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Original article

Helmintofauna of turbot *Scophthalmus maximus* (Linnaeus, 1758) from the southern Baltic Sea including new data

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Abstract

Turbot *Scophthalmus maximus* (Linnaeus, 1758) is a fish belonging to the *Pleuronectiformes* order. It is commonly observed in waters of the northern Atlantic, and also in the Baltic Sea. As an economically significant species, it is fished on an industrial scale, and also farmed in some European countries. Seventy-two turbots from the Gulf of Gdańsk (26th ICES zone) were examined for parasite presence in the years 2010-2012. The study revealed the presence of the tapeworm *Bothriocephalus scorpii* (Müller, 1776) and acanthocephalan *Corynosoma semerme* (Forssell, 1904). The overall (both parasites) prevalence of turbot infection was 100% with a mean intensity of 18.7. *C. semerme* is a parasite which has not been noted so far in turbot from the southern Baltic. The presence of *C. semerme* in turbot was emphasized in the context of possible infection of terrestrial mammals, including humans.

Key words: turbot, *Scophthalmus maximus*, parasites, *Bothriocephalus scorpii*, *Corynosoma semerme*, southern Baltic Sea

Introduction

Turbot *Scophthalmus maximus* (Linnaeus, 1758) is a marine fish commonly observed in Europe, belonging to the *Pleuronectiformes* order and *Scophthalmidae* family (Rodríguez-Villanueva and Fernández-Souto 2005). Its range of occurrence includes an area from the Mediterranean Sea, up to Lofoten, and also the North and Baltic Seas. Turbot is a benthos fish preferring sandy bedding. It is usually observed in the littoral zone up to a depth of 80 m. The mean length of an adult individual's body reaches 40-60 cm, while the maximum length is 100 cm in the Atlantic and 55 cm in the Baltic Sea. Males are smaller than females (Jackowski 2002). Turbot spawning takes place between May and July, except in the area of Mediterranean Sea, where it occurs earlier, between February and April (Rodríguez-Villanueva and Fernández-Souto 2005).

Turbot, as an economically significant species, is both fished and farmed in many European countries (Rodríguez-Villanueva and Fernández-Souto 2005). In 2013, 5512 tons of turbot were caught commercially in Europe, while the highest amount was caught in the

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year 1993 (9432 tons). In Poland, 72 tons of fished turbot was noted in 2013, with the highest recorded figure in 2008 (111 tons). Turbot farming plays a greater economic role in certain countries, utilizing coastal artificial water reservoirs. Currently, Spain is the largest turbot producer in Europe (6900 tons/year in 2013) (FAO 2010-2015).

Because of the threat they may pose to animals including humans, and the role that turbot plays in European countries' fishing industries, turbot parasites are of particular interest. Despite the fact that turbot parasitofauna is the subject of much research, the area of the south Baltic has been insufficiently investigated, and the data are based on information from the distant past and small samples (e.g. Markowski 1933, Rokicki 1975, Sulgostowska et al. 1987, 1998). Environmental factors including anthropogenic pollution are commonly known to affect the parasitic fauna of fresh water as well as marine fish (Khan and Thulin 1991, Mackenzie et al. 1995, Dzika and Wyżlic 2007). There are findings showing that water pollution may cause both increased abundance of some parasites with a coexisting decrease or even loss of other parasitic species (Overstreet 1997, Galli et al. 2001, Schmidt et al. 2003, Sasal et al. 2007). Also, environmental changes in the southern Baltic Sea impact upon parasitic fauna diversity and abundance among fish (Sulgostowska 1988, Chibani et al. 2001).

The goal of this study was to investigate and update knowledge about the state of the parasitic fauna of turbot from the southern Baltic Sea, as well as to highlight the possible threat that the turbot parasites may pose for terrestrial animals, including humans.

Materials and methods

Seventy-two turbots were acquired from the Gulf of Gdańsk (26th ICES zone) between April and May 2010-2012. In 2010 – 21 fish [25.0-33.3 (30.3) cm, 245-687 (470.0) g] were acquired, in 2011 – 25 fish [25.3-34.1 (29.9) cm, 251-760 (449.6) g] and in 2012 – 26 fish [25.0-34.4 (30.29) cm, 251-750 (441.6) g].

Standard parasitological dissections were performed. Integuments, eyes gills, body cavity, gonads and alimentary tract were examined using a stereoscopic microscope. Parasites found were preserved in 70% ethanol. Microscope specimens were then prepared from some of the collected tapeworms and acanthocephalans. For this purpose, the parasites were stained with borax carmine, dehydrated in an alcohol series, cleared in benzyl alcohol and embedded in Canada balsam (Rolbiecki 2010). Before embedding in balsam, the acanthocephalans' morphometric features were measured and photographic documentation was made.

The following indices were used in order to determine the level of turbot infection with particular parasites: prevalence (percentage of infected hosts), mean intensity (mean number of parasites per infected host) intensity range (minimum and maximum number of parasite individuals per host) and abundance (mean number of parasites per host in the examined sample). The condition of turbots with respect to tapeworm *B. scorpii* was examined, and Fulton's factor was used for this purpose ($F = Wx100/L^3$, W = body weight in g, L = body length in cm) (Lagler et al. 1962). Correlation of the intensity of infection with tapeworm *B. scorpii* was examined with respect to body length and body weight, as well as the condition index for the collection of all fish and with respect to subsequent years. Due to data divergence from the normal distribution (Shapiro-Wilk test, p = 0.05), non-parametric Spearman's r_s correlation coefficient (p = 0.05) was used. In one case, when a normal distribution of the compared collections was noted, both parametric Pearson's r coefficient and Spearman's r_s coefficient were used (in both cases p = 0.05). Also, mean intensities of turbot infection in subsequent years were compared. Due to the differences in standard deviations and low sample number, the Cochran-Cox t' test with a separate estimation of variance for p = 0.01 was used. Correlation strength was determined based on the scale proposed by Evans (1996).

Results

Two parasite species represented by tapeworm *Bothriocephalus scorpii* (Müller, 1776) and acanthocephalan *Corynosoma semerme* (Forssell, 1904) cystacanths were noted.

B. scorpii was found in turbot intestines and they represented mature development stages. Infection prevalence (taking into account all years) was 100%, with a mean intensity of 18.7 and an intensity range of 3-77, and for the given years – 2010, 2011, 2012 – the infection intensity was: 26.3, 3-77; 12.5, 4-26 and 18.4, 4-69, respectively.

Taking into account all the research periods, a significant but weak positive correlation was noted between the intensity of *B. scorpii* infection and fish condition (r_s = 0.33, p<0.05). A similar result was obtained in turbot caught in 2010, where the significant correlation was positive and moderate (r_s = 0.50, p<0.05). No significant correlations between the intensity of *B. scorpii* infection and turbot condition were observed in the years 2011 or 2012 (r = 0.13, p>0.05; r_s = 0.19, p>0.05 and r_s = 0.35, p>0.05, respectively).

A significant, moderately positive correlation was noted between *B. scorpii* infection intensity and turbot weight (r_s = 0.46, p<0.05) and length (r_s = 0.44, p<0.05) in the set of fish caught in the period 2010-2012. Similar results were obtained in the case of fish caught in 2010 (r_s = 0.51, p<0.05 oraz r_s = 0.69, p<0.05 respectively). In fish caught in 2011, no statisti-

Table 1. Morphometric features of *Corynosoma semerme* specimens found in turbot (measurements in μm).

Feature			Specimen 1	Specimen 2	Specimen 3
Body			2900 x 988	2683 x 862	2884 x 853
Proboscis			540 x 285	502 x 244	548 x 292
Hooks	number of rows / count per row		24 / 12	22 / 11-12	22 / 13
	hooks 1-5*	BL	31.7-43.4 (38.5)	34.0-49.1 (43.9)	39.6-55.7 (48.9)
		BW	5.8-9.3 (7.3)	4.3-11.3 (7.2)	6.3-12.1 (8.6)
		RL	23.1-40.6 (33.5)	25.2-42.8 (37.3)	29.5-50.9 (44.3)
	hooks 6-7	BL	44.8-67.7 (53.6)	47.5-63.4 (55.8)	39.6-55.7 (48.9)
		BW	10.7-16.4 (13.2)	8.5-20.6 (14.1)	6.3-12.1 (8.6)
		RL	38.6-59.9 (47.7)	38.4-60.3 (48.1)	41,6-64.9 (57.4)
	hooks 8-12**	BL	17.7-37.1 (27.1)	17.1-36.4 (24.9)	12.1-37.5 (22.0)
		BW	3.7-9.7 (6.1)	4.0-10.2 (6.8)	3.5-9.1 (5.7)
		RL	not visible	not visible	not visible
Neck			259 x 352	183 x 324	118 x 265
Receptacle			775 x 289	914 x 267	853 x 241
Lemnisci			549 x 82 560 x 72	670 x 80 541 x 74	559 x 49 517 x 57

BL – blade length, BW – basal width, RL – root length. *: Counting from anterior part of proboscis, **: Hooks 8-12 distinctly separated from others

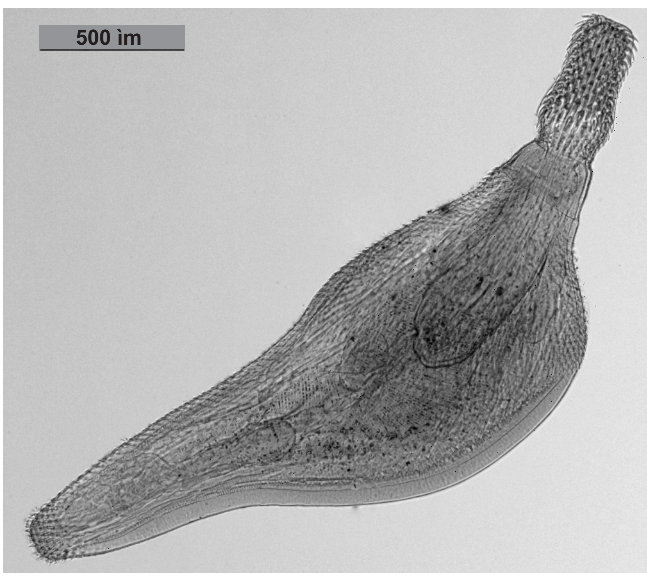

Fig. 1. *Corynosoma semerme* cystacanth, body view.

cally significant correlation was determined between the intensity of *B. scorpii* infection and fish weight (r_s = 0.31, p>0.05) and length (r_s = 0.29, p>0.05). In turbots caught in 2012, a statistically significant, moderate positive correlation was found between fish body length and weight, and the intensity of *B. scorpii* infection (r_s = 0.54, p<0.05 and r_s = 0.47, p<0.05 respectively).

Mean *B. scorpii* infection intensity differentiation was checked in the given years. It was observed that significant differences occur between sets of fish from 2010 and 2011 (t' = 3.04, p<0.01). No significant differences were noted between the years 2010 and 2012 (t' = 1.52, p>0.01), or 2011 and 2012 (t' = -1.85, p>0.01).

Three *C. semerme* cystacanths were noted in the intestine walls of two turbots (32.0 cm, 650 g and 34.4 cm, 665 g) from 2012. General infection (taking into account all years) of turbots with acanthocephalan *C. semerme* was as follows: prevalence 2.8%, mean intensity 1.5, intensity range 1-2, and abundance 0.04, while in the year 2012, the infection indices were 7.7%, 1.5, 1-2 and 0.12. Since *C. semerme* has not been noted so far in turbot from the southern Baltic, their morphometric features are presented (Table 1, Figs 1, 2).

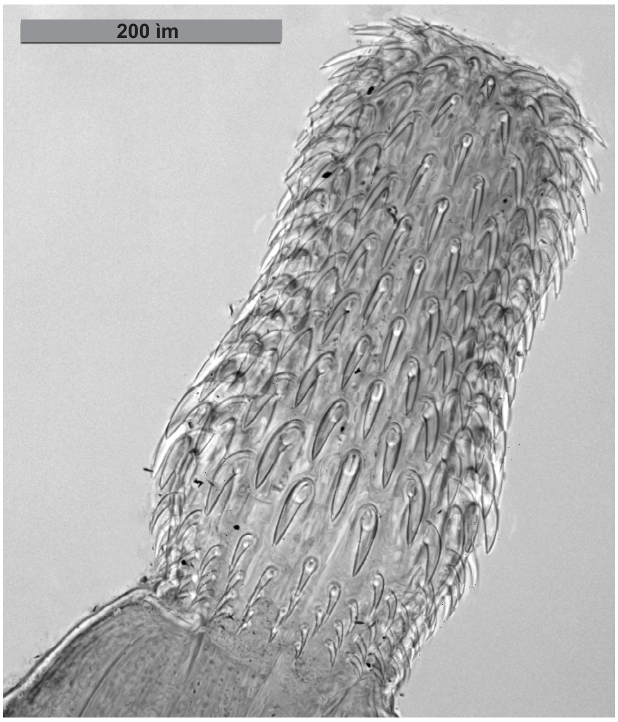

Fig. 2. *Corynosoma semerme* cystacanth, proboscis.

The mean value of the turbot condition index (taking into account all years) was 1.59. In subsequent years – 2010, 2011, 2012 – it was 1.65, 1.61 and 1.50, respectively.

Discussion

The current state of turbot parasitofauna recognition is insufficient. The occurrence of only 3 nematode species (*Cucullanus heterochorus* (Rudolphi, 1802), *Hysterothylacium aduncum (Rudolphi, 1802), Hysterothylacium* sp.) and 1 tapeworm species (*B. scorpii*) have been noted among wild marine populations (e.g. Robert et al. 1988, Sanmarťn-Durán et al. 1989a, 1989b, Cordero del Campillo et al. 1994). In turn, digenea *Diplostomum* sp., nematode *Hysterothylacium auctum* (Rudolphi, 1802), tapeworm *B. scorpii* and acanthocephalan *Acanthocephalus lucii* (Muller, 1777) have been found in turbots in the southern Baltic region (e.g. Markowski 1933, Sulgostowska et al. 1987, 1998). Moreover, *C. semerme* has also been noted in turbots in the Gulf of Riga (eastern Baltic Sea) (Kirjušina and Vismanis 2007). However, a range of parasitic protists have been observed among *Euglenozoa Ichthyobodo* sp. (=*Costia*), ciliates *Philasterides* sp., *Philasterides dicentrarchi* Dragesco, Dragesco, Coste, Gasc, Romestand, Raymond et Bouix, 1995, *Cryptocaryon* sp., *Trichodina* sp. and *Chromalveolata Amyloodinium ocellatum* Brown et Hovasse, 1946, in farming turbots (northern Spain and Portugal) (Novoa et al. 1992, Dyková and Figueras 1994, Ramos et al. 2007, Iglesias et al. 2001, Saraiva et al. 2011). Moreover, *Microsporidia Tetramicra brevifolium* Ralphs et Matthews, 1985 and *Myxozoa Enteromyxum scophthalmi* Palenzuela, Redondo et Alvarez-Pellitero, 2002 have been noted in them (Novoa et al. 1992, Dyková and Figueras 1994, Palenzuela et al. 2002). Since water pumped to breeding ponds is derived directly from the sea (Novoa et al. 1992) and turbot juvenile fish often originate from wild populations, it may be supposed that the mentioned parasites may occur in, or even originate from, wild turbot populations.

The most common parasite in turbot is the tapeworm *B. scorpii*. This is confirmed in this study and also in the research conducted by other authors. This tapeworm has been noted in turbot from the Atlantic Ocean, North Sea, English Channel, Mediterranean Sea and Baltic Sea (Markowski 1935, Davey and Peachey 1968, Groot 1971, Rokicki 1975, Sulgostowska et al. 1987, 1998, Robert et al. 1988, Novoa et al. 1992, Stankus et al. 2011). It should be added that, using molecular genetics methods, Renaud et al. (1984) noted that *B. scorpii* constitutes the complex of two sister-species *B. scorpii* and *Bothriocephalus gregarius* Renaud, Gabrion et Pasteur, 1983. Using their methodology, Robert et al. (1988) determined *B. scorpii* from turbot in the North Sea as *B. gregarius*.

The level of turbot infection with tapeworm *B. scorpii* is high. Most often, the prevalence reaches 100% with very high intensity. For example, in turbots from various parts of the North Sea and English Channel, the infection observed in the case of adult fish was 100%, 44.0 and 7-94, while in turbot juvenile fish during the first year of life in the British Islands coastal zone it was 29.0%, 13.0 and 1-66 (Davey and Peachey 1968). A very high infection rate for turbot with *B. scorpii* has also been noted in the Atlantic Ocean on the coast of Spain (Sanmartín-Durán et al. 1989b). *B. scorpii* is also common in turbots from brackish waters (Baltic Sea). Markowski (1936) noted an occurrence of the tapeworm *B. scorpii* in turbot in waters of the southern Baltic as early as in the 1930s, and infection in the case of 3.0-6.8 cm long fish was 19.0% and 1.3, 1-2, while in fish of a body length of 6.8-30 cm, the infection was 100% with intensity specified as „mass". The high infection rate for turbot with tapeworm *B. scorpii* in the southern Baltic was maintained in the years 1970-90 and prevalence was 100% with intensity at a level of a few tens of parasites in each infected fish (Rokicki 1975, Sulgostowska et al. 1987, 1998). Also, in the coastal regions of Lithuania, turbot infection reaches very high parameters – 97.5%, 25.0 and 2-178 (Stankus et al. 2011), while in turbots from maricultures tapeworm *B. scorpii* is observed more rarely (Novoa et al. 1992).

Tapeworm *B. scorpii* requires two intermediate hosts for its development: copepods from the order *Calanoida* – *Eurytemora affinis* (Poppe, 1880) (=*Eurytemora hirundo* Giesbrecht, 1881) (I intermediate host), which is a site of procercoid development, and fish, e.g. from *Nerophis*, *Pomatoschistus*, *Gasterosteus*, *Pungitius* genus (II intermediate host), being the site of plerocercoid development, which is an invasive stage for marine fish, mainly turbot (definitive host) (Markowski 1935, Pojmańska and Cielecka 2001). In the juvenile stage, turbot feeds on fine crustacea and molluscs, while in open seas it also feeds on polychaetes (Markowski 1935, Novoa et al. 1992, Jackowski 2002, Nissling et al. 2007). Nissling et al. (2007) report that fish of a length less than 3 cm, feed on copepods from *Calanoida* and *Harpacticoida* orders, while individuals of dimensions 3.0-6.7 cm feed mainly on crustacea belonging to the *Mysidacea* order, and those exceeding 5.5 cm have an increased proportion of isopods in their diet, and also started to feed on juvenile fish represented by gobies and three-spined sticklebacks *Gasterosteus aculeatus* (Linnaeus, 1758). Adult turbot, in turn, become entirely fish-eating, and they prey mainly on the greater sandeel *Hyperoplus lanceolatus* (Le Sauvage, 1824), gobies and fine sprats *Sprattus sprattus* (Linnaeus, 1758) (Markowski 1935, Jackowski 2002, Rodríguez-Villanueva and Fernández-Souto 2005). An increase in the proportion of fish in the turbot diet affects its infection with tapeworm *B. scorpii*.

Information concerning correlations between the conditions of infected turbots and their dimensions (body length, weight), and their intensity of infection with tapeworm *B. scorpii* seems to be equivocal. Stankus et al. (2011), examining turbots on the coasts of Lithuania, noted a negative correlation between turbot conditions and the intensity of parasite infection. In turn, Davey and Peachey (1968) did not observe any significant correlation between length, weight and age of turbots, and infection intensity. However, the present results demonstrate positive, although weak or moderate, correlations between the intensity of parasite infection and body length, weight and turbot condition. Low or no negative tapeworm effect on turbot condition suggest long-standing relationships between parasite and host. *B. scorpii,* being a specific parasite for turbot, has been able, during its evolution, to minimize its unprofitable effect on the host (Combes 2001). Moreover, Davey and Peachey (1968) report morphological (shape and folding) similarities between *B. scorpii* scolex and the intestinal mucosa of turbot, which also shows high specificity in this host-parasite relation. Despite high infection rates, hosts often remain in good physical condition. This phenomenon is not rare and is observed among many host-parasite systems (Izdebska and Rolbiecki 2014).

The morphometric features found with respect to *C. semerme* correspond to data reported by other authors (Sokolovskaya 1964, Sinisalo and Valtonen 2003). Only total body length in all specimens is slightly lower than that proposed by Sokolovskaya (1964). Moreover, the number of hooks per row in the second specimen (see: Table 1) is lower in selected rows. These remarks show the possible differentiation of *C. semerme* cystacanths depending on the host it parasitizes.

C. semerme is a parasite which has not thus far been noted in turbot from the southern Baltic. The intermediate host for *C. semerme* is crustaceans, especially isopod *Monoporeia affinis* (Lindstrom, 1855), fish constitute the paratenic host, while marine mammals – especially seals – are the final hosts; sometimes larvae occur in birds (Petrochenko 1971, Sinisalo and Valtonen 2003, Kennedy 2006). This acanthocephalan is commonly observed in fish and marine mammals, as well as piscivorous birds in other regions (Helle and Valtonen 1980, Valtonen and Helle 1988, Nickol et al. 2002, Sinisalo and Valtonen 2003, Kennedy 2006, Kirjušina and Vismanis 2007). In the southern Baltic, it has been noted in smelt *Osmerus eperlanus* (Linnaeus, 1758), flounder *Platichthys flesus* (Linnaeus, 1758), cod *Gadus morhua* (Linnaeus, 1758), herring *Clupea harengus* (Linnaeus, 1758), and in zander *Sander lucioperca* (Linnaeus, 1758) in the Vistula Lagoon (Markowski 1933, Janiszewska 1938, Studnicka 1965, Rokicki 1975, Rolbiecki 2003). It is worth adding that mammals are observed more often in the Baltic Sea area, including its southern part, which may result in an increased occurrence of typically marine parasites, including acanthocephalan *C. semerme* (Härkönen et al. 1998, Skrzypczak et al. 2014).

It is worth paying attention to the fact that acanthocephalans from the *Corynosoma* genus may constitute a potential threat to human health and that of other terrestrial animals (Acha and Szyfres 2003, Kennedy 2006). For example, mature *C. semerme* have been noted in farmed American minks *Neovison* (=*Mustela*) *vison* Schreber, 1777 fed with fish derived from the Gulf of Gdańsk (Malczewski 1962). Special attention should thus be paid to the presence of these parasites during consumption of dishes prepared from turbot, and also during preparation of wild marine fish containing food for farm animals.

Over recent decades dynamic environmental changes mainly caused by varying agricultural, municipal and industrial pollution loads in the Baltic Sea have been reported. It is noteworthy that *B. scorpii* is the only common parasite infecting turbot in the southern Baltic Sea – other species have been noted infrequently and cannot be treated as reliable bioindicators. Relatively high and rising concentrations of polluting substances were observed in the 70s and 80s. This caused increasing eutrophication and – subsequently – increased saprobicity of large areas of the southern Baltic Sea. From the 90s a continuous decline in major pollutant concentrations occurred (HELCOM 1987, 2011, Heybowicz et al. 1998, Albiniak et al. 2010). Despite these changes, the infection rates of turbot in the southern Baltic Sea were high and did not change significantly throughout the whole of the 20th century and they are still observed nowadays. Nevertheless, as a result of the increasing eutrophication of the whole basin that was observed continuously between 1900-1989 in the whole Baltic Sea, an increased occurrence of planktonic and benthic invertebrates was noted, including crustaceans that may carry larval stages of parasites infecting fish. Among the possible parasite vectors, the *M. affinis* population has been growing constantly since it first appeared in the southern Baltic Sea in 1930-1959 (Żmudziński 2002). This may increase the possibility of *C. semerme* infection among economically important fish species, including turbot.

To sum up, it may be concluded that turbot parasitic fauna is relatively poor and not diversified across the European seas, including the Baltic Sea. *B. scorpii* is the only parasite that is common in all the areas considered in this study and this does not depend on environmental conditions – neither salinity nor water purity. *C. semerme* is a rare turbot parasite, but when ingested in food made from turbot it may pose a threat for terrestrial animals, including humans.

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