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Long-term dynamics of *Metagonimus* spp. (Trematoda; Digenea) in ayu fish (*Plecoglossus altivelis*) in Shizuoka Prefecture, Japan

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静岡県内のアユ（***Plecoglossus altivelis***）におけるメタゴニムス属吸虫の長期変動

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Abstract

The digenetic parasites of the genus *Metagonimus* are one of the major human intestinal parasites in Japan. The long-term fluctuations in the their metacercarial density of *Metagonimus* spp. (*Metagonimus miyatai* and *M. yokogawai*) on the scales of ayu fish were analyzed in eight rivers in Shizuoka Prefecture, Japan, in relation to several environmental parameters including temperature, water level, and biotic/abiotic conditions in the previous year, using a generalized linear mixed model (GLMM). Our results indicated that water temperature in summer positively affects metacercarial abundance to some extent, as predicted from a previous experimental study. The effects of water level were only detected in the river with small fluctuation, but the coefficient values varied in the other rivers indicating that the effect is unpredictable. The effects of metacercarial abundance in the previous year at a local scale were limited, possibly due to the large home range of the definitive hosts (birds). Flood levels in the previous autumn had a significant negative effect in one river, and the coefficients were negative but non-significant in most of the rivers. Although our analysis included a limited number of parameters, the results suggest the possibility that metacercarial abundance of *Metagonimus* is predictable to some extent from river-environmental parameters.

Keywords: *Metagonimus* spp., ayu fish, parasite, river environment, long-term dynamics

摘　要

メタゴニムス属 *Metagonimus* は，日本における主要な人体寄生虫の一つである．静岡県内の８河川から得られたアユ鱗寄生のメタゴニムス属メタセルカリア（宮田吸虫 *M. miyatai* および横川吸虫 *M.*

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yokogawai と考えられる）感染量の長期変動を，水温/気温，水位，前年のメタセルカリア量，前年秋の出水レベルを説明変数とし，一般化線形混合モデルを用いて解析した．本属は水温の上昇に伴って第一中間宿主からの湧出量が増えることが知られているが，野外でも夏期の水温は正の係数を採ることが多く，３河川では有意であった．水位は比較的変動の小さい１河川で負の影響が検出されたが，その他の河川では係数が正負どちらになる場合もあり，影響は不明瞭であった．前年のメタセルカリア量の影響は限定的であったが，終宿主（鳥類）の生息域が広いためであると考えられた．前年秋の出水は負の係数を採ることが多く，１河川では有意であった．本研究で分析に含めることのできた説明変数は限られていたが，メタゴニムス属のメタセルカリア量は，水温等の環境要因によってある程度説明可能であることが示された．

キーワード：メタゴニムス属，アユ，寄生虫，河川環境，長期変動

Introduction

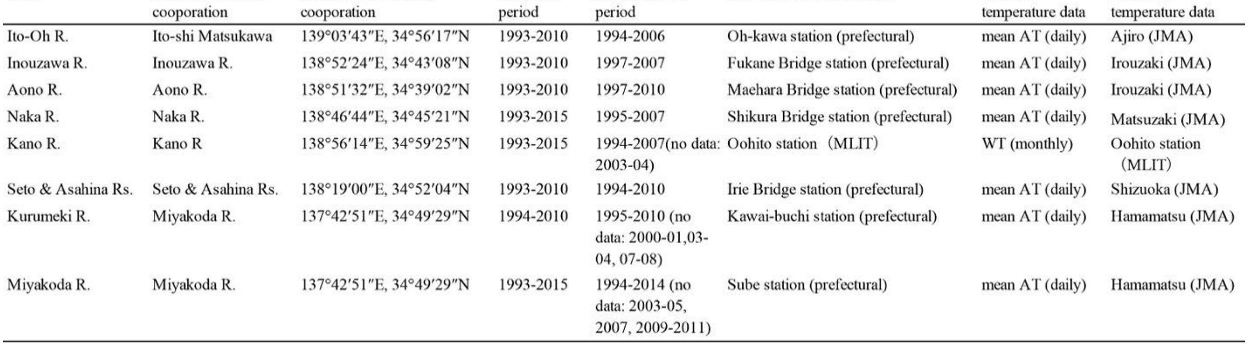
Most parasitic helminths have complex life cycles, and their density can be affected by numerous biotic and abiotic factors such as definitive host density, intermediate host density, predation efficiency of intermediate hosts by definitive hosts, and environmental factors affecting the survival of free-stage parasites. Several studies have shown that parasite density is related to environmental factors, such as water level (Yurlova et al., 2006; Urabe et al., 2009) and climate oscillations (Doi and Yurlova, 2011). Understanding such relationships is important for estimating the epidemiological dynamics of infectious diseases and the effects of parasite infection on host populations.

The genus *Metagonimus* comprises flukes that infect birds and mammals, including humans, and is distributed widely in East Asia (Shimazu and Kino, 2015). Their first intermediate hosts are freshwater snails (*Semisulcospira* spp.), and their second intermediate hosts are freshwater fishes. In particular, *Metagonimus yokogawai* and *M. miyatai* are important as human parasites, because they use ayu fish (sweetfish; *Plecoglossus altivelis*) as the second intermediate host, which are often the source of infection in humans (Saito, 2003; Kino et al., 2006). In addition to ayu fish, *M. yokogawai* also uses *Tribolodon hakonensis* (Saito et al., 1997) and *Salangichthys microdon* (Murata et al., 2004) as its second intermediate hosts, while *M. miyatai* also uses *Opsariichthys platypus* (syn. *Zacco platypus*), *Candidia temminckii* (syn. *Zacco temminckii*) and *Rhynchocypris lagowskii* (syn. *Moroko steindachneri*) (Saito et al., 1997). However, ayu is the most important fish species as a source of infection to humans. In the 1970s, the total prevalence of *Metagonimus* and *Heterophyes* was approximately 0.2–0.7 %, but local prevalence was over 50% in some communities near lakes or rivers (Saito, 2003). In 2005, 0.04% of fecal samples were positive for *Metagonimus* eggs in Japan, and *Metagonimus* remains the most prevalent parasitic helminth in Japan (Japan Association of Health Science, 2006).

In Japan, several public health institutes perform the epidemiological monitoring of *Metagonimus*. In Shizuoka Prefecture, the Shizuoka Health Service Association monitors for metacercariae (the stage infectious to humans) in ayu fish from 20 rivers throughout the prefecture (Shizuoka Health Service Association, unpublished). These data are suitable for determining the extent to which parasite densities are affected by external environmental conditions.

Cercarial shedding in *Metagonimus* is influenced by water temperature (WT). Kagei (1966) showed that *Metagonimus* shed cercariae massively at WTs of 20–26 °C, equivalent to summer temperatures in Japan. Therefore, the metacercarial density in ayu is likely to be relatively high in years with higher summer WTs. Moreover, cercarial density in water is likely affected by water volume, as revealed in the cercariae of the bucephalid trematode *Parabucephalopsis parasiluri* (Urabe et al., 2009), and metacercarial density is likely high in years with low water levels in summer. Conversely, particularly low water levels could negatively affect on the metacercarial density if low discharge in drought years limits the population size of intermediate hosts in the river. In addition to these factors that directly affect cercarial density, several conditions in the previous year could have relatively long-term or subsequent influences on the host populations, which in turn could affect cercarial density.

Table 1. Data sets analyzed.　　表１．分析に用いたデータセット．



River	Name of fisheries cooporation	Location of fisheries cooporation	Sampling period	Data analyzed period	Source of water level data	Type of temperature data	Source of temperature data
Ito-Oh R.	Ito-shi Matsukawa	139°03′43″E, 34°56′17″N	1993-2010	1994-2006	Oh-kawa station (prefectural)	mean AT (daily)	Ajiro (JMA)
Inouzawa R.	Inouzawa R.	138°52′24″E, 34°43′08″N	1993-2010	1997-2007	Fukane Bridge station (prefectural)	mean AT (daily)	Irouzaki (JMA)
Aono R.	Aono R.	138°51′32″E, 34°39′02″N	1993-2010	1997-2010	Maehara Bridge station (prefectural)	mean AT (daily)	Irouzaki (JMA)
Naka R.	Naka R.	138°46′44″E, 34°45′21″N	1993-2015	1995-2007	Shikura Bridge station (prefectural)	mean AT (daily)	Matsuzaki (JMA)
Kano R.	Kano R	138°56′14″E, 34°59′25″N	1993-2015	1994-2007(no data: 2003-04)	Oohito station （MLIT）	WT (monthly)	Oohito station （MLIT）
Seto & Asahina Rs.	Seto & Asahina Rs.	138°19′00″E, 34°52′04″N	1993-2010	1994-2010	Irie Bridge station (prefectural)	mean AT (daily)	Shizuoka (JMA)
Kurumeki R.	Miyakoda R.	137°42′51″E, 34°49′29″N	1994-2010	1995-2010 (no data: 2000-01,03-04, 07-08)	Kawai-buchi station (prefectural)	mean AT (daily)	Hamamatsu (JMA)
Miyakoda R.	Miyakoda R.	137°42′51″E, 34°49′29″N	1993-2015	1994-2014 (no data: 2003-05, 2007, 2009-2011)	Sube station (prefectural)	mean AT (daily)	Hamamatsu (JMA)

In this study, we analyzed the long-term fluctuations in metacercarial density in eight rivers in Shizuoka Prefecture, Japan, in relation to various environmental parameters such as temperature, water level, and biotic/abiotic conditions in the previous year. Several studies have examined the long-term dynamics of natural digeneans (Yurlova et al., 2006; Doi and Yurlova, 2011; Levakin et al., 2013), but none have examined digenean populations in river habitats, which are characterized by unique environmental conditions, such as flow regimes and floods/droughts, that are uncommon in other aquatic habitats such as lakes and coasts. In particular, rivers in Japan have high interseasonal and interannual variations in discharge, which is regarded as a natural disturbance. Thus, it is likely that the river flow regimes influence parasite population dynamics.

No data are available on host (intermediate or definitive) densities, which are correlated with metacercarial density (Levakin et al., 2013). Thus, we applied a generalized linear mixed model (GLMM) to consider random effects on the annual variation of metacercarial density. Based on the results, we discuss the extent and trends of the environmental factors that affect metacercarial abundance in Japanese rivers.

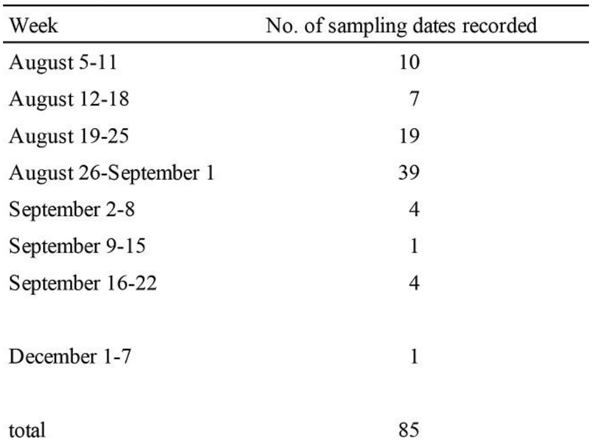
Materials and Methods

Metagonimus metacercariae monitoring

Metagonimus metacercariae monitoring data were collected by the Shizuoka Health Service Association from 1993 to 2015. We used the data from eight rivers out of a total of 20 rivers with high metacercarial densities throughout the sampling years and with environmental parameter datasets for at least 10 years (Table 1). Ayu fish were sampled in each river by local fishery cooperatives in Shizuoka Prefecture. The sampling dates were not recorded for all sites and years, but according to the dates recorded (n = 85), the earliest date of sampling was August 5 and the latest was December 3 (Table 2). Almost half of the samples were collected in the last week of August. Ayu is an amphidromous fish with an annual life cycle (Nishida, 2001); it hatches in autumn in the midreaches of rivers, and fry immediately flow down to the sea. Young fish migrate upstream in the following spring, remain in the river until they breed, and die in late autumn. In Shizuoka Prefecture, breeding peaks from October to December (Nishida, 2001; Suzuki et al., 2014). Thus, the abundance of metacercariae in the collected fish indicates the cumulative number of metacercariae that infected the fish during the summer of each year.

All scales of collected fish (raw or frozen) were removed and pooled by sampling location. The scales were digested in artificial gastric juice, and metacercariae in the digest were collected using the filtration method (Kino, 2016) and counted.

Table 2. Frequency of sampling dates of ayu fish recorded (n=85) from 20 rivers in Shizuoka Prefecture.
表２．静岡県内の20河川におけるアユのサンプル採集日の頻度．採集日記録のある85データに基づく．



Week	No. of sampling dates recorded
August 5-11	10
August 12-18	7
August 19-25	19
August 26-September 1	39
September 2-8	4
September 9-15	1
September 16-22	4
December 1-7	1
total	85

Two species of *Metagonimus* (*M. yokogawai* and *M. miyatai*) can infect ayu fish (Saito et al., 1997); however, most of the metacercariae surveyed in this study were likely *M. miyatai*, because only scales were investigated (Saito et al., 1997; Kino et al., 2006). Regardless, in this study, we describe the species of metacercariae as *Metagonimus* spp. because they were not identified individually, and some samples may have included *M. yokogawai*.

Environmental data

The environmental data were obtained from public databases. Water level data were provided by the Ministry of Land, Infrastructure, Transport and Tourism (MLIT) or the Public Works Control Division of Shizuoka Prefecture (Table 1). WT data were not available for most sites, so we used air temperature (AT) data from the Japan Meteorological Agency (JMA) at the observatory nearest to the sampling stations (Table 1).

WT is the only environmental factor known to affect cercarial shedding in *Metagonimus*. Kagei (1966) showed experimentally that the ratio of snails that shed cercariae increased linearly with WT up to 26°C (Fig. 1). WT data were available at only one site (Oohito station, Kano River) with a low measuring frequency (once per month). For the other sites, we estimated WT from AT based on the relationship between the former at the Oohito station and the latter at the Mishima observatory. A regression equation of AT to WT was calculated using monthly data from 1989 to 2015 ($n = 324$, $WT = 0.605AT + 3.739$; $r = 0.9663$). Considering Kagei's (1966) data, we assumed that cercarial shedding of *Metagonimus* increased with AT up to 36.8°C, which was higher than the maximum daily average AT in Shizuoka Prefecture. Thus, we used the average AT from June to August as an indicator of WT in each year assuming that AT affects cercarial abundance monotonically.

No data were available on the abundance/density of hosts. Although there is a public database for ayu catch in rivers in Shizuoka (Statistics Bureau, Ministry of Internal Affairs and Communications of Japan), the data are not representative of actual ayu density, and we did not apply this data to our analysis.

Statistical analysis

First, we determined the yearly change in metacercarial density to assess stationary and long-term trends in the data. When any long-term trends appear, data should be transformed into an appropriate form to correct for bias (Kitagawa, 2005). In our study, the data were not transformed due to a limitation of the problem of the statistical program. Instead, the metacercarial abundance in the previous year was added as an indicator of autocovariance in the time-series data.

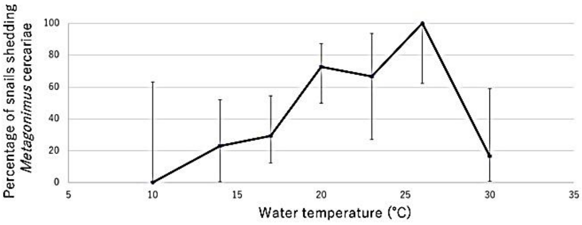


Fig. 1. Relationship between water temperature and the *Metagonimus*-infected snail ratio shedding cercariae, with 95% confidence interval. Data after Kagei (1966).

図 1. 水温とセルカリアを湧出するメタゴニムス属感染貝の比率の関係．影井(1966)のデータによる．縦線は 95％信頼限界を示す．

To analyze the effects of environmental conditions on metacercarial abundance, we applied a generalized linear mixed model (GLMM). The mean abundances of metacercariae per fish (number of pooled metacercariae/number of examined fish) were rounded into integers for application to a Poisson regression. The studied years were added as a random effect. The average AT from June to August was used as an indicator of WT for each year. Water levels measured in summer (June–August) at the station nearest to the sampling sites were used as an indicator of discharge. In Japanese rivers, the coefficients of river regime (i.e., maximum/minimum discharge) are generally very high (~100 to ∞; Saito et al., 1990). Thus, a few prominent spikes in water level (i.e., floods) would greatly influence average water levels. Therefore, to estimate water levels under normal conditions, we removed the highest 5% of data points before performing the analysis.

In addition, we considered flooding in the previous autumn, which could be related to metacercarial abundance. Japan often experiences typhoons from late summer to autumn (mainly August–October), during or just before the peak ayu spawning season, and flooding due to typhoons likely affects ayu population size and breeding success. Thus, we included the maximum water level during August and October in the previous year as an environmental factor that could affect the metacercarial abundance. We considered only the main effect of each variable and did not consider their interactions. All analyses were performed using R (ver. 3.3.2) and the glmmML package (R Development Core Team, 2016).

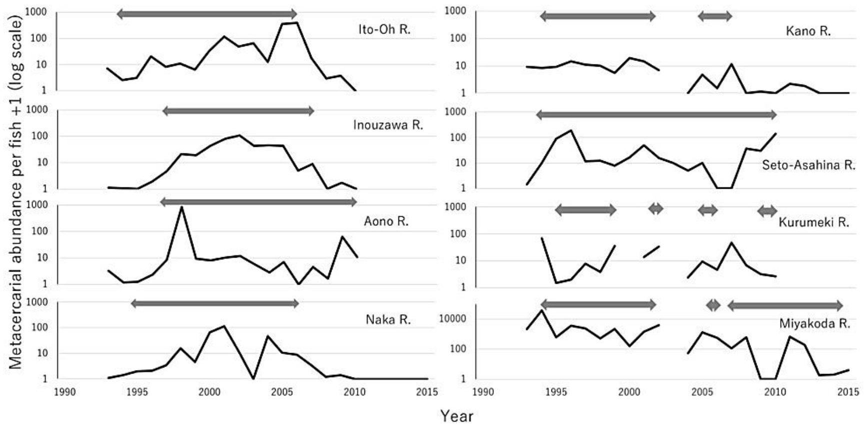


Fig. 2. Long-term trends of metacercarial abundance (in log scale) at eight rivers in Shizuoka Prefecture. All data was added by 1 for the log transformation. Arrows indicate the range of years used for the GLMM analysis.
図 2. 静岡県内の８河川におけるメタセルカリア量（対数表示）の長期変動. 対数変換を可能にするため，全データに１を足している. 矢印はデータを GLMM 解析に用いた年の範囲を示す.

Results

Figure 2 shows the long-term trends in metacercarial abundance per fish at the eight studied rivers. All locations showed large fluctuations in metacercarial abundance among years. In the Kano and Miyakoda Rivers, metacercarial abundance gradually decreased throughout the monitored years, and was almost zero in recent years in the Kano River. A recent decrease in metacercariae was also observed in the Ito-Oh, Inouzawa and Naka Rivers. In the Aono River, a prominent peak (>13 times higher than the second-most abundant year) in metacercarial abundance was observed in 1998.

We selected years for the GLMM analysis based on these results. To reduce bias due to factors that were not included in our analysis, data were removed from the analysis when low metacercarial densities (<1.0 per fish) continued for more than two years. One exception was the data from the Ito-Oh River, from which the data in 2007 (metacercarial abundance: 16.7 per fish) were also deleted. Trends in the Ito-Oh River changed markedly after 2006, and the results of the data analysis differed greatly when the data from 2007 were included. Moreover, some years were eliminated from the analysis due to the lack of some environmental data. Table 1 and Fig. 2 show the dataset used for the analysis.

Table 3 shows the results of the GLMM based on these trimmed data. Prior to the GLMM, correlations between independent variables were tested. Several correlations were

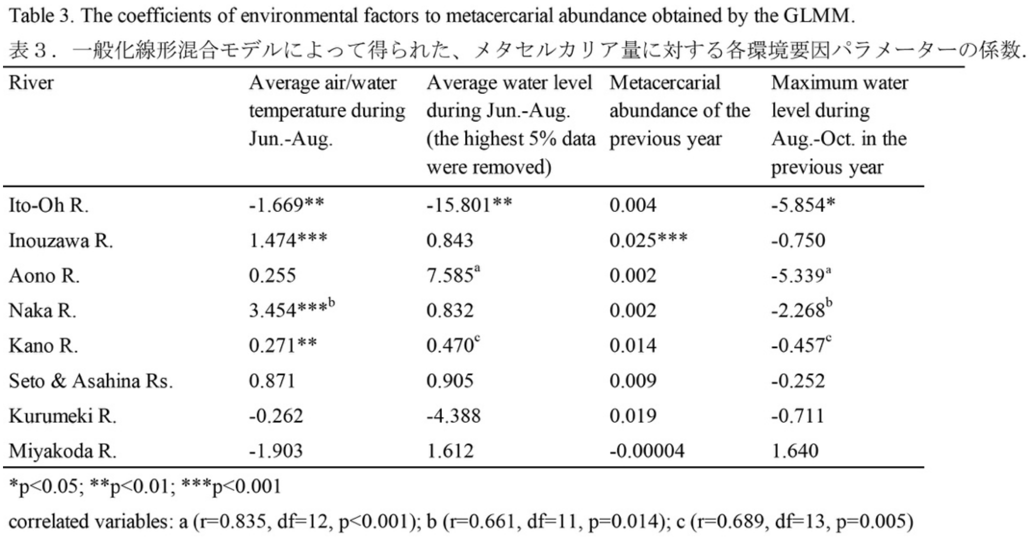


Table 3. The coefficients of environmental factors to metacercarial abundance obtained by the GLMM.
表３．一般化線形混合モデルによって得られた、メタセルカリア量に対する各環境要因パラメーターの係数.

River	Average air/water temperature during Jun.-Aug.	Average water level during Jun.-Aug. (the highest 5% data were removed)	Metacercarial abundance of the previous year	Maximum water level during Aug.-Oct. in the previous year
Ito-Oh R.	-1.669**	-15.801**	0.004	-5.854*
Inouzawa R.	1.474***	0.843	0.025***	-0.750
Aono R.	0.255	7.585[a]	0.002	-5.339[a]
Naka R.	3.454***[b]	0.832	0.002	-2.268[b]
Kano R.	0.271**	0.470[c]	0.014	-0.457[c]
Seto & Asahina Rs.	0.871	0.905	0.009	-0.252
Kurumeki R.	-0.262	-4.388	0.019	-0.711
Miyakoda R.	-1.903	1.612	-0.00004	1.640

*p<0.05; **p<0.01; ***p<0.001

correlated variables: a (r=0.835, df=12, p<0.001); b (r=0.661, df=11, p=0.014); c (r=0.689, df=13, p=0.005) detected among variables in three of eight rivers (the Aono, Naka and Kano Rivers) (Table 3). However, we used all variables in the analyses of these three rivers, although their effects may be underestimated, because we could not detect any direct logical reason for these correlations (average AT or average water level during Jun.-Aug., and the maximum water level during Aug.-Oct in the previous year), and could not determine which variables should be omitted from the analysis.

Figure 3 shows the single correlations between metacercarial abundance and environmental parameters with a significant effect in the GLMM. AT (WT) during the summer season positively affected with metacercarial abundance in three rivers, and negatively affected abundance in one river (Table 3). The average water level in the summer season negatively affected metacercarial abundance in one river (Ito-Oh River). Metacercarial abundance in the previous year had a significant positive effect on the metacercarial abundance in one river (Inouzawa River), and the coefficients were positive in seven of the eight rivers. Meanwhile, the maximum water level in the previous autumn had a significant negative effect on the metacercarial density in one river (Ito-Oh River), and the coefficients were negative in seven of the eight rivers.

Discussion

Metacercarial abundance had a positive relationship with AT(WT) in summer in some rivers, as expected from the experimental results of Kagei (1966). One exception was the Ito-Oh River, where metacercarial abundance had a weak but significant negative relationship with AT (Fig. 3), although the reason for this is unclear.

Water level was negatively correlated with metacercarial abundance only in the Ito-Oh River. River discharge can directly affect the cercarial density in water due to dilution (Urabe et al., 2009). Discharge data were not available for most of the rivers in this study; however, the range in interannual average water level in summer, and the difference between the maximum and average water level under the normal conditions, suggested that water level fluctuations in the Ito-Oh River were relatively small compared to the other studied rivers (Table 4). In the other rivers, excluding the Kurumegi River, large water level fluctuation events (i.e. floods and droughts) could affect parasite density in various and substantial ways. Large flood events would not only dilute free cercariae in water, but would also disturb river habitats and could limit the population size of the first and second intermediate hosts for

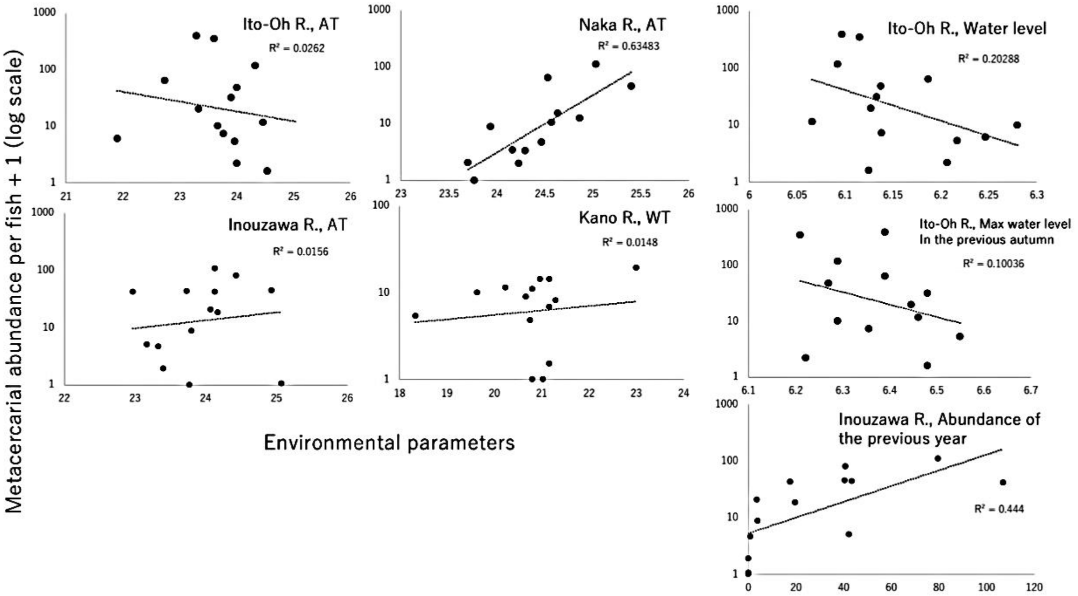


Fig. 3. Single correlations between metacercarial abundance and the environmental parameters that have a significant effect as the result of the GLMM analysis (in log scale).

図 3. GLMM において有意な効果が見られた環境パラメーターとメタセルカリア量の単相関．メタセルカリア量は対数表示で示す．

several years, which in turn would lead to a decline in parasite abundance. Similarly, extremely low water levels due to droughts might cause a decline in host populations due to the loss of their aquatic habitat. Therefore, large flood or drought events could have various unpredictable effects on parasite abundance.

Metacercarial abundance in the previous summer had a significant positive effect in only one river, and six of the other seven rivers showed positive, but non-significant, relationships. Because ayu is an annual fish, *Metagonimus* overwinters in the first intermediate hosts or in the definitive hosts, which become the source of metacercariae for the following year. Therefore, it is plausible that the metacercarial abundance of one year could influence that in the next year, but our results indicated that this effect was not strong. *Metagonimus* must pass through definitive hosts and first intermediate hosts before they can infect ayu again in the following year. Both *M. yokogawai* and *M. miyatai* exploit mammals and birds as definitive hosts, although their natural definitive hosts have not been fully clarified. In the present-day Japan, mammals including humans, would be minor hosts of *Metagonimus* due to the decline in wild carnivorous mammals and the hygienic treatment of human excreta. The major natural definitive hosts of *Metagonimus* are presumed to be piscivorous/scavenger birds such as the night heron (*Nycticorax nycticorax*) and the black kite (*Milvus migrans*) (Uchida et al., 1991) in the present day. Birds usually have large home ranges and often travel between rivers; thus, they can disperse parasite eggs widely. Moreover, these definitive hosts take in *Metagonimus* metacercariae not only through ayu fish but also through other second-intermediate hosts such as *Tribolodon hakonensis* or *Opsariichthys platypus* (Saito et al., 1997; Murata et al., 2004), which can live and carry metacercariae for more than one year. These are possible explanations for why the parasite abundance in one year had less of an effect than expected on that in the next year at a local scale.

The maximum water level in the previous autumn had a significant negative effect only on the Ito-Oh River, while six of the other seven rivers exhibited also a negative, but non-significant, relationship. However, this variable was significantly correlated with other variables in three of the six rivers, and its effect may be underestimated in these three rivers. The Ito-Oh River had the second-lowest fluctuation in water level (Table 4). As discussed above, large-scale floods can have unpredictable effects on parasite abundance. In the Miyakoda River, which had the largest fluctuation in water level among the eight studied rivers, the coefficients showed inverse effects in other rivers with the maximum water level in the previous autumn (Table 3).

Table 4. Maxmimus water level changes in summer under normal condition among studied years, and those between normal conditon in summer and the maximum water level in the typhoon season. Refer to Table 1 for the data source.
表４．調査河川の夏期平均水位（平水時）の最大年較差、および夏期平均水位（平水時）と台風期の最高水位との最大年較差．使用したデータソースについてはTable 1を参照.

Station No.	River	Estimated high-water dischage (m^3/s)	Max interannual change of mean water level in summer (Jun. - Aug.) under normal condition	Max difference between mean water level under normal condition in summer (Jun. - Aug.) and the max water level in the typhoon season (Aug. - Oct.)
1	Ito-Oh R.	550	0.215	0.275
2	Inouzawa R.	970	0.347	1.233
3	Aono R.	1,000	0.604	1.667
4	Naka R.	850	0.185	0.786
5	Kano R.	5,500	0.465	1.973
6	Seto & Asahina Rs.	1,900	0.443	1.978
7	Kurumeki R.	331	0.900	0.059
8	Miyakoda R.	1,600*	1.174	2.334

*Max discharge from Miyakodagawa Dam

Our results suggest that WT in summer affects the metacercarial abundance of *Metagonimus* to some extent. The effects of water level were detectable in one river, but they were unpredictable in most of the rivers, particularly in rivers with large water level fluctuations. The effects of metacercarial abundance in the previous year at a local scale were limited, possibly due to the large home range of definitive hosts (birds). The flood level in the previous autumn appeared to have a negative effect on metacercarial density in general. Although our analysis included a limited number of parameters, the results indicate the possibility that metacercarial abundance of *Metagonimus* is predictable from river-environmental parameters to some extent. Many factors, particularly biotic factors, could not be included in the present study. Levakin et al. (2013) reported on the effects of definitive host populations on parasite prevalence in intertidal snails in an area where long-term climatic conditions were relatively constant. Several unexplained trends in this study, such as the long-term trends of metacercarial decline (Fig. 1), may be related to biotic factors, and it should be clarified through further investigation.

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