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Microhabitat Distribution of *Pseudodactylogyrus anguillae* (Monogenea), *Ergasilus gibbus* and *Ergasilus lizae* (Copepoda) on the Gills of European Eels (*Anguilla anguilla*, L.)

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Abstract: Microhabitat distribution of *Pseudodactylogyrus anguillae* (Yin & Sproston, 1948) Gussev, 1965 (Monogenea: Dactylogyridae), *Ergasilus gibbus* von Nordman, 1832 and *Ergasilus lizae* Kroyer, 1863 (Copepoda: Ergasilidae) was studied on the gills of European eel *Anguilla anguilla* from Lake Köyceğiz-Dalyan, Estuarine Channel System, Turkey. A total of 69 *A. anguilla* were examined between December 2009 and March 2010. A total of 1421 *P. anguillae*, 143 *E. gibbus* and 63 *E. lizae* specimens were collected from the fish host. A prevalence of 81.15% and mean infection intensity of 25.16 for *P. anguillae*, 50.72%, 4.0 for *E. gibbus* and 27.53%, 3.31 for *E. lizae* were found. Gill arches II, III, IV were preferred by *P. anguillae*, gill arches I, II, III by *E. gibbus* and gill arches III, IV by *E. lizae.* In general occurrence of the parasite species, *P. anguillae* preferred proximal-dorsal segments, *E. gibbus* distal-dorsal segments, whereas *E. lizae* exhibited a rather homogenous distribution. Site of attachment was the inner surface of the gill arches for *E. gibbus* and the outer surface for *E. lizae. P. anguillae* was found mostly on the inner surface of the hemibranches. Bispecific infections of *P. anguillae* with *E. gibbus* and *E. lizae* were also analysed individually. Finally, single species infections of the three parasite species were analysed.

Key words: *gill parasite, microhabitat, Monogenea, Copepoda, fish host, ectoparasites*.

Introduction

The monogenean gill parasites *P. anguillae* and *P. bini* are known to occur on the gills of the Asian eel *Anguilla japonica* and the Australian eel *Anguilla reinharditi*. *P. bini* was first reported in 1929 by Kikuchi in Japan and *P. bini* and *P. anguillae* were reported in 1948 by Yin & Sproston 1948 in China. *P. anguillae* and *P. bini* are rather pathogenic to *Anguilla* spp. and can cause mortality in heavily infected fish (Gelnar *et al*. 1996). Both these monogeneans were apparently introduced via the Japanese eel *A. japonica* and were first reported in *Anguilla anguilla in* the Soviet Union (Golovin 1977). They have been reported on the gills of both wild and cultured eels from Hungary (Molnar 1984), France (Lambert *et al*. 1985), Italy (Saroglia *et al*. 1985), Denmark (Buchmann *et al*. 1987) and Poland (Dzika *et al*. 1995). Cone, Marcogliese (1995) were the first to record *P. anguillae* on the gills of the American eel *Anguilla rostrata* in North America. *A. anguilla* is an economically valuable fish species in Turkey. However it is not farmed, all harvesting is done by fishing. The number of studies on the parasites of *A. anguilla* in Turkey is limited; Altunel (1990) investigated the parasite fauna in *A. anguilla* in the Ekinli Lagoon, Genç *et al*. (2005) studied the occurrence of the swimbladder parasite *Anguillicola crassus* in the same fish host in the Ceyhan River.

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The coexistence of gill monogeneans has been studied widely (El-Hafidi 1998, Dzika 1989, Koskivaa *et al.* 1991, Simkova *et al.* 2000, Turgut *et al.* 2006). Numerous studies have investigated the spatial distribution and microhabitats selection of the monogenean gill parasites of *Anguilla anguilla* (Buchmann 1989; Rodrigues, Saraiva 1996, Dzika 1999, Matejusova *et al.* 2003). However, only a few studies have investigated co-occurrent copepods and monogeneans (Ramasamy *et al.* 1985, Baker, Cone 2000, Baker *et al.* 2005). The present study investigated the microhabitat distribution of the monogenean *Pseudodactylogyrus anguillae* in co-occurrence with the copepods *Ergasilus gibbus* and *Ergasilus lizae* on the gills of the European eel *Anguilla anguilla*.

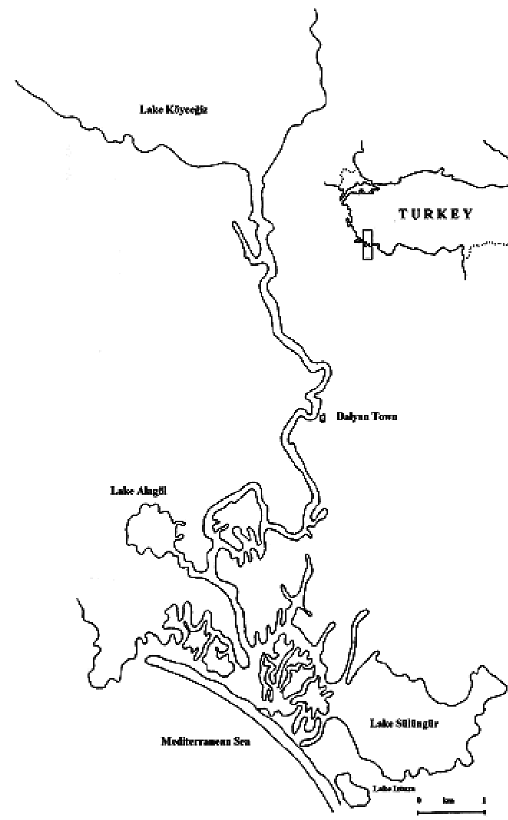

Fig. 1. Map of the study area.

Materials and Methods

The Köyceğiz-Dalyan Nature Reserve is an important wetland area in South-western Turkey (36° 45' and 37° 15' N, 28° 22' 30" and 28° 52' 30" E). The outflow of Lake Köyceğiz, the Dalyan River enlarges into a labyrinth-like channel system, discharging into the Mediterranean Sea (Fig. 1). The estuarine area includes three lakes (Alagöl, Sülüngür and Sülüklü). The Dalyan channel system is fed by Lake Köyceğiz.

The Mediterranean Sea has many sulphuric thermal springs located both on the bottom and around it. The Köyceğiz-Dalyan estuarine channel system is 14 km in length and connects the meromictic Lake Köyceğiz and the Mediterranean Sea. The Dalyan water mass consists of a mixohaline upper layer from Lake Köyceğiz and a lower layer of the Mediterranean Sea saline water mixed with the sulphuric thermal spring water (Kazanci *et al.* 2003).

A total of 69 *Anguilla anguilla* of mean (± SD) total length 52.5 ±10.24 cm (range 33.2-78.0 cm) and mean (± SD) weight 312.3 ±199.99 g (range 67.7-906.0 g) were examined between December 2009 and March 2010. Fish were caught by local fishermen and transferred alive to the laboratory in aerated lake water. After each eel was sacrificed all branchial arches from the left and the right sides were removed and placed in 4% formalin solution for further studies. Each gill arch was placed in a separate Petri dish filled with water, when examined. The gill arches were numbered I to IV from anterior to posterior. Each arch was divided into three gill segments: dorsal, medial and ventral; two gill areas: proximal and distal; two gill surfaces: outer and inner; two gill hemibranches: anterior and posterior, as a niche for parasites (Fig. 2). All monogenean and copepod parasites on the gills were collected one by one from each sector separately under a stereomicroscope at 20X magnification and the exact location of the parasites was recorded before removal. The monogenean parasites were cleared in glycerine or lactophenol and identified on the basis of their chitinous elements according to Pugachev *et al.* (2010). Copepod parasites were roughly sorted into two groups on the basis of their second antenna, and preserved in 70% ethanol for further identification on the basis of the keys of Bauer (1987).

Kruskal-Wallis tests were used to test the significance of the differences in the number of parasites between the dorsal, medial and ventral segments. The differences in the parasite numbers between the proximal and distal parts, left and right sides, and gill arches were tested using the Mann Whitney *U*-test. Differences of $P < 0.05$ were considered significant.

Results

A total of 69 *Anguilla anguilla* were examined, 4 of which (5.8%) were not infected at all, 56 (81%)

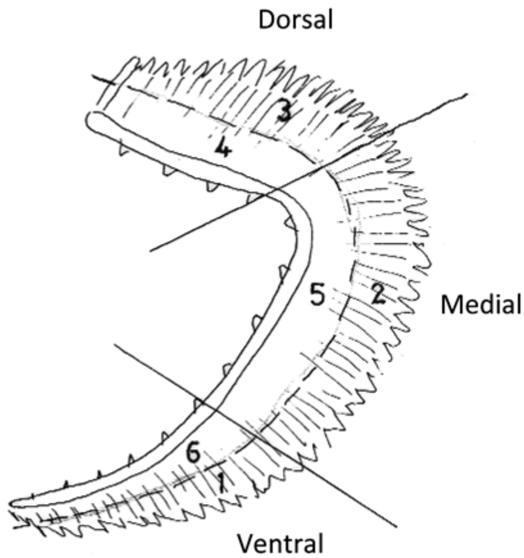

Fig. 2. Division of branchial arch (1-2-3 Distal part, 4-5-6 Proximal part).

were infected by *Pseudodactylogyrus anguillae*, 34 (49%) by *Ergasilus gibbus* and 19 (28%) by *E. lizae*. In only 5 of the infected fish were there simultaneous infections of *E. gibbus* and *E. lizae* with *P. anguillae*. A total of 1421 *P. anguillae*, 143 *E. gibbus* and 63 *E. lizae* individuals were recorded in general occurrence. The overall prevalence, mean intensity and mean abundance for *P. anguillae* were found to be 81.2%, 25.4, 20.6, for *E. gibbus* 49.3%, 4.2, 2.1 and for *E. lizae* 27.5%, 3.3, 0.9 respectively. The infection intensities (per eel) of *P. anguillae*, *E. gibbus* and *E. lizae* respectively ranged between 1-202, 1-41 and 1-14 individuals.

General occurrence of the parasites

Branchial distribution of *P. anguillae*, *E. gibbus* and *E. lizae* on the gills of *Anguilla anguilla* was examined. Of the 69 dissected fish, 56 were infected with *Pseudodactylogyrus anguillae* (prevalence 81.2%). The distribution of 1421 *P. anguillae* in general occurrence is shown (Table 1). The differences were not found to be significant between the number of *P. anguillae* on the left and right gill arches (P=0.861 > 0.05). Gill arches II, III and IV were preferred over I. The differences were significant between the numbers of *P. anguillae* found on the segments (P=0.00 < 0.05), *P. anguillae* preferred dorsal segments (84.1%), namely 55.9% of the *P. anguillae* settled in sector 4 and then 28.2% in sector 3. There were no statistically significant differences in the number of the parasites between the proximal and distal parts (P=0.225 > 0.05), 64.9% of *P. anguillae* were recorded on the proximal part, 80.0% of *P. anguillae* preferred the inner surface, 52.6% settled in the anterior hemibraches.

Of the 69 examined fish, 34 were infected with *Ergasilus gibbus* (prevalence 49.3%). A total of 143 *E. gibbus* were recorded. *E. gibbus* did not show preference for the left or right side of the gill (P

Table 1. General occurrence of *Pseudodactylogyrus anguillae*, *Ergasilus gibbus* and *Ergasilus lizae* on the gills of *Anguilla anguilla*.

	P. anguillae		***E. gibbus***		***E. lizae***	
Number of infected eels	56		34		19	
Mean intensity	25.4		4.2		3.3	
	Number	%	Number	%	Number	%
Left side	733	51.6	78	54.5	35	55.6
Right side	688	48.4	65	45.5	28	44.4
Gill arch I	224	15.8	43	30.0	2	3.2
Gill arch II	425	29.9	36	25.2	7	11.1
Gill arch III	407	28.6	40	28.0	25	39.7
Gill arch IV	365	25.7	24	16.8	29	46.0
Dorsal segment	1195	84.1	94	65.7	22	34.9
Medial segment	192	13.5	41	28.7	26	41.3
Ventral segment	34	2.4	8	5.6	15	23.8
Proximal part	922	64.9	29	20.3	31	49.2
Distal part	499	35.1	114	79.7	32	50.8
Anterior hemibranch	748	52.6	75	52.4	37	58.7
Posterior hemibranch	673	47.4	68	47.6	26	41.3
Inner surface	1137	80.0	136	95.1	3	4.8
Outer surface	284	20.0	7	4.9	60	95.2

=0.341 > 0.05), *E. gibbus* preferred gill arches I, II and III. A significantly greater number of *E. gibbus* occurred on the dorsal segment of the gills (65.7%) than on the medial and ventral segments (P=0.00 < 0.05). *E. gibbus* preferred distal part of the gill arches (79.7%) and significant differences were found with the proximal part (P=0.001 < 0.05). *E. gibbus* did not show preference for anterior or posterior hemibranches, and 95.1% of the parasites occupied the inner surface of the gill hemibranches.

Of the 69 examined fish, 19 were infected with *Ergasilus lizae* (prevalence 27.5%). A total of 63 *E. lizae* were recorded. *E. lizae* did not show preference for the left or right side of the gills (P=0.283 > 0.05), gill arches IV and III were preferably infected, differences between the number of *E. lizae* on gill arches IV-III and I-II were found to be significant (P=0.00 < 0.05). But distribution of *E. lizae* both on dorsal, medial, ventral segments and proximal, distal parts was found rather homogenous. *E. lizae* preferred outer surface (95.2%) of the hemibranches. *E. lizae* did not show preference for anterior or posterior hemibranches and the left or right side.

Bispecific infections

Bispecific infections of *A. anguilla* were examined with *P. anguillae – E. gibbus* and *P. anguillae – E. lizae* combinations. Of the 69 eels examined, 24 were infected with only *P. anguillae – E. gibbus* (prevalence 34.8%). In these eels, 849 *P. anguillae* and 133 *E. gibbus* were recorded. *P. anguillae* preferred gill arches II, III and IV, dorsal segments (86.0%), and proximal part (60.7%). *E. gibbus* preferred gill arches I and II, dorsal segments (64.7%), distal part (77.4%) and inner surface (94.0%) of the hemibranches (Table 2).

Of the 69 eels examined, 10 were infected with only *P. anguillae – E. lizae* (prevalence 14.5%). In these eels, 175 *P. anguillae* and 44 *E. lizae* were recorded. *P. anguillae* did not show a right or left side preference. *P. anguillae* preferred gill arches II and III, dorsal segment (84.0%), proximal part (71.4%) and inner surface (88.6%). In these bispecific infections, *E. lizae* preferred the left side (prevalence 61.4%). *E. lizae* preferred gill arches IV and III, dorsal (40.9%) and medial (36.4%) segments. *E. lizae* were evenly distributed over both proximal and distal parts, and they all (100%) were found on the outer surface of the hemibranches (Table 2).

Monospecific infections

A total of 17 eels (prevalence 24.6%) were infected with only *P. anguillae*, 5 eels (7.2%) with only *E. gibbus* and 4 eels (5.8%) with only *E. lizae.*

A total of 397 *P. anguillae* were recorded in these monospecific infections. *P. anguillae* settled in gill arches II, III and IV more frequently. The species predominantly occurred on dorsal segments (79.1%), significant differences were found between

Table 2. Distribution of *Pseudodactylogyrus anguillae – Ergasilus gibbus* and *Pseudodactylogyrus anguillae – Ergasilus lizae* on the gills of *Anguilla anguilla* in bispecific infections.

	P. anguillae		***E. gibbus***		***P. anguillae***		***E.lizae***	
Number of infected eels	24		24		10		10	
Mean intensity	35.4		5.5		17.5		4.4	
	Number	**%**	Number	**%**	Number	**%**	Number	**%**
Left side	444	52.3	74	55.6	98	56.0	27	61.4
Right side	405	47.7	59	44.4	77	44.0	17	38.6
Gill arch I	127	15.0	42	31.6	33	18.9	1	2.2
Gill arch II	259	30.5	43	32.3	55	31.4	5	11.4
Gill arch III	248	29.2	28	21.1	58	33.1	18	40.9
Gill arch IV	215	25.3	20	15.0	29	16.6	20	45.5
Dorsal segment	730	86.0	86	64.7	147	84.0	18	40.9
Medial segment	106	12.5	42	31.5	22	12.6	16	36.4
Ventral segment	13	1.5	5	3.8	6	3.4	10	22.7
Proximal part	515	60.7	30	22.6	125	71.4	21	47.7
Distal part	334	39.3	103	77.4	50	28.6	23	52.3
Anterior hemibranch	473	55.7	68	51.1	92	52.6	25	56.8
Posterior hemibranch	376	44.3	65	48.9	83	47.4	19	43.2
Inner surface	712	83.9	125	94.0	155	88.6	0	0
Outer surface	137	16.1	8	6.0	20	11.4	44	100

the numbers recorded on the dorsal and the other two segments (P=0.00 < 0.05). Although there were no statistically significant differences in the number of *P. anguillae* between proximal and distal parts (P=0.195 > 0.05), the parasite preferred proximal parts (72.0%), inner surface of the hemibranches (75.8%), but no differences were found between the left and right side (P=0.975 > 0.05).

A total of 10 *E. gibbus* were recorded in these monospecific infections. *E. gibbus* preferred gill arch I (40.0%) and no parasite was found on arch IV. Dorsal segments were mainly preferred by *E. gibbus* (70.0%), the parasite completely occupied the distal part (100%), inner surface (100%), and it was mainly found on the right side (70.0%).

Dorsal segments mainly preferred by *E. gibbus* (70.0%), parasite totally occupied distal part (100%), inner surface (100%) and of the (70.0%) found on the right side.

A total of 11 *E. lizae* were recorded in these monospecific infections. *E. lizae* preferred gill arch IV, with no parasite being found on gill arch I. *E. lizae* mostly preferred medial segments (63.6%). There were no significant differences in the number of *E. lizae* between the proximal and distal parts (P=0.949 > 0.05), but the parasite exhibited preference for the left side (P=0.027 < 0.05); 81.8% of the *E. lizae* found were on the left side and 90.9% were on the outer surface of the hemibranches (Table 3).

The numbers of *P. anguillae*, *E. gibbus* and *E. lizae* between bispecific and monospecific infections were also examined. The numbers of *P. anguillae* showed no statistically significant difference between bispecific infections with *E. gibbus*, bispecific infections with *E. lizae* and monospecific infections (Kruskal-Wallis test P=0.404; p > 0.05). There was no statistically significant difference in the numbers of *E. gibbus* between bispecific infections with *P. anguillae* and monospecific infections (Mann-Whitney U test P=359 > 0.05). There were no statistically significant differences in the numbers of *E. lizae* between bispecific infections with *P. anguillae* and monospecific infections (Mann-Whitney U test P=0.663 > 0.05).

Table 3. Distribution of *Pseudodactylogyrus anguillae*, *Ergasilus gibbus* and *Ergasilus lizae* on the gills of *Anguilla anguilla* in monospecific infections.

	P. anguillae		***E. gibbus***		***E. lizae***	
Number of infected eels	17		5		4	
Mean intensity	23.4		2.0		2.8	
	Number	%	Number	%	Number	%
Left side	187	47.1	3	30.0	9	81.8
Right side	210	52.9	7	70.0	2	18.2
Gill arch I	77	19.4	4	40.0	-	0
Gill arch II	112	28.2	3	30.0	3	27.3
Gill arch III	95	23.9	3	30.0	2	18.2
Gill arch IV	113	28.5	-	0	6	54.5
Dorsal segment	314	79.1	7	70.0	1	9.1
Medial segment	65	16.4	-	0	7	63.6
Ventral segment	18	4.5	3	30.0	3	27.3
Proximal part	286	72.0	-	0	5	45.5
Distal part	111	28.0	10	100.0	6	54.5
Anterior hemibranch	212	53.4	6	60.0	7	63.6
Posterior hemibranch	185	46.6	4	40.0	4	36.4
Inner surface	301	75.8	10	100.0	1	9.1
Outer surface	96	24.2	0	0	10	90.9

Table 4. Number of *Pseudodactylogyrus anguillae*, *Ergasilus gibbus* and *E. lizae* according to fish size.

Total length (cm)	***P. anguillae***		***E. gibbus***		***E. lizae***	
	Number	%	Number	%	Number	%
33.2-44.9	322	22.7	45	31.5	6	9.5
45.0-54.9	456	32.1	72	50.3	9	14.3
55.0 ≤	643	45.2	26	18.2	48	76.2

P. anguillae, *E. gibbus* and *E. lizae* were recorded in simultaneous infections in only 5 fish hosts but were not taken into account because of the low sample size.

The prevalence and the number of *P. anguillae* increased with the size of the host and reached 45.2% and 643 individuals in the 55.0 cm and higher size classes. The highest prevalence of *E. gibbus* 50.3% and 72 individuals was found in the 45.0-54.9 cm size classes. The highest prevalence of *E. lizae* 76.2% and 48 individuals was found in the 55.0 cm and higher size classes (Table 4).

Discussion

Statistical analysis showed that *P. anguillae* preferred the dorsal segments; this preference was observed in the general occurrence of the three parasites, in *P. anguillae* – *E. gibbus* and *P*. *anguillae* – *E. lizae* bispecific infections and moreover in monospecific infections. *P. anguillae* significantly preferred dorsal segments, proximal halves and inner surfaces of the hemibranches in general occurrence, bispecific and monospecific infections. According to Buchmann (1988), *P. anguillae* which has large hamuli, are never found embedded in a tissue reaction and thus they preferably attach more to intact gills and the more sheltered base of gill filaments. In contrast, *P. bini* attach more effectively to gills, as it appears that the filaments are ones with small hamuli, and it prefers distal parts (Buchmann 1987). Llewellyn (1956) and Suydam (1971) both stated that parasite distribution over the arches is highly affected by respiratory current flow rate distribution. The greatest volume of water in the gill ventilation current pass over the second and third gill arches (Paling 1968). The dorsal segments, proximal halves and inner surface of the primary filaments of each hemibranch are more sheltered than the outer parts (Wootten 1974). The findings mentioned above, evidence the microhabitat preference of *P. anguillae.* In the present study, the significant preference for arches II, III, and IV over arch I, dorsal segments, proximal halves, inner surface of the hemibranches and no preference for the left or right side was observed for *P. anguillae*. Prevalence and mean intensity of *P. anguillae* were higher when it coexisted with *E. gibbus* in bispecific infections. In general occurrence and bispecific infections, *E. gibbus* had no significant left or right side preference, but in monospecific infections *E. gibbus* preferred the right side. *E. gibbus* preferred gill arches I, II and III in general occurrence, and arches I and II in bispecific infections with *P. anguillae*. All *E. gibbus* were found on the inner surfaces of the hemibranches except for 4.9% on the outer surface in general occurrence. There were no significant differences of *E. lizae* on the left or right side of the branchial apparatus in general occurrence and bispecific infections with *P. anguillae*, but *E. lizae* preferred the left side in monospecific infections, however these findings are most likely a reflection of a smaller sample size, like the right side preference of *E. gibbus* in monospecific infection. But on the other hand, *E. lizae* did also show left side preference in bispecific infections with *P. anguillae*. *E. lizae* preferred gill arches III and IV, dorsal medial and ventral segments in general occurrence and in bispecific infections, but in monospecific infections *E. lizae* was found in arch IV, and the medial segment. In all infections, *E. lizae* were recorded on both proximal and distal segments, strongly preferring the outer surfaces of the hemibranches and not being found at all on the inner surface. In conclusion, our data indicate that the distribution of *P*. *anguillae* over the gill arches was not random. Even when numbers of *P. anguillae* were high, vacant niches were clear, and the parasite showed significant preference for the dorsal segment to the median and ventral segments. This aggregation of *P. anguillae* on the dorsal segments suggests the absence of intraspecific competition. From the parasite distribution observed on the gills of *A. anguilla*, it showed that niches of *P. anguillae* and *E. gibbus* overlapped each other and that there was no interspecific competition. *E. gibbus* preferred anterior, *E. lizae* – posterior gill arches. *E. gibbus* preferred inner, *E. lizae* – outer surface and *E. gibbus* preferred arch I, *E. lizae* – arch IV. Besides these, a record of *E. gibbus* and *E*. *lizae* coexisting with *P. anguillae* in simultaneous infections in only 5 eels indicate negative interactions between them.

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