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RESEARCH ARTICLE **ARAŞTIRMA MAKALESİ**

The occurrence of *Pomphorhynchus laevis* (Acanthocephala) in cage-reared rainbow trout (*Oncorhynchus mykiss*) from Işıklı Spring, Çivril, Turkey

Işıklı Kaynağı, Çivril'de ağ kafeslerde yetiştirilen gökkuşağı alabalığı (*Oncorhynchus mykiss*)'nda *Pomphorhynchus laevis* (Acanthocephala) varlığı

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Abstract: Occurrence of *Pomphorhynchus laevis* (Müller, 1776) in cage-reared rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) and the effects of parasitism on the fish condition were studied between July and November 2014 in Işıklı Spring. A total of 221 *O. mykiss* specimens were examined. In addition to *P. laevis*, three other parasite species; *Trichodina* sp., *Ichthyophthirius multifiliis* Fouquet, 1876 and *Diplostomum* sp. were also recorded. *P. laevis* was numerically the predominant species with the highest prevalence (77.8%), mean intensity (6.74), and mean abundance (5.24). In total 1160 *P. laevis* specimens were collected. The mean abundance of *P. laevis* increased over a period of months from 2.5 individual parasites per fish in July to 7.1 in November. The mean intensity of *P. laevis* increased with increasing fish host length from 1.5 in the 5.0–6.9 cm length class to 18.4 in the 25.0–26.9 cm length class. Although there was no statistically significant bias in the spatial distribution of *P. laevis* within the gut ($p > 0.05$), the parasite tended to prefer the pyloric caeca (47.9%). The larval stage (cystacanth) of the acanthocephalan parasite were obtained from the haemocoel of *Gammarus obnixus* Karaman and Pinkster, 1977. The value of Fulton's condition factor (K) ranged from 0.84 to 1.66.

Keywords: *Oncorhynchus mykiss, cage-reared, parasites, Pomphorhynchus laevis*

Öz: Ağ kafeslerde yetiştirilen gökkuşağı alabalığı *Oncorhynchus mykiss* (Walbaum, 1792)'nda *Pomphorhynchus laevis* (Müller, 1776) varlığı ve balık kondüsyonu üzerindeki paraziter etki Işıklı Kaynağı'nda Temmuz ve Kasım 2014 süresinde çalışıldı. Toplam 221 *O. mykiss* örneği incelendi. *P. laevis* den başka üç diğer parazit türü; *Trichodina* sp., *Ichthyophthirius multifiliis* Fouquet, 1876 ve *Diplostomum* sp. da kaydedildi. *P. laevis* yaygınlık (%77.8), ortalama yoğunluk (6.74) ve ortalama çokluk (5.24) ile sayısal olarak baskın parazitti. Toplamda 1160 *P. laevis* örneği toplandı. *P. laevis* ortalama çokluğu, aylık devrelerde Temmuz'da her balıkta 2.5 parazit bireyinden Kasım'da 7.1'e yükseldi. *P. laevis* ortalama yoğunluğu, artan balık uzunluğu ile 5.0-6.9 cm lik boy sınıfında 1.5'den 25.0-26.9 cm lik boy sınıfında 18.4'e yükseldi. Sindirim kanalında *P. laevis*'in uzamsal dağılımında istatistik olarak belirgin bir eğilim olmamasına karşın ($p > 0.05$) parazit tercihi pilorik çekaya (%47.9) yönelik olmuştur. Acanthocephalan parazitin larval dönemi (cystacanth), *Gammarus obnixus* Karaman and Pinkster, 1977' un vücut boşluğunda bulunmuştur. Fulton Kondisyon Faktörü değerleri (K), 0.84 ile 1.66 arasına yayılmıştır.

Anahtar kelimeler: *Oncorhynchus mykiss, kafes yetiştiriciliği, parazit, Pomphorhynchus laevis*

INTRODUCTION

Acanthocephalans of the genus *Pomphorhynchus* Monticelli, 1905 (Echinorhynchida: Pomphorhynchidae) are intestinal, non-specific parasites of a number of marine and freshwater fishes being their definitive or paratenic hosts (Kirin et al., 2014; Taraschewski, 2000). Like all fish acanthocephalans, they require trophic transmission to complete their life cycle using water amphipods as their intermediate hosts (Dezfuli et al., 2008). The arthropod intermediate host becomes infected by eating the acanthocephalan egg, and the acanthor larva is free into the host's digestive tract. The larva bores through the gut wall into the body cavity where it develops from acanthella to cystacanth, which can infect the vertebrate host (Dezfuli et al., 2011). The proboscis and bulb of *Pomphorhynchus* acanthocephalans deeply penetrate the entire gut wall of the fish host and lead to extensive damage to the digestive tract

(Dezfuli et al., 2002). The density of the parasite burden and the depth of penetration of the acanthocephalans are two main factors for their pathogenicity (Bullock, 1963). In amphipods, *P. laevis* has been recorded as the most abundant larval helminth (Dezfuli et al., 1999).

Recently, two genetically distinct but morphologically close species of *Pomphorhynchus*; *P. laevis* Müller, 1776 and *P. tereticollis* (Rodolphi, 1809), have been detected throughout Europe (Perrot-Minnot, 2004; Bombarova et al., 2007; Špakulová et al., 2011). *P. tereticollis* was treated as a synonym of *P. laevis* for a long time, but the species has been resurrected and re-described by Špakulová et al., (2011) on the basis of some morphological and molecular features. In Turkey, some authors have recorded *P. laevis* in freshwater fishes and also amphibian hosts (Yıldız and Çavuşoğlu 2003). On the other hand, Smales et al., (2012) recorded *P. tereticollis* in Great Beyşehir Spined Loach *Cobitis bilseli* Battalgil, 1942 (Cobitidae) from Lake Beysehir, Turkey. Düşen and Oğuz (2008), recovered *P. laevis* from Marsh frog (*Rana ridibunda*) in Lake Işıklı. Heckmann et al., (2010) identified *P. spindletruncatus* in the intestine of the marsh frog *Pelophylax ridibundus* (Pallas, 1771) from Işıklı Lake. In the present study, we observed the frequent occurrence of *Pomphorhynchus* infection in cage-reared rainbow trout *Oncorhynchus mykiss*. Although the structure of the proboscis hooks in the presently reported species reveals close similarity to *P. tereticollis*, the first molecular analysis suggests *P. laevis* (Špakulová and Perrot-Minnot, personal communication).

MATERIALS AND METHODS

The fish specimens were sampled between July and November 2014 from net-cages close to Işıklı Spring (38º 18' 55.67" N, 29º 51' 38.29" E). In total, 221 *Oncorhynchus mykiss* specimens of mean (± SD) total length 14.79 ± 4.65 cm (ranging from 5.1 to 27.8 cm) and mean (± SD) weight 53.90 ± 54.87 g (ranging from 1.9 to 287.1 g) were examined. The fish were transported to the laboratory alive, where they were weighed and measured. Fish were anaesthetised using MS-222 and their spinal cords cut with dissecting scissors. During the dissection, the skin, vitreous humour, eye lens, mouth and nasal cavities, gills, gonads, spleen, digestive tract, kidneys, swim bladder, peritoneum and muscles were examined for parasites. The digestive tracts were removed, opened longitudinally and examined for parasites, which were recorded by number and location. The condition factor of the fish was calculated using Fulton's formula: $K=W \times 100 / L^3$, where W= fish weight in grams, L= total length of fish in centimetres. Differences in the spatial distribution of *P. laevis* in the digestive tract and the differences in the number of P. laevis and the condition factor between the size classes were analysed using the Kruskal-Wallis H test. One-way analysis of variance (ANOVA) was used to determine differences in condition factors between the fish size classes. A total of 617 specimens of *Gammarus obnixus* were collected, using a hand net (2 mm mesh) sweeping over submerged plants. Amphipod specimens fixed with 4% formalin and cleared in lactic acid-glycerine-water. They were measured and sexed and infected individuals were separated for the counting of cystacanths. Identification of acanthocephalan specimens was performed as described by Špakulová et al., (2011) using unfixed fresh material and then by mitochondrial and nuclear sequencing. Identification of the other parasites were made according to Niewiadomska, (2003) and Bykhovskaya-Pavlovskaya et al., (1962). Species names of *Gammarus obnixus* and *G. balcanicus* Schaferna, 1922 were based on (Aygen and Balık, 2005). The prevalence, mean intensity and abundance were determined as defined by Bush et al. (1997).

RESULTS

During the study period, a total of 221 *O. mykiss* specimens were examined. The water temperature ranged from 10.5º – 19.3ºC, and dissolved oxygen concentrations ranged 5.7 mg/l in mid summer and increase to 13.8 mg/l in late fall at the cage area. Four parasite species were identified on/in the fish host: *Trichodina* sp., *Ichthyophthirius multifiliis*, *Diplostomum* sp. and *Pomphorhynchus laevis* (Figs 2, 3).

Figure 2. Male of *Pomphorhynchus laevis* in *Oncorhynchus mykiss* from Işıklı Spring (bar =2 mm)

Identification of acanthocephalan specimens was performed on living or fresh worms and the following morphological features found: (first four to five hooks longest, fifth or sixth hooks stoutest and significantly shorter (Fig 4), hooks on the posterior half of proboscis have proximal projections on the base (Fig 5), last hook row stands at the posterior-most end of the proboscis (Fig 6) reveals close similarity to *P. tereticollis*.

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SQ   Sequence 614 BP; 138 A; 92 C; 182 G; 202 T; 0 other;
     ggtctgatgt atgttttggt tggtgtgtga ggggggctaa tgggattttc tataaggcta        60
     ttaattcgat tagagctagg gagaggaggg gtttggatag gaagagaggc tgtgtataat       120
     gttttagtga ctagacatgc tgttataata gtatttttc tagtaatacc agtatttatg       180
     ggaggatttg gtaattggct catgccagtt atgttaggat tgagggacat ggccctccca       240
     cgactgaata atttgaggct tattctactt atcgctaggt tgggaattat aggagtatcc       300
     ctgcttttag gaggggtgg ggctggttgg acaatgtatc cacccctcat gttgggggat       360
     tacaggtctg gtgtagctgt tgacctaatg atcctgaggt tgcatgtagt aggtctttcc       420
     tctatcctag gctcaatcaa catcctgatt acatgggtag ccgggaggag ggtggtgtat       480
     agagtagaac aggcacctct gtttgtatgg gctttagtaa cgaccgctgg cttagtggtt       540
     ttaacggtcc cagtcttggc ggcagcttta acgatgcttt tgatagaccg taatttgaat       600
     gccagatttt ttga                                                        614
Pomphorhynchus laevis mitochondrial partial COI gene for cytochrome oxidase subunit 1
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Figure 1. Sequences of mitochondrial partial COI gene for cytochrome oxidase subunite 1 of the acanthocephalan specimen from Işıklı Spring

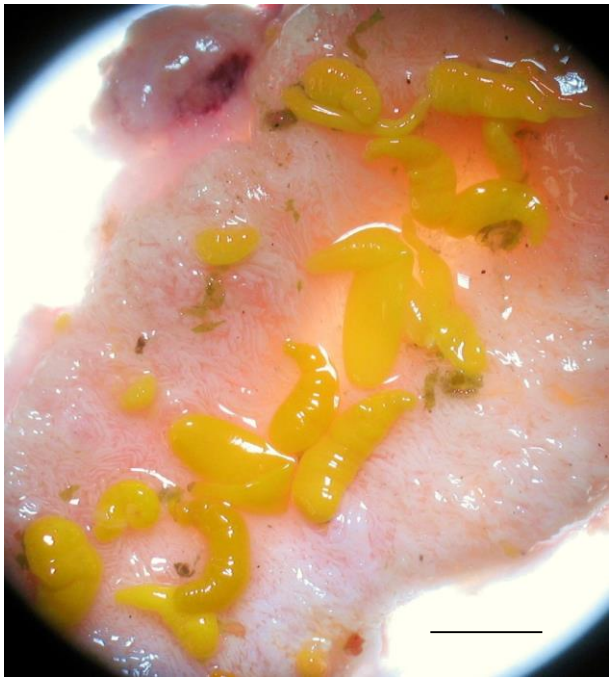

Figure 3. *Pomphorhynchus laevis* in the intestine of *Oncorhynchus mykiss* from Işıklı Spring (bar =5 mm)

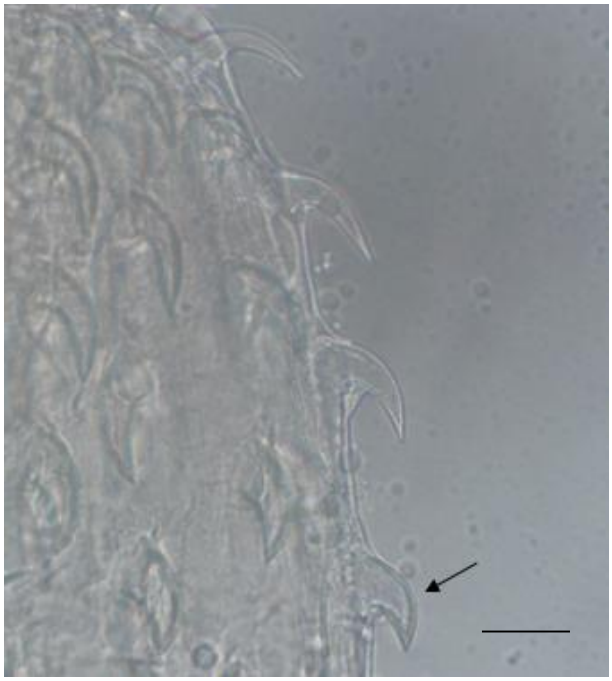

Figure 4. The first four to five hooks longest, fifth or sixth hooks (arrow) stoutest on the proboscis of *Pomphorhynchus laevis. (bar = 25 µm)*

But the first molecular analysis of mitochondrial partial COI gene for cytochrome oxidase subunite 1 determined the parasite specimens as *Pomphorhynchus laevis* (Figure 1).

The parasite infracommunity of the *Oncorhynchus mykiss* was strongly dominated by *P. laevis* and 172 fishes were found to be infected by a total of 1160 *P. laevis* individuals. The overall prevalence, mean intensity and mean abundance were determined as 77.8%, 6.74 and 5.24 respectively. The other three parasite *species: I. multifiliis* (prevalence 5.88%), *Diplostomum* sp. (0.45%) and *Trichodina* sp. (1.80%) were very scarce. The mean intensity of *P. laevis* in the fish host increased from 2.5 in July, 2.7 in August, 5.2 in September and 4.4 in October to 7.1 in November and the infection intensity ranged from 1 to 63 worms per host. The mean intensity of *P. laevis* increased with increasing host total length, from 1.5 in the 5.0 – 6.9 cm length class to 18.4 in the 25.0 – 26.9 cm length class.The mean intensity and the range of intensity for each length class are shown in Table 1.

The values of Fulton's condition factor (K) for the fish host ranged from 0.84 to 1.66 (average 1.22). Statistically significant differences were found in condition factors between fish size classes (one-way ANOVA) ($F=3.101$; $p=0.002$). The condition factor from each size group was computed to analyze the influence of *P. laevis* on fish condition; no significant differences were found (Kruskal Wallis *H test* $p > 0.05$). Even though the mean abundance of *P. laevis* increased with increasing fish size, the mean condition factor also increased. There was no statistically significant difference in spatial distribution of *P. laevis* among the different parts of the digestive tract was observed (*Kruskal-Wallis H test* $p > 0.05$), but the parasite tended to prefer (47.9%) the pyloric caeca.

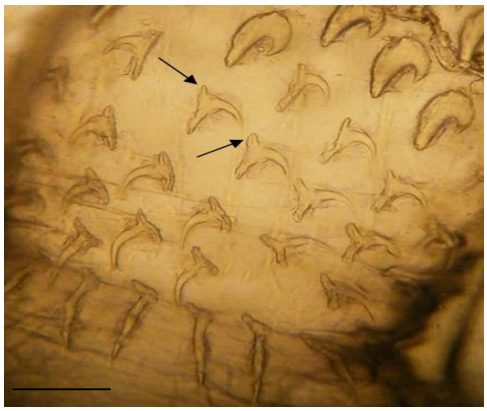

Figure 5. Hooks on the posterior half of the proboscis of *Pomphorhynchus laevis* have proximal projections on the base (arrows) from unfixed fresh material (bar = 25 µm)

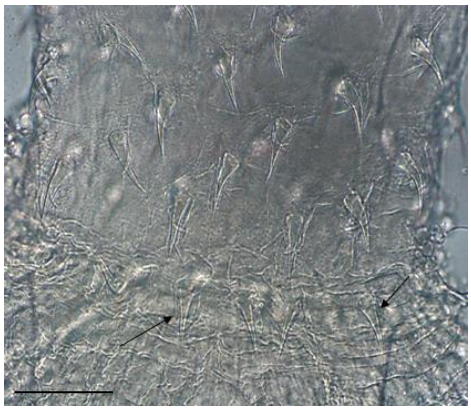

Figure 6. The last hook row (arrows) stands at the posterior-most end of the proboscis of *Pomphorhynchus laevis* (bar = 50 µm)

The spatial distribution and infection parameters of the *P. laevis* within the digestive tract are shown in Table 2. *Gammarus obnixus* was observed as intermediate host of *P. laevis* in the net cage area. *P. laevis* cystacanths infected 119 (19.3%) of the 617 *G. obnixus* specimens (Fig. 7) examined in November. The mean intensity and mean abundance of cystacanths was found to be 2.05 and 0.36 respectively. Intensities of infection ranged from one to nine cystacanths per *G. obnixus*.

Figure 7. *Pomphorhynchus laevis* cystacanths (arrows) in the haemocoel of *Gammarus obnixus* from Işıklı Spring (bar = 2 mm)

Table 1. Size classes of cage-reared *Oncorhynchus mykiss* and parameters of infection by *Pomphorhynchus laevis* in Işıklı Spring

Length (cm)	Number of fish examined	Infected fish number	Total number of parasites	Mean abundance	Mean intensity	Parasite intensity min - max
5.0–6.9	6	2	3	0.5	1.5	1 – 2
7.0–8.9	15	12	51	3.4	4.25	1 – 8
9.0–10.9	28	19	42	1.5	2.21	1 – 5
11.0-12.9	27	22	104	3.9	4.72	1 – 20
13.0–14.9	55	43	230	4.2	5.34	1 – 17
15.0–16.9	31	22	145	4.6	6.59	1 – 18
17.0–18.9	19	14	91	4.7	6.5	1 – 31
19.0–20.9	12	11	160	13.3	14.5	4 – 34
21.0–22.9	11	11	98	8.9	8.9	1 – 17
23.0–24.9	12	11	144	12.0	13.09	2 – 45
25.0–26.9	5	5	92	18.4	18.4	2 – 63

Table 2. Spatial distribution and infection parameters of *Pomphorhynchus laevis* within the digestive tract of *Oncorhynchus mykiss* in Işıklı Spring

Location of parasite	Total parasite number	Prevalence (%)	Mean intensity	Mean abundance	Parasite intensity min-max
Pyloric stomach	60	5.2	3.2	0.3	1–18
Pyloric caeca	556	47.9	4.3	2.5	1–24
Anterior intestine	143	12.3	3.2	0.6	1–18
Mid intestine	207	17.8	2.7	0.9	1–11
Posterior intestine	191	16.5	2.6	0.9	1–17

DISCUSSION

Turkey is the largest producer of farmed trout in Europe with an annual production of 85,250 tons. 78,150 tons come from inland aquaculture with the remaining 7,100 tons from seawater production (Bozoğlu et al., 2007). *Oncorhynchus mykiss* is the main freshwater fish species cultured in Turkey and cage farming of rainbow trout has become widespread in lakes and reservoirs during the last few decades. However, few studies have reported on parasites of trout in Turkey. Soylu, (1996) recorded *Ichthyobodo necator* (Henneguy, 1883), *Trichodina* sp., *Chilodonella cyprini* (Moroff, 1902) and *Ichthyophthirius multifilis* Fouquet, 1876 on farmed rainbow trout from the Marmara region. Altunay and Yıldız (2008) found *Trichodina* sp., *Epistylis* sp., *Chilodonella* sp., *Costia* sp., *Apiosoma* sp. and *Tripartella* sp. on cage-cultured *O. mykiss* from Kesikköprü Dam Lake. Özer et al. (2010) recorded *I. multifiliis*, *Trichodina* sp. and *Chilodonella* sp. and Öğüt and Parlak (2014) recorded *Hexamita salmonis* in the same fish host. At least 23 protozoan and 169 metazoan parasites are known to occur in rainbow trout throughout the world (Lom and Dykova, 1992; Buchmann et al., 1995). Protozoan parasites represent one of the most important groups of pathogens that negatively affect the health of cultured and feral fish (Scholz, 1999). Outside of the cage-rearing system, when *Oncorhynchus mykiss* are maintained in concrete ponds, artificially fed and treated with therapeutic products, cleaning and sterilizing ponds are the effective ways of reducing the numbers of the intermediate hosts of some parasites. When fish are fed with processed food under farmed conditions, transmission of many indirectly transmitted parasites is blocked (Johansen et al., 2011). In the present study the parasite infracommunity of the fish host was dominated by a single species, *Pomphorhynchus laevis.* Morphological analysis of proboscis hooks of the specimens are very similar to *P. tereticollis*, but there are several differences. The last row of hooks is not situated in bulbus, and hooks shorter. As a result, the morphology is slightly different from *P. tereticollis* and it is different apparently also from *P. laevis* (M. Spakulova, personal communication). Phylogeography of *P. laevis* and *P. tereticollis* appear rather complex especially for *P. laevis* with rather old lineages in the peri-Mediterranean area genetically more distant to *P. tereticollis*. There is a rather large genetic differentiation within *P. laevis* at pan-European scale, Mediterranean lineages seem to be older and more differentiated. Phylogeographic analysis shows that this lineages of *Pomphorhynchus* bracnhes is between *Pomphorhynchus* from Italy (oldest lineages) and those other parts of Europa (Danube-Volga lineages and west Europe lineages). If mentioning these clusters, Italian and Turkey and may be Danubian are quite distant (J-M. Perrot-Minnot, personal communication). Cages constitute an open system, which allows free exchange between wild and caged organisms, leading to the exposure to disease-causing agents (Merella et al., 2006). Some parasite species with complex life cycles cause phenotypic changes in their intermediate hosts that appear to enhance trophic transmission to their final hosts (Cezilly et al., 2014). Infected hosts often show alterations in behaviour in addition to changes in their appearance (Kaldonski et al., 2009). For example, the orange colouring of the *P. laevis* cystacanths coincides with the changed behaviour of the intermediate host (gammarids) that swims near the surface of the water, maximising their chances of transmission to the next host (Theo et al., 1997). *Gammarus obnixus* infected with cystacanths of *P. laevis* were found on the leaves of densely populated aquatic plants around the net cages. The mean intensity of *P. laevis* in our study increased along with the size classes of *O. mykiss*. This is an usual process because larger fish accumulate more parasites and can feed on larger amphipods that harboured high number and larger cysthacanths than small amphipods. Dezfuli *et al.* (2002) found *P. laevis* in the posterior part of trout middle intestine.

The preferred attachment site of *P. laevis* in our study was the pyloric caeca of the fish alimentary tract. *Trichodina* sp., *Ichthyophthirius multifiliis* and *Diplostomum* sp. were found scarcely, probably due to therapeutic chemical treatments. Use of formalin and Chloramine-T have been observed in control of ectoparasites in this trout farm during study period. According to the results, there was no appreciable effect of *P. laevis* on the condition factor of the fish host.

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