

Component community dynamics of larval trematodes in the freshwater snail *Semisulcospira nakasekoe* in the Uji River, central Japan

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Abstract

The component community of larval trematodes in the freshwater snail *Semisulcospira nakasekoe* (Caenogastropoda: Sorbeoconcha: Pleuroceridae) was surveyed over 13 months from April 1996 to April 1997 inclusive. Temporal and spatial fluctuation of trematode prevalence, the frequency of multiple infections, and the duration of cercarial shedding were examined as factors that might affect trematode community structure. The spatial prevalence of some species varied significantly, but the dynamics were too small to allow an explanation of the overall pattern. The prevalence of sanguinicolid flukes fluctuated temporally, despite a stable size distribution in the host populations (> 6.0 mm shell width), suggesting the life-cycle phenology of this species. Some pairs of species had statistically positive associations, but no pairs had negative associations. This shows the importance of positive association possibly as a result of suppression of the host defensive response on trematode community structures in molluscan hosts. The length of the patent period, which is part of the persistent period, varied among trematode species, suggesting it to be one of the factors determining prevalence in the host population.

Introduction

The component communities of larval trematodes in molluscan hosts have been much studied by community ecologists as a convenient set of systems to quantify resource (= host) density, the ratio of occupied (infected) and unoccupied (uninfected) resource patches, and the intensity of competition. The temporal and spatial variations of both host and parasite populations are the primary factors that determine the community structure of parasites. Interspecific interactions at the infracommunity level, for example, interspecific competition and predation, have also been found to organize the

component communities of trematodes in intertidal non-pulmonate snails, such as *Cerithidea californica* and *Ilyanassa obsoleta* (Sousa, 1993; Lafferty *et al.*, 1994). Interspecific interaction among trematodes also exists in freshwater pulmonate snails, but it is not thought to be an important factor in community organization (Fernandez & Esch, 1991b; Snyder & Esch, 1993; Yonder & Coggins, 1998). Instead, other factors, such as the temporal and spatial heterogeneity of infective trematodes (eggs or miracidia) and the population dynamics of their hosts, are considered important for trematode community dynamics (Fernandez & Esch, 1991a,b; Williams & Esch, 1991; Snyder & Esch, 1993; Esch *et al.*, 1997). The relative importance of interspecific interaction between parasites is affected by factors such as host life span, size (age) distribution, the phenology of trematodes, and the

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heterogeneous distribution of trematodes in infective stages. Among freshwater pulmonates with a short lifespan, the trematode community structure is governed more by population recruitment of hosts (Williams & Esch, 1991; Yonder & Coggins, 1998) than is the case among intertidal non-pulmonate snails, which are principally affected by parasite accumulation over their longer life spans (Abdul-Salam & Sreelatha, 1999).

In the present study, the component community of freshwater snail *Semisulcospira nakasekoe* (Kuroda, 1929) (Caenogastropoda: Sorbeoconcha: Pleuroceridae) was surveyed for 13 months from April 1996 to April 1997 inclusive, focusing on the interspecific interactions of trematodes in each host snail. There have been many epidemiological studies on the trematode fauna of *Semisulcospira* spp. (e.g. Ito *et al.*, 1959; Kagei, 1966; Tomimura *et al.*, 1989), some of which include important information about the community ecology of larval trematodes. However, discussions from an ecological viewpoint have been insufficient. *Semisulcospira* requires about one to two years to mature (Mishima, 1973; Takami, 1994, 1998) and can live for more than four to five years in the laboratory (Takami, 1994, 1998). Therefore, one can expect that the impact of interspecific interaction between trematodes on the trematode community of *Semisulcospira* may be greater than those of short-lived pulmonate snails. One interesting case is the lung fluke *Paragonimus westermani*, which can parasitize snails successfully when the host has been occupied by another trematode species (Hamajima *et al.*, 1981, 1989), suggesting that pre-suppression of the host immune response by other parasites is essential for the colonization of some species and for the organization of the trematode community in *Semisulcospira*.

Two common methods have been used to monitor infections in previous studies, namely the dissection of host snails and examination of free-emerged cercariae in the laboratory (Williams & Esch, 1991; Sousa, 1993). Dissection reveals the exact prevalence of infection and provides direct evidence of the interspecific interaction between parasites that is commonly observed among parthenitae. On the other hand, the examination of emerged cercariae allows for the fate of a host to be traced. This latter method is commonly used in the field in combination with the mark-recapture method, allowing researchers to investigate the replacement of cercariae species and the mortality and reproductive success of infected hosts. Under laboratory conditions where new infections can be prevented, the persistence of parasites in a host that contributes to the parasite prevalence in the host population can be measured. However, parasites in the prepatent period cannot be monitored by this method (Curtis & Tanner, 1999) and the incidence of multiple infection tends to be underestimated.

In the present study, the monthly prevalence of *S. nakasekoe* was investigated by the dissection method. The frequency of multiple-species infections was also examined for potential interspecific interference. Spatial and seasonal heterogeneities of infected snails were also analysed to test the effects of these factors on the overall community pattern. Some infected snails were also kept individually in the laboratory to investigate the persistence period of trematodes and the fate of hosts.

The importance of seasonal and spatial factors, interspecific interaction and parasite persistence in the component community dynamics of *S. nakasekoe* are discussed.

Materials and methods

Study site and snail hosts

The study was conducted at Ingen Bridge on the Uji River, at Makishima, Uji City, Kyoto Prefecture, central Japan (135°48'E and 34°55'N). The Uji River is the only outlet of Lake Biwa. The water level is controlled by two dams above Ingen Bridge. Normally, the river is about 70 m wide. The riverbed consists mostly of sand and cobbles, with an artificial rubble protection around the bridge piles.

Semisulcospira occurs at high density at this site (> 100 m⁻²). Three species, *S. nakasekoe*, *S. habei* and *S. reiniana* are dominant. The target species *S. nakasekoe*, which is endemic to the Uji River (Kuroda, 1929; Davis, 1969), has the richest trematode fauna among the three dominant species (Urabe, 2003). Therefore, we focused on the component community of trematodes in the *S. nakasekoe* population. The other two species have almost the same parasite fauna as that of *S. nakasekoe* except one species specific to *S. reiniana* (*Notocotylus magniovatius*; Urabe, 2003), but their prevalence was much lower than *S. nakasekoe* (Urabe, unpublished data) and their effect on parasite prevalence of *S. nakasekoe* is negligible.

Snails were collected from three habitats within 25 m of the south riverbank: within a cove in the riverbank (area A); from a sandy-pebble substratum in medium currents (area B); and from an artificial rubble area subject to fast currents (area C). Adult *S. nakasekoe* would be able to migrate between these areas within several days judging from the moving ability of congeneric species *S. reiniana* and *S. libertina* (Urabe, 1998). Therefore, the variance of trematode infection among these three sites was assumed to be that found between microhabitats for *S. nakasekoe*. From December 1996 to April 1997, almost all snails were collected from area C because an increase in water level had changed the conditions at areas A and B, and prevented further collection of snails from these areas.

Monthly survey of trematode infection

To monitor the dynamics of parasites, different cohorts of hosts must be analysed separately, because older cohorts are exposed to infective parasites for longer periods and inevitably accumulate more parasites (Fernandez & Esch, 1991a). Body size is often used as an indicator of host age (Lafferty *et al.*, 1994). In *Semisulcospira*, parasite prevalence is usually much higher in large and older snails than in small ones (Ito *et al.*, 1959) as expected. Because *S. nakasekoe* has a relatively long life span (>4–5 years) and a long reproductive season (Takami, 1994, 1998), cohorts are difficult to separate by size, especially among mature snails. Therefore, we only excluded juvenile snails from the analysis to lessen the effects of the dynamics or local heterogeneity of host population on parasite prevalence.

Juvenile snail densities vary greatly, both temporally (Mishima, 1973) and spatially (Urabe, 2000).

Snails with a shell width exceeding 6.0 mm were collected from April 1996 to April 1997 at the three sampling areas. Individuals of *S. nakasekoe* having a shell width of 6.0 mm are estimated to be 4 months old for juveniles born in spring, and 8–9 months old for juveniles born in autumn (Takami, 1998). Under experimental conditions, male *S. nakasekoe* start to produce spermatophores at 5 months, when they have a shell with an average of 6.1 mm, and females produce juveniles at 10–12 months with shell widths of 8.0–8.4 mm (Takami, 1998). Thus, snails having a shell width less than 6.0 mm are expected to be immature.

Snail sampling and water temperature measurements were carried out between 1030 and 1300 h. Snails were brought to the laboratory and their shell widths measured parallel to the shell axis using calipers. Shells were then crushed and the soft bodies dissected in 0.4% NaCl solution to obtain parthenitae and cercariae. Living cercariae and parthenitae were observed with a microscope using Nomarski optics. Neutral red and Nile blue sulphate were used for vital staining to help with gland cell observation. When only parthenitae were present, species identification was difficult. The sex and maturity of snails were also recorded as indicators of their developmental stage, although parasitic castration in infected snails often made this impossible.

Statistical analysis

Temporal and spatial fluctuations in parasite prevalence were investigated for the six major trematode species (see Results) to clarify phenology and microscale heterogeneity. Data collected between April and November 1996 were used for this analysis. Data collected during and after December 1996 were omitted because snails were not collected from all three areas during this period. To test whether the presence/absence of parasites was independent of sampling areas and sampling month, three-dimensional contingency tables were analysed for each parasite species. The probability of mutual independence of the three parameters and the partial independence of presence/absence of parasites were calculated by the 10,000 randomization test. When the presence/absence of parasites was not independent of sampling area and month, partial independence of the presence/absence of the other two variables was tested using a log-linear model. The significance of partial independence was decided using two criteria: probability by G-test when the corresponding interaction term is deleted from the hierarchical model, and by improvement of the model, namely diminishing Bayesian Information Criterion (BIC) (Powers & Xie, 2000) when the interaction term was added. The probability by G-test is affected by the sample size, but BIC is an indicator that deducts the sample-size effect from the G-value. For the calculation of log-linear models, 0.5 was added to all cells when the contingency table included zero cells (Everitt, 1977).

The frequency of multiple-species infection was analysed for all species pairs every month, to investigate interspecific interference. Fisher's exact test was used to analyse frequency, and the simulation procedure used by

Sousa (1993) was applied to resolve the independence of each test. The experimental error rate was set at 5% for each month. In this method, the frequency of multiple infection without interspecific interaction was estimated directly by the simulation using the prevalence of each trematode species and host number obtained from the field data. Therefore, one need not consider the effects of prevalence and sample size on the estimated frequency of positive or negative associations (Lotz & Font, 1994).

The computer programs for these randomization and log-linear models were written according to Tachibana (1997).

Cercarial shedding

Between May 1999 and June 2000, *S. nakasekoe* were collected several times at Ingen Bridge to study the duration of cercariae shedding. Snails were kept in water in 250 ml plastic cups each containing one to three individuals, with O₂-generating stones (Japan Animal Medicine Inc., Tokyo, Japan), in the laboratory maintained at 25°C. Every 2–3 days, snails were fed on Tetramin (Tetra, Germany), the water was changed, and emerged cercariae were checked. In several cases, snails were dissected after shedding had ceased for over 26 days, to check for the presence of trematodes. Uninfected snails were included in the data for the duration of cercariae shedding, while those containing trematodes were excluded. Snails that died during the experiment were usually too decomposed for any observations on the parasites. The total experimental period varied according to the shedding period of each snail, and ranged from four to 382 days.

The prevalence of each trematode species was also determined among shedding snails. This value, however, underestimates trematode prevalences when compared with the exact values among dissected snails, because non-shedding snails that had died during the experiment might have been infected with parthenitae or immature cercariae.

Results

Snail size distribution

Water temperatures and the monthly size distribution of *S. nakasekoe* from all three sampling areas are shown in figs 1 and 2, respectively. The size distribution pattern was unimodal, with peaks in the 8–9- or 9–10-mm classes from April to August in 1996 and from March to April in 1997 (fig. 1). From September 1996 to January 1997, the frequency of immature snails, which are estimated to have been born in the spring and summer of 1996, increased and the peaks shifted to 6–7 mm or 7–8 mm. The mean shell width of infected snails (8.8 mm) was larger than that of uninfected snails (8.5 mm; t-test for unequal variances: $P < 0.0001$).

Species of cercariae

Fifteen trematode species was detected from 4891 individuals of *S. nakasekoe*. Six of these were common: a sanguinicolid ($n = 105$); *Genarchopsis goppo* (= *Cercaria*

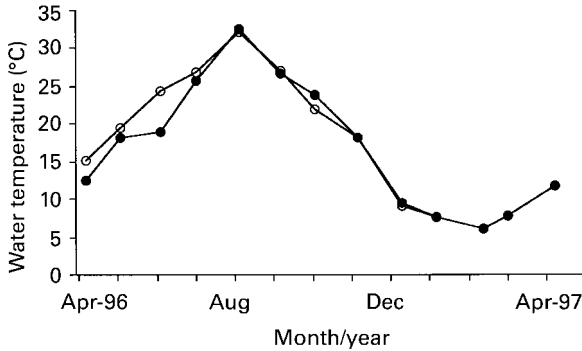


Fig. 1. The daytime water temperatures at Ingen Bridge from April 1996 to April 1997. ○, Area A (cove within riverbank); ●, area C (artificial rubble substrate with fast currents).

yoshidae; Urabe, 2001a) ($n = 37$); an opoecoid ($n = 333$); *Cercaria innominata* (originally spelled *C. innominatum*) ($n = 29$); cercaria of *Metagonimus* with four oral spines which may include *M. yokogawai*, *M. hakubaensis* and *M. otsurui* (Shimazu, 1999a; Shimazu & Urabe, 2002) ($n = 71$) and *Pseudexorchis major* ($n = 49$). The sanguinicolid and the opoecoid species possess only sporocysts as the parthenitae, whereas the others have rediae. Less frequent species included *Cercaria longicercus* ($n = 12$),

Cercaria problematica ($n = 1$), *Cercaria introverta* ($n = 5$), *Echinochasmus* sp. ($n = 2$), *Paragonimus westermani* ($n = 1$), *Cercaria monostyloides* ($n = 2$), *Cercaria* D (a cercarium; Urabe, 2001b) ($n = 1$), *Metagonimus takahashii* ($n = 5$) and *Centrocestus* spp. ($n = 8$).

Temporal and spatial fluctuation in prevalence of infection

Temporal and spatial fluctuations in parasite prevalence were investigated for the six major trematode species. Table 1 shows the number of *S. nakasekoe* collected from each sampling area by month. The *Metagonimus* species were placed in a single category, because species identification in this genus is not possible for immature cercariae. Figure 3 shows the monthly prevalence of the major trematodes. Table 2 summarizes the partial independence of the presence/absence of parasites and the sampling area and/or month. Figure 4 shows the additional data on prevalence obtained during the shedding experiment in 1999 and 2000.

Randomization tests using data generated during April and November 1996 showed that the three variables were dependent on each other among the six major parasite species (table 2). The presence/absence of *Pseudexorchis major* was independent of the sampling area and month, whereas that of the other five species was not independent.

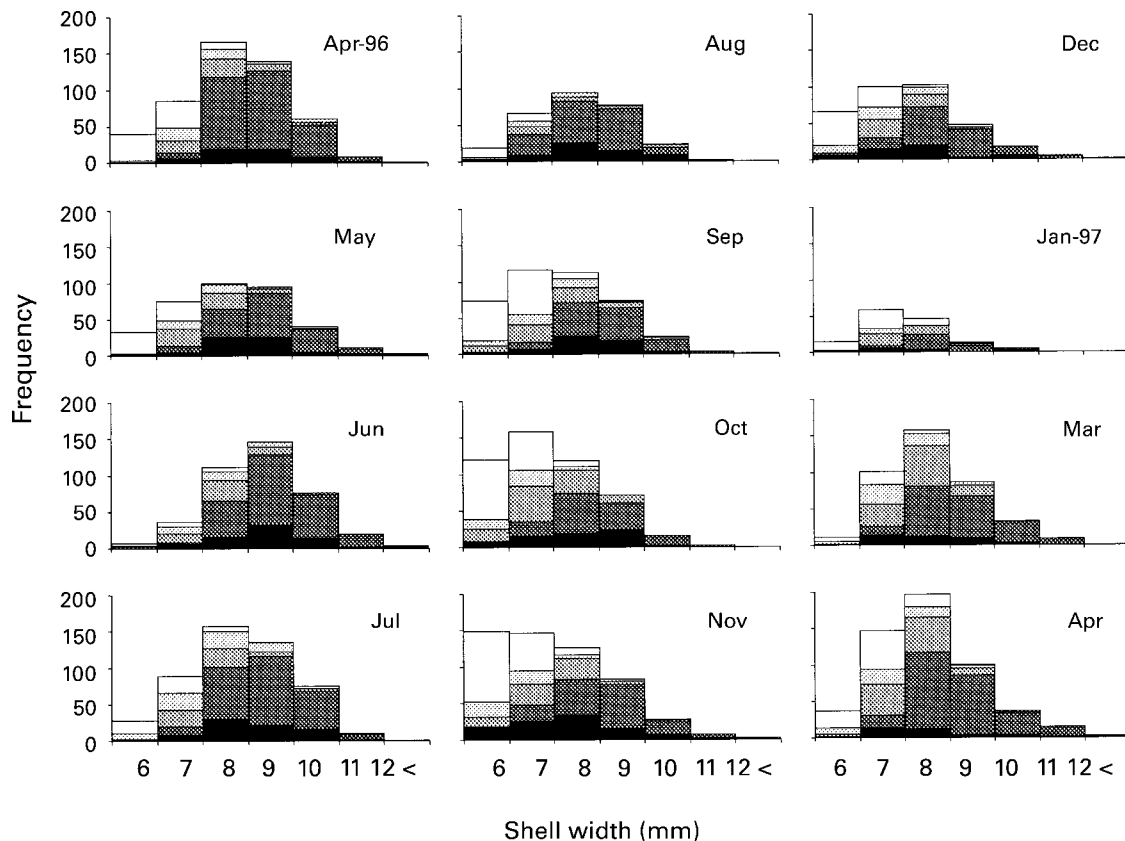


Fig. 2. The monthly size distribution of *Semisulcospira nakasekoe* from April 1996 to April 1997. Open bar, immature snails; pale grey bar, males; mid grey bar, non-gravid females and females with eggs or unshelled embryos; dark grey bar, females with shelled embryos; black bar, infected snails.

Table 1. Number of *Semisulcospira nakasekoe* examined at each sampling area, from April 1996 to April 1997.

Year	Month	Sampling areas*			Total
		A	B	C	
1996	Apr.	149	153	200	502
	May	88	153	115	356
	Jun.	22	287	94	403
	Jul.	109	184	204	497
	Aug.	63	175	48	286
	Sep.	86	132	188	406
	Oct.	47	181	261	489
	Nov.	78	185	278	541
	Dec.	6	14	316	336
	1997	Jan.			
Feb.					7
Mar.					398
Apr.					534

* A, within a cove in the riverbank; B, sand-pebble substratum in medium current; C, artificial rubble area subject to fast currents.

The log-linear model analysis revealed that the presence/absence of sanguinicolid depended on both the sampling area and month, and that the presence/absence-month interaction term was effective for the diminution of BIC. Clear prevalence peaks occurred in April–May and November in 1996 (fig. 3), in areas A and B. During 2000, the prevalence peaked in March (fig. 4) and was low from summer to early autumn in both 1996 and 1999 (August–October 1996, September 1999). In November 1996, the prevalence suddenly increased in all areas and all detected cercariae were young and immobile, suggesting they had been recruited just before sampling.

The presence/absence of *Genarchopsis goppo* was not independent of site and month, but the log-linear model did not reject partial independence between presence/absence and other variables (table 2). In the opecoelid cercariae, the presence/absence of the parasites was dependent on both area and/or month. In 1996, the seasonal fluctuation of prevalence was very different among the three areas (fig. 3). This was also observed in 1999. For *Cercaria innominata*, only the partial independence of the parasite presence and area was rejected statistically. The presence/absence of the *Metagonimus* spp. was dependent on both area and month. Their prevalence was high in the summers of 1996 and 1999 (May–October 1996 and June 1999), but the peak varied between areas and years. From June to August, all snails harbouring *Metagonimus* had mature cercariae.

The log-linear model analysis revealed that the interaction was significant in some cases, but BIC was improved only in a single case, suggesting that the effect of seasonal/spatial factors on the dynamics of prevalence was generally smaller than the unmeasured noise effects in the study area.

Analysis of multiple-species infections

From 1996 to 1997, 26 multiple-species infections were detected. Twenty-three were double infections: three sanguinicolid + *G. goppo*; seven sanguinicolid +

opecoelid; two sanguinicolid + unidentified redia; two *G. goppo* + opecoelid; two *C. longicerca* + opecoelid; one *C. introverta* + opecoelid; one opecoelid + unidentified redia; one *C. innominata* + four-spine *Metagonimus*; two opecoelid + *P. major*; and two four-spine *Metagonimus* + *P. major*. There were two triple infections: two *C. introverta* + opecoelid + four-spine *Metagonimus*, and one quadruple infection: *C. introverta* + opecoelid + four-spine *Metagonimus* + *Centrocestus* sp. Direct interspecific interference was observed between *C. innominata* and *Metagonimus*, i.e. the rediae of the former preyed on the rediae and cercariae of the latter.

The frequency analysis for all pairs of trematode species detected positive associations in several cases, but no significant negative associations were found. Table 3 shows the result of monthly frequency analysis. The species pairs having positive associations in one month or more are shown in the table. The simulation procedure for the experimental error rate revealed that the positive association among some species pairs was significant in June 1996 (sanguinicolid + *G. goppo*) and in November 1996 (sanguinicolid + opecoelid; *C. introverta* + opecoelid; *C. introverta* + *Metagonimus* spp.; *C. introverta* + *Centrocestus* spp. and *Metagonimus* spp. + *Centrocestus* spp.). In June, the sanguinicolid was detected from two snails, both of which were doubly infected with *G. goppo*. Four of five infections with *C. introverta* were multiple infections with other species; three cases were of triple or quadruple infections also involving the opecoelid and four-spine *Metagonimus*.

Cercariae shedding

Table 4 shows the results of the cercariae shedding experiment, with four trematode species presenting sufficient data for analysis. Nine snails were monitored for sanguinicolid until they died, and four snails were dissected 27–71 days after they had stopped shedding. Two of the dissected snails retained dead sporocysts, whereas the remaining snails were parasite-free. For the opecoelid, 33 snails were monitored until they died, one was 'taken over' by *C. innominata*, and two were dissected before they died. One dissected 90 days after shedding ceased was parasite-free, whereas the other dissected 34 days after shedding ceased, contained living sporocysts. For *C. innominata*, three hosts that shed only *C. innominata* were monitored until they died. In addition, two snails were observed in which *C. innominata* was the dominant species. The first case involved a snail that shed opecoelid cercariae for 49 days, ceased shedding for 42 days, shed *C. innominata* for 11 days, ceased shedding for 11 days and then died. In the second case, a snail shed *Metagonimus* sp. for 138 days, stopped shedding for 15 days, shed *C. innominata* cercariae once, before dying 4 days later. The duration of shedding of these two individuals is shown separately in table 4. In these snails, the entire patent period of *C. innominata* was monitored, whereas it was assumed to have been monitored from the mid-point of the patent period in the other snails. For *Metagonimus* spp., five snails were followed until they died; one was 'taken over' by *C. innominata*, and five were dissected alive. One snail dissected 155 days after shedding was parasite-free, whereas the other four snails dissected

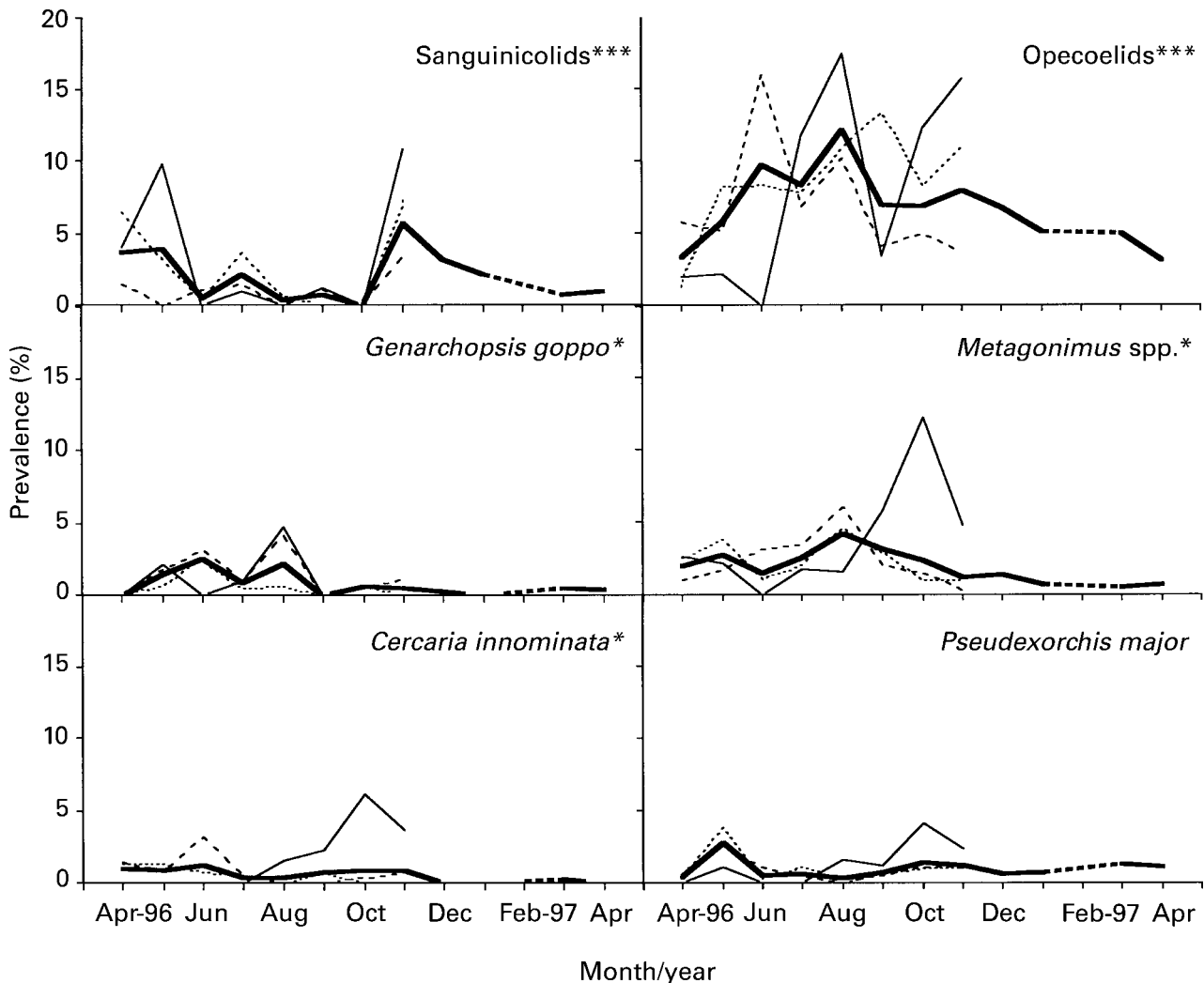


Fig. 3. Monthly prevalences of the six common trematode species at the three sampling areas (—, area A; ···, area B; ---, area C; —, total) at Uji in 1996–1997. Asterisks show the probability of partial independence of the presence/absence of trematodes and month/area (* $P < 0.01$, *** $P < 0.0001$).

44–63 days after shedding no longer retained living rediae (table 4).

When data from the snails in which *C. innominata* was dominant, and also from the five snails in which *Metagonimus* remained as rediae at the end of the experiment were excluded, the length of the patent period was significantly different among the four trematode species (ANOVA; $F = 4.500$, d.f. = (3, 51), $P = 0.0071$). A post-hoc test revealed a difference between the sanguinicolid and the opecoelid (Scheffe's test; $P = 0.0164$).

Discussion

Temporal and spatial fluctuation in the prevalence of infection

In this study, seasonal dynamics of the prevalence of infection of some trematodes were detectable as separate from the seasonal dynamics of the host populations

themselves. In particular, the seasonal change in prevalence of the sanguinicolids was effective for the diminution of BIC. Spatial heterogeneity was also detected in some trematode species, but the distribution pattern among the three sampling sites varied between months and none of them improve BIC. Therefore, spatial heterogeneity in prevalence among the sampling sites can be excluded from the analysis of component community pattern at this study site.

The sanguinicolid was the only species showing clear seasonal fluctuation. Because its definitive host species (a fish) is unknown, the ecological significance of its phenology is uncertain. The prevalence of *Metagonimus* also varied among seasons, with development of the cercaria showing remarkable seasonal changes. According to the data of *M. yokogawai* that produces cercariae at 20–26 °C (Kagei, 1966), the shedding season of *Metagonimus* is thought to be from May to October at Ingen Bridge. The shedding peak is expected to be from June to August based

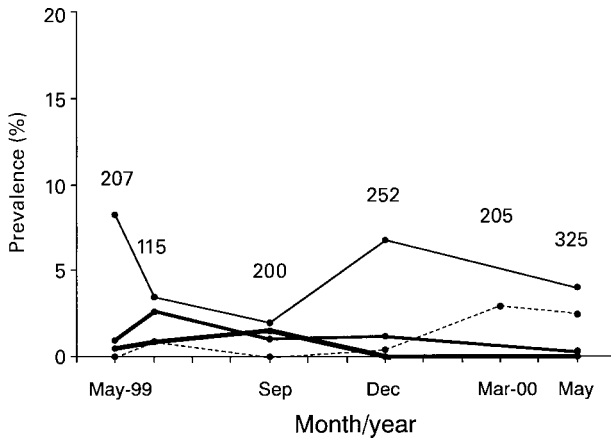


Fig. 4. Additional data of the prevalence of trematode infections in *Semisulcospira nakasekoe* obtained in the shedding experiment from May 1999 to May 2000. Numbers within the graph are the numbers of snails examined. ···, sanguinicolids; —○—, opecoelids; —■—, *Metagonimus* spp.; —▲—, *Cercaria innominata*.

upon cercarial development in the snails examined. Results from the shedding experiment suggest that hosts may suspend shedding during periods of low temperature, harbouring only parthenitae and immature cercariae.

Interspecific interaction among larval trematodes

Makita *et al.* (1996b) showed that *C. introverta* co-parasitized host snails with other species at a significantly higher rate than that expected by chance. *Cercaria introverta* may be an obligate second invader, as in the case of *Paragonimus westermani* (Hamajima *et al.*, 1981, 1989) and of *Austrobilharzia terrigallensis* (Walker, 1979), or it may suppress the host's defence to such an extent that it becomes vulnerable to infection by other parasites. *Metagonimus* spp. and *Centrocestus* spp. co-occurred in the quadruple infection in November 1996. The significant positive association between them is likely to be a side-effect of parasite accumulation accompanying the infection with *C. introverta*.

Table 2. Probability of partial independence between the presence/absence of trematodes and month and/or the sampling area.

Trematode species	Randomization test		Log-linear regression		
	All variables	Partial independence of presence/absence vs. area and month	Partial independence of presence/absence		
			vs. area	vs. month	vs. both
Sanguinicolids	<0.0001	<0.0001	0.0083	<0.0001*	<0.0001
<i>Genarchopsis goppo</i>	<0.0001	0.0092	0.9149	0.1113	0.1446
Opecoelids	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001
<i>Cercaria innominata</i>	<0.0001	0.0056	0.0339	0.2579	0.1553
<i>Metagonimus</i> spp.	<0.0001	0.0013	0.0124	0.0337	0.0098
<i>Pseudexorchis major</i>	<0.0001	0.1257			

*The result of log-linear regression analysis shows the probability by G-test when the corresponding parameter is deleted from the hierarchical model. The asterisk shows the variable pair that always improves the model (diminishing BIC) by adding the interaction term.

Table 3. Analysis of double-infection frequency by the Fisher's exact test among some trematode species of *Semisulcospira nakasekoe*.

Trematode combination	1996									1997		
	Apr.	May	Jun.	Jul.	Aug.	Sep.	Oct.	Nov.	Dec.	Jan.	Mar.	Apr.
Sanguinicolid vs. <i>Genarchopsis goppo</i>	–	n.s.	0.0006*	n.s.	n.s.	–	–	n.s.	n.s.	–	–	–
Sanguinicolid vs. opecoelid	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	–	0.0404*	n.s.	n.s.	n.s.	n.s.
<i>Cercaria longicercæ</i> vs. opecoelid	–	–	–	–	n.s.	n.s.	0.0287	n.s.	–	–	n.s.	n.s.
<i>Cercaria introverta</i> vs. opecoelid	–	–	–	n.s.	–	n.s.	n.s.	0.0068*	–	–	–	–
<i>Cercaria introverta</i> vs. <i>Metagonimus</i> spp.	–	–	–	n.s.	–	n.s.	0.0245	0.0002*	–	–	–	–
<i>Cercaria introverta</i> vs. <i>Centrocestus</i> spp.	–	–	–	n.s.	–	–	–	0.0111*	–	–	–	–
<i>Metagonimus</i> spp. vs. <i>Centrocestus</i> spp.	n.s.	–	–	n.s.	n.s.	–	–	0.0437*	–	–	n.s.	–

*Asterisks indicate the experimental error rates ($P < 0.05$) calculated by the procedure of Sousa (1993). All significant results show positive associations.

Table 4. The duration of cercarial shedding by trematode species in *Semisulcospira nakasekoe* during 1999 and 2000.

Trematode species	Mean shedding duration (days)	Max shedding duration (days)	Mean survival duration (days) after shedding ceases
Sanguinicolids	19.9 (13)	23	12.7 (9)
Opecoelids	103.2 (33)	374	12.3 (33)
<i>Cercaria innominata</i>	15.0 (3)	22	6.2 (5)
(host take-over)	6.0 (2)	11	
<i>Metagonimus</i> spp.	61.5 (6)	130	23.0 (5)
(snails harbouring rediae after the experiment)	212.3 (3)	325	

Numbers in parentheses indicate the sample size.

The sanguinicolids and *G. goppo* had a positive association in June 1996, and both snails infected with sanguinicolids were also infected with *G. goppo*. It is difficult to explain why a positive association was detected only in this month. One possible explanation is that multiple infections are promoted when a trematode species recruits to hosts because pre-infected hosts are more susceptible due to immune suppression, and when this decreases one species might be excluded by another. This process is more suggestible by the positive association of sanguinicolids with the opecoelids in November 1996, when sanguinicolids were newly recruited. At this time, both species were most prevalent in area A, whereas almost all double-infected snails (5/6) were collected from area B. Therefore, the microhabitat overlap of infected snails can be excluded from the factor causing the co-infection of these species. Parasite accumulation through the suppression of the host's defence system is thought to have been the most likely cause of the multiple infection. This positive association also disappeared in the next month, suggesting that exclusion through antagonistic interspecific interaction leads to a decrease in the frequency of co-occurrence. Knowledge of the exact temporal pattern of recruitment for each trematode species is needed to demonstrate the above process.

Double infections with *C. innominata* and *Metagonimus* sp. were observed in July, with rediae and cercariae of *Metagonimus* observed in the intestine of rediae of *C. innominata*. This indicates that *C. innominata* preyed on *Metagonimus*, explaining the 'take-over' process observed in the shedding experiment. Whereas *C. innominata* clearly dominates the other trematodes, a negative association was not detected between them during any month. This is partly due to the low prevalence of *C. innominata*, but may also in part be due to the slow process of exclusion by *C. innominata* of other species. In the shedding experiment, *Metagonimus* co-parasitizing with *C. innominata* in the prepatent period produced cercariae for 138 days.

Component community structure of trematodes

The duration over which the host harbours parasites has not been examined as a factor affecting prevalence in previous studies. However, the variation in the patent period revealed in this study suggests that it contributes to differences in trematode prevalences. The two taxa with the highest prevalence year-round (the opecoelid and *Metagonimus* spp.) were found to shed cercariae over

a long period without new recruitment. The duration of shedding in the sanguinicolids was relatively short (approximately 40 days, assuming the true shedding period to be twice the present result), but a high prevalence was observed in some seasons. In the field, therefore, the sanguinicolid must recruit at a high rate. Paradoxically, in Japan, only one adult sanguinicolid has been recorded from a bitterling *Acheilognathus tabira* (Shimazu, 1999b), although the cercariae occur frequently (Makita *et al.*, 1996a), and the recruitment rate is thought to be high.

In the present study, positive associations among the different trematode species were detected, whereas no negative associations were detected. In particular, *C. introverta* is thought to contribute to the community structure of trematodes in *S. nakasekoe* due to its infection characteristics that might accelerate multiple-species infection.

Direct interaction between larval trematodes within a host has been suggested as a factor determining the organization of trematode communities (e.g. Kuris, 1990; Fernandez & Esch, 1991a; Sousa, 1993). Antagonistic interactions such as predation and competition have been particularly well documented and analysed (Lafferty *et al.*, 1994). Usually, trematode species with a redial stage are considered dominant over species having only a sporocyst (Kuris, 1990; Fernandez & Esch, 1991a).

In the present study, no negative associations were detected between any pairs of trematode species. However, this does not mean that there was no antagonistic interaction between the trematodes of *S. nakasekoe*. *Cercaria innominata* obviously can exclude other species, but such an antagonistic interaction did not affect the community structure in the host population at the low prevalence observed here. Antagonistic interactions between trematodes may modify the community structure in host populations at higher prevalences of infection.

On the other hand, positive associations among trematodes have also been investigated. Some studies have shown that trematode species having only a sporocyst stage are often dominant in host snails. Walker (1979) reported the dominance of *Austrobilharzia terrigena*, which only has a sporocyst stage, over other trematodes. Well-known studies on *Schistosoma mansoni* by Lie *et al.* (1977) and Lie & Heyneman (1979) have shown that *S. mansoni* can invade the resistant strain of *B. glabrata* in the presence of the larvae of some species of *Echinostoma*. Sporocysts of *S. mansoni*, however, are often

eaten by rediae of *Echinostoma*, causing both positive and negative effects in their association (Lie *et al.*, 1977). *Schistosoma mansoni* is therefore not a good competing species but still benefits from *Echinostoma* when colonizing resistant hosts. In the present study, *C. introverta*, which has sporocysts only as parthenitae, co-occurred with other species at a high rate, indicating its need for hosts to be pre-parasitized by other species, or its ability to accumulate without being excluded. In fact, non-random colonization and the rate of exclusion are principal determinants of the component community structures regardless of the developmental pattern of trematodes.

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