INFESTATION OF *ARGULUS FOLIACEUS* L. ON FISH FRY REARED IN ILLUMINATED CAGES

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Summary. The rearing of fish fry in illuminated cages is one of the methods used before fish release into water bodies. The aim of the study was to investigate the peculiarities of infestation with *Argulus foliaceus* L. of the fry of pike *Esox lucius* (L.), pike-perch *Sander lucioperca* (L.), bream *Abramis brama* (L.), vimba *Vimba vimba* (L.) and peled *Coregonus peled* (Gmel.) reared in illuminated cages. The experiment was carried out in a mesotrophic lake in May-August. The fry of different fish species were reared in a separate net cages. Their density reached 10000 ind. m^{-3}. *A. foliaceus* was detected on all fish fry species investigated. Infestation of fish with this parasite started when the total length of fish reached 1.8–2.0 cm. Correlation between the prevalence of *Argulus* and the total length of fish fry (r = 0.96, p = 0.049) and water temperature (r = 0.95, p = 0.045) was found to be reliable. The prevalence of this parasite was higher on peled than on bream (t-test = -5.748, p = 0.029) and vimba (t-test = -5.167, p = 0.035) of the same length. At the end of rearing period 0.01% of pike, 0.3% of bream, 1.4 % of vimba, 14.9% of pike-perch and 77.8 % of peled fry were infected with *A. foliaceus*.

Keywords: *Argulus foliaceus*, fish fry, rearing, cages.

ŽUVŲ JAUNIKLIŲ, AUGINAMŲ APŠVIESTOSE VARŽOSE, UŽSIKRĖTIMAS *ARGULUS FOLIACEUS* L.

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Santrauka. Žuvų jaunikliams paauginti, prieš išleidžiant juos į vandens telkinius, taikomi įvairūs metodai, tarp jų – auginimas apšviestose varžose. Šio darbo tikslas buvo ištirti lydekos *Esox lucius* (L.), sterko *Sander lucioperca* (L.), karšio *Abramis brama* (L.), žiobrio *Vimba vimba* (L.) ir peledės *Coregonus peled* (Gmel.) jauniklių, auginamų apšviestose varžose, užsikrėtimo karpine utėle *Argulus foliaceus* L. ypatumus. Bandymas buvo atliekamas gegužės–rugpjūčio mėnesiais Lietuvos mezotrofinio tipo ežere. Skirtingų rūšių žuvų jaunikliai buvo auginami atskirose tinklinėse varžose. Jų tankis buvo 10 tūkst. ind./m^3. *A. foliaceus* rastas ant visų tirtų rūšių žuvų jauniklių. Šis parazitas pradėjo pulti žuvis, kai jos pasiekė 1,8–2,0 cm ilgį. Gauta patikima teigiama koreliacija tarp žuvų jauniklių užsikrėtimo ekstensyvumo *A. foliaceus* ir jų ilgio (r = 0,96; p = 0,049) bei vandens temperatūros (r = 0,95; p = 0,045). Peledės šiuo parazitu užsikrėtė ekstensyviau negu to paties dydžio karšiai (t-test = – 5,748; p = 0,029) ir žiobriai (t-test = – 5,167; p = 0,035). Auginimo pabaigoje *A. foliaceus* buvo užsikrėtę 0,01 proc. lydekų, 0,3 proc. karšių, 1,4 proc. žiobrių, 14,9 proc. sterkų ir 77,8 proc. peledžių jauniklių.

Raktažodžiai: *Argulus foliaceus***,** žuvų jaunikliai, auginimas, varžos.

Introduction. Argulus, *Argulus foliaceus* L., or fish louse, is one of the most widespread crustacean ectoparasites of freshwater fish in the world in terms of both distribution and wide variety of fish it infests (Kennedy, 1974; Holland, Kennedy, 1997; Evans, Matthews, 2000; Kearn, 2004; Walker *et al*., 2004; Schram *et al*., 2005; Рауцкис, 1988). Argulus kill fish in two different ways: 1) damage fish tissue by "sucking" on the fish, and 2) by secondary bacterial infection. Moreover, argulus inject a toxin that kills smaller fish and leaves reddened, inflamed lesions on larger fish (Taulescu *et al*., 2010; Рауцкис, 1988). All argulids are described as obligate ectoparasites of fish, but they are also frequently encountered swimming freely in the water column as they seek out new hosts, mates or when females detach from their hosts to deposit eggs (Mikheev *et al*., 1998; Bandilla *et al*., 2007). Argulus is a blood sucker and can survive for only a few days without the host fish (Mikheev *et al*., 1998). The mechanisms of host searching in *A. foliaceus* (e.g. olfaction, vision, mechanoperception) have been investigated previously by Mikheev *et al*. (1998, 2000, 2003, 2004). Many authors have commented on the lack of specificity of argulid parasites, sharing the opinion that individual species from this group can infect a wide range of host species (Kearn, 2004, Walker *et al*. 2004; 2008 a). However, some apparent host preferences have been demonstrated by Valtonen *et al*. (1997), Mikheev *et al*. (1998, 2000) and Pasternak *et al*. (2000).

The rearing of fish fry in illuminated cages is one of the methods of stocking material production (Jäger *et al*., 1980; Schlumpberger, Zieborth, 1981; Schiljukene *et al*., 1983; Mamcarz 1995; Žiliukienė, 2005; Žiliukienė, Žiliukas, 2006 a). This method is based on drawing passive planktonic organisms into cages by water movement and attracting plankton into cages at night with electric light. This allows production of the stocking material of various

fish species without additional feeding.

The parasites of fish play a major role in the economic success of both fisheries and aquaculture operations. The aim of this study was to investigate infestation of *Argulus foliaceus* L. on the fry of pike *Esox lucius* (L.), pike-perch *Sander lucioperca* (L.), bream *Abramis brama* (L.), vimba *Vimba vimba* (L.) and peled *Coregonus peled* (Gmel.) reared in illuminated cages.

Materials and Methods. Investigations took place in Lake Rubikiai (55°30'0"N and 25 °18'0"E), which is located in northeast Lithuania. The lake is a mesotrophic bream-type water body. Its surface area is 968 ha, average depth 5.7 m, maximum depth 16.1 m. Secchi disk visibility ranges from 150 to 210 cm. The fish fauna of the lake consists of 19 species (belonging to 8 families), among which roach *Rutilus rutilus* (L.), perch *Perca fluviatilis* (L.), bream *Abramis brama* (L.), rudd *Scardinius erythropthalmus* (L.) and bleak *Alburnus alburnus* (L.) predominate (Žiliukienė, Žiliukas, 2006 b).

The experiment was carried out during late spring and summer months, the seasons when *Argulus foliaceus* is known to be the most abundant (Walker *et al*., 2004). Pike, pike-perch, bream, vimba and peled fry were reared separately in five net cages (size 1.5 m^3) attached to a floating deck anchored in one of the lake's bays at a distance of 100 m from the coast. The cages were stocked with fish fry, which had just passed on to the exogenous feeding stage, at a density of 10 thaus. ind. m^{-3}. To attract zooplankton, each cage was illuminated with two 12 V/60 W bulbs put into cages at a depth of 0.5 m. The light was switched on at 21.00 and switched off at 7.00. Surface water temperature was measured three times per day (at 8.00, 15.00 and 22.00). The mean day temperature was calculated. Fish were individually measured for total length (TL).

The fry rearing period varied between different fish species subject to their feeding peculiarities and rate of infection with *A. foliaceus*. Pike and pike-perch fry rearing lasted the shortest, i.e. until cannibalism was noticed (Table 1). As it is reported in literature (Žiliukienė, Žiliukas, 2006 a), further rearing of pike and pike-perch fry in illuminated cages has no point as their output begins to considerably decrease due to cannibalism.

Table 1. **List of examined fish species and their rearing period in illuminated cages**

Fish species	Rearing period	Average total length (cm) of fish	
		At the beginning of rearing	at the end of rearing
Pike *Esox lucius* (L.)	5 May–19 May	1.2	2.2
Pike-perch *Sander lucioperca* (L.)	19 May–3 July	0.5	4.3
Bream *Abramis brama* (L.)	7 June–14 August	0.8	2.8
Vimba *Vimba vimba* (L.)	14 June–4 August	0.9	2.9
Peled *Coregonus peled* (Gmel.)	1 May–4 August	0.9	6.6

The external surfaces of fish were examined thoroughly for *Argulus* individuals. Parasite prevalence (= the proportion of the host sample that was infested) and mean intensity (= the average number of lice per infested fish, excluding the zero values for uninfected fish) were calculated (Walker et al., 2008).

Statistical analysis of the data was performed with the Statistica 6.0 program. The correlation coefficient (r) was used for calculating the significance of relationships between the prevalence of *Argulus* and the total length of fish and water temperature. Cluster analysis was performed using the Bray-Curtis coefficient with the Cluster Program of the Primer package. Differences among groups in the clustering scheme were tested for significance by Student's *t*-test.

Results. The rearing of pike fry lasted 14 days — from 5 to 19 of May. Water temperature of the lake during the experimental period varied from 7.9 to 13.4°C (mean 9.0°C) (Fig. 1). Infestation with *A. foliaceus* was noticed only at the end of the rearing period when fry total length was 1.9-2.6 (Table 2). Argulus was detected only on pike of the total length no less than 2.0 cm. The infected fish usually had only one parasite per fish and the prevalence did not exceed 0.01%.

Fig. 1 shows that during pike-perch fry rearing, which lasted from 19 of May to 3 of July, water temperature fluctuated form 10.0 to 21.6°C (mean 17.4°C). The first *A. foliaceus* on fry was observed on the 31st day of rearing, i.e. on 19 of June when fish total length reached 1.6-3.3 cm (Table 2). The smallest pike-perch on which *Argulus* was detected was 1.8 cm TL. The estimated infection prevalence was 3.8%, and intensity was 1.0 louse per fish. At the end of the rearing period, the infected pike-perch fry of the total length of 3.5-5.5 cm (4.3 cm on average) had from 1 to 2 lice per fish (mean intensity 1.04), and the prevalence of argulus increased to 14.9%.

Bream fry rearing lasted longer compared to pike and pike-perch fry, i.e. from 7 of June to 14 of August. The average water temperature during this period was 19.0°C (min 17.0, max 24°C). The data obtained shows low prevalence and intensity of *A. foliaceus* on bream fry throughout the rearing period (Table 2). Infestation with *A. foliaceus* was noticed on the 54th day of the experiment, i.e. on 31 of July when bream reached 1.7-2.4 cm TL (2.1 cm on average). The total length of bream infested with lice was above 1.9 cm. For bream fry, argulus prevalence did not exceed 0.3% and intensity was 1 parasite/fish.

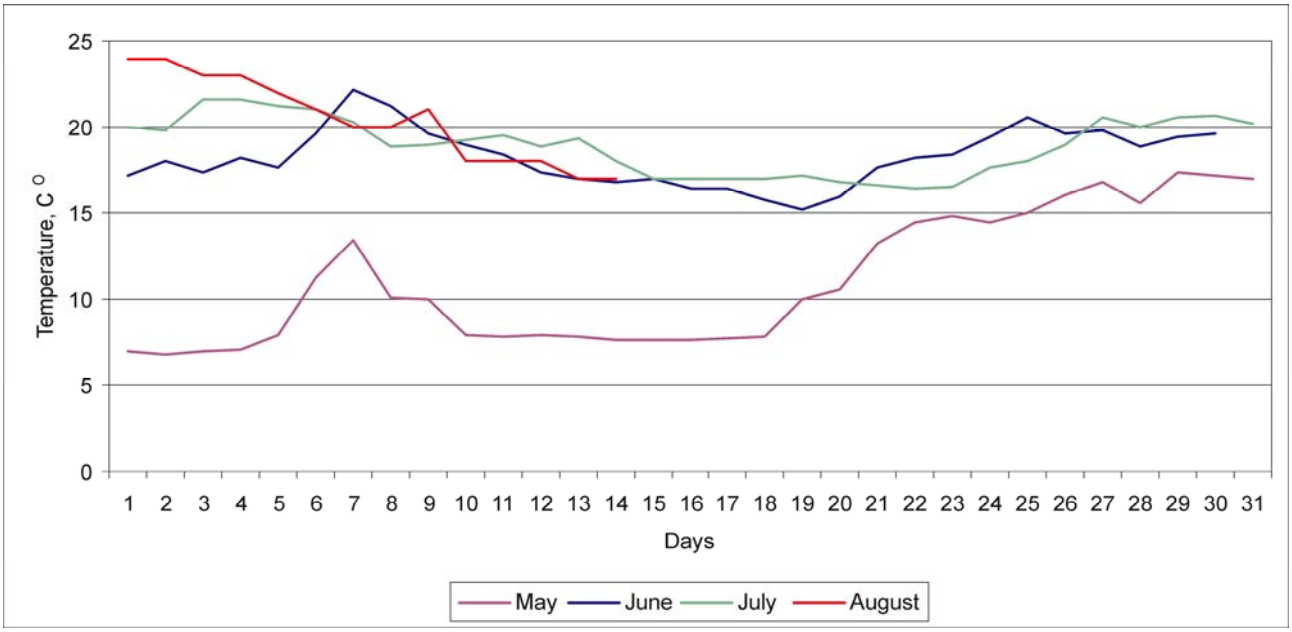

Figure 1. **Water temperature during fish rearing in illuminated cages**

Table 2. **Infection rate of *Argulus foliaceus* L. on the fry of various fish species reared in illuminated cages** (N – number of fish examined for *A. foliaceus*, * –argulus observed for the first time)

Fish species	Data	N	Fish total length (cm)			Louse prevalence (%)	Mean louse intensity
			mean	min-max	±SD		
Pike	19 May	9500	2.2*	1.9-2.6	0.20	0.01	1.0
Pike-perch	19 June	26	2.4*	1.6-3.3	0.44	3.8	1.0
	3 July	475	4.3	3.5-5.5	0.90	14.9	1.04
Bream	31 July	1000	2.1*	1.7-2.4	0.20	0.1	1.0
	14 August	1000	2.8	2.3-3.4	0.48	0.3	1.0
Vimba	16 July	1000	1.9*	1.6-2.3	0.27	0.2	1.0
	4 August	500	2.9	2.3-3.4	0.34	1.4	1.0
Peled	2 June	100	2.0*	1.8-2.3	0.19	9.0	1.0
	30 June	300	3.4	2.7-4.0	0.34	12.7	1.08
	15 July	270	4.2	3.5-5.2	0.64	28.9	1.28
	4 August	320	6.6	5.7-7.3	0.53	77.8	1.31

Vimba fry rearing in illuminated cages lasted from 14 of June to 4 of August. During the study period, water temperature varied from 16.8 to 24.0^{O}C (19^{O}C on average). Infestation of fish with *A. foliaceus* was observed on the 32nd day of rearing. Louse prevalence and intensity on vimba fry of 1.6-2.3 cm TL (1.9 cm on average) were 0.2% and 1, respectively (Table 2). Prevalence increased with fish length increasing, and at the end of the experiment it reached 1.4%.

Water temperature during the rearing period of peled fray (1 May – 4 August) ranged from a minimum of 6.8 ^{O}C at the beginning of rearing to a maximum of 23-24^{O}C at the end of rearing (mean 16.4^{O}C). The first fish infected with *A. foliaceus* appeared on the 32nd day of rearing (2 June). Their total length varied from 1.8 to 2.3 cm (2.0 cm on average). Louse prevalence was comparatively high and reached 9.0% (Table 2). At the end of rearing, more than half of the peled sampled were infected with *A. folliaceus.* Of 320 fish examined, 77.8% were infected with this parasite. Some fry of 5.7-7.3 cm TL (6.6 cm on average) harboured four lice. Infection intensity was 1.31 parasites per fish.

The results obtained showed that *Argulus* start attaching fish fry when their total length reaches 1.8-2.0 cm. The most rapidly growing pike fry was found to be infested with fish louse as early as after two weeks from the beginning of rearing, pike-perch fry, vimba fry and peled fry were infested after a month, and bream fry as late as on the 54th day after the start of rearing.

Statistical analysis of data showed reliable correlation between the prevalence of *Argulus* and the total length of fish fry (r = 0.96, p = 0.049) and water temperature (r = 0.95, p = 0.045). Cluster analysis revealed that fish fry with the total length ranging from 1.9 to 3.4 can be arranged into three groups according to louse prevalence, differences between which are significant ($p \leq 0.042$). As we can see from Fig. 2, the pike fry 2.2 cm in length, bream fry 2.1 and 2.8 cm in length, and vimba fry 1.9 cm

in length, which were the least infested with *Argulus,* formed group one. The pike-perch fry 2.4 cm in length and vimba fry 2.9 cm in length, which were more attractive to the ectoparasite, formed group two. Group three was basically constituted by peled fry infested with louse. The prevalence of argulus was significantly higher on peled than on bream (*t*-test = -5.748, p = 0.029) and vimba (*t*-test = -5.167, p = 0.035) of the same length.

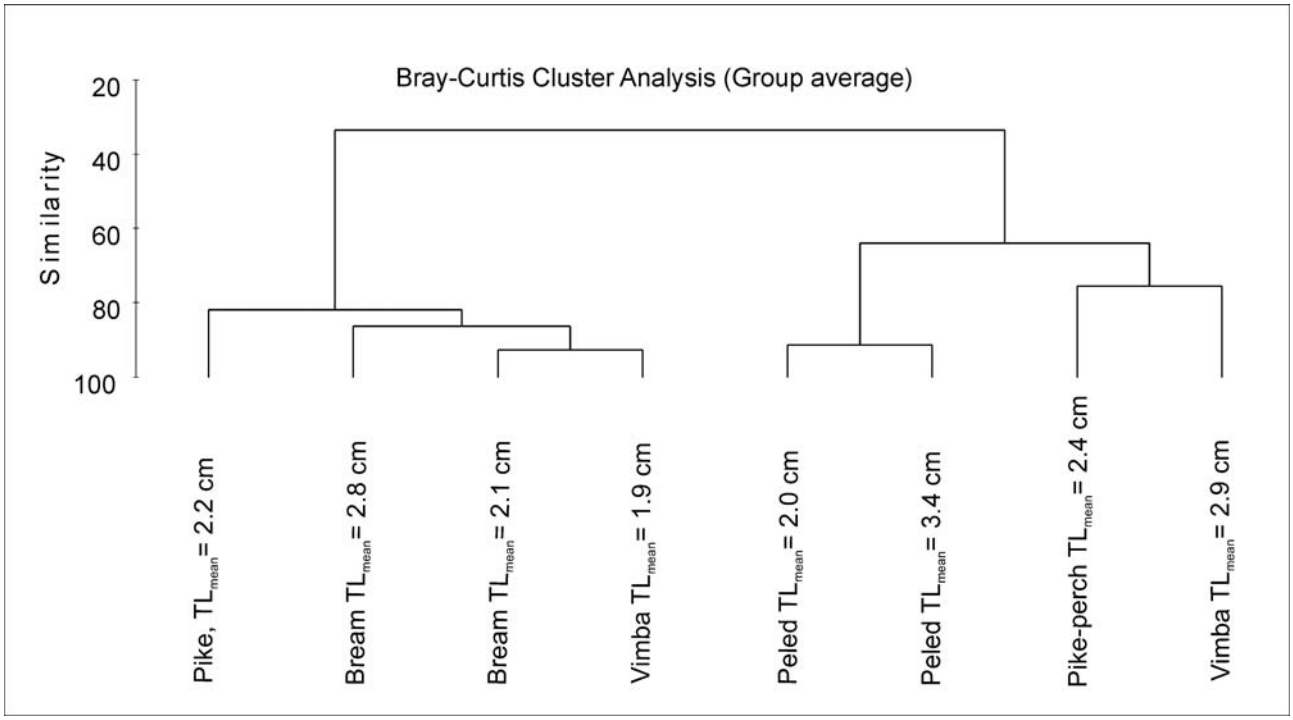

Figure 2. **Similarity between fry of various fish species according to the prevalence of *A. foliaceus***

Discussion. Kennedy (1974) indicates that *Argulus foliaceus* L. is regarded as a non-host specific parasite which has been recorded from practically every freshwater fish species within its natural range. Analogous results have been obtained in the present study. The fry of all five fish species (pike, pike-perch, bream, vimba and peled) reared in illuminated cages were infected with argulus. According to our data, infestation of fish fry with *A. foliaceus* started when fish fry were 1.8-2.0 cm TL. To our knowledge, this is the first paper to report the smallest size of fish infested with *A. foliaceus*.

Walker *et al.* (2008 b) indicate that in situations where equal numbers of equal sized hosts are present at the same time, host species preferences by *A. foliaceus* may become more apparent. Valtonen *et al*. (1997) studied this situation on roach, *Rutilus rutilus* L., and perch, *Perca fluviatilis* L. Extensive field studies in a number of lakes in Finland have clearly demonstrated higher levels of infection in perch than in roach in terms of both prevalence and intensity. According to our data, louse infection was obviously higher on peled than on all other fish species reared in illuminated cages. Vimba and pike-perch were more attractive to the ectoparasite than bream, whereas pike-perch exhibited higher infection levels than vimba.

During pike rearing, water temperature was often below 8^{O}C (mean 9^{O}C). That is why pike fry were not practically infected with argulus (prevalence = 0.01%). According to Craig (2000), eggs of *A. foliaceus* will not develop below 10^{O}C. The author reported that the optimum temperature for egg development is 25-26^{O}C. Laboratory observations have shown that *A. foliaceus* eggs can hatch in 28 days at 15^{O}C and in 18 days at 20^{O}C (Walker et al., 2008 a). References demonstrate that the level of infection increased in summer and decreased in cooler months (Shafir, Oldewage, 1992; Öztürk, Erdem, 1999; Harrison *et al*., 2006). In our experiment, water temperature at the beginning of August reached 24^{O}C, which evoked a very high infection level of peled with *A. foliaceus* (prevalence increased to 77.8%, mean intensity to 1.31 lice per fish). For this reason the rearing of peled fry was broken.

Öztürk and Bulut (2006), Walker *et al*. (2008 b) reported that *A. foliaceus* shows higher prevalence and intensity on larger fish. This is due to the fact that lager fish have a greater surface area and therefore are easier for *A. foliaceus* to locate on and attach to. We also found that larger fish fry are more likely to be infested with *A. foliaceus*.

Mikheev *et all*. (1998) indicate that host-searching and reproduction of *A. foliaceus* should be more successful in dense fish populations. This must be taken into account when fish are reared in cages where their concentration can reach thousands individuals per m^{-3}. Furthermore, net cages are a suitable substrate for egg depositing by *A. foliaceus* females. In our experiment, illuminated cages at night attracted fish living in the lake, some of which were infected with *A. foliaceus*. According to literature data (Stammer, 1959; Бауер, 1959) *Argulus* can also be characterized by positive phototaxis. All this conditions the level of lice infestation on fish fry in cages.

Conclusion. The fry of all five fish species (pike, pike-perch, bream, vimba and peled) reared in illuminated cages were infected with *Argulus foliaceus* L. Infestation of fish by this parasite started when fish total length reached 1.8-2.0 cm. Reliable correlation between the prevalence of *Argulus* and the total length of fish fry (r = 0.96, p = 0.049) and water temperature (r = 0.95, p = 0.045) was found. Low water temperature (mean 9^{O}C) prevented pike fry from being infected with *A. foliaceus* (prevalence = 0.01%), whereas high water temperature (23-24^{O}C) caused a very high infection level of peled with the parasite (prevalence increased to 77.8%). The prevalence of argulus was significantly higher on peled than on bream (t-test = -5.748, p = 0.029) and vimba (t-test = -5.167, p = 0.035) of the same length reared in illuminated cages.

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