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Studies on the population biology of helminth parasites of fish species from the Caspian Sea drainage basin

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Abstract

The northern section of the Caspian Sea and lower reaches of the Zhaiyk (Ural) River is an important fishery for Kazakhstan. In the present study, a total of 1597 individuals of ten fish species were analysed. The fish were caught over three years, from 2018 to 2020. For each species studied – *Abramis brama*, *Alosa saposchnikowii*, *Atherina boyeri caspia*, *Carassius gibelio*, *Chelon auratus*, *Cyprinus carpio*, *Leuciscus aspius*, *Rutilus caspius*, *Sander marinus* and *Sander volgensis* – between 100 and 200 individuals were examined. A series of generalized linear models (GLMs) were used to examine the association between individual parasite intensity of infection and the Fulton index, age, year the fish was captured, where the fish was captured (northern Caspian or Zhaiyk River) and sex. For each GLM, the best-fitting probability distribution was used –either Poisson, zero-inflated Poisson, negative binomial or zero-inflated negative binomial. For some fish/parasite species, an increased Fulton index was associated with higher intensities of parasite infection, whilst, for others, the Fulton index decreased with the intensity of parasite infection. This was also true of age-related intensity of infection, with some parasites having an increased intensity of infection with age whilst others had a decreased intensity of infection with age. There was also some evidence of variation in intensity of parasite infection between different years when the fish were caught. For some species of fish that are endemic to both the fresh waters of the Zhaiyk River and the low-saline waters of the northern Caspian, there were variations in intensity of parasite infection between the two environments. The best-fitting probability distribution also gave some information about the dynamics of infection. No fish species had a Poisson distribution of parasites, which is consistent with an entirely random infection process, with all fish being potentially exposed. For some parasites, the distribution was a zero-inflated Poisson, which is consistent with either the fish being exposed to parasite infection or not; and, if exposed, infection was a random process. Other parasites had a negative binomial distribution, consistent with the entire fish population being exposed, but the infection process was clumped or there were variations in the susceptibility of infection between fish. Finally, some of the parasites had a zero-inflated negative binomial distribution, which can be interpreted as part of the fish population not being exposed and the remainder of the population being exposed to a clumped or aggregated infection process and/or a variation in individual susceptibility to infection.

Introduction

Fish parasitoses act as a potential factor restraining the growth of the fish productivity. Some helminths of fish may also be zoonoses and, therefore, represent a public health problem. The Caspian Sea has a north–south positive gradient of water salinity, from freshwater salinity in the north basin, which has most of the freshwater inflow, to an almost homogeneous 12.5–13.5 parts per thousand surface water salinity in the middle and south basins (Leroy et al., 2007). The north Caspian Sea and associated drainage basin is the most important fishery of Kazakhstan, with about 0.3 million tons of fish caught annually. In total, 80 species or subspecies of fish are found in the northern part of the Caspian Sea (Naseka & Bogutskaya, 2009). These include freshwater species found close to the coast and rivers of the drainage basis, as well as marine species found in areas of higher salinity. Of these, 20 species are being developed by industry. Over a ten-year period, from 2010 to 2019, between 4.5 and 14.5 thousand tons of fish were caught per annum by Kazakhstani fishermen from the Caspian Sea. The largest catches were for bream (25%), carp (18.8), roach (15.9%) and pike perch (9.8%). For 2019, in the Zhaiyk River commercial fish catches included 1970 tons of bream, 670 tons of roach, 340 tons of pike perch, 250 tons of asp, 180 tons of Prussian carp and 138 tons of carp

(Assylbekova et al, 2020). Therefore, it is important to understand the diseases of fish from this region. Such studies may also contribute to ameliorating the public health risk of some helminths as some species such as the asp and pike perch may be heavily infected with *Anisakis* spp. (Abdybekova *et al.*, 2020).

Previous studies on the parasites of fish of commercial importance include a number of studies from the southern sectors of the Caspian, mainly from Iran (Khara *et al.*, 2011; Mazandarani *et al.*, 2016). Studies of fish parasites in the Soviet sector of the Caspian Sea were first carried out in 1931–1932 by Dogel & Bykhovsky (1939) and, more recently, by Tokpan & Rakhimov (2010).

There is less information on the distribution of parasites within host species of fish and how these may provide clues to the dynamics of parasite infection. The simplest of these is the Poisson model. This would be consistent with the fish becoming infected by individual parasites at random. Such a distribution of parasites in their hosts are rarely seen, although they have been described with adult *Taenia* spp. cestodes in dogs (Lahmar *et al.*, 2001). Generally, parasite infection of fish is overdispersed, with a small proportion of the population being heavily infected and the remainder having few or no parasites (see, e.g., Burrough, 1978). This is seen in common parasites of terrestrial vertebrates, which also tend to have an overdispersed parasite distribution (Grenfell *et al.*, 1995). Various probability models can be used to describe this overdispersed distribution of parasites in hosts. The zero-inflated Poisson distribution is an overdispersed distribution where there is an excess of zeros compared to a Poisson distribution. Here, this can be hypothesized to be due to two processes: a proportion of the host population is not exposed to the infectious stage of the parasite, whilst the rest of the population is exposed at random. Again, there are limited examples of parasites of terrestrial vertebrates that are consistent with a zero-inflated Poisson distribution (e.g. Abdybekova & Torgerson, 2012). A negative binomial distribution is consistent with a clumped infection process, although other processes such as variations in resistance to infection between individual hosts may also play a role. A negative binomial model is the most commonly used model and is the extension of a Poisson model for integer counts and can be used to model distributions where the variance of the mean is in excess of the mean abundance. There are numerous examples where parasite distributions have been modelled as a negative binomial distribution both within fish species (e.g. Burrough, 1978; Balling & Pfeiffer, 1997) and with terrestrial vertebrates (e.g. Grenfell *et al.*, 1995; Wilson *et al.*, 1996). The zero-inflated negative binomial distribution is when there is an excess of zeros compared to the negative binomial distribution, and, again, this may occur if the host population is partitioned into one group that is not exposed and another that is exposed to a clumped infection process, or there is a variation in resistance amongst exposed hosts. There are a limited number of studies in fish using the zero-inflated negative binomial, but do include attached sea lice on sea trout (Vollset *et al.*, 2018). For terrestrial vertebrates, such models have been used, for example, to model the distribution of helminths of wild carnivores (Ziadinov *et al.*, 2010) and for parasitic faecal egg counts in agricultural animals (Denwood *et al.*, 2008). A better understanding of such dynamics leads to a deeper understanding of transmission biology.

A previous, more limited study failed to find any association between the condition of the fish and the intensity of infection of individual fish (Abdybekova *et al.*, 2020). Using additional data collected over a further two years, we were able to explore these effects more fully. We were also able to analyse any association with the age of fish, which might give further clues on the infection dynamics and relative resistance to infection. Furthermore, we aimed to identify potential pathogenic effects by analysing the association of Fulton's condition index with the intensity of infection with parasites identified. In these analyses, it was essential to utilize the best generalized linear model (GLM), taking into account the aggregated or zero-inflated distribution to gain inferences with regard to any of these associations.

Materials and methods

In total, 1597 individuals of ten fish species were investigated from the Kazakhstan sector of the Caspian Sea from 2018 to 2020. All fish were collected in late spring to early summer. These included 450 fish collected in 2018 reported in a preliminary study (Abdybekova *et al.*, 2020). The species composition of the fish was determined on the basis of taxonomic descriptions according to Berg (1949), Kazancheyev (1981) and Reshetnikova (2002). A complete biological analysis of the fish was carried out with the determination of the length, mass, sex and maturity stages of the gonads (Pravdin, 1966). The ages of the fish were determined by rings on the scales or otoliths, or by cuts of the marginal rays of the pectoral fins (Chugunova, 1959; Konoplev, 1975). The body length of all fish was measured from the top of the snout to the end of the scaly cover and to the end of the caudal fin. Fish were weighed on an electronic scale with an accuracy of 1 g. For small fish, this was with an accuracy of 0.1 g. Fulton's condition index (F) was calculated for each fish as:

$$F = 100 \times W/L^3$$

where W = the weight in grams and L is the length in cm (Nash *et al.*, 2006).

In the field, a complete parasitological dissection of fish was carried out according to the standard classical method (Skriabin, 1928; Dogel, 1933; Bykhovskaya-Pavlovlskaya, 1969). The results of the autopsies of the fish were recorded. These included the fish species, the place of investigation, sex, age, weight of the fish and the number, species and localization of detected parasites. Fish muscles and all internal organs were examined under a KRUSSMSZ5000 stereomicroscope (Krüss Optronic, Hamburg, Germany) with a range of 7–45×. Parasites were fixed in various fixatives: monogeneans, trematodes, cestodes and parasitic crustaceans in 700 alcohol, and nematodes in Barbagallo fluid. For species identification, nematodes were placed in a solution of glycerol with water (1:1) in order to clear them and then view the internal structure of helminths. This, therefore, enabled taxonomic identification based on the morphological features of the parasites.

Four different models of the probability distribution were analysed. The Poisson model assumes fish are randomly infected, whilst the negative binomial model models overdispersion and is consistent with fish being infected with a clumped infection pressure. The zero-inflated model also models overdispersion, but is consistent with fish belonging to two classes. With the zero-inflated Poisson model, fish are not exposed to infection with parasites and, so, the zero-inflated component consists of having no parasites. The other class are fish which are exposed at random and, consequently, the non-zero inflated partition will have a Poisson distribution of parasites. Finally, the negative zero-inflated binomial distribution has a zero-inflated partition, where fish are not infected, whilst the fish in the other partition have an overdispersed distribution of parasites consistent with a clumped infection process or variability between susceptibility of infection in different individual fish. The data were fit to these four different models using the pscl library in R (Zeileis *et al*., 2008). To investigate any effects of parasitism on the fish, a GLM was used to analyse the association of the intensity of infection of each individual fish using the appropriate probability distribution. For each parasite, a multivariable GLM investigated the association of parasite abundance with the Fulton index, age, gender, year and location of where the fish was caught. Likewise, for the zero-inflated models, the magnitude of the zero inflation was examined for an association with each of these variables. A backward selection method was used with all variables included in the initial model, with each non-significant variable with a $P > 0.15$ being removed sequentially, and only significant variables remaining in the final model. If two or more independent variables remained in the model, it can be interpreted that all these variables had an association with the dependent variable. The regression model with the lowest Akaike information criterion was used to select the most parsimonious model. The relative difference in the odds of the zero-inflated proportion of the infection of fish with parasites according to the risk factors is reported as an odds ratio. The relative difference of abundances between fish according to the risk factors in the non-zero inflated proportion is reported as the incidence rate ratio (IRR). All analyses were undertaken in R (R Core Team, 2019).

Results

The results are summarized in table 1. The prevalences of infection ranged from 1% for the common bream with the parasite *Philometra abdominalis* to 64% for infection of carp with *Anisakis schupakovi*. Abundances were also highly variable, ranging from 0.12 parasites per fish (i.e. about one parasite for every eight fish) for *Khawia sinensis* infection of the common bream to 66 per fish for *A. schupakovi* in the carp. The most likely frequency distribution of each parasite according to host species is given in table 1.

An increase in parasite abundance was associated with an increase in the Fulton index (IRR > 1) for *Diplostomum helveticum* and *P. abdominalis* infection of the common bream; *Diplostomum spathaceum* infection of the Volga pike perch; and *Ligoforus vanbenedenii* and *Tylodelphis clavata* infection of the golden grey mullet. In contrast, increasing parasite abundance was associated with a decrease in the Fulton index for *Diplostomum gobiorum* infection in carp; *D. spathaceum* infection in the Caspian roach; and *Contracecum microcephalum* infection in the Prussian carp.

Variations in parasite abundance with age were seen in a number of the fish species. The abundance of *D. gobiorum* in the common bream decreased with increasing age; *D. spathaceum* in carp and the Caspian roach; and *T. clavata* in the marine pike perch. In contrast, increases in abundance associated with increasing age were seen with *D. gobiorum* in carp; *Dactylogyrus tuba* in the asp; *D. spathaceum*, *D. gobiorum* and *A. schupakovi* in the Volga pike perch; *C. microcephalum* in the Prussian carp; and *L. vanbenedenii* in the golden grey mullet.

Variations in the abundance according to sex of the fish were seen with *T. clavata* infection of the marine zander and with *Porrocaecum reticulatum* infection in the asp. In both instances, the males were more heavily infected than the females.

Variations in the abundance of parasites with the year in which the fish were recovered were seen on a number of occasions. The year 2020 had the most intensely infected bream with *D. gobiorum*; the most intensely infected carp with *D. spathaceum*; and the most intensely infected marine zander with *A. schupakovi*. The year 2018 had the most intensely infected asp with *D. tuba* and *A. schupakovi*; the most intensely infected Volga pike perch with *D. spathaceum*; and the most intensely infected Saposhnikovi shad with *Mazocraes alosae*. The year 2019 had the most intensely infected asp with *D. spathaceum* and the most intensely infected Saposhnikovi shad with *P. reticulatum*.

Higher abundances of *K. sinensis* in bream and *D. spathaceum* in asp were found in fish from the Zhaiyk River compared to the northern Caspian.

Discussion

In the present study, we have examined four models to describe the frequency distribution of various parasites in a sample of a number of different species of fish. We did not observe any parasite species whose distribution was consistent with a Poisson distribution and, thus, a completely random distribution. We observed a zero-inflated Poisson distribution in several fish species infected with parasite (see table 1). The zero-inflated Poisson distribution suggests that the infection is not clumped and would be consistent with intermediate hosts being infected with single larvae, given that the host is exposed to possible infection.

A negative binomial distribution is consistent with a clumped infection process and all fish potentially being exposed. This type of distribution was seen in a number of fish species infected by various parasites. Finally, the zero-inflated negative binomial distribution is consistent with a clumped infection process, but only a proportion of the fish population being exposed. This distribution was the most commonly seen distribution.

Diplostomum spp. are trematode parasites and fish are infected by cercariae which are released into the water by snail intermediate hosts. Infected snails can release large numbers of cercariae into the water – for *D. spathaceum*, a mean number of 5199 cercariae per snail per day was reported by Vyhlídalová & Soldánová (2020). For a Poisson process to result in infection of fish, it can be hypothesized that the cercariae are likely to be dispersed in the water and encounter the fish at random. Alternatively, there could be parasite-induced mortality, which removes heavily infected fish from the population; in which case, the infection process is not random, but the resulting distribution in the fish appears to be random. For a negative binomial process, it can be hypothesized that the cercariae are not randomly dispersed in water but may be at a greater density in certain habitats with heavily infected fish more likely to frequent these habitats. Since *D. spathaceum* cercariae are not actively host-seeking and are often patchily distributed, behavioural avoidance of these sources of infestation is the prime defence mechanism displayed by fish hosts (Karvonen *et al*., 2004a). Thus, the zero-inflated distributions of some of these parasites are consistent with the avoidance of these patches of cercariae by some of these wild-caught fish. Alternatively, the overdispersed distribution in fish may be related to variations in susceptibility of the fish to the parasite, resulting in a proportion

Table 1. Probability distributions and factors associated with parasite abundance from ten species of fish.

Fish species (sample size)	Parasite	Frequency distribution	Significant covariates associated with parasite abundance and the IRR (CI)	Significant covariates associated with zero inflation and the OR (CI)	Prevalence (CI)	Abundance (CI)
Common bream[1] *Abramis brama* (180)	*Diplostomum spathaceum*	Zero-inflated negative binomial		Age, OR = 1.69 (1.09–2.61), *P* = 0.019	21% (15–27%)	2.6 (1.5–4.8)
				Fulton index OR = 14.43 (2.74–75.86), *P* = 0.002		
				Origin Zhaiyk River OR = 6.32 (1.32–30.22), *P* = 0.02		
	D. helveticum	Zero-inflated negative binomial	Fulton index, IRR = 10.15 (1.05–98.27), *P* = 0.045		3% (0.9–6%)	0.4 (0.11–3.29)
	D. gobiorum	Zero-inflated negative binomial	Age, IRR = 0.39 (0.22–0.70), *P* = 0.0015.		9% (5–14%)	2.1 (1.0–5.0)
			Year 2020, IRR = 38.55 (3.32–4.47e2), *P* = 0.0035	Year 2020, OR = 42.47 (5.23–3.44e2), *P* = 0.0004		
	Khawia sinensis	Negative Binomial	Origin Zhaiyk River IRR = 15.00 (2.11–213.42), *P* = 0.015		3% (1–7%)	0.12 (0.04–0.46)
Carp[1] *Cyprinus carpio* (200)	*Tylodelphys clavata*	Zero-inflated negative binomial			8% (4–12%)	0.78 (0.34–2.38)
	Diplostomum spathaceum	Zero-inflated negative binomial	Age, IRR = 0.75 (0.60–0.92), *P* = 0.0067	Year 2019, OR = 9.06 (3.62–22.69)	23% (17–29%)	1.7 (1.1–2.9)
			Year 2020, IRR = 2.94 (1.57–5.50), *P* = 0.0008	Year 2020, OR = 7.10 (2.64–19.06), *P* = 9.9e-5		
	Diplostomum gobiorum	Zero-inflated Poisson	Age, IRR = 3.21 (1.19–8.68), *P* = 0.021	Age, OR = 7.28 (1.44–36.7), *P* = 0.016	3% (0.8–6%)	0.22 (0.06–2.0)
			Fulton index, IRR = 6.29e-5 (6.79e-3–5.83e-7), *P* = 5.12e-5			
Caspian roach[1] *Rutilus caspicus* (180)	*Diplostomum spathaceum*	Zero-inflated Poisson	Age, IRR = 0.31 (0.16–0.62), *P* = 0.0008		4% (2–8%)	0.80 (0.17–3.8)
			Fulton index, IRR = 1.9e-3 (8.8e-7–4.4e-2), *P* = 0.002			
	Anadonta sp.	Negative binomial	Fulton index, IRR = 6.7e-5 (8.3e-8–5.4e-5)		4% (2–8%)	0.13 (0.04–0.39)
Marine zander[1] *Sander marinus* (150)	*Tylodelphys clavata*	Zero-inflated Poisson	Sex male, IRR = 2.89 (1.31–6.34), *P* = 0.008		4% (1–9%)	0.22 (0.07–1.25)
			Age, IRR = 0.81 (0.67–0.98), *P* = 0.038			
	Anisakis schupakovi	Zero-inflated negative binomial	Date 2019, IRR = 3.76 (1.48–9.65), *P* = 0.005		18% (12–25%)	1.45 (0.81–2.97)
			Date 2020, IRR = 4.09 (1.66–10.13), *P* = 0.002			

Asp[1] *Leuciscus aspius* (180)	*Dactylogyrus tuba*	Zero-inflated Poisson	Date 2019, IRR = 0.17 (0.04–0.739), *P* = 0.02	Date 2020, OR = 0.15 (0.03–0.73), *P* = 0.02	13% (8–19%)	0.58 (0.32–1.16)
			Date 2020, IRR = 0.14 (0.04–0.44), *P* = 0.0008			
			Age, IRR = 2.28 (1.39–3.73)			
	Diplostomum spathaceum	Zero-inflated negative binomial	Origin Zhaiyk River, IRR = 4.83 (2.16–10.83), *P* = 0.0001	Date 2019, OR = 6.95 (2.33–20.74), *P* = 0.0005	21% (15–28%)	2.49 (1.47–4.69)
				Date 2020, OR = 9.38 (3.35–26.22), *P* = 0.00002		
	Diplostomum mergi	Zero-inflated negative binomial			5% (2–9%)	0.66 (0.23–3.35)
	Diplostomum gobiorum	Negative binomial			4% (2–9%)	0.34 (0.19–1.60)
	Anisakis schupakovi	Zero-inflated negative binomial	Date 2020, IRR = 0.46 (0.27–0.77), *P* = 0.0035	Date 2019, OR = 7.25 (2.73–19.29), *P* = 7.1e-5	64% (56–71%)	66 (19–93)
	Contracaecum micropapillatum	Zero-inflated negative binomial			32% (26–40%)	27 (16–47)
	Porrocaecum reticulatum	Zero-inflated negative binomial	Sex male, IRR = 7.26 (2.73–19.29), *P* = 0.0001		19% (14–26%)	12 (5.6–24)
Volga pike perch[1] *Sander volgensis* (128)	*Diplostomum spathaceum*	Zero-inflated negative binomial	Age, IRR = 2.32 (1.57–3.42), *P* = 2.3e-5		22% (15–30%)	2.4 (1.3–4.7)
			Fulton index, IRR = 17.12 (2.59–113),*P* = 0.003			
			Date 2019, IRR = 3e-4 (1e-5–1e-2), *P* = 4e-6			
	Diplostomum gobiorum	Zero-inflated Poisson	Age, IRR = 1.72 (1.24–2.37), *P* = 0.001		5% (2–11%)	0.31 (0.09–1.1)
	Diplostomum chromataphorum	Zero-inflated negative binomial	Fulton index, IRR = 0.02 (0.001–0.28), *P* = 0.004	Age, OR = 0.62 (0.42–0.91), *P* = 0.02	11% (6–18%)	4.0 (1.3–12)
	Tylodelphys clavata	Zero-inflated Poisson	Fulton index IRR = 0.03 (0.004–0.18), *P* = 0.0002	Date 2019, OR = 13 (1.6–109), *P* = 0.02	7% (3–13%)	0.34 (0.12–0.99)
	Anisakis schupakovi	Negative binomial	Age, IRR = 1.32 (1.04–1.67), *P* = 0.02		47% (38–57%)	3.7 (2.5–5.4)
			Fulton index, IRR = 0.40 (0.18–0.87), *P* = 0.02			
Persian carp[1] *Carassius gibelio* (178)	*Diplostomum spathaceum*	Zero-inflated negative binomial		Origin Zhaiyk River OR = 0.33 (0.11–0.97), *P* = 0.045	11% (7–16%)	0.79 (0.39–1.91)
	Contracaecum microcephalum	Zero-inflated Poisson	Age, IRR = 1.49 (1.01–2.18), *P* = 0.04		2% (0.6–6%)	1.29 (0.24–54)
			Fulton index, IRR = 0.009 (0.003–0.03), *P* = 5e-13			
Golden grey mullet[2] *Chelon auratus* (150)	*Ligoforus vanbenedenii*	Negative binomial	Age, IRR = 2.77 (1.28–5.99), *P* = 0.01		6% (3–11%)	0.19 (0.08–0.56)
			Fulton, IRR = 1.3e7 (4.8e3–3.4e10), *P* = 4.7e-5			

(*Continued*)

Table 1. (*Continued.*)

Fish species (sample size)	Parasite	Frequency distribution	Significant covariates associated with parasite abundance and the IRR (CI)	Significant covariates associated with zero inflation and the OR (CI)	Prevalence (CI)	Abundance (CI)
	Tylodelphis clavata	Zero-inflated Poisson	Fulton index, IRR = 20 (2.24–176), $P = 0.007$		4% (1–9%)	0.24 (0.08–1.36)
Saposhnikovi shad[3] *Alosa saposchnikowii* (150)	*Mazocraes alosae*	Zero-inflated negative binomial	Date 2020, IRR = 0.19 (0.10–0.37), $P = 5.8e\text{-}7$	Date 2020, OR = 14.8 (4.97–44.1), $P = 1.3e\text{-}6$	59% (51–68%)	21 (15–30)
	Porrocaecum reticulatum	Zero-inflated negative binomial	Date 2019, IRR = 8.89 (3.37–23.4), $P = 9.9e\text{-}6$	Fulton index, OR = 15.6 (1.54–158), $P = 0.02$	19% (13–27%)	1.3 (0.74–2.5)
			Date 2020, IRR = 3.30 (1.17–9.32), $P = 0.02$			
	Raphidascaris acus	Zero-inflated negative binomial		Sex male, OR = 0.18 (0.06–0.51), $P = 0.001$	11% (7–18%)	0.61 (0.41–2.17)
Big-scale sand smelt[3] *Atherina boyeri* (101)	*Diplostomum spathaceum*	Negative binomial			2% (0.2–7%)	0.16 (0.02–9.95)
	Anisakis schupakovi	Zero-inflated Poisson			5% (2–11%)	0.06 (0.02–0.14)

[1]Seven of these are found in the low-saline environment of the north Caspian and Zhaiyk rivers.
[2]One marine species has been introduced (Naseka & Bogutskaya, 2009).
[3]Two marine species are endemic to the Caspian.
IRR, incidence rate ratio; CI, confidence interval; OR, odds ratio.

of the fish population having a lower or zero burden of the parasite despite exposure.

With ocular trematodes, parasite metacercariae induce cataracts due to mechanical destruction of the lens and metabolic products excreted by the parasites, thus reducing the host's vision (Karvonen *et al.*, 2004b; Seppälä *et al.*, 2004). Consequently, an induced increase in susceptibility to predation with fish having heavier infections of *Diplostomum* species is due to reduced predator avoidance behaviour (e.g. Brassard *et al.*, 1982a; Flink *et al.*, 2017). In addition, *Diplostomum* have been reported as having a direct pathogenic effect, with an exponential increase in mortality as infection intensity increases (Brassard *et al.*, 1982b). Likewise, infection with *T. clavata* has been suggested to negatively affect the feeding behaviour of perch, which is hypothesized to increase the risk of predation (Vivas Muñoz *et al.*, 2019). Rainbow trout, *Oncorhynchus mykiss*, infected with *D. spathaceum* engage in behaviours that put them at greater risk of predation. For example, those harbouring the parasite appear to be more vulnerable to a simulated predator attack (Seppälä *et al.*, 2004, 2008). Additionally, when provided with the choice of a dark or light background, infected trout spent more time over the light area, which makes them visually more conspicuous (Seppälä *et al.*, 2005). Thus, by injuring important sensory organs, *D. spathaceum* is able to alter fundamental fish anti-predator mechanisms, such as crypsis and shoaling behaviour, in a way that is likely to increase the vulnerability of the intermediate host to predation by the definitive host (Krause & Ruxton, 2002; Seppälä *et al.*, 2004, 2005, 2008). Closely related eye flukes, *Tylodelphys* spp., are also found as metacercariae in the eyes of fish, but these parasites are comparatively understudied for their behavioural impacts (e.g. Vivas Muñoz *et al.*, 2017, 2019; Ruehle & Poulin, 2019).

The pathological effects of diplostomiasis have seldom been documented in wild fish, partly because heavily infected individuals are removed from the population through predation (Pennycuick, 1971). However, *D. spathaceum* epizootics can be serious in captive fish, particularly farmed salmonids, such as rainbow trout (*Oncohynchus mykiss*) (Betterton, 1974). Where there is a significant decrease in the Fulton index with increasing levels of parasitism (IRR < 1), then this might indicate a pathogenic effect of the parasite. In the present study, we found that the intensity of infection of carp, Caspian roach and Volga pike perch with *Diplostomum* spp. was inversely associated with the Fulton index. Other parasites such as *A. schupakovi* and the Volga pike perch also had an inverse association of infection abundance with the Fulton index. In contrast, the reason for an association between an increasing Fulton index and increasing parasites is not clear.

For a number of parasites, there is a relationship between parasite abundance and age and/or between the proportion that is zero-inflated and age. In some instances, such as the infection of carp with *D. spathaceum*, the IRR decreases with increasing age. This may be due to age-related resistance or pathogenic effects of the parasite, with heavily infected fish having an excess mortality rate compared to lightly or non-infected fish. The experimental infection of rainbow trout (*O. mykiss*) suggested an increase in resistance to reinfection following repeated infection (Karvonen *et al.*, 2004a). Alternatively, there may be a behavioural reason to explain why older fish are less exposed to the parasite. We also found, for some parasitic infections of fish, that IRR increases with age – an example of this is *A. schupakovi* infection of the marine sander. This could be due to the parasite being long lived with little concomitant resistance to reinfection; thus, the host accumulates parasites over its lifetime. This age-related dynamic of infection is seen in certain parasitic infections of terrestrial vertebrates such as *Echinococcus* infection of sheep (Torgerson & Heath, 2003).

Of interest is the different patterns of the same parasite distribution in different hosts. For *D. gobiorum*, the abundance decreases with age in bream (IRR < 1), but increases according to age in carp and the Volga pike perch. The reasons for this are somewhat speculative. This may be due to relative resistance or development of an immune response in bream, which is absent in the other species, or variations in infection pressure that could be due to different behaviours of the host species. In different hosts the same parasite also sometimes had different frequency distributions. For example, *D. spathaceum* was distributed as a zero-inflated negative binomial distribution in bream and as a zero-inflated Poisson distribution in Caspian roach. This may, in this case, be a statistical artefact. The prevalence of this parasite in bream was 21%, whilst in the Caspian roach it was just 4%. When the prevalence of parasites in the sample is low, the probability of having the individual fish with a large intensity of infection is quite low, even if the underlying distribution is negative binomial. Thus, a small sample size might suggest a Poisson distribution even if the population has a negative binomial distribution. Otherwise, it is necessary to consider variations in host resistance of behaviour between different host species to explain these differences.

The reasons behind the variations in parasite abundance in different years is unknown. Fish were sampled at the same time of year, so seasonal differences in transmission appear unlikely. Likewise, potential differences in the dynamics of the Zhaiyk River compared to the open Caspian Sea are unlikely to be confounding factors as the origin of each fish is considered as a covariate in the analysis. There was a higher abundance for *K. sinensis* in bream and *D. spathaceum* in asp in the Zhaiyk River compared to the northern Caspian. This may be due to differences in habitat, temperature or salinity, which promote the transmission of the parasites or affect the abundance of intermediate hosts. For ocular trematodes, increases in intensity were found in the roach from late summer to early winter, with another increase in the spring (Burrough, 1978), so seasonal transmission is possible, but such variations could not be analysed in our data.

Where we found differences in abundances of parasites between male and female fish, it was the males that were more heavily infected. This is in agreement with the general pattern of higher infestation in males across a range of host–parasite systems (Poulin, 1996; Klein, 2004), and has been linked to relative immune competence between males and females, which may be linked to hormones. However, there are a few results where female fish have been more heavily parasitised (see, e.g., Karvonen & Lindström, 2018).

Whilst the management of parasites in wild populations of fish would not be feasible, there could be opportunities for management of parasitism in aquaculture with a focus on those parasites that appear to be pathogenic or of zoonotic significance.

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Conflicts of interest. None.

Ethical standards. The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional guides on the care and use of laboratory animals.

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