

Can a nest associate fish use an introduced host?—Brood parasitism by *Pungtungia herzi* toward introduced *Coreoperca kawamebari*

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Abstract

The minnow *Pungtungia herzi* is a nest associate spawner that utilizes the spawning nests of other species, including freshwater perch, goby, and catfish. We investigated whether the minnow can utilize a nonindigenous host that has been recently introduced by human activity. In the Makuni River, Kii Peninsula, central Japan, the minnow has originally used the nests of the catfish *Pseudobagrus nudiceps*, which are located beneath rocks. Our field observations revealed that the minnow also uses the nests of the recently (~5 years ago) introduced perch, *Coreoperca kawamebari*, which are located at the stems of submerged plants. However, the frequency of utilization was lower than that in the native range of *C. kawamebari*, suggesting that the minnow has not yet fully used the new host. The new host may positively affect the population size of this brood parasite through the extension of the successful reproductive season of the minnow.

Keywords Nest association · Brood parasitism · Freshwater fish · Alien fish · Wakayama Prefecture

Introduction

In recent decades, human activities have increasingly spread artificially introduced species worldwide. Introduced species directly or indirectly impact native biological communities, often causing harmful effects on biodiversity through the extinction of species and alteration of ecosystem processes (Gurevitch and Padilla 2004; Vitule et al. 2009). On the other hand, the introduction of species can provide opportunities to study evolutionary and biodiversity processes under different selection regimes with newly constructed interspecific interactions (Cox 2004; Sax et al. 2005).

The freshwater perch *Coreoperca kawamebari* (Percichthyidae) is a typical alien fish that has invaded several regions in Japan (Ecological Society of Japan 2002; Matsuzawa and Senou 2008). The species was originally distributed in western Honshu, northern Shikoku, and northern Kyushu, Japan and in southern parts of the Korean Peninsula (Saitoh and Uchiyama 2015). However, due mainly to intentional, arbitrary introductions by hobbyists, the species has established many populations outside of its native range (e.g., in eastern and central Honshu). Keeping and releasing the species are hence prohibited in several regions (i.e., Shiga Prefecture 2006).

Coreoperca kawamebari, along with several other species that exhibit parental care, is used as a host in a heterospecific nest association by the minnow *Pungtungia herzi* (Cyprinidae) (Baba et al. 1990). At least three species, *C. kawamebari*, the goby *Odontobutis obscura*, and the catfish *Pseudobagrus nudiceps*, are known to foster eggs of *P. herzi* in their spawning nests (Nagata and Machata 1991; Yamane et al. 2004) despite the fact that the reproductive ecology and behavior of these hosts are somewhat diverse (Yamane et al. 2013). As a result of the nest association, the hosts *C. kawamebari* and *O. obscura* suffer negative impacts on their own eggs from the minnow's spawning (i.e., brood parasitism; Baba and Karino 1998; Yamane et al. 2013), whereas *P. nudiceps* is positively affected via predation on

the minnow eggs by the male parent and juveniles (Yamane et al. 2016). Based on the spawning behavior and reproductive tactics of *P. herzi*, Yamane et al. (2009) inferred the following evolutionary scenario of its interspecific brood adoption: (1) the ancestral mode of the spawning of *P. herzi* (or its ancestor) was substratum spawning in crevices formed by stones and rocks, likely without parental care; (2) *P. herzi* occasionally shared spawning sites with crevice spawners that engaged in parental care (e.g., *O. obscura* and *P. nudiceps*), providing opportunities for nest association; (3) the specialized brood adoption behavior using some stimulus from the host species evolved through strong selection involving the high survival rates of *P. herzi*'s eggs spawned in host nests; and (4) the stimulus from host species showing parental care caused the expansion of minnow hosts to those utilizing different environments for spawning sites (e.g., on the surfaces of submerged reed stems or similar structures in open spaces protected by *C. kawamebari*).

It is generally difficult to empirically examine the process of acquiring nest associations and novel hosts, and only a few studies have addressed the utilization of novel hosts following dispersal in brood parasitic birds, such as cuckoos (Nakamura 1990). However, observations of the response to artificially introduced hosts may provide an opportunity to study the acquisition process of novel hosts. Because *C. kawamebari* has been introduced to *P. herzi* habitats in which the former was not naturally distributed, these two species in such habitats allow examination of the process by which a novel interspecific interaction involving nest association occurs.

To explore the interaction between *P. herzi* and nonindigenous *C. kawamebari*, we conducted a quantitative survey of their nest association in several sections of a river where the latter species was recently introduced (Doi 2008). We then compared our results from the initial stage of their cohabitation (5 years or a little longer) to the situation observed in their native range (Baba et al. 1990). The rapid establishment of the nest association would imply that egg adoption by the minnow is simply triggered by some stimulus shared by the hosts.

Alternatively, if the nest association were incomplete in the short term, one might infer that the establishment of nest association would require adaptation to the unexperienced nest type of the novel host. The present report supports the latter possibility. Based on our findings, we discuss the process and consequences of the extension of host utilization by *P. herzi* when it encounters a new community.

Materials and methods

The field study was conducted in the Makuni River, a tributary of the Kinokawa River, in the northern part of Wakayama Prefecture, central Japan, in 2009. *Pseudobagrus nudiceps* is the only native species that *Pungtungia herzi* potentially uses as a host at the study sites. Since 2004 or just prior, *Coreoperca kawamebari* began to appear in this river (Kentarou Hirashima, pers. comm.). The river contains several small dams (weirs; 2–3 m in height) that prevent the upstream migration of fish (Fig. 1). In the preliminary survey, we searched for areas with high densities of both *C. kawamebari* and vegetation that provides its spawning substrate.

Subsequently, we selected three river sections with similar environmental conditions (from upstream: St. 1 with 100 m in river course length, St. 2 with 95 m, and St. 3 with 55 m; Fig. 1). These sections were separated by 380–640-m intervals, and one or more weirs were located between sections. St. 1 was located at nearly the uppermost limit where *C. kawamebari* was found.

Field observations were conducted eight times during May and September, the spawning season of *C. kawamebari*, at intervals of 12–24 days. The intervals were determined based on the hatching time of *C. kawamebari* and *P. herzi* (both around two weeks; Baba 1994; Yamane et al. 2013). During underwater observations, we exhaustively searched for and counted egg masses of fish attached to reed stems and other substrates. When any egg

masses were found, they were collected along with their substrate and transported to the laboratory. Hence, most egg masses found during a particular survey were assumed to have been spawned after the previous survey. In the laboratory, eggs were identified as those of either *C. kawamebari* or *P. herzi* based on size and color (Baba et al. 1990), and the number of eggs in each mass was counted for each species. The identification of *P. herzi* eggs was confirmed using mitochondrial cytochrome *b* sequences ($n = 8$ from three egg masses collected on 22 May 2009; DDBJ/EMBL/GenBank Accession number, LC425145–LC425152).

The density of nests of *C. kawamebari* and the relative density of *P. herzi* individuals were estimated in each river section. The former was estimated as the number of *C. kawamebari* nests per meter of the river course. The latter was evaluated as the number of adult *P. herzi* fish captured per minute. *Pungtungia herzi* were collected once at each station by two investigators using hand nets at night just after the spawning season of *C. kawamebari* (90 min on 22 Sept. at St.1, 125 min on 6 Sept. at St.2, and 85 min on 6 Sept. at St. 3). The density of *C. kawamebari* nests was compared among river sections using Friedman test followed by Steel–Dwass multiple comparisons using the functions “friedman.test” and “pSDCFlig” (NSM3 package), respectively, for the statistical software R 3.5.1 (R Development Core Team 2018). The density of the native host, *P. nudiceps*, appeared to be very low based on the rare day and night observations of this fish during the survey. Hence, the density of this species could not be quantitatively evaluated.

The rates of parasitized nests were compared among sites and with the data reported from a native range of *C. kawamebari* (the Ibo River system, Hyogo; Baba et al. 1990) using Fisher’s exact test. Also, the number of *P. herzi* eggs in each egg mass was compared with that reported from the native range (Baba et al. 1990) using Welch’s *t* test. A logistic regression analysis was performed to estimate the determinants of the brood parasitism of *C. kawamebari* nests by *P. herzi*. The criterion variable was the presence (1) or absence (0) of

parasitic spawning at each nest, and the explanatory variables were the density of *C. kawamebari* nests, the relative density of *P. herzi* individuals, the survey date (number of days since 30 April 2009), and the river section (St. 1–3, as ordinal). Interaction terms were not included because of the simplicity of the analysis. The analysis was performed using the function “glm” in the MuMin package for R 3.5.1. The best model was selected from all the possible models that did not include interaction terms using the Akaike information criterion (AIC). Other higher ranking models were also compared.

Results

Egg masses of *Coreoperca kawamebari* were observed in all eight surveys from May to September, and a total of 215 was recorded (Fig. 2). The peak of the spawning season occurred in May, and the majority of egg masses (56.3% in total) were found during the two surveys in May.

Pungtungia herzi eggs were found in 17 of the 215 egg masses of *C. kawamebari* (the rate of brood-parasitized nests was 7.9% in total). Eggs of *P. herzi* were mainly observed during the period from May to early June (Fig. 2), and the maximum rate of parasitized nests was 19.0% on 22 May (mean $\pm$ SD = $4.2 \pm 6.8\%$ for each survey, $n = 8$ surveys). These rates were all significantly lower than the values reported from the native range of *C. kawamebari* (65.5%, $n = 116$ nests; Baba et al. 1990; Fisher’s exact test, $P < 0.001$). The number of *P. herzi* eggs in each egg mass ranged from 1 to 161 (40 ± 52 , $n = 17$ egg masses), which was smaller than that observed in the native range of *C. kawamebari* (192 ± 294 , range: 2–1, 298, $n = 76$; Baba et al. 1990) (Welch’s t test, $t = -47.2$, $df = 17$, $P < 0.001$).

The density of *C. kawamebari* nests significantly differed among the three river sections (Friedman test, $P = 0.014$; Fig. 3a), and the density at St. 3 (lowermost section) tended to be

higher than that at the other two stations. However, no significant pairwise differences were observed among the three sections (Steel–Dwass test, $P > 0.41$). The relative density of *P. herzi* did not largely differ among sections, but it was slightly higher in the lowermost section (St. 1, 0.23; St. 2, 0.14; St. 3, 0.27 individuals captured/min; Fig. 3b). On the other hand, the rate of parasitized nests significantly differed among river sections (1.3–17.4% during the entire period; Fisher’s exact test, $P = 0.001$), with the highest rate occurring in the uppermost section (Fig. 3c). The selected logistic regression model for the occurrence of parasitic spawning included two explanatory factors: the density of *C. kawamebari* nests and the river section (Table 1). This model indicated that parasitic spawning of *P. herzi* tended to occur at higher densities of host nests in the upper river sections (Table 1). All seven models with AIC values smaller than the full model included the river section as an explanatory variable, whereas only three models of them included the density of *C. kawamebari* nests [Electronic Supplementary Material (ESM) Table S1]. The variable “date” was also included in three of the seven higher ranking models; i.e., parasitic spawning of *P. herzi* tended to occur in the earlier season (ESM Table S1; also see Fig. 2).

Discussion

The present study revealed that *Pungtungia herzi* can use an introduced species as a host for brood parasitism, but the degree of utilization, in terms of the rate of parasitized nests and the number of parasitic eggs, appeared to be lower than that observed in naturally cohabitating populations. This finding could be attributed to the low population density of *P. herzi* relative to that of the host species (native *Pseudobagrus nudiceps* and introduced *Coreoperca kawamebari*). However, although no direct data were available for comparing density among localities, our underwater observations did not support the presumed remarkably low density

of *P. herzi*. Additionally, considering the high fecundity of *P. herzi* (about 5,000 eggs in a 134-mm female; Nakamura 1969) and its group spawning behavior (Baba et al. 1990), the low rate of parasitism (1.3–17% in each river section vs. 65.5% in a native habitat; Baba et al. 1990) and the small number of parasitic eggs (average of 40 per nest vs. 192 in a native habitat; Baba et al. 1990) suggest that *P. herzi* did not fully use the available host resources at this site. Also, very high rates of parasitism by *P. herzi* for the nests of *P. nudiceps* (90.9%) and *Odontobutis obscura* (95.3%) in the same river system (Yamane et al. 2013) suggest potentially high parasitic pressure by *P. herzi*. Collectively, our result implies that the brood parasitism of *P. herzi* on the introduced host has not yet been completely established.

However, quantitative evaluation of the relative dependence on the new host is difficult as no data are available on the extent of brood adoption on the natural host (*P. nudiceps*) at this site.

The rate of parasitic spawning tended to be higher in the upper river section, which may reflect the time elapsed since colonization by *C. kawamebari*. Although the parasitic rate may be expected to be higher when the densities of the available host and the parasite itself are higher, no relationship or even the opposite relationship was observed in our case. On the other hand, *C. kawamebari* is inferred to have spread from upper to lower reaches, because several weirs hinder the upstream movements of fish. Hence, *P. herzi* and introduced *C. kawamebari* may have cohabitated for a relatively long time in the upper reaches. This hypothesis supports the idea that *P. herzi* cannot immediately start to utilize *C. kawamebari* nests, which occur in open spaces rather than in concealed spaces like the nests of the native host. Conversely, some *P. herzi* had already begun to parasitize nests within several years of first encountering *C. kawamebari*, which may reflect the expansion process of host utilization. Alternatively, *P. herzi* might utilize *C. kawamebari* nests at a higher frequency in the upper section because of a more severe shortage of the native host (*P. nudiceps*) in that section. The probability of this explanation cannot be completely negated because no population size data are available for *P. nudiceps*.

Introduced *C. kawamebari* has the potential to strongly affect the population dynamics of *P. herzi* via the extension of the latter's successful reproductive season. The period from May to June, when *P. herzi* eggs were most often observed, occurs before the spawning season of the native host *P. nudiceps* in this region (July to August; Yamane et al. 2004, 2013). *Pungtungia herzi* occasionally performs non-parasitic spawning when host availability is low, but the survival rate of eggs without alloparental care is quite low (2.4% of eggs, data from the same river system as this study; Yamane et al. 2009). These facts imply that introduced *C. kawamebari* individuals contribute to the increased reproductive success of *P. herzi*, particularly during the early spawning season.

The question remains whether this period of cohabitation with *C. kawamebari* represents the historically first such event for the *P. herzi* population in the study area. Considering the similar but narrower distribution of *C. kawamebari* (Fig. 1, top-left map), the species may have been extinct at the eastern edge of its distribution (including the study site), or only *P. herzi* may have expanded its range to the eastern area from the area of cohabitation. In either case, their cohabitation at the present location via the artificial introduction of *C. kawamebari* cannot be strictly considered a case of an acquisition of a new host. Even in such a case, however, further research on the dynamics of their interaction (i.e., temporal changes in the strength of parasitism and the responses of host and parasite populations) will provide an important opportunity to examine the (re)construction process of interspecific relationships.

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Figure legends

Fig. 1 Location of study sites and distribution of host species [*Odontobutis obscura* (*Oo*), *Pseudobagrus nudiceps* (*Pn*), introduced *Coreoperca kawamebari* (*Ck**)] in the Kinokawa River system, Wakayama Prefecture, central Japan (St. 1, 34.2209°N, 135.5071°E; St. 2, 34.2222°N, 135.5032°E; St. 3, 34.2255°N, 135.4967°E). The associate species *Pungtungia herzi* is distributed widely in this river system. Top-left inset represents the natural distributions of *P. herzi* and *C. kawamebari*

Fig. 2 Seasonal changes in the number of *Coreoperca kawamebari* nests with (*open*) and without (*solid*) *Pungtungia herzi* eggs. Photograph: A spawning nest of *C. kawamebari* containing parasitic eggs of *P. herzi* (9 May 2009)

Fig. 3 Average density of *Coreoperca kawamebari* nests (**a**), relative density of *Pungtungia herzi* (**b**), and average rate of brood-parasitized nests of *C. kawamebari* (**c**) at each study section (St. 1–St. 3). *Bar* standard deviation. The number of surveys in **c** includes only those in which *C. kawamebari* nests were found

Table 1 Results of logistic regression analyses on the occurrence of parasitic spawning in *Coreoperca kawamebari* nests by *Pungtungia herzi*

Best selected model (AIC = 108.0)				
	Estimate	Std. error	<i>z</i> value	Pr(> <i>z</i>)
(Intercept)	−0.9229	0.7754	−1.1900	0.2340
Density of <i>C. kawamebari</i> nests	7.1849	4.3468	1.6530	0.0984
Section	−1.7788	0.5131	−3.4670	0.0005
Full model (AIC = 111.4)				
	Estimate	Std. error	<i>z</i> value	Pr(> <i>z</i>)
(Intercept)	−0.1417	1.8743	−0.0760	0.9398
Density of <i>C. kawamebari</i> nests	5.2281	5.1088	1.0230	0.3061
Density of <i>P. herzi</i>	−0.5562	6.9325	−0.0800	0.9361
Date	−0.0121	0.0169	−0.7170	0.4735
Section	−1.7424	0.5713	−3.0500	0.0023

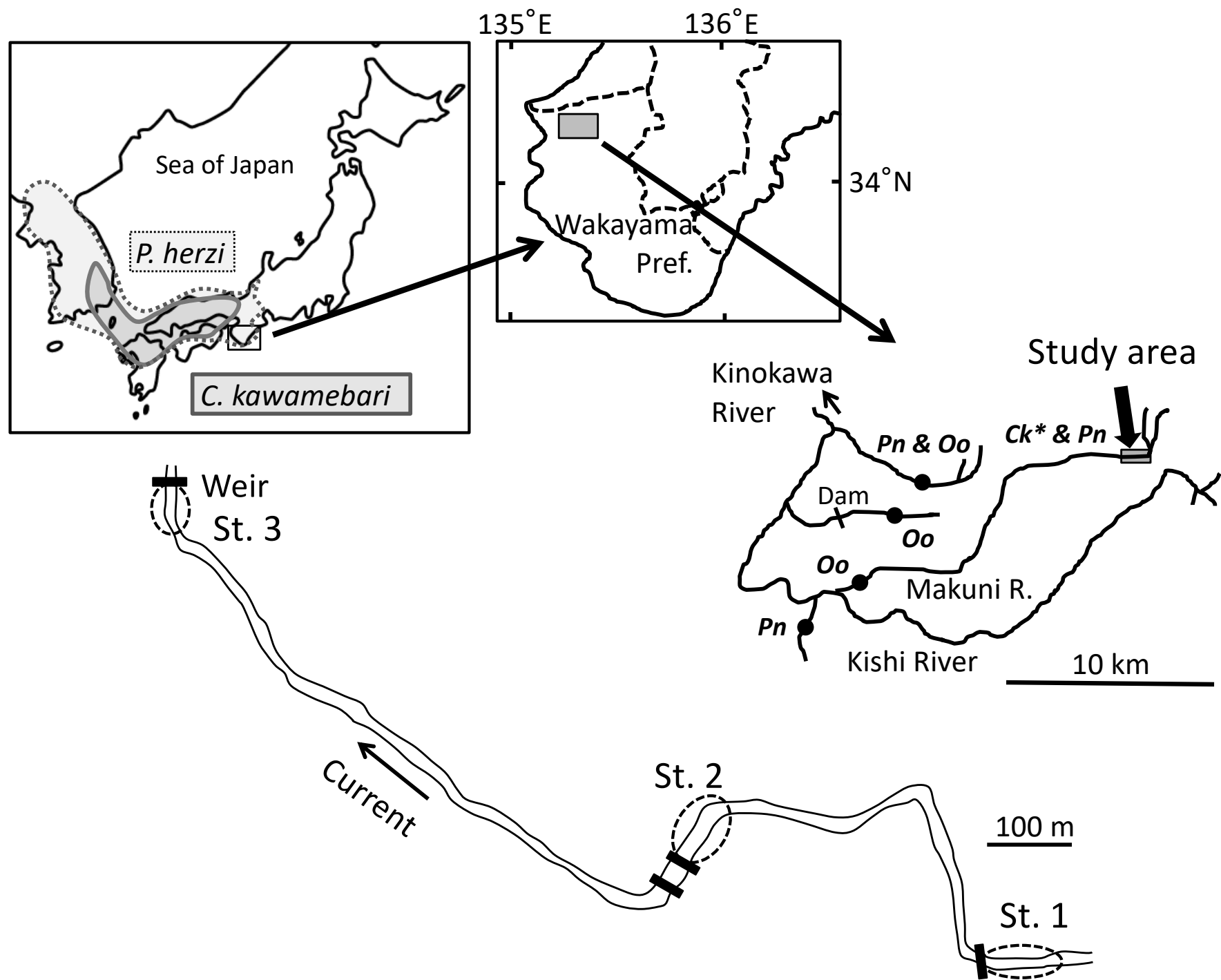


Fig. 1

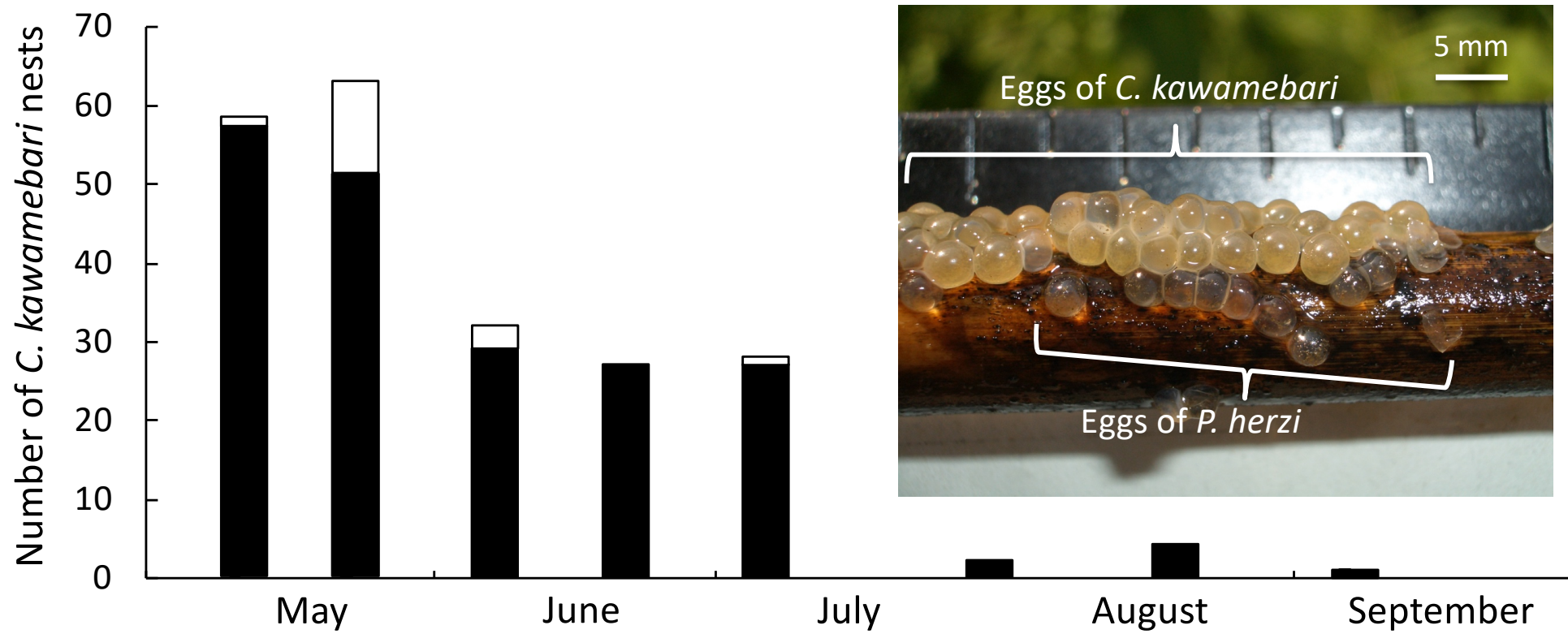


Fig. 2

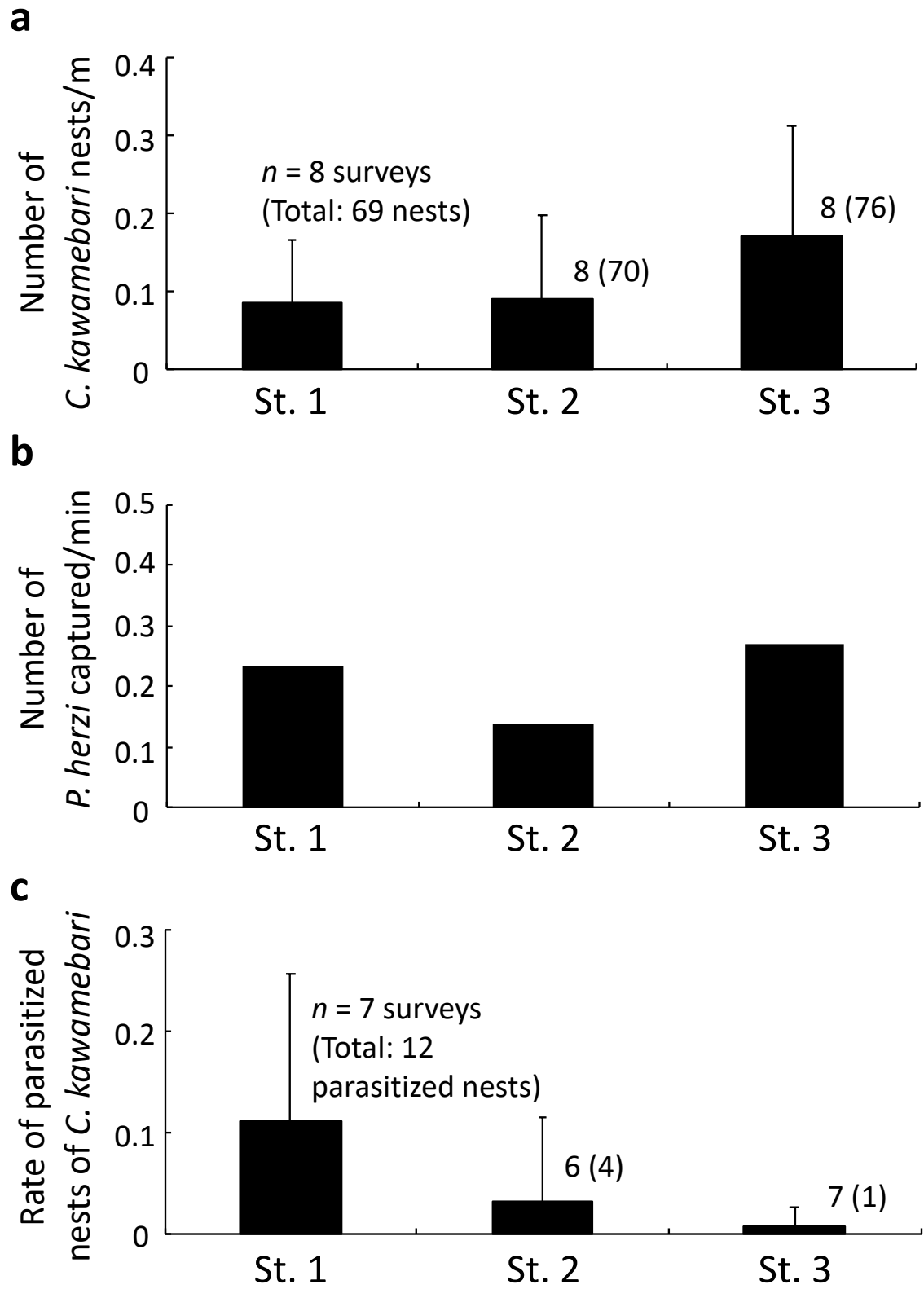


Fig. 3

ESM Table S1 The comparison of all possible models without interaction terms for the occurrence of parasitic spawning in *Coreoperca kawamebari* nests by *Pungtungia herzi* and the result of the model selection by AIC

Model	(Intercept)	Density of <i>C. kawamebari</i> nests	Density of <i>P. herzi</i>	Date	River section	df	Log likelihood	AIC	ΔAIC
Best	-0.9229	7.185	—	—	-1.779	3	-51.017	108.0	0.00
	0.5296	—	—	-0.02092	-1.422	3	-51.264	108.5	0.49
	-0.2194	—	—	—	-1.326	2	-52.554	109.1	1.07
	-0.2602	5.167	—	-0.01239	-1.727	4	-50.717	109.4	1.40
	-0.4819	7.235	-1.8450	—	-1.823	4	-50.979	110.0	1.92
	0.4147	—	0.4849	-0.02106	-1.410	4	-51.261	110.5	2.49
	0.0782	—	-1.2220	—	-1.355	3	-52.537	111.1	3.04
Full	-0.1417	5.228	-0.5561	-0.01208	-1.742	5	-50.714	111.4	3.39
	-0.3432	-5.890	—	-0.03138	—	3	-57.139	120.3	12.24
Null	-2.4550	—	—	—	—	1	-59.446	120.9	12.86
	-1.9910	—	—	-0.01612	—	2	-58.596	121.2	13.16
	-0.7796	-6.343	2.7850	-0.03338	—	4	-56.987	122.0	13.94
	-2.1430	-1.527	—	—	—	2	-59.285	122.6	14.54
	-2.1740	—	-1.3200	—	—	2	-59.404	122.8	14.77
	-1.9630	—	-0.1388	-0.01605	—	3	-58.596	123.2	15.16
	-1.9880	-1.432	-0.8194	—	—	3	-59.270	124.5	16.51