

Research Article

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Table S1. Species composition of fish in Lake Medard (Peterka et al. 2022) and Lake Otakar (Czech Anglers Union, pers. comm.).

Lake	Family	Fish species	Common name
Medard	Leuciscidae	<i>Abramis brama</i> (Linnaeus) ^a	Common bream
	Leuciscidae	<i>Alburnus alburnus</i> (Linnaeus) ^a	Common bleak
	Leuciscidae	<i>Blicca bjoerkna</i> (Linnaeus) ^a	Silver bream
	Leuciscidae	<i>Rutilus rutilus</i> (Linnaeus) ^a	Common roach
	Leuciscidae	<i>Scardinius erythrophthalmus</i> (Linnaeus) ^a	Common rudd
	Leuciscidae	<i>Squalius cephalus</i> (Linnaeus)	European chub
	Tincidae	<i>Tinca tinca</i> (Linnaeus) ^a	Tench
	Esocidae	<i>Esox lucius</i> Linnaeus ^a	Northern pike
	Percidae	<i>Gymnocephalus cernua</i> (Linnaeus)	European ruffe
	Percidae	<i>Perca fluviatilis</i> Linnaeus ^a	European perch
	Percidae	<i>Sander lucioperca</i> (Linnaeus)	Pikeperch
Otakar	Salmonidae	<i>Coregonus maraena</i> (Bloch) ^a	Maraena whitefish
	Salmonidae	<i>Coregonus</i> sp.	Whitefish
	Xenocyprididae	<i>Ctenopharyngodon idella</i> (Valenciennes) ^b	Grass carp
	Cyprinidae	<i>Cyprinus carpio</i> Linnaeus ^b	Common carp
	Esocidae	<i>Esox lucius</i> ^b	Northern pike
	Siluridae	<i>Silurus glanis</i> Linnaeus ^b	Wels catfish

^aPotential second intermediate hosts of *Tylodelphys clavata* (von Nordmann, 1832) (Gibson et al. 2005); ^bPotential definitive hosts of *Sanguinicola* sp. (Gibson et al. 2005, Kirk 2012, Zhokhov et al. 2021)

*Address for correspondence: Miroslava Soldánová, Biology Centre of the Czech Academy of Sciences, Institute of Parasitology, Branišovská 31, 370 05 České Budějovice, Czech Republic. E-mail: soldanova@paru.cas.cz; ORCID-iD: 0000-0002-5277-3799

Table S2. The size of experimental snails, proportion of the snail hepatopancreas occupied by trematode larvae (infection intensity) and daily output rates of *Tylodelphys clavata* (von Nordmann, 1832) and *Sanguinicola* sp. from first snail intermediate hosts pooled across three experimental days. *Abbreviation:* T-A, *T. clavata* in August 2021; T-S, *T. clavata* in September 2021; S-A, *Sanguinicola* sp. in August 2021; S-J, *Sanguinicola* sp. in July 2022.

Trematode	Season/snail code	Length (mm)	Width (mm)	Infection intensity (%)	Output rates Mean $\pm$ SD	Total
<i>T. clavata</i>	August				n^a = 11	
	1T-A	9.56	5.50	70	88 $\pm$ 46	264
	2T-A	8.38	5.54	50	437 $\pm$ 196	1,311
	3T-A	7.16	5.65	30	170 $\pm$ 55	511
	4T-A	6.68	4.68	30	74 $\pm$ 34	221
	5T-A	6.88	4.31	60	— ^b	— ^b
	6T-A	7.00	4.22	50	184 $\pm$ 34	552
	7T-A	7.17	4.31	100	459 $\pm$ 180	1,377
	8T-A	6.83	4.26	60	320 $\pm$ 201	961
	9T-A	9.01	5.75	50	112 $\pm$ 23	336
	10T-A	6.66	4.43	20	205 $\pm$ 44	615
	11T-A	4.88	3.64	30	24 $\pm$ 26	71
	September				n^a = 11	
	2T-S	7.85	4.60	70	985 $\pm$ 200	2,955
	3T-S	9.90	6.08	80	— ^b	— ^b
	4T-S	8.00	4.90	100	1,373 $\pm$ 318	4,119
	5T-S	8.92	5.74	40	256 $\pm$ 291	768
	6T-S	8.88	5.56	80	703 $\pm$ 218	2,109
	7T-S	10.04	6.60	70	1,568 $\pm$ 487	4,704
	8T-S	9.94	6.17	100	2,120 $\pm$ 713	6,360
	9T-S	9.32	5.72	80	1,505 $\pm$ 100	4,515
	10T-S	9.48	6.24	40	— ^b	— ^b
	11T-S	11.61	6.83	100	1,625 $\pm$ 312	4,875
	12T-S	11.39	6.97	80	3,087 $\pm$ 74	9,261
<i>Sanguinicola</i> sp.	August/July^c				n^a = 2/11	
	2S-A	8.89	5.01	70	1,390 $\pm$ 324	4,170
	3S-A	8.12	4.19	80	1,593 $\pm$ 677	4,779
	1S-J	10.79	6.39	80	4,154 $\pm$ 539	12,462
	2S-J	10.08	6.10	90	— ^b	— ^b
	3S-J	10.69	6.08	85	4,404 $\pm$ 690	13,212
	4S-J	10.63	5.97	100	3,143 $\pm$ 632	9,429
	5S-J	11.42	6.83	100	8,676 $\pm$ 2,245	26,028
	6S-J	9.34	5.55	90	3,060 $\pm$ 1,496	9,180
	7S-J	7.83	4.32	100	1,640 $\pm$ 592	4,920
	8S-J	11.47	6.92	100	5,425 $\pm$ 3,245	16,275
	9S-J	12.89	8.09	100	8,189 $\pm$ 1,193	24,567
	10S-J	11.36	7.01	100	3,070 $\pm$ 1,129	9,210
	11S-J	11.04	7.02	90	5,711 $\pm$ 433	17,133

^aNumber of infected snails entered into each experiment; ^bSnail died during the experiment; ^cPooled data from August 2021 and July 2022 considered as August

Table S3. Summary data for the newly generated sequences of *Tylodelphys clavata* (von Nordmann, 1832) ex *Ampullaceana balthica* (Linnaeus) from Lake Medard and *Sanguinicola* sp. ex *Radix auricularia* (Linnaeus) from Lake Otakar.

Trematode	Isolate	28S	GenBank accession number	
			cox1	ITS1-5.8S-ITS2
<i>T. clavata</i>	E1	—	PQ736934	—
	E2	—	PQ736935	—
	E3	PQ738921	PQ736936	PQ736521
	E4	—	PQ736937	—
	E5	—	PQ736938	—
	E6	—	PQ736939	—
	E7	—	PQ736940	—
	E8	—	PQ736941	—
	E9	—	PQ736942	—
	E10	—	PQ736943	—
	E11	—	PQ736944	—
	E12	—	PQ736945	—
	E13	—	PQ736946	—
	E14	—	PQ736947	—
	E15	—	PQ736948	—
	E16	—	PQ736949	—
	E17	—	PQ736950	—
	E18	—	PQ736951	—
	E19	—	PQ736952	—
<i>Sanguinicola</i> sp.	E27	PQ738922	—	—
	E28	PQ738923	—	—
	E29	PQ738924	—	—
	E30	PQ738925	—	—
	E31	PQ738926	PQ738634	—
	E32	PQ738927	—	—
	E33	PQ738928	—	—
	E34	PQ738929	—	—
	E35	PQ738930	—	—
	E37	PQ738931	—	—
	E38	PQ738932	—	—
	E39	PQ738933	—	—
	E40	PQ738934	—	—

Table S4. Summary data of newly generated ITS-2 sequences of snail hosts infected with *Tylodelphys clavata* (von Nordmann, 1832) ex *Ampullaceana balthica* (Linnaeus) and *Sanguinicola* sp. ex *Radix auricularia* (Linnaeus).

Snail host	Locality	Isolate	GenBank accession number
<i>A. balthica</i>	Medard	RA1	PQ778950
		RA2	PQ778951
		RA3	PQ778952
		RA4	PQ778953
<i>R. auricularia</i>	Otokar	RA5	PQ778954
		RA6	PQ778955
		RA7	PQ778956
		RA8	PQ778957

Table S5. Results of statistical pairwise comparison assessing the daily periodicity in cercarial emergence of *Tylodelphys clavata* (von Nordmann, 1832) and *Sanguinicola* sp. from *Ampullaceana balthica* (Linnaeus) and *Radix auricularia* (Linnaeus) snails, using Tukey's Honest Significance Difference *post-hoc* tests in repeated measures ANOVA.

Trematode	Season	Daily interval comparison					
<i>T. clavata</i>	August	Sunrise = Day ^d	Sunrise < Sunset ^c	Sunrise < Night ^b	Day < Sunset ^b	Day < Night ^a	Sunset = Night ^d
	September	Sunrise = Day ^d	Sunrise < Sunset ^b	Sunrise < Night ^b	Day < Sunset ^c	Day < Night ^b	Sunset = Night ^d
	August & September	Sunrise = Day ^d	Sunrise < Sunset ^c	Sunrise < Night ^c	Day < Sunset ^c	Day < Night ^c	Sunset = Night ^d
<i>Sanguinicola</i> sp.	August/July ^e	Sunrise = Day ^d	Sunrise = Sunset ^d	Sunrise < Night ^c	Day = Sunset ^d	Day < Night ^c	Sunset < Night ^b

^aStatistically significant results at $P < 0.05$; ^bStatistically significant results at $P < 0.01$; ^cStatistically significant results at $P < 0.001$; ^dNot significant;

^ePooled data from August 2021 and July 2022 considered as August

Table S6. Results of Pearson's correlation analyses testing the relationship between the number of emerged cercariae of *Tylodelphys clavata* (von Nordmann, 1832) and *Sanguinicola* sp. from first intermediate snail hosts and water temperature, light intensity, snail length and intensity of infection. Results are shown for each experimental day and pooled across days. Statistically significant results ($P < 0.05$) are indicated in bold.

Trematode	Season/ Day	No. of snails	Temperature		Light intensity		Snail length		Intensity of infection	
			r^a	P^b	r	P	r	P	r	P
<i>T. clavata</i>	August									
	Day 1	10	0.750	0.250	-0.797	0.203	0.218	0.545	0.344	0.331
	Day 2	10	0.449	0.551	-0.869	0.131	0.424	0.222	0.490	0.151
	Day 3	10	0.478	0.522	-0.867	0.134	0.349	0.323	0.422	0.224
	Pooled data	10	0.556	0.061	-0.687	< 0.01	0.321	0.366	0.477	0.164
	September									
	Day 1	9	0.714	0.494	-0.544	0.456	0.516	0.155	0.414	0.268
	Day 2	9	0.691	0.310	-0.773	0.227	0.511	0.160	0.776	< 0.05
	Day 3	9	0.666	0.335	-0.707	0.293	0.415	0.267	0.800	< 0.01
	Pooled data	9	0.350	0.292	-0.644	< 0.05	0.537	0.136	0.524	0.147
<i>Sanguinicola</i> sp.	August/July^c									
	Day 1	12	0.532	0.469	-0.773	0.227	0.794	< 0.01	0.564	0.056
	Day 2	12	0.916	0.084	-0.173	0.828	0.907	< 0.001	0.376	0.229
	Day 3	12	-0.352	0.648	0.594	0.165	0.810	< 0.001	0.420	0.175
	Pooled data	12	0.485	0.111	-0.333	0.290	0.889	< 0.001	0.497	0.100

^aPearson's correlation coefficient; ^bProbability value; ^cPooled data from August 2021 and July 2022 considered as August

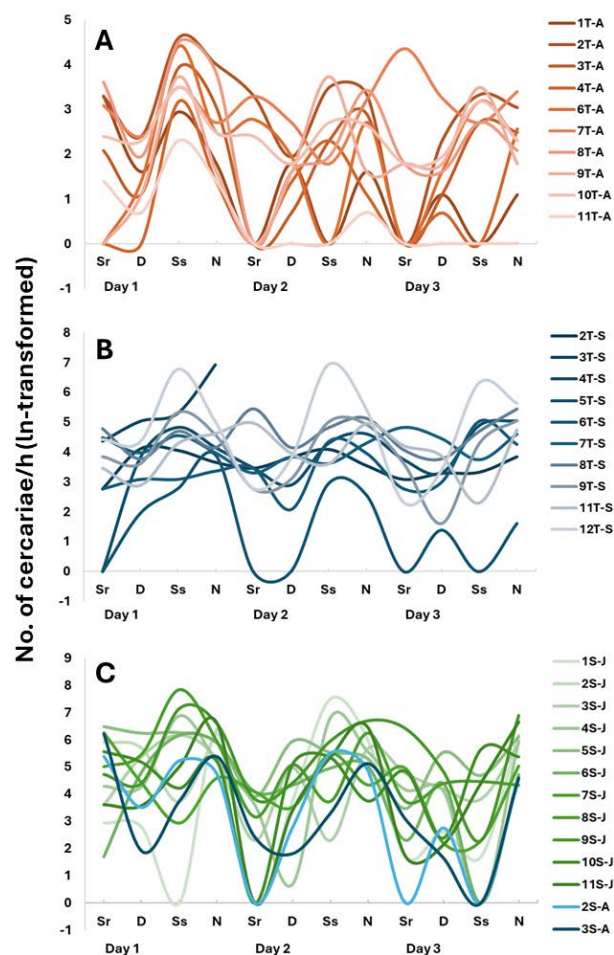


Fig. S1. Patterns in daily cercarial emergence of experimental trematodes from their snail hosts (converted to 1 h). **A** – *Tylodelphys clavata* (von Nordmann, 1832) ex *Ampullaceana balthica* (Linnaeus) in August 2021; **B** – *T. clavata* (ex *A. balthica*) in September 2021; **C** – *Sanguinicola* sp. ex *Radix auricularia* (Linnaeus) in August 2021 and July 2022. Lines represent number of emerged cercariae for each daily interval (Sr, sunrise; D, day; Ss, sunset; N, night) from an individual snail host across three experimental days. Abbreviation: T-A, *T. clavata* in August 2021; T-S, *T. clavata* in September 2021; S-A, *Sanguinicola* sp. in August 2021; S-J, *Sanguinicola* sp. in July 2022.

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