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# *Myoxocephalus scorpius* – liver nematodes and diet (pilot studies from Polish waters)

Katarzyna Nadolna-Ałtyn, [1,*], Joanna Pawlak [1], Marcin Kuciński [2]

**Abstract**

Research on the parasitology of the shorthorn sculpin (*Myoxocephalus scorpius*) from the Baltic Sea is presently limited. As a predatory fish, the species primarily acquire nematode parasites through the ingestion of infected prey. The main objectives of the current study were to (1) evaluate the presence of nematodes in the livers of shorthorn sculpin from the southern Baltic Sea and (2) investigate the dietary composition of this species. Accordingly, 32 fish from the north-western Polish waters of the Baltic Sea (ICES rectangle 39G6) were caught in November 2020 and subjected to standard ichthyological analyses. Moreover, liver samples were dissected from each fish and frozen for further parasitological investigation. The presence of the parasites was detected in 5 of the 32 analyzed livers, with a prevalence of 15.6%, intensity of infection from 2 to 99 parasites per fish and abundance of 3.9. Co-occurrence of *Contracaecum* sp. and *Hysterothylacium* sp. nematodes was observed in all infected fish. Stomach content analysis revealed that *Crangon crangon*, *Bylgides sarsi* and *Gammarus* spp. were the most abundant components of the shorthorn sculpin's diet.

**Keywords**

*Myoxocephalus scorpius*; Shorthorn sculpin; Dietary composition; Nematodes; Baltic Sea

[1] *National Marine Fisheries Research Institute, Kołłątaja 1, 81–332, Gdynia, Poland*
[2] *University of Gdańsk, Marszałka Piłsudskiego 46 Av., 81–378 Gdynia, Poland*
***Correspondence**: knadolna@mir.gdynia.pl (K. Nadolna-Ałtyn)

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## 1. Introduction

*Myoxocephalus scorpius*, commonly known as the shorthorn sculpin or bull-rout, is a demersal fish species belonging to the Cottidae family. It is distributed across the Northern Atlantic, including adjacent subarctic and Arctic seas, with a preference for lower water temperatures. Recent observations have shown a steady decline in shorthorn sculpin numbers in the Baltic Sea, which has been linked to a reduction in water salinity (Sonne et al., 2020).

The shorthorn sculpin is characterized by a relatively sedentary lifestyle, making it suitable as a model species for assessing the accumulation rate of the sealworm population in local waters (Aspholm et al., 1995; Midtgaard et al., 2003; Lunneryd et al., 2015). Analysis of the internal organs of various fish species by Valtonen et al. (1988) indicated that the shorthorn sculpin was one of the most parasitologically infected species in the Bothnian Bay, with a prevalence of 20%, during the years 1977–1980 and 1982. A later study focused only on the presence of nematodes in the muscle tissue of shorthorn sculpin collected along the Swedish coast (Lunneryd et al., 2015). However, there has been no survey of nematodes on or in the intestines or livers of *M. scorpius* within the last years.

So far, research on shorthorn sculpin from the Baltic Sea has garnered limited interest, missing comprehensive scrutiny of its parasitology (Valtonen et al., 1988; Sulgostowska et al., 1990; Lunneryd et al., 2015). This contrasts with other fish species inhabiting the Baltic Sea, for example, sprat *Sprattus sprattus* (Nadolna-Ałtyn et al., 2018; Nadolna-Ałtyn et al., 2023a), great sandeel *Hyperoplus lanceolatus* (Nadolna-Ałtyn et al., 2017), cod *Gadus morhua* (Nadolna and Podolska, 2014) and Atlantic salmon *Salmo salar* (Nadolna-Ałtyn et al., 2023b; Nadolna-Ałtyn et al., 2024), in which research has shown a marked increase in the number of Anisakidae nematode infections in recent years. The majority of these parasites has been found in the liver of fish, for example in cod (Nadolna and Podolska, 2014), leading the International Council for the Exploration of the Sea (ICES) to implement a routine visual inspection of Baltic cod livers surface during standard ichtyological analyses while Baltic International Bottom Trawl Surveys (BITS) (ICES, 2021). It was the reason why

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**Table 1.** Length distribution of the shorthorn sculpin (*Myoxocephalus scorpius*) analysed under the current study, including infected individuals.

| Length class [cm] | No analysed fish | No infected fish |
|---|---|---|
| 15 | 1 | 0 |
| 16 | 1 | 0 |
| 17 | 2 | 0 |
| 18 | 2 | 0 |
| 19 | 7 | 0 |
| 20 | 2 | 0 |
| 21 | 8 | 2 |
| 22 | 5 | 2 |
| 23 | 2 | 1 |
| 25 | 2 | 0 |
| **total** | **32** | **5** |

the liver was chosen during the presented studies.

Shorthorn sculpin, like other predatory fish, primarily acquire nematode parasites by eating infected prey. However, to date, there is only a single report (Raciborski, 1984) describing the diet of *M. Scorpius* in Gdańsk Bay, Polish marine waters. Moreover, Cardinale (2000) reported changes in prey items composition during ontogenesis for fish caught in the south-western Baltic Sea in 1997. Presently, the shorthorn sculpin is classified by Norderhaug et al. (2005) as a generalist that feeds on a wide range of kelp-associated invertebrates. Therefore, the available data on the dietary composition of shorthorn sculpin are insufficient, significantly limiting our understanding of the species' nematode infection routes and dynamics in the Baltic Sea.

The aims of the present study was (1) to evaluate the presence of nematodes in the livers of shorthorn sculpin from north-western Polish waters of the Baltic Sea and (2) to investigate the dietary composition of this species.

## 2. Material and methods

### 2.1 Sampling

A total of 32 specimens of shorthorn sculpin were collected during a survey conducted in November 2020 in north-western Polish waters of the Baltic Sea (ICES rectangle 39G6) (Figure 1). The fish were caught using bottom trawls and the current study was carried out in strict accordance with currently applicable law regarding ethical issues in animal research. All caught shorthorn sculpins were subjected to standard ichthyological analyses onboard immediately after the catch, including measurements of total body length and weight, sex determination, gonad developmental stage assessment (in the Meier scale: 1–8) and stomach fullness examination (scale 0–4). Furthermore, otoliths have been collected for age determination. The fish in our sample varied in length from 15 to 25 cm – length distribution of analysed fish is presented in Table 1, with individual weights ranging between 50 and 265 g. Of these, 59.4% were males (16–23 cm) and 40.6% were females (15–25 cm). The age of the fish ranged between 3 and 5 years. Length and mean weight in each age group of analysed fish are presented in Table 2. During ichthyological analyses digestive tracts and livers were dissected from each fish and stored at −20°C for further investigation.

### 2.2 Parasitological inspection of livers

Thawed livers were digested in artificial digestive juice (water solution of pepsine and hydrochloric acid) at room temperature for 24 h. All parasites extracted by this procedure were subjected to species identification at the genus level based on anatomo-morphological features. Subsequently, parasites were preserved in 96% ethanol for further molecular analyses. Parasitological descriptors, including prevalence, abundance and intensity of infection were calculated according to methodology described by Bush et al. (1997).

To verify the taxonomic identification, a subsample of parasites was subjected to molecular analysis. Genomic DNA was isolated from whole preserved parasite specimens using a Genomic Mini DNA kit (A&A Biotechnology, Poland) according to the recommended protocol. The 18S small subunit ribosomal RNA gene (18S rRNA) was PCR amplified using primers (18S_F: GCGAATRGCTCATTA CAACAGC, 18S_R: GGGCGGTATCTGATCGCC and SSU18A: AAAGATTAAGCCATGCATG, SSU22R: GCCTGCTGCCTTCCTT GGA) designed by Floyd et al. (2005) and Blaxter et al. (1998). PCR amplification was carried out using GoTaq® DNA Polymerase (Promega GmbH, Germany). Reaction mixtures were prepared in a total volume of 25 $\mu$l composed of 1X Hot Start GoTaq®Master Mix (Promega), 3.0 $\mu$M $MgCl_2$, 0.3 $\mu$M each primer and around 10 ng DNA template. Amplification was performed with a Thermal Cycler SimpliAMP (Applied Biosystems, Foster City, California, USA) under the following conditions: initial denaturation at 95°C for 5 min, succeeded by 35 cycles at 95°C for 30 s, annealing at 57°C for 30 s, elongation at 72°C for 70 s, with a final extension step at 65°C for 7 min. The PCR products were checked on ethidium bromide-stained 2.0% agarose gels and subsequently sent to the biotechnology company Genomed for sequencing by the Sanger dideoxy method. The DNA sequences obtained were aligned using MegaX software and manually checked against raw data by eye (Kumar et al., 2018). All DNA consensus sequences were then compared with NCBI GenBank resources using the BLAST search tool. Every newly acquired DNA sequence in our study was deposited in the NCBI GenBank (see Results for accession numbers).

### 2.3 Determination of dietary composition

Stomachs from the shorthorn sculpin sampled were defrosted and carefully opened. All prey animals and their remains were removed and subjected to taxonomic identification by anatomo-morphological observation under a light microscope. The prey organisms were identified to

**Table 2.** Length and mean weight in each age group of analysed in the present study shorthorn sculpin (*Myoxocephalus scorpius*) individuals.

| Age group [years] | Length class [cm] | Mean weight [g] | n fish | n *Contracaecum* sp. | n *Hysterothylacium* sp. | n nematodes |
|---|---|---|---|---|---|---|
| 3 | 15 | 82.0 | 1 | – | – | – |
| | 16 | 50.0 | 1 | – | – | – |
| | 17 | 62.5 | 2 | – | – | – |
| | 18 | 75.0 | 1 | – | – | – |
| | 19 | 85.8 | 6 | – | – | – |
| | 21 | 119.6 | 5 | 3 | 6 | 9 |
| | 22 | 130.0 | 1 | – | – | – |
| | 23 | 182.0 | 1 | – | – | – |
| 4 | 18 | 55.0 | 1 | – | – | – |
| | 20 | 98.0 | 2 | – | – | – |
| | 21 | 104.7 | 3 | – | – | – |
| | 22 | 115.0 | 4 | 3 | 15 | 18 |
| 5 | 19 | 80.0 | 1 | – | – | – |
| | 23 | 145.0 | 1 | 97 | 2 | 99 |
| | 25 | 225.0 | 2 | – | – | – |

the lowest possible taxonomic level depending on the degree of their decomposition. Each prey item was counted, and its mass and length were determined if the digestion of the prey had not progressed too far. All prey were also inspected for the presence of nematodes.

## 3. Results

### 3.1 Liver parasites and descriptors of infection

Parasites were detected in 5 out of 32 livers analyzed, resulting in a prevalence of 15.6% (intensity of infection between 2 and 99 parasites per fish; abundance 3.9). Two parasite species, *Contracaecum* sp. and *Hysterothylacium* sp., were identified and in all cases they co-occurred. In total, 103 individuals of *Contracaecum* sp. (intensity of infection between 1 and 97 parasites per fish; abundance 3.2) and 23 representatives of *Hysterothylacium* sp. (intensity of infection between 1 and 15 parasites per fish; abundance 0.7) were noted. The basic ichthyological characteristics of each infected fish are presented in Table 3. Infected fish were present in each age group; however, all the individuals less than 21 cm in length were free of liver nematodes, regardless of the age of the fish (Table 2). In the 21 cm length class, a prevalence of 25% was noted, rising to 40% in the 22 cm length class. In fish of the 23 cm

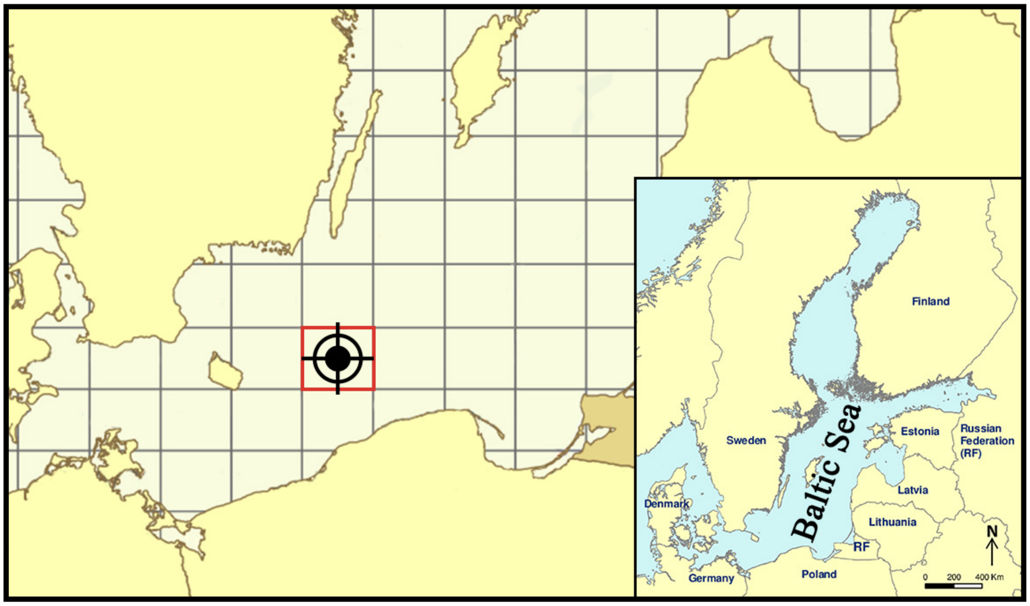

**Figure 1.** Map of the shorthorn sculpin (*Myoxocephalus scorpius*) sample collection site located on ICES rectangle 39G6 (marked by a red box and a circled cross).

**Table 3.** Basic ichthyological characteristics of each infected shorthorn sculpin (*Myoxocephalus scorpius*) specimens spotted under the present study.

| No. | Total length [cm] | Weight [g] | Sex | Gonad developmental stage (1–8) | Stomach fullness (0–4) | Age [years] | n *Contracaecum* sp. | n *Hysterothylacium* sp. | n nematodes |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 21.0 | 120 | M | 4 | 0 | 3 | 2 | 5 | 7 |
| 2 | 22.0 | 115 | F | 5 | 1 | 4 | 1 | 15 | 16 |
| 3 | 23.0 | 145 | F | 5 | 0 | 5 | 97 | 2 | 99 |
| 4 | 22.0 | 105 | M | 5 | 0 | 4 | 2 | 0 | 2 |
| 5 | 21.0 | 130 | M | 5 | 0 | 3 | 1 | 1 | 2 |

length class, which included two specimens, a prevalence of 50% was recorded. For fish in the length class of 25 cm ( two specimens), liver nematodes were not detected.

### 3.2 Molecular verification of parasites following taxonomical identification

To verify the identities of the parasite species, a subsample was subjected to molecular analysis. The presence of *Contracaecum* sp. and *Hysterothylacium* sp. in the livers of the sampled shorthorn sculpin was confirmed by partial sequencing of 18S rDNA and BLAST analysis with a probability > 99.5%. Newly identified sequences have been deposited in GenBank under accession numbers OR852747 and OR835589, corresponding to *Hysterothylacium* sp. and *Contracaecum* sp. annotations, respectively.

### 3.3 Dietary composition

In total, 24% of the fish stomachs were empty. The main component of the shorthorn sculpin diet was *Crangon crangon* (Decapoda), constituting almost 50% of the total weight of the food recovered from the specimens studied. The most abundant food items were *C. crangon*, *Bylgides sarsi* (Phyllodocida) and *Gammarus* spp. (Amphipoda). Other invertebrates in the diet represented *Mytilus* spp. (Mytilida), *Mysis mixta* (Mysida), *Pontoporeia femorata* (Amphipoda), constitute only a small weight percentage of food, accordingly 0.44%, 0.02%, 0.01%. The most frequent in the stomach were *B. sarsi* and *C. crangon*, in 15 and 12 analysed samples, respectively. Only one representative of fish was found – Gobiidae. The significant degree of decomposition of fish remains did not allow for precise identification of the taxonomic level of the individuals. None of the prey investigated contained parasites in the body cavity.

## 4. Discussion

The current extent of shorthorn sculpin infection with Anisakidae nematodes in Polish waters was previously unknown, but the results of the pilot studies presented here indicate that the prevalence of liver infection with nematodes is 15.6%. An earlier investigation into the presence of parasites in shorthorn sculpin from Polish waters was conducted approximately 40 years ago on specimens caught near the Hel peninsula (between August 1983 and March 1984). It revealed the presence of *Thynnascaris adunca* (current name: *Hysterothylacium aduncum*) in the liver of the individuals analyzed (Sulgustowska et al., 1990). However, the infection level (with a prevalence of up to 10.6% and an intensity of infection ranging from 1 to 10 parasites per fish) was lower than that observed in the pilot study presented here. Therefore, our investigation reveals a notable increase in the prevalence of *Contracaecum* sp. in shorthorn sculpin from the Baltic within recent decades. Similar observations relating to the presence of *Contracaecum* sp. have also been reported for other fish species sampled in the Polish marine waters of the southern Baltic Sea, such as great sandeel (Nadolna-Ałtyn et al., 2017), sprat (Nadolna-Ałtyn et al., 2018), cod (Nadolna and Podolska, 2014) and Atlantic salmon (Nadolna-Ałtyn et al., 2023b). Moreover, *Contracaecum* and *Hysterothylacium* nematodes have been recorded on/in the livers of other fish species caught in the Gulf of Gdańsk (Polish Baltic zone) e.g. *Contracaecum* in the saithe *Pollachius virens* (Rolbiecki et al., 2008), twaite shad *Alosa fallax* (Rokicki et al., 2009), garfish *Belone belone* (Rolbiecki et al., 2020); while *Hysterothylacium aduncum* in the European anchovy *Engraulis encrasicolus* (Kulikowski et al., 2012).

The increase in the grey seal population in the Baltic Sea within recent decades is most likely associated with an elevated load of *Contracaecum* sp. eggs to the environment, resulting in higher levels of fish infection. In the Baltic Sea grey seals are the final host of *Contracaecum* sp., where larval stage L4 of the parasite transforms to the dioecious mature stage, which can produce fertilized eggs, transported via the feces of the marine mammals to the water environment. Transformation to the larval stages L1, L2 or even L3 occurs within the egg (Køie and Fagerholm, 1995), which might be eaten by crustaceans – the intermediate host, where the transformation from L2 to L3 can potentially take place. Infected crustaceans are then eaten by pelagic fish, and these by predatory fish. Infected fish are prey for marine mammals, and the life cycle is completed. Thus, grey seals are indispensable for the completion of the life cycle of *Contracaecum* sp. in the Baltic Sea (Zuo et al., 2018). Available observations reveal that these marine mammals are highly infected with nematodes in the Baltic Sea, with a number counted up to hundreds of nematode individuals per seal (e.g. Skrzypczak et al., 2014; Gabel et al., 2021). Each single female of *C. osculatum* produces on average about 9000 eggs (Herreras et al., 2007). By multiplying the increasing number of seals, the number of parasites infecting each seal, and the number of eggs produced by each female parasite, we can estimate a substantial load of *Contracaecum* sp. eggs introduced into the environment, thereby increasing the potential for infection across all trophic levels. There are also other aspects favorable to the increasing infection level of marine organisms with this parasite. The observed decrease in water salinity is considered a potential factor favoring the dispersion of *C. osculatum* in the Baltic Sea (Lunneryd et al., 2015). Indeed, Fiorenza et al. (2020) showed that there was a significant change in *Anisakis* spp. abundance over the period 1978–2015 in the North East Atlantic driven by climate change. Populations of *Contracaecum* parasites may be also increasing due to anthropogenic factors (Shamsi, 2019).

*H. aduncum* is the most abundant fish nematode species of the genus *Hysterothylacium*, and is an extremely common digestive tract parasite of teleosts, especially in the marine environment (Navone et al., 1998). Its life cycle is similar to that described above for *Contracaecum* sp., but the final hosts are fish, e.g. eepout (*Zoarces viviparous*) or cod (Jackson et al., 1997), where sexually mature individuals develop in the digestive tract. The first intermediate hosts are crustaceans, such as copepods, amphipods, shrimps, and isopods (Køie, 1993). Eggs of the nematode with developed larvae ingested by benthic and/or pelagic crustaceans hatch in their intestine and attain their haemocoel. Larger invertebrates, such as chaetognaths and crustaceans, are important second intermediate hosts and various fish species play the role of paratenic or final hosts, acquiring *H. aduncum* through the food chain (Køie, 1993). Thus, fish that are planktonic feeders do have many encapsulated larvae in their viscera (Køie, 1993). Yoshinaga et al. (1987) demonstrated experimentally a freshwater life cycle of *H. aduncum*. Therefore, the brackish environment of the Baltic Sea creates favorable conditions for dispersion of that parasite.

Shorthorn sculpin infected with Ascaridoidea nematodes has been also reported outside the Baltic Sea. Presence of Anisakidae nematode *Pseudoterranova decipiens* in shorthorn sculpin sampled in the outer Oslofjord (North Sea) has been reported in 1990s (Andersen, 2001; Lähdekorpi, 2011). Parasite abundance increased significantly with host age and length within given age groups of sculpins (Andersen, 2001). Regarding the western area of the Atlantic Ocean, only protozoa and trematode parasites (without nematodes) were detected in the liver of shorthorn sculpin sampled East Greenland (Dang et al., 2017). However, studies on the fish sampled in the southern coast of Newfoundland in 1990s revealed the presence of *Hysterothylacium aduncum*, *Contracaecum osculatum*, *Anisakis simplex* sensu lato and *Pseudoterranova decipiens* (Khan, 2011). Studies conducted in Canada at the beginning of the 2000s revealed the presence of Anisakidae nematodes (*Pseudoterranova decipiens* and *Anisakis* sp. larvae), however, the location of parasites in the fish body was not precisely presented (Dick et al., 2009). It is also noteworthy to highlight that the aforementioned study revealed several new host records for parasites and reported that mean numbers of parasite species increased with shorthorn sculpin age (Dick et al., 2009). In our studies shorthorn sculpins infected with nematodes in the liver have been found in each age group. Moreover, the most severely infected fish has been noted among the oldest of the analyzed individuals (5 years old). It is most probably related with the fact, that older fish were exposed to the possibility of consuming infected food for a longer period and had more time to accumulate the parasites in their body. Regardless the age class of the fish, we did not observe liver infection with nematodes in fish smaller than 21 cm. It might be related with feeding preferences and different ability to prey between smaller (< 21 cm) and bigger (≥21 cm) individuals. However, due to the absence of 1- and 2-year-old individuals among the sampled fish, our research lacks information on infection prevalence and intensity in the youngest fish, highlighting the need for further study in the future. For predatory fish, the primary mode of nematode parasite infection is largely influenced by the consumption of infected prey. Our research reveals that *C. crangon* and *Gammarus* spp. are the most abundant prey items in the diet of shorthorn sculpin living in the north-western Polish waters of Baltic Sea. These species may serve as intermediate hosts for nematode parasites, carrying their larval stages. Indeed, it was recently shown that *C. crangon* is a transmitter of *H. aduncum* (Pawlak, 2021) and *Gammarus* sp. of *C. osculatum* (Pawlak et al., 2019) in the Baltic Sea. Thus, our study not only confirms previous findings but also provides novel insights into potential infection routes by parasitic nematodes that affect shorthorn sculpin in the Baltic Sea.

A previous study of the dietary composition of shorthorn sculpin conducted more than 40 years ago on fish sampled in the Gdańsk Bay (Polish marine waters) revealed that *C. crangon* was the main prey species (Raciborski, 1984), similar to our results. Similarly, analysis conducted on the fish from Elbe estuary (German waters) found that *C. crangon* was the main prey species followed by *Carcinus maenas* (Kühl, 1961). Crustaceans, mainly *Macropipus holsatus* and *Crangon* spp., were also the major prey species (with some fish and bivalves) present in the diet composition of shorthorn sculpin sampled in German waters in 1980s (Ebeling and Alshuth, 1989). In turn, the research conducted on shorthorn sculpins sampled in south-western Swedish waters of the Baltic Sea over two seasons, February–March and October–November 1997 revealed that the isopod *Mesidotea entomon* (current name:

*Saduria entomon*) and herring *Clupea harengus* were the predominant dietary items by weight (Cardinale, 2000). *Mysis mixta* was also frequently observed in the diet of shorthorn sculpin, particularly in smaller fish, but to a less significant extent. The most pronounced ontogenetic shift in the diet of the shorthorn sculpin was observed in individuals $> 21$ cm in length, where herring was eaten almost exclusively (Cardinale, 2000). This shift in the species' diet appears to coincide with the presence of liver nematode parasites, as recorded in our research, which were only found in fish $\geq 21$ cm. In our studies, neither *S. entomon* nor herring were detected; however, these prey items might have been eaten earlier in the life span of the fish we analyzed.

Studies on the diet of shorthorn sculpin sampled in Canada in the early 2000s show, that although a wide range of diet items were present in the stomachs of the sampled fish, 99.5% of the total food consumed consisted of only 2–3 diet items (Dick et al., 2009), similarly to our results. Also, the dominating amphipods were present in the stomachs in similar proportions among all age classes of shorthorn sculpin (Dick et al., 2009), that has been also noted in our samples. Research conducted in the Arctic revealed that smaller individuals of the shorthorn sculpin fed predominantly on invertebrates while larger fed more on fishes (Landry et al., 2018), which can explain the low level of fish prey in our samples. Moreover, the mentioned study indicated that shorthorn sculpin had high % reliance on pelagic resources, thus coupling benthic and pelagic sources (Landry et al., 2018).

Interestingly, among our samples 24% of stomachs were empty. Since shorthorn sculpin belongs to fish species that do not feed during spawning time (Luksenburg et al., 2004), the empty stomachs may be attributed to the fact that the examined fish were caught in November, a peri-spawning period characterized by advanced gonadal development (stages 4–5). Additionally, sudden changes in pressure due to depth variations while fishing can cause stress of the fish, leading to regurgitation as observed in other demersal fish species (e.g., cod), which may also explain this phenomenon.

Another interesting aspect of shorthorn sculpin feeding is the spawning and sex composition, with males dominating our sample at nearly 60%. Luksenburg et al. (2004) suggested that during the spawning period, male shorthorn sculpins are more mobile in search of unmated females, making them easier to catch than females. Contrary, after spawning male guards the eggs, while the female is more mobile during preying (Luksenburg et al., 2004). To conclude, our study represents the first attempt to delineate the current status of parasitological infection with nematodes and the dietary composition of shorthorn sculpin in north-western Polish waters of the Baltic Sea. It is worth emphasizing that our results based on a single sampling effort, reflect the specific time of year and particular area where the study was conducted. It is important to remember, that fish undertake feeding and spawning migration during the year and their ability to prey and food availability might differ across seasons. For a more comprehensive understanding of these issues, further studies are essential, encompassing spatio-temporal changes in the infection profile and dietary composition of the species.

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## Declaration of competing interest

None declared.

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