FIGURE 3. Range in the occurrence of oligochaete species in Japanese lakes on the trophic axis. Rare species, including all naidines, were excluded. L, Lumbriculidae; H, Haplotaxidae; E, Enchytraeidae; R, Rhyacodrilinae; T, Tubificinae.

Azoic zone

Practical surveys in the present study found that six lakes (Nos 4, 13, 14, 21, 49, 50) have azoic zones in their deepest profundal, where no zoobenthos was found. The bottom sediments of these lakes were deep black with a smell of H₂S, suggesting anoxic conditions. In addition, no macrozoobenthos including any oligochaetes was found in the profundal of Lake Tazawa (No. 17) as noted by Ohtaka and Martin (2011) although the bottoms were highly aerobic and harbored a small number of nematodes.

Discussion

Environmental factors determining oligochaete composition

All Japanese lakes except Lake Biwa are young and originated during or subsequent to the most recent glacial maximum (Saijo and Sakaguchi 1978), thus it is expected that their profundal bottom fauna is represented by a part of that distributed in the surrounding area. Most species recorded from profundal bottoms in the present study are certainly widely distributed species and they could be derived from surrounding waters. *Yamaguchia toyensis* and *K. towadensis* have been recorded only from L. Shikotsu and Toya (Fend and Ohtaka 2004), and L. Towada (Ohtaka 2004), respectively. However, these two stenotherm and oxyphilic species have been thought to be primarily subterranean (Fend and Ohtaka 2004; Ohtaka 2004), and they could occur in surrounding underground waters, too. Although enchytraeids are diverse and primarily inhabit terrestrial or marine littoral environments, a series of species have been recorded from profundal habitats of many lakes of the world, mainly in high latitudes (Timm, 1996). *Marionina klaskisharum*, recorded from Lakes Shikotsu and Toya in the present study, is one such species. It was originally described from the Pacific northeastern coast in Canada (Coates 1983) and subsequently recorded from the littoral of Lake Kurilskoe in Kamchatka Peninsula, Russia (Timm and Vvedenskaya 2006). Occurrence of a Gondwanan-originated phreodrilid, *Astacopsidrilus ryuteki*, from profundal Lake Biwa (Martin and Ohtaka 2009) is mysterious. But because there is no sign of former zoogeographical connection between Japan and Gondwanan landmasses, the Japanese record could be based on an introduction (Martin and Ohtaka 2009).

There has been wide recognition that trophic level as well as geographical region regulate oligochaete composition of lakes, and that profundal oligochaetes have been used for assessment of the lake trophic status (Aston 1973; Brinkhurst 1974; Brinkhurst and Kennedy 1965; Milbrink 1978, 1980, 1983; Milbrink et al. 2002; Lang 1985, 1998; Rodriguez and Reynoldson 2011). As in European lakes, two tubificines with high tolerance for oxygen deficiency, *Tubifex tubifex* and *Limnodrilus hoffmeisteri*, were widely distributed in trophic scale in Japan. Five lakes in which *T. tubifex* occurred as a single oligochaete were relatively small but deep and oligotrophic or mesotrophic like similar European lakes (Timm 2012). On the other hand, in Japan, *T. tubifex* has never been found from the hypertrophic lakes with the mTSI values exceeded 60, where *L. hoffmeisteri* was invariably dominating, accompanied by *B. sowerbyi* and *Aulodrilus* species. *Tubifex tubifex*, like most other tubificines, has been regarded to need at least periodically low temperatures for spermatogenesis, and it was absent in tropical regions (Timm 2012). Therefore, absence of *T. tubifex* in hypertrophic lakes in the present study could be due to high temperature, because these hypertrophic lakes are shallow and easily mixed and the bottom temperature exceeded 15 °C in summer, although the bottoms become as cool as in deep lakes in other seasons. The absence of *T. tubifex* and dominant occurrence of *L. hoffmeisteri* followed by *Aulodrilus* species and *B. sowerbyi* found in shallow hypertrophic lakes are also widely encountered in rice paddies in Japan, where the water is very shallow and the bottom temperature exceed 30 °C in summer and close to freezing in winter (Ohtaka unpublished). The present observation for the absence of *T. tubifex* in the lake bottoms with the temperature higher than 15 °C in summer appears to contradict the rearing temperature of higher than 15 °C in many laboratory studies (e.g. Timm 1984; Poddubnaya 1984; Anlauf 1994; Reynoldson et al. 1996; Arrate et al. 2004). This discrepancy might depend on genotypic variants in *T. tubifex* (Anlauf 1994; Beauchamp et al. 2001). It is also probable that other environmental parameters, for example oxygen or diet deficiency, can reduce tolerance to high temperature.

Similar to *L. hoffmeisteri*, adaptation to relatively higher temperatures is also suggested for *L. grandisetosus*, *Aulodrilus* spp. and *Branchiura sowerbyi*. Some *Branchiura sowerbyi* were found in an exceptionally ever-cool (7-8 °C) habitat in the profundal Lake Biwa, where the temperatures are lower than the threshold value for cocoon production (Aston 1968) or embryonic development (Bonacina et al. 1994) of usual *B. sowerbyi* (estimated at 10°C). The profundal L. Biwa population of *B. sowerbyi*, once described as

Kawamura japonica by Stephenson (1917), reveals only weakly developed gills or is completely devoid of them, and differs from other *B. sowerbyi* populations in the chaetal morphologies too (Ohtaka and Nishino, 1999). A recent study using molecular characters showed that the profundal Lake Biwa *B. sowerbyi* can be regarded as a distinct lineage (Lindström et al. unpublished). It is highly probable that the profundal population of *B. sowerbyi* has adapted to life in the constantly cool lake profundal environment. This is the only oligochaete endemic to the present Lake Biwa as well as the only authentic oligochaete specialized to profundal environment in Japan.

Acidity of lake water can also be an important factor regulating composition of the oligochaete fauna. Roff and Kwiatkowski (1977) studied acid tolerance of several oligochaetes in six lakes in Northern Ontario, and they found that *L. hoffmeisteri* was missing from lakes with pH lower than 5.5-6.6, being more sensitive than *Stylodrilus heringianus* and *Rhyacodrilus montana* whose tolerance reached as low as pH 4. Occurrence of *R. coccineus* as a single oligochaete representative in Lake Inawashiro (No. 20) could be related to the acidic condition of the lake water (pH 4.4-5.5; Tanaka 1992). Another acidic Lake Tazawa (No. 17) used be neutral and harbored oligochaetes throughout the depths (Miyadi 1932f; Uéno 1940), but since 1940 the lake water has become acidic, with pH 4.4-5.3, due to the artificial inflow of very acidic water from Tama-gawa River into the lake for irrigation and power generation (Tanaka 1992). Accordingly, the benthic macroinvertebrates, including oligochaetes, have disappeared since that time (Kitagawa 1978). *Tubifex (Peloscolex) nomurai* Yamamoto and Okada, 1940, was originally described from the deep profundal of L. Tazawa, but it had gone extinct there and the taxonomic status remain unknown (Ohtaka and Martin 2011).

Rhyacodrilus hiemalis has been recorded from six shallow lakes in central Japan with high productivity (Table 1), and summer aestivation in the deep layer of bottom sediments has been known in at least four lakes: Lakes Suwa (No. 33), Kasumi-ga-ura (No. 34), Kita-ura (No. 35) and south basin of Lake Biwa (No. 46) (Takada et al. 1992 under the name of *Rhyacodrilus* sp; Ohtaka 1995; Narita 2006). *Rhyacodrilus hiemalis* needs aerobic and cool bottoms with a temperature lower than 15 °C for growth and reproduction as well as abundant food for rapid growth (Ohtaka and Kikuchi 1997; Narita 2006) and such environments can be found only in shallow eutrophic lakes where stratification does not last long.

Naidines are generally diverse and prosper in lake littoral with aquatic vegetation, and not common in lake profundals. Among 11 naidine species recorded from profundal in Japanese lakes, seven species (*Specaria josinae*, *Uncinai uncinata*, *Nais communis*, *Slavina appendiculata*, *Vejdovskyella simplex*, *Stephensoniana trivandranana*, *Dero digitata*) were collected from the depth exceeding 50 m. These records were concentrated on the most frequently studied north basin of Lake Biwa (No. 45) where oxygen never disappears from the bottom. Diverse and abundant naidines have been recorded from profundal bottoms in the Finnish Lake Päijänne (Särkkä 1989). Among 8 naidines listed by Särkkä (1989), three species (*S. appendiculata*, *U. uncinata*, *D. digitata*) are common with the Japanese deep profundal naidines listed above.

Comparison with other regions

Timm (2012) demonstrated that the profundal oligochaete assemblage in the oligotrophic L. Kurilskoe in Kamchatka displays a remarkable parallel to those in European lakes. Oligochaete composition in Japanese oligotrophic lakes is different from those in the Western Palaearctic and Kamchatkan lakes, although counterpart species are also recognized. In the Scandianvian lakes, *S. heringianus*, *Rhynchelmis limosella*, *Spirosperma ferox*, *T. tubifex*, *Lamprodrilus isoporus* etc. are typical of oligotrophic lake profundals, *Psammoryctides barbatus*, *Limnodrilus profundicola*, *R. coccineus*, *B. vejdovskyanum* etc. are typical of mesotrophic lakes, and *Potamothrix hammoniensis*, *L. claparedianus*, *I. templetoni* and *Aulodrilus pluriseta* etc. are typical of eutrophic lakes (Milbrink 1978, 1983). Among them, occurrences of *Tubifex tubifex* in oligotrophic lakes, *L. profundicola* and *R. coccineus* in mesotrophic ones, *I. templetoni* and *A. pluriseta* in eutrophic ones are common with the Japanese lakes. On the other hand, several lumbriculids in European oligotrophic lakes such as *S. heringianus* are replaced by *S. japonicus* and *Y. toyensis*, and the papillate tubificine *S. ferox* is replaced by *E. nikolskyi* in Japanese oligotrophic lakes. No *Potamothrix* species have been recorded in Japan, whereas, in Japanese eutrophic or hypertrophic lakes *R. hiemalis*, *Aulodrilus* sp. and *Branchiura sowerbyi* occurred in place of *Potamothrix* in addition to great abundance of *L. hoffmeisteri*.

Long-term changes in profundal oligochaete assemblages

During these several decades, many Japanese lakes have been polluted and a part of them have been recovering. However, long-term succession in species composition of the profundal oligochaete communities

is mostly unclear due to absence of past studies, except for a few cases. Lake Biwa used to be oligotrophic, but it has suffered from anthropogenic eutrophication in past 40 years and a steady decrease in dissolved oxygen has been observed in the hypolimnion (Nishino and Ohtaka unpublished). As well as in Yamaguchi (1964), repeated surveys in Lake Biwa in recent decades could not find any lumbriculids and criodrilids (*Biwadrilus bathybates*) from the profundal bottoms, both of which were recorded in the earliest oligochaete research in 1915 (Stephenson 1917). This could have resulted from the environmental changes (Ohtaka and Nishino 2012). It has been widely recognized that as the trophic level rises, profundal oligochaete assemblages become simpler with some oxygen-tolerant species, while the density increases, and acceleration of eutrophication induces an anoxic bottom harboring no zoobenthos in the profundal. Increase in the profundal oligochaete density was recorded in many Japanese lakes such as the eutrophic Lake Yunoko (Ohtaka and Iwakuma 1993) and meso-eutrophic L. Kawaguchi (Hirabayashi et al. 2012), and this can be related to ongoing eutrophication. Among the Japanese lakes harboring oligochaetes throughout the depths in the past, azoic zones have been recorded in Lake Akan since 1970s (Kitagawa 1975; Ito and Uno 1980), L. Numazawa (present study), L. Ikeda since 1990s (Ohtaka et al. 2006) and L. Unagi (present study). In these lakes the last oligochaetes just before disappearing were *T. tubifex* and/or *L. hoffmeisteri*. In some lakes, for example L. Tazawa and L. Biwa, the water quality has been recovered recently, however oligochaete assemblages have not recovered accordingly, as reported in Lake Geneva (Lang 1985, 1998).

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Bathymetric distribution of aquatic Oligochaeta in Lake Kizaki, Central Japan

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Abstract

Bathymetric distribution of aquatic oligochaetes was studied at 8 stations with different depths (mean depth 16.7 ± 9.4 m, min. 5.3 m, max. 29.4 m) in July 2012 in mesotrophic Lake Kizaki, Nagano Prefecture, Central Japan. The average density was 10424 ± 5346 individuals m^{-2} . Oligochaetes were numerically dominant at all sampling stations. High density, sometimes more than 10000 individuals m^{-2} , was recorded at 3 stations (St. 4, 5, and 6) deeper than 18 m. Maximum density was 20561 ± 13864 individuals m^{-2} at Station 4 (depth 29.4 m, ignition loss of sediment 13.5%, bottom water temperature 8.1 °C and DO of bottom water 0.12 mg l^{-1}). We identified a total of 5 genera and 7 species belonging to three subfamilies: i.e., 1 species of Naidinae, 2 species of Rhyacodrilinae and 4 species of Tubificinae. Dominant species (in order of abundance) were *Tubifex tubifex* (Müller, 1774) (8550 ± 6317 individuals m^{-2} ; 82.0%) and *Limnodrilus* spp. (*L. hoffmeisteri* Claparède, 1862 and *L. claparedeianus* Ratzel, 1868) (1710 ± 1383 individuals m^{-2} ; 16.4%). *Tubifex* and *Limnodrilus* were obtained from every station, and the density of *T. tubifex* tended to be higher as the depth became greater, while *Limnodrilus* spp. was also widely distributed, but with a peak value at a depth of 5.3 m in the shallowest station. From the submerged plant zone, *Ophidonais serpentina* (Müller, 1773) was collected. An earlier study by Hirabayashi & Hayashi (1994) showed that in 1985 the average density of aquatic oligochaetes was 435 ± 428 individuals m^{-2} , oligochaetes dominated in only four locations and were distributed over the whole lake approximately equally. Comparisons of our data with the earlier studies have shown that oligochaete density has increased greatly according to Hirabayashi & Hayashi (1994). We suggest that a decrease in dissolved oxygen concentration of bottom water was due to an increase in organic matter content of the sediment. As a result, *T. tubifex* could expand their habitats in the profundal zone.

Key words: aquatic Oligochaeta, density, bathymetric distribution, Lake Kizaki, *Tubifex tubifex* (Müller, 1774)

Introduction

It has been reported that aquatic Oligochaeta play an important role in the material flows in lake ecosystems, especially the detrital food chain (Brinkhurst & Jamieson 1971). In Japan, however, there have been few ecological studies on aquatic Oligochaeta in lakes up to the present, because of the difficulty of species identification.

In Lake Kizaki, Miyadi (1931) conducted a descriptive study of aquatic Oligochaeta. Kitagawa (1973) and Hirabayashi & Hayashi (1994) reported on the horizontal distribution of total aquatic Oligochaeta. However, their studies left something to be desired from the ecological point of view. Recently, Hirabayashi *et al.* (2007) reported the seasonal changes in depth distribution of aquatic oligochaeta in the southern part of Lake Kizaki. They indicated that the dominant species were *Tubifex tubifex* (Müller, 1774) (9737 individuals m^{-2} ; 81.0%) and *Limnodrilus* spp. (1876 individuals m^{-2} ; 15.6%). *T. tubifex* was obtained from all three investigation stations, and its density tended to be higher as the depth was greater. However, Hirabayashi *et al.* (2007) only collected the samples at three different depth sampling stations in the southern part of Lake Kizaki. Thus, the purpose of this study is to clarify the characteristics of oligochaetes and their depth distribution throughout Lake Kizaki.

Materials and methods

Study site

Lake Kizaki is located at the eastern foot of Japan's Northern Alps (764 m above sea level) along the tectonic zone or Fossa Magna which traverses the main island (Honshu) of Japan (36° 33' N, 137° 50' E). It has an area of 1.4 km², a maximum depth of 29.5 m, and a mean depth of 17.9 m. There are two main inflowing rivers, the Nakanogu and Inaosawa, while the Shimonogu is the only river flowing out of the lake's southern end (Fig. 1). The lake's eastern shore is partly surrounded by cultivated land. Villages occupy its northwestern and southern shores. The lake is covered by ice from late January to mid-March with a relatively stable summer thermocline.

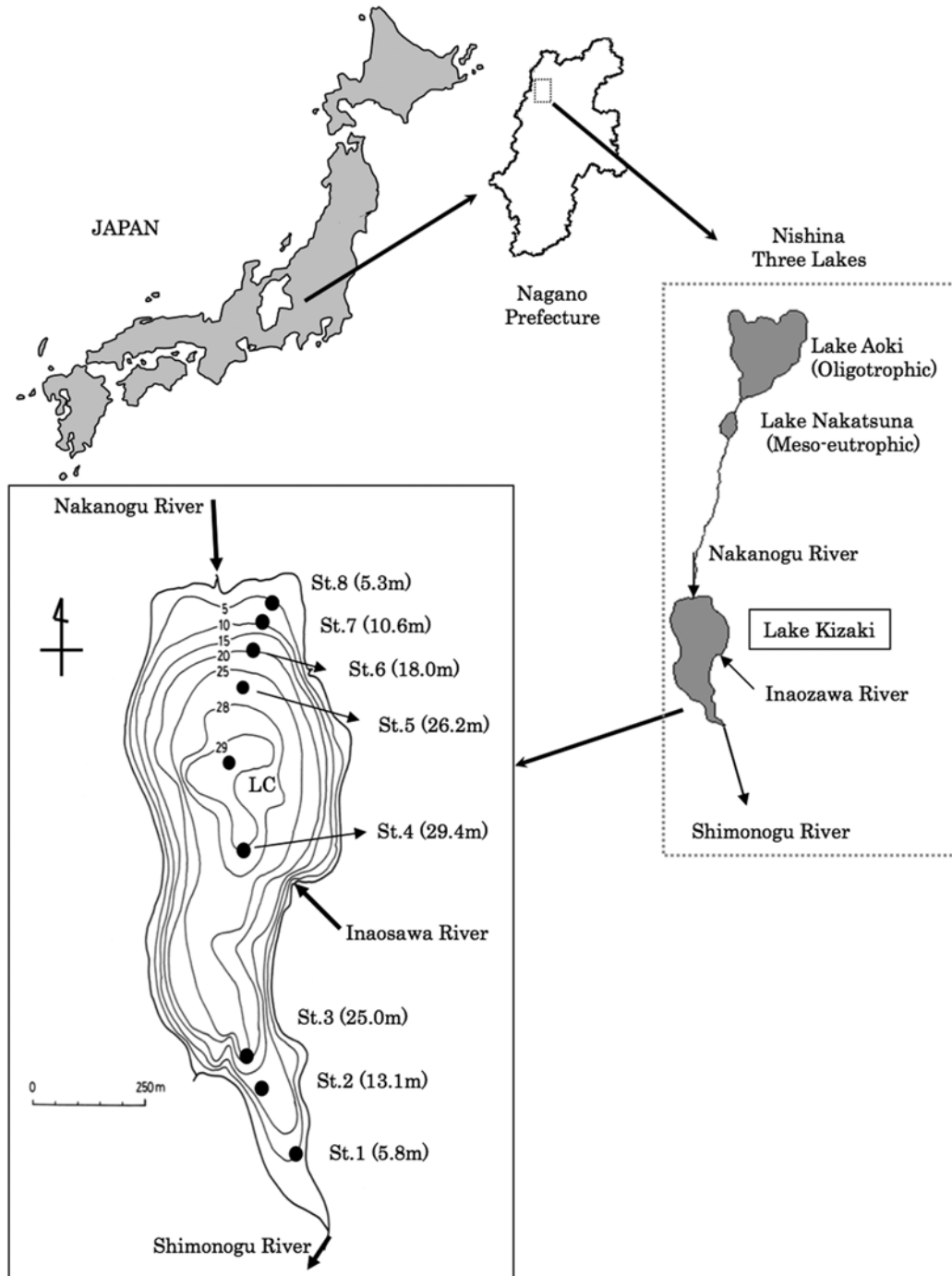


FIGURE 1. Maps showing location of Lake Kizaki, isopleths of depth (m), and sampling stations in the lake.

Many limnological studies have been done since the first major work by Tanaka (1930). Transparency averaged about 5 m until 1950 (Saijo 1983), but had decreased to approximately 3 m by 1970 (Hayashi 1989). From the end of the 1970's to the 1980's the biota and the environment of the lake changed drastically (Hayashi 1990). Since the end of the 1970's, *Anabaena* has bloomed annually in July-August (Watanabe *et al.* 1985), and the changes in water coloration have increased in frequency due to an abundance of microorganisms which graze upon *Anabaena* (Funakoshi *et al.* 1985). In the autumn of 1987, the dinoflagellate *Peridinium bipes* grew explosively, forming a freshwater red tide (Park & Hayashi 1993). Aizaki *et al.* (1981) ranked this lake as mesotrophic, using the modified Carlson's trophic state index based on chlorophyll-a, total phosphorus, and transparency. Such an alteration of the lake environment must have caused some changes in the bottom fauna, especially dominant Chironomidae and Oligochaeta (Hirabayashi & Hayashi 1994; Hirabayashi *et al.* 2007).

Collection of Oligochaeta

A survey of Oligochaeta was carried out on 13 July 2012, using a standard Ekman-Birge grab (15 x 15 cm). Three replicate sediment samples were taken at each of 8 sampling sites in Lake Kizaki (5.3–29.4 m depth; Fig. 1). The sampling points were determined with a Global Positioning System (GPS). After sieving the sediment through a Surber net (NGG 66: 250 μm mesh size), Oligochaeta were arranged against a white and / or black background using a binocular microscope (magnification 10 x and 20 x) and put into a glass bottle with 10% formaldehyde solution. They were used for species identification, and the individual numbers of each species were counted and their wet weight measured. For species identification, Amman's lactophenol preparation method (Brinkhurst 1971) was adopted. They were identified using the keys of Brinkhurst (1971), and Kathman & Brinkhurst (1999). In addition, chironomid and chaoborid larva were picked up and counted in the laboratory.

Physical environmental factors

Bottom sediment samples for organic matter content analysis were collected with a core sampler (three cm inner diameter) at each sampling site. The mud in the upper three-cm layer of each core was oven-dried at 110 °C for 2 days and ignited in a muffle furnace at 550 °C for three hours to determine the ignition loss (IL). The core sampler was also used to measure dissolved oxygen concentrations (DO) and water temperature in the water at the mud-water interface. The water near the mud surface in the core sampler (which remained above the sediment in the core sampler when it was pulled from the water) was siphoned carefully into a glass bottle. The dissolved oxygen concentration was measured with the Winkler method with azide modification. The water temperature (WT) also was measured, using a thermistor thermometer. Correlations between environmental variables such as depth, IL, WT and DO and oligochaetes densities were examined with the Kendal rank correlation test, utilizing a computer program package (NAP Ver. 4, Igaku-Shoin, Tokyo, Japan).

Results

Physical environmental factors

Table 1 shows the physical environmental factors in the bathymetric sampling survey in Lake Kizaki, 13 July 2012. The bottom water temperature ranged from 6.8 (St. 3) to 22.3 °C (St. 8). In sites shallower than 6 m, i.e., at St. 1 and 8, the bottom water temperature was over 20 °C. On the other hand, in sites deeper than 20 m, it ranged below 8 °C. The dissolved oxygen concentrations in bottom water ranged from 0.1 (St. 4) to 7.7 mg l⁻¹ (St. 7). In sites shallower than 6 m, the dissolved oxygen concentrations were over 7.0 mg l⁻¹ and in sites deeper than 25 m they were below 3 mg l⁻¹. The values of loss on ignition of the upper 3 cm of the sediment taken from 8 locations ranged from 1.2% (St. 1, sand) to 14.8% (St. 5, mud). Most of the lake basin consisted of soft bottom with organic matter contents higher than 10%. Sediments at St. 5 contained the highest levels of organic matter (14.8%) of all the stations.

TABLE 1. Physical environmental factors in the bathymetric sampling survey in Lake Kizaki, 13 July 2012.

| Station No. | Depth (m) | Bottom water Temp. (°C) | D.O. (mg/L) | Ignition loss (%) | Remarks |
|-------------|-----------|-------------------------|-------------|-------------------|----------------------------------|
| 1 | 5.8 | 20.3 | 7.7 | 1.2 | Sandy mud, <i>Elodia nuttari</i> |
| 2 | 13.1 | 9.9 | 6.9 | 11.3 | Mud |
| 3 | 25 | 6.8 | 2.5 | 14.5 | Mud |
| 4 | 29.4 | 7.9 | 0.1 | 13.5 | Mud |
| 5 | 26.2 | 7.6 | 0.8 | 14.8 | Mud |
| 6 | 18 | 9.7 | 5.4 | 14.5 | Mud |
| 7 | 10.6 | 12.1 | 6.9 | 10.1 | Mud |
| 8 | 5.3 | 22.3 | 7.4 | 1.8 | Sandy mud, <i>Elodia nuttari</i> |

Mean values and standard deviations of the densities of Oligochaeta

In our study, based on environmental factor observation (Table 1) and recent oligochaete observations (Hirabayashi *et al.* 2007), we divided the lake into two zones: a littoral zone covered with submerged plants, *Elodia nuttallii*, (6 m <) and another area (> 6 m). A list of the benthic macroinvertebrates (chironomid and chaoborid larvae and oligochaetes) collected at 8 stations in Lake Kizaki on 13 July 2012 is presented in Table 2. The average densities of chironomid and chaoborid larvae and oligochaetes for all the stations were 2261 ± 1823 (17.8%), 20 ± 25 (0.2%) and 10424 ± 5346 (82.0%) individuals m^{-2} , respectively. Chironomid larvae and oligochaetes were collected at all 8 stations, but chaoborid larvae were not collected at Stations 2, 6 and 8. The highest Oligochaeta densities were measured at St. 4 (20561 ± 13864 individuals m^{-2}) and the fewest were collected at St. 1 (3422 ± 2252 individuals m^{-2}) with an approximate ratio of 6 : 1. We identified a total of 5 genera and 7 species belonging to three subfamilies, i.e., 1 species of Naidinae, 2 species of Rhyacodrilinae and 4 species of Tubificinae. The most abundant genus was *Limnodrilus* (3 species). In this study, aquatic Oligochaeta in Lake Kizaki were dominated by two species (in order of abundance); *T. tubifex* (8550 ± 6317 individuals m^{-2} ; 82.0%), *Limnodrilus hoffmeisteri* Claparède, 1862 and *Limnodrilus claparedeianus* Ratzel, 1868 (1710 ± 1383 individuals m^{-2} ; 16.4%). In *L. hoffmeisteri* and *L. claparedeianus*, immature worms are difficult to distinguish; they are shown as *Limnodrilus* spp. in Table 3. These two groups accounted for 98.4% of the overall total individual numbers. Seven species were present at St. 1 and 8 (< 6 m), making these the stations with the highest number of species, against only 2 species from St. 2 to 7 (> 6 m). Less than 6 m depth, *Limnodrilus* spp. (3755 ± 1233 individuals m^{-2} ; 84.1%) were dominant, while *T. tubifex* was dominant at more than 6 m (> 6 m) (11382 ± 4167 individuals m^{-2} ; 91.7%). *T. tubifex* and *Limnodrilus* spp. were common to all of the stations. On the other hand, the other 4 species were only collected at St. 1 and 8.

The population density of each Tubificidae species differed among the sampling stations. As shown in Figure 2, the bathymetric distribution of mean densities (\pm SD) of the total oligochaetes, *T. tubifex* and *Limnodrilus* spp. in Lake Kizaki was measured on 13 July 2012. Oligochaetes were abundant at all stations with a peak value at 29.4 m at the deepest station (St. 4). *T. tubifex* was widely distributed, with a peak value at the deepest station. *Limnodrilus* spp. was also collected from all stations, but with a peak value at a depth of 5.3 m in the shallowest station.

Table 3 presents the correlation matrix of the densities of total oligochaetes, *T. tubifex*, and *Limnodrilus* spp., as well as the environmental factors. The densities of *T. tubifex* exhibited a positive correlation with water depth and ignition loss, and a negative correlation with bottom water temperature and dissolved oxygen concentration. The density of *Limnodrilus* spp. exhibited a positive correlation with bottom water temperature, and a negative correlation with ignition loss.

TABLE 2. Mean values and standard deviations (SD) of the densities and proportion of Oligochaeta, and chironomid and chaoborid larvae in Lake Kizaki, 13 July 2012.

| Tubificidae | < 6 m (St. 1 and St. 8) Mean densities (Ind. m ⁻²) | Proportion (%) | > 6 m (from St. 2 to St. 7) Mean densities (Ind. m ⁻²) | Proportion (%) | Total (all stations) Mean densities (Ind. m ⁻²) | Proportion (%) |
|------------------------------------|---|-------------------|---|-------------------|--|-------------------|
| Naidinae | | | | | | |
| <i>Ophidonais serpentina</i> | 360±118 | (8.1) | 0±0 | (0.0) | 90±172 | (0.9) |
| Rhyacodrilinae | | | | | | |
| <i>Bothrioneurum vejdovskyanum</i> | 266±88 | (6.0) | 0±0 | (0.0) | 67±128 | (0.6) |
| <i>Branchiura sowerbyi</i> | 1.5±0.7 | (0.03) | 0±0 | (0.0) | 0.4±0.7 | (0.004) |
| Tubificinae | | | | | | |
| <i>Tubifex tubifex</i> | 54±17.7 | (1.2) | 11382±4167 | (91.7) | 8550±6317 | (82.0) |
| <i>Limnodrilus hoffmeisteri</i> | 3755±1233 | (84.1) | 1028±377 | (8.3) | 1710±1383 | (16.4) |
| <i>Limnodrilus claparedeianus</i> | | | | | | |
| <i>Limnodrilus grandisetosus</i> | 27±8.5 | (0.6) | 0±0 | (0.0) | 6.8±13 | (0.07) |
| Others | 1.5±0.7 | -0.03 | 0±0 | 0 | 0.4±0.7 | -0.004 |
| Total mean oligochaete density | 4465±1467 | (100.0) | 12410±4543 | (100.0) | 10424±5346 | (100.0) |
| Total mean chironomid density | 2200±1267 | - | 2281±2033 | - | 2261±1823 | - |
| Total mean chaoborid density | 7±18 | - | 25±41 | - | 20±25 | - |

TABLE 3. Correlation matrix for environmental variables and densities of oligochaetes in Lake Kizaki on 13 July 2012, based on Kendal rank correlation test.

| | Depth | DO | WT | IL | Total Oligochaeta | <i>Tubifex tubifex</i> | <i>Limnodrilus</i> spp. |
|------------------------|-------|---------|---------|---------|-------------------|------------------------|-------------------------|
| Depth | - | -0.96** | -0.87** | 0.81* | 0.86** | 0.88** | -0.56 |
| DO | | - | 0.69 | -0.65 | -0.80* | -0.77* | 0.32 |
| WT | | | - | -0.98** | -0.72* | -0.85** | 0.88** |
| IL | | | | - | 0.72 | 0.81* | -0.76* |
| Total Oligochaeta | | | | | - | 0.97** | -0.43 |
| <i>Tubifex tubifex</i> | | | | | | - | -0.63 |

DO: dissolved oxygen concentration, WT: bottom water temperature, IL: ignition loss

Discussion

One Naidinae species, *Ophidonais serpentina* (Müller, 1773), was captured only at St. 1 and 8 (Table 2). This species is well known to inhabit an aquatic plant zone and live among macrophytes (Timm & Veldhuijzen van Zanten 2003). Ohtaka & Iwakuma (1993), and Ohtaka & Nishino (1995; 1999; 2006) reported this species was collected at a submerged plant area in Lake Yunoko and Lake Biwa, Japan. According to Nagasaka (2004), patchy *E. nuttallii* cover was observed around St. 1 and 8 in Lake Kizaki, and we collected *E. nuttallii* with the benthic samples. Higuchi *et al.* (2005) also reported high densities of *Vallisneria asiatica* Miki (< 2 m depth) and *Nitella flexilis* (Linnaeus) C. Agardh, 1824 were observed in the western and northern parts of Lake Kizaki, recently. *O. serpentina* might use these submerged plant body surfaces as a habitation place. Higuchi *et al.* (2005) also reported that the vertical growth limits of submerged plants was c.a. 9 m in Lake Kizaki and *E. nuttallii* mainly inhabited from 2 - 5 m and *N. flexilis* inhabited from 6 - 9 m (mixed in 5 - 6 m), so the distribution of *O. serpentina* would depend on the distribution pattern of submerged plants in Lake Kizaki. According to Hirabayashi *et al.* (2007), they described two Naidinae species, *Uncinais uncinata* and *Pristina aequiseta*, from the submerged plant zone, and *U. uncinata* was captured only in August and October, while *P. aequiseta* was captured during the winter (only in December and March). In our study, we did not collect these species. In regions shallower than St. 8 and 1, there were many kinds of submerged plants (Nagasaka 2004; Higuchi *et al.* 2005) which provided many types of habitats for Oligochaeta. Thus, it is necessary to investigate in more detail the distribution of aquatic Oligochaeta in shallower regions (<5 m) of this lake.

At St. 1 and 8 (shallower regions, < 6 m), *T. tubifex* comprised 1.2% of all oligochaetes against 91.7% from St. 2 to St. 7 (deeper regions, > 6 m) (Table 2). At the deeper regions (> 6 m) dissolved oxygen concentration decreased with increasing water depth. In particular, the oxygen concentration of bottom water fell below 1 mg l⁻¹ at St. 4 and 5 (Table 1). At these stations, *T. tubifex* was the most abundant species (Figure 2). Berg *et al.* (1962) reported that *T. tubifex* tolerated low oxygen concentration conditions: i.e., it was able to regulate its rate of oxygen consumption down to a concentration of only 0.6 mg l⁻¹. Thus, it might be that this species could expand their habitats in the profundal zone in Lake Kizaki.

We compared our results with those of Hirabayashi & Hayashi (1994), and tried to clarify the relationship between the change of the organic matter contents (IL) of the sediments and the densities of the total oligochaetes in Lake Kizaki. Hirabayashi & Hayashi (1994) showed that in 1985 the average density of aquatic oligochaetes was 435 ± 428 individuals m⁻², oligochaetes dominated in only four locations and were distributed over the whole lake approximately equally (> 20 m depth stations; IL was 12.0 ± 1.5%). In our results, the average density of aquatic oligochaetes was 10424 ± 5346 individuals m⁻², oligochaetes dominated at all stations (> 20 m depth stations; IL was 14.3 ± 0.7%) and oligochaete densities increased greatly (ca. 24 times) compared with those of Hirabayashi & Hayashi (1994). Brinkhurst (1974) reported that the organic

matter contents of sediments and the dissolved oxygen concentration near the bottom are the most important factors determining the abundance of oligochaetes in lakes. According to Newrkla & Wijegoonawardana (1987), oligochaetes are able to increase in number with increasing sediment organic matter content, mainly because such an increase results in an increased number of bacteria. These bacteria can play a role in transforming relatively refractory detritus into more nutritious form or can serve as a direct food for oligochaetes (Baker & Bradnam, 1976), that is, the dissolved oxygen was consumed by bacterial activity near the bottom. More recently, Hirabayashi *et al.* (2007) reported the dissolved oxygen concentration near the bottom decreased to 0.1 mg l⁻¹ from August to December at the center of lake (< 25 m depth). This may be why the oligochaete density, especially *T. tubifex*, in the lake, was about 24 times the level previously observed in 1985. We suggest that the decrease in dissolved oxygen concentration of bottom water was due to an increase in organic matter content of the sediment. As a result, *T. tubifex* could expand their habitats in the profundal zone.

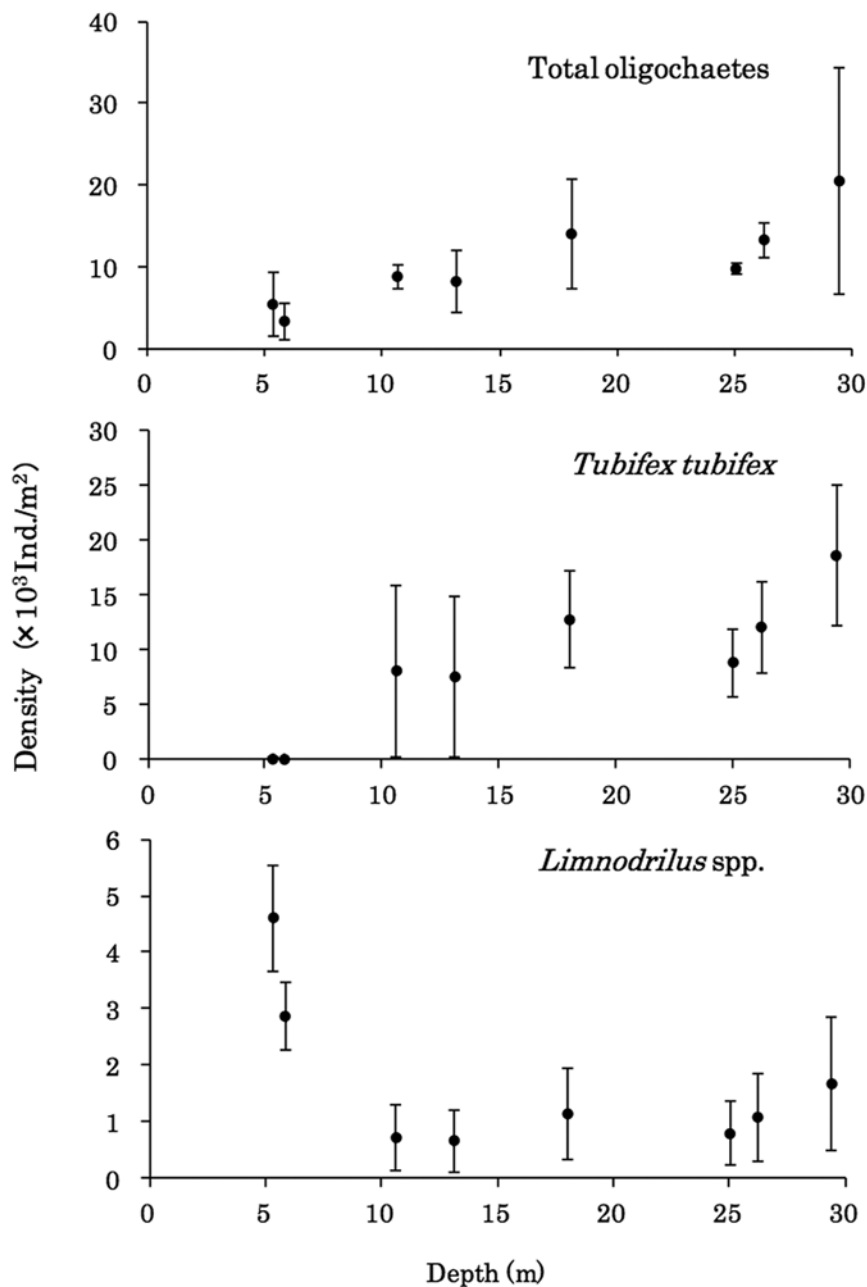


FIGURE 2. Bathymetric distribution of mean densities (\pm SD) of the total oligochaetes, *T. tubifex* and *Limnodrilus* spp. in Lake Kizaki, 13 July 2012.

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Toxicity and bioaccumulation of fluoride ion on *Branchiura sowerbyi*, Beddard, (Oligochaeta, Tubificidae)

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Abstract

Fluoride concentrations are increasing significantly in many aquatic ecosystems as a consequence of human activities (agrochemicals, pharmaceuticals, refrigerants, pesticides, surfactant compounds). Several investigations have revealed that sensitivity to fluorides and safe concentrations vary greatly within classes, families and genera. Aquatic oligochaetes have often been used for pollution assessment and accumulation testing, but no information has been given about tolerance to fluoride ion. Among endobenthic tubificids *Branchiura sowerbyi* is easily identifiable (evident posterior gills, large size) and particularly useful for tissue requirements in chemical analysis. The purpose of this study was to examine the tolerance of this tubificid to fluoride ion and its bioaccumulation capacity by performing short (LC50 96h) and long-term (18 day) experiments at different temperatures (17°C and 22 °C). LC₅₀ values (91.3 and 61.7 mg/L for 17°C and 22°C respectively), especially in the presence of sediment (267.6 and 80.1 mg/L for 17°C and 22°C respectively) showed that *B. sowerbyi* is more resistant to fluoride than other freshwater invertebrates. Fluoride became more toxic with increased temperature, demonstrating that seasonal temperature changes could influence the sensitivity of this freshwater tubificid. Bioaccumulation was lower when the organisms were exposed to sodium fluoride in the absence of sediment, indicating that this animal also accumulates fluoride by ingesting sediment.

Key words: *Branchiura sowerbyi*, Fluoride ion toxicity, Bioaccumulation, Temperature influence

Introduction

Natural water fluoride concentrations are mostly dependent on the occurrence of fluoride-bearing minerals in groundwater (Barbier et al. 2010). Fluorides also enter many water bodies via the waste products of anthropogenic activities, such as heavy industry and agriculture. As a result, the fluoride level in many aquatic ecosystems is increasing (Chlubek 2003). The effects of fluoride exposure have been revealed by several studies on many animal and plant phyla: Camargo's review (2003) focused on fluoride toxicity in aquatic organisms, revealing that safe concentrations are significantly different within classes, families, genera and species. The risks for vertebrates are known; most significant are the effects on bone cells that can lead to the development of skeletal fluorosis. However, it is now recognized that fluoride also affects soft tissues cells, such as renal, gonadal, and neurological cells (National Research Council 2006). The main toxic effect of fluoride in cells, involves its interaction with enzymes; it acts as an enzyme inhibitor and interrupts metabolic processes, such as glycolysis and protein synthesis (Kessabi 1984; Casellato et al. 2012). Aquatic invertebrates living in soft waters are more affected by fluoride pollution than those living in seawater, because the bioavailability of fluoride ions (and consequently their toxic action) is reduced with increasing water hardness (Camargo 2003). Various investigators have focused on fluoride toxicity in soft water organisms, but no data have been reported for aquatic oligochaetes, although they have a long history of use in pollution monitoring and in relevant toxicity and bioaccumulation tests (Chapman 2001). In particular, oligochaetes are widely used to assess the toxicity and bioaccumulation of sediment-associated contaminants (Ingersoll et al. 1995). Endobenthic tubificids are particularly appropriate because of their intimate contact with the substrate for their entire life cycle (Reynoldson 1987) and *Branchiura sowerbyi* can be easily

collected and identified due to their large size and prominent posterior gills (Ducrot et al. 2007). They are widely distributed and have already been used for both toxicity and bioaccumulation tests (Chapman et al. 1982; Casellato & Negrisolo 1989 Casellato et al. 1992; Marchese & Brinkhurst 1996; Bhunia et al. 2000) as they have a low natural mortality rate in the laboratory compared to other tubificids (Bonacina et al. 1994). These worms feed themselves down in the sediment, a behaviour that involves the intake of large amounts of substrate (Wang & Matisoff 1997). Since there are specific tissue requirements for chemical analyses, *B. sowerbyi* is more appropriate for bioaccumulation tests compared to other smaller oligochaete species (Chapman et al. 1980). *B. sowerbyi* dominates other bottom macroinvertebrates in terms of abundance and biomass (Ducrot 2007) and is a significant part of the diet of some fish and crustaceans. The main goal of this research is to provide missing information about the toxic effects of fluoride on *B. sowerbyi* and to investigate the bioaccumulation capacity of this species.

Materials and methods

A rich population of *B. sowerbyi*, living in a lily tank at the Botanic Garden, Padova University, has been monitored for a long time, in order to study its life cycle and the regeneration of the sexual apparatus (Casellato 1984; Casellato et al. 1987). The tank is filled with thermal water with temperatures ranging between 17°C and 22°C throughout the year. Original water and sediment (both with a concentration less than 0.1 mg F/L) were collected together with animals in order to set up an optimal laboratory culture and test conditions. The worms were gradually acclimatized to the test temperatures in glass aquaria (1L) under controlled oxygen conditions. All the experiments were performed at 17°C and 22°C in order to determine if temperature has an influence on fluoride toxicity and bioaccumulation rate. The aquaria were covered to prevent fluoride evaporation. Daily observations of mortality and differences in the animals' behavior and morphology were made. Worms were considered dead when there was no response to physical stimulation; dead individuals were removed and kept in 4% formaldehyde for accumulation analysis. At the end of the experiments, surviving animals were also put in formaldehyde and analyzed. Fluoride accumulation in *B. sowerbyi* soft tissues (dried at 80 °C for 24 h) and in the sediment was evaluated using the alkali fusion method, according to Malde et al. (2001) and McQuacker & Gurney (1977), respectively. Analyses were conducted at pH 5.5 after adding a total ionic strength adjustment buffer (TISAB III) and using an ion selective electrode ISE F800 (0.02 mg F/L to saturation, accuracy 0.1%, WTW).

Statistical analysis

An analysis of variance (ANOVA) was carried out to determine differences in bioaccumulation values (Software R ver. 2.15.0). Data relationships were assessed using simple linear regression. The daily mortality percentage was used to estimate LC₅₀ values at the 95% confidence level, using multifactor probit analysis (MPA; SAS software ver.9.1.3) (Lee 1991). Concentrations between the no observed effect concentration (NOEC) and the lowest observed effect concentration (LOEC) values, were noted and used to set up long term experiments.

Short-term experiments

Acute toxicity bioassays were performed at 17±0.5°C and 22±0.5°C, for 96 h. For each temperature 15 individuals (three replicates of five worms) were exposed to nominal sodium fluoride concentrations of 80, 160, 320 and 640 mg F/L, vs. control (tank water with no added fluoride), either with 125g of original sediment or without sediment.

Long-term experiment

Long term tests were carried out both at 17±0.5°C and 22±0.5°C, for a period of 18 days. All animals were exposed to nominal fluoride concentrations of 80, 120 and 160 mg F/L (between NOEC and LOEC) in the presence of sediment. Not only were mortality and behaviour checked in these experiments, but any decrease in water fluoride concentration was also noted. Each week, 10 ml of water were taken from each aquarium and combined with 10 ml of TISAB III for ion-selective electrode analysis. At the end of the experiment, fluoride content analysis was measured for both *B. sowerbyi* (as total body) and the sediment.

Results

Short-term experiments

B. sowerbyi demonstrated great tolerance to fluoride ion toxicity, especially at 17°C. The mortality of *B. sowerbyi* was reduced in the presence of sediment: LC₅₀ at 17°C was 91.3 mg F/L for animals in water without sediment, and 267.6 mg F/L when sediment was added. The same effect of presence of sediment was observed at 22°C (Table 1). The NOEC and LOEC for this species were 80 and 160 mg F/L respectively, when sediment was present. Animals treated at 22°C accumulated greater amounts of fluoride in their bodies than did those treated at 17°C. Sediment enhanced bioaccumulation values: at 22°C, animals exposed to 640 mg F/L had 1400.9 µg F/g when kept in sediment (Fig.1A), while those not kept in sediment had only 790 µg F/g (Fig.1B). The accumulation rate increased with increasing fluoride concentration (p<0.001, ANOVA). The animals treated with sediment accumulated double the amount of fluoride.

TABLE 1: LC50 values at 96 hours for *B. sowerbyi* at different temperature; 95% confidence limits are in parentheses.

| Temperature°C | 17 ± 0.5 | 22 ± 0.5 | 17 ± 0.5 | 22 ± 0.5 |
|--------------------------------|------------------------|----------------------|--------------------|---------------------|
| Sediment | yes | yes | no | no |
| LC ₅₀ (mg F/L) 96 h | 267.63 (257.75–277.51) | 80.07 (62.10–111.55) | 91.28 (84.5–98.05) | 61.68 (47.83–90.11) |

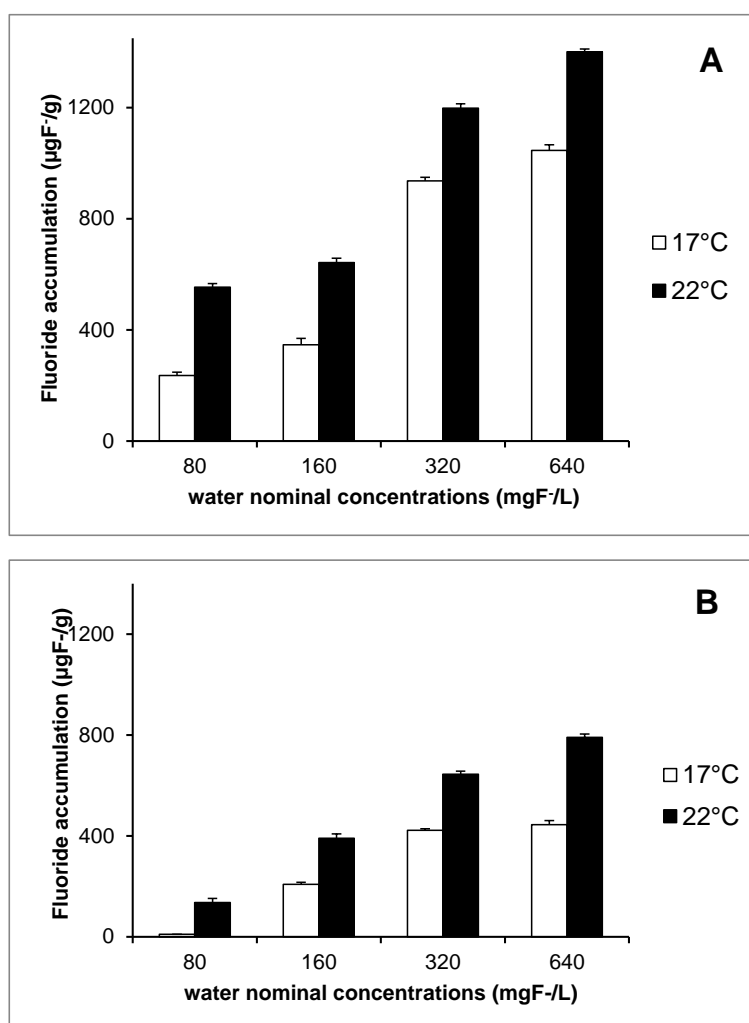


FIGURE 1: Fluoride accumulation in *B. sowerbyi* during short term experiments (96h) (A) With sediment (B) Without sediment.

Long-term experiment

The influence of temperature on fluoride toxicity in *B. sowerbyi* was evident in the long term mortality tests: 6.67% mortality was found for all concentrations tested at 17°C, whereas mortality was higher when the test population was exposed at 22°C and increased ($r^2 = 0.97$) with increasing fluoride concentration (Fig. 2). At 22°C mortality increased by 1.2% for every 10 mg F/L added ($r = 0.97$) and more than half the population died when exposed to the highest concentration (160 mg F/L) (Fig. 2).

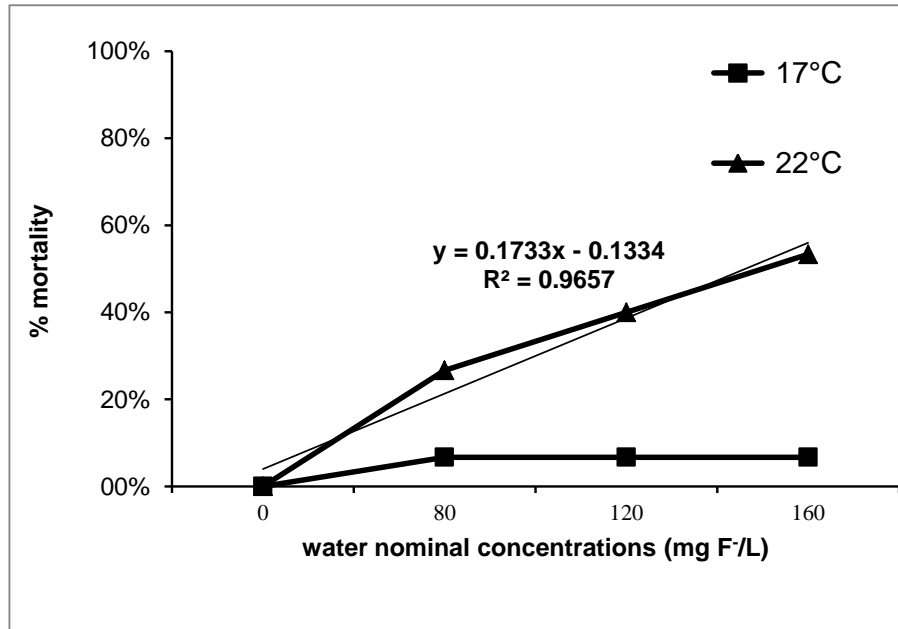


FIGURE 2: Mortality of *B. sowerbyi* during the long term experiments (18 days).

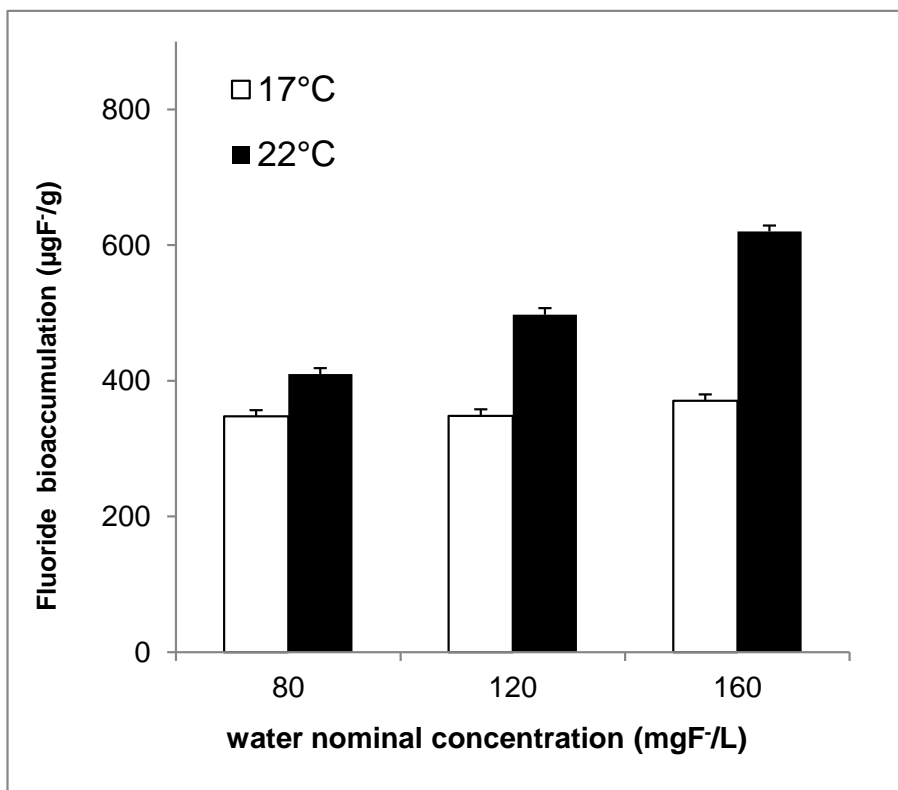


FIGURE 3: Fluoride accumulation in *B. sowerbyi* during the long term experiments (18 days).

Fluoride bioaccumulation showed a statistically significant relationship ($p < 0.001$) with increasing test concentration. The highest values were reached by test populations at 22°C (Fig.3); the exposure of *B. sowerbyi* to 160 mg F⁻/L resulted in accumulation at 22°C 40.25% higher than at 17°C. Sediment accumulation was directly proportional to test concentration ($p < 0.01$), with peak values of 722 and 815 µg F⁻/g, for experiments at 17°C and 22°C respectively (Fig.4). Fluoride ion was rapidly absorbed by animals and sediment, as shown by declining fluoride concentrations in water: the highest percentage decrease (26%) was reached in 6 d at 22°C from the initial nominal concentrations (Fig.5).

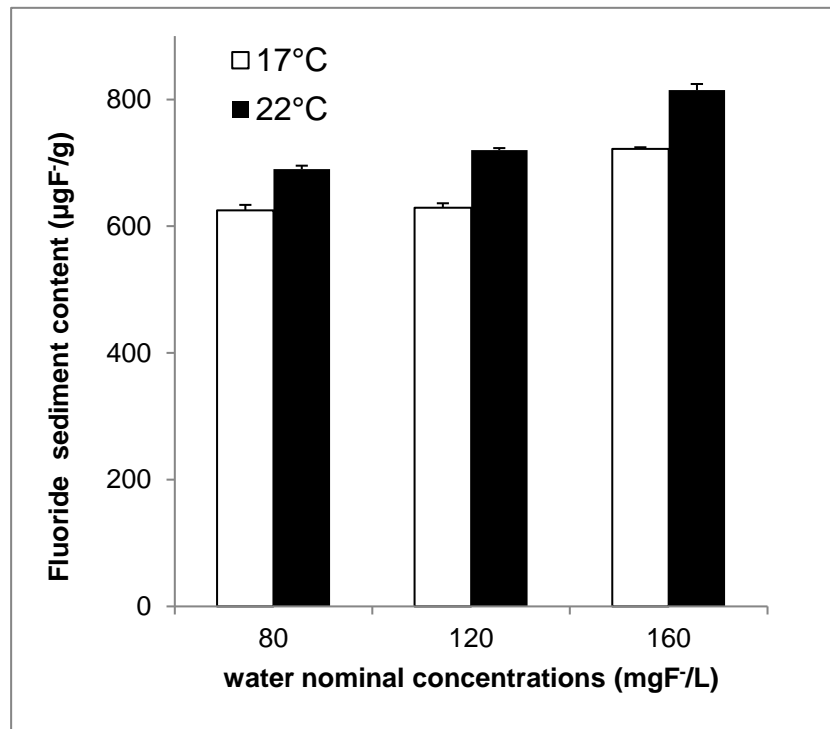


FIGURE 4: Fluoride content in sediment after the long term experiment (18 days).

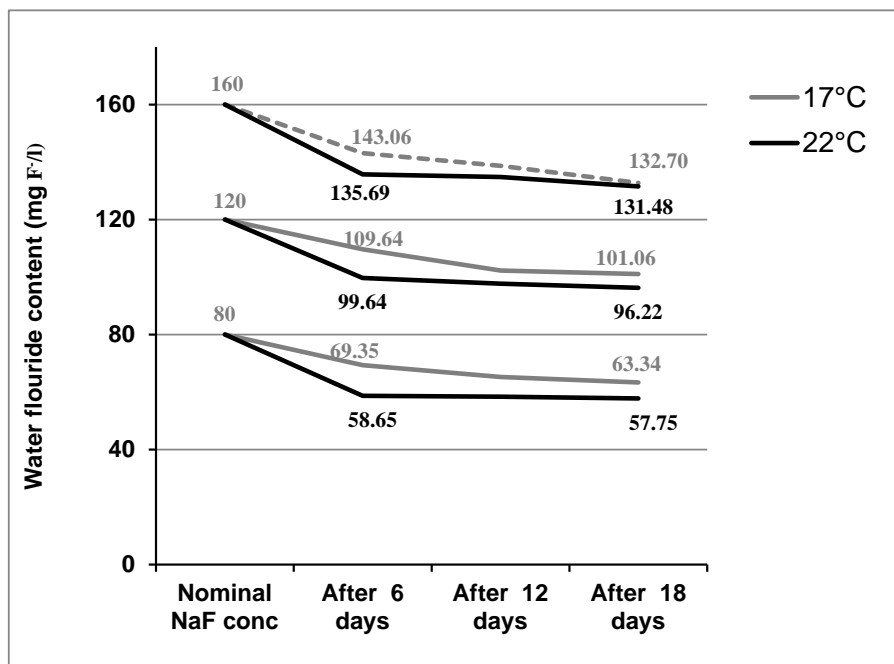


FIGURE 5: Decreasing concentration of fluoride in water during the long term experiment (18 days).

Discussion

This research clearly indicated that *B. sowerbyi* is very resistant to fluoride ion dissolved in water, especially in presence of sediment, much more than other aquatic invertebrates. Gonzalo et al. (2010) reported a much lower LC₅₀ value (5.8 mg/L at 17°C) for the gammarid *Dikerogammarus villosus*. Camargo (2003) summarized many literature results regarding fluoride toxicity in freshwater invertebrates, showing that the mean 96h LC₅₀ ranges from 10.5 mg F/L for some species of crustaceans to 45 mg F/L for the most resistant species of caddisfly (*Chimarra marginata*). *Branchiura sowerbyi* survival is two times higher compared to various species of *Hydropsyche* (Janice et al. 2003) and showed a 96h LC₅₀ value close to the 24 h LC₅₀ value of *Daphnia magna*, which is considered one of the less sensitive invertebrate species (Dave 1984). In addition, our results indicate a temperature –dependent effect: fluoride toxicity increases with increasing temperature, probably because the temperature increases the rate of metabolism, resulting in faster fluoride absorption and faster poisoning, as reported by Angelovic et al. (1961).

A similar effect was recently described for the mollusc *Dreissena polymorpha* (Del Piero et al. 2012).

A further consideration with respect to the presence or absence of sediment in the bioassays is that the resistance of *B. sowerbyi* during short term exposure was enhanced by the presence of sediment, probably because this presence prevents an additional stress for the tubificid worm, which is a limicolous species, feeding on sediment. In his “*ad libitum*” feeding test involving *B. sowerbyi*, Ducrot (2007) found that part of the total amount of the food ingested by this worm is the sediment itself. Moreover, Casellato & Negrisola (1989), Chapman et al. (1982) and Naqui (1973), using various toxicity bioassays, demonstrated that sediment reduces the bioavailability of the contaminants, limiting the toxic effects on organisms exposed to pollutants dissolved in water. But this is true for *B. sowerbyi* only in the short term bioassays. When the duration of the test was extended to 18 days, the prolonged feeding on the contaminated sediment increased fluoride bioaccumulation, particularly at 22°C (Fig.3). Lee et al (2000); Volparil & Mayer (2000); Weston et al (2000) suggested that the contaminated sediments ingested by deposit-feeding animals should be considered the major source of absorbed contaminants.

Thus, fluoride absorption through ingestion could explain the difference in the bioaccumulation values between the performed bioassays with and without sediment, both at 17°C and 22°C (Fig1).

Finally, as far as we know, no study has been previously conducted to examine fluoride bioaccumulation in deposit-feeding freshwater invertebrates at different times and exposures. Our research on *B. sowerbyi*, an important component in terms of abundance and biomass of the diet of some fish and crustaceans, is not exhaustive but can represent a first contribute to evaluate properly capacity of an invertebrate species to take up and retain fluoride ions from the environment.

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Effects of sodium fluoride on the gametogenesis of the tubificid oligochaete *Branchiura sowerbyi* Beddard

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Abstract

Fluoride concentration is significantly increasing in many aquatic ecosystems because of human activities (agrochemical, pharmaceutical, refrigerant, pesticide and surfactant compounds). This study aims to examine the effects of fluorides on gametogenesis by exposing *Branchiura sowerbyi* (Oligochaeta) to sodium fluoride concentrations 50 to 100 times above normal ones in freshwater (0.1–0.3 mg/L) and observing the effects over time. Because non-toxic concentrations determined in short-term experiments could induce effects as exposure times increase, *B. sowerbyi* were exposed to sodium fluoride from the end of February 2012 to the end of July 2012. They were continuously monitored until the cocoon deposition stage to investigate any possible effects. Weekly observations were conducted on treated and untreated specimens to monitor gamete maturation in both male and female germinal lines. The results revealed temporal differences in the gametogenesis phases of treated specimens, such as incomplete spermatozoa maturation. At the end of the experiment, the exposed animals were thinner and shorter than the controls and cocoon deposition did not occur.

Key words: fluoride effects, gametogenesis, *Branchiura sowerbyi*, freshwater oligochaete

Introduction

Many authors have previously studied the life cycle and gametogenesis of *Branchiura sowerbyi* and the organism's reaction to xenobiotic exposure (Aston 1966, Casellato 1984; Casellato et al. 1987; Casellato et al. 1992; Casellato & Negrisolò 1989; Hirao 1973; Lobo & Alves 2011; Naqui 1973; Reynoldson et al. 1991). Fluoride (F⁻) concentration is significantly increasing in many aquatic ecosystems as a consequence of human activities. These activities include the manufacture and use of agrochemicals, pharmaceuticals, refrigerants, pesticides and surfactant compounds. The main goal of this study is to examine the effects of fluorides on gametogenesis by exposing *B. sowerbyi* to fluoride concentrations in water 50 to 100 times above normal (0.1–0.3 mg/L) and observing the effects over time. A recent study by Del Piero et al. (in this issue) showed that *B. sowerbyi* is more resistant to fluoride than other freshwater invertebrates (Camargo, 2003; Gonzalo et al., 2010; Del Piero et al., 2012), especially in the presence of sediment. These studies indicated NOEC (no-effect concentration) and LOEC (lowest effect concentration) values to be between 80 and 120 mg/L for a short exposure time (96 h).

Considering that non-toxic concentrations in short- and medium-term experiments could induce effects as exposure time increases, numerous *B. sowerbyi* were exposed to fluoride concentrations of 15 mg/L and 30 mg/L (lower than the NOEC), and were monitored weekly beginning late February 2012, to investigate the possible effects of fluoride on male and female gamete maturation.

Materials and methods

At the end of February, many *B. sowerbyi* specimens were collected in a water lily tank in the Botanical Garden of Padua University. The tank was filled with thermal water that was maintained at a temperature range

between 17°C and 24°C throughout the year. The period of late winter was chosen for the beginning of the experiment because the sexual apparatus of *B. sowerbyi*, which degenerates in mid-summer after cocoon deposition, partially regenerates in the following autumn when the ovaries and testes increase in size and show clear signs of germ cell renewal. The organisms are without a clitellum during this period. At the beginning of winter, the testes and ovaries undergo a quiescence period for about two months and normal gonad activity resumes with an increase in testis and ovary size at the end of winter (Casellato 1984; Casellato et al. 1987). In this period it is easy to distinguish the two-year-old specimens (9–12 cm long) with a completely regenerated sexual apparatus from the one-year-old specimens (5–6 cm long) that will reach maturity for the first time. Only animals of the first cohort (the larger specimens) were considered for the experiments.

Small aquaria (10 L) were prepared to mimic the conditions of the original sampling site. These conditions included using the original water at its normal temperature range, the original sediment at a depth of 8 cm, and the original population density. Numerous specimens ($n = 50$) were reared and exposed to water containing either 15 or 30 mg/L of F^- (prepared with NaF solution), which was renewed weekly, and compared with a control without fluoride. We chose to perform the experiment at $23 \pm 1^\circ\text{C}$ because this temperature is optimal for gamete maturation. The treated and untreated specimens were checked weekly to monitor gamete maturation in both male and female germinal lines. To observe gamete maturation, individual samples were numbed with drops of 70% alcohol and segments 5 to 17, which contain the entire sexual apparatus, were dissected using micro-scissors. The sexual apparatus was placed on a slide and gently compressed with a coverslip to extract germinal cysts from the coelomatic fluid, which could be observed outside the body wall. In this manner, cytomorphological changes during the transformation of spermatogonia to mature spermatozoa, and of oogonia to mature eggs could be observed under a light microscope (Leica Application Suite software with a Leica HQ DFC480 camera) over the course of five months. This method was used to observe changes in the entire sexual apparatus.

The animals were exposed to the nominal fluoride concentrations in the water, which were maintained by weekly renewal for all treatment periods. Moreover, fluoride accumulation in the sediment was periodically tested because it was important to quantify the real exposure level of the endobenthic animals to the substance over time. The fluoride concentration was determined from aliquots of water from the aquaria at the beginning of the experiment, and before every weekly renewal, using an ion-selective electrode ISE F800 (0.02 mg/L to saturation, accuracy 0.1%). The sediment was also monitored by taking 0.5 mg samples and analysing them using the alkali fusion method as described by McQuaker & Gurney (1977).

During the experiment the temperature of the aquaria was continuously monitored and the data showed an almost constant temperature during the 5-month period ($24 \pm 1^\circ\text{C}$). Chemical analyses were conducted periodically (monthly) during the experiment to ensure optimal fluoride content in the water and sediment, and to measure organic matter content and dissolved oxygen concentration.

Results

By monitoring the F^- concentration range in the water tanks during the experiment, variations from 15 mg/L to 19 mg/L, and from 30 mg/L to 39.56 mg/L, respectively, were observed throughout the week, suggesting a portion of the fluoride was absorbed by the sediment and then released back into the water. The fluoride concentration in the sediment increased from an initial value of 2.59 ppm to 95.87 ppm in the tank filled with 15 mg/L of fluoride, and reached 122.01 ppm in the tank with 30 mg/L of fluoride. This observation indicates that the animals were exposed to a higher concentration of fluoride than the nominal value in water during the experimental period. The amount of dissolved oxygen varied between 3.9 mg/L and 5.3 mg/L, which corresponded to an average saturation percentage of 68% at 25°C. The mean organic matter content never exceeded 4%, which is consistent with the original sampling site.

When *B. sowerbyi* individuals (average length 9–12 cm) were first exposed to fluoride at the end of February, they revealed normal gonad activity, indicated by increased volume in the testes and ovaries, as well as by the appearance of 4–8 spermatogonial cysts in the sperm sacs and large oogonia in the ovaries (Fig. 1). None of the specimens had developed a clitellum yet, which only appeared after a month of fluoride exposure in both treated animals and controls. At the beginning of March, the first 16-cell spermatogonial cysts (22 μm) (Fig. 2A) appeared in the control specimens, which was a month before than in the treated animals. On the contrary, free oocytes in the coelom (51 μm long) were found in all treated and untreated specimens at the



FIGURE 1: Large peripheral oogonia in the ovary, sign of germ cell renewal.

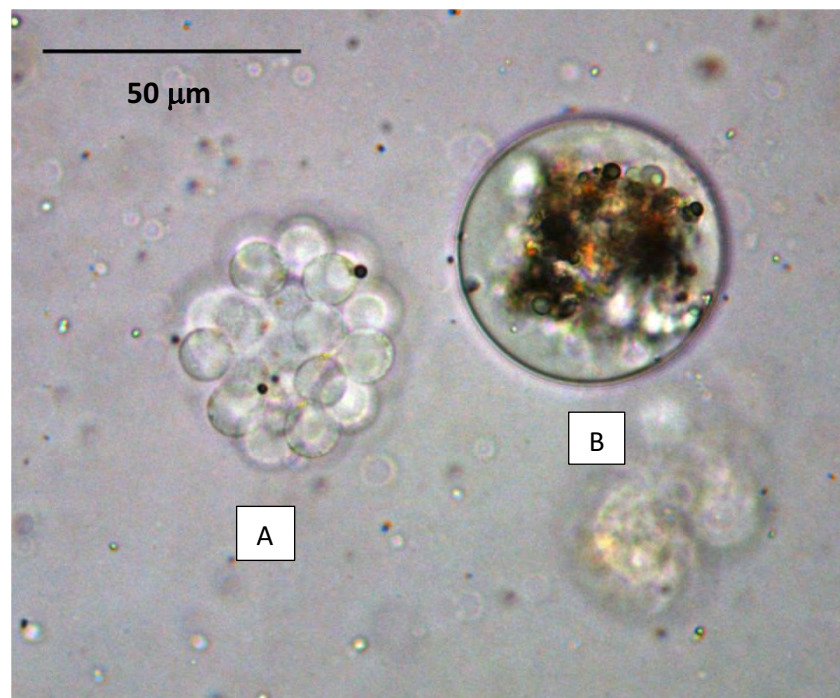


FIGURE 2: Spermatogonial cysts of 16 cells (A) and a free oocyte in coelomatic cavity (B).

beginning of March (Fig. 2B). Free oocytes appearing greatly enlarged (56 μm), showed more englobing and more yolk globules in their cytoplasm (Fig. 3). The first 32-cell spermatocyte cysts (42 μm) (Fig. 4) appeared at the beginning of April in the controls, but were a month late in the specimens treated with either 15 mg/L or 30 mg/L of fluoride. The first mature eggs were observed in both the controls and the specimens treated with 15 mg/L of fluoride (Fig. 5), but were observed a month later for those treated with 30 mg/L of fluoride. The development of the male germinal line occurred normally in controls. The 64-cell spermatocytes and the 128-cell spermatids cysts (Fig. 6) were present in large numbers in the coelom cavity. At the beginning of June, free spermatozoa were present in some of the controls (Fig. 7). However, the first spermatid cysts were

observed a month later in the treated specimens, and presented certain deformities (Fig. 8). There were also no free spermatozoa found in these specimens. At the end of May, mature sperm could be observed in the seminal funnels of the control specimens and a month later, spermatozeugmata were found in the spermathecae of these animals, which confirmed they had copulated (Fig.9). These structures have been described for other tubificid species (Braidotti & Ferraguti 1982; Ferraguti et al. 1989). Mature spermatozoa and spermatozeugmata were never observed in the treated specimens, which also presented fewer mature eggs when compared with the controls.

At the end of the experiment, the treated animals appeared to be shorter and thinner than the controls.



FIGURE 3: Free oocyte in advanced stage of maturation.

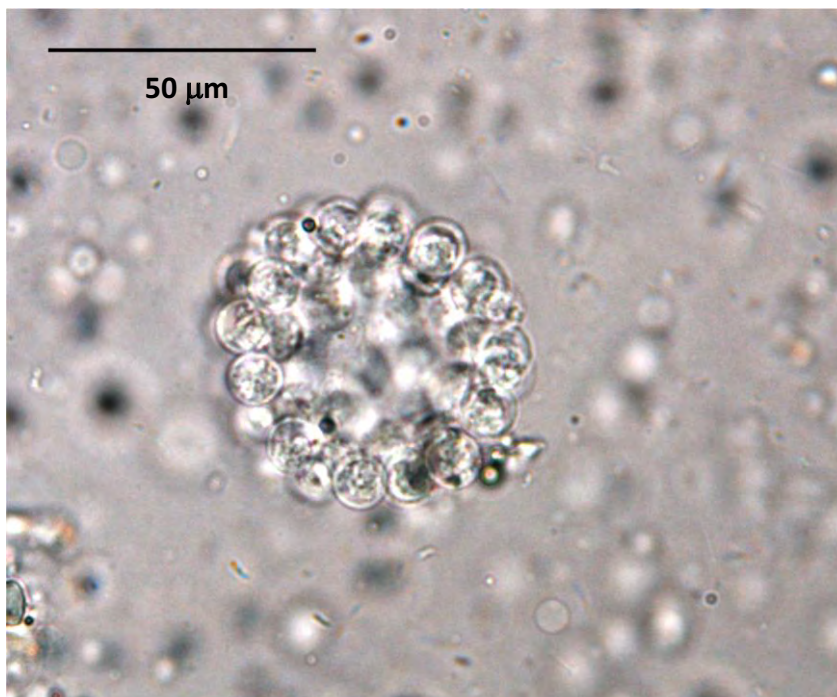


FIGURE 4: 32 cell spermatocyte cyst in a control individual.

Discussion and conclusions

The experiment indicated that prolonged exposure to fluoride damages the gametogenic cycle, particularly the spermatogenic cycle, in *B. sowerbyi*, even if the animals are exposed to concentrations that do not show an effect in the short- or medium-term. These results revealed temporal differences in the gametogenic phases of treated specimens when compared with controls. Treated animals did not complete spermatozoan maturation in either the 15 or 30 mg/L NaF concentrations, and the number of mature eggs produced was greatly reduced. The male line stopped maturation at the spermatid stage, which appeared abnormal and regressed. Moreover, the treated animals were thinner and shorter than the controls at the end of the experiment and cocoon deposition did not occur.

No previous studies have been performed to study the effects of fluoride on aquatic Oligochaeta. Only data for some mammals are available in the literature. For example, El-Iethey et al. (2011) demonstrated that the relative weights of reproductive organs were lower in adult rat males that were administered sodium fluoride (NaF). Histopathological examination showed degenerative changes in the testes, seminal vesicles and prostate glands of NaF-exposed males. The severity varied according to the dose, which was administered in the drinking water (0.50 ppm to 100 ppm). Other studies have reported delayed maturation of male cells in relation to testicular oxidative stress, with decreased testicular testosterone in the plasma of fluoride-exposed rats when compared with controls. Ghosh et al. (2002) and Bataineh & Nusier (2006) investigated the effects of sodium fluoride on adult male rats that ingested the substance in drinking water (100 ppm and 300 ppm) for 12 weeks. The rats' spermatozoa showed decreased motility and density, and females impregnated by these males showed increased foetal resorption. Susheela & Kumar (1991) administered 10 mg of fluoride per kg of body weight in rabbits for 29 months and observed the disruption of spermatogenic cells in the seminal funnels. Reddy et al. (2007) indicate that exposure to NaF during gestation and lactation affects the reproduction of male rats born from treated females by decreasing spermatogenesis and steroidogenesis. Other authors (Elbetieha et al. 2000; Inkielewicz-Stepniak & Czarnowski 2010; Kour & Singh 1980; Jhala et al. 2004) report abnormal development of both male and female germinal lines in animals treated with fluoride, which confirms that NaF can adversely affect fertility in mammals. Other published studies report that fluoride can induce oxidative stress and modulate intracellular redox homeostasis, lipid peroxidation and protein carbon content, as well as alter gene expression and cause apoptosis (Ahotupa & Huhtaniemi 1992; Barbier et al. 2010; Casellato et al. 2012). These data may help explain the anomalies observed in the gametogenic processes of *B. sowerbyi* that were exposed to NaF for a long time.

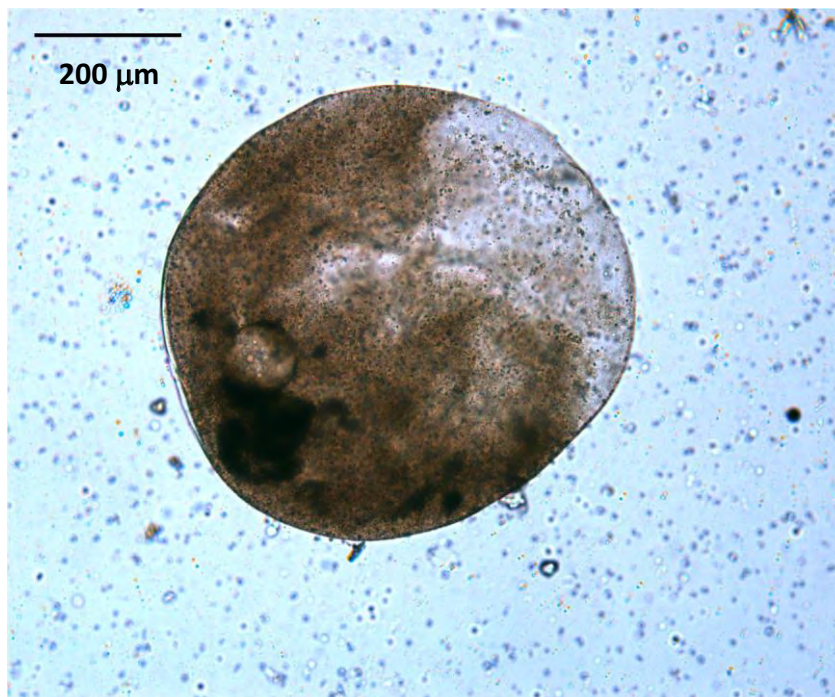


FIGURE 5: Ripe egg in a control individual.

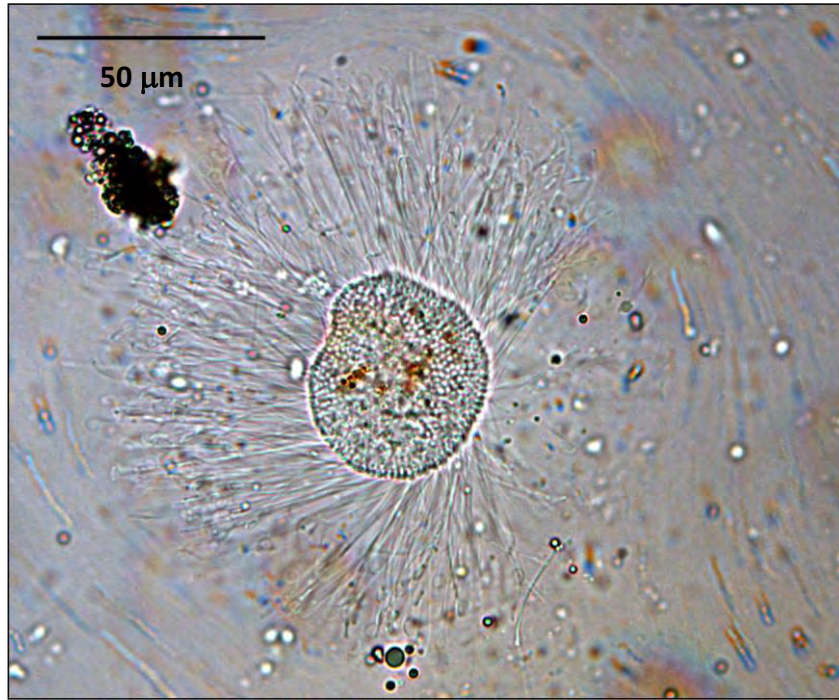


FIGURE 6: 128 spermatid cyst in a control individual.



FIGURE 7: Free spermatozoa in a control individual.

Finally, our results suggest that fluoride treatment is associated with gametogenic disorders and induction of oxidative stress in the reproductive organs of both mammals and invertebrates, but while data for mammals are numerous, those for invertebrates are completely lacking. Our research emphasizes the necessity of investigating other animal groups to explain fluoride's exact mode of action and monitor its toxic potential.



FIGURE 8: Abnormal spermatid cysts in a treated individual.

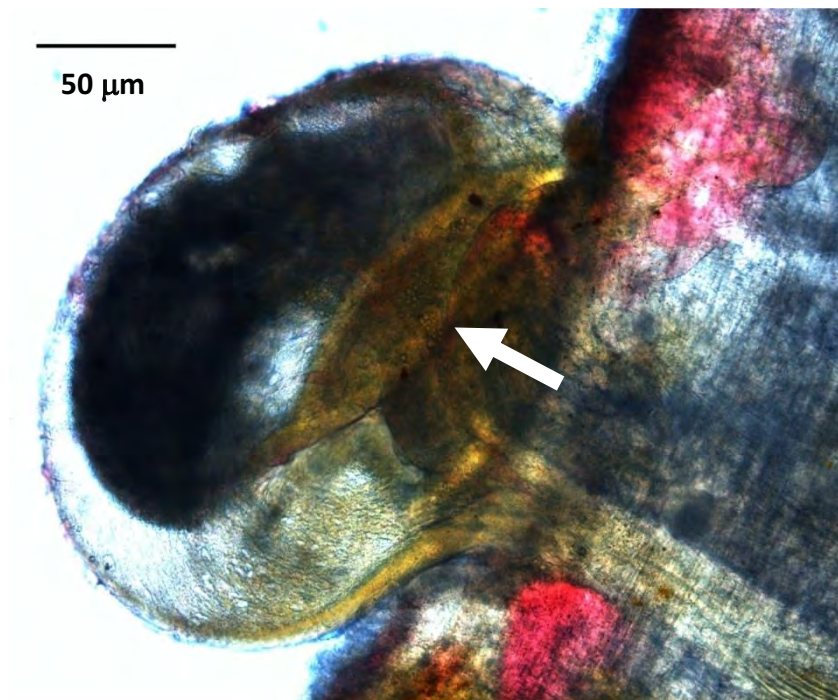


FIGURE 9: Spermatozugma (indicated by the arrow) in the spermatheca out of the body wall obtained by squashing.

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***Branchiura sowerbyi* Beddard, 1892 (Oligochaeta: Naididae) as a test species in ecotoxicology bioassays: a review**

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Abstract

Branchiura sowerbyi (Oligochaeta; Naididae; Rhyacodrilinae) is an oligochaete with great potential to be used as a test-species in toxicology bioassays. Since 1950, its life cycle has been studied and nowadays it is well described in the literature. *B. sowerbyi* has a cosmopolitan distribution and can be found in places where *Tubifex tubifex* (normally used in toxicity bioassays) does not occur, especially in tropical regions. Due to its high individual biomass, *B. sowerbyi* is suitable for use in bioaccumulation bioassays. The present study reviews papers that have used this species in toxicology bioassays and were published between 1950 and the first semester of 2012. In the first part, a brief overview is provided of the biology and the life-cycle parameters of *B. sowerbyi*. In the second part, the bioassays are presented and discussed, and in the third and last part, conclusions about research to date and perspectives about future studies are presented. Throughout the investigation, it was possible to find a total of 30 papers that tested approximately 65 different substances (insecticides, metals, industrial chemicals and others). The majority of the bioassays run are 96-h acute water-only tests, only five were chronic bioassays and two involved bioaccumulation. The lack of research and the small number of tested substances in comparison with the standardized *T. tubifex* species (i.e. approximately 9 and 44 tested metals for *B. sowerbyi* and *T. tubifex*, respectively) can be explained by the absence of a bioassay protocol for *B. sowerbyi*, causing researchers to choose other species. It is necessary to undertake more methodological research in order to find a better and common methodology for bioassays.

Key words: bioassay; Oligochaeta; sediment quality; toxicology

Introduction

Aquatic oligochaetes have long been used in bioassays. They were probably collected by Aristotle, who first used them in toxicity tests when rudimentary bioassays were conducted to verify the effects of salt water on such organisms (Chapman 2001; Martin *et al.* 2008). Throughout the last century, their use for testing environmental impact has grown markedly and, over the last decades, with developments in ecotoxicology, these animals have earned a great reputation as potential test-organisms.

According to Warren *et al.* (1998), Chironomidae and Oligochaeta are the most suitable taxa for assessing the effects of contaminants on the environment because their exposure to chemicals occurs not only through water but also sediment intake. Chapman (2001) underscores that oligochaetes are suitable for such assessments because they are key players in water ecosystems; a number of species are cosmopolitan; many have been used in chronic toxicity tests; they are often exposed to contaminants through water and sediments; many species present enough biomass to allow for bioaccumulation tests; they can be easily grown and handled; and they can resist unique physical and chemical conditions.

However, only a few oligochaete species have been studied so far by international agencies for which toxicology-test protocols have been established. This is the case for *Lumbriculus variegatus* (Müller) (Lumbriculidae), *Pristina leidy* Smith (Naididae) and *Tubifex tubifex* (Müller) (Naididae) (USEPA 2000; ASTM 2005; OECD 2008). The first species is typically found in temperate-climate areas, the second shows a high rate of asexual reproduction—which makes data analysis complicated (i.e. survival), and the last one presents a cosmopolitan distribution despite its low frequency in tropical regions.

Other species have been considered as alternative organisms for testing toxicity, such as *Limnodrilus hoffmeisteri* Claparède (Naididae) and *Branchiura sowerbyi* Beddard (Naididae). The latter is an option for tests in tropical areas both because it is a native species and also because of its higher individual biomass in comparison with other oligochaete species (Marchese & Brinkhurst 1996; Raburu *et al.* 2002), which makes it suitable for bioaccumulation assays (Chapman 2001; OECD 2008). However, there is no protocol for testing toxicity with *B. sowerbyi*, which causes researchers to resort to other species.

In light of the current demand, this study aims to provide a review of papers published over the last 50 years on the use of *B. sowerbyi* in ecotoxicological tests, and thus present a compilation of methodological information as well as a discussion about the next steps towards the establishment of a protocol for the species. The review was based on a search of the following platforms: Scielo, SpringerLink and Elsevier.

Life-cycle

B. sowerbyi reproduces by cross-fertilization, during which there is mutual gamete exchange and, a few days after mating, relatively large transparent cocoons are laid (2–4 mm long), which contain between one and eight eggs (Aston 1968). The embryos present early development of gill filaments (one pair per segment) in the rear part of the body, which is a particular taxonomic trait in the species (Bonacina *et al.* 1994). In addition to the respiratory specialization, this species also shows sensorineural specializations that maximize the detection of vibrations in the substrate, water movement and contact, which make them more successful in escaping predators (Drewes & Zoran 1989). This specialization is extremely important because this species, as well as species of the subfamily Tubificinae (Naididae), leave the rear portion of their bodies exposed on the sediment surface and, by moving it, together with the gills, maximize oxygen intake.

Even though it is a tropical-climate species, it is also found in rivers and lakes of some temperate countries, where it is usually used as an indicator of thermal pollution (Aston 1968; Bonacina *et al.* 1994). According to Aston (1968), it is not clear where the species originated, whether in South America or Asia, probably the latter, since there is no record of the species in Amazonia. In Europe, this species was introduced together with water plants from tropical countries, which accounts for its abundance in botanical gardens across the continent. This author has found that *B. sowerbyi* growth and reproduction are affected by temperature, and that no reproductive activity takes place at temperatures lower than 10°C, a finding confirmed by Bonacina *et al.* (1994). This threshold (T_0) is a high temperature in comparison with values for cosmopolitan species such as *L. hoffmeisteri* ($T_0 = 4^\circ\text{C}$) and *T. tubifex* ($T_0 = 0^\circ\text{C}$) (Bonacina *et al.* 1987), which restricts the species' distribution to rivers and lakes in which the average temperature exceeds 10°C.

Aston *et al.* (1982) and Bonacina *et al.* (1994) showed that higher growth and reproduction rates for *B. sowerbyi* are attained at 25°C, at which temperature the population can double in less than two weeks. Marchese & Brinkhurst (1996) found that a higher rate of cocoon production was achieved at 30°C, and a larger number of juveniles per cocoon at 25°C. Among cocoon cultures, at this temperature, full embryonic development and subsequent hatching take nearly three weeks (Nascimento & Alves 2008; Lobo & Alves 2011).

Besides temperature, the concentration of organic matter in the sediment is a factor that affects reproduction and growth in this species. Aston and Milner (1982) found that activated sludge, which is the result of treated effluents, added to sand at a 33% concentration, yields good conditions for the cultivation of these organisms. On the other hand, high concentrations of activated sludge (equal to or higher than 66%) reduce survival, reproduction and growth among individuals, which, according to these authors, may be associated with lower dissolved oxygen due to the high decomposition rate of the organic matter.

This species is usually more abundant in tropical aquatic environments (Alves & Strixino 2000; Raburu *et al.* 2002; Alves & Strixino, 2003; Pamplin *et al.* 2005; Dornfeld *et al.* 2006; Pamplin *et al.* 2006; Jorcín & Nogueira 2008). Dornfeld *et al.* (2006), for instance, reported different densities throughout sampled parts of the Salto Grande reservoir (Americana, São Paulo, Brazil), ranging between 44 and 8711 ind.m⁻².

When cultivated in laboratory conditions (25°C), this species exhibits low hatching rate: 34.4%—Marchese & Brinkhurst (1996), 31.0%—Ducrot *et al.* (2007) and 44.93%—Nascimento & Alves (2008). However, the survival rate among juveniles is high and can reach 96% (Ducrot *et al.*, 2007). Sexual maturity was reached in approximately 35 days, with an average daily growth of 0.58 mg.day⁻¹ (Marchese & Brinkhurst 1996). After becoming sexually mature, the organisms lay an average of 0.17 cocoons.day⁻¹ (Ducrot *et al.* 2007), during two yearly egg-laying cycles lasting approximately 20 weeks each (Ducrot *et al.*

2007; Lobo & Alves 2011). Life expectancy for this species remains unclear but is estimated to lie between 18 (Aston 1968) and 36 months (Ducrot *et al.* 2007). This uncertainty may be related to the difficulty of monitoring such organisms over a long period of time.

Table 1 shows the biological data available in the literature for *B. sowerbyi*.

TABLE 1. *Branchiura sowerbyi* life-cycle parameters (mean values and 95%-confidence interval or standard deviation) (Adapted from Ducrot *et al.* 2007).

| Stage of development | Life-cycle parameter | Values | Reference |
|------------------------------------|--------------------------------|------------------------------------|---|
| Embryos | Cocoon size | 2–4 mm | Aston 1968 ^a |
| | | Hatching time | 7–8 days |
| | Hatching rate | 21 days | Marchese & Brinkhurst 1996 ^c |
| | | 17 ± 7 days | Ducrot <i>et al.</i> 2007 ^d |
| | | 14–16 days | Nascimento & Alves 2008 ^e |
| | | 12–18 days | Lobo & Alves 2011 ^f |
| | | 18% | Aston <i>et al.</i> 1982 ^g |
| | Hatching rate | 50% | Casellato 1984 |
| | | 21% | Marchese & Brinkhurst 1996 |
| | | 31% | Ducrot <i>et al.</i> 2007 |
| 44.93% | | Nascimento & Alves 2008 | |
| 33.08% | | Lobo & Alves 2011 | |
| Juveniles | Initial weight | 0.44 mg | Liang 1984 ^h |
| | | 1.82 mg | Marchese & Brinkhurst 1996 |
| | | 0.5 [0.3–0.8] mg | Ducrot <i>et al.</i> 2007 |
| | | 1.04 mg | Lobo & Alves 2011 |
| | Survival rate | 96.7 [57–62] % | Ducrot <i>et al.</i> 2007 |
| | | 100% | Lobo & Alves 2011 |
| | Growth rate | 0.58 mg·day ⁻¹ | Marchese & Brinkhurst 1996 |
| 2.4 [1.8–1.7] mg·day ⁻¹ | | Ducrot <i>et al.</i> 2007 | |
| 0.41 ± 0.09 mg·day ⁻¹ | | Lobo & Alves 2011 | |
| Adults | First reproduction | 42 days | Aston & Milner 1982 ⁱ |
| | | 28–35 days | Marchese & Brinkhurst 1996 |
| | | 60 [57–62] days | Ducrot <i>et al.</i> 2007 |
| | | 40.83 ± 6.88 days | Lobo & Alves 2011 |
| | Weight at first reproduction | 40 mg | Aston & Milner 1982 |
| | | ±23 mg | Marchese & Brinkhurst 1996 |
| | | 84.1 [77.5–90.6] mg | Ducrot <i>et al.</i> 2007 |
| | | 17.56 ± 4.57 mg | Lobo & Alves 2011 |
| | Growth rate | 1.3 [0.9–1.7] mg·day ⁻¹ | Ducrot <i>et al.</i> 2007 |
| | Number of eggs per cocoon | 1.94 | Marchese & Brinkhurst 1996 |
| | | 2.2 [1.8–2.6] | Ducrot <i>et al.</i> 2007 |
| | | 1.21 ± 0.08 | Nascimento & Alves 2008 |
| | | 1.73 ± 0.57 | Lobo & Alves 2011 |
| Mean fertility | 0.17 cocoons day ⁻¹ | Marchese & Brinkhurst 1996 | |
| | 0.16 cocoons day ⁻¹ | Ducrot <i>et al.</i> 2007 | |
| | 0.13 cocoons day ⁻¹ | Nascimento & Alves 2008 | |
| | 0.12 cocoons day ⁻¹ | Lobo & Alves 2011 | |
| Life expectancy | > 18 months | Aston 1968 | |
| | 1100 days | Ducrot <i>et al.</i> 2007 | |

Conditions for tests shown in Table 1

^a – Data collected at 20°C, substrate from the Thames, no food added, 32 days' monitoring.

^b – Data collected at 27°C, substrate made up of silt, decomposing lettuce as food, 365 days' monitoring.

^c – Data collected at 25°C, substrate from Lake Eric, no food added, 56 days' monitoring.

^d – Data collected at 21°C, sterilized natural substrate, *ad libitum* food (3.2 mg of Tetramin[®] fish food per oligochaete per day), 365

^e – Data collected at 25°C, muddy substrate, *ad libitum* food (fish food), 30 days' monitoring.

^f – Data collected at 25°C, sandy substrate, *ad libitum* food (fish food, 20 mg per oligochaete per week), 365 days' monitoring.

^g – Data collected at 25°C, activated sludge for substrate, no food added, 12 weeks' monitoring.

^h – Data collected following field observation.

ⁱ – Data collected at 25°C, activated sludge mixed with river mud for substrate, no food added, 14 days' monitoring.

Branchiura sowerbyi as a test-species

Thirty-one papers have been found that report on the use of *B. sowerbyi* as a test-species in ecotoxicology assays (Table 2). Results of assays involving single chemicals are presented in Table 3 and results of assays involving combinations of chemicals are presented in Table 4.

TABLE 2. Use of *B. sowerbyi* as a test-species, tested substances and testing procedure

| References | Tested substances | Type of test |
|------------------------------|--|---|
| Konar & Mullick 1993 | Detergent parmol-J, n-hexane, Endosulfan, DDVP, Urea, Phosphate, Zinc, Copper, Iron, Lead (mixed) | Acute 48h (water-only) |
| Naqvi 1973 | Aldrin, Monocrotophos, Dicrotophos, Chlordane, DDT, Dieldrin, Chlorfenethol, Chlorpyrifos, Endrin, Ethion, Azinphosmethyl, Heptachlon, Diofol, Lindane, Melathion, Methoxychlor, Methyl parathion, Parathion, Perthane, Mevinphos, Strobane, Carbaryl, Toxophene | Acute 72h (water-only and with sediment) |
| Inoue & Kondo 1962 | NaPC, Parathion, EPN, Diazinon, DDT, Lindane, Endrin, Dieldrin, Rotenone, Pyrethrins | Acute 96h (water-only) |
| Ghosh & Konar 1980 | Sodium sulfate, Sodium hydroxide, Sodium sulfide, Sodium carbonate, Calcium hypochlorite, Magnesium bisulfite (mixed) | |
| Kaviraj & Konar 1982 | Mercury, Chromium, Cadmium | |
| Sarkar & Konar 1982 | Urea, Ammonium sulphate, Single superphosphate, Muriate of potash, Lime | |
| Kaviraj & Konar 1983 | Cadmium, Mercury, Chromium (mixed) | |
| Ghosh & Konar 1983 | Formalin | |
| Kaviraj <i>et al.</i> 1985 | Spent bark of Cinchona | |
| Ghatak <i>et al.</i> 1988 | n-heptane, n-hexane | |
| Ghatak & Konar, 1990 | Cadmium, DDVP, Detergent parmol-J, N-heptane (mixed) | |
| Mullick & Konar 1991 | Zinc, Copper, Iron, Lead, n-hexane, detergent parmol-J, endosulfan, DDVP, Urea, Superphosphate (mixed) | |
| Das & Kaviraj 1994 | Cadmium, Potassium Permanganate, Cobalt Chloride (single and mixed) | |
| Sarkar & Konar 1995 | Thiodan, chromium, alkaline FI (mixed) | |
| Ghosal & Kaviraj 1996 | Cadmium (effects of poultry manure on Cd toxicity) | |
| Dutta & Kaviraj 1996 | Cadmium (effects of lime acclimation on Cd toxicity) | |
| Sarkar & Konar 1997 | Thiodan (Endosulfan), Chromium (single and mixed) | |
| Bhunia <i>et al.</i> 2000 | Thiocyanate (tested at 3 temperatures: 20, 25 and 28°C) | |
| Ghosal & Kaviraj 2002 | Cadmium, composted manure (mixed) | |
| Bhunia <i>et al.</i> 2003 | Aniline (C ₆ H ₇ N) | |
| Kaviraj <i>et al.</i> 2004 | Methanol | |
| Das & Das 2005 | Copper, CaO (single and mixed) | |
| Saha <i>et al.</i> 2006 | Lactic acid, acetic acid, benzoic acid | |
| Mondal & Kaviraj 2008 | Jute-retting water | |
| Saha & Kaviraj 2008 | Cypermethrin | |
| Mukherjee & Kaviraj 2011 | Cobalt | |
| Chapman <i>et al.</i> 1982 | Salinity, Temperature, pH, NaPCP, Black Liquor, Mercury, Cadmium, Sewage | Acute 96h (water-only and with sediment) |
| Kaviraj & Ghosal 1997 | Cadmium | Bioaccumulation of Cd after 96h of exposure in contaminated sediments |
| Casellato & Negrisolo 1989 | LAS | Acute 96h (water-only and with sediment), and chronic (140 days) |
| Casellato <i>et al.</i> 1992 | LAS (sediment spiked in the laboratory) | Chronic (220 days) |
| Ducrot <i>et al.</i> 2010 | Zinc (sediment spiked in the laboratory) | Chronic (partial life cycle and full life cycle tests) |

Insecticides were found to be the chemical group with the largest number of tests. Inoue & Kondo (1962) studied the acute effect of a number of insecticides on the mortality of *B. sowerbyi*, and Naqvi (1973) carried out various acute and chronic bioassays to assess toxicity of more than 23 insecticides (Table 2). The acute bioassays were conducted in finger bowls containing only tap water (control) or a solution with toxic substances at 4.4, 21 and 32.2°C. According to this author, temperature had a significant impact on insecticide toxicity: at the extreme values it caused 100% mortality for all tested substances (19 and 18 insecticides, at 4.4 and 32°C respectively), whereas only 8 insecticides (Chlorpyrifos, Ethion, Azinphosmethyl, Dicofol, Parathion, Perthane®, Mevinphos, Toxaphene) led to 100% mortality at 21°C. The author completed bioassays in order to establish sediment influence on the toxicity of pesticides DDT, endrin, toxaphene and chlordane, and it was found that the presence of sediment increases organism resistance to toxic agents and that this species can accumulate large amounts of organochlorine compounds, thus causing toxicity among fish that feed on it. Another interesting finding by this author is their release capacity (detoxification): after a 48h-exposure to 4 ppm Endrin, 500 oligochaetes were transferred to a container containing 5 L of dechlorinated water for 24 h in, and they released enough insecticide to kill 15 mosquitofish, 15 crayfish and 15 shrimps.

Chapman *et al.* (1982) also conducted acute bioassays (96 h), among other substances, with the insecticide/fungicide NaPCP and found different LC50 values from those reported by Inoue & Kondo (1962) (Table 3). The authors also found that the presence of substrate increased the tolerance to contaminants in the water and to changes in environmental variables (pH and salinity).

In addition to insecticides, a number of metals have been subjected to acute bioassays with *B. sowerbyi* as a test-species, and LC50 values can be found in the literature for cadmium (Chapman *et al.* 1982; Kaviraj & Konar 1982; Das & Kaviraj 1994; Ghosal & Kaviraj 1996; Ghosal & Kaviraj 2002), chromium (Kaviraj & Konar 1982; Sarkar & Konar 1997), cobalt (Mukherjee & Kaviraj 2011), copper (Das & Das 2005) and mercury (Chapman *et al.* 1982; Kaviraj & Konar 1982). Kaviraj and Konar (1982) tested acute toxicity (96 h) of mercury, chromium and cadmium and reported LC50 values of 0.007; 10.362 and 4.631 mg.L⁻¹ (for Hg, Cr and Cd, respectively). In another study (Kaviraj & Konar 1983), the effects of a mixture of these metals at the ratio of 0.001:0.46:1.0 (Hg, Cr and Cd respectively) was tested using values based on LC50 for metals in isolation (Table 4). Acute tests were conducted in 300 mL beakers with 10 individuals per replication and found LC50 (96 h) of 15 mg.L⁻¹. The mixture was more toxic than the metals in isolation. Metal concentration in the mixture was nearly one-fifth of lethal concentrations for the metals tested in isolation.

In another study of mixed substances, Konar & Mullick (1993) conducted bioassays using *B. sowerbyi*, among other organisms, in order to study the influence of detergent endosulfan, organophosphate pesticide DDVP and fertilizers (nitrogen and phosphate) on the toxic behavior and safe elimination of mixtures of zinc, copper, iron and lead. A solution was prepared containing 10 times the calculated LC50 value for zinc, copper and DDVP, and the LC50 value for the other components (LC50 values were based on tests performed with zooplankton) (Table 4). LC50 values were calculated for 10 different admixtures (Table 4). The authors found that the tested compounds present very different toxicity levels depending on the mixture in which they are found and concluded that, in order for a mixture of substances to be safely eliminated in the environment, it is not only the toxicity of the compounds in isolation that needs to be taken into account but, in fact, the toxicity they present when mixed at specific concentrations.

Other studies have also shown the effect of substances on metal toxicity. Ghosal & Kaviraj (1996) studied the influence of turkey manure (not toxic at the maximum tested concentration; 250 mg.L⁻¹) on the toxicity of cadmium and found that large amounts of manure reduce the toxicity for the species. Dutta & Kaviraj (1996) investigated the effect of lime acclimatization on the susceptibility of organisms to Cd. In a first test, organisms were acclimatized in lime solutions at 50, 75, 100, 125, 150, 175, 200 and 125 ppm for 96 h before bioassays were carried out with Cd and found that, when they had been acclimatized in concentrations 100 and 150 ppm, their tolerance to Cd increased markedly. In a second test, the authors found a reduced susceptibility to the metal when the organisms had been acclimatized for a period of eight days in a constant 75 ppm solution in comparison to a four-day period (different acclimatization periods were tested: 4, 6, 8 and 12 days). Findings suggest that the Ca⁺² ion fills the binding sites in the cells of the organism, thus preventing Cd⁺² from binding and then causing toxicity. However, excess calcium might also prevent oxygen (O₂) attachment, then causing anoxia in the organisms and their death. The authors recommend eight-day acclimatization in a 75-ppm lime solution for 96 h in a solution of 100 to 150 ppm as being ideal to increase resistance to Cd.

TABLE 3. Chemicals and respective LC50 values for species *B. sowerbyi*

| Chemicals | LC50 (mg·L ⁻¹) | | Temp. °C | References | |
|--------------------------------------|----------------------------|----------|----------------------|----------------------------|-----------------------------|
| | Water | Sediment | | | |
| Metals | | | | | |
| Cadmium (Cd) | 0.24 | 5.7 | 10 | Chapman <i>et al.</i> 1982 | |
| | 4.63 | | ? | Kaviraj & Konar 1982 | |
| | 36.98 (27.58 – 48.47) | | ? | Das & Kaviraj 1994 | |
| | 58.02 (55.78 – 60.34) | | 25 | Ghosal & Kaviraj 1996 | |
| | 58.02 (55.78 – 60.34) | | 25 | Ghosal & Kaviraj 2002 | |
| Chromium (Cr) | 10.36 | ? | ? | Kaviraj & Konar 1982 | |
| | 207.5 | | 28 | Sarkar & Konar 1997 | |
| Cobalt (Co) | 179.00 (140.00 – 228.00) | ? | 34 | Mukherjee & Kaviraj 2011 | |
| Cooper (Cu) | 0.08 (0.07 – 0.10) | | 20-21 | Das & Das 2005 | |
| Mercury (Hg) | 0.08 | 3.2 | 10 | Chapman <i>et al.</i> 1982 | |
| | 0.007 | | ? | Kaviraj & Konar 1982 | |
| Insecticides | | | | | |
| NaPCP | 2.2 | 0.56 | 25 | Inoue & Kondo 1962 | |
| | 0.28 | | 10 | Chapman <i>et al.</i> 1982 | |
| Parathion | 3.5 | 0.56 | 25 | Inoue & Kondo 1962 | |
| EPN | 2.06 | | 25 | Inoue & Kondo 1962 | |
| Diazinon | 4.95 | | 25 | Inoue & Kondo 1962 | |
| DDT | 19.91 | | 25 | Inoue & Kondo 1962 | |
| Lindane | 11.6 | | 25 | Inoue & Kondo 1962 | |
| Endrin | 7.66 | | 25 | Inoue & Kondo 1962 | |
| Dieldrin | 4.12 | | 25 | Inoue & Kondo 1962 | |
| Rotenone | 0.25 | | 25 | Inoue & Kondo 1962 | |
| Pyrethrins | 0.56 | | 25 | Inoue & Kondo 1962 | |
| Thiodan (Endosulfan) | 0.88 | | 28 | Sarkar & Konar 1997 | |
| Cypermethrin | 71.12 (64.00 – 78.00) | | 20 | Saha & Kaviraj 2008 | |
| Detergents | | | | | |
| LAS | 4.38 (3.75 – 5.13) | | 10.82 (9.27 – 12.64) | 10 | Casellato & Negrisollo 1989 |
| | 4.82 (3.75 – 6.19) | | | 20 | Casellato & Negrisollo 1989 |
| Effluents | | | | | |
| Black liquor (%) | 0.79 | 1.4 | 10 | Chapman <i>et al.</i> 1982 | |
| Sewage (%) | 2.5 | 7.6 | 10 | Chapman <i>et al.</i> 1982 | |
| Others | | | | | |
| Low pH | 3.7 | 2.6 | 10 | Chapman <i>et al.</i> 1982 | |
| High pH | 10.5 | 11.3 | 10 | Chapman <i>et al.</i> 1982 | |
| Salinity (‰) | 7.5 | 12 | 10 | Chapman <i>et al.</i> 1982 | |
| Temperature (°C) | 35 | 35 | 10 | Chapman <i>et al.</i> 1982 | |
| Spent Bark of Cinchona | 13800 | | 27 | Kaviraj <i>et al.</i> 1985 | |
| n-heptane | 2500 | | 27.8 | Ghatak <i>et al.</i> 1988 | |
| n-hexane | 3286.5 | | 27.8 | Ghatak <i>et al.</i> 1988 | |
| Cobalt Chloride (CoCl ₂) | 132.62 (115.16 – 152.73) | | ? | Das & Kaviraj 1994 | |
| Potassium Permanganate | 0.03 (0.23 – 0.38) | | ? | Das & Kaviraj 1994 | |
| Thiocyanate | 217.79 (194.46 – 243.92) | | 20 | Bhunja <i>et al.</i> 2000 | |
| | 186.45 (161.82 – 214.82) | | 25 | Bhunja <i>et al.</i> 2000 | |
| | 166.88 (136.03 – 204.72) | | 28 | Bhunja <i>et al.</i> 2000 | |
| Aniline | 586.50 (498.78 – 691.56) | | ? | Bhunja <i>et al.</i> 2003 | |
| Methanol | 54890 (53200 – 56630) | | ? | Kaviraj <i>et al.</i> 2004 | |
| Quicklime (CaO) | 83.00 (72.10 – 95.52) | | 20-21 | Das & Das 2005 | |
| Lactic acid | 50.82 (48.40 – 53.24) | | ? | Saha <i>et al.</i> 2006 | |
| Acetic acid | 14.90 (14.48 – 15.43) | | ? | Saha <i>et al.</i> 2006 | |
| Benzoic acid | 39.47 (38.92 – 40.03) | | ? | Saha <i>et al.</i> 2006 | |

? – Not provided

Das & Das (2005) also tested lime effect (CaO) on the toxicity of another metal for water organisms including *B. sowerbyi*. Acute tests were conducted for 96 h, in two test batteries, the first to determine LC50 for copper and lime separately, and the second to find the effect of variations of lime concentrations on Cu

LC50. The findings revealed that Cu is toxic at low concentrations for *B. sowerbyi* (0.08 mg.L⁻¹), which is also sensitive to CaO (LC50 = 83.00 mg.L⁻¹) when compared to the fish species *Cyprinus carpio* L., which in turn did not present mortality for any CaO concentration lower than 500 mg.L⁻¹. The authors have also noted that the presence of Cu reduces lime toxicity; LC50 for lime in the absence of the metal was 83 mg.L⁻¹; however, with the addition of 0.08 mg.L⁻¹ Cu (LC50 value), no organism mortality was found even when they were exposed to 450 mg.L⁻¹ of CaO, which points to an antagonistic relation between both substances. According to the authors, the reduced toxicity may be caused by the same factor reported by Dutta & Kaviraj (1996): the competition between Ca⁺² and Cu⁺² ions for binding sites in the cells of the organisms.

Ghosal & Kaviraj (2002) studied the effects of cadmium combined with manure made up of *Pistia stratiotes* leaves, mixed with bovine manure at the ratio of 1:1, decomposed anaerobically for 85 days. The authors found that LC50 for cadmium increases with an increment in the amount of decomposed manure added to the solution. They also found that the oligochaetes accumulated more metal in the presence of the manure.

Also with regard to mixtures with metals, Sarkar & Konar (1997) studied the effect of insecticide Thiodan (Endosulfan) interacting with chromium in 96-h acute bioassays. The mixture was obtained based on the LC50 calculated for the individual substances. The authors found that when they are mixed, LC50 is much lower in comparison with LC50 for the components in isolation (mixture LC50: 166.50 mg.L⁻¹ – 166.4 mg Cr + 0.00209 mg.L⁻¹ Thiodan; LC50 Cr: 207.5 mg.L⁻¹; LC50 Thiodan: 0.882 mg.L⁻¹).

Kaviraj & Ghosal (1997) performed bioassays with natural sediments from brackish water lagoons (Sundarban, India), contaminated with Cd. Twelve samples were collected at different spots in the lagoons for laboratory bioassays carried out at 25°C for a period of 96 h. The organisms were placed in 3 L flasks (2.5 g of wet weight) containing 0.125 L of sediment and 2.5 L of Cd-free water (sediment-water ratio: 1:20). *B. sowerbyi* accumulates large amounts of Cd, and the highest reading was 10.50 ± 2.51 µg.g⁻¹ (organisms kept in sediment containing 5.76 ± 3.23 mg.kg⁻¹ cadmium).

Another substance that has been studied well is Linear Alkylbenzene Sulphonate (LAS), an anionic surfactant used in detergents. Casellato & Negrisola (1989) carried out acute (96 h) and chronic (140 days) tests to assess its effects and have found, like Naqvi (1973) and Chapman *et al.* (1982), that, in acute tests, sediment presence significantly increases this species' tolerance to the tested compound (10.8 mg.L⁻¹ and 4.38 mg.L⁻¹, LC50 with and without sediment, respectively). The chronic tests, however, showed that low concentrations of LAS dissolved in water (0.5 and 2.5 mg.L⁻¹) influenced the species' reproductive behavior. At such concentrations, the organisms presented a discontinuous reproductive period which was clearly altered by the presence of LAS. Fewer cocoons were found within concentrations 0.5 and 2.5 mg.L⁻¹ in comparison to the control. The 5 mg.L⁻¹ concentration was very similar to the control for all aspects under observation; however, the authors were not able to account for these unexpected results. Still on the study into the effects of long exposure to LAS, Casellato *et al.* (1992) conducted an experiment with contaminated sediment in the laboratory and exposed the species for 220 days. No differences were found between the treatment (25.87 mg.kg⁻¹) and the control for the cocoon degeneration rate, hatching rate, number of eggs per cocoon and embryo development time; only the total numbers of cocoons and eggs were slightly larger in the treatment than the control. The authors concluded that, at that concentration, when LAS is present in the sediment, it has a weaker effect on the studied species than when it is dissolved in water. This was the first long-term toxicity experiment using *B. sowerbyi*.

Ducrot *et al.* (2010) also performed chronic bioassays to assess long-term Zn effects on the partial (28 days) and full (179 days) life-cycles of *B. sowerbyi*. In order to assess the effects of the metal on the partial life-cycle (PLC), the authors ran five bioassays: one with young organisms (14 days old), one with young-adults (40 days old), two with adults (60 days old) and one with cocoons. Two kinds of PLC test were performed, one to check the effects on survival and growth (28 days long) and another to check reproduction/hatching (42 days long). As for the effect on the full life-cycle (FLC), a single bioassay was conducted with young, 14-day-old organisms. In the PLC, the authors found that, for young and adult organisms, there was a significant difference for survival only for the higher concentration tested (3317 mg.kg⁻¹, a value 25 times lower than the average value for French water bodies, which is nearly 130 mg.kg⁻¹). For young organisms, on the other hand, lower survival was found among organisms exposed to Zn concentrations higher than 1819 mg.kg⁻¹. As far as growth is concerned, the PLC test for all concentrations showed that young organisms grow

less when they are exposed to the metal. No significant differences were found in reproduction or hatching tests across the tested concentrations (419–1651 mg.kg⁻¹) and the control. The authors found that 67 ± 34% of the organisms born in sediments with Zn concentration higher than 551 mg.kg⁻¹ presented deformed gills; however, they were unable to confirm whether the metal was to blame for such deformities due to lack of replications in the experimental design. Following the FLC assays, it was found that the organisms exposed to concentrations between 409 and 1050 mg.kg⁻¹ showed lower body weight in comparison to the control. No significant differences have been reported for the other parameters (mortality, maturation time and reproduction). According to the findings, the authors conclude that the species is sensitive to Zn only in the first month of life.

The range of other substances (pure or mixed) that have already been used in bioassays with *B. sowerbyi* can be found in table 2, and calculated LC50 values are shown in tables 3 and 4.

TABLE 4. Mixtures and respective LC50 values for species *B. sowerbyi*.

| Mixtures | LC50 (mg·L ⁻¹) | References |
|--|----------------------------|-----------------------|
| Hg + Cr + Cd | 15.0 (0.80 – 21.00) | Kaviraj & Konar 1983 |
| Zn + Cu + Fe + Pb | 17.07 (1.00 – 35.84) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane | 63.69 (12.45 – 114.93) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + PJ | 14.58 (1.00 – 41.29) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane + PJ | 44.57 (7.85 – 81.29) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane + PJ + Endosulfan | 49.80 (18.26 – 81.34) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane + PJ + DDVP | 50.16 (1.00 – 50.16) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane + PJ + Endosulfan + DDPV | 32.37 (1 – 32.37) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane + PJ + Endosulfan + DDPV + Urea | 36.13 (19.91 – 61.35) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane + PJ + Endosulfan + DDPV + SSP | 65.68 (31.11 – 100.25) | Konar & Mullick 1993 |
| Zn + Cu + Fe + Pb + <i>n</i> -hexane + PJ + Endosulfan + DDPV + Urea + SSP | 31.89 (1 – 31.89) | Konar & Mullick 1993 |
| Cd + KMnO ₄ | 41.17 (44.90 – 51.67) | Das & Kaviraj 1994 |
| Cd + CoCl ₂ | 65.01 (51.63 – 58.38) | Das & Kaviraj 1994 |
| Cd + 0.25g·L composted manure | 70.80 (65.92 – 76.03) | Ghosal & Kaviraj 2002 |
| Cd + 0.50 g·L composted manure | 77.47 (71.43 – 84.26) | Ghosal & Kaviraj 2002 |
| Cd + 1.00 g·L composted manure | 78.75 (72.98 – 85.00) | Ghosal & Kaviraj 2002 |
| Cd + 6.70 g·L composted manure | 85.33 (79.60 – 91.46) | Ghosal & Kaviraj 2002 |
| Cd + 30 mg·L poultry litter | 48.55 (45.71 – 51.56) | Ghosal & Kaviraj 1996 |
| Cd + 65 mg·L poultry litter | 68.73 (61.86 – 76.36) | Ghosal & Kaviraj 1996 |
| Cd + 125 mg·L poultry litter | 77.56 (67.67 – 88.9) | Ghosal & Kaviraj 1996 |
| Cd + 250 mg·L poultry litter | 85.76 (76.57 – 96.12) | Ghosal & Kaviraj 1996 |
| Thiodan + Cr | 166.5 (122.5 – 212.5) | Sharkar & Konar 1997 |

Review of bioassay methods

It has been noted that the vast majority of studies included acute bioassays in which only water was used. Some tests were run in Petri dishes and the significant majority in beakers between 250 to 600 mL, in which 200 to 300 mL solutions were assessed, by exposing between 3 and 10 organisms per replication.

Another significant variation in methodology that has been found across the studies concerns test temperatures. Some experiments tested species sensitivity at 10°C (Chapman *et al.* 1982) and others as high as 34°C (Mukherjee & Kaviraj 2011). Temperature is known to influence substance toxicity (Naqvi 1973;

Casellato & Negrissolo 1989; Bhunia *et al.* 2000) and to play a key role in bioassays, in addition to its impact on the biology of reproduction and species growth (Aston 1968; Aston *et al.* 1982; Bonacina *et al.* 1987; Bonacina *et al.* 1994). Chapman *et al.* (1982) have found that the LC50 for temperature is 35°C, which is very close to the temperature (34°C) used by Mukherjee & Kaviraj (2011) in their acute bioassays with cobalt, which may cause uncertainty as to whether the actual cause of mortality among the organisms is due to temperature, to the chemical or to a combination of both.

In chronic bioassays, the most noticeable difference concerns length of exposure, which ranged between 28 (Ducrot *et al.* 2010) and 220 days (Casellato *et al.* 1992), and other variations noted included container size, sediment and water volume, and organism density. In most cases, such methodological discrepancies make the comparison of results impossible. The design of a protocol for bioassays with *B. sowerbyi* should promote methodological standardization which, in turn, may allow for the comparison of results across different laboratories.

Concluding remarks

As we have shown, a number of papers have already been published on ecotoxicity assays with *B. sowerbyi*; however, the lack of a specific protocol for the species makes the comparison of results difficult and causes researchers to choose other species for which there is already a protocol, such as *T. tubifex* (ASTM 2005; OECD 2008) and *L. variegatus* (USEPA 2000; OECD 2008).

According to ASTM (2005), in order for a species to be used in ecotoxicological tests with substrates, it must (1) possess an ecotoxicity database that shows sensitivity to a number of chemicals concerning the sediment, (2) possess a database for comparison across different laboratories, (3) be in physical contact with the sediment, (4) always be available for testing, either in cultures or field samples, (5) be easily kept in the laboratory, (6) be easily identified, (7) be ecologically or economically important, (8) have broad geographic distribution, be native to the site to be assessed or be of a similar niche as that of native organisms (9) be tolerant to physical and chemical variations in the sediment, (10) be compatible with the exposure methods and selected endpoints, (11) be reviewed and (12) be confirmed by answers from natural populations of benthic organisms.

B. sowerbyi has been known to have great potential for use as a test-species; however, a bioassay protocol is required in order for this species to become another tool in programs to assess and monitor the health of tropical water environments.

For chronic tests, we recommend using the methods described in protocol E1706–05 (ASTM, 2005), described for *T. tubifex*: 250 mL flasks with 100 mL of sediment and 100 mL of dechlorinated water, temperature adjusted to 25 or 30°C, instead of 21°C, and maybe, in order to allow for a larger number of juveniles born in the control, a 7-day extension to the final duration of the chronic test if the temperature is 25°C. Test duration would then become 35 days, as recommended by Marchese and Brinkhurst (1996). For acute tests with no sediments (water-only), we recommend using 200 mL flasks with 100 mL of water/solution and 4 or 5 individuals per replication, at 25°C. However, further studies are required in order for a specific protocol for *B. sowerbyi* to be produced.

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Aquatic Oligochaeta (Annelida) of Dam Lakes Çatören and Kunduzlar (Turkey)

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Abstract

Çatören and Kunduzlar Dam Lakes, located on Seydi River, represent the main irrigation water resources of the Seyitgazi District (Eskişehir Province), in west-central Turkey. The river and the reservoirs are both under the threat of pollution primarily originating from several domestic point source discharges and land-based runoff. The numerical and proportional distributions of oligochaetes in Çatören and Kunduzlar Dam Lakes were surveyed seasonally in 2010 and 2011 at two stations on each lake. According to the results of this study, the benthic invertebrate fauna of Çatören Dam Lake consisted of Oligochaeta (40.2 %), Chironomidae larvae (32.2 %) and the varia (27.6 %); the benthic invertebrate fauna of Kunduzlar Dam Lake consisted of Oligochaeta (56.7 %), Chironomidae larvae (18.2 %) and the varia (25.1%). By evaluating the data via a Shannon-Wiener index it was found that the Çatören Dam Lake had an index of 2.32; while Kunduzlar Dam Lake had an index of 3.27. Several physicochemical water quality parameters were also analyzed during this study. The relationships between the dynamics of organisms and environmental parameters were supported by Pearson correlation index. It was determined that Çatören and Kunduzlar Dam Lakes waters were polluted and slightly polluted, respectively. The dominance and abundance of oligochaete species and low species richness showed that similar studies should be carried out periodically in Çatören and Kunduzlar Dam Lakes for the future monitoring of the dam lakes.

Key words: Oligochaeta, Çatören and Kunduzlar Dam Lakes, Turkey

Introduction

Benthic macroinvertebrate assemblages are very important for aquatic system food chains and may serve as biological indicators of various environmental stresses on aquatic ecosystems (Richardson and Kiffney 2000). Invertebrate communities are also trustworthy indicators of water and ecosystem conditions (Chao et al. 1996). Oligochaetes have a worldwide distribution, with species adapted to every kind of body of water, such as brackish water, fresh water or salt water. In addition, certain species of Oligochaeta are abundant in organically polluted waters because of lack of competition and an abundant food supply coupled with a tolerance to reduced oxygen condition (Brinkhurst and Jamieson 1971) and they are considered an interesting indicator of water quality because of their ubiquity and abundance in aquatic ecosystems (Sæther 1979; Pinder 1986). However, there have been few studies on the invertebrate fauna and water quality of Çatören and Kunduzlar Dam Lakes (CDL and KDL, respectively) (Altındağ and Özkurt 1998; Özkurt 2000) and, to date, there have been no studies related to their oligochaete faunas and environmental parameters. This study is aimed at investigating the qualitative and quantitative characteristics of the oligochaete faunas in the CDL and KDL. In this study, scales of distribution of oligochaete species and relationships with environmental variables were examined. Data have also been used to identify correlative relationships between physicochemical features and the dynamics of oligochaete fauna.

Materials and methods

Study Area

Çatören and Kunduzlar dam lakes are located in the Kırka basin of Eskişehir (Figure 1) and have been used for irrigation, erosion control and energy supply since 1984 and 1986, respectively. The surface areas and maximum depths of the dam lakes are 2.64 km² and 4.04 km², and 28 m and 38 m, respectively. Kümbet and Akin are the main streams that carry water to CDL and KDL, respectively (Figure 1).

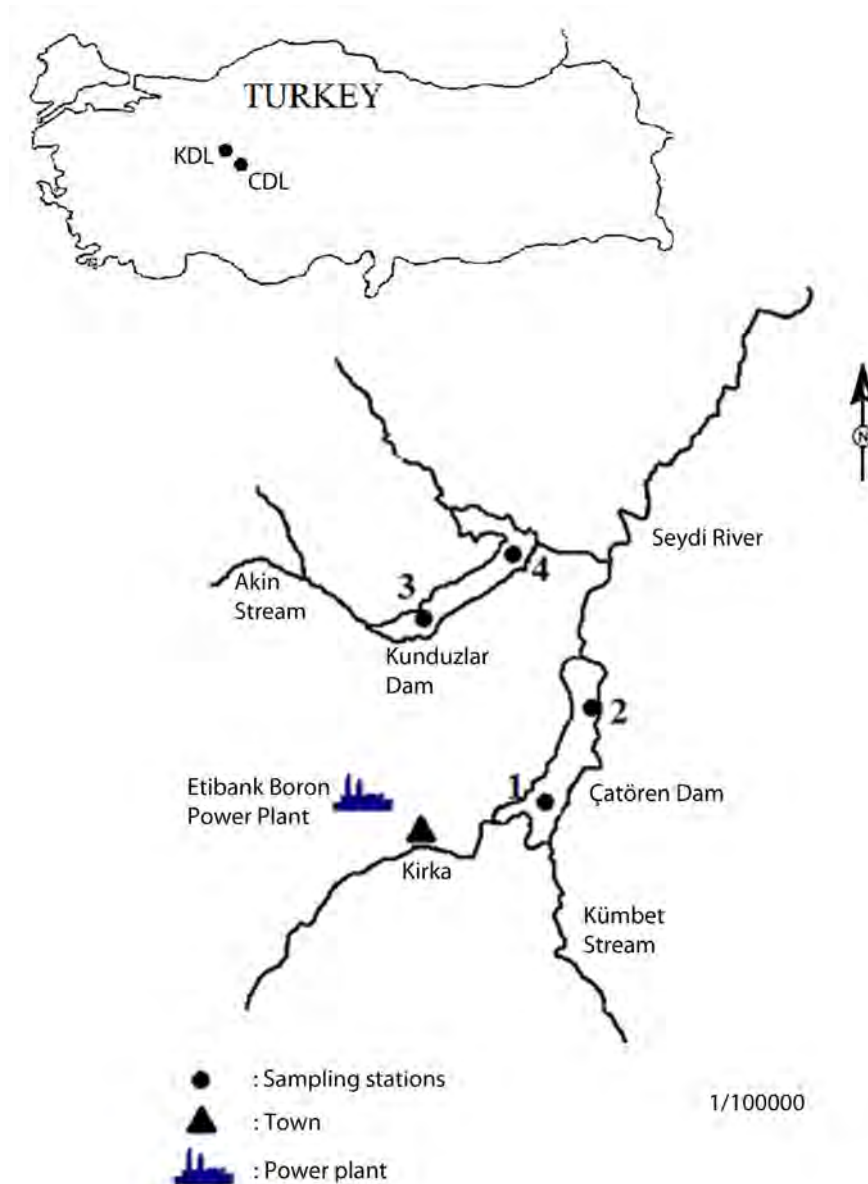


FIGURE 1: Location of sampling sites on the Çatören (CDL) and Kunduzlar (KDL) dam lakes, Seydi River (Seyitgazi District, Eskişehir Province) in west-central Turkey, where surveys for aquatic oligochaetes and other benthic macroinvertebrates were conducted during 2010 and 2011.

Sampling

This study was carried out between March 2010 and June 2011. Two replicate benthic samples were taken seasonally at four different stations (Figure 1) using an Ekman grab. Samples were washed in situ using a sieve of 200µm mesh size. The material was preserved in 4% formalin in the field, taken to the laboratory and sorted under a stereomicroscope. All samples were identified to family, order or class level and preserved in

70% alcohol. Taxa were grouped as Oligochaeta, Nematoda, Chironomidae larvae, Gastropoda and Bivalvia, plus low numbers of Hirudinea, Odonata, Ephemeroptera, Coleoptera and Hemiptera which were grouped as "Varia". Only oligochaete samples were identified to genera or species level. Oligochaete specimens were either studied as whole mounts in glycerine or mounted in polyvinyl lactophenol (for chaetal characters), and finally mounted as whole specimens in Canada balsam after dehydration. The identification of the oligochaete species was completed using the four identification keys (Brinkhurst and Jamieson 1971; Sperber 1950; Timm 1999; Timm and Veldhuijzen van Zanten 2002).

Ten physico-chemical variables were measured at each of the four stations (Table 2). Water temperature, hydrogen ion concentration (as pH), dissolved oxygen (mg/L) and conductivity (mSiemens) were measured in the field. Two replicates of water samples from each station were stored in polyethylene bottles (1000 ml). The chemical and biological oxygen demand (BOD and COD), SO_4 , NO_3-N , NO_2-N were all determined in accordance with the standard method procedures (APHA 1992). The water samples were kept in a refrigerator at a temperature below 4 °C and were analyzed within 24 hours. The highest and lowest values of the measured physico-chemical parameters of four sampling sites of CDL and KDL during the study period are given in Table 2.

Statistical Analysis

Shannon index was based on PAST 1.75b (Hammer, Harper and Ryan 2001). Bellan-Santini's (1969) quantitative dominance index (Bellan-Santini 1969) of each species was estimated by; $D_i = N_i/N_x \times 100$, where N_i = number of individuals of species i ; and N_x = total number of macrobenthic specimens. Also, relationships between the distribution of the oligochaete species and environmental variables were determined using Spearman correlation in SPSS 9.0 for Windows.

RESULTS and DISCUSSION

During this study, 24 invertebrate taxa were recorded, collectively, from the Çatören and Kunduzlar dam lakes; however, since the scope of this study focused on the aquatic oligochaete fauna in these lakes, only the oligochaetes were identified to the genus or species level (Table 1). A total of 2524 individuals were counted, comprising 15 species of Oligochaeta and nine taxa representing Gastropoda, Chironomidae, Bivalvia, Nematoda and varia. A taxonomic list of the oligochaete species and other benthic invertebrates determined in the CDL and KDL during the study, with their individual numbers and dominance (D %) values were given in Table 1. We found that the CDL and KDL's zoobenthos was dominated by oligochaetes and Chironomidae larvae. Oligochaeta was composed of a high value of dominance (40.2% and 56.7% for CDL and KDL, respectively); the second taxon was Chironomidae (32.2 % and 18.2 %).

Oligochaete species richness varied between 10 and 15 in the study area (Table 1). The highest Oligochaeta species number was found in KDL (15 species) while the lowest oligochaete species (10 species) were found in CDL. *Psammoryctides albicola* was found to have the highest dominance value (8.3 %D) and it was followed by *Potamothrix hammoniensis* (7.8 %D) and *Limnodrilus hoffmeisteri* (6.6 %D) and *Limnodrilus claparedianus* (5.9 %D) in KDL; while in CDL, *Stylaria lacustris* was found to have the highest dominance value (8.2 %D) and it was followed by *Pristina jenkinsae* (5.1 %D), *Nais elinguis* (5.5 %D) and *Potamothrix hammoniensis* (4.7 %D). In addition, *Potamothrix bavaricus*, *Psammoryctides barbatus*, *Psammoryctides albicola*, *Uncinails uncinata*, and *Paranais frici* were found in only KDL. According to Shannon index, the species diversity in KDL was found as 3.27 while in CDL 2.32 (Table 2). It is clearly shown that the number of species (N), taxa number and Shannon (H) are higher in KDL than in the CDL.

Pearson's correlation coefficients between abundance of selected oligochaete species (which were shown common distribution or high abundance) and examined environmental parameters of CDL and KDL water were examined. In KDL especially tubificine species, *P. albicola*, *P. hammoniensis*, *L. hoffmeisteri* and *L. udekemianus* showed significant positive correlations to the BOD ($p < 0.01$); *Potamothrix hammoniensis* and *L. hoffmeisteri* showed positive correlations to the SO_4 and NO_2-N respectively ($p < 0.05$). In CDL, it was found that the abundance of naidid species *Nais elinguis* and *Aulophorus furcatus* showed a negative correlation to the DO level of the water ($p < 0.05$) while *P. jenkinsae* showed a positive correlation ($p < 0.05$).

In Turkey, surface water quality is classified according to: a) physical and inorganic-chemical parameters, b) organic parameters, c) inorganic parameters, and d) bacteriological parameters. In addition, each group contain four water quality classes; surface water quality classes are defined as: class I: high quality waters, class II: slightly contaminated waters, class III: contaminated waters, and class IV: severely polluted waters (SSKY 2008). According to SSKY (2008), the values of pH, conductivity, and temperature were found at normal levels during the course of this present study (Table 2). When the water quality was evaluated for nutrients, the values of SO₄ and NO₂N were found at third and second quality level, respectively, in CDL; while the values of SO₄ and NO₂N were found at first and second quality level, respectively, in KDL. According to the Turkish water quality classes (SKKY 2008) CDL sampling sites during the 4 sampling months in 2010–2011 (Table 2) were categorized as Class III (in terms of physical and inorganic-chemical parameters) and Class II (in terms of organic parameters) while KDL sampling sites were categorized as Class II and Class I respectively. According to SSKY (2008), Class II waters are used for: (a) drinking purposes (after advanced treatment), (b) recreational purposes, (c) fish farming (except trout), (d) irrigation, and (e) other purposes not covered by Class I; Class III waters can be used for industrial purposes (except food and textile industries).

TABLE 1: Taxonomic list of the oligochaete species and other benthic invertebrates determined in the Çatören (CDL) and Kunduzlar (KDL) dam lakes, Seydi River (Seyitgazi District, Eskişehir Province) in west-central Turkey, during the present study, with their individual numbers and dominance (D %) values.

| | CDL | | | KDL | | | |
|--------------------|--|-----------------|----|-----------------|-----------------|-----|------|
| | 1 st | 2 nd | D | 3 rd | 4 th | D | |
| OLIGOCHAETA | | | | | | | |
| 1 | <i>Limnodrilus claparedianus</i> Ratzel, 1869 | 25 | 0 | 3.5 | 63 | 39 | 5,6 |
| 2 | <i>Limnodrilus hoffmeisteri</i> Claparède, 1862 | 15 | 15 | 4.2 | 78 | 42 | 6,6 |
| 3 | <i>Tubifex tubifex</i> (Müller, 1774) | 12 | 0 | 1.7 | 27 | 2 | 1,6 |
| 4 | <i>Potamothrix hammoniensis</i> (Michaelsen, 1901) | 15 | 18 | 4.7 | 63 | 78 | 7,8 |
| 5 | <i>Potamothrix bavaricus</i> (Oschmann, 1913) | 0 | 0 | 0.0 | 48 | 0 | 2,6 |
| 6 | <i>Psammoryctides barbatus</i> (Grube, 1861) | 0 | 0 | 0.0 | 42 | 18 | 3,3 |
| 7 | <i>Psammoryctides albicola</i> (Michaelsen, 1901) | 0 | 0 | 0.0 | 72 | 78 | 8,3 |
| 8 | <i>Nais elinguis</i> Müller, 1773 | 24 | 15 | 5.5 | 42 | 33 | 4,1 |
| 9 | <i>Nais variabilis</i> Piguet, 1906 | 6 | 9 | 2.1 | 48 | 9 | 3,1 |
| 10 | <i>Aulophorus furcatus</i> (Müller, 1774) | 15 | 6 | 3.0 | 51 | 30 | 4,5 |
| 11 | <i>Dero digitata</i> (Müller, 1773) | 6 | 9 | 2.1 | 15 | 18 | 1,8 |
| 12 | <i>Uncinaiis uncinata</i> (Orsted, 1842) | 0 | 0 | 0.0 | 6 | 12 | 1,0 |
| 13 | <i>Stylaria lacustris</i> (Linnaeus, 1767) | 25 | 33 | 8.2 | 9 | 57 | 3,6 |
| 14 | <i>Paranais frici</i> Hrabě, 1941 | 0 | 0 | 0.0 | 15 | 0 | 0,8 |
| 15 | <i>Pristina jenkinsae</i> (Stephenson, 1931) | 15 | 21 | 5.1 | 36 | 0 | 2,0 |
| | Number of oligochaete taxa at the stations | 10 | 8 | - | 15 | 12 | - |
| 16 | Gastropoda | 45 | 66 | 15.7 | 48 | 24 | 4,0 |
| 17 | Bivalvia | 0 | 6 | 0.8 | 21 | 33 | 3,0 |
| 18 | Nematoda | 3 | 18 | 3.0 | 36 | 27 | 3,5 |
| 19 | Chironomidae | 159 | 69 | 32.2 | 210 | 120 | 18,2 |
| VARIA | | | | | | | |
| 20 | Coleoptera | 24 | 9 | 4.7 | 21 | 15 | 2,0 |
| 21 | Hemiptera | 0 | 0 | 0.0 | 15 | 24 | 2,1 |
| 22 | Odonata | 6 | 12 | 2.5 | 42 | 63 | 5,8 |
| 23 | Ephemeroptera | 0 | 6 | 0.8 | 42 | 24 | 3,6 |
| 24 | Hirudinea | 0 | 0 | 0.0 | 6 | 15 | 1,2 |
| | Shannon H' | 2.32 | | | 3.27 | | |

TABLE 2: Some parameters of the water in Çatören (CDL) and Kunduzlar (KDL) dam lakes, Seydi River (Seyitgazi District, Eskişehir Province) in west-central Turkey by season (W: winter, December 2010, Sp: Spring, April 2011, Su: Summer, July 2011, Au: Autumn, October, 2011).

| Parameters/Sampling | CDL | | | | | KDL | | | | |
|--|------------|----------|----------|----------|--------------|-----------|----------|----------|----------|-------------|
| | W. 2010 | Sp. 2011 | Su. 2011 | Au. 2011 | Average ± SD | W. 2010 | Sp. 2011 | Su. 2011 | Au. 2011 | Average ±SD |
| Physical inorganic-chemical parameters | | | | | | | | | | |
| Temperature (°C) | 7.8 | 12.4 | 17.3 | 19.7 | 14.3 ± 5.2 | 9.2 | 13.3 | 18.6 | 18.2 | 14.8 ± 4.4 |
| pH | 7.9 | 8.1 | 7.8 | 8.6 | 8.1 ± 0.31 | 8.2 | 8.4 | 8.1 | 8.3 | 8.2 ± 0.1 |
| DO (mg L ⁻¹) | 7.6 | 6.8 | 4.3 | 5.4 | 5.7 ± 1.3 | 8.3 | 8.1 | 6.2 | 6.8 | 7.3 ± 1 |
| SO ₄ ²⁻ (mg L ⁻¹) | 285 | 210 | 225 | 315 | 258.7 ± 49.5 | 86 | 55 | 105 | 110 | 89 ± 24.9 |
| NO ₃ N (mg L ⁻¹) | 2.63 | 2.45 | 3.38 | 4.16 | 3.1 ± 0.7 | 0.75 | 1.19 | 0.41 | 0.58 | 0.7 ± 0.3 |
| NO ₂ N (mg L ⁻¹) | 0.026 | 0.0071 | 0.0058 | 0.029 | 0.01 ± 0.01 | 0.011 | 0.014 | 0.023 | 0.026 | 0.01 ± 0.01 |
| Water Quality Class | III | | | | | II | | | | |
| Organic parameters | | | | | | | | | | |
| BOI (mg L ⁻¹) | 3.6 | 7.2 | 3.2 | 3.1 | 4.2 ± 1.80 | 2.8 | 3.1 | 4.4 | 4.6 | 3.72 ± 0.9 |
| KOI (mg L ⁻¹) | 10.9 | 15.6 | 18.5 | 19.4 | 16.1 ± 3.83 | 9.9 | 14.2 | 17.5 | 18.4 | 15 ± 3.8 |
| Water Quality Clasis | II | | | | | I | | | | |
| Other | | | | | | | | | | |
| Total hardness (mg L ⁻¹ CaCO ₃) | 196 | 208 | 226 | 218 | 212 ± 12.9 | 267 | 270 | 274 | 284 | 273.7 ± 7.4 |
| Conductivity (µmhos cm ⁻¹) | 325 | 368 | 412 | 395 | 375 ± 37.9 | 416 | 397 | 401 | 408 | 405.5 ± 8.3 |
| Water Quality Clases (SKKY, 2008) | | | | | | | | | | |
| | I | II | III | IV | | I | II | III | IV | |
| DO (mg L ⁻¹) | 8 | 6 | 3 | < 3 | | 8 | 6 | 3 | < 3 | |
| SO ₄ (mg L ⁻¹) | 200 | 200 | 400 | > 400 | | 200 | 200 | 400 | > 400 | |
| NO ₃ N (mg L ⁻¹) | 5 | 10 | 20 | > 20 | | 5 | 10 | 20 | > 20 | |
| NO ₂ N (mg L ⁻¹) | 0.002 | 0.01 | 0.05 | > 0.05 | | 0.002 | 0.01 | 0.05 | > 0.05 | |
| BOI (mg L ⁻¹) | 4 | 8 | 20 | > 20 | | 4 | 8 | 20 | > 20 | |
| KOI (mg L ⁻¹) | 25 | 50 | 70 | > 70 | | 25 | 50 | 70 | > 70 | |

Conclusions

It was found that the zoobenthos of Dam Lakes were dominated by two groups of invertebrates, Oligochaeta and Chironomidae, which is typical of many freshwater systems. Dam lakes oligochaete fauna consist mainly of taxa with wide ecological tolerances and extensive geographical ranges. The oligochaetes in KDL were mainly represented by three species, *P. hammoniensis*, *P. albicola*, and *Limnodrilus hoffmeisteri*, each of which was present throughout the year. The naidine species, *Stylaria lacustris* and *N. elinguis*, were the dominant species present in CDL. *Limnodrilus hoffmeisteri* and *L. claparedianus* are commonly collected in organically enriched waters. In addition, these tubificine species are often found in large numbers in highly polluted waters (Brinkhurst and Jamieson 1971; Mason 1996) and *Potamothrix hammoniensis* is a reliable indicator of eutrophy or local organic enrichment when occurring in considerable densities (Milbrink 1980). In the study area, these tubificine species were the most abundant. The pollution of the dam lake KDL could be characterized by the species *T. tubifex*, *L. hoffmeisteri*, *L. claparedianus* and *P. hammoniensis*. The most common oligochaete species in KDL were *Stylaria lacustris*, *Nais elinguis*, *Pristina jenkiniae* and *Aulophorus furcatus*. These naidine species, common inhabitants of sewage filter beds, ponds, lakes and streams, have also been found in brackish water (Davis 1982). In addition, *Pristinella jenkiniae* has been collected from a wide range of current velocities and within a wide temperature range by Pascar-Gluzman and Dimentman (1984) who reported euryoic and eurythermal species. These four species are reported to be tolerant of severe pollution and have been found in a stream degraded by industrial effluents (Maciorowski et al. 1977; Harman 1979). *Stylaria lacustris*, *Nais elinguis*, *Pristina jenkiniae* and *Aulophorus furcatus* were reported to occur in abundance in stony bottomed, muddy substratum, organically enriched streams and lakes in Turkey (Çamur-Elipek et al. 2006; Arslan and Şahin 2006; Akbulut et al. 2009). This fact can be explained by the euryoic character of four naidine species, as reported by Pascar-Gluzman and Dimentman (1984) and Davis (1982).

The distribution, species composition, and development of aquatic oligochaetes depends on many factors such as water temperature, chemical and physical properties of the water (Grigelis et al. 1981), sediments, bottom microflora, and vegetation cover. In the present study, there were no significant differences from the point of view of the dam lakes substrate type. However, the main and important differences between the dam lakes stations were the vegetation. High naidine species abundance in the CDL may be partly due to their rich littoral vegetation.

The relationship between the number of tubificine taxa and BOD was directly proportional ($p < 0.01$), while the relationship between the number of some naidine taxa (*Nais elinguis* and *Aulophorus furcatus*) and DO was inversely proportional ($p < 0.05$).

The dominant oligochaete species collected during this study, *P. hammoniensis*, *P. albicola*, and *L. hoffmeisteri*, are commonly collected from eutrophic lakes (Brinkhurst and Jamieson 1971; Milbrink 1980). Most of the naidine taxa recorded during this study were found in CDL stations, which contain a high density of aquatic vegetation. The subfamily Naidinae contains species with a wide variety of environmental preferences. In both dam lakes, oligochaete diversity was low, but abundance was high. Generally, tubificine members were widespread in all year during the study period. Several authors (Milbrink 1980; Pascar-Gluzman and Dimentman 1984; Särkkä 1994; Mason 1996) indicated that some species of tubificine and naidine were an indicator of organic loading in lakes. All of our results during this present study support these findings.

As we mentioned above, according to physical and inorganic-chemical and organic parameters, it was determined that CDL and KDL waters were polluted and slightly polluted respectively (Table 2). Emiroğlu et al. (2010) investigated boron concentration of Seydi Stream water, its sediment, and different organisms from surrounding Kirka county. They examined inflow and outflow waters of CDL and KDL dam lakes. They found that boron levels in water flowing into CDL was 3.45 mg L^{-1} , but lower (2.51 mg L^{-1}) in water flowing into KDL. Their results showed that boron concentrations of the Seydi Stream water is higher than the Turkish Environmental Guidelines standard ($>1 \text{ mg L}^{-1}$). In comparison, mean values of boron in surface waters throughout Europe are typically below 0.6 mg L^{-1} .

According to the boron concentrations found in their study, Emiroğlu et al. (2010) concluded that water of Seydi Stream was included to the class IV ($>1 \text{ mg L}^{-1}$) heavily polluted water that should not be used at all (for drinking, recreational, fish farming and irrigation purposes).

Consequently, irrigation, sewage system, variable flow rate and temperature affect the quality of waters in CDL and KDL. The structure of benthic invertebrate fauna in the CDL and KDL changes with effects of environmental variables. The dominance by, and abundance of, oligochaete species showed that similar monitoring studies should be carried out periodically in CDL and KDL for the future of the dam lakes.

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Oligochaete (Annelida, Clitellata) communities in lakes of the Ural Mountains (Russia)

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Abstract

The oligochaete fauna of 25 lakes located in the Polar and Subpolar regions of the Ural Mountains in Russia was studied. In total, 46 oligochaete taxa were collected, 32 of them identified to species level. Naididae had the highest number of taxa (19); the other taxa belonged to Pristinidae (2), Enchytraeidae (6), Tubificidae (10), Lumbriculidae (8) and Lumbricidae (1). Canonical correspondence analysis (CCA) was used to characterize the relationship between abundance of oligochaete species, abundance and biomass of all oligochaetes, abundance of separate families and environmental factors. Latitude, elevation, silt, detritus, moss and algal periphyton and depth were found to be factors influencing the distribution of oligochaetes.

Key words: oligochaetes, fauna, distribution, mountain water bodies, Ural Mountains, Komi Republic, Russia

Introduction

The European high-mountain water bodies represent an important resource of unpolluted water for the future but are becoming increasingly threatened by local (e.g. tourism and water extraction) and global (climate change) environmental impacts (McGregor et al. 1995; Lencioni et al. 2004). The geographical position of the Ural Mountains on the border of Europe and Asia contributes to a great interest in the fauna of this region. The investigated lakes are situated within the National Park “Yugyd Va”, one of the largest territories protected as natural areas in the European Northeast, and also included on the list of the UNESCO’s World Heritage sites.

The local benthic fauna has been regularly studied over the last two decades by researchers at the Institute of Biology of the Komi Branch of Ural Division of the Russian Academy of Sciences within the framework of two projects: “Biodiversity of terrestrial and aquatic ecosystems of the Subpolar Ural Mountains: mechanisms of formation, a modern condition, the forecast of natural and antropogenic dynamics” and the UNDP/GEF project “Strengthening Protected Areas System of the Komi Republic to Conserve Virgin Forest Biodiversity in the Pechora River Headwaters Region” (2008–2013).

In this paper, we compare the oligochaete fauna of 25 lakes in the Polar and the Subpolar Ural Mountains. We also assessed some ecological factors responsible for differences in the distribution of oligochaete communities (e.g., depth, elevation, bottom sediment).

Study area

The 25 lakes under study are located in the Komi Republic of the Russian Federation on the western slope of the Polar Urals (PU area, 10 lakes) and Subpolar Urals (SPU area, 15 lakes), in the extreme Northeast of Europe. All of these lakes are located in areas ranging from latitude 64°00' to 68°08' N, and longitude 59°40' to 65°21' E; the elevation of the PU lakes range from 200 to 500 m a.s.l. in PU, while the elevation of the SPU lakes range from 200 to 1000 m a.s.l.

Two of these lakes belong to the Kara River basin while the others are drained by tributaries of the Pechora River (Fig. 1, Table 1).

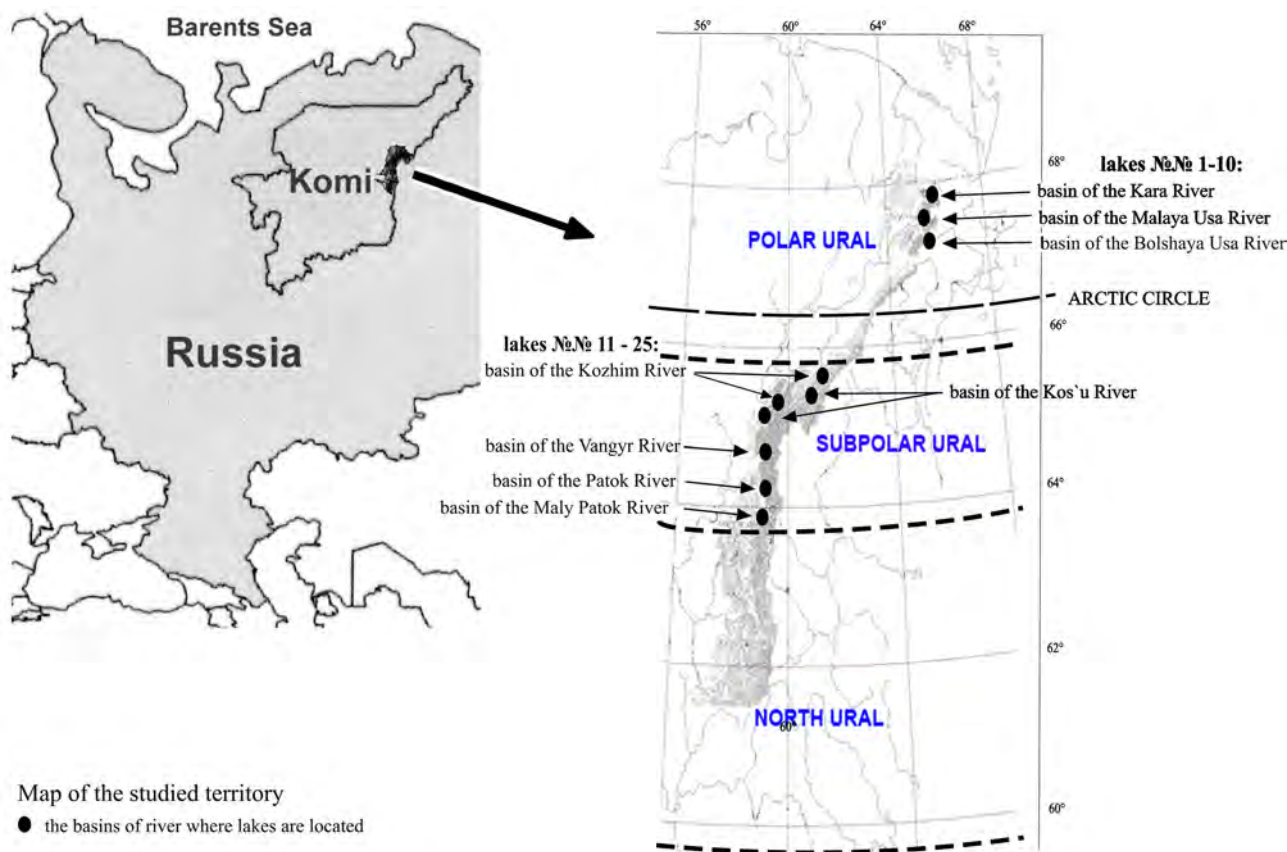


FIGURE 1. Location of the study area.

These lakes are of diverse origin: tarns, glacial, thermokarst, floodplain lakes. They are frozen for most of the year; only ice-free during the months of June through September. The surface water temperature of most of these lakes ranges from 10 to 15 °C in summer, although the temperature in floodplain lakes can reach 25 °C. The lakes vary in size (in PU 0.22–1.5 km², SPU 0.05–1.45 km²), depth (in PU 1.3–37 m, SPU 2.0–21 m), and water transparency (5–11 m). The substrates of these lakes vary, including rocks, pebbles, gravel, sand and mud (silt). They are ultraoligotrophic and oligotrophic. In the investigated lakes the hydrogen ion concentration (as pH) measured usually fluctuates from slightly acidic to weakly alkaline. For example, during the sampling period the lowest pH (4.6) was observed just after the end of snowmelt (early in June) in a lake of the Maly Patok River basin, while the highest recorded pH values (8.6) were seen in August. Water mineralization and conductivity are very low; saturation with oxygen is typical, during the observation period dissolved oxygen being always present in the profundal zone of the lake.

Most of the lakes are pristine, without any direct human impact. They can be reached only with helicopter or special cross-country vehicles. Severe climate conditions and inaccessibility are the reasons that the fauna of these local lakes has not been investigated until the recent time.

Methods

The Polar and Subpolar Ural lakes were studied during summer seasons of 1998 through 2010. Quantitative zoobenthos samples (46 from PU lakes and 54 from SPU lakes) were taken with a Petersen dredge (sampling area 0.040 m²) on soft bottoms and with a hydrobiological scraper ($S = 0.09$ m², 230 µm mesh size net) on stony and gravel bottoms. The zoobenthos samples were taken from different depths. After the samples had been sorted, oligochaetes were prepared for identification. The worms were mounted in a glycerin-water solution and identified to species level under a light microscope (Leica DM 4000 B), following the methodologies and keys presented in Timm (2009). Some part of the material remained unidentified, including several taxa in the family Enchytraeidae and juvenile individuals. These individuals were determined only to family level. Oligochaete biomass was determined as a wet weight using a torsion balance (WT-250, Poland).

TABLE 1. List of the lakes under study.

| N ^o | Name of lake | River basin | Coordinates | Area (km ²) | Elevation above sea level (m) | Depth (max / widespread) | Substrate type |
|----------------------|----------------------------|------------------------------------|--------------------|-------------------------|-------------------------------|--------------------------|----------------|
| Polar Ural | | | | | | | |
| 1 | Lake N ^o 1-B.U. | Bol'shaya Usa | N 67°28'; E 65°45' | 0.25 | 154.7 | 7 / 3.5 | rk, pb |
| 2 | Bol'shoye Kuz'ty | Bol'shaya Usa | N 67°36'; E 65°39' | 1.5 | 289.1 | 4.6 / 2 | rk, pb |
| 3 | Chan-ty | Malaya Usa | N 67°42'; E 65°42' | 0.5 | 244.3 | 37 / 20 | rk, pb |
| 4 | Maloe Shuch'e | Malaya Usa | N 67°48'; E 66°10' | 4.0 | 270.0 | 36 / 20–30 | sd, rk |
| 5 | Usva-ty | Malaya Usa | N 67°44'; E 65°59' | 1.8 | 245.4 | 12 / 1.3 | rk, pb, sd |
| 6 | Gnet-ty | Kara | N 67°58'; E 65°34' | 0.8 | 189.7 | 7 / 3 | rk, pb, sd, sl |
| 7 | Koma-ty | Kara | N 68°08'; E 65°21' | 0.9 | 91.5 | 4 / 2 | sd, sl |
| 8 | Plaun-ty | Malaya Usa | N 67°45'; E 65°40' | 0.44 | 175.8 | 13 / 3.5 | rk |
| 9 | Bezmyannoe-U | Malaya Usa (near Lake Usva-ty) | N 67°28'; E 65°38' | 0.22 | 154.1 | 5.2 / 3 | rk, sd |
| 10 | Protochnoe | Malaya Usa | N 67°46'; E 65°35' | 1.31 | 172.5 | 1.3 / 0.8 | rk, pb, sl |
| Subpolar Ural | | | | | | | |
| 11 | Bol'shoye Balban-ty | Kozhim | N 65°12'; E 60°14' | 0.92 | 654.9 | 15 / 8 | sl, rk |
| 12 | Maloe Balban-ty | Kozhim | N 65°09'; E 60°13' | 0.5 | 687.6 | 10 / 8 | sd, sl, rk |
| 13 | Verkhnee Balban-ty | Kozhim | N 65°03'; E 60°09' | 0.12 | 1007.0 | 12 / 8 | sl, sd, rk |
| 14 | Trubka | Kozhim (small river of Limbeko-yu) | N 65°05'; E 60°08' | 0.1 | 850.0 | 2 / 1 | sd, pb |
| 15 | Padezha-ty | Kozhim (small river of Limbeko-yu) | N 65°11'; E 60°03' | 1.45 | 694.3 | 21 / 9 | sl, rk |
| 16 | Forel'noe | Kozhim (small river of Limbeko-yu) | N 65°16'; E 59°55' | 0.42 | 757.4 | 14 / 4 | rk, sl |
| 17 | Lake N ^o 1-K | Kos'u (small river of Lomes-vozh) | N 64°54'; E 59°54' | 0.2 | 1048.3 | 5.7 / 2.5 | rk, sl, sd |
| 18 | Lake N ^o 2-K | Kos'u (small river of Lomes-vozh) | N 64°55'; E 59°54' | 0.34 | 1010.2 | 6.3 / 2 | rk, sl, sd |
| 19 | Lake N ^o 3-K | Kos'u (small river of Lomes-vozh) | N 64°56'; E 59°55' | 0.17 | 785.2 | 11 / 8.5 | rk, pb, sd, sl |
| 20 | Nom-ty | Maly Patok | N 64°39'; E 59°40' | 0.08 | 562.0 | 16 / 9 | rk, sd |
| 21 | Patok | Maly Patok | N 64°40'; E 59°41' | 0.24 | 560.6 | 16 / 5 | rk, pb, sd, sl |
| 22 | Losinoe | Vangyr | N 64°58'; E 59°10' | 0.06 | 274.8 | 2 / 1 | sl |
| 23 | Lake N ^o 1-V | Vangyr | N 64°59'; E 59°10' | 0.05 | 278.5 | 8.5 / 6 | rk |
| 24 | Lake N ^o 2-V | Vangyr | N 64°59'; E 59°09' | 0.15 | 278.0 | 2 / 1 | rk |
| 25 | Mezhgornoe | Kos'u (small river of Nidysej) | N 65°15'; E 59°41' | 0.03 | 601.7 | 9 / 2.4 | rk, sl, sd |

Abbreviation used: sl – silt, sd – sand, pb – pebbles, rk – rocks (boulders)

During the sampling period, water depth, temperature, hydrogen ion concentration (as pH), conductivity and oxygen content were measured using the portable analyzer Multi 340i/SET (Germany). The substrate components were classed according to grain-size as silt (0.01–0.1 mm), sand (0.1–1.0 mm), gravel (1.0–10.0 mm), pebbles (10.0–100.0 mm) and boulders (100.0–1000 mm) (Konstantinov 1972). Substrates with a mixture of several different fractions are defined as follows – silted sand, sand-gravel, boulder-pebble, and boulder-gravel.

The similarity of the oligochaete fauna identified from each of these lakes was estimated according to the Sørensen's similarity coefficient (Sørensen 1948). For the characterization of community evenness, the Pielou index (Pielou 1969) was used. Species' domination was characterized using the Kownacki index (d): $d > 10$ – dominant, $1 < d < 10$ – sub-dominant (Kownacka 1971).

The relationships between oligochaetes and environmental variables were investigated with canonical correspondence analysis (CCA) followed by Monte Carlo tests and cluster analysis with Euclidean distance measure and Ward's group linkage method using PC-ORD for Windows (McCune & Mefford 1999). Eleven environmental variables were included in these analyses. These included substrate sizes, sampling depth (m), maximum depth (m) and area of lakes (m²), as well as the qualitative characteristics such as presence and absence of visually distinguishable plants, moss, algae, detritus, and silt, and the geographical coordinates (elevation, latitude and longitude). Abundance of 31 identified species of oligochaetes, the total abundance and biomass of oligochaetes, abundance and number of Naididae, Tubificidae, Lumbriculidae, Enchytraeidae and Lumbricidae were used. The species identified only to family or genus level as "sp." were included, too. Species found in only one lake were omitted.

Results

Species composition

A total of 46 oligochaete taxa were found, 32 of them identified to species level (Table 2). Naididae was the most diverse group (with 19 taxa); the other taxa belonged to Pristinidae (2), Enchytraeidae (6), Tubificidae (10), Lumbriculidae (8) and Lumbricidae (1).

Numerous juvenile specimens of Enchytraeidae and Tubificidae (with hair chaetae) were found but remained unidentified due to the absence of genital organs. The majority of oligochaete species were cosmopolitan or widely distributed. There were 33 oligochaete taxa in the PU lakes, and 32 taxa in the SPU lakes, but similarity of their oligochaete fauna was low, the Sørensen index of similarity being only 0.36 between the all lakes of Polar and Subpolar Urals. The total number of species per lake was low, between 5 and 16 in PU and between 1 and 13 in SPU.

The greatest species number in both groups of lakes belonged to Naididae (17 species in PU and 11 in SPU). The number of Naididae species per lake varied from 1 (mostly in SPU lakes) to 8 (e.g., in the lakes of Gnet-ty and Koma-ty—PU, and Bol'shoe Balban-ty—SPU). *Chaetogaster diaphanus*, *Nais pseudobtusa*, *N. barbata* and *N. communis* were the most frequent species in PU, *N. variabilis* and *Uncinaiis uncinata* in SPU.

Enchytraeidae (5 taxa) and Lumbriculidae (6 taxa) were most diverse in the SPU lakes. The genus *Cernosvitoviella* was the most frequently encountered enchytraeid taxon in SPU lakes while *Cognettia glandulosa* and *Mesenchytraeus armatus* were found in PU lakes.

Only 10 taxa of Tubificidae were found in the Ural lakes, 1–5 species per lake. No tubificids were found in the samples of SPU lakes Bol'shoe Balban-ty and Trubka (Kozim River basin) and Lake № 1-K (Kos`u River basin). *Spirosperma ferox* was the most common tubificid in both PU and SPU lakes.

The Pielou index varied in different lakes (Table 3). In some lakes of Subpolar Ural (Maloe Shuch`e, Usva-ty, Plaun-ty, Bezymyannoe-U) and Polar Ural (Losinoe, Verkhnee Balban-ty, Patok) the evenness of community was greater. The index of domination "d" indicated that oligochaete communities had one or two dominants and a large number of subdominants. In these lakes the share of dominating species varied from 20 to 35 % in oligochaete abundance, while abundance of the other oligochaete species was more than 50 %. In the lakes with low evenness of community, Lake № 1-B.U. and Chan-ty in the SPU lake group, and Lake № 1-K, Lake № 2-K, and Maloe Balban-ty in the PU lake group, the share of the dominating species varied from 50 to 80 % from the abundance of all oligochaetes.

TABLE 2. List of oligochaete taxa found in the Polar and Subpolar Ural Mountain lakes under study (taxonomy follows Timm 2009).

| Species | Polar Ural lakes (n = 10) | | Subpolar Ural lakes (n = 15) | |
|---|---|--|---|--|
| | Average abundance in all lakes (min – max, ind. m ⁻²) | Numbers of the lakes (from Table 1) where the species were found | Average abundance in all lakes (min – max, ind. m ⁻²) | Numbers of the lakes (from Table 1) where the species were found |
| Naididae | | | | |
| <i>Chaetogaster diaphanus</i> (Gruithuisen, 1828) | 64.6 (0.0 – 456.0) | 2 | 13.1 (0.0 – 166.8) | 11 |
| <i>Ch. diastrophus</i> (Gruithuisen, 1828) | 14.5 (0.0 – 75.1) | 4 | | 16 |
| <i>Nais alpina</i> Sperber, 1948 | 2.7 (0.0 – 26.7) | 4 | 1.3 (0.0 – 18.9) | 11 |
| <i>N. behningi</i> Michaelsen, 1923 | | | 2.1 (0.0 – 31.7) | 21 |
| <i>N. communis</i> Piguet, 1906 | 20.7 (0.0 – 79.6) | 1 | | |
| <i>N. elinguis</i> Müller, 1774 | 16.1 (0.0 – 17.3) | 7 | | |
| <i>N. pseudobitusa</i> Piguet, 1906 | 66.2 (0.0 – 425.5) | 1, 2 | 37.4 (0.0 – 222.3) | 11 |
| <i>N. variabilis</i> Piguet, 1906 | 25.3 (0.0 – 194.3) | 1, 2 | 39.8 (0.0 – 360.8) | 11 |
| <i>N. parvalis</i> Piguet, 1906 | 22.1 (0.0 – 176.6) | 1, 2, 3 | 0.7 (0.0 – 11.1) | 25 |
| <i>N. barbata</i> Müller, 1774 | 121.2 (0.0 – 549.5) | 3 | | |
| <i>N. bretscheri</i> Michaelsen, 1899 | | 6, 7 | 0.4 (0.0 – 5.6) | 25 |
| <i>N. simplex</i> Piguet, 1906 | | 4, | 22.3 (0.0 – 258.3) | 11 |
| <i>Nais</i> sp. | 8.6 (0.0 – 70.3) | 6, 7 | | |
| <i>Specaria josinae</i> (Vejdovský, 1884) | 2.7 (0.0 – 30.0) | 1 | | |
| <i>Piguetiella blanci</i> Piguet, 1906 | 0.3 (0.0 – 3.7) | 7 | 0.9 (0.0 – 13.3) | 11 |
| <i>Uncinaxis uncinata</i> (Oersted, 1842) | 12.9 (0.0 – 50.1) | 2 | 68.9 (0.0 – 513.8) | 11, 12 |
| <i>Vejdovskya comata</i> (Vejdovský, 1884) | 17.9 (0.0 – 114.7) | 7, 8, 9, 10 | 1.8 (0.0 – 26.7) | 20 |
| <i>V. macrochaeta</i> (Lastockin, 1921) | 36.4 (0.0 – 400.0) | 6, 7 | | 11 |
| <i>Slavina appendiculata</i> (Udekem, 1855) | 4.0 (0.0 – 400.0) | 7 | | |
| Pristinidae | | | | |
| <i>Pristina bilobata</i> (Bretscher, 1903) | 0.6 (0.0 – 6.7) | 5 | 0.9 (0.0 – 13.3) | 12 |
| <i>Pristina</i> sp. | | | | |
| Enchytraeidae | | | | |
| <i>Cognettia glandulosa</i> (Michaelsen, 1888) | 12.9 (0.0 – 101.0) | 8, 9, 10 | 15.1 (0.0 – 111.0) | 15 |
| <i>Mesenchytraeus armatus</i> (Levinsen, 1884) | 13.2 (0.0 – 103.6) | 6, 7 | 2.7 (0.0 – 40.0) | 14 |
| <i>Cernosvitoviella</i> sp. | | | 91.8 (0.0 – 680.0) | 14, 15 |

| Species | Polar Ural lakes (n = 10) | | Subpolar Ural lakes (n = 15) | |
|--|---|--|---|--|
| | Average abundance in all lakes (min – max, ind. m ⁻²) | Numbers of the lakes (from Table 1) where the species were found | Average abundance in all lakes (min – max, ind. m ⁻³) | Numbers of the lakes (from Table 1) where the species were found |
| <i>Fridericia</i> sp. 1 | | | 0.7 (0.0 – 11.1) | 23 |
| <i>Fridericia</i> sp. 2 | | | 4.4 (0.0 – 66.6) | 23 |
| Enchytraeidae gen. spp. | 104.8 (0.0 – 313.4) | 1, 2, 3, 4, 5, 6, 7 | 119.0 (0.0 – 614.0) | 11, 12, 13, 17, 18, 19, 20, 21 |
| Tubificidae | | | | |
| <i>Autodrilus</i> sp. | 0.3 (0.0 – 3.7) | 8 | | |
| <i>A. limnobioides</i> Bretscher, 1899 | | | 0.7 (0.0 – 11.1) | 23 |
| <i>A. plurisetia</i> (Pignatelli, 1906) | | | 0.9 (0.0 – 13.3) | 20 |
| <i>Rhyacodrilus coccineus</i> (Vejdovsky, 1875) | 0.3 (0.0 – 3.7) | 6 | 10.8 (0.0 – 134.1) | 15, 25 |
| <i>Limnodrilus</i> sp. | 1.2 (0.0 – 13.3) | 6 | | |
| <i>Alexandrovina ringulata</i> (Sokol'skaja, 1961) | 0.6 (0.0 – 3.7) | 2, 7 | | |
| <i>Spirosperma ferrox</i> Eisen, 1879 | 116.3 (0.0 – 311.8) | 1, 2, 4, 6, 7, 8, 9, 10 | 132.7 (0.0 – 632.8) | 11, 12, 15, 16, 20, 21, 22, 23, 24, 25 |
| <i>Lophochaeta ignota</i> Štolc, 1886 | 2.5 (0.0 – 13.3) | 1, 6, 7 | 30.9 (0.0 – 360.0) | 11, 18, 19, 21 |
| <i>Tubifex tubifex</i> (Müller, 1774) | 63.4 (0.0 – 485.6) | 2, 3, 7, 10 | 31.5 (0.0 – 213.7) | 11, 12, 16, 20, 21, 22, 24 |
| <i>Tubificidae</i> gen. sp. juv. with hair chaetae | 68.6 (0.0 – 241.6) | 1, 2, 4, 5, 6, 7, 9 | 4.5 (0.0 – 55.3) | 15, 20 |
| Lumbriculidae | | | | |
| <i>Rhynchelmis</i> sp. (<i>limosella</i> ?) | | | 0.3 (0.0 – 3.7) | 21 |
| <i>Lumbriculus variegatus</i> (Müller, 1774) | 9.5 (0.0 – 52.9) | 1, 2, 5, 6, 7, 8 | 15.6 (0.0 – 93.3) | 11, 12, 15, 17, 22, 25 |
| <i>Tarriella slovenica</i> Hrabě, 1939 | | | 20.6 (0.0 – 160.0) | 14, 15 |
| <i>Stygodrilus heringianus</i> Claparède, 1862 | | | 2.3 (0.0 – 34.4) | 20 |
| <i>Lumbriculus</i> sp. | 11.5 (0.0 – 126.1) | 9 | 3.3 (0.0 – 22.3) | 19, 20, 25 |
| ? <i>Lamprodrilus</i> sp. | 5.7 (0.0 – 63.5) | 2 | | |
| ? <i>Stygodrilus</i> sp. | | | 4.2 (0.0 – 64.6) | 18 |
| Lumbricidae | | | | |
| <i>Eiseniella tetraedra</i> (Savigny, 1826) | | | 0.7 (0.0 – 11.1) | 23 |

TABLE 3. The average abundance (N, ind. m⁻²) and biomass (B, mg.m⁻²) of oligochaetes, Pielou Index (E), dominant species (d>10).

| N ^o | Lake | N | B | E | Dominant species |
|----------------|----------------------------|--------|--------|------|--|
| 1 | Lake N ^o 1-B.U. | 851.0 | 367.7 | 0.52 | <i>S. ferox</i> <i>V. macrochaeta</i> |
| 2 | Bol'shoe Kuz-ty | 786.3 | 480.9 | 0.68 | <i>S. ferox</i> <i>Ch. diaphanus</i> |
| 3 | Chan-ty | 645.6 | 179.4 | 0.51 | <i>T. tubifex</i> |
| 4 | Maloe Shuch'e | 918.0 | 122.9 | 0.82 | <i>S. ferox</i> |
| 5 | Usva-ty | 143.7 | 111.5 | 0.89 | <i>N. variabilis</i> |
| 6 | Gnet-ty | 2973.8 | 386.4 | 0.80 | <i>Ch. diaphanus</i> |
| 7 | Koma-ty | 2113.9 | 508.0 | 0.74 | <i>N. pseudobtusa</i> <i>N. barbata</i> |
| 8 | Plaun-ty | 394.7 | 234.4 | 0.9 | <i>S. ferox</i> <i>N. elinguis</i> |
| 9 | Bezemyannoe-U | 1052.8 | 321.7 | 0.89 | <i>S. ferox</i> <i>N. pardalis</i> |
| 10 | Protochnoe | 144.3 | 79.9 | 0.68 | <i>S. ferox</i> |
| 11 | Bol'shoe Balban-ty | 2581.7 | 735.6 | 0.79 | <i>N. simplex</i> <i>N. variabilis</i> |
| 12 | Maloe Balban-ty | 1242.5 | 909.5 | 0.54 | <i>S. ferox</i> |
| 13 | Verkhnee Balban-ty | 120.0 | 12.0 | 0.91 | <i>N. pseudobtusa</i> |
| 14 | Trubka | 1080.0 | 340.0 | 0.59 | <i>Cernosvitoviella</i> sp. <i>Tatriella</i> sp. |
| 15 | Padezha-ty | 2509.8 | 1245.2 | 0.78 | <i>S. ferox</i> <i>Cernosvitoviella</i> sp. |
| 16 | Forel'noe | 486.8 | 176.7 | 0.83 | <i>Ch. diaphanus</i> <i>N. pseudobtusa</i> <i>S. ferox</i> |
| 17 | Lake N ^o 1-K | 181.5 | 65.8 | 0.50 | <i>L. variegatus</i> |
| 18 | Lake N ^o 2-K | 476.5 | 111.4 | 0.32 | <i>L. variegatus</i> |
| 19 | Lake N ^o 3-K | 772.0 | 248.4 | 0.81 | <i>L. ignotus</i> |
| 20 | Nom-ty | 1281.4 | 368.3 | 0.76 | <i>N. elinguis</i> <i>U. uncinata</i> <i>T. tubifex</i> <i>S. heringianus</i> |
| 21 | Patok | 214.1 | 115.3 | 0.87 | <i>P. hammoniensis</i> <i>S. ferox</i> |
| 22 | Losinoe | 600.0 | 1120.0 | 0.92 | <i>T. tubifex</i> <i>L. variegatus</i> |
| 23 | Lake N ^o 1-V | 3391.5 | 595.0 | 0.84 | <i>N. pseudobtusa</i> <i>C. glandulosa</i> <i>Cernosvitoviella</i> sp. <i>Fridericia</i> sp. <i>S. ferox</i> |
| 24 | Lake N ^o 2-V | 2776.0 | 1526.0 | 0.65 | <i>S. ferox</i> <i>T. tubifex</i> |
| 25 | Mezhgornoe | 2342.1 | 2295.2 | 0.83 | <i>S. ferox</i> |

Average abundance and biomass of oligochaetes

Zoobenthos of the studied lakes consisted of 28 higher taxa of invertebrates, with 14–21 in each lake. The most widespread of these were Chironomidae, oligochaetes, Mollusca, Nematoda, Cladocera, Copepoda, Ostracoda, Hydracarina and Trichoptera. The total abundance of zoobenthos usually varied between 3,000–5,000 ind.m⁻², rarely over 10,000 ind.m⁻², and was lowest at the highest elevations. Biomass was low (1–5 g.m⁻²).

Oligochaetes represented an important component of the zoobenthos, both in average abundance (11.1 and 16.5 % in the PU and SPU lakes respectively) and average biomass (12.5 and 21.9 %). However, this percentage varied in the individual lakes. In the PU lakes oligochaetes represented 0.6–58.9 % of abundance and 0.6–99.6 % of biomass. Their contribution to the benthic fauna in SPU lakes was 0.8–61.7 % of abundance and from 0.1–95.8 % of biomass. Their highest percentages were recorded in Lake Padezha-ty (SPU), with prevailing mud bottom. The average abundance and biomass of oligochaetes were both somewhat higher in the PU than in the SPU lakes (Table 4).

TABLE 4. Average abundance and biomass of oligochaetes in the samples from Polar (n=39) and Subpolar Ural Mountain lakes (n=49).

| | Polar Urals | | Subpolar Urals | |
|----------------|---------------------|--------------------|---------------------|--------------------|
| | ind.m ⁻² | mg.m ⁻² | ind.m ⁻² | mg.m ⁻² |
| average | 1229.4 | 701.0 | 1059.3 | 490.8 |
| ± m | 247.9 | 306.7 | 224.1 | 126.6 |

Proportional abundance of the separate families differed somewhat between regions (Table 5). In PU lakes the Naididae was the most abundant family, forming 53.7 % of all oligochaetes (from 23.1% in Lake Bol'shoe Kuz-ty to 60.2 % in the Lake № 1-B.U.). Among them, *Chaetogaster diaphanus*, *Nais barbata* and, less frequently, *N. pseudobtusa* were most abundant in the PU lakes, while *Uncinaiis uncinata* and *N. variabilis* were the most abundant species in the SPU lakes.

TABLE 5. Average abundance of different families of oligochaetes in the samples from Polar (n=39) and Subpolar Ural lakes (n=49).

| | Polar Urals | | | Subpolar Urals | | |
|----------------------|---------------------|------|----|---------------------|------|----|
| | ind.m ⁻² | % | n | ind.m ⁻² | % | n |
| Naididae | 495.3 | 53.7 | 17 | 186.3 | 26.0 | 10 |
| Pristinidae | 1.0 | 0.1 | 1 | 0.8 | 0.1 | 1 |
| Enchytraeidae | 137.3 | 14.9 | 3 | 253.5 | 35.4 | 6 |
| Tubificidae | 258.6 | 28.0 | 8 | 230.7 | 32.2 | 7 |
| Lumbriculidae | 30.4 | 3.3 | 4 | 44.7 | 6.2 | 6 |
| Lumbricidae | | | | 0.2 | 0.03 | 1 |

On the contrary, Tubificidae and Enchytraeidae were numerically dominant in the SPU lakes (more than 60 % of all oligochaetes). Enchytraeidae were dominant in some lakes (e.g. 93.3 % in the Lake № 2-V). The maximum share of Tubificidae in the abundance (60.8 % of all oligochaetes) was registered in Lake Maloe Balban-ty (the Kozhim River basin). In both PU and SPU lakes, *Spirosperma ferox* was the dominant tubificid. *Tubifex tubifex* and *Lophochaeta ignota* were abundant in SPU lakes only. Enchytraeidae were represented in most lakes mainly by immature individuals (more than 50 %) forming an important part of the oligochaete community. In some lakes juveniles of the genus *Cernosvitoviella* were the dominant taxa. Among Lumbriculidae, *Lumbriculus variegatus* was most abundant in both PU and SPU lakes. *Tatriella slovenica* was found in the SPU lakes only.

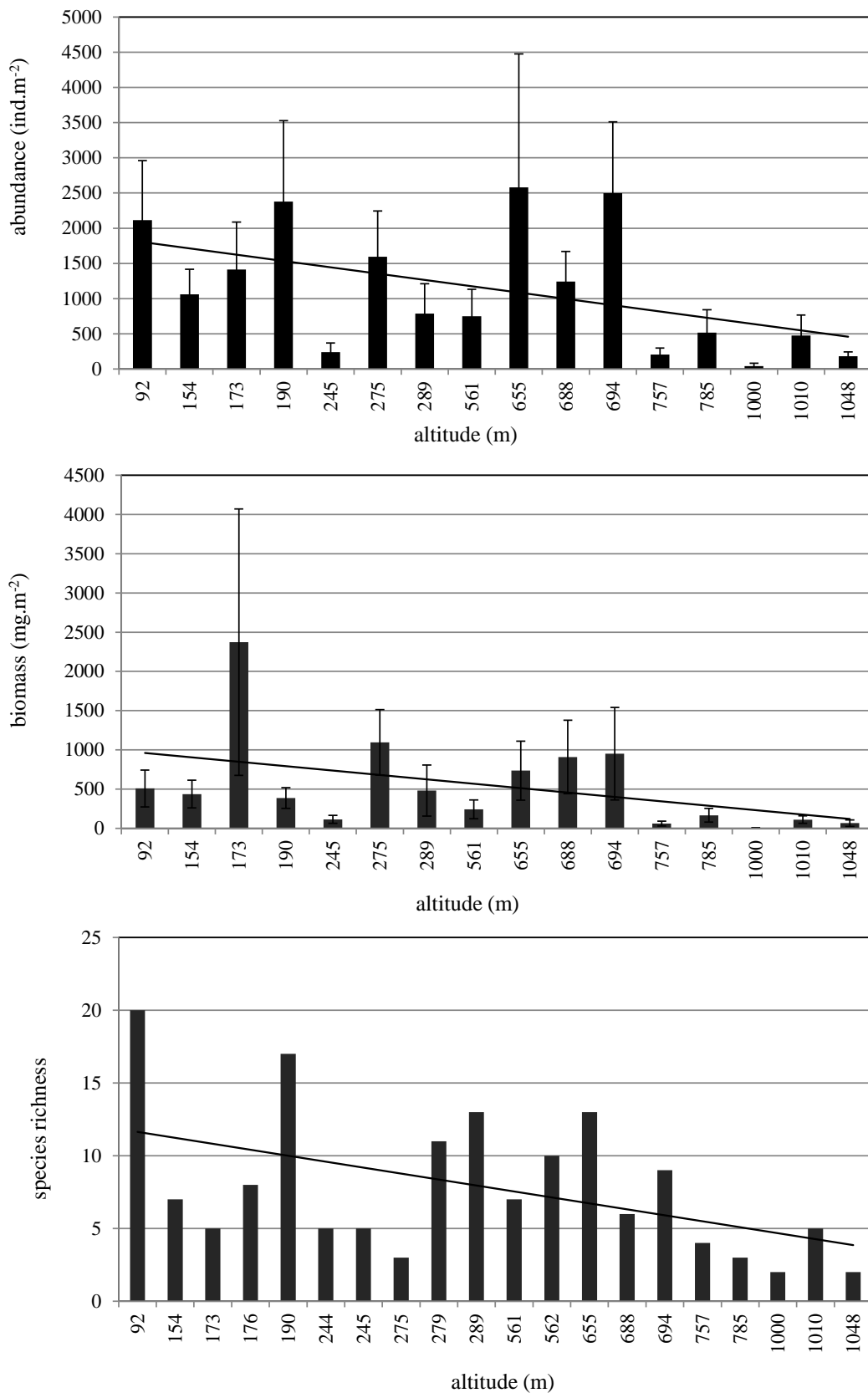


FIGURE 2. Average abundance (top), biomass (middle) and number of oligochaete species (bottom) at different elevations. The lakes located at similar elevations were combined into one group. The mean-square error was calculated from all samples collected from lakes at that elevation. “—” is the linear trend line.

Relationships with environmental variables

Elevation. We found that elevation influences the species composition, average abundance and biomass of oligochaetes in the Urals. All these parameters decrease with elevation (Fig. 2). At the highest elevations, number of oligochaete species was low and structure of communities was distinct. Although Naididae formed more than 50% of the abundance of oligochaetes at elevations up to 300 m a.s.l., this number decreased to less than 20 % in the higher lakes, up to 1000 m a.s.l. in elevation. The share of Tubificidae plus Enchytraeidae at these higher elevations was more than 60 %.

Water depth. Total biomass of zoobenthos and the number of species were significantly lower in mountain lakes where the bottom was formed by boulders, with small amount of mud. Lakes with mud bottoms at depths of 1.5–2.0 m were quantitatively the richest, here the biocenoses were dominated by mollusks and chironomids. The littoral zone in most of the lakes was less productive, yet the zoobenthos was more diverse due to variety of biotopes (e.g. different sediments and the presence of macrophytes). There were usually four dominant invertebrate groups here: mollusks, chironomids, small crustaceans and oligochaetes.

In an earlier investigation of lakes of the Bolshezemelskaya Tundra and foothill area of Urals, we found the most diverse and abundant oligochaete community in the littoral zone, at 0–3 m depth (Baturina & Loskutova 2010). In the present study we compared the distribution of oligochaetes at depths 0–3 m and 3–7 m in the Polar and Subpolar Urals (Figs 3 and 4).

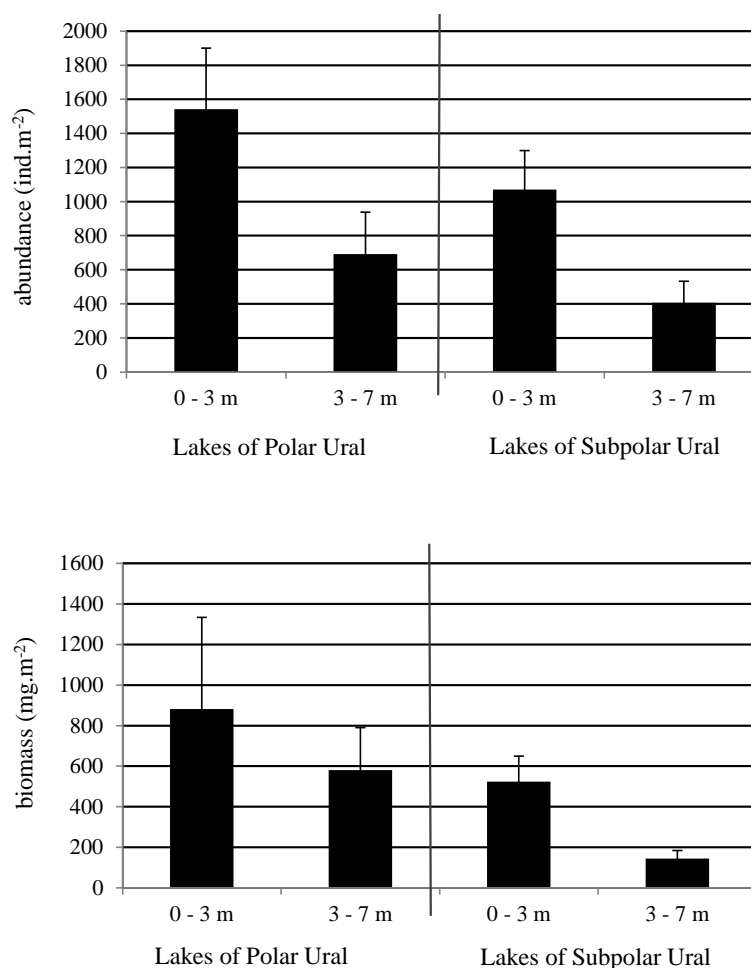


FIGURE 3. Average abundance (top) and biomass (bottom) of oligochaetes in the shallower (0–3 m) and deeper (3–7 m) zone of the Polar Ural and Subpolar Ural lakes.

The abundance and biomass of oligochaetes were also higher in the shallower zone of the lakes and decreased with increasing depth (Fig. 3). In the PU lakes, Naididae (mainly *Chaetogaster diaphanus*, *Nais barbata* and *N. pseudobtusa*) were abundant, representing more than 50 % of oligochaetes in the shallow zone (Fig. 4). The average abundance of oligochaetes decreased in the deeper zone where Tubificidae dominated (*Spirosperma ferox*, *Tubifex tubifex*, *Lophochaeta ignota* and unidentified immature Tubificidae, altogether 75 %).

In SPU lakes, the oligochaete fauna at the depths of 0–3 m consisted mainly of Enchytraeidae (37.0 % of abundance), Naididae (30.3%) and Tubificidae (25.4%) (Fig. 4). Among the Enchytraeidae, juveniles and the genus *Cernosvitoviella* dominated. *Nais variabilis* and *U. uncinata* were the most abundant naidids in the littoral zone of SPU lakes. As in the PU lakes, tubificids became dominant in the deeper zone (more than 80% of average abundance). *Spirosperma ferox* was the most common tubificid present, while *L. ignota* was nearly as common.

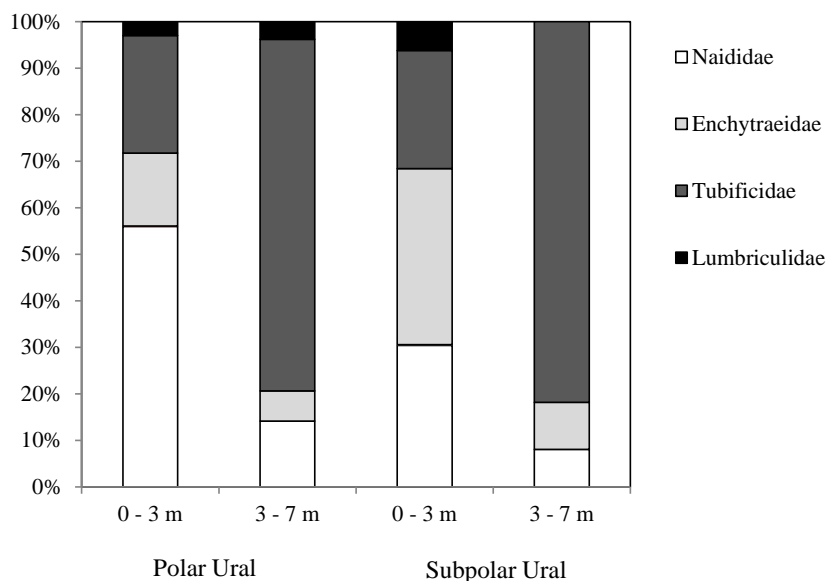


FIGURE 4. Proportional contribution of families to oligochaete abundance in the shallower (0–3 m) and deeper (3–7 m) zone of the Polar Ural (left) and Subpolar Ural lakes (right).

Bottom sediments. The most of studied lakes have soft sediments (mud (silt), and sand) in the deeper zones, but different sediment types in the littoral: either soft or hard (boulders, pebbles and gravel, often covered with algae, moss or silt).

In PU lakes the abundance and biomass of oligochaetes reached their maximum on hard substrata (stones and gravel) (Fig. 5A). Oligochaetes formed here 0.7–50 % of abundance and 0.1–64 % of biomass of the zoobenthos. Naididae, comprising up to 50 % of the total oligochaete abundance (Fig. 5B), were represented by 11 species (57 % of the fauna), of which *Chaetogaster diaphanus* and *Nais barbata* dominated. Enchytraeidae and Tubificidae (mainly *S. ferox* and *T. tubifex*) comprised on average 44% of oligochaete abundance on hard substrata. Mud bottoms both in littoral and deep-water zones were dominated by Tubificidae (62.5% of abundance; *S. ferox* forming more than 40 %). On the sandy bottom in the littoral, *N. barbata* prevailed among the Naididae.

In SPU lakes, average abundance of oligochaetes was similar on all bottom types, while biomass was highest on sand. The faunal composition of oligochaetes differed among the different sediment types (Fig. 5). On hard bottoms in the littoral, Enchytraeidae (mainly immatures and *Cernosvitoviella*) dominated the abundance (39.4%). Naididae (mainly *N. pseudobtusa* and *N. variabilis*) and Tubificidae (mainly *S. ferox*) were similarly abundant (respectively 26.7 and 26.8 %). On the mud bottom, generally in deeper water, Naididae and Tubificidae formed 81 % of total abundance of oligochaetes; with *S. ferox*, *T. tubifex*, *N. variabilis* and *U. uncinata* dominating. In contrast to PU lakes, Tubificidae (*S. ferox* and *L. ignota*) were important (more than 60% of the abundance of oligochaetes) on the sand bottom of littoral zone in SPU lakes.

Canonical Correspondence Analysis. For ordination plot of species (Fig. 6A), lakes (Fig. 6B) and environmental factors the eigenvalue for the first axis was 0.248, the second 0.156 and the third 0.134. The percent variance in species–environment relations was 18.3 for the first axis, 11.5 for the second and 9.9 for the third axis. For the ordination plot of species (Fig. 7A) and lakes (Fig. 7B) and geographical factors the eigenvalue for the first axis was 0.208, the second 0.160 and the third 0.009. The percent variance in species–environment relations was 15.3 for the first axis, 11.8 for the second and 6.8 for the third axis. The statistical significance of the relationships between all species, parameters and all variables was tested by a Monte Carlo permutation test.

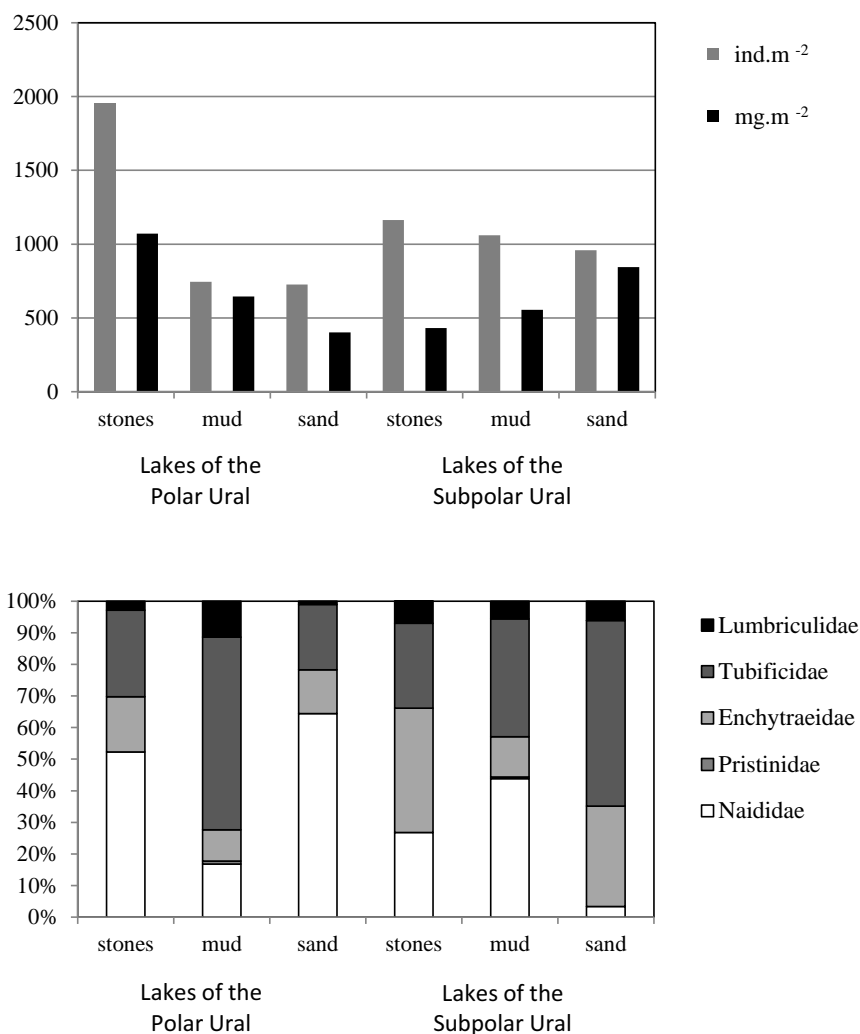


FIGURE 5. Average abundance and biomass of oligochaetes on the different bottom sediments (top) and proportional contribution of families to oligochaete abundance on the different bottom sediments (bottom).

Classification of the environmental conditions in the lakes showed that axis 1 is associated with detritus, substratum and silt (negatively) and macrophytes, moss, algae, sampling depth and maximum depth (positively). The following factors were correlated with axis 2: negatively with detritus, sampling depth and algae, and positively with substrate, macrophytes, moss and maximum depth. A group of lakes (10, 22, 24, 25; 12, 15, 23; 2, 8, 9; 14) was negatively correlated with the axis 1 in CCA (Fig. 6A). These lakes are characterized by hard bottom (boulders) with detritus or silt. The other groups of lakes (Pr. 3, 16, 17, 21; 13; 4, 11, 20 and Pr. 6, 7, 16) had positive relationships with this axis. In these lakes the bottom was often covered either with soft sediments (silt or sand) or boulders with algae or moss. Also, in these lakes the average depths were similar. As for the taxa, biomass, abundance and species number of oligochaetes, the environmental variables on plot (Fig. 6B) showed several groups. Some species (*Cognettia glandulosa*, *Lumbriculus variegatus*, *Spirosperma ferox*, *Nais pardalis*, *Cernosvitoviella* sp., *Tatriella slovenica* and Lumbriculidae spp.), as well as the biomass of all oligochaetes had negative relationship with axis 1. *Cernosvitoviella* sp. and *Tatriella slovenica* plotted in the upper left in Fig. 6B (negative correlation with axis 1 and positive with axis 2) revealed high abundance on the boulders in Lake Trubka (14). The distribution of the other species can be explained by the presence or absence detritus (negative correlation with axis 1 and axis 2). The lower right part of Fig. 6B unites the species depending on presence or absence of algae in the shallow zone of lakes. The groups of species and abundance of Tubificidae, Enchytraeidae, the number of species of Tubificidae, Lumbriculidae and Enchytraeidae on the upper right of Fig. 6B indicate that their distribution is probably influenced by the depth, as well as by presence of moss or macrophytes in the shallow zone of lakes.

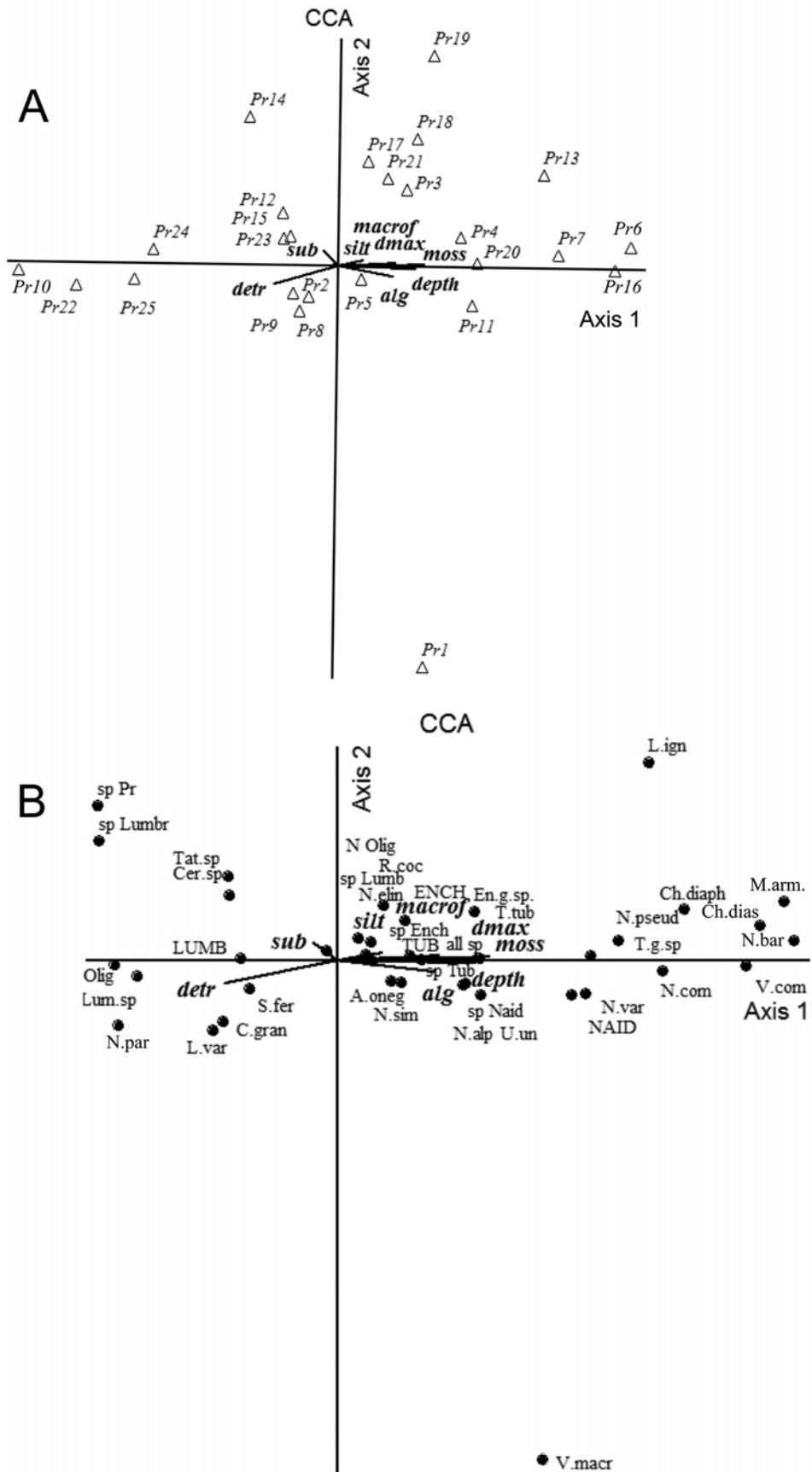


FIGURE 6. CCA diagrams of axes 1 and 2 of the lakes (A), species (B), and environmental factors. Pr. 1–25 are identical with Lakes 1–25 in Table 1. For abbreviations of species see Table 6.

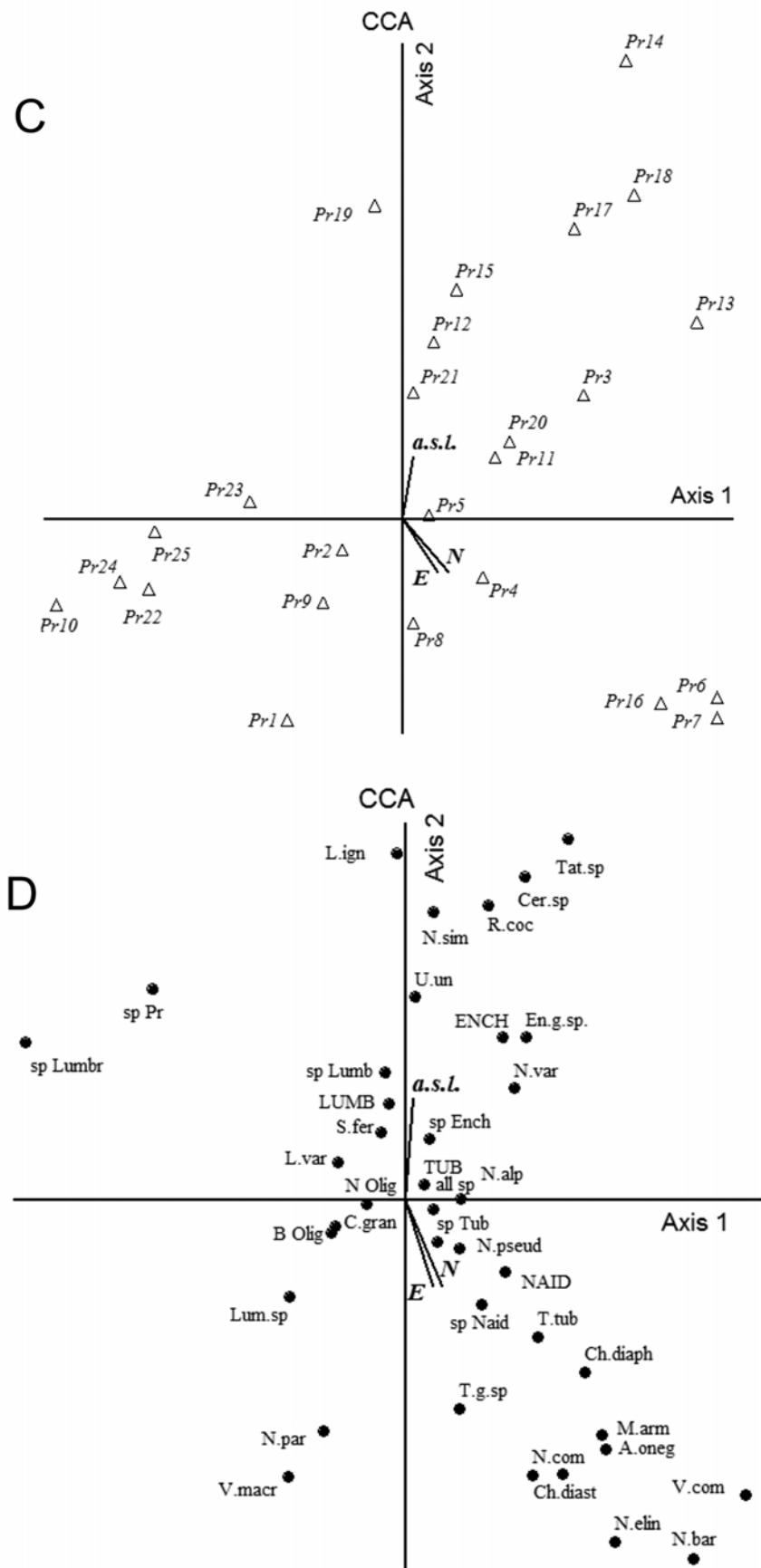


FIGURE 7. CCA diagrams of axes 1 and 2 of the lakes (C), species (D) and geographical coordinates. Pr. 1–25 are identical with Lakes 1–25 in Table 1. For abbreviations of species see Table 6.

The geographical factors were found to be statistically significant: the latitude (N), longitude (E), and elevation above sea level (a.s.l.). CCA indicated a positive relationship between all factors and the axis 1; a negative for the latitude and longitude, and a positive for a.s.l. with axis 2. In the upper right quadrant (Fig. 7A) are the lakes (7, 8, 11, 12, 13, 14) revealing high positive correlation with both axis 1 and 2. Some of these lakes are placed high in the mountains. The lakes 16, 6, 7 (low right quadrant in Fig. 7A) had a negative relationship with axis 1 and positive with axis 2. Among them, the PU lakes Gnet-ty (6) and Koma-ty (7) had very similar coordinates: N 68°08', E 65°21', and N 67°58', E 65°34', respectively. The Lake Forel`noe having negative correlation with a.s.l. CCA showed a negative correlation with both axis 1 and 2 for group of lakes 1, 2, 9, 10 (PU) and lakes 22, 24 (SPU). The elevation of each of these lakes is less than 300 m a.s.l. As for the abundance of oligochaetes and biomass, abundance and number of species, the geographic coordinate variables on plot (Fig. 7B) showed several groups, too. The distribution of *L. ignota*, *Cernosvitoviella* sp., *T. slovenica*, *N. alpina*, *N. simplex*, *N. variabilis*, *U. uncinata* and *R. coccineus* can be explained by the a.s.l. Other species (the lower right quadrant) and the species number of Naididae and Tubificidae, as well as the abundance of Naididae probably depend on the latitude (N) and longitude (E). The third group (lower left in Fig. 7B) was composed of species present in the lower elevation PU and SPU lakes.

TABLE 6. Abbreviations associated with Fig. 6, 7 (Canonical Correspondence Analyses in the Lakes 1–25)

| Variable | Abbreviation |
|---------------------------------|--------------|
| Coordinates: latitude | N |
| longitude | E |
| elevation, m | a.s.l. |
| substratum type | sub |
| silt (presence/ absence) | silt |
| detritus (presence/ absence) | detr |
| moss (presence/ absence) | moss |
| algae (presence/ absence) | alg |
| macrophytes (presence/ absence) | macrof |
| depth (average), m | depth |
| depth (maximum), m | dmax |
| biomass of oligochaetes | B Olig |
| <i>Chaetogaster diaphanus</i> | Ch. diap |
| <i>C. diastrophus</i> | Ch. dias |
| <i>Nais alpina</i> | N. alp |
| <i>N. communis</i> | N. com |
| <i>N. elinguis</i> | N. elin |
| <i>N. pseudobtusa</i> | N. pseud |
| <i>N. variabilis</i> | N. var |
| <i>N. pardalis</i> | N. par |
| <i>N. barbata</i> | N. bar |
| <i>Vejdovskyella comata</i> | V. com |
| abundance of Naididae | NAID |
| Enchytraeidae gen. spp. | Ench gen |
| <i>Cognettia glandulosa</i> | C. gran |
| <i>Mesenchytraeus armatus</i> | Mes. arm |
| <i>Cernosvitoviella</i> sp. | Cer. sp |
| abundance of Enchytraeidae | ENCH |
| number of species Enchytraeidae | sp ENCH |
| <i>Spirosperma ferox</i> | S. fer |
| <i>Lophochaeta ignota</i> | L. ign |
| <i>Rhyacodrilus coccineus</i> | R. coc |
| <i>Lumbriculus variegatus</i> | L. var |
| <i>Tatriella slovenica</i> | Tat. sl |
| abundance of Lumbriculidae | LUMB |
| number of species Lumbriculidae | sp LUMB |

Discussion

Some authors (Lencioni et al. 2004) have observed that literature on the oligochaete fauna of high mountain habitats is scarce. There exist data on streams and lakes in the Scandinavian Mountains (Piguet 1919 a, b); Swiss Alps (Malard et al. 2001); Italian Alps (Dumnicka & Boggero 2007); Carpathians (Hrabě 1939; Dumnicka 1976, 2000); Pyrénées (Juget & Giani 1974); Šar Planina massif (Živić et al. 2010) and Balkan Range (Uzunov & Varadinova 2000) on the Balkan Peninsula; and Eastern Black Sea Mountains in Turkey (Yıldız et al. 2012).

Many authors have regarded elevation as the most important factor influencing the distribution of oligochaetes in the remote alpine lakes across Europe (Dumnicka & Galas 2002; Kownacki et al. 2006; Schenková et al. 2001 and others). A significant correlation was also found between their abundance and the elevation in Turkish lakes (Yıldız et al. 2012). Our study identified several factors (latitude and longitude, elevation, depth, presence of algae or moss) being in significant correlation with the distribution of oligochaetes. In the lakes of Ural situated at up to 500 m a.s.l. (mainly PU lakes), the dominant group by average abundance included Naididae (*Nais barbata*, *N. pseudobtusa*, *Chaetogaster diaphanus*, and others), Enchytraeidae (Enchytraeidae gen. sp.) and Tubificidae (*Spirosperma ferox*). In the higher elevation lakes (mainly in SPU), a community comprised less the dominating Naididae (*Uncinaiis uncinata*, *N. pseudobtusa*) but more Enchytraeidae (*Cernosvitoviella* sp., Enchytraeidae gen. spp.), Tubificidae (*Spirosperma ferox*) and Lumbriculidae.

The oligochaete fauna found in the Ural lakes, with a high number of species, particularly in the family Naididae, is distinguished from that of the other, previously studied European high-mountain lakes that are less diverse, and lower numbers of naidid taxa. Most naidid species are grazers, positively correlated with periphyton (Schenková & Helešic 2006); thus abundance of the Naididae depends on the amount of periphyton. The algae may be particularly lush and productive during the long polar days, supporting summer development of naidids. The number of species and abundance of Naididae decreased considerably in the higher elevation lakes, up to 1000 m a.s.l., suggesting that a complete bottom freezing also may limit the number of naidids because their hibernating individuals and cocoons are sensitive to long-term freezing (Dumnicka & Galas 2002).

The littoral zone of most PU lakes was characterized by variable bottom types (frequently with moss or algal cover) and macrophytes. These conditions support the development of Naididae, particularly *Chaetogaster diaphanus*, *Nais pseudobtusa* and *N. barbata*, which have been found elsewhere in North Europe in similar habitats. The results from CCA showed that distribution of these species, as well as general abundance of Naididae was related to elevation, algae, moss and latitude. In the more stony, shallow littoral areas of the SPU lakes, *N. variabilis* prevailed. However, on the mud bottom in deeper water, *N. variabilis* was also dominant, together with *Uncinaiis uncinata*. Dominance of *N. variabilis* has been also shown in other high-mountain lakes of Europe (Piguet 1919; Hrabě 1939; Dumnicka & Boggero 2007).

Enchytraeidae was the most frequently encountered family in the European alpine water bodies. In particular, the genus *Cernosvitoviella* has been found both in mountain streams and standing waters; some authors have described increasing abundance of *Cernosvitoviella* with increasing elevation (Giani 1979; Dumnicka & Galas 2002; Dumnicka & Boggiero 2007). On the contrary, this genus was not found in the high-mountain lakes of East Siberia studied by Kaygorodova et al. (2012). In our investigation, *Cernosvitoviella* was the most frequently encountered genus of Enchytraeidae in the SPU but not in the PU lakes. Abundance of *Cernosvitoviella* sp. as well as the species number of Enchytraeidae depends on several parameters of environment (for example, substratum and elevation).

Some authors relate the distribution of Tubificidae to their preference for substrata with high organic content and fine particle size (Schenková et al. 2001). Among tubificids, *Spirosperma ferox* prevailed both in PU and SPU lakes, mainly on soft bottom. This species, as well as other tubificids, have been recorded as most abundant in sediment with medium silt content in Germany (Sauter & Güde 1996), but other authors have described *S. ferox* as an opportunistic species in high mountain ecosystems, having wide adaptive abilities (Dumnicka & Galas 2002). The distribution of *S. ferox* has been positively correlated with elevation and negatively with water depth and dissolved oxygen in Turkish alpine lakes (Yıldız et al. 2012). According to our data, abundance of *S. ferox* was positively associated with elevation and presence of detritus, and negatively responded to presence of moss and algae. The tubificids *S. ferox*, *Tubifex tubifex* and *Lophochaeta ignota* prevailed in different biotopes, especially in the SPU lakes. These species are represented in all studied mountain massifs of Europe.

Lumbriculidae is a thermophobe family of the northern temperate zone (Timm 1980; Popchenko 1988). *Lumbriculus variegatus* has been found as negatively correlated with temperature and positively with water depth in Turkish lakes (Yıldız et al. 2012). In our study, *L. variegatus* as well as the species number of Lumbriculidae were negatively correlated with some unknown environmental factors. Another lumbriculid, *Tatriella slovenica*, was positively correlated with elevation. *T. slovenica* is known in Europe from a few localities and in small number only (Dumnicka & Galas 2002).

Acknowledgements

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Where have all the "vejdovskies" gone (*Potamothrix vej dovskyi* Hrabe)? Ponto-Caspian tubificid oligochaete species in Lake Mälaren, south-central Sweden, in a 100 year perspective

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Abstract

Potamothrix vej dovskyi is one of several alien Ponto-Caspian species presently invading Europe and the New World. Its invasive success has been suggested to be dependent upon the trophic degree of the water, and this species is considered to be particularly indicative of mesotrophy (Lang & Reymond 1996). Bottom fauna surveys performed by the Swedish Fisheries Board in 1915 and 1916 and in 1933–1935 did not reveal the existence of *P. vej dovskyi* in Lake Mälaren, whereas extensive bottom fauna programs in the lake in 1967–1976 demonstrated a wide distribution of the species in the central, least polluted basins. Strict quantifications of the distribution of oligochaetes in those basins in 1974 showed that *P. vej dovskyi* then made up on average 28 % of the total abundance of oligochaetes. However, in later bottom fauna surveys in Lake Mälaren, performed by the Swedish Environment Protection Agency in 1981 and in 2009–2011), *P. vej dovskyi* could not be found at all. Sewage treatment around Lake Mälaren accelerated in the late 60's and the phosphorus load is nowadays considerably lower than before treatment (about 30 % less in terms of total-P concentrations in the water). The question of why this species seems to have more or less disappeared from the lake remains unsolved.

Key words: Ponto-Caspian species, alien species, invasions, *Potamothrix vej dovskyi*, Lake Mälaren, distribution

Introduction

The present European distribution of several alien Ponto-Caspian oligochaete species of the genus *Potamothrix* originating in the Black Sea-Caspian Sea areas is today fairly well-known (Milbrink 1999, Milbrink & Timm 2001). Modes of dispersal are also discussed in these works. In most cases the world distribution of the same species is more uncertain, due to the fact that the oligochaete fauna in great parts of the world is on the whole little known. Most characteristically, however, several *Potamothrix* species have managed to cross the Atlantic Ocean and reached the Great Lakes of North America. It is only natural to think of such east-west dispersal via the ballast water of ships. In other cases there is a most typical north-south gradient in the dispersal of the same species. Some Ponto-Caspian *Potamothrix* species have actually reached localities in South-America and other species places on the African continent. In those cases birds (see below) are likely to be the vectors of dispersal (Milbrink, 1999).

Most of these Ponto-Caspian oligochaetes are known to be particularly invasive once natural physical obstacles like mountain ridges and oceans have been crossed. The massive construction of canals for transportation in the 19th century, the regulation of lakes and major rivers for energy production, and, in the 20th century, the use of ballast water tanks in large vessels, has greatly facilitated dispersal (Bij de Vaate *et al.* 2002).

Whereas the world distribution and modes of dispersal of *Potamothrix heuscheri* and *Potamothrix moldaviensis* over the world have previously been described, not much, however, has been said about *Potamothrix vej dovskyi* in that respect although briefly commented upon in Milbrink & Timm, 2001. This paper is devoted to the known presence of *P. vej dovskyi* in Lake Mälaren in southern Sweden between 1966 and 1980. After that period there are no records at all from Sweden of this species. Why is that so?

Results

World distribution of *P. vej dovskyi* and other alien species of the genus *Potamo thr ix*

All available world distribution data on alien species of the genus *Potamo thr ix* have been compiled by Dr. Tarmo Timm, Tartu University, Estonia, and transformed into world maps, such as for *P. hammoni ensis* (Fig. 1) and *P. vej dovskyi* (Fig. 2). Similar distribution maps for *P. heuscheri* and *P. moldavi ensis* have previously been published in Milbrink 1999 and Milbrink & Timm 2001, respectively. The distribution pattern covering Europe (and parts of Western Asia) is rather similar for several *Potamo thr ix* species, whereas the world distribution of the different species vary considerably.

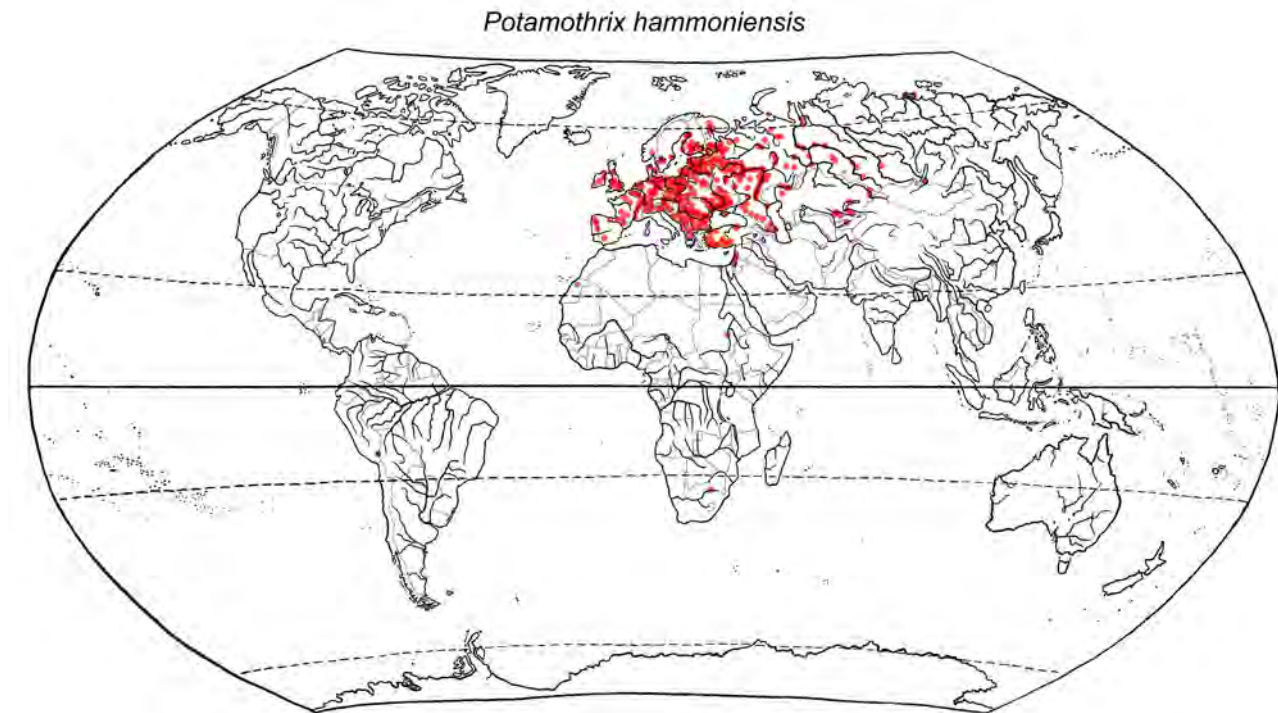


FIGURE 1. World distribution of *Potamo thr ix hammoni ensis* (courtesy Dr. Tarmo Timm).

The world distribution of *P. hammoni ensis*, by far the most frequently occurring *Potamo thr ix* species in Europe, covers most of Europe with extensions far into Asia (Fig 1). This species is steadily on the move to the north and north-west but has not reached north-western Scandinavia (Milbrink, 1999) and obviously not yet crossed the Atlantic Ocean. There is, however, one slightly mysterious record from the New World—Lake Titicaca in Bolivia—Peru (Juget & Lafont 1994) and there are also records from the Middle East and from three localities in Africa. In that respect its distribution pattern reminds of that of *P. heuscheri* which has also been found in Lake Titicaca (Juget & Lafont 1994) and in a few localities in Africa (Milbrink, 1999). *Potamo thr ix vej dovskyi* (Fig. 2), the target species for this study, seems to be fairly much bound to Europe. Though not shown in Fig. 2 there is, however, one record of this species from Ethiopia (Dr. Tarmo Timm, personal information). *Potamo thr ix moldavi ensis*, *P. vej dovskyi*, *P. bedoti* and *P. bavari cus* have all also reached the Great Lakes of North-America. *P. bavari cus* has an even wider distribution in the U.S.A. than the other species mentioned, and there are also records from Australia and surrounding islands.

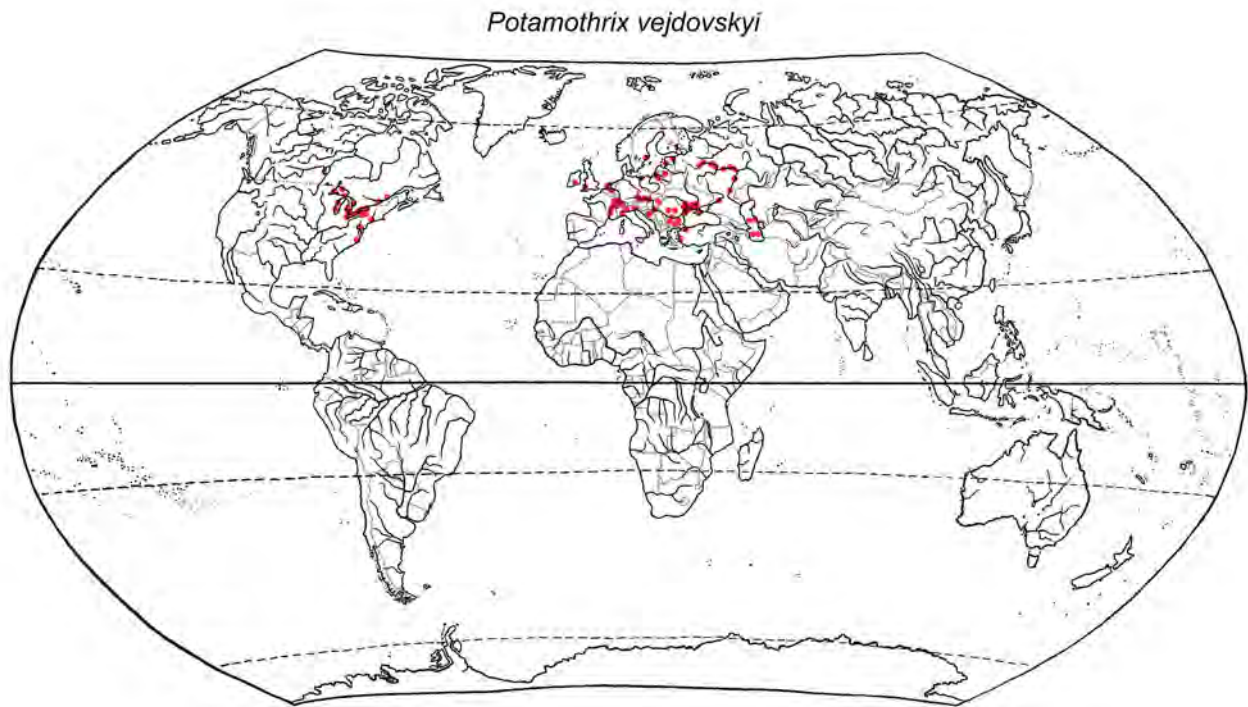


FIGURE 2. World distribution of *Potamothenix vej dovskyi* (courtesy Dr. Tarmo Timm).

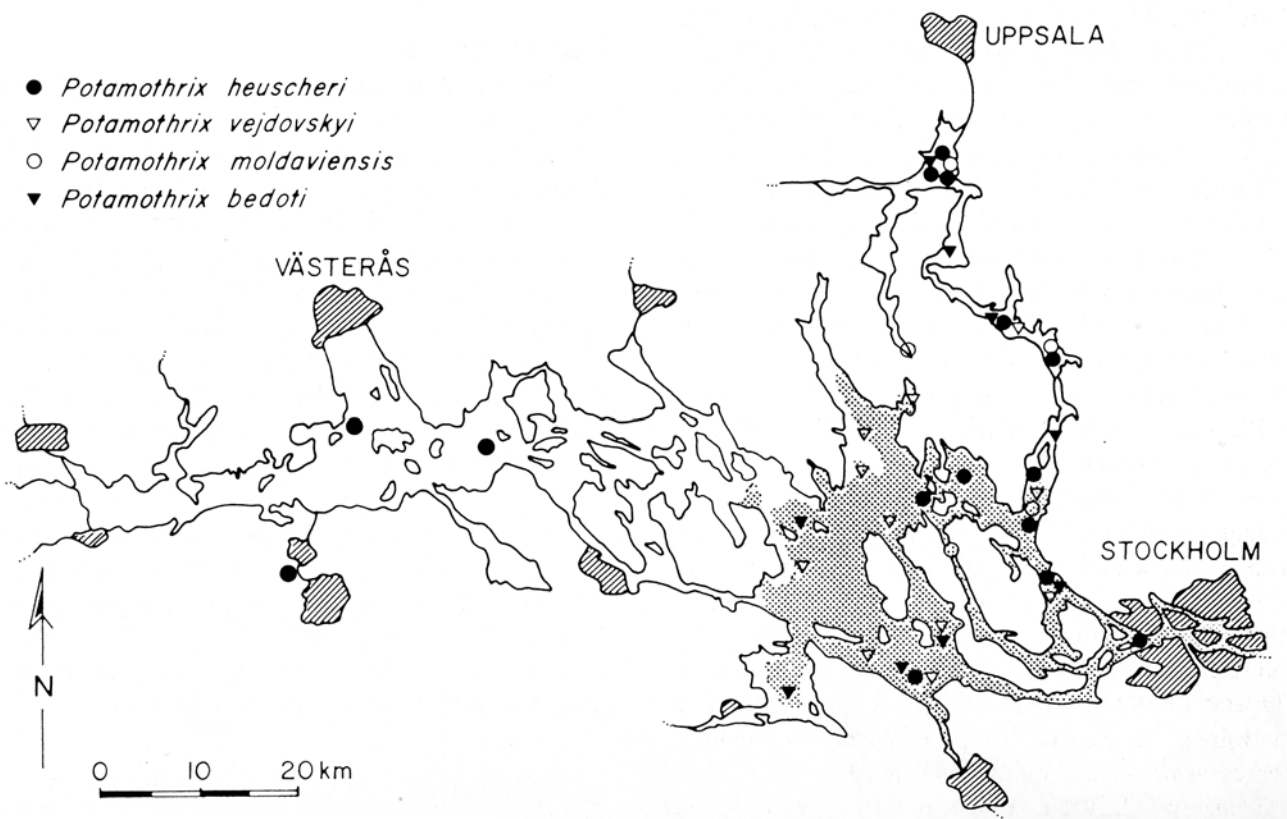


FIGURE 3. Distribution of all Ponto-Caspian species of the genus *Potamothenix*, except *P. hammoniensis* (species frequently occurring nearly everywhere in the lake) found in Lake Mälaren in the early 1970's. The full extension of brackish water in the profundal in the 1930's is indicated in grey (Dr. Sten Vallin, personal information; from Milbrink, 1980).

The Swedish distribution of *P. vej dovskyi*

In several early faunistic surveys over Lake Mälaren, i.e. the first ones in 1915 and 1916 and later in 1933–1935, *P. vej dovskyi* was not present (Milbrink, unpublished). In 1966, however, this species was suddenly present all over the central, northern and eastern basins (Milbrink 1999). *Potamo thrix vej dovskyi* is likely to have reached Lake Mälaren in the 1950ies. The known distribution of all species of the genus *Potamo thrix* in Lake Mälaren in the early 1970's (except *P. hammoni ensis* which is abundantly present everywhere in the lake) is shown in Fig. 3. As an illustrative example of the fairly dense populations of *P. vej dovskyi* in the central basins of Lake Mälaren around 1970 the following study in the basin of Hovgårdsfjärden may be mentioned (Milbrink *et al.* 1974). It was primarily a study on the horizontal distribution of the oligochaete fauna. Accordingly, in February 1970 a grid measuring 100 m x 50 m was marked on the ice over a mudflat at a depth of 30 meters, and tube bottom and Ekman grab samples were randomly taken within the grid (Fig. 4). Thus in all 38 tube bottom samples (aperture about 38cm²) and 6 Ekman grab samples (aperture about 225 cm²) were obtained. Here *P. vej dovskyi* made up about 28% of all oligochaetes, *P. hammoni ensis* about 34%, *Limnodrilus hoffmeisteri* about 8%, *Spirosperma ferox* and *Psammoryctides barbatus* about 2–3% each, etc. (Fig. 5). In total, no less than 19 different oligochaete species were recorded. *P. vej dovskyi* was actually present in all samples obtained except one. The specific horizontal distribution of oligochaetes was in accordance with the negative binomial, i.e. a slightly aggregated distribution, whereas chironomids showed a random (Poisson) distribution (Milbrink *et al.* 1974). High species diversity and presence of the sensitive species *S. ferox* and *P. barbatus* reveal mesotrophic conditions so characteristic of the central basins of Lake Mälaren.

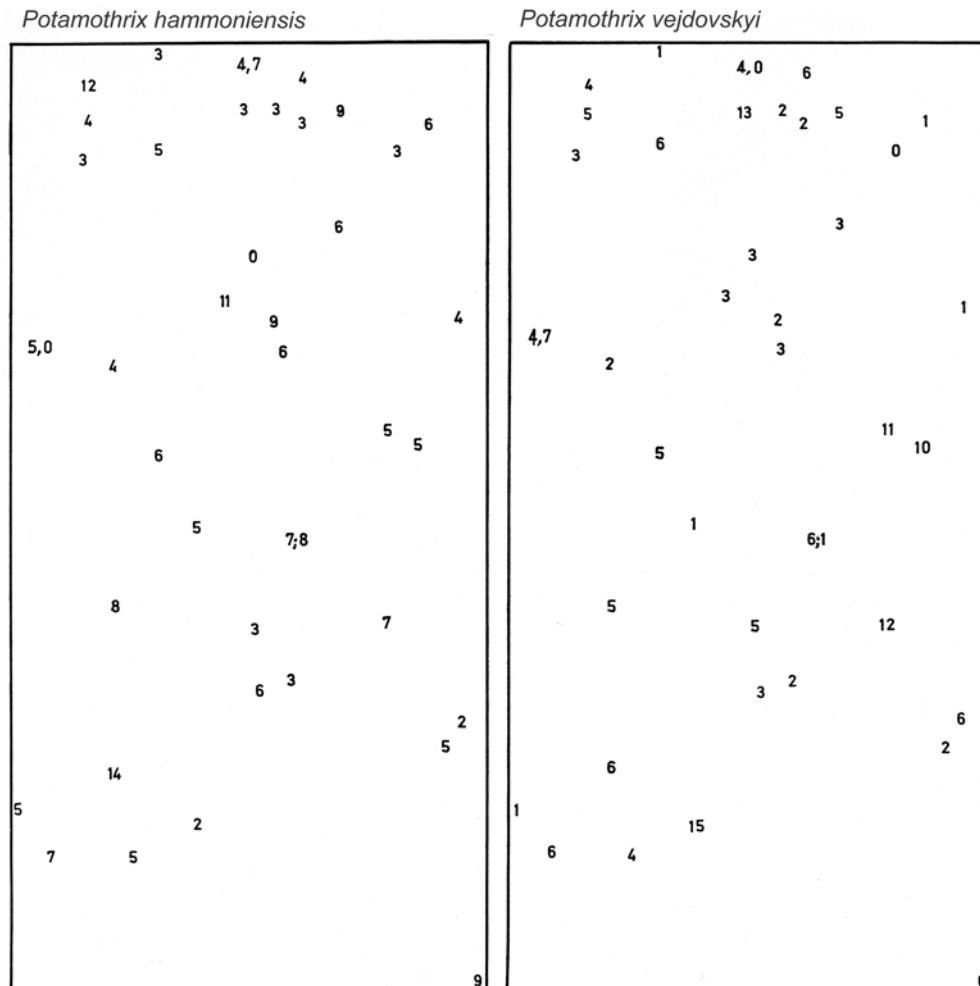


FIGURE 4. Horizontal distribution of the Ponto-Caspian oligochaete species *Potamo thrix vej dovskyi* and *Potamo thrix hammoni ensis* in a grid measuring 100 x 50 m² in the basin of Hovgårdsfjärden in central Lake Mälaren. Samples obtained with a core sampler (38 samples) and the larger Ekman grab sampler (6 samples) at a depth of 30 meters. The total numbers of oligochaetes in the same samples are also given in this figure. For strict comparisons abundance values obtained with the Ekman grab sampler are divided by a factor of 6 (from Milbrink *et al.* 1974).

Potamothenrix vej dovskyi had its strongholds in the central basins, such as in Prästfjärden and Södra Björkfjärden. Abundance in the profundal was generally high and steadily around 30% of all oligochaetes (see below). Even in 1980 abundance was constantly high, but thereafter no specimens of *P. vej dovskyi* have been observed in the lake or elsewhere in Scandinavia. Other species of the genus *Potamothenrix*, however, are still present in Lake Mälaren after 1980 (Milbrink, unpublished).

Potamothenrix vej dovskyi has been found to be rather erratic in its appearance in Sweden, Switzerland and France and has mostly been associated with mesotrophic conditions (Lang & Reymond 1996). Modes of dispersal of the Ponto-Caspian oligochaete fauna are further discussed below.

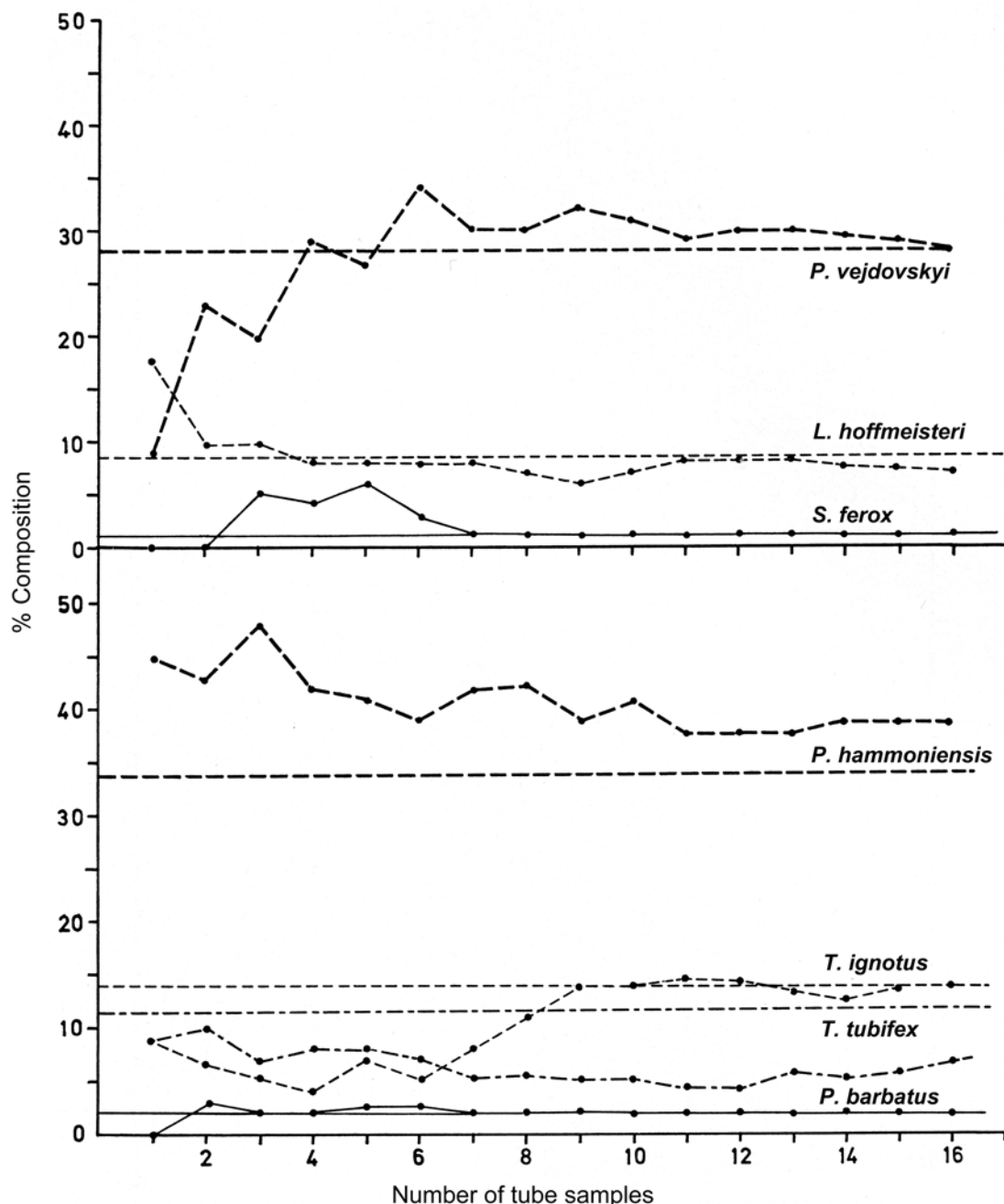


FIGURE 5. Percentage composition of oligochaete species inside the sampling net (Fig. 4) for different numbers of tube bottom samples (accumulative values). Horizontal lines are the percentages obtained after 38 samples (from Milbrink *et al.* 1974).

Discussion

Distributional pathways for species of the genus *Potamothenix* and modes of dispersal of the Ponto-Caspian tubificid oligochaete fauna

According to Timm (1980) and Milbrink & Timm (2001) *P. hammoniensis* was one of the first species to disperse from the Black Sea-Caspian Sea area over Eurasia in a first invasion wave. *Potamothenix heuscheri*, *P. moldaviensis*, *P. bavaricus*, and *P. bedoti* are supposed to have dispersed over the world in a second invasion wave. *P. vejdvoskyi* is likely to be in the midst of a third wave rather than in the second wave (Milbrink & Timm 2001) presently having reached Lake Mälaren in Scandinavia and, for example, lakes in Switzerland and France (Lang 1994; Lang & Reymond 1996).

Most Ponto-Caspian species of the genus thus originated in the Black Sea-Caspian Sea area and have gradually dispersed over Europe along three main pathways described by, for instance, Bij de Vaate *et al.* (2002) primarily for amphipod species—the Northern, the Central and the Southern Pathways. The Northern Pathway goes to the north-west along Rivers Don and Volga, the Central mainly follows Rivers Dnjeper, Bug, Wistula, etc. and via canal systems reaches the River Rhine estuary in the Netherlands. The Southern Pathway stretches mainly to the west along Rivers Danube and Rhine. There are obviously also other pathways facing south over Turkey and Italy.

There is little doubt that the Ponto-Caspian fauna has spread over Europe via major rivers and canals mainly dug in the 19th century. Several species of amphipods—in all about 20—have thus spread in a "Central corridor" and via a number of waterways connecting Rivers Dnjeper and Rhine reached Holland and there partly outcompeted the indigenous fauna (Bij de Vaate *et al.* 2002). The ballast water of ships trafficking these rivers and dense networks of connecting canals has, of course, greatly facilitated the dispersal of the Ponto-Caspian fauna. Carlton & Geller (1993) and Hallegraeff & Bolch (1991) have stated that the ballast water of ships is perhaps the overall most efficient agent of dispersal of aquatic invertebrates and planktonic algae. We are talking about "conveyor belts" across, for instance, the Atlantic Ocean (Carlton & Geller, 1993). Centres of further dispersal of oligochaete species are harbours—often in estuaries, where ballast water is likely to be exchanged. Ballast water often containing stirred sediment and most likely live oligochaetes and cocoons is thus pumped into ships which after having reached distant harbours is again exchanged (Timm 1980). Timm has beautifully described the gradual dispersal of the oligochaete species *Tubifex (Isochaetides) newaensis* in Russia via Rivers Don and Volga eventually reaching the Finnish Bay in the Baltic Sea.

The profundal of the central and eastern basins of Lake Mälaren was not long ago connected with the Baltic Sea (indicated in grey in Fig. 3), and there are still some brackish water invertebrate species of other phyla present in the bottom fauna (Milbrink, 1980). The Ponto-Caspian *Potamothenix* species are believed to have reached ports in Lake Mälaren via ocean-going ships from ports in the Baltic states.

Passive dispersal via harbours and the ballast water of ships has naturally made "sudden leaps" across the Atlantic possible. Since the Great Lakes of North-America have since long been fully accessible for ocean-going ships, there have been no physical obstacles for the Ponto-Caspian fauna (four *Potamothenix* species so far) to reach these lakes (Ricciardi & MacIsaac, 2000). Other distant destinations like the Australian continent, Tasmania and New Zealand are also likely to have been reached by ships (*P. bavaricus*).

Some destinations for Ponto-Caspian oligochaete species could not, however, be explained by ships as vectors of dispersal, since the physical obstacles for dispersal are so huge, for instance Lake Titicaca in South-America (*P. hammoniensis* and *P. heuscheri*; Juget & Lafont 1994), various inland sites in Africa—Kenya, Congo, Botswana (*P. heuscheri*) and Ethiopia (*P. vejdvoskyi*), etc. (cf. Milbrink, 1999). The author feels inclined to suggest migratory birds, especially wading and swimming birds, as vectors of dispersal (Milbrink 1999). Here "stepping-stone" dispersal along north-south gradients is most likely. There could thus be a logical explanation for the presence of *P. heuscheri* in Lake Tiberias in Israel, which is a well-known resting spot for wading birds on their yearly migration to East Africa and further south. The same explanation may go for the dispersal of oligochaete species in inland South-America, such as in Lake Titicaca in Bolivia-Peru (*P. hammoniensis*, *P. heuscheri*; Juget & Lafont 1994). Based on laboratory experiments performed by the present author not only the cocoons of oligochaetes but also live young specimens could be carried in droplets of water contained in the plumage of migratory birds even over fairly long distances, provided temperatures are low enough (around +4°C) at cruising altitudes (Milbrink, unpublished).

Possible explanations to the sudden disappearance of *P. vej dovskyi* in Lake Mälaren

In the author's opinion there are no risks for misidentifications involved, or this species having been overlooked at any time. The setal set-up is so very characteristic with all bifid setae having equally long broad prongs and dorsal hair seta being short and characteristically bent. The setal bundles are also organized in fan-like arrangements easy to observe (Fig.6).

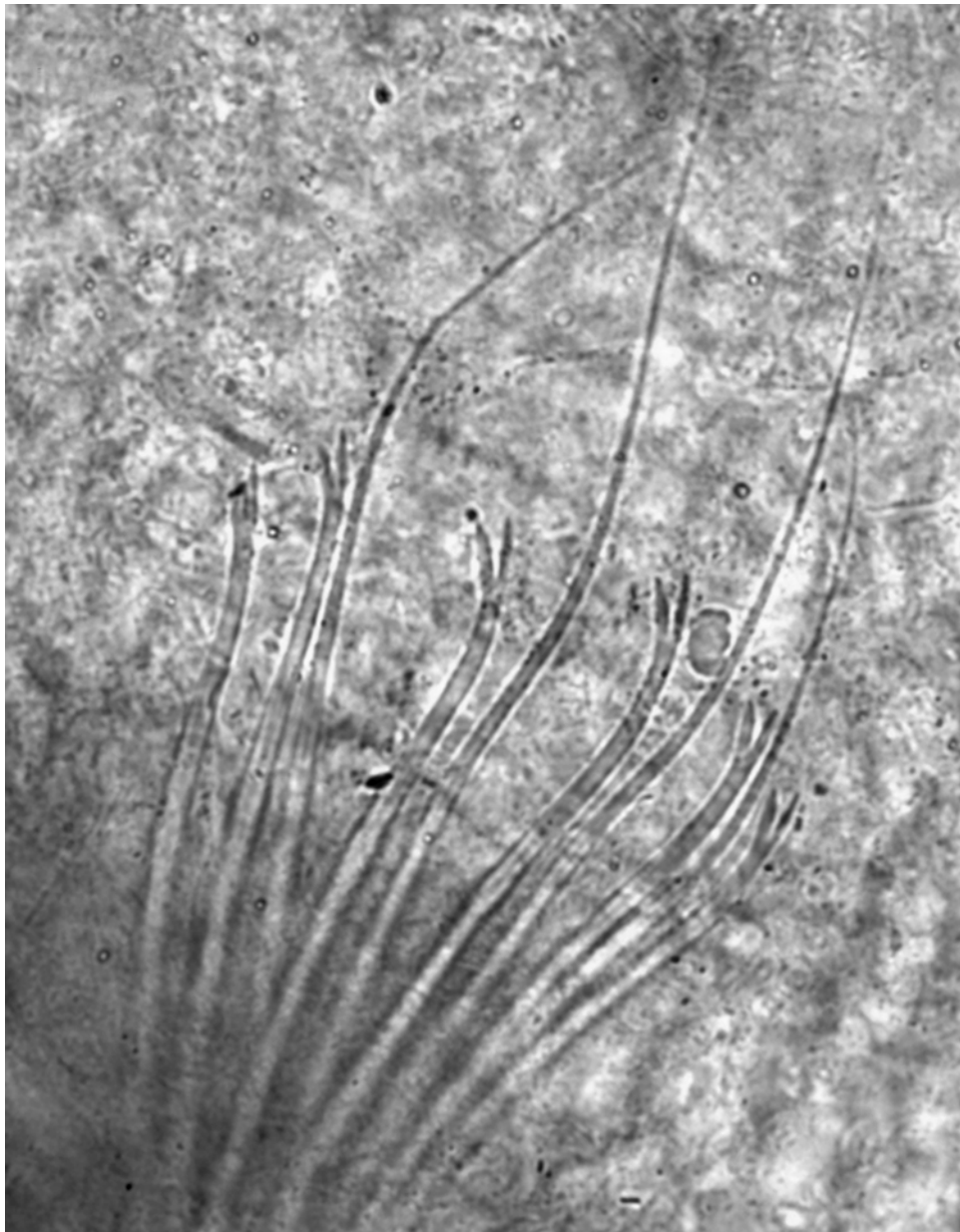


FIGURE 6. Dorsal setae of *Potamothenis vej dovskyi* from central Lake Mälaren in 1967 (photo G. Milbrink).

We know from the Lake of Geneva and Lake Neuchatel in Switzerland that *P. vej dovskyi* may be quite erratic in its appearance almost disappearing in localities affected by efficient sewage treatment (Lang & Reymond 1996). *P. vej dovskyi* may be particularly sensitive to changes in water quality over time such as has taken place in the large lakes in South Sweden. Eutrophication of Lakes Mälaren, Vänern and Vättern culminated in the 1960s and 1970s, after which conditions have improved fairly rapidly due to general sewage treatment. As said before *P. vej dovskyi* is mostly associated with mesotrophic conditions (Lang & Reymond 1996).

There are no indications from Lake Mälaren of Ponto-Caspian species excluding other indigenous species by species segregation for common nutrient resources. On the contrary there may actually be positive mutualistic interactions involved, because wherever *P. vej dovskyi* occurred in Lake Mälaren—often in great abundance—other species were mostly also abundant (Milbrink, unpublished). There are indications that

similar effects could locally be seen in the Lake of Geneva (Lang & Reymond, 1996). Laboratory experiments with *P. hammoniensis*, *L. hoffmeisteri* and *Tubifex tubifex* from the basin of Görvåln in eastern Lake Mälaren have actually given results that suggested possible mutualistic interactions with references to bacterial resources involved (Milbrink 1993), similar to results obtained with species assemblages from Toronto Harbour in Ontario in Canada (Wavre & Brinkhurst 1971). Such mutual advantages would rather promote higher abundance than the opposite.

In the disappearance of *P. vej dovskyi* there might be viruses or pathogens involved. *Potamoatrix hammoniensis* and *P. vej dovskyi* were both quite often parasitized by the cestode *Caryophyllaeus laticeps* in Lake Mälaren in the 1960s and 1970s (Milbrink 1975; Milbrink, unpublished), and other parasites we know little of may be involved and affect survival.

Could the fact that the number of stations visited each year by the Swedish Agricultural University for bottom fauna sampling has been reduced considerably during the last decades be of significance, and could therefore *P. vej dovskyi* still be present, but now in low abundance? Again in the author's opinion this scenario is not very likely since at least one of those stations visited on a yearly basis is in Prästfjärden—one of the deep central basins of Lake Mälaren, where this species was always found between 1966–1980.

It may be a matter of sheer chance. Species may come and go. However, there are no indications that other alien Ponto-Caspian species have fluctuated in abundance that way in Lake Mälaren. Why only *P. vej dovskyi* in that case? A future comprehensive bottom sampling program carried out in the central basins of Lake Mälaren would certainly reveal whether there are still remaining populations of *P. vej dovskyi* in the lake.

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