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Investigation of *Ichthyophthirius multifiliis* infection in fish from natural water bodies in the Lhasa and Nagqu regions of Tibet

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ABSTRACT

This study aimed to examine the prevalence of *Ichthyophthirius multifiliis* in fish inhabiting natural water bodies in the Lhasa and Nagqu regions of Tibet in September 2020 and August 2021. The results showed that *Schizopygopsis selincuoensis* had the highest prevalence of *I. multifiliis* at 33.73% (56/166), followed by *Triplophysa tibetana* at 30.00% (6/20), *Triplophysa brevicauda* at 27.91% (12/43) and *Schizopygopsis thermalis* at 23.66% (31/131). No infection with *I. multifiliis* was observed in exotic fish species. In addition, the prevalence of *I. multifiliis* in Boqu Zangbo (river), Selincuo Lake and Cuona Lake in the Nagqu region was found to be significantly higher than that in Lalu Wetland and Chabalang Wetland in the Lhasa region ($P < 0.05$). The study revealed a significantly lower prevalence in Lhasa River than in Cuona Lake ($P < 0.05$). Notably, our findings revealed instances of *I. multifiliis* infections even in saline water bodies, thereby emphasizing the potential threat that this parasite poses to the preservation of indigenous fish resources in Tibet. Consequently, immediate and effective countermeasures are imperative. This study represents the first systematic investigation of *I. multifiliis* infection in natural water bodies in Tibet.

1. Introduction

Ichthyophthirius multifiliis is a freshwater fish parasite that is widely distributed and known for the significant harm it causes to fish populations. This highly pathogenic ciliate exhibits a broad range of host compatibility and is capable of infecting nearly all species of freshwater fish. It primarily infects the epithelial tissues of the host's gills and body surface, resulting in the formation of conspicuous white spots, commonly known as "white-spot" disease or ichthyophthiriasis (Li et al., 2023). The life cycle of *I. multifiliis* comprises three distinct stages: the trophont, which parasitizes the surface of fish; the tomont, which undergoes asexual reproduction; and the theront, which has a brief period of free-living and infectious activity (Tange et al., 2020). However, some scholars have also defined a fourth stage, the protomont, to describe the parasite in the period when it has fallen off the fish epidermis but has not yet transformed into a tomont (Li et al., 2023; Ewing et al., 1985).

Ichthyophthiriasis not only inflicts significant harm on freshwater aquaculture fish globally (Matthews, 1994; Gholami et al., 2016) but also presents a substantial risk to the preservation of rare aquatic resources in natural water bodies. For instance, *Coreius guichenoti*, a second class nationally protected fish species in China, exhibits a high vulnerability to *I. multifiliis* infection (Zhang and Chen, 2005). Consequently, this infection has led to a substantial decrease in the population of *C. guichenoti*. Furthermore, Tian et al. (2017) demonstrated the susceptibility of *Gymnocypris przewalskii*, a native fish species in Qinghai Lake, to *I. multifiliis*, which highlights one of the challenges in conserving native fish populations on the Qinghai-Tibet Plateau.

Tibet has unique natural conditions and complex hydrological patterns that nurture endemic fish resources. The Lhasa River in the Yarlung Zangbo River basin is one of the highest rivers in the world, with diverse topography and geomorphology. However, with rapid population growth and economic development in recent years, this region is facing increasing environmental pressures due to excessive exploitation and utilization of its resources (Lin et al., 2021). These pressures pose a

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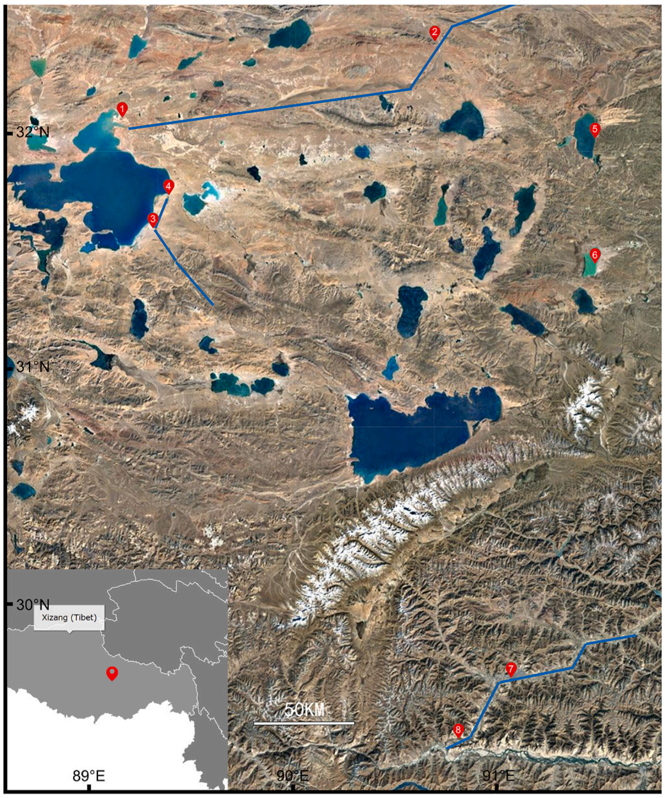

Fig. 1. The map shows the sampling locations of fish in Tibet: 1. North shore of Selincuo (89.112679E, 32.102116N, 4558 m altitude); 2. Za'gya Zangbo (river) (90.723403E , 32.437662N , 5060 m altitude); 3. Boqu Zangbo (river) (89.292002E , 31.606048N , 4579 m altitude); 4. Boqu estuary (89.354410E , 31.762732N , 4554 m altitude); 5. Cuona lake (91.547087E, 32.012987N, 4726 m altitude); 6. Cuoe lake (91.545788E, 31.474142N, 4541 m altitude); 7. Lalu Wetland (91.099541E, 29.668837N, 4989 m altitude); 8. Chabalang Wetland (90.832207E, 29.380565N, 3590 m altitude).

threat to the survival of native fishes. In contrast, the Nagqu region is situated amidst the Tanggula, Nyainqentanglha and Gangdise mountain ranges within the Qinghai-Tibet Plateau's hinterland. This area boasts abundant water resources with minimal human disturbance.

The fish species found in Tibet exhibit a relatively simple composition, encompassing 3 orders, 5 families and 22 genera. The dominant groups are the Schizothoracinae, Nemacheilinae and Cyprinidae (Pan et al., 2019). The subfamily Schizothoracinae is the most diverse and abundant, as well as the most economically valuable, group of native fish species in the plateau regions; the subfamily Nemacheilinae can thrive at altitudes up to 5600 m above sea level; and members of the subfamily Cyprinidae primarily inhabit the lower reaches of the Yarlung Zangbo River and are known for their delicious meat and high fisheries value. Most of these native fish are adapted to cold-water environments, typically showing slow growth rates and low fecundity (Liu et al., 2020). Among them, *Schizothorax waltoni*, *Schizothorax macropogon* , *Oxygymnocypris stewartii* and *Glyptosternum maculatum* hold the status of national second class protected species in China.

The origin and evolution of these species is closely related to the unique environment and natural isolation of the Qinghai-Tibet Plateau. The Tibetan region is characterized by its fragile alpine wetlands, which are highly susceptible to environmental and other changes and require urgent conservation measures. In these wetlands, the preservation and propagation of native fish species faces various challenges, including the spread of fish diseases (Chen, 2022; Li et al., 2008; Pan et al., 2022) and the invasion of exotic fish species (Zhu et al., 2022). For example, exotic fish species possess characteristics, such as their small body size, short generation cycle and early maturation, that allow them to adapt to the variable and vulnerable environment of the plateau (Sun et al., 2022). Consequently, they can negatively impact native fish populations through predation, competition, and other mechanisms, resulting in a decline in the numbers of native species. In addition, these exotic fish species have the potential to transmit foreign pathogens to native fish species that have not yet acquired resistance (Salgado-Maldonado and Pineda-López., 2003; Reading et al., 2012; Lymbery et al., 2010). This phenomenon has the potential to cause significant harm to native fish populations. However, there is currently a lack of research regarding the occurrence of *I. multifiliis* infection in fish inhabiting natural water bodies in Tibet. The aim of this study was therefore to conduct a systematic survey on the prevalence of *I. multifiliis* infection in natural water bodies in Tibet. The survey results will provide crucial, basic data to inform risk assessment, monitoring, and early warning systems for ichthyophthiriasis in fish populations in Tibet. Furthermore, the findings of this survey will lay the groundwork for the conservation and protection of rare fish resources in Tibet.

2. Materials and methods

2.1. Sampling sites

The sampling sites are in the interior of the Qinghai-Tibet Plateau (Fig. 1), mainly encompassing Selincuo Lake, Za'gya Zangbo (river), Boqu Zangbo (river), Cuona Lake, and Cuoe Lake in the Nagqu Prefecture, and the Lhasa River, Lalu Wetland and Chabalang Wetland in the Yarlung Zangbo River Basin of the Lhasa Prefecture. The geographical coordinates are N29.37°–32.92°, E89.01°–91.98°, with altitudes between 3600 and 5000 m.

2.2. Collection of fish samples

With the authorization of the Science and Technology Department of Tibet and the Forestry and Grass Department of Nagqu City, we collected wild fish, using gill-nets, sticky nets, and traps, from the Lhasa River, Lalu Wetland and Chabalang Wetland in the Yarlung Zangbo River basin and Za'gya Zangbo (river), Boqu Zangbo (river), and Selincuo Lake in the Nagqu region, Tibet, during September 2020 and August 2021.

2.3. Measurement indicators and data analysis

The live fish were transported to the laboratory and examined within 48 h. A numerical label was assigned to each fish based on its location information, followed by taxonomic identification of the respective species. Subsequently, measurements were taken to determine the body length and weight of each fish (Table 1). Each fish host was examined comprehensively under a stereomicroscope, including external surfaces, caudal fin, gill filaments, and mucus. The number of *I. multifiliis* observed in each fish was recorded according to the methods of Hu (2023). Prevalence (proportion of fish that are infected within the same species, expressed as a percentage), mean intensity of infection (mean number of *I. multifiliis* per infected fish) and mean abundance (mean number of *I. multifiliis* per fish sampled within the same fish species) were calculated according to Bush et al. (1997) (Table 2). Due to constraints imposed by regional and local policies during the data collection process, the sample size was relatively limited. Therefore, no data correction was performed on the infection rate data, as the results of correct analysis on such constrained data would be meaningless.

To test the normality of the *I. multifiliis* prevalence data across different locations, we conducted the Shapiro–Wilk test. The measured variables did not meet the assumption of normality ($P < 0.05$) (Table 3). Therefore, a non-parametric method (Kruskal–Wallis test) was used to analyze the prevalence data after grouping them according to different regions.

We also measured the salinity of the water at sampling locations for genus *Schizopygopsis* in the Nagqu region (Table 4), as we had observed a

Table 1
Freshwater fish species recorded in the Lahsa River basin and Nagqu regions, Tibet, and the number of fish infected with *Ichthyophthirius multifiliis*.

Family	Species	Number collected	Number infected	Prevalence (%)	Mean length (mm)	Mean weight (g)	Species authority
Cobitidae	*Misgurnus anguillicaudatus*	23	0	–	84.0 (31.0–150.0)	4.6 (0.3–27.9)	Cantor, 1842
	Paramisgurnus dabryanus	20	0	–	106.4 (104.3–108.5)	12.1 (10.9–13.2)	Sauvage, 1878
Cyprinidae	*Carassius auratus*	192	0	–	85.2 (34.0–219.2)	20.7 (1.2–134.5)	Linnaeus, 1758
	Cyprinus carpio	7	0	–	189.0 (100.0–420.0)	356.5 (32.8–2086.7)	Linnaeus, 1758
	Ptychobarbus dipogon	6	0	–	355.7 (343.0–366.0)	422.6 (395.1–454.3)	Regan, 1905
	Pseudorasbora parva	29	0	–	60.1 (28.0–85.1)	4.5 (0.2–68.3)	Temminck and Schlegel, 1846
	Schizothorax macropogon	19	0	–	421.0 (415.0–427.0)	1280.7 (1088.0–1473.4)	Regan, 1905
	Schizothorax oconnori	19	0	–	376.4 (277.0–489.0)	798.7 (219.5–1533.6)	Lloyd, 1908
	Schizothorax waltoni	22	0	–	389.8 (64.0–511.0)	948.4 (4.1–1288.1)	Regan, 1905
	Schizopygopsis thermalis	131	31	23.66	279.6 (28.0–554.0)	329.4 (0.3–1055.0)	Herzenstein, 1891
	Schizopygopsis selincuoensis	166	56	33.73	307.0 (161.0–460.0)	322.6 (78.9–777.0)	Chen & Cao, 2000
	Schizopygopsis younghusbandi	78	10	12.82	108.5 (37.0–342.0)	42.2 (0.5–485.4)	Regan, 1905
Nemacheilidae	*Triplophysa brevicauda*	43	12	27.91	65.5 (28.0–139.0)	4.4 (0.3–21.3)	Herzenstein, 1888
	Triplophysa tibetana	20	6	30.00	89.9 (40.0–163.0)	8.0 (0.4–41.7)	Regan, 1905
	Triplophysa orientalis	52	1	1.92	73.9 (29.0–122.0)	4.8 (0.4–16.3)	Herzenstein, 1888
	Triplophysa stenura	5	1	20.00	58.0 (33.0–91.0)	1.6 (0.5–4.4)	Herzenstein, 1888
	Triplophysa stewartii	13	0	–	69.9 (47.0–101.0)	3.9 (0.8–8.5)	Hora, 1922
	Triplophysa stoliczkae	8	0	–	89.3 (42.0–136.0)	6.7 (0.8–18.7)	Steindachner,1866
Siluridae	*Silurus asotus*	13	0	–	217.1 (149.2–256.0)	75.6 (32.0–128.9)	Linnaeus, 1758

Fish lengths and weights expressed as mean (minimum–maximum).

Table 2
Prevalence of freshwater fish species recorded in different localities in the Lhasa River basin and Nagqu region, Tibet, and the number, intensity, and abundance of *I. multifiliis* parasites infecting them.

Species	Locality	FN (n)	IH (n)	PN (n)	MA	P (%)	MI
Schizopygopsis selincuoensis	ZZ	94	31	825	8.78	32.98	26.61
	BZ	47	23	1172	24.94	48.94	50.96
	SL	25	2	44	1.76	8.00	22.00
Triplophysa tibetana	ZZ	17	6	202	12.63	35.29	33.67
	BZ	3	0	0	0.00	0.00	–
Triplophysa brevicauda	ZZ	15	0	0	0.00	0.00	–
	BZ	12	3	302	25.17	25.00	100.67
	CNL	16	9	383	23.94	56.25	42.56
Schizopygopsis thermalis	CEL	39	7	220	5.64	17.95	31.43
	CNL	63	23	1274	20.22	36.51	55.39
	SL	15	1	101	6.73	6.67	101.00
	ZZ	14	0	0	0.00	0.00	–
Triplophysa stenura	LR	5	1	20	4.00	20.00	20.00
Schizopygopsis younghusbandi	LR	78	10	80	1.03	12.82	8.00
Triplophysa orientalis	CNL	16	1	10	0.63	6.25	10.00
	ZZ	5	0	0	0.00	0.00	–
	LR	18	0	0	0.00	0.00	–
	LW	7	0	0	0.00	0.00	–
Paramisgurnus dabryanus	LW	11	0	0	0.00	0.00	–
	LR	9	0	0	0.00	0.00	–
Carassius auratus	LW	174	0	0	0.00	0.00	–
	LR	4	0	0	0.00	0.00	–
	CW	14	0	0	0.00	0.00	–
Schizothorax macropogon	LR	19	0	0	0.00	0.00	–
Schizothorax waltoni	LR	18	0	0	0.00	0.00	–
	LW	4	0	0	0.00	0.00	–
Cyprinus carpio	LW	3	0	0	0.00	0.00	–
	LR	2	0	0	0.00	0.00	–
	CW	2	0	0	0.00	0.00	–
Pseudorasbora parva	CW	5	0	0	0.00	0.00	–
	LW	24	0	0	0.00	0.00	–
Misgurnus anguillicaudatus	CW	14	0	0	0.00	0.00	–
	LW	9	0	0	0.00	0.00	–
Silurus asotus	CW	7	0	0	0.00	0.00	–
	LW	5	0	0	0.00	0.00	–
	LR	1	0	0	0.00	0.00	–
Ptychobarbus dipogon	LW	6	0	0	0.00	0.00	–
Triplophysa stoliczkae	CNL	8	0	0	0.00	0.00	–
Schizothorax oconnori	LR	19	0	0	0.00	0.00	–
Triplophysa stewartii	CEL	13	0	0	0.00	0.00	–

Localities and Codes: BZ, Boqu Zangbo (river); CEL, Cuoe lake; CNL, Cuona lake; CW, Chabalang Wetland; LR, Lhasa River; LW, Lalu Wetland; SL, Selincuo Lake ZZ, Za'gya Zangbo (river). IH, number of infected hosts by each species; P (%) prevalence of the location; PN, number of parasites; FN, number of examined fishes; MI, mean intensity; MA, mean abundance.

Table 3
Normality test of prevalence data.

	Statistic	df	Significant
Prevalence	0.578	41	<0.001

gradient of salinity at these sites, and we calculated the prevalence of genus *Schizopygopsis* in these samples. Given that both the prevalence and salinity data conformed to a normal distribution ($P > 0.05$) (Table 5), we employed Pearson correlation analysis to investigate the relationship between prevalence and salinity.

All the above analyses provided confidence intervals with a significance level of 95%. A p-value below 0.05 was considered indicative. All analyses were performed using SPSS 27.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Infection status of I. multifiliis in Tibetan fishes

A total of 866 fish of 19 species were examined in Tibet (Table 1), including two Cobitidae, ten Cyprinidae, six Nemacheilidae, and one Siluridae species. With *C. auratus*, *S. selincuoensis* and *S. thermalis* being collected in the highest numbers.

The fish from the subfamily Schizothoracinae, such as the genus *Schizopygopsis*, and the family Nemacheilidae, such as the genus *Triplophysa*, exhibited a relatively higher prevalence of *I. multifiliis* than the other fish examined (Table 1). *Schizopygopsis selincuoensis* (33.73%) exhibited the highest prevalence, followed by *Triplophysa tibetana* (30.00%) and *Triplophysa brevicauda* (27.91%). The other two species of *Schizopygopsis*, *Schizopygopsis thermalis* and *Schizopygopsis younghusbandi*, were also found to be infected but at a lower level. In terms of parasite infection intensity and abundance (Table 2), the mean intensity and mean abundance in fish in the Boqu Zangbo (river), such as *S. selincuoensis* (MA = 24.94, MI = 50.96) and *T. brevicauda* (MA = 25.17, MI = 100.67), were relatively high. Conversely, the mean intensity and mean abundance in fish in Selincuo Lake and Lhasa River, such as *S. selincuoensis* (MA = 1.76, MI = 22.0), *T. stenura* (MA = 4.0, MI = 20.0), and *S. younghusbandi* (MA = 1.03, MI = 8.0), were comparatively low. In addition, varying degrees of infection were also observed in Cuona Lake, Cuoe Lake, and Za'gya Zangbo (river). This indicates that *I. multifiliis* is widespread in Cuoe Lake, Cuona Lake, Za'gya Zangbo (river) and Boqu Zangbo (river) in the Nagqu region and more inclined to parasitize the genera *Schizopygopsis* and *Triplophysa*. No instances of infection were observed in exotic species, such as *C. auratus*, *C. carpio*, *P. parva*, *M. anguillicaudatus*, and *S. asotus*.

Table 4
Salinity in Nagqu region.

Locality	Salinity (‰)
BZ1	0.17
BZ2	0.18
BZ3	0.19
BZ4	0.71
Boqu Estuary 1	7.09
Boqu Estuary 2	6.04
Boqu Estuary 3	6.82
Boqu Estuary 4	6.78
Boqu Estuary 5	7.06
Boqu Estuary 6	0.71
SL1	4.82
SL2	4.62
SL3	4.57
SL4	4.58
ZZ1	0.09
ZZ2	0.31
ZZ3	0.29
ZZ4	0.28
ZZ5	4.26
Northern CEL1	3.54
Northern CEL2	4.47
Eastern CEL 1	3.54
Eastern CEL 2	3.63
Southern CEL1	3.42
Southern CEL2	4.64
Western CEL1	4.38
Western CEL2	4.44
Northern CNL1	0.27
Northern CNL2	0.3
Eastern CNL 1	0.3
Eastern CNL 2	0.3
North Eastern CNL1	0.27
North Eastern CNL2	0.24
Southern CNL1	0.27
Southern CNL2	0.27

Table 5
Normality test of Prevalence and Salinity.

	Statistic	df	significance
Prevalence	0.923	6	0.528
Salinity	0.833	6	0.114

3.2. *I. multifiliis* infection from different localities

The prevalences of *I. multifiliis* in Selincuo Lake, Boqu Zangbo (river) and Cuona Lake in the Nagqu region were significantly higher than those in Lalu Wetland and Chabalang Wetland in Lhasa ($P < 0.05$) (Table 6; Fig. 2). In addition, the prevalence in Lhasa River was significantly different from that in Cuona Lake ($P < 0.05$). The highest prevalence in Boqu Zangbo (river) was found in *S. selincuoensis* (48.94%; Table 2). In Cuona Lake, *S. thermalis* and *T. brevicauda* had high prevalences (36.51% and 56.25%, respectively). In Cuoe Lake, the highest prevalence was also found in *S. thermalis* (17.95%). In Za'gya Zangbo (river), high prevalences were observed in *T. tibetana* (35.29%) and *S. selincuoensis* (32.98%). The other species of *Triplophysa* and *Schizopygopsis* were not infected. Moreover, *I. multifiliis* infection was rare or absent in fish from Lalu Wetland and Chabalang Wetland.

3.3. Correlation analysis of salinity and *I. multifiliis* prevalence

The prevalence of *I. multifiliis* in the genus *Schizopygopsis* varied depending on the salinity of the sampling sites in Za'gya Zangbo (river), Boqu Zangbo (river), and Selincuo Lake. The prevalence was higher in areas with low salinity, such as the upper reaches of Za'gya Zangbo, Boqu Zangbo and Cuona Lake (Table 7; Fig. 3), and significantly lower in high salinity areas, such as the north and south shores of Selincuo Lake

Table 6
Pairwise comparisons of locality.

Sample 1-Sample 2	Test Statistic	Standard Error	Standrad Test Statistic	Significant
CW-BZ*	15.333	7.033	2.180	0.029
LW-BZ*	15.333	6.340	2.419	0.016
CW-CEL	9.500	8.058	1.179	0.238
LW-CEL	9.500	7.460	1.273	0.203
CW-CNL*	16.250	6.460	2.515	0.012
LW-CNL*	16.250	5.698	2.852	0.004
CW-LW	0.000	5.275	0.000	1.000
CW-LR	−3.800	5.275	−0.720	0.471
CW-ZZ	−9.000	6.091	−1.478	0.140
CW-SL*	−16.500	8.058	−2.048	0.041
LW-LR	−3.800	4.307	−0.882	0.378
LW-ZZ	−9.000	5.275	−1.706	0.088
LW-SL*	−16.500	7.460	−2.212	0.027
LR-ZZ	−5.200	5.275	−0.986	0.324
LR-CEL	5.700	7.460	0.764	0.445
LR-BZ	11.533	6.340	1.819	0.069
LR-CNL*	12.450	5.698	2.185	0.029
LR-SL	12.700	7.460	1.702	0.089
ZZ-CEL	0.500	8.058	0.062	0.951
ZZ-BZ	6.333	7.033	0.900	0.368
ZZ-CNL	7.250	6.460	1.122	0.262
ZZ-SL	7.500	8.058	0.931	0.352
CEL-BZ	5.833	8.792	0.664	0.507
CEL-CNL	−6.750	8.340	−0.809	0.418
CEL-SL	−7.000	9.631	−0.727	0.467
BZ-CNL	−0.917	7.356	−0.125	0.901
BZ-SL	−1.167	8.792	−0.133	0.894
CNL-SL	−0.250	8.340	−0.030	0.976

Each row tests the null hypothesis that the Sample 1 and Sample 2 distributions are the same. Asymptotic significances (2-sided tests) are displayed. The significance level is 0.050.
*: Indicates a significant difference between sample 1 and sample 2.

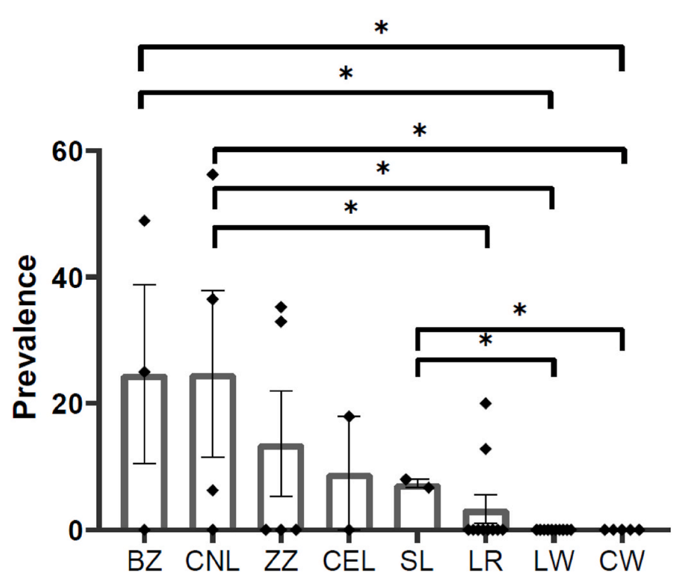

Fig. 2. Pairwise comparisons of locality. Localities: BZ, Boqu Zangbo (river); CEL, Cuoe lake; CNL, Cuona lake; CW, Chabalang Wetland; LR, Lhasa River; LW, Lalu Wetland; SL, Selincuo Lake ZZ, Za'gya Zangbo (river). *: Indicates a significant difference between location 1 and location 2.

(Boqu Estuary) and Cuoe Lake. However, it is noteworthy that there was still a relatively high prevalence (17.95%) in Cuoe Lake, where the salinity was 4.01 ± 0.02 ppt. The Pearson correlation analysis revealed a significant negative association between prevalence and salinity ($P < 0.05$; Table 8).

Table 7
Ichthyophthirius multifiliis infection in *Schizopygopsis* at different salinities.

Location	FN (n)	IH (n)	P (%)	MS (‰) ± SD
Za'gya Zangbo	94	31	32.98%	0.24 ± 0.27
Cuona Lake	63	23	36.51%	0.28 ± 0.1
Boqu Zangbo	47	23	48.93%	0.31 ± 0.1
Cuoe Lake	39	7	17.95%	4.01 ± 0.02
North shore of Selincuo	15	1	6.67%	4.64 ± 0.52
Boqu Estuary	25	2	8.00%	6.76 ± 0.42

Codes: IH, number of infected hosts by each species; P (%), prevalence; FN, number of examined fishes; MS, mean salinity. SD, standard deviation.

4. Discussion

Studies on fish parasites in Tibet are still in a nascent phase (Chen, 2022), with only a few works published to date (Kuang, 1964; Li et al., 2008; Pan et al., 2022). Prior to this study, no information was available on the prevalence of *I. multifiliis* in natural water bodies in Tibet, with only two cases of mass infection reported in *S. macropogon* (Li et al., 2022) and *O. stewartii* (Yang et al., 2020) under artificial breeding conditions. Considering the enormous harm that *I. multifiliis* inflicts on fish populations in other parts of the world, and for the purpose of protecting the indigenous fish resources of Tibet, we conducted the present survey of the prevalence of *I. multifiliis* in natural water bodies in Tibet. This is the first systematic investigation of *I. multifiliis* infection in natural fish populations in the Tibetan region.

The introduced fish species examined in this study, including *C. auratus*, *C. carpio*, *P. parva*, *M. anguillicaudatus*, and *S. asotus*, did not exhibit any signs of parasitism by *I. multifiliis*. The potential reason for this phenomenon may be the limited sampling number of certain species, such as carp (*Cyprinus carpio*) and catfish (*Silurus asotus*), resulting in no diseased fish being captured. Introduced fish are primarily found in regions characterized by high levels of human activity, such as the middle reaches of the Yarlung Zangbo River (Yang et al., 2010), Chabalang Wetland, and Lalu Wetland in the Lhasa River basin (Zhu et al., 2022; Chen and Chen, 2010). Among them, *C. auratus*, *P. parva*, and other dominant species have successfully established natural populations (Hu et al., 2019; Zhu et al., 2022). In these particular localities, ichthyophthiriasis among indigenous fish is infrequent, with lower infection intensity and abundance than in the Nagqu region. The possible reason for this could be that the fish residing in the Lhasa River basin have developed a survival mechanism to adapt to coexistence with *I. multifiliis* to some extent. This mechanism may involve the activation of both innate and adaptive immune responses (Teixeira Alves and Taylor, 2020) and may have ultimately led, over time, to a reduction in overall infection prevalence. In contrast, the fish species in the rivers and lakes of the Nagqu region in the northern Tibetan Plateau are predominantly native Tibetan fish (Chen et al., 2001), mainly the genus *Schizopygopsis* and *Triplophysa*. The response mechanisms of these fishes to infection with *I. multifiliis* appear to be either underdeveloped or in the process of gradual development and the fish appear to lack an evolutionary co-adaptation process with the parasite (Honka and Sures, 2021), leaving them vulnerable to severe damage by the parasite. It is therefore evident that *I. multifiliis* presents a significant potential hazard to indigenous fish species.

When sampling in Za'gya Zangbo (river), Boqu Zangbo (river) and Selincuo Lake, we noticed that the salinity changed according to the flow direction of the river. Elevated salinity levels in natural aquatic environments are known to impede the survival of *I. multifiliis* (David, 2012) and our correlation analysis supports this assertion. Previous studies have shown that a salinity of 5 ppt will completely inhibit the normal development of *I. multifiliis* (Aihua and Buchmann, 2001), so this could potentially account for the low intensity and abundance of *I. multifiliis* observed in Cuoe Lake and Selincuo Lake. However, *I. multifiliis* infections could still be observed in Boqu Estuary with salinity levels above 4 ppt. This could be attributed to two possible factors. Firstly, the genus *Schizopygopsis* has been found to have high salt tolerance and migratory characteristics (Chen et al., 2002; Huang et al., 2022). After being infected by *I. multifiliis* in low salinity areas, these fish could therefore have migrated to high salinity areas, where they were subsequently captured prior to the detachment of the trophonts from their body surface. Secondly, it is possible that the *I. multifiliis* commonly found in these areas has become adapted to high salinity environments. If the latter is the case, this will pose a potential threat to the preservation of native fish species in Tibet. Further studies will therefore be carried out to investigate whether *I. multifiliis* in these regions can indeed withstand high salinities.

Table 8
Correlation analysis between salinity and prevalence.

		Prevalence	Salinity
Prevalence	Pearson correlation	1	−.914*
	Significant (2-tailed)	–	.011
	N	6	6
Salinity	Pearson correlation	−.914*	1
	Significant (2-tailed)	.011	–
	N	6	6

*Correlation is significant at the 0.05 level (2-tailed).

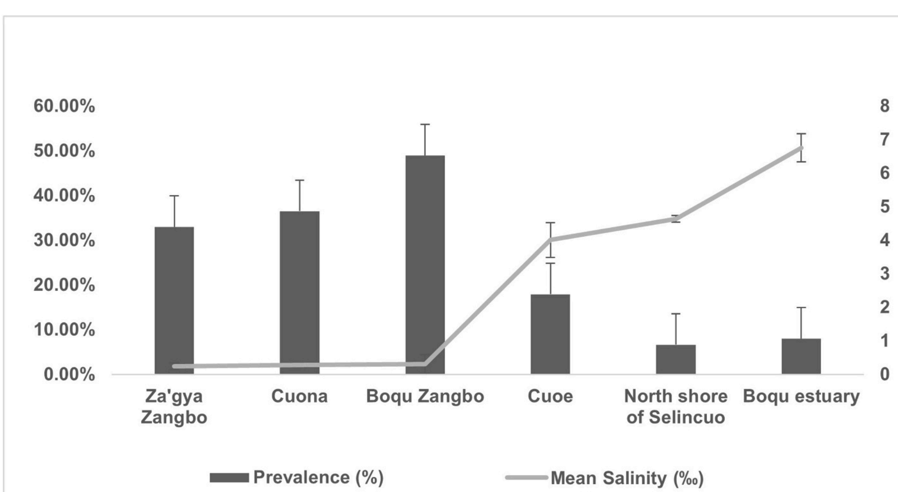

Fig. 3. Prevalence of I. mutlifillis infection in Schizopygopsis at locations of differing salinity.

5. Conclusions

This study represents a pioneering investigation into the prevalence of *I. multifiliis* infection in fish inhabiting natural water bodies in the Lhasa River, certain wetlands and the Nagqu region of Tibet. The variations in infection among different rivers, lakes, and wetlands were also analyzed. Our survey revealed that the prevalence of *I. multifiliis* was higher in the genera *Schizopygopsis* and *Triplophysa*. It also varied greatly across different regions, being significantly higher in Selincuo Lake, Boqu Zangbo (river), and Cuona Lake in Nagqu than in Lalu Wetland and Chabalang Wetland in Lhasa ($P < 0.05$) (Table 6; Fig. 2). This indicates that the *I. multifiliis* infection situation in the Nagqu region may be more severe than in the Lhasa region. By contributing to our knowledge of fish parasites in these regions, this survey has also established a fundamental basis for the assessment of risks to, and preservation of, valuable fish resources in Tibet.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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