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WYDZIAŁ OCEANOLOGII I GEOGRAFII

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TRANSMISSION OF PARASITES BY MARINE  
CRUSTACEANS WHICH CONSTITUTE FOOD OF COD,  
*GADUS MORHUA* FROM THE SOUTHERN BALTIC

TRANSMISJA PASOŻYTÓW PRZEZ SKORUPIAKI  
MORSKIE STANOWIĄCE POKARM DORSZA,  
*GADUS MORHUA* Z POŁUDNIOWEGO BAŁTYKU

**The doctoral dissertation**

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## SUMMARY

Cod (*Gadus morhua*) Linnaeus, 1758, is an important fish species due to its commercial exploitation in many parts of the world, for example, in the fisheries of northern Europe. In the Baltic Sea there are two biologically distinct stocks: western Baltic cod and eastern Baltic cod (ICES, 2021a). For many years, the eastern Baltic cod stock was a very important part of the fishing industry in Poland, but over the last four decades there has been a massive drop in biomass and a contraction of its distribution to the southern areas (Orio *et al.*, 2019). The poor status of eastern Baltic cod is characterised by biological changes in the fish: growth, condition (weight-at-length), size-at-maturation and recruitment have all substantially declined (ICES, 2021b). Changes in the ecosystem, such as poor oxygenation conditions, can affect cod both directly by altering their metabolism and indirectly by resulting in a shortage of benthic prey (Conley *et al.*, 2009; Carstensen *et al.*, 2014; Haase *et al.*, 2020). In addition, the reduced availability of food in the main distribution area of cod may have a negative impact on fish (Eero *et al.*, 2012). Indeed, the feeding levels of small cod in recent years are consistent with severe growth limitation and increased starvation-related mortality (Neuenfeldt *et al.*, 2020). In the same time period, sprat and herring, major prey species of adult cod, have developed a more northerly distribution, and there is less overlap with the distribution of the cod stock. It is, however, unclear whether the limited remaining cod stock would be impacted by this shift in distribution (ICES, 2021b). Importantly, increasing infection with parasites likely also affects the health and condition of fish (Haarder *et al.*, 2014; Mehrdana *et al.*, 2014; Horbowy *et al.*, 2016), while intensive exploitation of marine resources probably also has a negative impact (Lindgren *et al.*, 2009). All these factors contribute to the fact that the population of eastern Baltic cod stock has decreased dramatically over the years and the European Commission decided to close the cod stock fishery from July 2019 (EC, 2019; COM, 2019). The International Council for the Exploration of the Sea (ICES) has advised fisheries focused on cod in the Baltic Sea (in subdivisions 24-32) to cease fishing in 2020, 2021 and 2022 (ICES, 2019; ICES, 2020; ICES, 2021b).

In ecological terms, cod is a predator at the top of the trophic pyramid, alongside the salmonids and marine mammals. During spawning, cod concentrate in offshore waters and migrate to coastal waters for feeding (Bagge *et al.*, 1994). Young individuals mainly occupy the shallow water of coastal zones, which provide optimal conditions for growth, such that cannibalism does not occur. Older individuals migrate to deeper, offshore areas. Feeding preferences differ between small and larger fish and depend on their ability to catch and eat specific prey species. Cod is predator during almost its whole lifetime. Young fish prey on invertebrates, for example *Bylgides sarsi*, *Pontoporeia femorata*, *Gammarus* sp., *Mysis mixta*, *Crangon crangon*, *Saduria entomon* and also on small fish e.g. Gobiidae (Załachowski *et al.*, 1975; Załachowski, 1977; Pachur and Horbowy, 2013; Haase *et al.*, 2020). Adult fish feed on Clupeidae fish (sprat *Sprattus sprattus*, herring *Clupea harengus*), but also on larger crustaceans (Załachowski *et al.*, 1975; Załachowski, 1977; Pachur and Horbowy, 2013; Haase *et al.*, 2020). These changes in cod diet therefore reflect both fish age and the biodiversity of prey species in the specific areas where fish are feeding.

Diet is not only a source of nutrients, but may also be a source of infection by parasites. The type of parasite species a fish might contain depends on its age and developmental stage. Different parasite species occur in small/young versus larger/older cod. The phenomenon of organ specificity (topospecificity) appears to limit the occurrence of parasites to specific organs. In young fish, the acanthocephalan parasite *Echinorhynchus gadi* (Pilecka-Rapacz and Sobecka, 2004), which resides in the digestive tract, is often recorded. Acanthocephalans also occur in older fish (Studnicka, 1965; Sobecka, 2007), but the dominant parasitic fauna are the nematodes *Anisakis simplex*, *Contracaecum osculatum*, *Pseudoterranova decipiens* and *Hysterothylacium aduncum* (Szostakowska *et al.*, 2005; Buchmann and Kania, 2012; Nadolna and Podolska, 2014). *H. aduncum* is an intestinal parasite, while *C. osculatum* prefers liver, and *A. simplex* and *P. decipiens* are most often located in muscle tissue. Cod may play various roles in parasite life cycles. For example, cod might be an intermediate host in which the transformation of the parasite to the next developmental stage occurs. Fish may also act as a paratenic host, i.e. it hosts the larval stage, but this role is not necessary for completion of the parasite life cycle because there is no transformation of the parasite to the next developmental stage

(Pojmańska *et al.*, 2016). Lastly, fish may be the final host, in which transformation from the larval stage to the adult form takes place.

The life cycles of *A. simplex*, *C. osculatum* and *P. decipiens* are very similar: marine mammals are the final hosts for these parasites (McClelland *et al.*, 1990; Klimpel and Palm, 2011) and there is a clear host specificity, with particular species of marine mammal being responsible for closing the life cycle of a particular parasite species. Thus, the final hosts for *A. simplex* are cetaceans, which in the Baltic Sea are represented by the harbor porpoise, *Phocoena phocoena* (Herrerias *et al.*, 2004); for *C. osculatum*, the grey seal, *Halichoerus grypus* (Fagerholm, 1990); and for *P. decipiens*, the harbor seal, *Phoca vitulina* (Aspholm *et al.*, 1995) and the grey seal (Hauksson, 2011). In the final host, larval stage L4 transforms to the dioecious mature stage, which is capable of producing fertilized eggs. Parasite eggs enter the water environment via the feces of their final hosts, i.e. marine mammals. Transformation to the larval stages L1, L2 or even L3 occurs within the egg (Køie and Fagerholm, 1995). L2 or L3 larvae are eaten by crustaceans, which then play the role of intermediate host where transformation from L2 to L3 can take place. Such infected crustaceans are eaten by pelagic fish (e.g. sprat, herring), which in turn are eaten by predatory fish (e.g. cod). Marine mammals represent the last link in the trophic chain: they feed on infected fish and thus become the definitive, i.e. final host and subsequently the life cycle is complete (Køie and Fagerholm, 1995; Klimpel *et al.*, 2004; Mouritsen *et al.*, 2010). The life cycle of *H. aduncum* is similar to that described above, 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.

Marine crustaceans - Amphipoda, Cirripedia, Copepoda, Decapoda, Euphausiacea, Isopoda and Mysidacea - very commonly act as intermediate or paratenic hosts of parasites. Studies on this issue have been conducted in Canada, Norway and Scotland. However, until now, research on the detection of intermediate hosts for nematode and acanthocephalan parasites in the Baltic Sea has been limited to western areas (e.g. Kiel Bay and Lübeck Bay in Germany) or has focused on experimental approaches.



Examples of Baltic Sea crustaceans infected with nematodes and acanthocephalans are listed below:

- *Acartia bifilosa* - *Hysterothylacium* sp. (Lick, 1991; Zander *et al.*, 1994);
- *Acartia tonsa* - *H. aduncum*, *A. simplex*, *C. osculatum* (Køie, 1993, 2001; Køie and Fagerholm, 1995);
- *Temora longicornis* - *C. osculatum* (Køie and Fagerholm, 1995);
- *Oithona similis* - *A. simplex* (Køie, 2001);
- *Balanus* sp. - *A. simplex*, *C. osculatum* (Køie, 2001; Køie and Fagerholm, 1995);
- *Neomysis integer* - *H. aduncum*, *P. decipiens*, *C. osculatum* (Gibson, 1972; Lick, 1991; Køie and Fagerholm, 1995);
- *Gammarus salinus* - *Hysterothylacium* sp., *E. gadi* (Zander *et al.*, 1994, 2000, 2002);
- *Gammarus zaddachi* - *Hysterothylacium* sp., *E. gadi* (Zander *et al.*, 1994, 2002);
- *Gammarus oceanicus* - *E. gadi*, *H. aduncum* (Zander *et al.*, 1994; Fagerholm, 1987; Zander *et al.*, 2002);
- *Gammarus locusta* - *Hysterothylacium* sp. (Zander *et al.*, 2000);
- *Crangon vulgaris* (*C. crangon*) - *H. aduncum* (Gibson, 1972);
- *Hyperia galba* - *H. aduncum* (Klimpel and Rückrt, 2005);
- *Idotea* sp. - *H. aduncum* (Køie, 1993).

In addition, the last studies in the Baltic area were performed almost 20 years ago, and therefore it is timely to check for, and determine the nature of any changes that have occurred in the marine environment. To the best of my knowledge there have been no reports on the details of the life cycle of the above parasites, except in the western part of the Baltic Sea. For these parasites, intermediate hosts in the southern and central Baltic sea are unknown.

The presence of the nematodes *H. aduncum*, *A. simplex*, *C. osculatum*, and the acanthocephalan *E. gadi*, has been noticed in a variety of crustaceans, many of which also occur in the Baltic Sea, but to date there are no reports of individual crustaceans from the Baltic Sea containing these parasites. Indeed, the life cycles of the above parasites are only described in a general way for the Baltic Sea and there is no information on which specific crustacean species are potentially intermediate hosts. The only studies performed are those for areas bordering the western part of the Baltic Sea, i.e. the Danish Straits and German waters.

The lack of studies involving direct detection of parasites in invertebrates that are predated by fish and that also may be a transmission route for parasites reflects the technical difficulty of the research and its time-consuming, laborious and expensive nature. Moreover, the abundance of invertebrates in the marine environment and their unequal distribution makes it impossible to examine a whole body of water for infected organisms. Such studies require experience, knowledge and patience.

The parasite fauna of cod in the Baltic Sea is well known. Less clear, however, are the specific organisms that act as intermediate hosts for particular parasite species. Invertebrates that represent an important component of the cod diet may be crucial for the life cycles of their parasites. This study is the first investigation of the presence of parasites *in situ* in the diet of cod from the Baltic Sea.

**Aim of the study:**

To determine possible sources of cod infection with parasites based on an analysis of the parasite fauna of invertebrates present in the diet of cod.

The following research **hypotheses** were proposed:

- invertebrates eaten by cod can also be a source of parasite infection;
- various invertebrate species act as intermediate hosts in the life cycle of different parasite species occurring in cod.

Invertebrates infection with parasites were described using the following parameters (Bush *et al.*, 1997):

- prevalence - the number of individuals infected with parasites as a proportion of all individuals examined in a given area;
- intensity of infection - the number of individuals of a particular parasite species in a single host.

The biological material for research (digestive tracts of cod) was collected between 2012 and 2016 during commercial and research cruises in the Polish Exclusive Economic Zone of the Baltic Sea. In total, the food composition of 2,695 fish was examined. Parasitological analyses were performed on almost 25,000 invertebrates, of which 2,899 were *S. entomon*, 7,119 were *C. crangon*, and 1,977 were *Gammarus* sp., with approximately 13,000 other species or genera (Crustacea, Polychaeta, Bivalvia) also catalogued. Initially, each invertebrate food component was reviewed for the presence of parasites using a stereoscopic microscope. In case parasites were not visible during this initial analysis, the invertebrates were digested using artificial gastric juice (a solution of pepsin and hydrochloric acid) and each food component was checked again for parasites in the body cavity. This additional digestion was only necessarily for crustaceans. The parasites collected were subjected to taxonomic identification based on anatomical and morphological characteristics determined using a stereomicroscope. The initial taxonomic identification of the parasites was confirmed by DNA sequencing. Parasite DNA was purified and then amplified by polymerase chain reaction using specific primers. Amplicons were analyzed by DNA sequencing, which identified parasites to the species level.

The parasite-host system, *H. aduncum* - *S. entomon*, was described here for the first time (Fig. 1) (**publication 1**). L4 (n=1) and adult (n=1) stage *H. aduncum* were found in *S. entomon* for the first time. Molecular analyses of the parasites confirmed the nematode species, with the two sequences being deposited in GenBank. The presence of adult-stage *H. aduncum* in a crustacean suggests that invertebrates can act as final host for this parasite. On the other hand, the adult stage was found inside the undigested individual *S. entomon* in the cod stomach, suggesting that the transformation from larval L4 to adult stage took place in *S. entomon*, but after the crustacean's death in the cod stomach. The occurrence

of a L4 larva in this Isopoda species might also indicate migration of the parasite to the crustacean in the fish stomach. Experimental studies have shown that pepsin, a component of both natural and artificial gastric juice, may drive the transformation of larvae to the next developmental stage (Iglesias *et al.*, 2002; Adroher *et al.*, 2004). In addition, in the body cavity of *S. entomon* four more *H. aduncum* individuals were observed. The presence of these parasites was only detected after additional digestion in artificial gastric juice. Unfortunately, the condition of the parasites did not allow for identification of the larval stage. Parasites were also found inside the cod stomach, where the nematode *H. aduncum* and the acanthocephalan *E. gadi* were predominant.



Fig. 1 *S. entomon* infected with *H. aduncum*

A completely new and at the same time very important discovery is the demonstration of the presence of *A. simplex* in *C. crangon* (**publication 2**). In the Baltic Sea, brown shrimp has never been indicated as an intermediate host for nematode parasites. Nevertheless, one individual was found to be infected a single L3 larva. Furthermore, three *Gammarus* sp. individuals were found to be infected with nematode *C. osculatum* L3 larvae (**publication 2**). Taxonomic identification was confirmed by molecular analyses and DNA sequences were deposited in GenBank. Individuals from the Anisakidae family (*A. simplex* and *C. osculatum*) have the ability to migrate in host tissues and may spread to various organs. *C. osculatum* is noticed most frequently in the liver, which may

negatively influence the condition of fish. Cod stores energy reserves in the liver in the form of lipids. These reserves are used during the spawning period or when there is limited food in the environment. The parasite may damage liver tissue and feed on the nutrients, potentially leading to dysfunction of the liver. Consequently, the intensity of infection may negatively impact fish condition and could increase natural mortality (Horbowy *et al.*, 2016). Generally, *A. simplex* migrate to muscle tissue and occasionally to the liver. In the Baltic Sea cod is important for fisheries and fish processing, therefore food safety is related with economic aspect. This represents a food safety issue, since the presence of parasitic nematodes in fish and fish products may pose a risk to the human health. Ingestion of fish products containing *A. simplex* and other anisakid larvae can cause a disease called anisakidosis. Human health may also be compromised by allergic reactions to parasite antigens (hypersensitivity) (Alonso-Gómez *et al.*, 2004; Audicana and Kennedy, 2008; Valero *et al.*, 2003; Mehrdana and Buchmann, 2017). Although adequate thermal processing kills the parasites (Wharton and Aalders, 2002), allergens present in Anisakidae are thermostable (Audicana *et al.*, 2002; Moneo *et al.*, 2005). It is also important to note that *A. simplex* larvae are tolerant to a wide range of temperatures and thus may survive freezing at -20°C (Podolska *et al.*, 2019).

During the parasitological analyses it was found that *C. crangon* may also be an intermediate host for nematode *H. aduncum* (Fig. 2) (**publication 3**). This host-parasite system have never been observed before in the Baltic Sea. Based on anatomical features, nine larvae were identified as *Hysterothylacium* sp. (L3). Molecular identification was confirmed for seven larvae. Examples of sequences have been deposited in GenBank. *Hysterothylacium* sp. is one of the most numerous nematode species in fish and is common in the marine food chain. More than 70 different invertebrate species have been reported as intermediate hosts for this parasite (Lick, 1991).

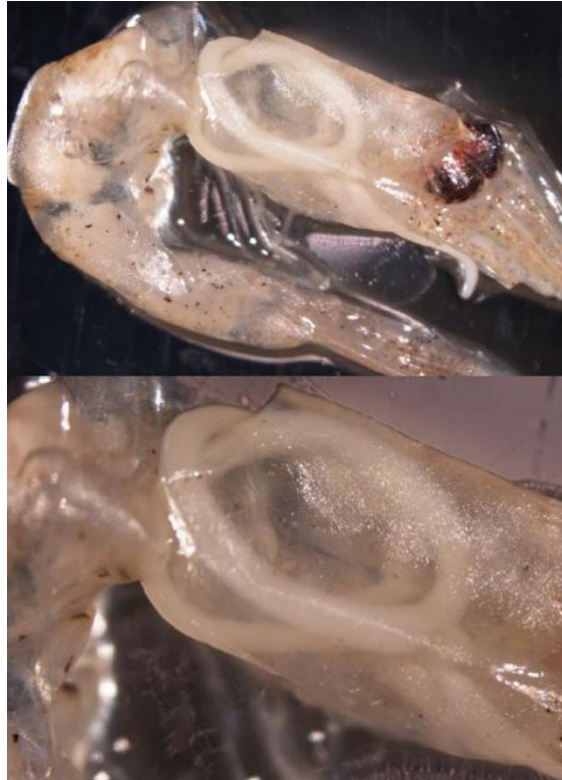


Fig.2 *C. crangon* infected with *H. aduncum*

The intended goal of this study was achieved: possible sources of cod infection with parasites were determined by analyzing the parasite fauna of invertebrates that form part of the diet of cod. The initial hypotheses were verified: invertebrates that are components of the cod diet may also be a source of infection with fish parasites; different species of invertebrate serve as intermediate hosts for different species of cod parasite.

Parasite-host systems that have not been recorded so far in the Baltic Sea were described for the first time. Thus, *S. entomon* and *C. crangon* may act as intermediate hosts in the *H. aduncum* life cycle, as shown in **publications 1** and **3**. A very important finding, in terms of food safety, is the demonstration of *C. crangon* infected with *A. simplex*, and of *Gammarus* sp. infected with *C. osculatum*. These relationships are described in **publication 2**.

The research results obtained during my studies have contributed to a broadening of basic knowledge about the sources of cod infection. Moreover, this work allows for a more detailed description of the life cycle of cod parasites in the Baltic Sea. Finally, it will also enable a better understanding of the role of particular food components in the transmission of certain parasite species.

The marine environment is currently experiencing progressive changes in climate, physicochemistry, the range of occurrence and condition of marine organisms, the occurrence of alien species, etc. Permanent environmental change is a challenge, but also creates new opportunities for parasites to modify their life cycle. Accordingly, further monitoring of this phenomenon is advisable and particular attention should be paid to intermediate hosts. The dominant crustaceans in the diet of cod are also part of the diet of other fish in the Baltic Sea. Therefore, it seems highly likely that such parasites will be transferred to new hosts. Climate change may also contribute to the migration of parasites, which then can occupy new hosts.

Bearing in mind the above facts, I would like to continue my research work in this topic using the rich biological data and new biological material collected so far. An important point that I would like to verify is whether there are any spatio-temporal changes in intensity and prevalence of infection in organisms constituting the diet of fish. I would ask whether the intensity and prevalence of infected food components vary depending on the biological parameters of the host (e.g. length or age of the fish). Since environmental conditions change over time, the diets of other fish should be considered and checked for the presence of parasites. In addition, it would be interesting to investigate areas located near seal colonies, e.g. the Vistula River estuary or near Bornholm island, for infected invertebrates.

## STRESZCZENIE

Dorsz (*Gadus morhua*) Linnaeus, 1758, jest jednym z najczęściej poławianych komercyjnie gatunków ryb w wielu krajach i gatunkiem znaczącym dla rybołówstwa w północnej Europie. W Morzu Bałtyckim występują dwa biologicznie odrębne stada: zachodnie i wschodnie stado dorsza bałtyckiego (ICES, 2021a). Polska jest jednym z krajów, dla którego połowy dorsza skoncentrowane na stadzie wschodnim były bardzo ważną częścią przemysłu rybnego. W ostatnich latach bałtyckie rybołówstwo dorszowe przeżywa poważny kryzys. Wiąże się to ze słabą kondycją i stanem populacji ryb stada wschodniego. W ciągu ostatnich czterech dekad wykazano znaczny spadek biomasy dorszy ze stada wschodniego Bałtyku a zasięg ich rozmieszczenia ograniczył się do obszarów południowych (Orio i in., 2019). Zły stan dorsza wschodniego jest w dużej mierze spowodowany zmianami biologicznymi - tempo wzrostu, kondycja i rozmiar w okresie dojrzewania płciowego znacznie się zmniejszyły (ICES, 2021b). Zmiany w ekosystemie, takie jak niekorzystne warunki tlenowe, które mogą wpływać na dorsza zarówno bezpośrednio poprzez zmianę jego metabolizmu, jak i pośrednio poprzez niedobór bentosowego pokarmu (Conley i in., 2009; Carstensen i in., 2014; Haase i in., 2020), jak również przetrwanie potomstwa. Ponadto zmniejszona dostępność pokarmu rybnego w głównym obszarze występowania dorsza może mieć negatywny wpływ na kondycję i stan zasobów ryb (Eero i in., 2012). Obecnie, małe dorsze wykazują poważne ograniczenie tempa wzrostu i zwiększoną śmiertelność, która może być efektem głodowania (Neuenfeldt i in., 2020). W ostatnich latach wykazano, że rozmieszczenie szprota i śledzia (główne gatunki stanowiące pokarm dorosłego dorsza) w północnych obszarach Bałtyku w mniejszym stopniu pokrywało się z rozmieszczeniem zasobów dorsza. Nie jest jednak jasne, czy ta zmiana rozmieszczenia wpłynie na stado dorsza (ICES, 2021b). Ponadto intensywna eksploatacja zasobów, może negatywnie wpływać na ich stan (Lindgren i in., 2009). Co ważne, rosnąca ekstensywność i intensywność zarażenia pasożytami, także może mieć niekorzystne konsekwencje dla zdrowia i kondycji dorszy (Haarder i in., 2014; Mehrdana i in., 2014; Horbowy i in., 2016). Wszystkie te czynniki wpływają na to, że populacja wschodnia dorsza bałtyckiego drastycznie zmalała na przestrzeni lat, dlatego Komisja Europejska zdecydowała o zamknięciu



połowów dorszy stada wschodniego od lipca 2019 roku (EC, 2019; COM, 2019). Międzynarodowa Rada Badań Morza (ICES) zaleciła rybołówstwu, które skupia się na połowach dorsza w Morzu Bałtyckim (w podobszarze 24-32) zaprzestania połowów w 2020, 2021 oraz 2022 roku (ICES, 2019; ICES, 2020; ICES, 2021b).

Pod względem ekologicznym dorsz pełni rolę drapieżnika znajdującego się na szczycie piramidy troficznej, zaraz obok ryb łososiowatych oraz ssaków morskich. Dorsz jest gatunkiem, który w czasie tarła koncentruje się w wodach głębszych, natomiast w celu odżywiania migruje do wód przybrzeżnych (Bagge i in., 1994). Młode osobniki występują głównie w pobliżu wybrzeża, w płytkich wodach, co zapewnia im optymalne warunki do wzrostu oraz unikają tym samym kanibalizmu. Starsze osobniki migrują do głębszych obszarów morza. Preferencje pokarmowe różnią się między młodymi a dorosłymi rybami i są uzależnione od dostępności pokarmu i zdolności do jego zdobywania. Dorsz jest rybą drapieżną przez większość swojego życia. Młode osobniki polują na bezkręgowce, takie jak *Bylgides sarsi*, *Pontoporeia femorata*, *Gammarus* sp., *Mysis mixta*, *Crangon crangon*, *Saduria entomon*, oraz małe ryby, np. ryby babkowate (Załachowski i in., 1975; Załachowski, 1977; Pachur i Horbowy, 2013; Haase i in., 2020). Dorosłe osobniki odżywiają się przede wszystkim rybami śledziowatymi (szprot *Sprattus sprattus*, śledź *Clupea harengus*), ale także większymi skorupiakami (Załachowski i in., 1975; Załachowski, 1977; Pachur i Horbowy, 2013; Haase i in., 2020). W związku z tym, zmienność w diecie dorsza odzwierciedla wiek ryb i bioróżnorodność gatunków, którymi żywi się ryba w zajmowanych siedliskach.

Dieta jest nie tylko źródłem składników odżywczych, ale także może być źródłem zarażenia pasożytami. Obecność i różnorodność gatunków pasożytów jest również uzależniona od okresu rozwoju ryb. Inne gatunki pasożytów występują u małych/młodych, a inne u większych/starszych dorszy. U wielu gatunków pasożytów występuje zjawisko specyficzności topicznej (topospecyficzności), czyli preferencji do określonej lokalizacji w organizmie żywiciela. Najczęściej u młodych osobników występują kolcogłowy z gatunku *Echinorhynchus gadi* (Pilecka-Rapacz i Sobecka, 2004), obecne w przewodzie pokarmowym. Kolcogłowy występują też u starszych dorszy (Studnicka, 1965; Sobecka, 2007), lecz dominującą parazytofaunę stanowią nicienie *Anisakis simplex*, *Contracaecum osculatum* i *Pseudoterranova decipiens*, *Hysterothylacium aduncum* (Szostakowska i in., 2005; Buchmann i Kania, 2012; Nadolna i Podolska, 2014).

Nicienie *H. aduncum* są pasożytami jelitowym, *C. osculatum* preferują wątrobę, a *A. simplex* i *P. decipiens* tkankę mięśniową dorszy. Dorsz w cyklach życiowych pasożytów może pełnić różne role. Może być żywicielem pośrednim, czyli takim, w którym następuje przeobrażenie pasożyta do kolejnych etapów rozwojowych. Ryby pełnią także rolę żywiciela paratenicznego, u którego nie następuje przeobrażenie pasożyta do kolejnego stadium rozwojowego, a obecność ryb nie jest konieczna do zamknięcia cyklu życiowego pasożyta (Pojmańska i in., 2016). Dorsz może być również żywicielem ostatecznym, w którym następuje przeobrażenie postaci larwalnej do postaci dorosłej.

Cykle rozwojowe nicieni *A. simplex*, *C. osculatum* i *P. decipiens* są podobne, a ssaki morskie są żywicielami ostatecznymi (McClelland i in., 1990; Klimpel i Palm, 2011). Występuje specyficzność żywicielska tzn. określone gatunki ssaków morskich pełnią rolę żywicieli ostatecznych, u których dochodzi do zamknięcia cyklu rozwojowego określonego gatunku pasożyta: dla *A. simplex* są to walenie, które w Bałtyku reprezentuje morświn *Phocoena phocoena* (Herreras i in., 2004); dla *C. osculatum* - foka szara *Halichoerus grypus* (Fagerholm, 1990); dla *P. decipiens* - foka pospolita *Phoca vitulina* (Aspholm i in., 1995) i foka szara (Hauksson, 2011). W żywicielu ostatecznym następuje przeobrażenie postaci larwalnej L4 do rozdzielнопłciowych postaci dorosłych oraz do produkcji jaj i zapłodnienia. Jaja pasożytów trafiają wraz z odchodami ssaków do środowiska morskiego. W jajach dochodzi do przeobrażenia w postaci larwalne L1, L2 a nawet L3 (Køie i Fagerholm, 1995). Larwy L2 lub L3 wraz z pokarmem dostają się do skorupiaków, które pełnią rolę żywiciela pośredniego. W żywicielu pośrednim następuje przeobrażenie postaci larwalnej z L2 do L3. Następnie zarażone skorupiaki zjadane są przez małe ryby np. szprotki, śledzie, a te przez ryby drapieżne np. dorsze. Ssaki morskie stanowią ostatnie ogniwo w łańcuchu troficznym: żywią się zarażonymi rybami i w ten sposób stają się żywicielami ostatecznymi. Cykl życiowy pasożyta zostaje zamknięty (Køie i Fagerholm, 1995; Klimpel i in., 2004; Mouritsen i in., 2010). Cykl życiowy *H. aduncum* wygląda podobnie do opisanego powyżej, natomiast różni się tym, że żywicielem ostatecznym są ryby. Dojrzałe płciowo osobniki najczęściej bytują w przewodzie pokarmowym ryb np. węgorzycy (*Zoarces viviparus*) czy dorsza (Jackson i in., 1997).

Skorupiaki morskie - Amphipoda, Cirripedia, Copepoda, Decapoda, Euphausiacea, Isopoda i Mysidacea są opisane w literaturze naukowej jako żywicieli pośredni lub parateniczni pasożytów. Prace dotyczące tego zagadnienia prowadzono m. in. w Kanadzie, Norwegii i Szkocji. W Morzu Bałtyckim dotychczasowe badania poświęcone żywicielom pośrednim nicieni i kolcogłówów ograniczały się do badań z zachodniej części Morza Bałtyckiego (np. Zatoka Kilońska, Zatoka Lubecka - Niemcy) lub do badań eksperymentalnych.

Poniżej przedstawiono przykłady bałtyckich skorupiaków zarażonych nicieniami lub kolcogłowami:

- *Acartia bifilosa* - *Hysterothylacium* sp. (Lick, 1991; Zander i in., 1994);
- *Acartia tonsa* - *H. aduncum*, *A. simplex*, *C. osculatum* (Køie, 1993, 2001; Køie i Fagerholm, 1995);
- *Temora longicornis* - *C. osculatum* (Køie i Fagerholm, 1995)
- *Oithona similis* - *A. simplex* (Køie, 2001)
- *Balanus* sp. - *A. simplex*, *C. osculatum* (Køie, 2001; Køie i Fagerholm, 1995)
- *Neomysis integer* - *H. aduncum*, *P. decipiens*, *C. osculatum* (Gibson, 1972; Lick, 1991; Køie i Fagerholm, 1995);
- *Gammarus salinus* - *Hysterothylacium* sp., *E. gadi* (Zander i in., 1994, 2000, 2002);
- *Gammarus zaddachi* - *Hysterothylacium* sp., *E. gadi* (Zander i in., 1994, 2002);
- *Gammarus oceanicus* - *E. gadi*, *H. aduncum* (Zander i in., 1994; Fagerholm, 1987; Zander i in., 2002);
- *Gammarus locusta* - *Hysterothylacium* sp. (Zander i in., 2000);
- *Crangon vulgaris* (*C. crangon*) - *H. aduncum* (Gibson, 1972);
- *Hyperia galba* - *H. aduncum* (Klimpel i Rückrt, 2005);
- *Idotea* sp. - *H. aduncum* (Køie, 1993).

Ponadto, ostatnie badania parazytofauny bezkręgowców w rejonie Bałtyku wykonywano na początku XXI wieku, dlatego też ważna jest weryfikacja czy i jakie zmiany zaszły w środowisku na przestrzeni ostatnich lat. Na podstawie posiadanej przeze mnie wiedzy nie podejmowano próby dokładnego opisanie cykli życiowych wymienionych pasożytów poza zachodnim rejonem Bałtyku i dotychczas nie wykazano, które gatunki bezkręgowców są żywicielami pośrednimi pasożytniczych nicieni w południowym i centralnym Bałtyku.

Występowanie nicieni *H. aduncum*, *A. simplex*, *C. osculatum*, *P. decipiens* oraz kolcogłówów *E. gadi* opisano w wielu gatunkach skorupiaków, które występują także w Morzu Bałtyckim, ale nigdy nie stwierdzono przypadków zarażenia *in situ* u bezkręgowców z Bałtyku. Cykle życiowe tych pasożytów dla Bałtyku są przedstawione jedynie ogólnie. Nie opisano, który konkretnie gatunek bezkręgowca może być potencjalnym żywicielem pośrednim dla powyższych gatunków pasożytów. Wcześniej nie prowadzono prac poza zachodnim rejonem Bałtyku (cieśniny duńskie i wody niemieckie).

Dotąd nie prowadzono badań *in situ* tj. mających na celu wykrycie obecności pasożytów bezpośrednio w bezkręgowcach, które stanowią pokarm ryb i mogą być potencjalnym „nośnikiem” pasożytów. Jest to trudne, czasochłonne, pracochłonne i wymagające sporych nakładów finansowych przedsięwzięcie. Ponadto, liczebność organizmów w środowisku jest znaczna i są one nierównomiernie rozproszone w toni wodnej, co powoduje, że niemożliwym jest przebadanie całego akwenu w poszukiwaniu zainfekowanych organizmów. Dlatego też potrzebne jest duże doświadczenie, wiedza oraz cierpliwość.

Parazytofauna dorsza z Morza Bałtyckiego została dobrze poznana. Nie znane są jednak specyficzne organizmy, które pełnią rolę żywicieli pośrednich dla poszczególnych gatunków pasożytów. Bezkręgowce, stanowiące ważny element w diecie dorsza, mogą mieć kluczowe znaczenie w cyklach życiowych jego pasożytów. Dlatego po raz pierwszy przeprowadzono badania dotyczące obecności pasożytów *in situ* w pokarmie dorsza z Morza Bałtyckiego.

### **Cel naukowy badań:**

Celem badań było określenie możliwych źródeł zarażenia dorszy bałtyckich pasożytami, na podstawie analizy parazytofauny bezkręgowców morskich występujących w pokarmie tych ryb.

Założono następujące **hipotezy** badawcze:

- bezkręgowce stanowiące pokarm dorszy mogą być jednocześnie źródłem zarażenia ryb pasożytami;
- różne gatunki bezkręgowców pełnią rolę żywiciela pośredniego w cyklu rozwojowym różnych gatunków pasożytów występujących u dorszy.

Zarażenie bezkręgowców pasożytami opisano następującymi parametrami (Bush i in. 1997):

- ekstensywność zarażenia, czyli stosunek liczby osobników zarażonych pasożytami do wszystkich osobników badanych w danym rejonie;
- intensywność zarażenia, czyli liczba osobników określonego gatunku pasożyta występująca w jednym żywicielu.

W latach 2012-2016 pozyskano materiał biologiczny w postaci przewodów pokarmowych dorszy z rejsów badawczych i rejsów komercyjnych w Polskiej Strefie Ekonomicznej Morza Bałtyckiego. Łącznie przebadano pokarm z 2 695 dorszy. Pozyskano i przeprowadzono analizę parazytologiczną u prawie 25 000 bezkręgowców, w tym 2 899 *S. entomon*, 7 119 *C. crangon*, 1 977 *Gammarus* sp. i około 13 000 innych (Crustacea, Polychaeta, Bivalvia). Obecność pasożytów w bezkręgowcach sprawdzono przy użyciu mikroskopu stereoskopowego. W pierwszej kolejności wykonano przegląd każdego składnika pokarmowego. Jeżeli pasożyty nie były widoczne, a badany organizm nie uległ znacznemu nadtrawieniu w żołądku, w celu łatwiejszej detekcji, poddawano go dodatkowemu trawieniu w sztucznym soku żołądkowym (wodny roztwór pepsyny i kwasu solnego). Następnie, ponownie sprawdzano czy pasożyty uwidoczniły się pod powłokami ciała. Trawienie było konieczne tylko w przypadku skorupiaków. Znalezione pasożyty zostały poddane analizie parazytologicznej i określeniu przynależności gatunkowej na podstawie cech anatomicznych i morfologicznych

z wykorzystaniem mikroskopu stereoskopowego. W celu potwierdzenia przynależności gatunkowej pasożytów wykorzystano techniki molekularne - sekwencjonowanie DNA. DNA pasożytów zostało odpowiednio przygotowane tzn. oczyszczone i powielone przy wykorzystaniu starterów specyficznych (reakcja PCR) dla danego gatunku pasożyta, wstępnie zidentyfikowanego na podstawie cech anatomicznych i morfologicznych. Produkty reakcji PCR przygotowano do sekwencjonowania. Wyniki sekwencjonowania pozwoliły na jednoznaczne określenie przynależności gatunkowej pasożytów.

Po raz pierwszy opisano układ pasożyt - żywiciel: *H. aduncum* - *S. entomon* (rys. 1) (**publikacja 1**). Po raz pierwszy stwierdzono występowanie larwy L4 (n=1) i postaci dorosłej (n=1) *H. aduncum* w *S. entomon*. Wyniki analizy molekularnej pozyskanych nicieni, potwierdziły ich przynależność gatunkową, obydwie sekwencje zdeponowano w GenBanku. Obecność dorosłej postaci *H. aduncum* w skorupiaku sugeruje, że bezkręgowce mogą pełnić rolę żywicieli ostatecznych. Natomiast, biorąc pod uwagę fakt, że dorosłego pasożyta znaleziono u niestrawionego osobnika *S. entomon* wewnątrz żołądka dorsza, to mogło mieć miejsce przeobrażenie z larwy L4 do stadium dorosłego w *S. entomon*, ale już po śmierci skorupiaka w dorszu. Obecność larwy L4 i dorosłego nicienia we wspomnianym gatunku Isopoda może wskazywać na migracje pasożytów do skorupiaków w żołądku ryby. Badania laboratoryjne udowodniły, że pepsyna, która znajduje się w naturalnym jak i sztucznym soku trawiennym może przyspieszać przeobrażenie się larw z jednego do drugiego stadium (Iglesias i in., 2002; Adroher i in, 2004). Ponadto, pod powłokami ciała *S. entomon* zaobserwowano 4 osobniki *H. aduncum*, których obecność stwierdzono dopiero po dodatkowym trawieniu organizmów w sztucznym soku żołądkowym. Niestety, stan pozyskanych pasożytów nie pozwalał na zidentyfikowanie ich stadiów rozwojowych. Znaleziono także pasożyty we wnętrzu żołądków dorszy: dominowały nicienie *H. aduncum* oraz kolcogłowy *E. gadi*.



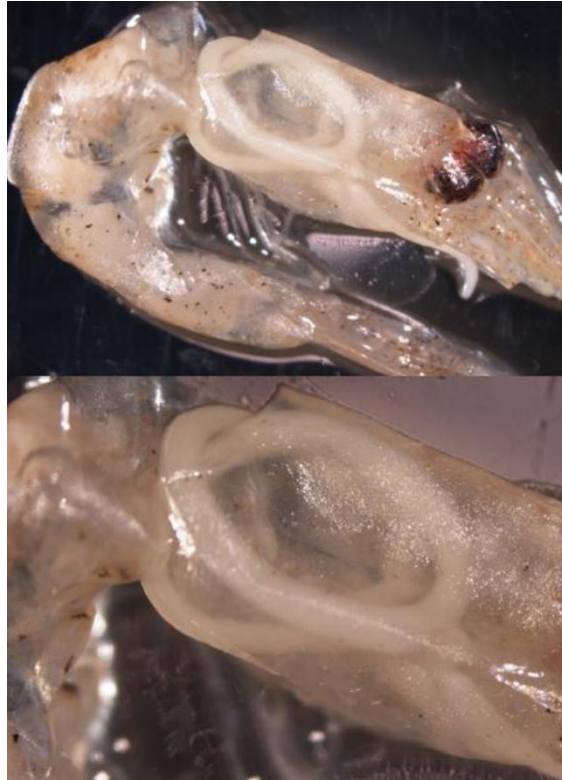
Rys. 1 *S. entomon* zarażony *H. aduncum*

Zupełnie nowym i jednocześnie bardzo ważnym odkryciem jest wykazanie obecności *A. simplex* w *C. crangon* (**publikacja 2**). W Bałtyku nigdy wcześniej nie wskazywano garneli (*C. crangon*) jako żywiciela pośredniego nicieni. Stwierdzono zarażenie larwą L3 u jednego osobnika. Ponadto u trzech osobników *Gammarus* sp. znaleziono nicienie *C. osculatum* w stadium L3 (**publikacja 2**). Przynależność taksonomiczną pasożytów potwierdzono na podstawie analiz genetycznych, uzyskane sekwencje zdeponowano w GenBanku. Przedstawiciele rodziny Anisakidae (*A. simplex* i *C. osculatum*) mają zdolność do migracji w tkankach żywiciela i mogą docierać do różnych narządów. Obecność gatunku *C. osculatum* notuje się najczęściej w wątrobie, co może mieć negatywny wpływ na kondycje ryb. U dorszy w wątrobie magazynowane są rezerwy energetyczne w postaci tłuszczu. Jest to materiał zapasowy, wykorzystywany w okresie tarła lub w sytuacji niedoboru pokarmu w środowisku. Pasożyty mogą uszkadzać tkankę wątroby oraz żywić się znajdującymi się w niej substancjami odżywczymi. Może dochodzić wówczas do zaburzenia funkcjonowania tego narządu. Konsekwencją obecności pasożytów jest spadek kondycji dorsza a wysoka intensywność zarażenia, może prowadzić do wzrostu śmiertelności naturalnej (Horbowy i in. 2016). W Morzu Bałtyckim dorsz jest ważnym gatunkiem dla rybołówstwa i przetwórstwa ryb. Bezpieczeństwo żywności jest zatem bardzo istotnym aspektem ekonomicznym. Obecność pasożytniczych nicieni w rybach i produktach rybnych może mieć negatywny wpływ na zdrowie ludzi, ponieważ nicienie *A. simplex* najczęściej migrują do tkanki mięśniowej ryb. Larwy *A. simplex* i inne larwy Anisakidae,

znajdujące się w produktach rybnych mogą być źródłem zarażenia człowieka, powodując chorobę zwaną anisakidozą. Może również występować reakcja alergiczna na antygeny pasożytów (nadwrażliwość), co może mieć negatywny wpływ na zdrowie człowieka (Alonso-Gómez i in., 2004; Audicana i Kennedy, 2008; Valero i in., 2003; Mehrdana i Buchmann, 2017). Obecność tych zoonotycznych pasożytów w bałtyckim dorszu budzi obawy związane z bezpieczeństwem żywności. Pomimo, że odpowiednia obróbka termiczna zabija pasożyta (Wharton i Aalders, 2002), alergeny występujące u nicieni Anisakidae są termostabilne (Audicana i in., 2002; Moneo i in., 2005). Należy podkreślić, że larwy *A. simplex* posiadają dużą tolerancję na zmiany temperatury i mogą przetrwać mrożenie w temperaturze -20°C (Podolska i in., 2019).

Podczas analiz parazytologicznych wykryto, że *C. crangon* może być żywicielem pośrednim także dla nicienia *H. aduncum* (rys. 2) (**publikacja 3**). Tego układu żywiciel-pasożyt nigdy wcześniej nie notowano w Morzu Bałtyckim. Na podstawie cech anatomicznych 9 larw określono je jako *Hysterothylacium* sp. (L3). Badania genetyczne pozwoliły na potwierdzenie przynależności gatunkowej 7 z nich. Przykłady sekwencji zdeponowano w bazie GenBank. *Hysterothylacium* sp. jest jednym z najliczniejszych nicieni występujących u ryb i niezwykle powszechnym pasożytem w morskiej sieci troficznej. Odnotowano ponad 70 różnych gatunków bezkręgowców w roli żywicieli pośrednich tego pasożyta (Lick, 1991).





Rys. 2 *C. crangon* zarażony *H. aduncum*

Zrealizowano założony cel tj. określono możliwe źródła zarażenia dorszy bałtyckich pasożytami, na podstawie analizy parazytofauny bezkręgowców morskich występujących w pokarmie tych ryb. Potwierdzono założone hipotezy badawcze: bezkręgowce stanowiące pokarm dorszy są jednocześnie źródłem zarażenia ryb pasożytami; różne gatunki bezkręgowców pełnią rolę żywiciela pośredniego w cyklu rozwojowym różnych gatunków pasożytów występujących u dorszy.

Wykazano istnienie nienotowanych dotąd w Morzu Bałtyckim układów pasożyt-żywiciel. Pierwszy raz stwierdzono, że *S. entomon* i *C. crangon* mogą być żywicielami pośrednimi w cyklu życiowym *H. aduncum* co udowodniono w **publikacji 1 i 3**. Bardzo ważnym odkryciem, z punktu widzenia bezpieczeństwa żywności, jest odnotowanie *C. crangon* zainfekowanego *A. simplex* i *Gammarus* sp. zarażonego *C. osculatum*. Te zależności opisano w **publikacji 2**.

Wyniki przeprowadzonych przeze mnie badań przyczyniły się do poszerzenia wiedzy podstawowej na temat źródeł zarażenia dorszy pasożytami. Ponadto, pozwolą na bardziej szczegółowe opisanie cykli rozwojowych pasożytów dorsza w Morzu Bałtyckim. Umożliwią lepsze zrozumienie roli poszczególnych składników sieci troficzej w transmisji określonych gatunków pasożytów.

W środowisku morskim obserwuje się ciągłe zmiany: klimatyczne, fizykochemiczne, zmiany zasięgu występowania i kondycji organizmów, występowanie gatunków obcych i inne. Nieustanne zmiany środowiskowe stanowią wyzwanie, ale i stwarzają nowe możliwości dla pasożytów do modyfikacji cykli życiowych. Ze względu na te permanentne zmiany w środowisku morskim, uzasadnione jest prowadzenie dalszych badań w tym kierunku. Należy zwrócić szczególną uwagę na żywicieli pośrednich. Dominujące w pokarmie dorsza skorupiaki są także składnikiem diety innych ryb w Morzu Bałtyckim. Można więc przypuszczać, że pasożyty będą przenoszone do nowych żywicieli. Zmiany klimatyczne mogą się przyczynić także do migracji pasożytów i zasiedlania przez nich nowych żywicieli.

Mając na uwadze powyższe fakty chciałabym kontynuować swoją pracę badawczą w tym zakresie z wykorzystaniem zebranych dotąd, bogatych danych i nowego materiału biologicznego. Ważną kwestią, którą chcę zweryfikować jest zagadnienie czy obserwuje się sezonowość występowania pasożytów w pokarmie oraz czy intensywności i ekstensywności zarażenia mogą różnić się w poszczególnych badanych rejonach. Interesujące jest również czy intensywność i ekstensywność występowania pasożytów w pokarmie, a tym samym ryzyko zarażenia różnymi gatunkami pasożytów, może być zależne od parametrów biologicznych żywiciela (np. długość, wiek). Skoro cały czas zmieniają się warunki środowiskowe, to należałoby się przyjrzeć diecie innych ryb i sprawdzić obecność pasożytów w ich pokarmie. Dodatkowo ciekawe rejony do poszukiwań bezkręgowców zarażonych pasożytami znajdują się w okolicach skupisk fok, np. przy Ujściu Wisły czy przy wyspie Bornholm.

## **PUBLICATION 1**

Pawlak J., Nadolna-Altyn K., Szostakowska B., Pachur M., Podolska, M. 2018. *Saduria entomon* infected with *Hysterothylacium aduncum* found *in situ* in the stomach of cod (*Gadus morhua*) from the Baltic Sea. Journal of Helminthology, 92, 645-648.

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## *Saduria entomon* infected with *Hysterothylacium aduncum* found *in situ* in the stomach of cod (*Gadus morhua*) from the Baltic Sea

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### Abstract

The parasite fauna of cod (*Gadus morhua*) is well described, but the life cycles of Baltic cod parasites are known only in general terms. Invertebrates commonly found in the stomach of cod are recognized as intermediate hosts in the life cycles of nematodes or acanthocephalans. The aim of this study was to determine the source of infection of Baltic cod with parasites found *in situ* in invertebrates present in the cod stomach. Our results indicate that *Saduria entomon* is both a source of infection of Baltic cod with parasites and an intermediate host in the life cycle of *Hysterothylacium aduncum* in the Baltic Sea.

### Introduction

Food is not only a source of nutrients for fish, but can also be a route of infection with parasites. In the latter case, a shift in the diet of small versus large fish results in a change of parasite fauna composition. In small Baltic cod the dominant parasites are acanthocephalans, particularly *Echinorhynchus gadi*, which occurs in the digestive tract (Pilecka-Rapacz & Sobecka, 2004). Larger cod may serve as intermediate or paratenic hosts for anisakid nematodes, as well as being the final host for the rhabditid nematode *Hysterothylacium* sp., which resides mainly in the digestive tract of fish. The parasite fauna of cod from the Baltic Sea is well known and has been studied by many authors (Myjak *et al.*, 1994; Buchmann, 1995; Møllergaard & Lang, 1999; Perdiguero-Alonso *et al.*, 2008; Haarder *et al.*, 2014; Mehrdana *et al.*, 2014; Nadolna & Podolska, 2014; Zuo *et al.*, 2016), but the life cycles of the parasites of Baltic cod have only been described in general.

Invertebrates have been noted previously as intermediate hosts in the life cycles of both nematode (Klimpel *et al.*, 2004) and acanthocephalan (Olson & Pratt, 1971) parasites. There is no *in situ* evidence that invertebrates are transmitters of parasites in the Baltic Sea ecosystem. The aim of this study was to determine the source of Baltic cod infections with parasites found *in situ* in invertebrates present in cod stomachs.

### Materials and methods

#### Sampling of fish

The research material consisted of the stomachs of 98 individual Baltic cod, collected during two commercial cruises in the southern Baltic Sea Polish Exclusive Economic Zone (depth 30 and 41 m) in February and May 2015. Samples were frozen at  $-20^{\circ}\text{C}$  until stomach content was analysed for dietary composition.

#### Species identification

All invertebrates found in each cod stomach were collected, and taxonomic identification of each prey item was performed to the lowest possible level, depending on the degree of its decomposition. All individual invertebrates were analysed for the presence of parasites. The majority of invertebrates were already partly digested, therefore parasites could be observed without additional treatment. For invertebrates such as *Saduria entomon* with a thick, non-transparent carapace, digestion in artificial stomach juice was carried out prior to parasitological analysis.

Preliminary taxonomic identification of parasites to the genus level was performed on the basis of anatomico-morphological features (Fagerholm, 1982; Berland, 1991). The microscopic identification was then verified using molecular methods described by Zhu *et al.* (1998). Polymerase chain reaction (PCR) products were sequenced directly using standard procedures

**Table 1.** Composition of cod diet and parasite fauna according to the length class of fish.

Length class (cm)	30–34	35–39	40–44	45–49	50–54	55–59	75–80*	Total
Number of analysed cod	4	37	36	11	8	1	1	98
Diet composition: number of fish with particular food item								
Polychaeta		10	3	1	1			15
Bivalva	2	9	5	2				18
<i>Gammarus</i> sp.	3	16	14	2	5		1	41
<i>Mysis mixta</i>	4	5	4	2	1			16
<i>Crangon crangon</i>	2	2	3		1			8
<i>Saduria entomon</i>	1	23	26	6	5			61
Other Malacostraca		10	5					15
<i>Sprattus sprattus</i>		2	10	2	2			16
<i>Clupea harengus</i>				1				1
<i>Zoarces viviparus</i>		1	3	1				5
<i>Gadus morhua</i>				1	1		1	3
Other Pisces	1	9	8	6	4			28
Parasite fauna analysis: number of individuals								
Number of analysed <i>Saduria entomon</i>	2	98	95	49	24			268
<i>H. aduncum</i> in <i>Saduria entomon</i>		2	2	2				6
<i>H. aduncum</i> in stomach	3	67	26	6	3			105
<i>E. gadi</i> in stomach		11	1		4			16

\*There were no fish in length classes 60–74 cm.

and amplification primers. The sequences obtained have been deposited in GenBank under accession numbers KY018600 and KY018601.

## Results and discussion

The results of stomach content analysis are shown in table 1. Macroscopic analysis revealed the presence of one nematode parasite in the body cavity of each of two individuals of *S. entomon* (fig. 1), identified as one adult male and one stage 4 larva (L4) of *Hysterothylacium* sp. The remaining *S. entomon* were digested using artificial stomach juice to allow easier detection of parasites, which were subsequently found in a further four individuals of *S. entomon*, but it was impossible to identify their developmental

stage due to the level of decomposition of the parasite. Digestion of other food items did not reveal the presence of parasites. Molecular analysis confirmed that all these nematodes were of the species *Hysterothylacium aduncum*. The rDNA sequences of both individuals were identical and the lengths of read fragments were 910 nt.

Individuals of *H. aduncum* were also found in the lumen of the stomach of 27 fish (prevalence 25%; intensity of infection 1–31) close to the remains of invertebrates. The acanthocephalan *E. gadi* was found in the stomachs of eight cod (prevalence 8.16%; intensity of infection 1–8), whose stomachs contained prey species *Gammarus* sp., *S. entomon* or *Mysis mixta*. The numbers of parasites found in stomachs, according to the length class of fish, are presented in table 1.



**Fig. 1.** *Hysterothylacium aduncum* in the body cavity of *Saduria entomon*.

The routes of Baltic cod's parasitic infection are described only in general terms and the role of particular organisms in the life cycles of these parasites is not fully understood. This is probably at least partially because research on the parasitic infection of invertebrates in the sea environment is complicated, laborious and expensive.

Food as a source of the parasitic infection of fish has been described for freshwater fish (Valtonen & Julkunen, 1995). In cod, the composition of the diet varies with the size of fish and the type of foraging area (Pachur & Horbowy, 2013). Cod is a migratory species (Sualamo & Neuman, 2002), therefore the stomach contents do not necessarily correspond to the food species available where fish are caught. Parasitic infection may have occurred during earlier feeding episodes. In our analysis, the parasites *H. aduncum* and *E. gadi* were found in the stomachs of cod in the vicinity of predominant food species. *Echinorhynchus gadi* has been found next to the remains of *S. entomon*, *Gammarus* sp. and sprat *Sprattus sprattus*, while *H. aduncum* has been found near *Gammarus* sp., *S. entomon* and *M. mixta*. If the parasites are present near the remains of the digested prey, this suggests that these particular food organisms might be the source of infection.

In our studies, *H. aduncum* (Rudolphi 1802) was found to be the dominant parasite in the cod stomach. In general, *H. aduncum* matures in fish, but the first intermediate hosts are crustaceans such as copepods, amphipods, decapods and isopods (Køie, 1993). Various fish species serve as paratenic or final hosts, acquiring *H. aduncum* through the food chain (Køie, 1993; Balbuena *et al.*, 1998); however, gadoids are considered to be the main final hosts for *Hysterothylacium* sp. (Berland, 1961).

During our analysis of well-preserved *H. aduncum* found in *S. entomon*, both adult ( $n=1$ ) and L4 larval stages ( $n=1$ ) were identified. The presence of the adult form of *H. aduncum* in the crustacean suggests that invertebrates (as well as fish) might function as final hosts, as described by Luque *et al.* (2007). On the other hand, the adult parasite was found in an undigested *S. entomon* individual inside a cod stomach. It is possible, therefore, that transformation from L4 larva to the adult stage took place inside the *S. entomon*, but after the death of the invertebrate in the cod. The initial digestion of the invertebrate by the stomach juices might drive the transformation of the parasite to the next developmental stage. However, the presence of an L4 larva and adult nematode in the isopod might be connected with post-mortem migration of the parasites to the crustacean in the fish stomach (Marcogliese, 1996). Iglesias *et al.* (2002) described the conditions for optimal development and survival of these nematodes. They proved that if the medium was supplemented with pepsin, all the larvae reached the adult stage. Similar conditions were described by Adroher *et al.* (2004).

To the best of our knowledge, no parasite species has been reported previously in *S. entomon*. More specifically, this is the first report of *H. aduncum in situ* in *S. entomon* found in the stomach of cod sampled from the Baltic Sea. Genetic identification confirmed the results of anatomo-morphological analysis. Cod is an opportunist when feeding and, as *S. entomon* is a major part of its diet, this invertebrate might be an important intermediate host in the life cycle of *H. aduncum* and thus a significant source of cod infection.

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**Conflict of interest.** None.

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## **Statements of co-authors**



Gdynia, dnia 17.11.2017

## O Ś W I A D C Z E N I E

Współautorzy artykułu pt. „*Saduria entomon* infected with *Hysterothylacium aduncum* found *in situ* in the stomach of cod (*Gadus morhua*) from the Baltic Sea” opublikowanego w czasopiśmie naukowym Journal of Helminthology, 1-4, doi:10.1017/S0022149X1700092X, zgodnie oświadczają i podpisami poświadczają, że wkład poszczególnych osób w powstanie publikacji i wynikający z tego procentowy udział był następujący:

### **Joanna Pawlak (45%)**

- przygotowanie ostatecznej koncepcji badań
- opracowanie metodyki badań
- analiza składu pokarmu dorszy i identyfikacja taksonomiczna pozyskanych organizmów
- analiza parazytologiczna bezkręgowców
- statystyczne opracowanie danych
- wiodący udział w pisaniu artykułu
- autor korespondujący z redakcją

### **Katarzyna Nadolna-Altyn (20%)**

- konsultacje na etapie opracowywania koncepcji badań i metodyki badań
- identyfikacja taksonomiczna pasożytów na podstawie cech anatomo-morfologicznych
- udział w pisaniu artykułu

### **Beata Szostakowska (15%)**

- identyfikacja taksonomiczna pasożytów na podstawie badań molekularnych i zdeponowanie uzyskanych sekwencji DNA w Banku Genów
- wprowadzenie ważnych poprawek merytorycznych do artykułu

### **Marzenna Pachur (10%)**

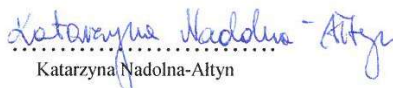
- analiza składu pokarmu dorszy i identyfikacja taksonomiczna pozyskanych organizmów
- wprowadzenie ważnych poprawek merytorycznych do artykułu

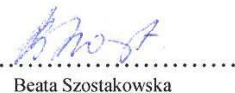
### **Magdalena Podolska (10%)**

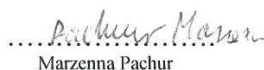
- konsultacje na etapie opracowywania koncepcji badań
- wprowadzenie ważnych poprawek merytorycznych do artykułu

Każdy z współautorów oświadcza, że zatwierdził ostateczną wersję artykułu i przyjmuje pełną odpowiedzialność za wszystkie aspekty pracy, zapewniając, że kwestie związane ze starannością i integralnością każdej części pracy zostały odpowiednio zbadane i wyjaśnione.

  
.....  
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Marzenna Pachur

  
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## **PUBLICATION 2**

Pawlak J., Nadolna-Ałtyn K., Szostakowska B., Pachur M., Bańkowska A., Podolska M. 2019. First evidence of the presence of *Anisakis simplex* in *Crangon crangon* and *Contracaecum osculatum* in *Gammarus* sp. by *in situ* examination of the stomach contents of cod (*Gadus morhua*) from the southern Baltic Sea. *Parasitology* 146, 1699-1706.

## Research Article

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*Anisakis simplex*; Baltic; cod; *Contracaecum osculatum*; *Crangon crangon*; *Gammarus* sp.

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# First evidence of the presence of *Anisakis simplex* in *Crangon crangon* and *Contracaecum osculatum* in *Gammarus* sp. by *in situ* examination of the stomach contents of cod (*Gadus morhua*) from the southern Baltic Sea

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**Abstract**

Cod (*Gadus morhua*), an important fish species in the Baltic Sea, is the paratenic host for many parasite species, including the zoonotic nematodes, *Anisakis* sp. and *Contracaecum osculatum*. We aimed to identify which invertebrate species (found *in situ* in the fish stomach) are responsible for infection of cod with zoonotic nematodes. We found that *Crangon crangon* and *Gammarus* sp., both invertebrate prey species of cod, were infected with *Anisakis simplex* and *C. osculatum*, respectively. These host–parasite systems are reported here for the first time, implicating *C. crangon* and *Gammarus* sp. as sources of infection of Baltic cod with zoonotic nematodes.

**Introduction**

Cod (*Gadus morhua*) is among the most commercially exploited species in the Baltic Sea and is popular as a food in many countries. In the Baltic there are two stocks, eastern and western, with a mixing zone around the Bornholm area. Cod is a migratory species, spawning in deeper water and migrating to shallow areas for feeding (Bagge *et al.*, 1994). It is one of the most important predators in the Baltic Sea after marine mammals and salmonid fish. Cod plays an important role in the ecosystem of the Baltic Sea where small, young individuals occur mostly in shallow, coastal waters, which provide optimal conditions for growth. This environment also offers reduced opportunities for cannibalism by larger, older fish, which migrate to deeper, off-shore waters. Horbowy *et al.* (2003) revealed that the highest abundance of young cod by depth was at 50–70 m, with the density of fish increasing almost linearly up to this depth range and decreasing in deeper areas. According to Sparholt *et al.* (1991), adult cod prefer 60–70 m depths compared to the 80–100 m depths suggested in the analysis of Horbowy *et al.* (2003). Feeding preferences differ between small (young) and larger (adult) cod and dietary composition reflects both the availability of food and its diversity in the environment. Cod is a predatory fish during most of its life span. Young individuals prey on invertebrates and small fish such as the following (in order of decreasing frequency): sprat *Sprattus sprattus*, *Saduria entomon*, Gobiidae fish, *Crangon crangon*, *Pontoporeia femorata*, *Bylgides sarsi* and *Mysis mixta*. Older cod feed mostly on sprat, herring *Clupea harengus*, cod, Ammodytidae fish and also on *S. entomon*. In the southern Baltic Sea an ontogenetic shift in dietary composition is observed in cod between 30 and 40 cm in length (Pachur and Horbowy, 2013). Analysis of cod food items can give much more than just information about the fish diet, however. It is well known that crustaceans eaten by cod are not only a source of nutrients, but may also transmit parasites to fish.

Marine crustaceans, including amphipods, decapods, copepods, mysids and euphausiids, are common intermediate or paratenic hosts of parasites. For example, the nematode *Hysterothylacium* sp. has been reported by Lick (1991) and Zander *et al.* (2000) in many gammarid species from the North Sea and Baltic Sea (studies limited to German coastal waters), including *Gammarus locusta*, *Gammarus salinus* and *Gammarus zaddachi*. Experimental research described in the literature confirms that invertebrates can serve as intermediate hosts. Thus, Copepoda (*Acartia tonsa* and *Oithona similis*) have been successfully infected with *Anisakis simplex* (Køie, 1993, 2001); Cirripedia (*Balanus* sp.) with *A. simplex* and *Contracaecum osculatum* (Køie and Fagerholm, 1995); and Copepoda (*Temora longicornis*) with *C. osculatum* (Køie and Fagerholm, 1995). For these studies, biological material was obtained from Baltic seals and eels.

The parasite fauna of cod from the Baltic Sea is well documented. Buchmann (1995) described the presence of acanthocephalan *Echinorhynchus gadi* in cod from the Baltic Sea. Parasites *Cryptocotyle lingua* and *Lernaocera branchialis* were recorded in cod in 11 areas

from the Mecklenburg Bight in the southwest to the Estonian coast in the northeast of the Baltic Sea (Møllergaard and Lang, 1999). The composition and structure of the parasite fauna of cod sampled in 2002–2003 in south-eastern Swedish waters were analysed by Perdiguero-Alonso *et al.* (2008). Recent interest has focused on the presence of nematodes in the Baltic cod, with Nadolna and Podolska (2014) reporting a marked increase in the prevalence of infection with anisakid nematodes in fish sampled in the southern Baltic in 2011. These authors reported an 11% infection rate, compared with 4% between 1987 and 1993 (Myjak *et al.*, 1994). In 2011, the dominant species among Anisakidae found in the cod liver was *Contracaecum* sp. at 95.2%, then *Anisakis* sp. 4.7% and *Pseudoterranova* sp. 0.1% (Nadolna and Podolska, 2014). Haarder *et al.* (2014) also conducted a parasitological study of cod from the southern Baltic Sea in spring 2012. They compared the results with data from 1982/1983 and also observed an increase in the prevalence of infection of cod with nematodes from 22 to 55.1%. Haarder *et al.* (2014) suggest that the elevated parasitism of cod liver is associated with the increased number of grey seals in the south-western Baltic. The level of cod infection with parasitic nematodes depends on the area of sampling, as discussed by Lunneryd *et al.* (2015). During sampling conducted east of Bornholm in 2013–2014, nematodes were found in fillets of cod, with *Pseudoterranova decipiens* at a prevalence of 28.7% and *A. simplex* at 8.1% (Mehrdana *et al.*, 2014), while in the area south-west of Bornholm (sampling 2013–2015) only 1.09% of cod examined were infected with *P. decipiens* (Zuo *et al.*, 2016).

The level of cod infection is determined by many factors and depends on the sampling area. In the Baltic Sea, genetically distinct stocks of cod follow different migration patterns (Ovegård *et al.*, 2012) and are therefore exposed to different infection risks (Lunneryd *et al.*, 2015). Nursery areas for cod in the Baltic Sea are located in coastal waters (Bagge *et al.*, 1994) and fish that stay near a coastline with a high seal density have a higher likelihood of infection (Hauksson, 2002, 2011). The life cycles of *A. simplex*, *C. osculatum* and *P. decipiens* are similar and involve marine mammals as the final hosts (McClelland *et al.*, 1990; Klimpel and Palm, 2011). The following important cetacean hosts for *A. simplex* have also been reported in the Baltic: the harbour porpoise, *Phocoena phocoena* (Herreras *et al.*, 2004), the white-beaked dolphin, *Lagenorhynchus albirostris*, and the common bottlenose dolphin *Tursiops truncatus* (Smith and Wootten, 1978). According to Fagerholm (1990), the grey seal, *Halichoerus grypus*, is considered to be the most important final host for *C. osculatum*, while the common seal (harbour seal), *Phoca vitulina*, is the most important final host for *P. decipiens* (Aspholm *et al.*, 1995). In the eggs of *A. simplex* and *P. decipiens*, two moults occur during larval development; therefore L3 larvae are likely to be present in sea water (Køie *et al.*, 1995) and are available for invertebrates, fish and mammals. As a consequence, in an area with high seal density the likelihood of cod food items being infected is higher than otherwise. The distribution of invertebrates (that serve as intermediate or paratenic hosts for these nematodes) is driven by salinity, because in the Baltic the number of invertebrate species declines with declining salinity (Bonsdorff, 2006). In some areas there could be a lack of suitable hosts to complete the life cycles of the parasites (Lunneryd *et al.*, 2015). Young cod, which stay near the coastline and feed mostly on invertebrates, have the highest risk of infection due to ingestion of infected food items. Many authors have reported a strong positive correlation between fish size (e.g. length) and the prevalence, intensity and/or abundance of infection with Anisakidae in different species of fish (Horbowy and Podolska, 2001; Podolska and Horbowy, 2003; Zuo *et al.*, 2016; Sokolova *et al.*, 2018). Such a correlation is explained by the accumulation of parasites during

the life of the fish. Anisakidae nematodes migrate from the digestive tract to the liver (*C. osculatum*, *A. simplex*) and muscle tissue (*A. simplex*, *P. decipiens*) of cod, where parasites are deposited and cannot be cleared by the host fish.

The most dangerous nematode parasites to human health found in Baltic cod are *A. simplex* and *Anisakis pegreffii* (Ishikura *et al.*, 1993; Audicana and Kennedy, 2008; Mattiucci *et al.*, 2013), *Pseudoterranova* spp. and *C. osculatum* (Torres *et al.*, 2007; Shamsi and Butcher, 2011; Mattiucci *et al.*, 2013; Mehrdana *et al.*, 2014; Shamsi and Suthar, 2016). Accidental ingestion by humans of viable, invasive anisakid larvae may cause anisakidosis: symptoms include nausea, diarrhoea, vomiting, and intense abdominal pain (Ishikura *et al.*, 1993; Hochberg and Hamer, 2010). To date, there are no published case studies of acute anisakidosis caused by eating infected cod, but the presence of these potentially zoonotic parasites in Baltic cod still raises concerns connected with food safety, because parasites do not need to be viable to endanger human health. Although adequate treatment kills the parasite (Wharton and Aalders, 2002), the allergenic proteins of anisakid nematodes are thermostable (Audicana *et al.*, 2002; Moneo *et al.*, 2005). Two examples of allergy related to anisakidosis linked with consumption of raw cod have been reported (Alonso-Gómez *et al.*, 2004).

The European Food Safety Authority (EFSA) stated that all wild-caught fish (intended to be eaten raw or nearly raw) must be considered at risk of containing viable parasites of concern to human health (EFSA, 2010). Cod is very popular as food in many countries. There is a particular risk with dishes prepared from fresh (neither frozen nor prepared at high temperature) fillets (e.g. sushi, ceviche, fish smoked in low temperature) or livers (e.g. marinated liver), because these tissues may contain live parasites. The liver is used for the production of cod-liver oil and the consumption of cod liver is popular in many European countries, e.g. the traditional North Norwegian dish mølje or canned cod livers/pate available in markets in Iceland, Latvia, Lithuania, Russia, Poland and Norway (Nadolna and Podolska, 2014). It must be emphasized that *A. simplex* larvae possess a high tolerance to a wide temperature range and can survive freezing under some conditions (Podolska *et al.*, 2019).

Cod plays the role of paratenic or definitive host for many parasite species (Hemmingsen and MacKenzie, 2001) in various habitats, including the Baltic Sea. However, the life cycles of particular species of these cod parasites in the Baltic sea are described only in general terms. Invertebrates are known to serve as intermediate hosts, but for any particular species of parasite the respective host invertebrate in the Baltic Sea is unclear. A single experimental study indicated the possibility of cod infection with *C. osculatum* (Køie and Fagerholm, 1995), although some marine invertebrate species have been noted as intermediate hosts in the life cycles of parasitic nematodes (Lick, 1991; Klimpel *et al.*, 2004). Because of the paucity of information in this area, we decided to conduct parasitological inspection of the food items in the stomachs of Baltic cod to investigate which invertebrate species are responsible for the infection of cod with zoonotic nematodes.

## Materials and methods

The research material consisted of cod stomachs collected during research surveys in November 2015 and February 2016, and a commercial cruise in February 2015, in the southern Baltic Sea Polish Exclusive Economic Zone (Polish EEZ). Cod is a demersal fish and during our studies was caught using the trawl method, but there were differences between research and commercial surveys, the most important being the selectivity of the fishing gear. In commercial fisheries, the minimum conservation reference size

**Table 1.** Dominant invertebrate species among food items in cod stomachs

	February 2015	November 2015	February 2016	Sum
Cod stomachs analysed	56	414	446	916
<i>Gammarus</i> sp.	156	328	631	1115
<i>Bylgides sarsi</i>	32	51	53	136
<i>Crangon crangon</i>	23	958	1392	2373
<i>Diastylis rathkei</i>	0	2444	100	2544
<i>Mysis mixta</i>	3	235	490	728
Other species	58	653	1194	1905
Total	272	4669	3860	8801

of cod caught in the Baltic Sea is 35 cm, whereas the gear used in research surveys allows much smaller fish to be caught. A sampling of these young, so-called undersized cod (<35 cm in length) allowed data on food composition and the presence of parasites in small fish to be obtained that would not be available from commercial fisheries. Furthermore, the trawling time in commercial fishing was 240 min, which was much longer than during research surveys (30 min). This might influence the nature of food items in the cod stomach due to longer residence times and the possibility of regurgitation under stress.

Basic ichthyological analysis of each fish was performed and age of fish was determined by examination of otoliths. The total number of stomachs collected was 916. All samples were frozen at  $-20^{\circ}\text{C}$  until analysis of stomach content. The taxonomic identification of each organism found in cod stomach was performed to the lowest possible level depending on the degree of decomposition of prey. Invertebrates were identified by morphology (Żmudziński, 1990; Hayward and Ryland, 1995). All invertebrates found in the stomach were collected and checked for the presence of parasites. If necessary, crustaceans were additionally digested in artificial digestive juice (aqueous solution of 10 g pepsin and 30 mL 35–38% hydrochloric acid in 1 L tap water; room temperature) to improve the level of detection of parasites, and were then re-analysed parasitologically. The duration of digestion depended on the species of crustacean and the degree of its decomposition. For partially digested crustaceans, which were not transparent, laboratory digestion took a few hours; for example, in the case of *Gammarus* sp., 4 h. For crustaceans that had only just been ingested by cod and showed no sign of decomposition, the digestion time was long; for example, in the case of *C. crangon* this was 12 h. The crustaceans were laboratory digested only when the organisms were well preserved, i.e. they had not been digested earlier by cod and the body was not transparent. Well-preserved crustaceans were analysed carefully, to avoid accidentally damaging any parasites that may have been present inside the body. When a parasite was visible in the body cavity during initial analysis, it was carefully, manually removed with tweezers. All parasites found in the stomach adjacent to prey items were also collected.

Taxonomic identification of parasites was performed on the basis of anatomico-morphological features (Fagerholm, 1982; Berland, 1989). Additionally, molecular analysis of parasites was conducted according to the procedures described by Zhu *et al.* (1998), which involved amplification and sequencing of the ITS-1 region of rDNA. Polymerase chain reaction (PCR) products were sequenced directly using standard procedures and amplification primers. DNA was isolated using a Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. Amplification was performed using the primers NC5 (forward) 5'-GTA GGT GAA CCT GCG GAA GGA TCA

TT-3' and NC13 (reverse) 5'-GCT GCG TTC TTC ATC GAT-3'. The reaction mixture consisted of 25  $\mu\text{L}$  PCR Master MixPlus High GC (ready-to-use PCR mixture containing Taq DNA polymerase, PCR buffer,  $\text{MgCl}_2$  and dNTPs; A&A Biotechnology), 0.2  $\mu\text{L}$  each primer (stock concentration 100  $\mu\text{M}$ ), 20  $\mu\text{L}$  DNA template, supplemented with deionized water up to 50  $\mu\text{L}$ . The PCR reaction conditions were as follows: 2 min at  $94^{\circ}\text{C}$  (initial denaturation) followed by 35 cycles of 30 s at  $94^{\circ}\text{C}$ , 30 s at  $60^{\circ}\text{C}$ , 30 s at  $72^{\circ}\text{C}$  and a final extension step of 5 min at  $72^{\circ}\text{C}$ . Some DNA fragments obtained as a result of the amplification reaction were purified using a Gel-Out Concentrator kit (A&A Biotechnology). PCR products were eluted with sterile water. Sequences were analysed using the software CLC Workbench and GeneStudio and confirmed by a BLAST search of GenBank.

## Results

We analysed 916 cod stomachs and found 8801 individual invertebrates, which we inspected for the presence of parasites. In February 2015, the most prevalent invertebrate species were *Gammarus* sp., *B. sarsi* and *C. crangon*; in November 2015, *Diastylis rathkei*, *C. crangon* and *Gammarus* sp.; in February 2016, *C. crangon*, *Gammarus* sp. and *M. mixta* (Table 1). The only invertebrate species found to be infected with parasites were members of Decapoda (*C. crangon*) and Amphipoda (*Gammarus* sp.).

One individual *C. crangon* was infected with a single *A. simplex* L3-stage larva, found in the stomach of a cod collected in November 2015 (cod 36 cm in length, 2 years old). This fish was caught at a depth of 19 m, ICES rectangle 38G7; Polish fisheries rectangle M6. The crustacean was well preserved, and not initially digested. The presence of the parasite only became apparent after it was rinsed out of the body of *C. crangon* following laboratory digestion in artificial gastric juice.

Each of three individuals of *Gammarus* sp. was infected with a single *C. osculatum* L3-stage larva in each of three sampling seasons (Table 2). These fish were caught in February 2015: cod 38 cm in length and 4 years old (depth 22 m; ICES 38G7; O7); November 2015: cod 34 cm in length and 3 years old (depth 19 m; ICES 38G7; M6); and February 2016: cod 21 cm in length and 2 years old (depth 59 m; ICES 38G8; R7). In the Baltic Sea a few species of *Gammarus* have been reported previously (Szaniawska, 2018). Characteristic elements of morphology, such as antennules, legs, telson and mandible, are used to distinguish the species, but these structures are fragile and are easily damaged when preyed on by cod during the passage of the food item to the stomach. It was therefore impossible to identify the species of *Gammarus* on the basis of these features. The body cavities of these individuals were well preserved and not initially

**Table 2.** Invertebrates infected with Anisakidae nematodes found in stomachs of Baltic cod

	February 2015	November 2015	February 2016	Sum
Cod stomachs analysed	56	414	446	916
Cod stomachs with <i>C. crangon</i>	4	162	144	310
Total no. <i>C. crangon</i>	23	958	1392	2373
No. <i>C. crangon</i> infected with <i>A. simplex</i>		1		1
Intensity of infection		1		
Prevalence of infection (%)		0.001		
Cod stomachs with <i>Gammarus</i> sp.	38	45	61	144
Total no. <i>Gammarus</i> sp.	156	328	631	1115
No. <i>Gammarus</i> sp. infected with <i>C. osculatum</i>	1	1	1	3
Intensity of infection	1	1	1	
Prevalence of infection (%)	0.006	0.003	0.002	

**Table 3.** Parasites found in stomachs of Baltic cod adjacent to prey items

	February 2015	November 2015	February 2016	Sum
Cod stomachs analysed	56	414	446	916
Nematoda: <i>Hysterothylacium</i> sp.	39	34	22	95
Acanthocephala: <i>E. gadi</i>	7	45	15	67
Cestoda		5	1	6

digested. In each case, the parasite was not detected until the *Gammarus* sp. individuals had been digested in artificial gastric juice and rinsed out.

Molecular identification of these larvae was performed and the results deposited in GenBank: *A. simplex* from *C. crangon* ID MK757430, *C. osculatum* from *Gammarus* sp. ID MK757431, MK757432, MK757433. In all cases, the *C. osculatum* and *A. simplex* sequences were identical to those previously recorded for these species in the GenBank.

Parasites found among the stomach content adjacent to prey items were also collected and identified; these comprised the nematode *Hysterothylacium* sp., the acanthocephalan *E. gadi* and a few individuals representing Cestoda (Table 3). No *A. simplex* or *C. osculatum* larvae were found next to prey items; these species of nematode were only present in infected invertebrates.

## Discussion

Cod is known to play a role as an intermediate, paratenic or definitive host for numerous species of parasites (Hemmingsen and MacKenzie, 2001). The most dangerous for consumer health is cod infected with zoonotic nematodes such as *Anisakis* spp., *Pseudoterranova* spp. or *C. osculatum*. Increasing levels of *G. morhua* infection with these parasites have been reported for the Baltic Sea in recent years (Mehrdana *et al.*, 2014; Nadolna and Podolska, 2014; Horbowy *et al.*, 2016; Zuo *et al.*, 2016), but the source of infection is unknown.

Despite the fact that the parasite fauna of Baltic cod is well documented, the life cycles of these parasites are described only in general terms for that area. Invertebrates are suspected to play a role as transmitters of parasites to cod, but which species of invertebrates are responsible for the transfer of particular parasite species in the Baltic Sea is unclear. There is a lack of reports from the Baltic on invertebrates that are infected with the nematode parasites *Pseudoterranova* spp., *Anisakis* spp. or *C.*

*osculatum*. Experimental infection of crustaceans with *A. simplex* has been conducted (Køie and Fagerholm, 1995; Køie, 2001). However, the detection of parasites in free-living invertebrates requires a large number of samples, and such studies are consequently laborious and expensive, which at least partially explains why these investigations are infrequent. In our study, we used a new approach, i.e. a parasitological analysis of the invertebrates found *in situ* in the stomachs of cod, which was proposed for the first time by Pawlak *et al.* (2018). The advantages of this method are that it both directly shows the route of infection of fish with parasites and also records the infection of invertebrate prey.

During our analysis, an individual of the species *C. crangon* was found to be infected with a single *A. simplex* larva, which represents the first record of this host–parasite system in general, and not only for the Baltic Sea. Indeed, *C. crangon* has never been reported previously as a host for any parasitic nematode (Busch *et al.*, 2012). To the best of our knowledge, before the current report there were no findings of invertebrates infected with *Anisakis* in the Baltic Sea. This raises the question of whether the life cycle of *A. simplex* can be completed in the Baltic Sea. Grabda (1974) stated that low salinity environmental conditions are unsuitable for *A. simplex*. According to Grabda (1974, 1976), herring that spawn along the Polish coast of the Baltic belong to the western spring herring group, which migrate from the Baltic Sea to the Danish Straits or even to the North Sea, and there become infected with *Anisakis* larvae while feeding upon euphausiids. The results obtained by Podolska *et al.* (2006) indicate that fish infected with *Anisakis* larvae represent a mixed group of western and central Baltic herring. *Anisakis simplex* has been used as an indicator of herring migration out of the Baltic Sea.

The main final hosts for *A. simplex* in the Baltic Sea are cetaceans, namely the harbour porpoise, *P. phocoena* (Herrerias *et al.*, 2004), the white-beaked dolphin, *L. albirostris*, and the common

bottlenose dolphin, *T. truncatus* (Smith and Wootten, 1978). The density and distribution of harbour porpoise in the central Baltic Sea or Baltic Proper considerably declined during the last century (Koschinski, 2001). Acoustic monitoring indicates a recent increase in porpoise registration rates in the Kadet Trench and Pomeranian Bay, but this does not necessarily represent a population increase (Benke *et al.*, 2014). Baltic porpoises have been examined by Rokicki and Berland (1995) and Rokicki *et al.* (1997), but *A. simplex* was not recorded. Later studies, conducted by Wünschmann *et al.* (2001), revealed the presence of *A. simplex* in the first and second gastric compartment of porpoises from the German North Sea and the Baltic Sea. Similarly, Lehnert *et al.* (2005) found *A. simplex* in the first stomach compartment in six (28%) of the Baltic Sea porpoises examined. Some individuals (6%) showed infections in the second compartment. Carlén *et al.* (2018) indicated the presence of three separate populations of harbour porpoises in the eastern part of the North Atlantic: one extending into the North Sea, Skagerrak and northern Kattegat; one in southern Kattegat, the Belt Sea and southwestern Baltic Sea; and one in the Baltic Proper. The authors revealed that harbour porpoise might migrate from the Belt Sea to the Danish straits. In the Danish straits, harbour porpoise may become infected with *A. simplex* and, after returning to the southwestern Baltic, shed parasite eggs, which would make it possible to complete the life cycle of *A. simplex* in the Baltic.

*Anisakis simplex* has been found in three species of Baltic seal: grey seal, *H. grypus* (Skrzypczak *et al.*, 2014; Zuo *et al.*, 2018); harbour seal, *P. vitulina* (Lunneryd, 1991; Skrzypczak *et al.*, 2014); and ringed seal, *Pusa hispida* (Skrzypczak *et al.*, 2014). The grey seal population in the Baltic Sea increased recently (Haarder *et al.*, 2014): according to HELCOM (2016), there were 32 000 grey seals in the Baltic in 2014, showing an annual rate of increase of 7.9% over the period 2000–2014. Larger numbers of seals in the environment potentially lead to more infected crustaceans, and a corresponding increase in infected fish. With such an increase in grey seal numbers and increasing levels of infection of fish with nematodes, completion of the *Anisakis* life cycle in the Baltic Sea could theoretically be possible. Despite the fact that there is no direct evidence for reproduction of *A. simplex* in seals (i.e. the presence of mature stage larva that is capable of producing fertilized eggs), hypothetically it cannot be excluded that these marine mammals may play the role of final host for *Anisakis* in the Baltic Sea.

According to some authors (Smith, 1971, 1983a, 1983b; Højgaard, 1999), euphausiids are regarded as the most important intermediate hosts for *A. simplex* in the marine environment. However, Makings (1981) reported the presence of *A. simplex* larvae in Mysidae (*Mesopodopsis slabberi*) from the west coast of Scotland. Also, other studies have indicated that euphausiids are not important for the successful transmission of *A. simplex* in particular areas; for example, Klimpel *et al.* (2004) found *Paraeuchaeta norvegica* (Copepoda) from the Norwegian Deep infected with an L3-stage *A. simplex* larva (coiled in the haemocoel of the invertebrate). The authors clarified the role of the different intermediate hosts in the life cycle of *A. simplex* in the study area. These findings confirm that *A. simplex* does not require a particular species of marine crustacean as an intermediate host, and therefore it is possible that *C. crangon* can play this role in the life cycle of the nematode. Furthermore, studies conducted by Køie *et al.* (1995) show that the invasive L3 larval stage can be reached by parasites even inside the egg, and therefore invertebrates are not needed as intermediate hosts to infect fish.

This is the first report of infection of *Gammarus* sp. from the Baltic Sea with a *C. osculatum* larva, in this case on three separate occasions. However, amphipods were indicated by Sudarikov and

Ryzhikov (1951) as transport hosts for endemic *C. osculatum baicalensis* in Baikal Lake. Representatives of different *Gammarus* species were earlier reported as hosts for parasitic nematodes. The presence of *Hysterothylacium* sp. was reported in *Gammarus duebeni* and *Gammarus inaequicauda* (Lick, 1991), and in *Gammarus finmarchicus* (Zander *et al.*, 1994), while *Ascarophis* sp., *Hysterothylacium* sp., *Paracuarria adunca*, *P. decipiens* and *Tetrameres* sp. were recorded in *Gammarus lawrencianus* (Lick, 1991; Jackson *et al.*, 1997). *G. locusta*, *G. salinus* and *G. zaddachi* were reported as hosts for nematodes *Ascarophis arctica* and *Hysterothylacium* sp. by Lick (1991) and Zander *et al.* (2002), while the nematodes *A. arctica*, *Hysterothylacium* sp., *P. adunca* and *Tetrameres* sp. were found in *Gammarus oceanicus* (Svendsen, 1990; Lick, 1991; Jackson *et al.*, 1997; Zander *et al.*, 2002). However, no *Gammarus* species has previously been indicated as a host for *C. osculatum* in the Baltic Sea. Despite the fact that Haarder *et al.* (2014) suggested the amphipod as transport host in the Baltic life cycle of *C. osculatum*, our finding is the first *in situ* evidence of this in a particular species, i.e. *Gammarus* sp.

During our studies, *C. crangon* infected with *A. simplex* was found in a 36 cm-long cod, while *Gammarus* sp. infected with *C. osculatum* was found in cod 21, 34 and 38 cm in length. According to Nadolna and Podolska (2014) and Zuo *et al.* (2016), the level of infection of small cod with *C. osculatum* is usually low. However, the rate of infection by anisakid nematodes of cod >30 cm long is increasing rapidly, in particular by *C. osculatum* larvae (Zuo *et al.*, 2016). Such increases in infection rate might be related to the shift in the composition of the cod diet, resulting from differences in environmental conditions between coastal and deeper waters. Münster *et al.* (2015) and Mouritsen *et al.* (2010) indicate, for cod from Greenland, that the diet changes from predominantly invertebrate (Crustacea) to vertebrate prey (fish) as the body length of the fish reaches approximately 20–30 cm. Pachur and Horbowy (2013) suggest that the shift in dietary composition for the Baltic cod occurs between 30 and 40 cm in length. Nevertheless, cod >30 cm can also prey on invertebrates.

In the Baltic Sea, the prevalence and intensity of infection of cod with *Contracaecum* sp. increased during 2011–2014 (Horbowy *et al.*, 2016). Several studies have shown the negative impact of parasitic infection on the condition of fish. Acha and Szyfres (1987) reported a decrease in the weight of cod infected with *Contracaecum*, and a significant reduction in the fat content of the liver if the number of larvae was large. Santoro *et al.* (2013) demonstrated an adverse effect of the presence of *C. osculatum* in the liver of the icefish *Chionodraco hamatus* on the body condition index of the host. Horbowy *et al.* (2016) revealed that the intensity of infection has a significant negative effect on the condition of cod. The poor body condition of fish infected with nematodes may be explained on physiological grounds. According to Lambert and Dutil (1997a, 1997b), the protein reserves of cod are stored in the trunk muscles, whereas the main energy reserves are stored in the form of lipids in the liver. A severe reduction in liver lipids may reduce the chance of cod surviving periods of food limitation. During starvation, cod first mobilize liver lipids, then muscle and hepatic glycogen, and finally muscle proteins (Guderley *et al.*, 2003).

The most important finding in this study was the demonstration of the presence of invertebrates infected with nematodes in the stomach of cod. The novel approach we used might raise the question of whether the invertebrates became infected, not in the marine environment, but in the cod stomach; i.e. the nematode larvae were already present in the digestive tract of the fish (as the remains of an earlier meal of infected fish, for example) before the arrival of the invertebrate food item. Several

observations should dispel these doubts. The infected invertebrates were well preserved (only slightly digested), which indicates that these food items had not spent a long time in the cod stomach. Załachowski (1977) stated that the time spent by food items in the cod stomach depends on many factors, including the length of the fish, and the type and weight of the food item; for organisms like *Gammarus* sp. and *C. crangon*, this does not exceed 8 h. Thus, there was a short time between the cod's last meal and sampling, invertebrates were not decomposed. Furthermore, *A. simplex* and *C. osculatum* larvae were found inside the body of the crustacean food items, indicating that parasites were not ingested by the invertebrates in the cod stomach. We did not find any 'free' larvae of either *Anisakis* sp. or *Contracaecum* sp. in the cod stomach; these parasites are known to migrate quickly from the digestive tract to the muscle tissue (*Anisakis* sp.) or liver (*Contracaecum* sp., *Anisakis* sp.) of their host. Thus, Wootten and Smith (1975) observed that *A. simplex* migrate very quickly from the digestive tract to the body cavity, in only 2 h after experimental infection of trout. There is a small possibility that *Contracaecum* sp. or *Anisakis* sp. larvae originated from a previous meal (invertebrate or fish) and were released into the cod stomach and then infected an invertebrate that was subsequently ingested. However, this is unlikely, at least in the case of an earlier fish meal, because the infected invertebrates were found in small cod. Infection of Baltic herring with *A. simplex* does not occur if the herring is smaller than 21 cm in length (Podolska and Horbowy, 2003) and cod <36 cm long are not able to eat such large fish. The above observations are therefore most consistent with our hypothesis, i.e. that the parasites did not infect the invertebrates in the cod stomach, but were already present within the body of the invertebrates when they were ingested by the cod.

This study reveals for the first time that Baltic cod may be infected with *A. simplex* via *C. crangon*, and with *C. osculatum* via *Gammarus* sp. Thus, our results provide new information about the life cycles of parasitic nematodes in the Baltic sea.

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## Statements of co-authors

Gdynia, dnia 02.10.2020

## O Ś W I A D C Z E N I E

Współautorzy artykułu pt. „*First evidence of the presence of Anisakis simplex in Crangon crangon and Contracaecum osculatum in Gammarus sp. by in situ examination of the stomach contents of cod (Gadus morhua) from the southern Baltic Sea*” opublikowanego w czasopiśmie naukowym Parasitology, 146, 1699–1706. <https://doi.org/10.1017/S0031182019001124>, zgodnie oświadczają i podpisami poświadczają, że wkład poszczególnych osób w powstanie publikacji i wynikający z tego procentowy udział był następujący:

### **Joanna Pawlak (50%)**

- przygotowanie ostatecznej koncepcji badań
- opracowanie metodyki badań
- analiza składu pokarmu dorszy i identyfikacja taksonomiczna pozyskanych organizmów
- analiza parazytologiczna bezkręgowców
- identyfikacja taksonomiczna pasożytów na podstawie badań molekularnych i zdeponowanie uzyskanych sekwencji DNA w Banku Genów
- statystyczne opracowanie danych
- wiodący udział w pisaniu artykułu
- autor korespondujący z redakcją

### **Katarzyna Nadolna-Altyn (25%)**

- konsultacje na etapie opracowywania koncepcji badań i metodyki badań
- identyfikacja taksonomiczna pasożytów na podstawie cech anatomo-morfologicznych
- udział w pisaniu artykułu

### **Beata Szostakowska (5%)**

- identyfikacja taksonomiczna pasożytów na podstawie badań molekularnych
- wprowadzenie ważnych poprawek merytorycznych do artykułu

### **Marzenna Pachur (5%)**

- analiza składu pokarmu dorszy i identyfikacja taksonomiczna pozyskanych organizmów
- wprowadzenie ważnych poprawek merytorycznych do artykułu


### **Anna Bańkowska (5%)**


- identyfikacja taksonomiczna pasożytów na podstawie badań molekularnych
- wprowadzenie ważnych poprawek merytorycznych do artykułu

### **Magdalena Podolska (10%)**

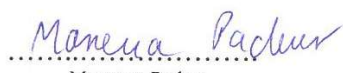
- konsultacje na etapie opracowywania koncepcji badań
- wprowadzenie ważnych poprawek merytorycznych do artykułu

Każdy z współautorów oświadcza, że zatwierdził ostateczną wersję artykułu i przyjmuje pełną odpowiedzialność za wszystkie aspekty pracy, zapewniając, że kwestie związane ze starannością i integralnością każdej części pracy zostały odpowiednio zbadane i wyjaśnione.

  
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Joanna Pawlak

  
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Katarzyna Nadolna-Altyn

  
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Beata Szostakowska

  
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Marzenna Pachur

  
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Anna Bańkowska

  
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Magdalena Podolska

### **PUBLICATION 3**

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# In situ evidence of the role of *Crangon crangon* in infection of cod *Gadus morhua* with nematode parasite *Hysterothylacium aduncum* in the Baltic Sea

## Research Article

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### Abstract

Cod was one of the most important fish species in the Baltic Sea, but its condition is deteriorating for several reasons, including an increasing parasite burden. The aim of this study was to determine the source of infection of Baltic cod with parasites by examination of invertebrates found *in situ* in the cod stomach. A total of 1681 cod were sampled during four research cruises in the southern Baltic Sea in 2012, 2013 and 2014 and the composition of their diet was analysed. Each prey item from cod stomach was identified to the lowest possible taxonomic level and a parasitological analysis of all invertebrates collected was performed. *Crangon crangon*, *Saduria entomon* and *Mysis mixta* were the most commonly represented invertebrates among food items. *Hysterothylacium aduncum* was found only in *C. crangon*. This host–parasite system is reported here for the first time *in situ* in the stomach of cod from the Baltic Sea, confirming the role of *C. crangon* in cod infection with *H. aduncum*.

### Introduction

Baltic cod (*Gadus morhua*) is one of the most commercially exploited fish species and is a popular food in several countries. Cod concentrates in deeper waters for spawning and migrates to the water column or shallow areas to feed (Bagge *et al.*, 1994). The dietary preferences of cod, which is a predatory fish throughout most of its life, depend on its ability to catch and eat specific prey species. Young cod mostly occur near the coast, in shallow water, and feed on invertebrates, especially Crustacea (e.g. *Crangon crangon*, *Mysis mixta* and *Gammarus* sp.). Older cod migrate to deeper, offshore waters where they prefer to eat fish (*Clupea harengus* and *Sprattus sprattus*) and larger invertebrates (*Saduria entomon*). Thus, the variability in the cod diet reflects the age of the fish and the biodiversity of prey species in the habitats occupied. Pachur and Horbowy (2013) revealed that a shift in dietary composition can be observed as cod reach between 30 and 40 cm in length. This shift in the cod's diet has consequences for the risk of infection with different parasite species.

The ecological status of the Baltic Sea is deteriorating and as a consequence the state of the cod population and the condition of individual cod grow progressively worse. This might be due to low availability of fish prey in areas where cod feed (Eero *et al.*, 2012); a lack of benthic prey because of lower dissolved oxygen or changes in salinity (Conley *et al.*, 2009; Carstensen *et al.*, 2014); intensive exploitation of resources, which negatively influences fish stocks (Lindegren *et al.*, 2009) and increasing infection with nematode parasites (Haarder *et al.*, 2014; Mehrdana *et al.*, 2014; Horbowy *et al.*, 2016). The Baltic cod population has decreased drastically over the years, and the International Council for the Exploration of the Sea (ICES) has advised fisheries focused on cod in the Baltic Sea to cease fishing in 2020 (ICES, 2019).

Studies conducted on Gadiformes fish, specifically the burbot (*Lota lota*), by Valtonen and Julkunen (1995) revealed that the type of diet was a key factor determining the risk of infection with particular species of parasite. Cod can be intermediate, paratenic or definitive hosts for a large number of parasite species (Hemmingsen and MacKenzie, 2001), and the parasite fauna of cod differs depending on the size of individual fish (Zuo *et al.*, 2016). In the Baltic Sea, the dominant group of parasites in small cod is acanthocephalans, especially *Echinorhynchus gadi*, which occur in the digestive tract (Pilecka-Rapacz and Sobiecka, 2004). In larger cod, the most abundant parasites are nematodes, particularly *Contracaecum osculatum* (Szostakowska *et al.*, 2005; Nadolna and Podolska, 2014; Mehrdana *et al.*, 2014) and occasionally *Anisakis* sp. (Nadolna and Podolska, 2014), which occupy the liver; rarely, *Pseudoterranova* sp. and *Anisakis* sp. accumulate in the muscle tissue (Mehrdana *et al.*, 2014). *Hysterothylacium* sp. occurs mainly in the digestive tract of larger cod. In spite of the fact that the parasite fauna of cod from the Baltic Sea is well known and has been studied by several authors (Myjak *et al.*, 1994; Buchmann, 1995; Møllergaard and Lang, 1999; Perdiguero-Alonso *et al.*, 2008; Haarder *et al.*, 2014; Mehrdana *et al.*, 2014; Nadolna and Podolska, 2014; Zuo *et al.*, 2016), the life cycles of Baltic cod parasites are described only in general.

Cod infection with the nematode parasite *Hysterothylacium* sp., as well as its basic life cycle, is documented for the Baltic Sea, but little is known about the crustacean species that play the

role of intermediate host. The aim of this study was to determine the source of infection of Baltic cod with parasites by examination of invertebrates found *in situ* in cod stomach.

### Materials and methods

The research material (cod stomachs) was collected during four research surveys in November (Q4) 2012 and 2013; as well as in February (Q1) 2013 and 2014. In total, 1681 cod stomachs were sampled in the Polish Exclusive Economic Zone, southern Baltic Sea. Ichthyological analysis of each individual cod was performed on board the survey ship. The stomachs were frozen at  $-20^{\circ}\text{C}$  for further food content analysis in the laboratory on land. Analysis of the cod diet was performed: prey items found in stomachs were sorted and classified to the lowest possible taxonomic level (Żmudziński, 1990; Hayward and Ryland, 1995). All invertebrates were collected, counted and analysed one by one for the presence of parasites. The organisms found had decomposed to different extents: some were partly digested, whereas others were not macerated at all. Well-preserved invertebrates were digested in the laboratory in artificial gastric juice (aqueous solution of pepsin and 35–38% HCl) to expose any parasites in the body cavity. This treatment increased the transparency of prey organisms and improved the level of detection of parasites. All parasites observed were collected and examined under the stereomicroscope for taxonomic identification on the basis of anatomomorphological features (Fagerholm, 1982; Berland, 1989). Infection with parasites is described according to the prevalence and intensity of infection (Bush *et al.*, 1997).

To confirm taxonomic identification, molecular analysis of parasites was performed. Analysis was conducted according to Zhu *et al.* (1998) and involved the amplification and sequencing of the ITS-1 (Internal Transcribed Spacer) region of rDNA. Polymerase chain reaction (PCR) products were sequenced directly using standard procedures and amplification primers. DNA was isolated using a Genomic Mini Kit (A&A Biotechnology, Gdynia, Poland) according to the manufacturer's instructions. Amplification was performed using the primers NC5 (forward) 5'-GTA GGT GAA CCT GCG GAA GGA TCA TT-3' and NC13R (reverse) 5'-GCT GCG TTC TTC ATC GAT-3'. The reaction mixture consisted of 25  $\mu\text{L}$  PCR Master MixPlus High GC (ready-to-use PCR mixture containing Taq DNA polymerase, PCR buffer,  $\text{MgCl}_2$  and dNTPs; A&A Biotechnology), 0.2  $\mu\text{L}$  each primer (stock concentration 100  $\mu\text{M}$ ), 20  $\mu\text{L}$  DNA template and supplemented with deionized water up to 50  $\mu\text{L}$ . The PCR reaction conditions were as follows: 2 min at  $94^{\circ}\text{C}$  (initial denaturation) followed by 35 cycles of 30 s at  $94^{\circ}\text{C}$ , 30 s at  $60^{\circ}\text{C}$ , 30 s at  $72^{\circ}\text{C}$  and a final extension step of 5 min at  $72^{\circ}\text{C}$ . Some DNA fragments obtained as a result of the amplification reaction were purified using a Gel-Out Concentrator Kit (A&A Biotechnology). PCR products were eluted with sterile water. Sequences were analysed using software CLC Workbench and GeneStudio and confirmed by a BLAST search of the GenBank.

### Results

The food content analysis revealed 15 467 invertebrates in cod stomachs (Table 1). The dominant (the most numerous) species were Crustacea, especially *C. crangon*, *S. entomon*, *M. mixta*, *Gammarus* sp.; the polychaete *Byligides sarsi* was also frequently observed. Parasitological analysis of the invertebrates found in cod stomachs revealed the presence of *Hysterothylacium* sp. L3 larvae, but only in the decapod *C. crangon* (Fig. 1). The first microscopic investigation of *C. crangon* was insufficient, and additional digestion in artificial gastric juice was necessary to determine the presence of nematodes in the body cavity. Among the

4731 *C. crangon* examined, parasites were found in nine individuals: five in the sample from November 2012; one from February 2013; two from November 2013 and one from February 2014 (Table 2). On the basis of anatomomorphological features, all the above parasites were identified as *Hysterothylacium* sp. Therefore, the prevalence of *C. crangon* infection with *Hysterothylacium* sp. was 0.0027% in November 2012; 0.0005% in February 2013; 0.0067% in November 2013 and 0.0018% in February 2014. The mean prevalence of infection was 0.0029%. The intensity of infection was 1 in every case.

Molecular analysis identified seven parasites as *Hysterothylacium aduncum*; two individuals were impossible to verify by DNA sequencing. The long process of obtaining parasites from under the carapace of crustaceans (digestion in stomach, freezing, additional digestion in artificial gastric juice) is likely to result in the partial degradation of the DNA in a significant proportion of cases and cause difficulties with molecular identification. Where parasites were successfully identified, the sequence similarity was 98.21–100% with *H. aduncum* compared to examples registered in the GenBank. Examples of the sequences obtained have been deposited in the GenBank (accession no. MW506285, MW506286, MW506287, MW506288 and MW506289).

Table 3 shows all parasites found in cod stomachs near to, but not within, food items (excluding samples from 2012, where they were not collected). The nematode *Hysterothylacium* sp. and the acanthocephalan *E. gadi*, as well as representatives of Trematoda, were found.

### Discussion

Several recent studies have revealed a remarkable increase in the prevalence of cod infection with Anisakidae nematodes (Haarder *et al.*, 2014; Mehrdana *et al.*, 2014; Nadolna and Podolska, 2014; Horbowy *et al.*, 2016; Zuo *et al.*, 2016). The negative effect of such an increase in the intensity of infection on the condition of fish has also been reported (Horbowy *et al.*, 2016). The parasite fauna of Baltic cod is well known (Myjak *et al.*, 1994; Buchmann, 1995; Mellergaard and Lang, 1999; Perdiguero-Alonso *et al.*, 2008; Haarder *et al.*, 2014; Mehrdana *et al.*, 2014; Nadolna and Podolska, 2014; Zuo *et al.*, 2016). Invertebrates, which represent important basic food items for Baltic cod in their early stages of development, are thought to be the first intermediate host and transmitter of pathogenic nematodes to fish (Bagge *et al.*, 1994; Horbowy *et al.*, 2016; Engelhardt *et al.*, 2020). The role of particular invertebrate species in the life cycles of specific parasites is not precisely defined. Clearly, a high-quality diet is essential for healthy fish development.

Similar to the research conducted by Pachur and Horbowy (2013), in the current study, the dominant invertebrate food items in cod stomach were Malacostraca, especially *C. crangon*, *S. entomon*, *M. mixta*, *Gammarus* sp. and Polychaeta *B. sarsi*. The presence of the parasite in the brown shrimp suggests that this invertebrate is not only a source of nutrients, but might also be a route of infection with parasites. However, parasites were found only in the brown shrimp, *C. crangon* (Decapoda), which frequently occurs in offshore sandy and sandy-muddy habitats in the Baltic and North Seas, and also along the north and west coasts of Europe and the American coastal waters of the North Atlantic (Żmudziński, 1967). Brown shrimp is a migratory species: in the autumn, when temperatures decrease, it migrates into deeper waters, returning to shallower waters in the spring (Żmudziński, 1961). Therefore, it is accessible to both large demersal cod in the colder part of the year and to small cod individuals present in shallow waters. In the Polish waters of the southern Baltic Sea, *C. crangon* reaches 55 (male) to 70 (female) mm in size (Szaniawska, 1991), making it easily

**Table 1.** Dominant invertebrate species among food items in cod stomachs (number of individuals)

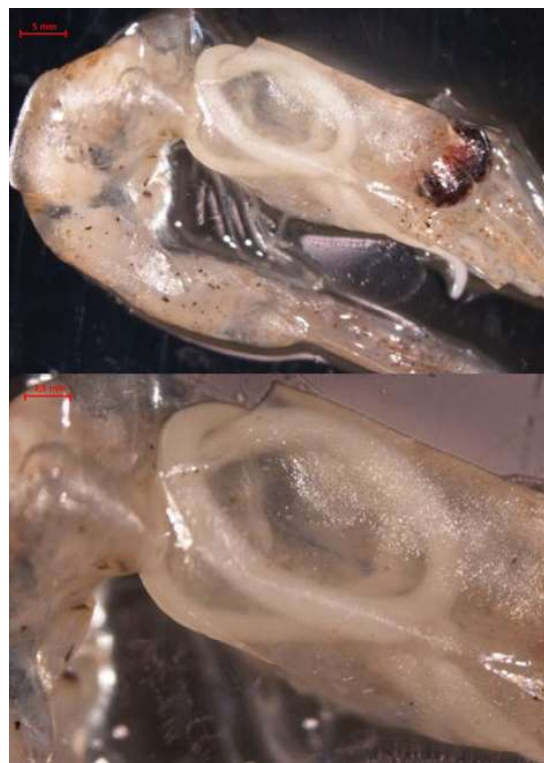
	2012 Q4	2013 Q1	2013 Q4	2014 Q1	Sum
No. of cod stomachs analysed	363	380	394	544	1681
<i>Crangon crangon</i>	1843	2030	299	559	4731
<i>Saduria entomon</i>	518	282	350	687	1837
<i>Mysis mixta</i>	184	990	1927	1357	4458
<i>Bylgides sarsi</i>	82	67	486	161	796
<i>Gammarus</i> sp.	169	184	209	534	1096
Other species	325	479	583	1162	2549
Total	3121	4032	3854	4460	15 467

available even to small cod; consequently, it is a common item in the cod diet (Pachur and Horbowy, 2013). The role of *C. crangon* as a transmitter of the nematode parasite *Anisakis simplex* to Baltic cod has been described by Pawlak *et al.* (2019). The results presented in this report are the first *in situ* confirmation that *C. crangon* may be a route for cod infection with *H. aduncum*.

In this study, *C. crangon* was found to be infected with L3-stage *H. aduncum* larvae. Genetic identification confirmed the results of anatomo-morphological analysis. To my best knowledge, this is the first evidence for this host–parasite system in the Baltic Sea. The prevalence of *C. crangon* infection with *Hysterothylacium* sp. was 0.0027% in November 2012; 0.0005% in February 2013; 0.0067% in November 2013 and 0.0018% in February 2014; the intensity of infection was 1 in all cases. Although the prevalence of infection is low, *C. crangon* is an important food item in the cod diet, and therefore this invertebrate may play a role as a transmitter of parasites to cod.

The nematode *Hysterothylacium* sp. (mainly *H. aduncum*), a member of the Raphidascarididae family, is a common parasite of marine fish throughout the world (Andersen, 1993; Rello *et al.*, 2008; Knoff *et al.*, 2012; Moravec *et al.*, 2012; Kong *et al.*, 2015; Morsy *et al.*, 2015; Shamsi *et al.*, 2015; Shamsi *et al.*, 2016; Shamsi, 2017; Ghadam *et al.*, 2018; Roca-Geronès *et al.*, 2018) and is the most frequently occurring parasite in invertebrates acting as intermediate hosts: it has been reported in 70 different invertebrate species (Lick, 1991). *Hysterothylacium aduncum* (Rudolphi 1802) has a circumpolar distribution in the Northern hemisphere (Deardorff and Overstreet, 1981) and has been found in the north-west Atlantic (Marcogliese, 1996), the North Sea and the Baltic Sea (Lick, 1991; Klimpel and Ruckert, 2005), but also in the Mediterranean Sea (Dural *et al.*, 2011; Abdel-Ghaffar *et al.*, 2015), the Black Sea (Pekmezci *et al.*, 2013) and the waters around Japan (Moravec and Nagasawa, 1986; Kong *et al.*, 2015). In the southern hemisphere, it has been found among other locations in the south-west Atlantic (Navone *et al.*, 1998), and around Australia (Shamsi *et al.*, 2015, 2018).

In general, in the *H. aduncum* life cycle, invertebrates play the role of intermediate hosts and fish are the final host. Natural infection with larval *H. aduncum* has been documented in seven phyla of both benthic and planktonic invertebrates in the north-western Atlantic (Norris and Overstreet, 1976; Marcogliese, 1996). Third-stage larvae of *H. aduncum* have been obtained from *C. crangon* in the Ythan estuary (Scotland)

**Fig. 1.** *Crangon crangon* infected with *Hysterothylacium aduncum* (photo: J. Pawlak).

(Gibson, 1972). In the Canadian Bras d'Or Lake, *H. aduncum* uses a variety of intermediate hosts including the mysids *Neomysis americana* and *M. stenolepis* and the chaetognath *Sagitta elegans*, as well as mixture of zooplankton: calanoid copepods, crab zoea and megalops and euphausiid larvae (Jackson *et al.*, 1997). *Hysterothylacium* sp. has been reported by Lick (1991) in several invertebrate species from the North Sea and Baltic Sea (studies limited to German coastal waters), including *Acartia bifilosa*, *Eurytemora affinis*, *Temora longicornis*, *Pseudocalanus elongatus* and *M. mixta*. Infection of gammarid species including *Gammarus locusta*, *G. salinus* and *G. zaddachi* with *Hysterothylacium* sp. in the same area has been noted by Lick (1991) and Zander *et al.* (2000). The same nematode parasite has also been recorded in representatives of Calanoida (Svendsen, 1990; Marcogliese, 1995), especially in *Hyperia galba* and *Idotea* spp. from the North Sea (Klumpel and Ruckert, 2005) and *Neomysis. integer* from both the North Sea and German coastal waters of the Baltic Sea (Lick, 1991; Klumpel and Ruckert, 2005). Pawlak *et al.* (2018) found *S. entomon* infected with *H. aduncum in situ* in cod stomach, which was the first evidence of such a host–parasite system, similarly to the *C. crangon* and *H. aduncum* system in the Baltic Sea presented here.

Experimental research described in the literature confirms that the role of intermediate host may be served by invertebrates, for example, Crustacea, Polychaeta, Ctenophora, Echinodermata, Chaetognatha and Mollusca (Koie, 1993; Münster *et al.*, 2015). The life cycles of *Hysterothylacium* sp. and potential intermediate hosts were also studied after experimental infection of *T. longicornis* (Koie and Fagerholm, 1995) and calanoid species (Hurst, 1984; Koie, 1993), especially *N. integer* (Koie and Fagerholm, 1995).

In the Baltic Sea, the life cycle of *H. aduncum*, and in particular which invertebrate species serve as intermediate hosts for this parasite, has been described only in general terms. The marine



**Table 2.** *C. crangon* infected with *Hysterothylacium aduncum*/*Hysterothylacium* sp. nematodes in stomachs of Baltic cod

	2012 Q4	2013 Q1	2013 Q4	2014 Q1	sum
No. of cod stomachs with <i>C. crangon</i>	197	84	76	76	433
Total no. of <i>C. crangon</i>	1843	2030	299	559	4731
No. of <i>C. crangon</i> infected with <i>H. aduncum</i> / <i>Hysterothylacium</i> sp.	5	1	2	1	9
Prevalence of infection (%)	0.0027	0.0005	0.0067	0.0018	

**Table 3.** Parasites found in stomachs of Baltic cod

	2012 Q4	2013 Q1	2013 Q4	2014 Q1	Sum
Nematoda: <i>Hysterothylacium</i> sp.	No data	23	19	19	61
Acanthocephala: <i>Echinorhynchus gadi</i>	No data	26	4	71	101
Trematoda	No data	4	5	1	10

environment is changing and new species that act as intermediate hosts might appear. It is known, however, that the eggs of this nematode (which contain developed larvae) can be ingested by both benthic and pelagic crustaceans. Eggs hatch in the intestine of these invertebrates and the parasite larvae migrate to the haemocoel of the intermediate host. Larger invertebrates can play the role of second intermediate hosts (Køie, 1993).

Pawlak *et al.* (2018) revealed that moulting and transformation from L4 larva to the adult nematode (*H. aduncum*) might take place inside invertebrate hosts (*S. entomon*). Iglesias *et al.* (2002) conducted *in vitro* cultivation of *H. aduncum* from L3 to egg-laying adults and described the conditions (temperature = 13°C; pH = 4; 5% CO<sub>2</sub> in the growth atmosphere, etc.) for optimal development and survival of these nematodes. The authors proved that if the medium was supplemented with pepsin, all larvae reached the adult stage. Similar conditions were used for *in vitro* cultivation of L3-stage *H. aduncum* larvae obtained from the fish host through a complete developmental cycle of the parasite to L3 larvae hatched from eggs obtained during the experiment (Adroher *et al.*, 2004).

In the life cycle of *H. aduncum* the final hosts are fish. The larval stages live in different tissues of several fish species and in numerous invertebrate species (Norris and Overstreet, 1976; Hurst, 1984; Marcogliese, 1996). The parasites enter the fish with food and are able to penetrate the stomach wall of the fish to get to the body cavity and internal organs, e.g. liver (Myjak *et al.*, 1994). Sexually mature adult individuals of *H. aduncum* are often found in the digestive tract of fish (Deardorff and Overstreet, 1981), such as eel-pout and Atlantic cod (Jackson *et al.*, 1997), including the Baltic Sea (Køie, 1993). *Hysterothylacium* sp. has also been reported in the Baltic in flatfishes (Køie, 1993), eel *Anguilla anguilla* (Køie, 1993), sea trout *Salmo trutta trutta* (Unger and Palm, 2016), sprat (Skrzypczak and Rolbiecki, 2015), sticklebacks (Køie, 1993) and Gobiidae fish (Zander *et al.*, 1993, 1994; Zander, 2004). Gadoids are considered to be the main final hosts for *Hysterothylacium* sp., however (Berland, 1961). Therefore *C. crangon* might also be the source of infection with this parasite for other listed fish species in the Baltic Sea that feed on the invertebrate.

The emergence of new intermediate hosts is an interesting development, to which the changing climate may be a contributory factor. Rokicki (2009) noted that, in general, environmental changes affect the occurrence and abundance of parasites either directly by their influence on the free-living larval stages of parasites or indirectly by their effect on the respective hosts (mainly invertebrate). This problem has already been noted in several parts of the world, such as Australia, where environmental changes

have negatively impacted the survival of early-stage parasite larvae in their first intermediate hosts. The absence of Anisakis larvae in fish collected in this area shows the importance of the role of zooplankton and crustaceans in the food chain and in the ecosystem more generally (Shamsi *et al.*, 2018). In Canada, the limited availability of specific food components may be one reason for the decreasing number of parasites in fish (Khan and Chandra, 2006).

In this study, the parasites *Hysterothylacium* sp., *E. gadi* and representatives of Trematoda were found in the stomachs of cod adjacent to food items. These parasites might therefore be present in the body cavity of prey before digestion in the stomach, which could be a source of infection.

It must be emphasized that when cod are caught in a particular area it does not unequivocally indicate that parasites found in its stomach or in its food items were obtained by the fish in the same area. Cod is a migratory species and the adult cod can migrate up to 1000 km (Saulamo and Neuman, 2002) without clear spatial or temporal distribution patterns (Aro, 2000).

In summary, to my best knowledge, *H. aduncum* has been reported in the Baltic Sea in *C. crangon* for the first time and this also represents the first report of this host–parasite system found *in situ* in cod stomach. Because *C. crangon* plays an important role in the food composition of cod, this invertebrate is likely to be an intermediate host in the life cycle of *H. aduncum* in the Baltic Sea. The results of this research are important for the development of the parasitology, fish biology and ecology of the Baltic Sea, in particular for an improved understanding of the function of individual components of the food web in the transmission of cod parasites.

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**Conflict of interest.** The author declares none.

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- Załączowski W. 1977. Ilościowa i ekologiczna analiza pokarmu użytkowanego przez populację dorsza w południowym Bałtyku w latach 1972–1974. [Quantitative and ecological analysis of food consumed by southern Baltic cod population in the years 1972–1974]. Szczecin, Poland: Akademia Rolnicza w Szczecinie, 53, 1–109. (in Polish)
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## **APPENDIX**

### **CURRICULUM VITAE OF THE AUTHOR**

#### **EDUCATION**

- 2015 - 2021 PhD studies  
University of Gdańsk, Faculty of Oceanography and Geography,  
Environmental doctoral studies
- 2010 - 2012 MSc studies  
University of Gdańsk, Faculty of Oceanography and Geography  
Oceanography, specialty: Protection and management of marine  
resources
- 2007 - 2010 BSc studies  
University of Gdańsk, Faculty of Oceanography and Geography  
Oceanography, specialty: Biological oceanography

#### **EMPLOYMENT**

- 2018 - present National Marine Fisheries Research Institute  
Department of Fisheries Resources: specialist
- 2014 - 2018 National Marine Fisheries Research Institute  
Department of Logistics and Monitoring: specialist
- 2013 - 2014 National Marine Fisheries Research Institute  
Department of Logistics and Monitoring: intern
- 2013 National Marine Fisheries Research Institute  
Department of Fisheries Resources: intern

## PUBLICATIONS

Author and co-authorship of 8 scientific papers, published in journals indexed on JCR list:

a) Series of publications constituting the doctoral dissertation:

1. **Pawlak, J.** 2021. *In situ* evidence of the role of *Crangon crangon* in infection of cod *Gadus morhua* with nematode parasite *Hysterothylacium aduncum* in the Baltic Sea. *Parasitology*, 148, 1691–1696. DOI:10.1017/S0031182021001414  
IF: 3.234, 5-year IF: 3.118, MSHE points: 100
2. **Pawlak, J.**, Nadolna-Ałtyn, K., Szostakowska, B., Pachur, M., Bańkowska A., Podolska, M. 2019. First evidence of the presence of *Anisakis simplex* in *Crangon crangon* and *Contracaecum osculatum* in *Gammarus* sp. by *in situ* examination of the stomach contents of cod (*Gadus morhua*) from the southern Baltic Sea. *Parasitology* 146, 1699-1706. DOI:10.1017/S0031182019001124  
IF: 3.234, 5-year IF: 3.118, MSHE points: 100
3. **Pawlak, J.**, Nadolna-Ałtyn, K., Szostakowska, B., Pachur, M., Podolska, M. 2018. *Saduria entomon* infected with *Hysterothylacium aduncum* found *in situ* in the stomach of cod (*Gadus morhua*) from the Baltic Sea. *Journal of Helminthology*, 92(5), 645-648. DOI:10.1017/S0022149X1700092X  
IF: 2.17, 5-year IF: 1.958, MSHE points: 25 (2019: 40)

b) Other papers published in journals indexed on JCR list:

1. Nadolna-Ałtyn, K., Podolska, M., **Pawlak, J.**, Szostakowska, B. 2022. Distribution of anisakid nematodes in the muscle tissue of cod (*Gadus morhua*) from the Norwegian Sea. *Oceanologia (accepted for publication)*  
IF: 2.427, 5-year IF: 2.671, MSHE points: 100
2. Białowąs, M., Jonko-Sobuś, K., **Pawlak, J.**, Polak-Juszczak, L., Dąbrowska, A., Urban-Malinga, B. 2022. Plastic in digestive tracts and gills of cod and herring from the Baltic Sea. *Science of The Total Environment*, 822, DOI:10.1016/j.scitotenv.2022.153333  
IF: 7.963, 5-year IF: 7.842, MSHE points: 200
3. Łopieńska-Biernat, E., Stryński, R., Polak, I., Pawlikowski, B., **Pawlak, J.**, Podolska, M. 2020. Effect of freezing on the metabolic status of L3 larvae of *Anisakis simplex* ss. *Infection, Genetics and Evolution*, 82, 104312. DOI:10.1016/j.meegid.2020.104312  
IF: 3.342, 5-year IF: 3.188, MSHE points: 100

4. Haase, K., Orio, A., **Pawlak, J.**, Pachur, M., Casini, M. 2020. Diet of dominant demersal fish species in the Baltic Sea: Is flounder stealing benthic food from cod? *Marine Ecology Progress Series*, 645, 159-170. DOI:10.3354/meps13360

IF: 2.824, 5-year IF: 3.090, MSHE points: 70

5. Podolska, M., Pawlikowski, B., Nadolna-Ałtyn, K., **Pawlak, J.**, Komar-Szymczak, K., Szostakowska, B. 2019. How effective is freezing at killing *Anisakis simplex*, *Pseudoterranova krabbei*, and *P. decipiens* larvae? An experimental evaluation of time-temperature conditions. *Parasitology Research*, 118, 2139–2147. DOI:10.1007/s00436-019-06339-1

IF: 2.289, 5-year IF: 2.403, MSHE points: 70

Total impact factor: 27.483, total 5-year IF: 27.388, sum of MHES points: 740+25 (2019: 780)

#### Other publications:

1. Podolska M., Nadolna-Ałtyn K., **Pawlak J.**, Różycki M., Pękala-Safińska A. 2019. Wykrywanie i identyfikacja nicieni Anisakidae - metody niedestrukcyjne. [Detection and identification of Anisakidae nematodes - non-destructive methods]. In: Różycki M. and Podolska M. (Ed.), *Zasady Dobrej Praktyki W Przetwórstwie Rybnym*. SeaQual 2019. Państwowy Instytut Weterynaryjny – Państwowy Instytut Badawczy, 100-108 p. (in Polish)
2. Podolska M., Bogusław Pawlikowski B., Katarzyna Nadolna-Ałtyn K., **Pawlak J.**, Komar-Szymczak K., Szostakowska B. 2019. Wpływ procesu mrożenia na przeżywalność larw nicieni z rodziny Anisakidae. [The impact of freezing process on the survival of larvae Anisakidae nematodes]. In: Różycki M. and Podolska M. (Ed.), *Zasady Dobrej Praktyki W Przetwórstwie Rybnym*. SeaQual 2019. Państwowy Instytut Weterynaryjny – Państwowy Instytut Badawczy, 246-254 p. (in Polish)
3. Pachur M. E., **Pawlak J.** 2016. Zmiany w składzie pokarmu i odżywianie się dorsza (*Gadus morhua*) w południowym Bałtyku na przestrzeni ostatnich lat. [Changes in the food composition and feeding of cod (*Gadus morhua*) in the Southern Baltic Sea over the past years]. In: Psuty I. (Ed.), *95-lecie Morskiego Instytutu Rybackiego: aktualne tematy badań naukowych Tom II – Stan środowiska południowego Bałtyku*. Morski Instytut Rybacki – Państwowy Instytut Badawczy, ISBN 978-83-61650-18-8, 87-96 p. (in Polish)
4. Nadolna-Ałtyn K., Podolska M., **Pawlak J.** 2016. Wysoki poziom zarażenia dorszy nicieniami Anisakidae w połowach komercyjnych z POM w 2015 r. [High level of cod infection with Anisakidae nematodes in commercial fisheries in PMA in 2015]. *Wiadomości Rybackie* 1-2 (209), 17-18 p. (in Polish)

5. Huwer B., Neuenfeldt S., Rindorf A., Andreassen H., Levinsky S.-E., Storr-Paulsen M., Dalmann Ross S., Haslund O.H., Horbowy J., Pachur M., **Pawlak J.**, Ustups D., Kruze E., Sics I., Uzars D., Velasco A., Kempf A., Eberle S., Floeter J., Temming A., van Hal R., de Boois I., Pennock I., Hoek R., Pinnegar J., Hunter E., Pliró A., Casini M., Belgrano A. 2014. Study on stomach content of fish to support the assessment of good environmental status of marine food webs and the prediction of MSY after stock restoration. Final report for EU contract No MARE/2012/02. 56 p.

### **RESEARCH GRANTS:**

Principal investigator of 1 research project and participant of over 20 projects.

#### **Principal investigator of project:**

Identification of sources of cod (*Gadus morhua*) infection with parasites, based on the analysis of parasite fauna in marine invertebrates present in the diet of these fish (Określenie źródeł zarażenia dorszy bałtyckich pasożytami, na podstawie analizy parazytofauny bezkręgowców morskich, występujących w pokarmie tych ryb)

Funder: National Science Centre, duration: 2016-2018, Grant No. 2015/19/N/NZ9/00173

#### **Participant of selected projects:**

1. Multilevel assessment of microplastics and associated pollutants in the Baltic Sea (Kompleksowa ocena występowania mikroplastików i stowarzyszonych z nimi zanieczyszczeń w Morzu Bałtyckim (MICROPOLL))

Funder: NCBiR, Bonus EEIG, duration: 2017-2020

2. Seafood safety and quality in terms of the zoonotic and toxicological hazard: risk assessment, monitoring and mitigation (Bezpieczeństwo i jakość żywności pochodzenia morskiego w aspekcie zagrożeń zoonotycznych i toksykologicznych: ocena ryzyka, monitoring i przeciwdziałanie (SeaQual))

Funder: NCBiR, BIOSTRATEG, duration: 2016-2019

3. Study on stomach content of fish to support the assessment of good environmental status of marine food webs and the prediction of MSY after stock restoration.

Funder: EU, No. MARE/2012/02, duration: 2012-2014

## **CONFERENCES:**

Co-authorship of 7 oral presentations and 43 posters presented at 16 scientific conferences, including 11 international and 5 national.

### **National:**

II Scientific Conference For Polish Marine Researchers (II Konferencja Naukowa Polskich Badaczy Morza), 24-25.09.2019, Gdynia

XXV Congress of the Polish Parasitological Society (XXV Zjazd Polskiego Towarzystwa Parazytologicznego), 9-12.09.2019, Warszawa

XV Symposium of Young Oceanographers (XV Sympozjum Młodych Oceanografów), 25.11.2016, Gdynia

XXIV Congress of the Polish Parasitological Society (XXIV Zjazd Polskiego Towarzystwa Parazytologicznego), 05-08.09.2016, Kraków

I Pomeranian PhD Students' Conference „BioMed Session” (I Konferencja Doktorantów Pomorza „BioMed Session”), 12.12.2015, Gdańsk

### **International:**

13<sup>th</sup> European Multicolloquium of Parasitology (EMOP), 12-16.10.2021, Belgrad, Serbia, online

20<sup>th</sup> International Conference on Diseases of Fish and Shellfish, 20-23.09.2021, online

Conference for Young Marine Researchers YOUMARES 10, 11-13.09.2019, Hamburg, Germany

19<sup>th</sup> International Conference on Diseases of Fish and Shellfish, 09-13.09.2019, Porto, Portugal

48<sup>th</sup> West European Fish Technologists Association, 15-18.10.2018, Lisbon, Portugal

14<sup>th</sup> International Congress of Parasitology, 19-24.08.2018, Daegu, South Korea

26<sup>th</sup> International Conference of the World Association for the Advancement of Veterinary Parasitology, 4-8.09.2017, Kuala Lumpur, Malaysia

18<sup>th</sup> International Conference on Diseases of Fish and Shellfish, 04-07.09.2017, Belfast, UK

7<sup>th</sup> Conference of Scandinavian-Baltic Society for Parasitology, 8-9.06.2017, Riga, Latvia

12<sup>th</sup> European Multicolloquium of Parasitology (EMOP), 20-24.-07.2016, Turku, Finland

9<sup>th</sup> International Symposium of Fish Parasites, 31.08 – 04.09.2015, Valencia, Spain

## **SCHOLARSHIPS**

- Doctoral scholarship awarded by the National Marine Fisheries Research Institute (2019-2020)
- Doctoral scholarship from the pro-quality grant awarded by the University of Gdańsk (2016-2018)
- Scholarship for the best PhD students awarded by the University of Gdańsk (2016-2018)

## **MEMBERSHIP IN SCIENTIFIC ORGANIZATIONS**

- 2016 - present            Polish Parasitological Society
- 2016 - present            European Federation of Parasitologists

## **WORKSHOPS AND COURSES**

1. Workshop on registrations of cod liver worm, Copenhagen, Denmark, 3-5.02.2020, DTU AQUA.
2. Workshop on Better Coordinated Sampling Stomachs (WKBECOSS), Santander, Spain, 3-6.09.2019, Spanish Institute of Oceanography.
3. Workshop - Nucleic acid isolation, basics of PCR technique and preparation of PCR products for sequencing (Izolacja kwasów nukleinowych, podstawy techniki PCR i przygotowanie produktów PCR do sekwencjonowania), Gdynia, 12-13.10.2017, A&A Biotechnology.
4. Workshop - Planning research and analysis of variance (Planowanie badań i analiza wariancji), Warszawa, Poland, 09-10.10.2017, StatSoft Polska.
5. Training in the welfare and humane handling of fish (Szkolenie w zakresie dobrostanu i humanitarnego obchodzenia się z rybami), Gdynia, 02.02.2017, Inland Fisheries Institute Olsztyn.
6. Workshop - Data analysis using R (Analiza danych w systemie R), Gdynia, 6-17.05.2016.
7. Training course in laboratory animal science: for people responsible for the design and conduct of animal experiments; for people performing procedures; for people killing animals used in the procedures (Szkolenie dla osób: odpowiedzialnych za planowanie procedur i doświadczeń oraz za ich przeprowadzenie; wykonujących procedury; uśmiercających zwierzęta wykorzystywane w procedurach), Olsztyn, 01-05.02.2016, Polish Laboratory Animal Science (PoLLASA).

8. Workshop - Pneumatic techniques for obtain spawn for people handling reproduction of sturgeon fish, wild and salmon fish (Techniki pneumatycznego uzyskiwania ikry dla osób zajmujących się rozrodem ryb jesiotrowatych, dzikich i łososiowatych), Tarnowo (Piła), 25-27.03.2015, Institute of Animal Reproduction and Food Research of Polish Academy of Sciences in Olsztyn.
9. Deck Hand Certificate (07.10.2015, Gdynia).
10. Junior Deck Fisherman Certificate Seagoing Fishing (07.09.2015, Gdynia).
11. Certificate of Basic Safety Training (05.09.2014, Gdynia):
  - Personal Survival Techniques,
  - Elementary First Aid,
  - Personal Safety & Social Responsibilities,
  - Fire Prevention & Fire Fighting.

## **INTERNSHIP**

Medical University of Gdańsk, Department of Tropical Medicine and Parasitology, National Centre for Tropical Medicine, Division of Tropical Parasitology, 16-20.10.2017, Gdynia.

## **TEACHING EXPERIENCE**

Assistance in university classes for students of Oceanography and Aquaculture - business and technology (University of Gdańsk):

- Basics of ichthyology (2016-2018)
- Ecological modeling, Marine and inland fisheries (2018)

Tutor of interns and volunteers in NMFRI (2014, 2015, 2020)

Workshop on procedures using in the detection of parasites in fish as part of the course "Quality and safety of fish products - biological threats". 18.04.2018, National Marine Fisheries Research Institute, Gdynia

## **RESEARCH CRUISES**

5 research cruises on r/v BALTICA (2014, 2016, 2018, 2019, 2021)