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Distribution of non-indigenous tubificid worm *Branchiura sowerbyi* (Beddard, 1892) in Bulgaria

ABSTRACT

The first record of *Branchiura sowerbyi* in Bulgaria was in 1964 at a fish farm on Belene Island (576-560 R. km) in the Danube River. During the last 50 years this species has become established downstream to the 530 R. km of the Danube River and has spread rapidly upstream of nearly all the Danube tributaries. Nowadays the distribution of this species is enlarged and is widespread in the Danube River Basin and the West-Aegean River Basin of Bulgaria. In the Black sea River Basin and the East-Aegean River Basin the species was found in isolated locations. As a component of the benthic macrozoobenthos of mostly standing waters, the species was found at altitudes in the range between 15 m a.s.l. (Danube R., u/s Russe = at Lyulyak Island, 496 R. km) and 950 m a.s.l. (Iskar R., u/s Samokov). The pathways of introduction and potential negative impact of the species to the native ecosystems and fish population were discussed.

Key words: *Branchiura sowerbyi*, invasive alien oligochaeta worm, distribution, Bulgaria

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Introduction

During the last century the scientific interest in the human-aided dispersal of species beyond their natural range of distribution has increased. The establishment and consequences of introduced alien species have been the objects of discussion since they are a large and quickly increasing threat causing dramatic changes in many ecological systems worldwide (Guveritch & Padilla, 2004; Didham et al., 2005). The aquatic ecosystems are among ones that suffer the most damages from this kind of distribution. All non-indigenous species are potentially harmful unless it is proven that the risks involved are low or none (Gollasch, 2006). One of the foremost scientific tasks is to predict the outcomes of the introduction of a particular species and the impact of invasions in general to specific ecosystems. Therefore, every finding of a non-indigenous species and the effort to understand the ways of their transportation, introduction, establishment and spread is valuable.

Beddard (1892) found and described *Branchiura sowerbyi* for the first time in the mud of the Victoria Regia tank in the Royal Botanical Society's Gardens, Regents Park in London (UK) (Beddard, 1892). This species is generally thought to be native to tropical and subtropical Asia (Mills et al., 1993) and was first identified in South-East Asia by Brinkhurst (1969). During the past century the species was found all over the world. Nowadays it can be found in 23 European countries (http://www.faunaeur.org/) where it occurs usually in shallow, warm, stagnant or slowly flowing waters. Aside from this *B. sowerbyi* has been found in some large European rivers as Rhone, Sava, Oder (Tockner et al., 2009) and is accepted as invasive by Raposeiro (2009). In the Elbe R. the worm was recognized as an introduced alien species (Nehring, 2006). In the Rhine R. the species was found for the first time in 1961 and with the opening of the Main-Danube canal in 1992, one other route for the exchange of biota between the Rhine R. and the other biogeographical regions was established (Bernauer & Jansen, 2006). Nowadays Arbačiauskas et al. (2008) declare that the species is recorded in the middle Danube R., along the entire lower Danube R. and in surveyed tributaries. According Uzunov (unpublished data) the species was represented in the macrozoobenthos of the Srebarna biosphere Reserve, located on the right Danube bank in North-Eastern Bulgaria.

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The next studies show a significant change in the species composition of the bottom macroinvertebrate community in comparison with the data of preceding studies (Michev et al., 1998 after Uzunov's unpublished data). 118 species recorded in the references concerning Srebarna Biosphere Reserve were not found recently and *B. sowerbyi* is one of them (Uzunov et al., 2001; Varadinova et al., 2011).

In Bulgaria the first record of *B. sowerbyi* was in 1964 (Dimitrov, 1966) in the benthos of the Danube R. In later studies the species was found in one site in the Danube R. and one in the Struma R. (Uzunov, 1976). Nowadays it can be found along the entire Bulgarian sector of the river (Figure. 1). In research carried out in 1987 in the Kresna Gorge Uzunov & Varadinova (2001) identified the species for the first time in this area of the river. In the Greek sector of the Strymonas R. (Struma R.) the species was registered for the first time in 2008 (Grabowski & Jabłońska, 2009) with abundance of 56 individuals on the sampling point near Neo Petritsi village in Serres Prefecture of Greece, which is close to the Bulgarian-Greek border.

In Serbia the worm was reported for the first time in 1972 in a fish pond in Vojvodina by Pujin (1978, after Paunovic, 2005) and now it is widely spread in high density (Paunovic, 2005). Nowadays the species is one of the constant-dominants in the macrozoobenthos of the dam lake Iron Gate I (Popescu-Marinescu, 2004). The species was reported as a non-native species with high frequency in the Serbian section of the Danube R. (Paunovic et al., 2008). In Greece the species was unknown till 2008 and reported by Grabowski & Jabłońska (2009). The species presence has never been reported in Turkey, Albania and Macedonia.

The present distribution of the tropical tubificid species *B. sowerbyi* in warm-water effluents in Europe and North and South America can be a result of the import of tropical plants to botanical gardens, from which waste-waters eventually could reach the recipients (Brinkhurst & Jamieson, 1971). European inland waterways have provided opportunities for the spread of non-native aquatic species (Panov et al., 2009) and in this connection Minchin et al. (2007) identified some of the main pathways of invasive alien species introduction in Europe as shipping, canals, wild fisheries, culture activities, thermal pollution and even research and education activities.

B. sowerbyi can be assumed a non-indigenous species that fits the Williamson's "Tens rule" (Williamson, 1996) as an introduced species, not only established but also spread in a non-native continent. The introduced alien species are a big threat to the aquatic ecosystems and their natural inhabitants. In this connection the aim of this study is to point out the progressive dispersal of the non-indigenous species *B. sowerbyi* in Bulgaria during a 50 years period of time. Only after the question of distribution and adaptation of the species is answered it will be possible to discuss different ways of early notification and environmental protection from invasive species.

Materials and Methods

The study was based on long-term investigations on the oligochaete fauna in Bulgaria, from 1964 until 2011 and used data mostly on the distribution of the species rather than its abundance. The material was collected by the authors and also provided by colleagues from the former Institute of Zoology (BAS), now merged into the Institute of Biodiversity & Ecosystem Research (Table 1). Part of the presented data has been included into the Vol. 7 of the Catalogus Faunae Bulgaricae (Uzunov, 2010) and involved collections up to 2008. The investigation was performed at main watercourses, as well as at tributaries and reservoirs in that country, in periods of high (April-June) and low water conditions (August-November). The material was obtained using a qualitative Petersen bottom sampler (ISO 9391:1993 compatible with the international standards EN ISO 9391:1995) and a quantitative hand-net sampling in accordance with ISO 7828/1985 (compatible with the international standards EN 27828:1994).

Recently published data were enriched with some new findings and unpublished data. In result there are 83 sampling points where the species was detected during the 50 years period of time but the most comprehensive study of zoobenthos was carried out in the periods 2004-2006 and 2009-2011. The localities are given with geographical names, coordinates and Distribution of non-indigenous tubificid worm *B. sowerbyi* (Beddard, 1982) in Bulgaria 6 river km (R.km) of the Danube R. (Table 1, Figure 1).

Studying the horizontal distribution of *B. sowerbyi*, a cartographic system UTM a.k.a. "Gauss-Krüger project", was used as represented by Lehrer & Delchev (1978). The drawing the map of distribution was done with ESRI software ArcGIS 10.

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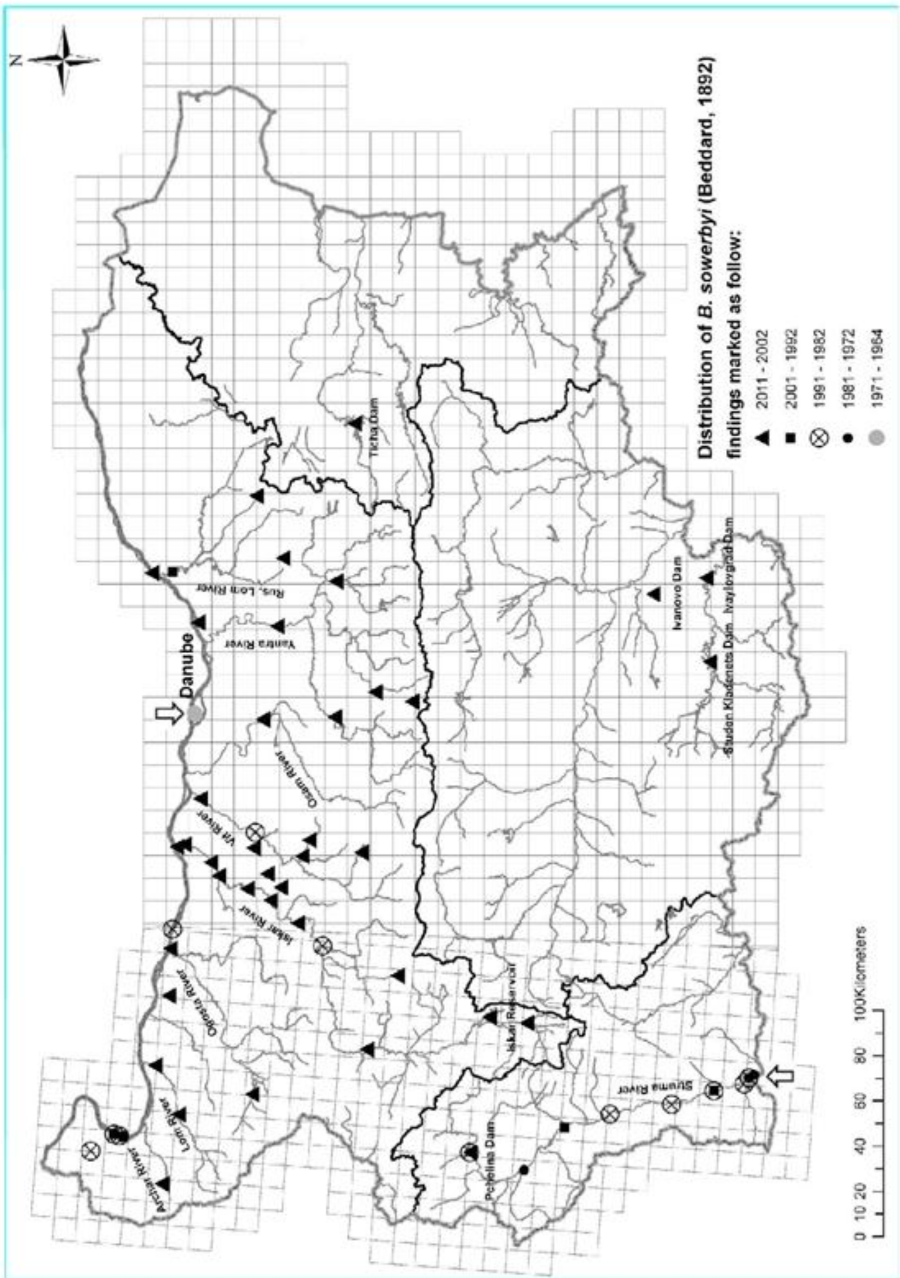

Figure 1. *General data on the distribution of B. sowerbyi in Bulgaria.*

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Table 1. *Localities of B. sowerbyi (Beddard, 1892) found in Bulgaria within the period 1964-2011.* (Notes: u/s - upstream the site, d/s - downstream the site)

River Basin	River/Dam-Lake; Locality; River km (R. km); Altitude (m a.s.l.)	Geographic Coordinates	Date of finding
	Danube River Basin		
Danube R., tributaries and lotic (stagnant) water bodies	Danube R. – Fish Pond on the Belene Island (576-560 R. km; 20 m. a.s.l.)	N 43°40'30.19" E 25°10'33.94"	08.1964
	Danube R., d/s Oryachovo (677 R.km; 26 m a.s.l.)	N 43°44'19.64" E 23°59'20.60"	06.1991
	Danube R., u/s Russe = at Lyulyak Island (496 R. km; 15 m a.s.l.)	N 43°51'20.15" E 25°56'30.31"	04.2005
	Danube R., at Batin Island (530 R. km; 15 m a.s.l.)	N 43°40'10.92" E 25°40'27.50"	10.2010
Vidbol R. Basin	Rabisha Reservoir - the dam (289 m a.s.l.)	N 43°43'57.9" E 22°35'09.4"	09.2009 09.2011
	Vidbol R., u/s the mouth in Danube R. (33 m a.s.l.)	N 43°56'8.45" E 22°51'3.38"	04.1986 10.1986 06.1992
Topolovets R. Basin	Topolovets R., at Gradets (56 m a.s.l.)	N 44° 1'28.17" E 22°45'6.23"	04.1986 10.1986
	Topolovets R., mouth in Danube R. (31 m a.s.l.)	N 43°53'53.26" E 22°50'16.91"	10.1992
Voynishka R. Basin	Voynishka R., u/s the mouth in Danube R. (31 m a.s.l.)	N 43°54'48.68" E 22°50'34.99"	10.1986
Lom R. Basin	Lom R., at Zamfirovo (40 m a.s.l.)	N 43°47'10.59" E 23°14'16.25"	08.2009
	Lom R., at Drenovets (120 m a.s.l.)	N 43°40'59.24" E 22°58'32.72"	09.2011
Ogosta R. Basin	Ogosta R., d/s Ogosta Reservoir (200 m a.s.l.)	N 43°23'46.80" E 23°6'18.19"	09.2009
	Skut R., mouth in Danube R. (30 m a.s.l.)	N 43°44'41.30" E 23°52'57.17"	08.2009
	Asparuhov Val Reservoir - the dam (95 m a.s.l.)	N 43°44'51.2" E 23°37'19.8"	10.2009
Iskar R. Basin	Iskar R., u/s Samokov (950 m a.s.l.)	N 42°19'9.32" E 23°33'14.46"	11.2006
	Iskar R., at Gara Roman (156 m a.s.l.)	N 43°8'25.74" E 23°55'42.05"	06.1987
	Iskar R., u/s Svoge (500 m a.s.l.)	N 42°56'55.09" E 23°22'32.77"	06.2004
	Iskar R., at Resselets (110 m a.s.l.)	N 43°14'31.99" E 24°2'48.03"	05.2004 08.2004
	Iskar R., at Koynare (73 m a.s.l.)	N 43°21'10.54" E 24°9'54.53"	05.2004 08.2004 11.2004
	Iskar R., at Pelovo (61 m a.s.l.)	N 43°26'48.17" E 24°13'25.25"	04.2004 08.2004 11.2004 05.2005
	Iskar R., at Stavertsi (44 m a.s.l.)	N 43°33'46.68" E 24°17'21.83"	11.2004
	Iskar R., at Orehovitsa (37 m a.s.l.)	N 43°35'48.49" E 24°21'40.93"	04.2004 08.2004 11.2004
	Iskar R., d/s Gigen (mouth in the Danube R.) (26 m a.s.l.)	N 43°43'54.99" E 24°26'29.44"	04.2004 08.2004 04.2005 06.2005 08.2009
	Iskar Reservoir - the dam (822 m a.s.l.)	N 42°26'54.6" E 23°34'14.1"	08.2011
	Bebresh Reservoir - the dam (501 m a.s.l.)	N 42°49'55.3" E 23°47'03.8"	10.2009 09.2011
Vit R. Basin	Vit R., at Yassen (57 m a.s.l.)	N 43°25'20.22" E 24°32'2.96"	04.1987
	Vit R., at Gulyantsi (mouth in the Danube R.) (26 m a.s.l.)	N 43°38'42.85" E 24°42'30.19"	09.2009 05.2011 11.2011
	Katunetska R., at Kamenka (213 m a.s.l.)	N 43°12'24.34" E 24°29'58.57"	09.2009
	Vit R., at Bejanovo (168 m a.s.l.)	N 43°13'51.70" E 24°24'22.77"	07.2011 11.2011
	Sopot Reservoir - the dam (360 m a.s.l.)	N 42°59'58.11" E 24°26'25.08"	10.2009
	Gorni Dabnik Reservoir - the dam (168 m a.s.l.)	N 43°22'26.55" E 24°19'06.2"	10.2009
	Dolni Dabnik Reservoir - the dam (100 m a.s.l.)	N 43°25'40.86" E 24°26'48.63"	09.2011
	Telish Reservoir - the dam (218 m a.s.l.)	N 43°18'30,6" E 024°14'13.7"	09.2011
Ossam R. Basin	Ossam R., at Kozarbelene (d/s Levski) (58 m a.s.l.)	N 43°24'18.58" E 25° 9'0.44"	08.2009
Yantra R. Basin	Eliyska R., u/d Yantra R., (P. Trumbesh) (40 m a.s.l.)	N 43°21'17.18" E 25°39'35.31"	10.2009
	Dzhulyunitsa R., u/s Uantra R. (70 m a.s.l.)	N 43° 7'23.04" E 25°54'32.89"	04.2005 08.2009
	Stamboliyski Reservoir - the dam (175 m a.s.l.)	N 43°06'51,8" E 025°08'43,8"	09.2009 10.2011
	Studena Reservoir, u/s Yantra R. (832 m a.s.l.)	N 42°32'38.16" E 23°9'32.83"	08.2009
	Hristo Smirnenski Reservoir (520 m a.s.l.)	N 42°48'47.81" E 25°15'53.31"	09.2009 09.2011
Rusenski Lom R. Basin	Rusenski Lom R., at Besarabovo (26 m a.s.l.)	N 43°46'22.35" E 25°57'0.71"	10.1992
	Malki Lom R., at Lomtsi (240 m a.s.l.)	N 43°26'38.56" E 26°22'12.38"	10.2009
	Kayadzhik R., u/s Boyka Reservoir (270 m	N 43°20'4.73"	10.2009

		E 24°17'21.83"	
	Iskar R., at Orehovitsa (37 m a.s.l.)	N 43°35'48.49" E 24°21'40.93"	04.2004 08.2004 11.2004
	Iskar R., d/s Gigen (mouth in the Danube R.) (26 m a.s.l.)	N 43°43'54.99" E 24°26'29.44"	04.2004 08.2004 04.2005 06.2005 08.2009
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Results

Figure 1 presents generalized data about the distribution of B. sowerbyi in Bulgaria found in the oligochaeta component of the macrozoobenthos of 17 river main basins (including 19 rivers; 15 dam-lakes and 1 fish farm), belonging to four catchment areas: (I) Danube River Basin; (II) Black Sea River Basin; (III) East-Aegean and (IV) West-Aegean River Basins. With arrows are marked the first sites of appearance.

As mentioned above, *B. sowerbyi* was observed for the first time in Bulgaria in a fish farm on the Belene Island (576-560 R.km) in the macrozoobenthos community of the Danube R. in 1964 (Dimitrov, 1966). Since then, the worm has rapidly spread its areal, and now it could be found in a lot of reservoirs and lower streams of the Bulgarian rivers. The highest frequency of finding is in altitude lower than 100 m (Table 1). In this connection the record of few fragments of the worm tail in the Iskar R. upstream the town of Samokov (950 m a.s.l.) can be given to the assumption that the worm does not normally live there, but it was transported by birds' excrements e.g. In the period 2002-2011 the study on macrozoobenthos began more comprehensive and new findings of the species were registered. In the Iskar R. the species is spread along almost the entire river length (Figure 1). The species is most frequent within the Danube R. Basin and lower stream river sections but during the years it can be found at sites at increasingly higher altitudes in the North Bulgaria.

Discussion

It can be assumed that the hot and dry climate in the southern Europe will become hotter and dryer, and will be a threat to the water transport; hydro-technical plants; agriculture and biodiversity. The dry climate in Bulgaria during the period 1982-1994 had a lot of negative effects on the environment (Radev et al., 2003). The lower rainfalls cause decreasing flow and the water velocity. The sediment concentration and sediment yield are correlated with slope and rainfall intensity and sediment concentration and sediment yield have negative and positive relationships with runoff rate, respectively (Defersha & Melesse, 2012). The structure and function of the benthic invertebrate community are in a dynamic balance with the abiotic environment and numerous features of the stream morphology, flow and discharge are strongly influenced by the hydrological factors. Changing the hydrological conditions generates series of biotic responses in stream communities along rivers and the available organic matter can be factor of the distribution of *B. sowerbyi*. The species has formed significant part of the bottom invertebrate community in Bulgaria in different periods and has colonized new biotopes according to the adaptation abilities of the species. It is a typical inhabitant of slow waters with intensive sedimentation so the hydrological conditions in the plane rivers contribute to the successful adaptation of the worm. The species is typical inhabitant for waters with current velocity under 0.5 m.s-1. In Serbia the species is more frequent in rivers with modified hydrological conditions (Paunovic, 2005) so if the construction of hydro power plants in the Bulgarian sector of Struma R. is allowed, the river bed will be modified and sediments will be deposited as the observed situation in Serbia.

On the other hand the Oligochaete worms *B. sowerbyi* together with *Limnodrilus hoffmeisteri* (Claparède, 1862) and

Tubifex tubifex (Müller, 1773) are important sediment particles transporters. They remove sediment particles and deposite them onto the sediment surface bringing deeper deposits to the surface and increasing solute transport within the lower water column. Paunovic (2005) suggests that the species potentially can have a large impact on the recipient environment since it is characterized by high adaptability to local conditions. Successful adaptation of *B. sowerbyi* to silt-clay dominated aquatic habitats in Bulgaria could be connected to its morphological adaptations and the fact that *B. sowerbyi* is highly tolerant to organic pollution (Ersèus, 2005). This statement can be supported by the fact that the saprobic state of the sampling points in the Danube tributaries in western Bulgaria (Topolovets R., Voynishka R. and Vidbol R.) in the period 1986-1992 is β-mesosaprobic (Russev, 1994) and *B. sowerbyi* is more typical of β-mesosaprobic zone (Uzunov, 1977). The same is the trophic state in the Struma R. where the species was found along the entire Bulgarian sector of the river (Soufi et al., 2002).

B. sowerbyi spread its areal and became frequent and abundant inhabitant of soft-bottom habitats in most of the lowland rivers in North Bulgaria. In the East-Aegean River Basin the species was found only in isolated sites.

For instance, it has never been recorded along the Arda R. so it can be assumed that *B. sowerbyi* spreads its areal to Studen Kladenets Dam, Ivaylovgrad Dam and Ivanovo Dam (Table 1) due to fish stocking the ponds for fish farming activities and angling sport and dissemination by waterfowl and/or by birds' excrements.

At present the Iskar R. is one of the best studied Bulgarian rivers and the investigations concern mainly the section downstream the city of Sofia. This fact must be taken in account along with the specific bottom substrate conditions in the lower stream of the Iskar R. to explain the high number of findings of *B. sowerbyi* in this section of the river. According Paunović et al. (2005) the distribution of this species generally is associated with hydro-morphological modification of rivers and the middle river section of the Iskar R. is heavily modified and with changed hydrological conditions by the constructed hydro power plants (Kenderov & Yaneva, 2009). Such interference in the river bed may result in lowing the river velocity and depositing more silt-clay substrate preferable of aquatic oligochaeta warms and *B. sowerbyi* in particular. In the lower stream of the Iskar R. between the villages Stavertzi, Orehovitza and Gigen the specific conditions (reduced stream velocity, mud substrate, etc.) induce natural transformations of the communities and class Oligochaeta is the dominant (Kenderov & Yaneva, 2009). The Danube tributary nearest to the Iskar R. is the Vit R. and the presence of the species is determined by the similar conditions of the river and the assumption that the species is distributed by fish-anglers and/or birds.

B. sowerbyi is a tubificid worm (aquatic oligochaete) that can easily be recognized by the tail gills that cover the posterior quarter of the body (Figure 2) (Chekanovskaya, 1962). Tubificid worms construct burrows, and feed on and excrete surface soil. Such a physical disturbance of soil by tubificid worm enhances soil-water interface area and accelerates nutrient diffusion from soil into overlying water (Ito & Hara, 2010).

Figure 2. *B. sowerbyi – posterior part of the body with gill filaments (after Grabowski & Jabłońska, 2009*.

In order to feed *B. sowerbyi* makes burrows to a depth of 20 cm. Once it has depleted an area of nutrients it moves on to create another burrow displacing a significant amount of sediment, bringing deeper deposits to the surface and increasing the solute transport within the lower water column (Wang & Matisoff, 1997). Measurements of chemical and biological features show that the tubificids accelerated the diffusion of dissolved substances increase the release of soil bacteria and enhance the production of algae and duckweed *Lemna sp.* in the overlying water. The tubificids enhance the microbial activity in the upper soil layer, which in turn produce large amounts of organic matter (Kikuchi & Kurihara, 1982).

The knowledge of the life habits and life cycle of the tubificid worms is basic of the determination of their secondary production. It is known that oligochaete worms are one of the main food sources for bentophagous fish (Hossain et al., 2011). As a freshwater worm with long body and high biomass *B. sowerbyi* is part of the feeding diet of most carp fish (Cyprininidae) such as *Labeo rohita* (Hamilton, 1822) and *Cyprinus carpio* (Linnaeus, 1758) (Rahman et al., 2006). Aquatic oligochaetes may potentionally become an important constituent in commercial aquaculture as a live fish food source (Lietz, 1987).

According to Carroll & Dorris (1972) *B. sowerbyi* is a thermal water species, with huge ability of adaptation to the local environmental conditions. Temperature affects reproductive activity and eggs viability of *B. sowerbyi* (Aston, 1968, after Carroll & Dorris, 1972). Considering the global warming and climate changes the suggestion that the area of distribution of the species developed in the upstream direction can be made, but the correlation between the years passing and the altitude of the sites of founding is very weak (Figure 3). The data only show that the number of findings and its altitudes for the last decades went up. The Pearson correlation coefficient is R2=0.0647 (R=0.254), so probably the available data is not enough and it is necessary further researches to be done so more convincing proof to be found.

Conclusion

The aquatic worm *B. sowerbyi* is an obvious example of anthropogenically introduced and fast dispersed invertebrate. Introduction of this worm in Europe could be connected with transport of plants, roots or ground particles from one part of the world to another, transplanting fish for stocking or dissemination of cocoons by bird excrements. The distribution of the species on the Bulgarian territory shows very weak correlation between the altitude and the years passing. One fact that must be taken in mind is that *B. sowerbyi*, with other oligochaetes, has been documented as a host of myxosporean parasites which cause fish diseases such as swim-bladder disease and haemorrhagic thelohanellosis in Asia and Europe, and its presence has been correlated to high levels of infection in fish (Liyanage et al., 2003). Two myxozoan parasites in *B. sowerbyi* - *Sphaerospora renicola* (Dykova & Lom, 1982) and *Thelohanellus hovorkai* (Ahmerov, 1960), prefer *Cyprinus caprio* (Linnaeus, 1758) as a fish host causing swim bladder inflammation. The species is a desired trophic resource for bentophagus fish (most Cyprinidae) and this is a positive aspect of its widely inner distribution in the Bulgarian water bodies. In spite of the positive effect that the species may have, resulting in increasing the fish biomass and number, the spreading of *B. sowerbyi* should be considered a serious threat, causing fish diseases, reducing the fish abundance and causing damages to the local fisheries.

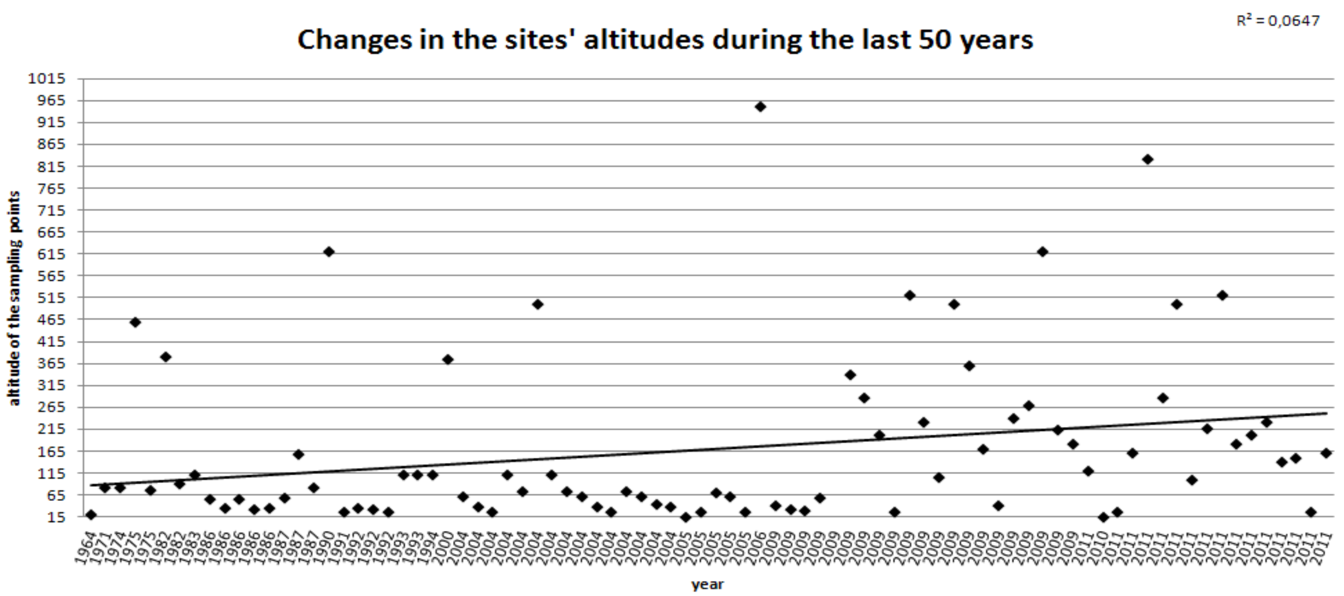

Figure 3. *Linear correlation between the years passing and the altitudes of localities*

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No effective treatment for the infected fish is known. Due to the fast dispersal and successful adaptation of B. sowerbyi to silt-clay dominated aquatic habitats in Bulgaria that was underlined in our work, the species could be characterized as a fast introducing alien species. According to presented results and the possible threat of fish diseases, further monitoring of distribution, population dynamics and possible effects to aquatic ecosystems is recommended.

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