Metazoan parasites from herring (*Clupea harengus* L.) as biological indicators in the Baltic Sea

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Abstract

Zoographical distribution of metazoan fish parasites in herring, *Clupea harengus*, from the Baltic Sea was analysed in order to use them as potential biological indicators. A total of 210 herring from six different sampling sites were investigated, harbouring 12 different parasite species [five digeneans (D), one cestode (C), three nematodes (N) and three acanthocephalans (A)]. The distribution of the parasite species differed according to region, with a distinct gradient of decreasing species richness towards the east of the Baltic Sea. The western localities at Kiel Bay, Rügen and Poland had the highest parasite diversity, including the marine parasite species *Anisakis simplex* (s.s.) (N), *Brachyphallus crenatus* and *Hemiurus luehei* (both D). The eastern localities had low parasite species richness, predominated by the freshwater digenean *Diplostomum spathaceum*. We could identify three different Baltic herring stocks, the spring-spawning herring of the western Baltic reaching from the Kattegat to the German and Polish coast, the stock of the central Baltic proper and the northern stock of *C. harengus* var. *membras* of the Gulf of Finland. The limited distribution of the herring parasites within the Baltic Sea enables their use as biological indicators for migration patterns and stock separation. The acanthocephalan *Pomphorhynchus laevis* that has already been used as an accumulation bioindicator for heavy metals was only recorded for the western herring stocks. However, the presence of mainly generalistic parasites and their uneven distribution patterns make their use as indicators for regional environmental and global change more difficult.

Keywords

Fish parasites, Atlantic herring, *Clupea harengus*, zoogeographic distribution, biological indicators, Baltic Sea

Introduction

Fishes demonstrate a wide variety of feeding habits and occupy numerous trophic levels and ecological niches. The fish community of the Baltic Sea is dominated by the planktivorous sprat (*Sprattus sprattus* L., 1758) and herring (*Clupea harengus* L., 1758) and the piscivorous cod (*Gadus morhua* L., 1758). These species make up more than 95% of the commercial catch (Sparholt 1994). During the late 1970s to mid-1980s the Baltic Sea fish community has switched from a cod-dominated system to a clupeid-dominated system from the early 1990s until today (Kornilovs et al. 2001). Herrings undertake extensive annual migrations between their feeding and spawning grounds. Because of their high salinity and temperature tolerance (Klinkhardt 1996) and their ability to feed on the lower trophic levels, this species has successfully colonised a wide range of habitats. Planktivores, mainly clupeids and mysid shrimps consume approx. 70% of the crustacean zooplankton production in the northern Baltic Sea (Hansson et al. 1990, Rudstam et al. 1992). In general, clupeids have the highest yield among the landed species in the global fisheries. The herring is of major importance in the North Atlantic (FAO Code 27), reaching a total catch of 2.7 mio to in 2010 (FAO 2012).

The Baltic Sea, with a total area of 415,023 km² including the Kattegat, is connected with the North Sea and the North Atlantic Ocean (Wasmund et al. 2001). A wide salinity range is characteristic for the Baltic Sea, with a gradient of 30 PSU in the Kattegat (western part), and one PSU in the northern Bothnian Bay. This gradient is caused by restricted salt-water influx into the Baltic Sea at the Darß Sill, between Mecklenburg-Western Pomerania, Germany and Nykobing, Denmark,

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Parasites are one of the most successful life forms on earth and play an important role for the health and reproduction of marine fishes (Palm and Klimpel 2007). Healthy ecosystems are characterised by a high parasite biodiversity, reflecting all possible transmission pathways in such habitats. Transition zones such as the Baltic Sea are far less diverse, with a species minimum in brackish waters of 6–8 PSU (Remane 1958). This is also true for parasites, with their often complex life cycles and high requirements to diverse and functional food webs (Palm and Klimpel 2007). According to Palm et al. (1999), 86 parasite species have been recorded for fish from the North Sea coast of Germany, compared with 142 species from the Baltic Sea along the German coastal waters. Arthur and Arai (1984) and MacKenzie (1987) recorded 31 parasite species for herring from the Baltic Sea.

The distribution of the fish parasite fauna within the Baltic Sea is not homogeneous. Fish parasites and their life cycle stages are dependent on the water quality, and have been consequently used as biological indicators for their environment (MacKenzie et al. 1995, Galli et al. 2001, Palm 2011). Also the salinity has effects on their species diversity (MacKenzie 1983, Landsberg et al. 1998). The zoonotic nematode *Anisakis simplex* has been recorded from herring in the Kattegat and Skagerrak at a prevalence of 60% (Ellemann 1989), while no records are known from the Gulf of Finland. The common freshwater parasite *Diplostomum spathaceum* occurred in the Gulf (52.8%) and is absent in the open water of the western Baltic Sea (Zander 1998). This exemplifies different zoogeographical distribution patterns of Baltic herring parasites, suggesting them being useful as biological indicators of herring migration in the region. The purpose of the present study is an assessment of herring fish parasites over its range of distribution within the Baltic Sea. Special emphasize is given to those species that have been recorded as indicators for stock identification and migration. For the first time a transect of six sampling sites from the Kiel Bay (western Baltic) to the Gulf of Finland (eastern Baltic) was sampled within two weeks. The potential use of herring parasites as biological indicators in the sense of Palm (2011) is discussed.

**Material and Methods**

**Sample collection and examination**

Fish were caught between 19 August and 3 September 2009 using either PSN 205 pelagic or 140 ft bottom trawl on Cruise...
325 of FRV Walther Herwig III. A total of 210 *Clupea harengus* from six different sampling sites in the Baltic Sea were studied for their metazoan parasite fauna. 35 specimens each were studied from waters off Kiel Bay (54°32.24N–10°42.52E), the Island Rügen (54°52.25N–14°01.63E), off Poland (55°03.36N–16°29.24E), Lithuania (55°46.42N–20°40.19E), the Gulf of Finland off Estonia (59°36.22N–24°10.04E), and Finland (60°12.09N–27°07.53E), respectively (Fig. 1). The specimens had a size range from 22.7 cm in the western Baltic to 13.6 cm in the Eastern Baltic Sea (Table I). The samples were deep-frozen immediately after catch for subsequent examination in the laboratory. At each sampling site, hydrographical conditions were recorded by using a CTD-probe with O₂ sensor. Morphometrical data including the standard length (SL), total length (TL), total weight (TW) and weight at slaughter (SW) were recorded to the nearest 0.1 cm and 0.1 g (Table I). The total length of the examined herring ranged between 12.3–32.5 cm, with a mean of 19.0–22.7 cm in the western and 13.6–14.2 cm in the eastern sampling sites.

**Parasitological Examination**

Skin, fins, eyes, gills, nostrils and buccal cavity of each herring were examined for ectoparasites. The body cavity was opened and rinsed with water to detect endoparasites. The liver, stomach, intestine, kidney, gall bladder, spleen, swim-bladder, pyloric caeca and gonads were examined microscopically, followed by an examination of the musculature. The isolated parasites were cleaned and fixed in 4% borax-buffered formalin and preserved in 70% ethanol/5% glycerine. Digesta, Cestoda and Acanthocephala were permanently mounted in 98% glycerine surrounded by paraffin (Riemann 1988). Subsamples of these parasites were stained with acetic carmine, dehydrated and mounted in Canada balsam. Nematoda were dehydrated in a graded ethanol series and stored in 99.6% ethanol for molecular analyses. Parasite identification literature included original descriptions. The parasitological terms follow Bush *et al.* (1997).

**Molecular identification of acanthocephalans and anisakid nematodes**

Following Verweyen *et al.* (2011) genomic DNA was extracted from individual acanthocephalan specimens by using an extraction kit from Peqlab, Erlangen. The region of nuclear rDNA was amplified by using polymerase chain reaction (PCR). Nearly complete SSU rDNA (~1800 bp) regions were amplified after Garey *et al.* 1996 (94°C four min initial denaturing followed by 30 cycles: 94°C 30 s, 60°C 30 s, 72°C 90 s) using primers corresponding to conserved regions at the extreme ends of the 18S rRNA gene (5'-AGATTAAGCCTAGCATGCGTAAG-3' and 5'-TGATCCTTCTGAGGTTCCACCTAC-3'), cloned into pCRH2.1-TOPOH vector (Invitrogen, Karlsruhe) and used to transform competent *Escherichia coli* (TOP 10, Invitrogen, Karlsruhe). Positive clones were identified by blue/white selection, and target inserts of white colonies were confirmed by PCR of bacterial DNA extracts. Liquid cultures for minipreps were grown in Luria broth containing 50 mg/ml of ampicillin following plasmid purification on the next day (MBI Fermentas, St. Leon-Rot). Orientation of cloned inserts was controlled by restriction mapping using 1% agarose gel. Both strands of the 18S rDNA were sequenced completely in both directions after Sanger *et al.* 1977 by Seqlab (Göttingen) using M13 universal primers (forward (220): 5'-GTAAAACGACGGCCAG-3', reverse: 5'-CAGGAAAACAGCTATGAC-3'). Site polymorphisms were recorded only when both alternative nucleotide peaks were present in all sequence reactions representing both DNA strands.

For anisakid nematodes, the rDNA region comprising the internal transcribed spacer ITS-1, 5.8S, ITS-2 and flanking sequences were amplified by using the primers TK1 (5'-GGCACAAAAGTCTGTAACGACCTAGCTAC-3') and TK2 (5'-AGGCTTCTGCAGGTTCAC-3') of Invitrogen. Site polymorphisms were recorded only when both alternative nucleotide peaks were present in all sequence reactions representing both DNA strands.

Table I. Morphometrical data of *Clupea harengus*. GOF, Gulf of Finland

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean length (range) [cm]</th>
<th>Mean weight (range) [g]</th>
<th>Adult</th>
<th>Juvenile</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kiel Bay</td>
<td>19.0 (15.5–23.7)</td>
<td>51.7 (25.3–92.1)</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Rügen</td>
<td>22.7 (17.6–25.9)</td>
<td>90.9 (38.2–116.9)</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>Poland</td>
<td>20.6 (16.0–24.9)</td>
<td>63.5 (34.4–110.8)</td>
<td>28</td>
<td>7</td>
</tr>
<tr>
<td>Lithuania</td>
<td>21.0 (16.2–32.5)</td>
<td>69.3 (30.3–258.4)</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>GOF Estonia</td>
<td>13.6 (12.6–14.5)</td>
<td>18.4 (13.1–21.9)</td>
<td>35</td>
<td>0</td>
</tr>
<tr>
<td>GOF Finland</td>
<td>14.2 (12.3–15.7)</td>
<td>20.7 (14.6–27.1)</td>
<td>35</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 2. Hydrographical conditions at the sampling sites in the Baltic Sea. Catch depths at each site are indicated by the inserted fish symbols. I; Kiel Bay; II, off Rügen; III, off Poland; IV, off Lithuania; V, off Estonia; VI, off Finland.
A 100bp ladder marker (Peqlab Biotechnology GmbH) was used to estimate the size of the PCR products.

**Species identification of both taxa**

To identify the parasite species, the PCR products were purified (EZNA Cycle-Pure Kit (Peqlab Biotechnology GmbH)) and double strand sequenced (Seqlab, Göttingen GmbH, Germany). All sequences were identified by BLASTN database search in GenBank and aligned with homologous sequences of *Anisakis simplex*, *Contracaecum osculatum*, *Corynosoma strumosum* and *Echinorhynchus gadi* using CLUSTAL_X (Thompson et al. 1997).

**Species richness and Diversity**

The diversity of the metazoan parasite fauna was estimated by using the Shannon–Wiener diversity index (H') and the evenness index (E) of Pielou (Magurran 1988):

\[ H' = H_s = -\sum_{i=1}^{s} p_i \ln p_i \]

\[ E = H_s / \ln s \]

where H' is the diversity index, \( p_i \) the proportion of the individual species to the total and s is the total number of species in the community (species richness).

**Results**

The hydrographical conditions (salinity, temperature, \( O_2 \)-saturation) at the 6 sampling sites in the Baltic Sea are summarized in Fig. 2. The different sites in the western Baltic are mesohaline (sites 1–4) (mean salinity 17.7–7.5PSU) while the eastern part (sites 5 and 6) is oligohaline (mean salinity 6.9–5.5PSU). Sites 2–6 show a distinct thermocline, a higher salinity and decreasing levels of oxygen saturation and temperature with increasing depth. A missing thermocline demon-

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**Table II.** Isolated metazoan parasite species of *Clupea harengus* in the Baltic Sea. A, abundance; a, adults; Be, body cavity; E, eye; El, eyelense; I, intensity; In, intestine; L, liver; l, larvae; ml, mean Intensity; P (%), prevalence; Py, pylorus; s.s., sensu stricto; Sf, surface; St, stomach

<table>
<thead>
<tr>
<th>Parasite species/-taxa</th>
<th>Stage</th>
<th>P (%)</th>
<th>ml (l)</th>
<th>A</th>
<th>Site</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Digenea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brachyphallus crenatus</em></td>
<td>a</td>
<td>8.1</td>
<td>2.2 (1–6)</td>
<td>0.18</td>
<td>St</td>
</tr>
<tr>
<td><em>Cryptocotyle lingua</em></td>
<td>l</td>
<td>4.8</td>
<td>9.0 (1–26)</td>
<td>0.43</td>
<td>Sf</td>
</tr>
<tr>
<td><em>Diplostomum spathaceum</em></td>
<td>l</td>
<td>14.8</td>
<td>2.6 (1–10)</td>
<td>0.38</td>
<td>E, El</td>
</tr>
<tr>
<td><em>Hemiurus luehei</em></td>
<td>a</td>
<td>18.1</td>
<td>13.3 (1–112)</td>
<td>2.34</td>
<td>In, Py, St</td>
</tr>
<tr>
<td><em>Lecithaster confusus</em></td>
<td>a</td>
<td>1.4</td>
<td>1.0 (1)</td>
<td>0.01</td>
<td>St</td>
</tr>
<tr>
<td><strong>Cestoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Proteocephalus sp.</em></td>
<td>l</td>
<td>1.0</td>
<td>2.5 (1–4)</td>
<td>0.02</td>
<td>I</td>
</tr>
<tr>
<td><strong>Nematoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anisakis simplex</em> (s.s.)</td>
<td>l</td>
<td>2.9</td>
<td>2.2 (1–7)</td>
<td>0.06</td>
<td>Be</td>
</tr>
<tr>
<td><em>Contracaecum osculatum</em> (s.s.)</td>
<td>l</td>
<td>4.3</td>
<td>1.9 (1–5)</td>
<td>0.08</td>
<td>Be, L</td>
</tr>
<tr>
<td><em>Paracuaria tridentata</em></td>
<td>l</td>
<td>1.0</td>
<td>3.5 (1–6)</td>
<td>0.03</td>
<td>Be, L</td>
</tr>
<tr>
<td><strong>Acanthocephala</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Corynosoma strumosum</em> (s.s.)</td>
<td>l</td>
<td>1.4</td>
<td>2.3 (1–3)</td>
<td>0.03</td>
<td>In</td>
</tr>
<tr>
<td><em>Echinorhynchus gadi</em> (s.s.)</td>
<td>a</td>
<td>3.6</td>
<td>2.3 (1–10)</td>
<td>0.38</td>
<td>In</td>
</tr>
<tr>
<td><em>Pomphorhynchus laevis</em></td>
<td>a</td>
<td>1.0</td>
<td>10.0 (1)</td>
<td>0.01</td>
<td>In</td>
</tr>
</tbody>
</table>

**Table III.** Local prevalence of selected parasite species which may act as biological indicators for stock discrimination or migration patterns of *Clupea harengus*. GOF, Gulf of Finland

<table>
<thead>
<tr>
<th>Parasite species/-taxa</th>
<th>Kiel Bay</th>
<th>Rügen</th>
<th>Poland</th>
<th>Lithuania</th>
<th>GOF Estonia</th>
<th>GOF Finland</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Digenea</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Brachyphallus crenatus</em></td>
<td>20.0</td>
<td>20.0</td>
<td>8.6</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Diplostomum spathaceum</em></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>17.1</td>
<td>71.4</td>
</tr>
<tr>
<td><em>Hemiurus luehei</em></td>
<td>68.6</td>
<td>37.1</td>
<td>2.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Nematoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Anisakis simplex</em> (s.s.)</td>
<td>14.3</td>
<td>2.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
Fig. 3. Shannon-Wiener diversity index (H), evenness index (E) and the number of parasite species at each sampling site and in total.

Table IV. Molecular analyses of nematode and acanthocephalan parasite species with the length and composition (base pairs) of the isolates. C, Cytosine; G, Guanine; KB, Kiel Bay; L, Lithuania; P, Poland; R, Rügen

<table>
<thead>
<tr>
<th>Species</th>
<th>Sampling site</th>
<th>Parts of 18S- sequence</th>
<th>ITS1</th>
<th>5.8S</th>
<th>ITS2</th>
<th>Parts of 28S- sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>length</td>
<td>G/C in %</td>
<td>length</td>
<td>G/C in %</td>
<td>length</td>
</tr>
<tr>
<td><strong>Nematoda</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anisakis simplex</td>
<td>KB</td>
<td>–</td>
<td>–</td>
<td>392</td>
<td>46.7</td>
<td>152</td>
</tr>
<tr>
<td>A. simplex</td>
<td>KB</td>
<td>–</td>
<td>–</td>
<td>392</td>
<td>46.7</td>
<td>157</td>
</tr>
<tr>
<td>A. simplex</td>
<td>KB</td>
<td>–</td>
<td>–</td>
<td>392</td>
<td>46.7</td>
<td>157</td>
</tr>
<tr>
<td>A. simplex</td>
<td>KB</td>
<td>–</td>
<td>–</td>
<td>392</td>
<td>46.7</td>
<td>157</td>
</tr>
<tr>
<td>Anisakis simplex</td>
<td>KB</td>
<td>–</td>
<td>–</td>
<td>394</td>
<td>47.0</td>
<td>157</td>
</tr>
<tr>
<td>A. simplex</td>
<td>KB</td>
<td>–</td>
<td>–</td>
<td>394</td>
<td>47.0</td>
<td>157</td>
</tr>
<tr>
<td>Echinorhynchus gadi</td>
<td>L</td>
<td>86</td>
<td>46.5</td>
<td>246</td>
<td>37.8</td>
<td>122</td>
</tr>
<tr>
<td>E. gadi</td>
<td>P</td>
<td>114</td>
<td>46.5</td>
<td>210</td>
<td>37.6</td>
<td>122</td>
</tr>
<tr>
<td>Contracaecum osculatum</td>
<td>P</td>
<td>151</td>
<td>52.3</td>
<td>454</td>
<td>45.6</td>
<td>157</td>
</tr>
<tr>
<td>C. osculatum</td>
<td>P</td>
<td>149</td>
<td>51.0</td>
<td>447</td>
<td>46.1</td>
<td>156</td>
</tr>
<tr>
<td>C. osculatum</td>
<td>P</td>
<td>–</td>
<td>–</td>
<td>448</td>
<td>46.0</td>
<td>156</td>
</tr>
</tbody>
</table>
strates complete mixing of the water body at the shallow western sites. The herring were caught in depths with sufficient O₂-saturations as illustrated in Fig. 2.

Parasite fauna and regional distribution

Fish parasitological studies of Clupea harengus from the Baltic Sea revealed 12 different metazoan parasite species, belonging to the taxa Digenea (five), Cestoda (one), Nematoda (three) and Acanthocephala (three). The information on prevalence, intensity, mean intensity, and mean abundance is summarized in Table II. In the western part, seven parasite species were found, whereas in the eastern parts, only three parasite species occurred. Most abundant were Hemiurus luehei, Diplostomum spathaceum (both Digenea) and Echinorhynchus gadi (Acanthocephala) in the intestine, with a prevalence above 10%. Most parasite species were not recorded from all over the Baltic Sea, but were found at distinct localities (see Table III). Metacercariae of Diplostomum spathaceum were abundant in the Gulf of Finland, infesting the eyes. In contrast, the digeneans Brachyphallus crenatus and Hemiurus luehei were present in the western part of the Baltic Sea, including Kiel Bay, Rügen and Poland. The distribution range of the nematode Anisakis simplex (s.s.) was limited to the Kiel Bay and off Rügen. Third stage larvae (L3) were found in the body cavity, but started to migrate into the musculature within a few hours after the death of their host.

Molecular analyses revealed the identity of the four parasite species Anisakis simplex (s.s.), Contracaecum osculatum (s.s.) (both Nematoda), Corynosoma strumosum and Echinorhynchus gadi (both Acanthocephala). The length (base pairs) of the single parts of the nuclear rDNA and the amount of Guanine and Cytosine is given in Table IV.

Ecological parameters

Differences in the diversity index (H’) and Evenness (E) as well as the number of isolated parasite species at each sampling site are shown in Fig. 3. The value of diversity index ranged between 0.47 and 1.70 and the value of Evenness ranged between 0.37 and 0.88. Of the 12 recorded fish parasites, 10 can be characterized as typical saltwater generalists with a wide range of common hosts. Two species are typical freshwater parasites, all of them are known as generalistic parasites.

Discussion

The distribution of the metazoan parasite species of Clupea harengus in the Baltic Sea was analysed in order to identify fish parasites as biological indicators in the Baltic Sea. By using a transect of six different sampling sites from the western to the eastern Baltic, 12 different metazoan parasite species could be isolated, four of them with a contrasting zoogeographical occurrence. The western localities at Kiel Bay, Rügen and Poland had the highest parasite diversity, with the predominant marine parasite species Anisakis simplex (s.s.), Brachyphallus crenatus and Hemiurus luehei. The eastern localities had low parasite species richness, dominated by the freshwater digenean Diplostomum spathaceum.

Parasite distribution

10 of the 12 recorded parasites species can be considered as mainly marine species, also being abundant in the North Sea or Atlantic Ocean. Of these, Anisakis simplex (s.s.) cannot complete its life cycle in the Baltic Sea. The larvae of this nematode are globally spread in cetaceans (Klimpel and Palm 2011), which serve as final hosts. The only cetacean inhabiting the Baltic Sea is the harbour porpoise, Phocoena phocoena. Anisakis simplex (s.s.) was reported in P. phocoena from the western Baltic Sea and the North Sea (Smith and Wootten 1978; Herreras et al. 1997; Siebert et al. 2001; Wunschmann et al. 2001). The distribution of P. phocoena is limited to the western Baltic Sea where only a few individuals live. In the north-eastern Baltic Sea, porpoises are considered to be virtually absent and in the Baltic proper, the population is critically endangered (Hammond et al. 2008). First obligate intermediate hosts of A. simplex are species of euphausiids (and less important copepods), with a distribution limited to a salinity above 20PSU (Köhn and Gosselck 1989; Szostakowska et al. 2005). Fishes like clupeids serve as second intermediate host. It can be assumed that the observed Anisakis larvae did not originate from the Baltic Sea but have been introduced with migrating herring from the western Baltic or the North Sea, as earlier suggested by Grabda (1974). This contrasts the other nematode, Contracaecum osculatum, a seal parasite from the North and Baltic Sea. Crustaceans and fishes serve as intermediate hosts, whereas calanoid copepods act as paratenic hosts, since they are ingested by small fishes including herring (Køie and Fagerholm 1995). This nematode has been recorded from the grey seal (Halichoerus grypus) as well as the copepods intermediate hosts have been recorded from different parts of the Baltic Sea. Consequently, this nematode might complete its entire life cycle at least within the western Baltic Sea.

The digeneans Brachyphallus crenatus, Hemiurus luehei and Lecithaster confusus are widespread generalistic species endemic to marine habitats (Køie 1992). In this study, the hemiurid species were distributed in the south-western Baltic (sampling site 1–3). According to Palm et al. (1999) 18 different fish species can serve as final hosts for B. crenatus. The first intermediate hosts of this digenean are marine gastropods, like Retusa obtusa and Rissoa sp. for B. crenatus, Philine denticulata for H. luehei and Odostomia sp. for L. confusus (Reimer 1970; Køie 1992; Zander and Reimer 2002). Zander and Reimer (2002) observed that the easternmost distribution range of R. obtusa in the Baltic was the Gotland Basin and the one of P. denticulata was the Bay of Mecklenburg. Thus, the
herring which were infected with *H. luehei* might have immigrated from more western areas to Rügen and Poland as the intermediate host is missing there. Metacercaria of the digenean parasite *Diplostomum spathacaeum* only occurred in the northernmost sampling sites (sampling site 5, 6), where the salinity was lowest (6.9 and 5.5PSU, resp.). The distribution limit is linked to the occurrence of their first intermediate host, the snail *Radix ovata* (*Zander et al. 2000*). *Zander and Reimer (2002)* reported a distribution range of *R. ovata* at a salinity below 8PSU in the Baltic Sea. With a prevalence of 71.4%, *D. spathacaeum* is a core species of herring from sampling site 6. This parasite was not found in the western parts of the Baltic Sea, which have a mean salinity over 8PSU. Metacercaria of *Cryptocotyle lingua* were found encysted in the skin and fins of herring from many sampling sites in the Baltic Sea (Sites 1, 2, 4, 6). First intermediate host is the snail *Littorina littorea* while fishes are second intermediate hosts. In the Baltic Sea, the snail *Hydrobia* spp. was integrated into the life cycle (*Zander 2002*) and areas with salinities down to 4PSU are sufficient. The marine acanthocephalan *Corynosoma strumosum*, *Echinorhynchus gadi* and *Pomphorhynchus laevis* are common generalists. Final hosts of *E. gadi* and *P. laevis* are fishes like Atlantic cod (*Gadus morhua*) for *E. gadi* and flounder (*Platichthys flesus*) for *P. laevis*. Seals serve as final hosts for *C. strumosum* (*Nickel et al. 2002, O’Neill and Whelan 2002*). Fishes are infected as the second intermediate hosts by preying upon amphipods. All of them are able to complete the entire life cycle within the Baltic Sea.

**Biological indicators**

According to Palm (2011), fish parasites can serve as a variety of different biological indicators. The first category is the use as a biological indicator for stock separation and migration patterns. The potential use of the different parasites depends on the infestation sites and the life cycles ecology. Digeneans are useful as biological indicators, but the infestation site (alimentary tract) and the short life span of many adults of less than one year limits their use (*Grabda 1974*). On the other hand, metacercariae in the fish might live longer. According to their natural range of distribution, the hemiurids as well as *Cryptocotyle lingua* characterise the western Baltic herring, while *Diplostomum spathacaeum* metacercariae occurred only in herring from freshwater dominated environments. Because the latter only was found in the eastern and the other species in the western Baltic nearly at the same time of the year, the respective herring stocks cannot have migrated in either region until that age. The herring from the Gulf of Finland represent a local, non migratory stock (smaller Baltic Herring, *C. harengus var. membras* L.). The explicit infestation site of the metacercariae of *D. spathacaeum* inside the fish eyes leads to a high stress tolerance of this species against shifting ecological parameters. Migrating fishes from the Gulf of Finland can be easily identified by having *D. spathacaeum* in the eyes because the parasite is sheltered against changing salinities. That fact demonstrates a high potential for its use as a biological indicator for *C. harengus var. membras*. Consequently, the recorded digeneans have the potential to serve as biological indicators for general as well as seasonal migrations patterns of herring stocks within the Baltic Sea. The dominance of generalistic parasites and planktonic species like hemiurid digeneans in general is indicating the pelagic feeding grounds as well as a high level of eutrophication in the Baltic Sea (*Zander and Reimer 2002*).

As exemplified in the present and earlier studies (e.g. Lang et al. 1990, Podolska et al. 2006), *Anisakis simplex* (s.s.) can be used as a biological tag for the western spring herring stock. According to our data, no other sibling species beside *A. simplex* (s.s.) was found. Weak infection rates for this species with a sharp gradient decreasing from west to east and the absence in the eastern parts prove the possible indicator function of *A. simplex*. All of the infected herrings seem to have migrated for spawning from the feeding grounds in the western parts of the Baltic like the Kattegat, Skagerrak or the North Sea. Thus, the stock of the spring-spawning western herring may interact and overlap with herring stocks in the North Sea. The Digenea *Hemirura luehei* and *Brachyphallus crenatus* reveal the same distribution patterns as *A. simplex* and they can be used for stock distinction and migration markers as well. One major key to determine the geographical range of helminth parasites is the occurrence of intermediate and final hosts, which are included into their life cycles (MacKenzie and Abaunza 2005). Hence, the completion of a parasite life cycle is only possible in areas where all required hosts are present (Hemmingsen and MacKenzie 2013).

The present study describes the situation within a time frame of two weeks, not allowing statements concerning seasonal or annual variation. However, *Anisakis simplex* (Nematoda) and *Diplostomum spathaceum* (Digenea) can survive a long time period in their intermediate hosts, increasing their potential as indicators for stock identification and discrimination. According to our parasitological results, we could identify the 3 different Baltic herring stocks, the western spring-spawning *C. harengus* reaching until the Polish coast, the stock of the Baltic proper and the northern stock of *C. harengus var. membras* of the Gulf of Finland. This concurs with the results by using other methodologies, such as meristic measurements like vertebrae counts (e.g. Gröger and Gröhsler 2001), pectoral finray counts (Dutt 1958), gillraker counts (Popiel 1958) or genic analyses of mitochondrial (e.g. Rajasilta et al. 2006), microsatellite DNA (e.g. Bekkevold et al. 2005) or allele frequencies (Ryman et al. 1984), analyses of otolith size and shape (e.g. Campana and Casselman 1993) and chemical analyses of fatty acids (e.g. Grahl-Nielsen and Ulvund 1990) or enzymes (e.g. King et al. 1987). According to Palm (2011), fish parasites can be also used as accumulation indicators, for regional environmental or possibly also global change. The acanthocephalan *P. laevis* accumulates heavy metals at concentrations many hundred to a thousand times higher than their hosts tissue (*Nachev et al. 2013*), and this parasite was recorded...
for both western stocks. Although this species was only rare within the present sampling, both western herring stocks can be compared according to their heavy metal contents, with possible interpretations for the environmental conditions and food safety. The occurrence of Corynosoma strumosum, Paracuaria tridentata and Proteocephalus sp. in total was too low to reach any statistical relevance. According to the observed ecological parameters, both Evenness (E) and diversity index (H') reached their highest values at the sampling site off Poland (E = 0.88; H' = 1.70), indicating a moderate number of different parasite species with an approximate even distribution. In contrast, in the waters off Finland, lower values of the Shannon-Wiener-index (0.47) and Evenness (0.43) display a parasite fauna which was dominated by a single digenean species (D. spathaceum). Similar conditions were found in Kiel Bay, where H. luehei was the predominating parasite species. Because the sampled herrings belonged to 3 different stocks (above) and their parasite fauna consisted of only few and generalistic parasite species, interpretations based on the observed ecological parameters concerning regional differences or possibly seasonal change in abundances are difficult.

Conclusion

Mainly generalistic parasites were recorded in the present study, that are able to cope with the environmental characteristics of the Baltic Sea. The changing salinity gradient and restrictions to complete the entire life cycles throughout the Baltic Sea replaces the respective parasite species from west to east, allowing herring stock discrimination and identification of migration patterns. The recorded acanthocephalan Pomphorhynchus laevis can function as an accumulation indicator, however, only for the western stocks. Because we did not find any specialized parasites and the observed ecological parameters and predominance of few generalist parasite species demonstrate a typical situation under harsh environmental conditions, detection of regional environmental change by using herring parasites seems to be difficult. Similarly, the young history of the Baltic Sea and a large temperature change throughout the year, coinciding with a wide distribution range of the observed generalists, indicate that the suitability of herring parasites as indicators of global warming in the Baltic Sea is limited.

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