MICROHABITAT PREFERENCE AND RELATIONSHIPS BETWEEN METAZOAN PARASITES ON THE GILL APPARATUS OF THE EUROPEAN EEL (ANGUILLA ANGUILLA) FROM FRESHWATERS OF LATVIA

Maksims Zolovs, Gunita Deksne, Janīna Daukšte, Jānis Aizups, Muza Kirjušina


Although fish gills represent a relatively homogenous habitat, parasites show preference to a certain area to attach. Fish gills are inhabited by different parasite groups which may potentially induce interspecific competition in limited space habitat. Parasite spatial distribution within the gill apparatus also is influenced by parasite intraspecific relationships. We investigated the spatial distribution of metazoan ectoparasites within the gill apparatus of eel from several freshwater bodies of Latvia from October 2013 to November 2014. The spatial distribution within gill apparatus was analyzed by Savage index. We determined parasite niche breadth and overlap and calculated parasite intra- and interspecific relationships. The obtained results indicate that pseudodactylogyrids are aggregated and show preference to a certain area of the gill apparatus while glochidia Anodonta sp. and copepoda Ergasilus sieboldi are randomly distributed. Intraspecific relationships influenced notably the microhabitat preference of Pseudodactylogyrus spp.

Key words: microhabitat, gills, intra/interspecific relationships, European eel, Pseudodactylogyrus sp..

INTRODUCTION

Many parasite species specialize to the host, organ and tissue level where they prefer a specific site to localize. The fish gills are one of the most functional and inhabited organ by different parasites groups (Gussev 1985). Previous ecological studies of gills parasites focus mainly on one parasite group with congener taxons (Paperna 1964, Buchmann 1988, Dzika 1999, Gutierrez & Martorelli 1999, Kadlec et al. 2003, Koskivaara et al. 2009) while other parasites species co-occur on the same host. For example, ecological studies of monogeneans focus on relationships of congener taxons. The limited data are available for monogeneans interaction
with other parasites taxons (Ramasamy et al. 1985). Surveys of ectoparasites microhabitat on the European eel’s gill apparatus were conducted mainly on pseudodactylogyrids where parasite preference to a certain site of the gill apparatus was analyzed considering fish size and seasons (Buchmann 1989, Dzika 1999, Matejusová et al. 2003). Monogeneans are an appropriate parasite group for investigating the adaptation of an organism, their morphological changes and relationship between organisms, as monogeneans have a direct life cycle, many species are narrowly host specific within species, genus or family and can occupy restricted specific sites (microhabitat) within their host (Bychowsky 1957). Thus, ecologists and evolutionist may use them as a model organism to study parasite relationships, adaptation to the environment, evolution etc. The aim of this study was to investigate aggregation, microhabitat and relationships of eel’s gills parasites.

MATERIAL AND METHODS

A total of 75 eels were collected from October 2013 to November 2014 in autumn (n=33) and spring (n=42). The eels were collected by traps from six freshwaters sample sites of Latvia and transferred to the laboratory alive in the water tanks. After euthanasia of fish (Algers et al. 2009) by spinal cord incision the gill apparatus was dissected and investigated from both sides. Arches were divided and numbered from anterior to the posterior end (1–4). Each arch was divided into three segments from dorsal to ventral end (I–III) (Fig. 1). The first left side arch from each eel was collected for histological studies within the project framework. Thus, 21 separate areas were used to record all gill ectoparasites. The absence of one gill arch in the gill apparatus was taken into account when processing statistical data analysis.

Each parasite individual was removed from gills with needles and a species was determined after morphological characteristics (Gussev 1985, Bauer 1987). No significant difference has been found between the number of individuals in each parasite species on the left and right sides of the gill arches (one-way ANOVA p>0.05). Therefore, we merged together obtained data of parasite microhabitat from both sides to calculate parasites preference to certain gill area and relationships.

Parasite prevalence, intensity of infection and abundance were calculated according to Bush et al. (1997). In the case of *Myxidium giardi* and *Trichodina* sp., we determined only its presence/absence and made no attempt to estimate intensity or abundance.

According to recorded distribution within the areas of the gill apparatus, we analyzed parasite preference or avoidance to a certain gill area, parasite niche breadth and overlap, parasite intra- and interspecific relationships. Parasite species preference to a certain area of the gill apparatus was calculated as proportion of parasite individual records in each area, the habitat selection was assessed by Savage index (Savage 1931):

\[
W_i = \frac{o_i}{p_i}
\]

where \(o_i\) is a proportion of parasite species in the gill area \(i\); and \(p_i\) – proportion of gill areas \(i\) available in the gill apparatus from one fish side.

We assumed that all the investigated gill areas are equal in their size for parasite attachment. Habitat selection indices above 1.0 indicate preference to a certain area of the gill apparatus; values less than 1.0 indicate avoidance. Habitat selection indices may range from 0 to \(\infty\). Thus, it was standardized on a scale of 0 to 1 with (Manly et al. 1993):

\[
W_s = \frac{w_i}{\sum w_i}
\]

where \(w_i\) is habitat selection index. Standardized ratios of \(\left(\frac{1}{\text{number of habitats}}\right)\) indicate no preference to a certain area of the gill apparatus. Values below \(\left(\frac{1}{\text{number of habitats}}\right)\) indicate relative avoidance; values above indicate relative preference. The Chi-square test of homogeneity was conducted to test hypothesis that parasites select their habitats randomly.
Parasite intraspecific interaction was analyzed by Levin’s niche breadth and intraspecific aggregation indexes. Niche breadth was calculated as follow (Colwell & Futuyma 1971):

$$B = \frac{1}{\sum(p_j^2)}$$

where $p_j$ is a proportion of a parasite species found on area of the gill apparatus (on one side of fish). Index was standardized on a scale of 0 to 1 with:

$$B_s = \frac{B - 1}{n - 1}$$

where $B$ is niche breadth and $n$ – a total number of investigated areas of the gill apparatus on one side of fish. Intraspecific aggregation was calculated according to Ives (1988):

$$I_r = \frac{V_r}{m_r} - 1$$

where $m_r$ is a mean number of parasite species $r$ individuals per gill area, $V_r$ is a variance in number of parasite species $r$. Intraspecific aggregation index value of $J=0$ indicates that parasites are randomly distributed within gill area. Values above indicate aggregated (clumped) and values below – regular (uniform) parasite distribution within gill area.

Parasite interspecific interaction was analyzed by parasite niche overlap and interspecific aggregation indexes. Niche overlap was assessed by Renkonen index (Renkonen 1938):

$$R = 1 - \frac{\sum | p_{ia} - p_{ja} |}{2}$$

where $p_{ia}$ is proportion of individuals of parasite species $i$ on the area $a$ and $p_{ja}$ – proportion of species $j$ on the area $a$. Interspecific aggregation was calculated according to Ives (1988):

$$C_{rn} = \frac{Cov_{rn}}{m_r m_n}$$

where $Cov$ is a covariance between a pair of parasite species $r$ and $n$. Values above indicate that two parasite species are positively associated; values below – negatively associated and the value $C_{rn}=0$ means parasites are randomly distributed within gill area.

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Fig. 1. The gill apparatus and sectors of gill arch.
RESULTS

We recorded parasite species that belong to the four systematic groups: protists *Trichodina* spp. and *Myxidium giardi*, monogeneans *Pseudodactylogyrus anguillae* and *P. bini*, molluscs *Anodonta* sp. glochidia and copepods *Ergasilus sieboldi*. The prevalence of *Trichodina* spp. and *M. giardi* were 1% and 8%, respectively. We did not analyze protists preference or avoidance to a certain gill area, parasite niche breadth and overlap, parasite intra- and interspecific relationships. Infection parameters of metazoan parasites: mean abundance and intensity of infection, prevalence and total niche breadth of parasites in gill apparatus of European eel *Anguilla anguilla* from freshwater bodies of Latvia

### Table 1. The mean abundance, intensity, prevalence of infection, and total niche breadth of parasites in gill apparatus of European eel *Anguilla anguilla* from freshwater bodies of Latvia

<table>
<thead>
<tr>
<th>Parasite species</th>
<th>Prevalence, %</th>
<th>Abundance ± SD</th>
<th>Intensity of infection ± SD</th>
<th>Total niche breadth ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Pseudodactylogyrus bini</em></td>
<td>49</td>
<td>18.67±33.03</td>
<td>39.03±38.71</td>
<td>0.44±0.23</td>
</tr>
<tr>
<td><em>Pseudodactylogyrus anguillae</em></td>
<td>55</td>
<td>9.49±37.10</td>
<td>15.98±47.27</td>
<td>0.16±0.13</td>
</tr>
<tr>
<td><em>Anodonta</em> sp.</td>
<td>11</td>
<td>0.39±1.54</td>
<td>3.37±3.37</td>
<td>0.14±0.13</td>
</tr>
<tr>
<td><em>Ergasilus sieboldi</em></td>
<td>22</td>
<td>0.64±1.50</td>
<td>2.75±1.98</td>
<td>0.05±0.05</td>
</tr>
</tbody>
</table>

### Table 2. The habitat selection index of *Pseudodactylogyrus anguillae* and *Pseudodactylogyrus bini* in gill apparatus of European eel *Anguilla anguilla*

<table>
<thead>
<tr>
<th>Parasite species</th>
<th><em>P. anguillae</em></th>
<th><em>P. bini</em></th>
<th><em>P. anguillae</em></th>
<th><em>P. bini</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>References</td>
<td>Present investigation</td>
<td>Dzika 1999*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Arches</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>0.78</td>
<td>0.65</td>
<td>0.83</td>
<td>0.07</td>
</tr>
<tr>
<td>2</td>
<td>1.09</td>
<td>0.09</td>
<td>1.25</td>
<td>0.1</td>
</tr>
<tr>
<td>3</td>
<td>1.22</td>
<td>0.1</td>
<td>1.21</td>
<td>0.1</td>
</tr>
<tr>
<td>4</td>
<td>0.92</td>
<td>0.08</td>
<td>0.71</td>
<td>0.06</td>
</tr>
<tr>
<td>Segments</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>2.29</td>
<td>0.19</td>
<td>1.23</td>
<td>0.1</td>
</tr>
<tr>
<td>II</td>
<td>0.52</td>
<td>0.04</td>
<td>1.04</td>
<td>0.09</td>
</tr>
<tr>
<td>III</td>
<td>0.19</td>
<td>0.02</td>
<td>0.73</td>
<td>0.06</td>
</tr>
</tbody>
</table>

(*number of habitats) = 0.083

The relative strength of intraspecific versus interspecific aggregation in a pair of species *r* and *n* was assessed with (Ives 1991):

$$A_{rn} = \frac{(r_n + 1)(n_r + 1)}{(r_n + 1)^2}$$

where values above 1 indicate that intraspecific aggregation is stronger than interspecific aggregation and values below – interspecific aggregation is stronger than intraspecific aggregation. The value *A*<sub><sup>rn</sup></sub>=1 indicate that intraspecific and interspecific aggregation is equal.

Differences among values were tested by the one-way ANOVA if data were normally distributed and were homogenous. Statistical significance for all tests was assessed at 5%. Statistical tests were carried out by SPSS Statistics Version 21 (IBM Corporation, Chicago, Illinois).
Microhabitat preference and relationships between metazoan parasites on the gill apparatus of the European eel.

The mean interspecific aggregation index of congeneric *Pseudodactylogyrus* species was $C=4.79$. Parasites associated more positively on the first and second segments and tended to independent distribution on the third segment. The pair of *P. bini* & *Anodonta* sp. showed weak positive interspecific aggregation for the whole gill apparatus ($C=0.09$). Parasites independently distributed on anterior arches (first, second and third) and on both the first and third segments. The positive parasite association increases on the fourth arch ($C=0.44$) and the third segment ($C=0.33$). Also, the pair of *P. bini* & *E. sieboldi* showed weak positive interspecific aggregation for the whole gill apparatus ($C=0.05$). The highest index value was for the first arches ($C=0.27$) and third segments ($C=0.24$). Parasites on other gill areas tend to independent distribution. The relative strength of intraspecific aggregation on interspecific aggregation was $3.49$, $4.73$ and $5.83$ for the pairs *P. bini* & *Anodonta* sp., *P. bini* & *E. sieboldi*.

*Pseudodactylogyrus bini* was the most abundant species that preferred the second and third gill arches and occupied mostly the first and second segments ($\chi^2=98.96$, df=11, $p<0.001$) (Fig. 2A and Table 2). The intensity of infection gradually decreased from the first to the third segments. Due to high intensity of infection and distribution pattern, *P. bini* show high degree of niche overlap: with *P. anguillae* 47%, with *Anodonta* sp. 31% and with *E. sieboldi* 28%. The mean value of $J=1.24$ of the whole gill apparatus indicate that *P. bini* had aggregated distribution. The index of intraspecific aggregation varied slightly between gill arches and segments.

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**Fig. 2.** The metazoan parasite prevalence in different gill area of European eel *Anguilla anguilla* from freshwater bodies of Latvia. A – *Pseudodactylogyrus bini*; B – *Pseudodactylogyrus anguillae*; C – *Ergasilus sieboldi*; D – *Anodonta* sp.
The gill apparatus demonstrates that monogeneans were more aggregated than molluscs and copepods. Although both pseudodactylogyrids preferred the first segments and the second and third arches, \textit{P. anguillae} showed stronger specificity to a certain site of the gill apparatus than \textit{P. bini}. For example, we recorded 74\% of \textit{P. anguillae} specimens on the first sectors versus 47\% of \textit{P. bini}, and niche breadth of \textit{P. anguillae} was twice shorter (Fig. 3 and Tables 1, 2). It means that \textit{P. anguillae} have a clearly defined microhabitat while \textit{P. bini} tends to occupy the entire gill apparatus.

The findings of \textit{P. anguillae} microhabitat partly coincide with several researches (Rodrigues & Saraiva 1996, Dzika 1999, Soylu et al. 2013) where parasites inhabit the second, third and fourth arches and occupy the first segment, and do not coincide with Buchmann data (Buchmann 1988, Buchmann 1989) where monogeneans inhabit two posterior arches. According to Matejusová et al. (2003) research data, \textit{P. anguillae} had not preference for gill arches in any of the seasons studied, and occupied the first segments only in summer.

The obtained data of \textit{P. bini} microhabitat greatly vary between studies (Buchmann 1988, Buchmann 1989, Rodrigues & Saraiva 1996, Dzika 1999, Matejusová et al. 2003). Dzika (1999) documented that monogeneans mainly occupy the three anterior arches but Matejusová et al. (2003) noted that \textit{P. bini} preferred only the second arches and – in summer season – the third segment. Also previous investigations of Buchmann reveal differences of \textit{P. bini} microhabitat considering fish size where parasites selected the first and second arches on small eels or the second and third arches on large fishes (Buchmann 1988, Buchmann 1989). According to Rodrigues and Saraiva (1996), \textit{P. bini} occupy two posterior arches and the third segment. The authors reported bilaterally asymmetrical distribution by recording preference of right side of eel’s gills for both \textit{P. anguillae} and \textit{P. bini} species.
The discrepancy between microhabitat preferences of pseudodactylogyrids could be caused by geographical disparities of investigated water bodies (Buchmann 1988, Buchmann 1989, Rodrigues & Saraiva 1996, Dzika 1999, Matejusová et al. 2003, Soylu et al. 2013). Probably a significant variation of abiotic conditions caused by geographical remoteness determines the monogenean microhabitat preference. However, Latvia is a relatively small country with many freshwater bodies with similar eutrophication level. At least 40% of national country territory is accessible for eel’s upstream and downstream migration within freshwaters as well as within sea and freshwaters (Latvia Eel Stock Management statement 2014 - 2020).

One of the first proposed factors that may influence the spatial distribution of parasites within gill arches were the water flow (Llewellyn 1956, Wootten 1974) and the area of arches that allowed parasite to attach to a fish (Wootten 1974, Buchmann 1989). Thus, the parasite preference to a certain microhabitat could be influenced by all these factors (Gutiérrez & Martorelli 1999). However, Dmitrieva (2000) suggests that the water flow influences parasite distribution only among gill arches, the distribution among gill segments is determined by intraspecific relationships of parasites. Buchmann (1989) explained the differences in preference of *P. anguillae* and *P. bini* to occupy a specific site of the gill apparatus by the morphology of parasite hamuli and parasite mobility where *P. bini* is more successful to occupy the entire gill apparatus while *P. anguillae* would be forced to attach to a specific gill site. To minimize hybridization, parasites select different microhabitats or tend to have different reproductive organs or attachment apparatus (Rohde 1977, Rohde 1994, Jarkovský et al. 2004). Congeners *P. anguillae* and *P. bini* have similar shape and size of reproductive organs but different attachment apparatus that induces spatial segregation of pseudodactylogyrids.

Our results show that pseudodactylogyrids had an aggregated distribution while copepods *E. sieboldi* and molluscs *Anodonta* sp. distributed randomly. Intraspecific aggregation is one of the strategies that increase the survival of various organisms by benefiting from several mechanisms. The predation, reproduction, feeding and competition seem to be the main beneficial mechanisms (Parrish & Edelstein-Keshet 1999). As the distribution among gill arches is influenced by various abiotic factors, the aggregation within one arch presumably may be affected by a complex of biotic factors with a single dominant one. Although monogeneans are hermaphroditic animals that are capable of self-fertilization, the cross-fertilization is more beneficial for parasites due to a greater range of gene variability for natural selection (Bychowsky 1957). Thus, the reproduction of monogeneans could contribute to intraspecific aggregation opposite to the copepods and mollusks that fertilize outside the host (Bauer 1987, Abdelhalim et al. 1991) and do not need to migrate for mating. Also, the infection of fish by copepods and molluscs has a seasonal pattern (Bauer 1987).

Usually one parasite species shares the host with another parasite species and induces interspecific competition in a limited space habitat. In the present investigation, we did not obtain the interspecific competition among parasites. This finding has been confirmed by several other studies. After investigation of metazoan ectoparasites from marine fishes, Rohde et al. (1995) concluded that the interspecific aggregation is rare. Morand et al. (1999) have investigated the ectoparasites of marine fishes from several localities and found that interspecific aggregation is reduced as compared to intraspecific aggregation. The analysis of dactylogyrids coexistence in roach also coincides with our findings (Simková et al. 2000).

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