

EFFECTS OF SNAIL SIZE AND AGE ON THE PREVALENCE AND INTENSITY OF AVIAN SCHISTOSOME INFECTION: RELATING LABORATORY TO FIELD STUDIES

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ABSTRACT: Both the prevalence and intensity of patent infection by avian schistosomes (*Trichobilharzia ocellata*) increase with increasing size of lymnaeid snails (*Stagnicola elrodi*) collected in Flathead Lake, Montana. Because the size and age of a snail are positively correlated, snails of different sizes may have experienced differential duration of exposure to and development of infection. Another possibility is that infection itself induces snail gigantism. Each of these possibilities could lead to increased prevalence and intensity of infection among the oldest–largest snails. To decouple size variation from many correlated effects of age and to test for parasite-induced gigantism, laboratory experiments standardized snail size–age-at-infection, exposure history, inoculating dose, and duration of infection. The positive relationship between size and prevalence was eliminated in the laboratory, but the relationship between size and infection intensity remained. Laboratory results thus suggest that infection intensity is related to snail size per se, whereas prevalence in the field is related to snail size only through the correlation between size and age. In addition, under these experimental conditions, infected snails were no larger than uninfected snails, so the patterns observed in the field might not be attributable to gigantism.

Transmission of digenetic trematodes from the snail to the next host in the life cycle depends largely on the proportion of snails that release cercariae, as well as the number of cercariae released from each infected snail (Anderson and May, 1991). These factors may be quantified, respectively, in terms of the prevalence and intensity of patent infection (Margolis et al., 1982; Bush et al., 1997). In field populations, both these factors tend to increase with increasing snail size. Among freshwater snails, for example, a positive correlation between snail size and the prevalence of infection was reported in 73% of the field studies reviewed by Sorensen and Minchella (2001); size may also be positively correlated with intensity of infection (e.g., Smith [1984]). For both mammalian and avian schistosomes, the prevalence and intensity of infection tend to be highest among the largest snails (Sturrock, 1973; Kulesa et al., 1982; Loker, 1983; Woolhouse, 1989; Niemann and Lewis, 1990).

Large snails are older, on average, than small snails, within a given population (Minchella et al., 1985). This age variation translates into differential exposure to and duration of infection among snails of differing sizes because larger–older snails may have been exposed to more miracidia or may have been infected for a longer period and thus have patent, highly productive infections. A greater cumulative risk of infection for older snails could account for the increased prevalence and intensity of infection with increasing size (Anderson and Crombie, 1985; Woolhouse, 1989); these effects could be compounded if larger–older snails also had more productive infections. The correlation between the size and age of a snail is not perfect, however. Freshwater snails exhibit marked intraspecific phenotypic plasticity in adult size (reviewed in Hutchinson, 1993), such that same-aged lymnaeid snails, for example, often differ greatly in size (Cort et al., 1940; Lam and Calow, 1989a, 1989b; Ward et al., 1997). Trematode-induced gigantism can likewise disrupt the correlation between the age and size of a lymnaeid by accelerating the growth of infected snails (McClelland and Bourns, 1969; Sluiter et al., 1980; Joosse and van Elk, 1986;

Ballabeni, 1995; Sorensen and Minchella, 1998; Zakikhani and Rau, 1999). Snail size itself (regardless of age) might in fact affect trematode development if, for example, a relatively large snail provides more space, greater energetic resources, or both, for production of cercariae.

Among same-aged snails, do prevalence and intensity of patent infection still increase with increasing snail size? Are size differences per se sufficient to generate the patterns observed in so many snail–trematode systems? These questions were explored in a study system in Flathead Lake, Montana, a freshwater lake with an endemic lymnaeid snail species, *Stagnicola elrodi* (Baker and Henderson, 1933; Russell, 1967), which serves as an intermediate host for the avian schistosome *Trichobilharzia ocellata* (Loken et al., 1995). Because it is difficult to estimate the age of a field-collected freshwater snail and because infected snails are relatively rare in the field, controlled infection experiments were used to assess the effects of snail size on schistosome infection in relative isolation from the correlated effects of age. The experiments also permitted investigation of the role of infection-induced gigantism in producing the patterns observed in the field.

MATERIALS AND METHODS

Parasitological examination of snails from natural populations

Individuals of the species *S. elrodi* were collected monthly, from early July to early September, at each of the 9 Flathead Lake sites in 1998 and 7 sites in 1999. Snails were collected by afternoon snorkels through permanent transect areas (50 m² of cobble substrate per site in 1998 and 45 m² in 1999) in water that was 1- to 1.5-m deep. All snails over 4 mm in shell length were collected, and sampling effort was standardized at 1 min/m².

In a laboratory at Flathead Lake Biological Station (FLBS), each snail was isolated in 10 ml of spring water and placed beneath a light source for 24 hr (35 W/m² of 400–700 nm light with a 16-hr photoperiod). Each snail that shed trematode cercariae under these conditions was considered to have a patent infection. Cercariae were identified to the family level according to Schell (1985); the observed fauna included Schistosomatidae, Diplostomatidae, Plagiorchiidae, and Strigeidae. In accordance with Loken et al. (1995), the Flathead Lake schistosomes are in this study considered to represent *T. ocellata*. Liquid iodine was used to kill and stain the cercariae in a gridded dish, and the cercariae in the central 16-mm² area of each of the 4 nonoverlapping fields of view were counted under a dissecting microscope. The counts were multiplied by a scaling factor to estimate the number of cercariae present in each sample. Four such estimates were obtained at 1-day intervals

Received 27 June 2002; revised 2 October 2002; accepted 13 December 2002.

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for each of 25 randomly chosen infected snails. The mean number of cercariae shed per 24-hr period by an infected snail was used as the estimate of infection intensity for that snail. Maximum snail length (from the shell's apex to the point on the apertural lip furthest from the apex, along the columellar axis) was measured to the nearest 0.05 mm with vernier calipers.

Two controlled infection experiments: 1998 and 1999

Field-collected snails were induced to reproduce in an FLBS growth room to obtain parasite-free cohorts for the experiments. Adults were collected from 4 source populations around Flathead Lake as soon as the snails emerged in late spring. Two of the source populations were used in both experiments (Big Arm State Park, the type locality for the species, and Wayfarers State Park), whereas the other 2 were changed because of extremely late 1999 snail emergence in the populations used previously: the FLBS Point and Yellow Bay source populations were replaced by Yenny Point and Woods Bay in 1999. The field-collected snails were maintained on lettuce in aerated spring water at 20–21 C on a 16:8 hr light–dark cycle (to induce reproduction, see Lundelius and Freeman, 1986; Rollo and Hawryluk, 1988). Once oviposition began, egg masses were collected every 2 days. For each experiment, 10–15 egg masses collected on a single day were pooled for each source population. Oviposition by individual lymnaeids in crowded conditions generally occurs at 3–4 day intervals (van Duivenboden et al., 1985), so each set of egg masses probably represented 10 or more parent snails (and as many as 20–30, depending on the rate of hermaphroditism). The egg masses were maintained at 20–21 C with weekly water changes, and the resultant hatchlings were kept at 20–21 C and fed boiled lettuce ad lib. When the experiments began, snails in 1998 were 6 wk posthatching and 2.40 ± 0.03 mm long, whereas those in 1999 were 4 wk posthatching and 1.65 ± 0.03 mm long.

Miracidia of *T. ocellata* were obtained from fresh feces of common mergansers (*Mergus merganser*) from Flathead Lake. The collection of fecal samples was much as described by Loken et al. (1995). To clear the guano and fish scales, each sample was sedimented 3 times in a 0.85% sodium chloride solution (Garcia and Bruckner, 1993) immediately on return to the laboratory. Schistosome hatching was then induced in spring water. In 1998, 11 infected birds, i.e., at least 11 pairs of adult schistosomes, provided miracidia for the experiment. At least 8 pairs of schistosomes produced the miracidia used in 1999. Each snail source population was matched against as many different schistosome sources, i.e., birds, as possible. An untested assumption of this work is that the mergansers of Flathead Lake only carry 1 species of schistosome.

Within 3 hr of miracidia hatching, snails were individually exposed to 1 miracidium each, in 10 ml (1998) or 7 ml (1999) of spring water at 20 C. Control snails were sham-exposed under the same conditions. Trematode infection can increase the mortality rate of laboratory snails (Loker, 1979; Sluiter, 1981; Smith, 1984), so, to buffer against mortality of the entire exposed group, the number exposed to infection (120 in 1998; 143 in 1999) was greater than the number sham-exposed (40 in 1998; 53 in 1999).

Snails were reared at different temperatures and densities in the 2 experiments. In 1998, snails were kept in isolation in 50 ml of spring water at 20–21 C, with weekly water changes. In 1999, after exposure and 10 days at 20–21 C, each snail was randomly assigned to a "Toby Tea Ball" (a cylindrical tea infuser; Tops Manufacturing Co., Inc., Darien, Connecticut) that had been attached to an acrylic tray with non-toxic adhesive. Each snail was no more than 6 cm from its neighbors. The trays were placed in a tank with slowly flowing 17–17.5 C spring water about 3 cm deep; tray position was shuffled weekly. The latter temperature regime was closer to natural conditions experienced by near-shore snails in Flathead Lake: data loggers at field sampling sites recorded mean 1999 summer temperatures of 16–16.5 C. Snails in both experiments were maintained under 16:8 hr light–dark conditions and fed fresh romaine lettuce ad lib, supplemented with artificial food (much as described by Lewis et al. [1986]). Sand and chalk were also provided.

Snails were tested for patent infection (at 20–21 C) 6 wk after exposure and biweekly thereafter. In 1998, they were shedding cercariae by that time, whereas they did not shed until 10 wk after exposure in the 1999 experiment. Infection intensity was estimated as described for field-collected snails, except that samples were taken at biweekly intervals. Snail size (defined as for field snails) was measured at the time of

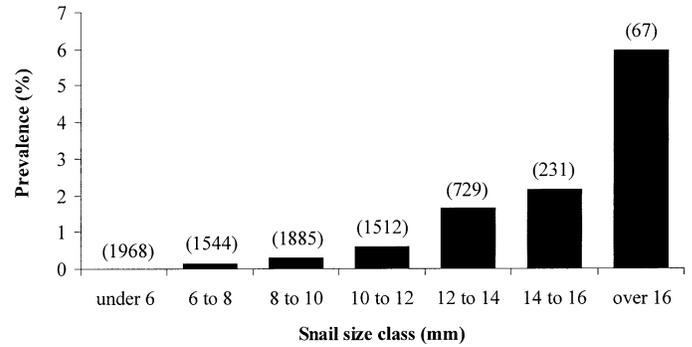


FIGURE 1. Prevalence of patent *Trichobilharzia ocellata* infection among field-collected snails of different size classes. Data from the summers of 1998 and 1999 are combined, and the number of snails in each size class is shown in parentheses.

exposure and then biweekly for the duration of the experiment (12 wk in 1998; 17 wk in 1999). Prevalence and intensity of infection were related to the final size reached by snails during the experiment.

Statistical analyses

To investigate size–prevalence relationships, snails were first binned into size classes. Logistic regression was then used to assess whether the proportion of snails with patent infection differed among size classes. Similar analyses were conducted for data on field-collected and laboratory-exposed snails. Sham-exposed snails were excluded from both sets of laboratory data. Bin widths were largely standardized for ease of graphical comparison among the 3 size–prevalence data sets, but only bins containing observations were analyzed.

Size–intensity relationships were investigated by linear regression, with size as a continuous predictor variable and intensity as the response variable. Too few infected snails were collected in 1998 to allow assessment of year effects in field data ($n = 5$ snails in 1998), but experimental effects were considered in the regression analysis of laboratory data. Intensity data were square root transformed to meet the assumptions of the analyses.

To test for infection-induced gigantism, the laboratory snails were classified by infection status: (1) sham-exposed; (2) exposed, but not patently infected; or (3) patently infected. Analysis of variance (ANOVA) can partition out-size variance attributable to different source populations and experiments and then allow assessment of variance due to the factor of interest, infection status (Snedecor and Cochran, 1989). ANOVA was thus used to assess the contribution of infection status to initial and final size of snails, after statistically controlling for the effects of experiment and source population. A Tukey adjustment was used to correct the P values for pairwise comparisons among groups within each experiment.

All the above analyses were conducted in SAS Systems 8.0, with $P < 0.05$ considered the baseline level of whole-model statistical significance.

Chi-square (χ^2) tests (Snedecor and Cochran, 1989) were used to assess the significance of differences between experiments and among source populations in the laboratory mortality rate and the proportion of exposed snails that developed infection.

RESULTS

In 1998 and 1999 combined, 38 of the 7,898 field-collected snails were infected with schistosomes. The prevalence of patent infection increased significantly with increasing snail size class (odds ratio = 2.23; $P < 0.0001$; Fig. 1). Intensity also increased with increasing size because snail size was a statistically significant predictor of the square root of infection intensity (slope = 0.49; $F_{1,23} = 5.79$; $P < 0.05$; untransformed data shown in Fig. 2).

Data on the survival and infection of laboratory snails allow

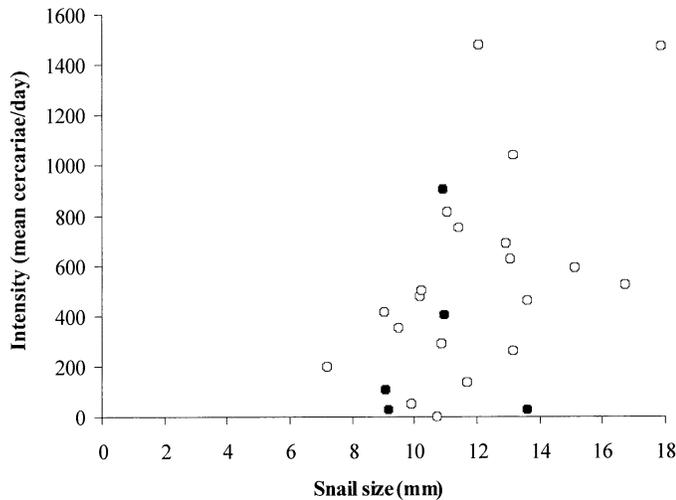


FIGURE 2. Intensity of patent *Trichobilharzia ocellata* infection, i.e., the mean number of cercariae shed per day by a given infected snail (n = 4 trials per snail), plotted against size of field-collected snails (1998, filled circles; 1999, open circles).

assessment of the comparability of the 2 experiments (Table I). Overall survival differed significantly between the 1998 (83%) and 1999 (59%) experiments ($\chi^2_1 = 24.02, P < 0.005$), perhaps driven by the fact that, in 1999, survival was significantly lower among truly exposed snails (53%) than among sham-exposed snails (75%) ($\chi^2_1 = 7.97, P < 0.005$). The proportion of surviving, exposed snails that developed patent infection did not differ significantly, however, between the 1998 (25%) and 1999 (36%) experiments ($\chi^2_1 = 2.51, P > 0.10$). All data presented below concern survivors only. Source population had no significant effect on mortality, the development of patent infection, or the relationship of prevalence or intensity to snail size (data not shown). None of the sham-exposed snails developed patent infection.

Among laboratory-exposed snails, the prevalence of patent infection was unaffected by snail size class (Fig. 3a, for 1998: odds ratio = 0.73 and $P > 0.15$; Fig. 3b, for 1999: odds ratio = 0.63 and $0.05 < P < 0.10$). Intensity of infection still increased with snail size, however, because size was again a significant predictor of the square root of infection intensity (slope = 0.81; $F_{1,48} = 27.08; P < 0.0001$; untransformed data shown in Fig. 4). This relationship was not due to differences between the 2 experiments because they generated statistically indistinguishable estimates of slope ($F_{1,48} = 1.63; P > 0.20$) and intercept ($t_1 = -1.91; 0.05 < P < 0.10$). Despite protocol differences, the 2 experiments thus produced similar results re-

TABLE I. For each experiment, the proportions of sham-exposed and truly exposed snails that survived and the proportion of surviving, truly exposed snails that developed patent infection.

Experiment	Proportion of sham-exposed snails surviving	Proportion of truly exposed snails surviving	Proportion of surviving, truly exposed snails that developed infection
1998	35/40 (88%)	98/120 (82%)	24/98 (25%)
1999	40/53 (75%)	76/143 (53%)	27/76 (36%)

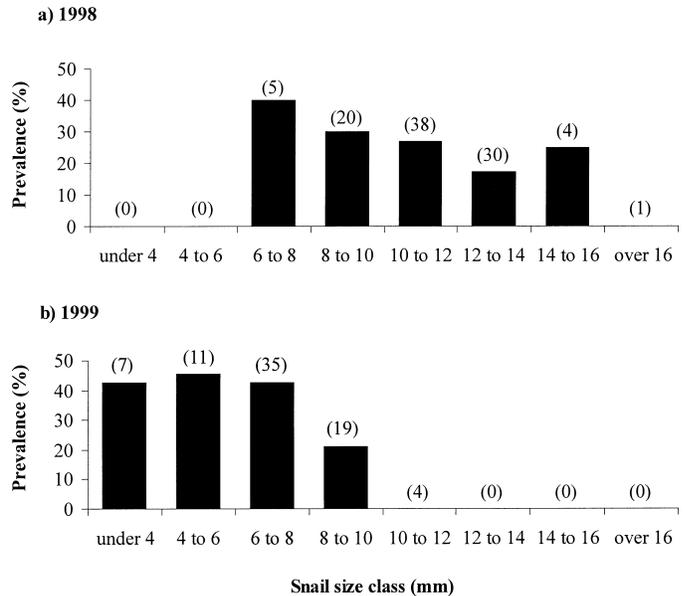


FIGURE 3. Prevalence of patent *Trichobilharzia ocellata* infection among laboratory-exposed snails of differing final size, for the experiment conducted in (a) 1998 and (b) 1999. The number of snails in each size class is shown in parentheses.

garding the influence of size on the prevalence and intensity of patent infection.

Growth curves for laboratory snails of differing infection status provide no evidence of infection-induced gigantism, however transient (Fig. 5). Only initial and final size data were statistically analyzed in this study. After controlling for source population and experiment, snails of differing infection status did not differ significantly in initial size, but they did differ in final size ($F_{2,239} = 4.90; P < 0.01$). Pair-wise comparisons indicated the following significant differences in final size. At the end of the 1998 experiment, exposed but uninfected snails were larger than patently infected snails ($P < 0.05$). In 1999, sham-

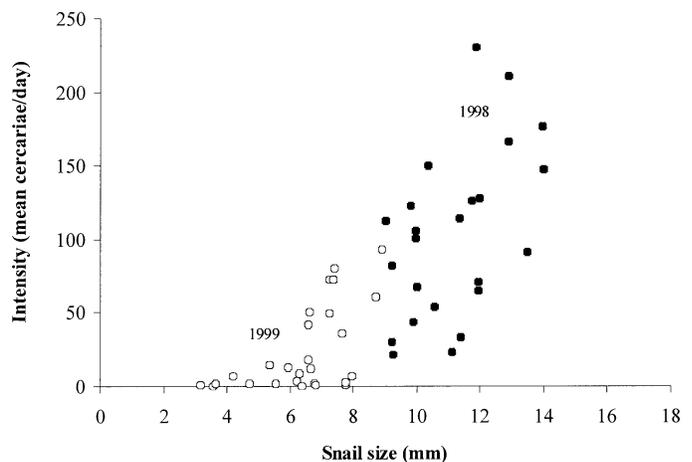


FIGURE 4. Intensity of patent *Trichobilharzia ocellata* infection plotted against final size of laboratory-exposed snails (filled circles = 1998; open circles = 1999). Intensity was again calculated as the mean number of cercariae shed per day by a given snail, based on 4 shedding events per snail.

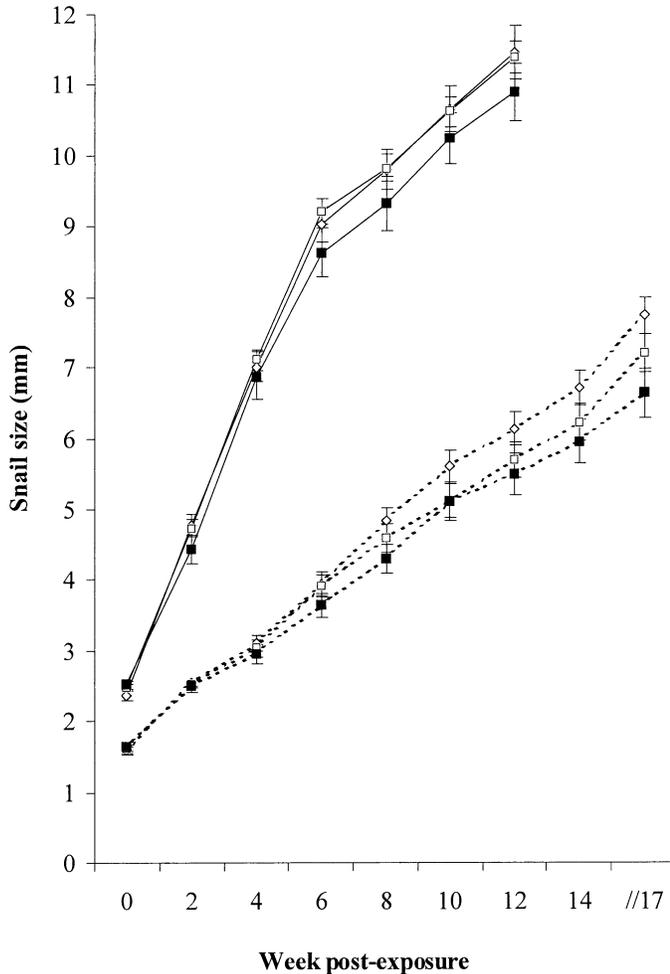


FIGURE 5. Growth curves for laboratory-reared snails of differing infection status (\diamond = sham-exposed; \square = truly exposed, but uninfected; \blacksquare = patently infected). Means and standard errors are plotted. Solid lines connect means from the 1998 experiment, whereas dashed lines connect means from the 1999 experiment.

exposed snails achieved greater final size than patently infected snails ($P < 0.05$).

DISCUSSION

In field populations of lymnaeid snails in Flathead Lake, Montana, both the prevalence and intensity of patent avian schistosome infections increased with increasing snail size. The field epidemiological patterns observed in many other snail-trematode systems thus hold for *S. elrodi* and *T. ocellata* as well. Although a complete decoupling of size and age variation would require further experimental work, the experiments reported in this study did standardize many of the variables that tend to confound the effects of age and size on infection in natural populations, i.e., snail size and age at infection, exposure history, inoculating dose, and duration of infection. Shell size variation and its correlates, e.g., volume of soft tissue, or hemolymph, were thus relatively isolated from age variation and its correlates, e.g., exposure to miracidia, or time for sporocyst and cercarial development, albeit over a limited range of experimental conditions. Still, in light of the experimental elim-

ination of the size-prevalence, but not the size-intensity, relationship, what can be inferred about the role of snail size variation per se in generating the epidemiological patterns observed in the field?

Inferences about causation often rely on the logic of sufficient and necessary conditions (see Sosa and Tooley [1993]). For example, a condition is said to be necessary for a given outcome if its absence prevents the occurrence of that outcome. If, in the absence of age variation among snails that vary in size, the prevalence and intensity of infection still increased with size, then age variation and its correlates would not be necessary to explain the patterns in the field. Causation is difficult to prove, but identification of sufficient and necessary conditions can suggest mechanisms generating patterns in the field.

In the *S. elrodi*-*T. ocellata* system, age variation and its correlates are indeed not necessary to generate increased infection intensity with increasing snail size. Instead, size variation among same-aged snails is sufficient, that is, the pattern is not observed solely because older snails have accrued multiple infections or because they have been infected for a longer period and hence produce more cercariae. These findings are similar to those of Sluiter et al. (1980) regarding *T. ocellata* and those of Ward et al. (1988) and Niemann and Lewis (1990) regarding *Schistosoma mansoni*. Intense infection may of course just be a correlate of whatever makes a snail large (Kendall, 1949; Keas and Esch, 1997), but the 2 might also be causally connected. Cercariae of avian schistosomes are produced in the hemocoel of the digestive-gonad gland, and shell length is probably a reasonable proxy for the size of this resource base; there is a strong, positive relationship between *S. elrodi* shell length and dry mass (mass = $0.00006 \times [\text{shell length}]^{2.89}$; $R^2 = 0.98$; R. Hauer, pers. comm.). Snail size could, through spatial or energetic constraints (Joosse and van Elk, 1986), cap cercariae production and, hence, intensity of patent infection. Evidence for such resource limitation includes increased replication in the absence of competition among larval genotypes (Kendall, 1949; Kuris, 1990) or when sporocysts can make use of multiple snails, as in the serial transplantation experiments of Donges and Gotzelmann (1988). The replicative potential of intramolluscan larvae may thus be greater than what is usually expressed, which is suggestive of resource limitation. In any case (whether or not the positive relationship between snail size and infection intensity is causal), size variation per se is sufficient to lead to the field pattern observed in this system.

In contrast, size variation in the absence of age variation is insufficient to generate the size-prevalence relationship observed in the field. Age-related differential exposure to miracidia, differential duration of infection, or both, has been invoked to explain high prevalence among big snails (Anderson and Crombie, 1985; Ward et al., 1988; Woolhouse, 1989; Sousa, 1990). These same correlates of age variation may be operating in the *S. elrodi*-*T. ocellata* system; the probability that a snail develops patent avian schistosome infection increases with exposure to increasing numbers of miracidia (Sluiter et al., 1980), and infections take 4–6 wk to become patent (Cort et al., 1940; Anderson et al., 1976; Sluiter et al., 1980), so older snails are more likely to have achieved patency. Other possibilities (also untested in this study) include enhanced survival of infected snails (Minchella et al., 1985), decreased resistance

to infection (but see Richards, 1975; Anderson et al., 1982), or enhanced apparency to miracidia (Theron et al., 1998) with increasing size. In any case, in this system, size variation per se did not generate the size-prevalence relationship observed in the field.

If *T. ocellata* infection induced snail gigantism, then infected snails would always be in the largest size classes, thereby increasing the prevalence and perhaps intensity of infection at that end of the size spectrum. However, under the experimental conditions described in this study, snails with patent infection were, if anything, smaller than uninfected snails. There was no evidence of even transient infection-induced gigantism, and most authors who have found such gigantism report that the growth acceleration is overcome on the time scales of these experiments (McClelland and Bourns, 1969; Loker, 1979; Minchella et al., 1985; but see Joosse and van Elk, 1986). It is thus tempting to conclude that gigantism does not underlie the increases in prevalence and intensity with increasing size in this field system.

Evaluation of the generality of the results reported in this study would require experimental work on snails of a range of ages and sizes, tested over a range of fieldlike conditions of exposure, temperature, and snail density, over a period comparable to the lifespan of lymnaeid snails in temperate lakes. Such factors can determine, for example, whether infection induces gigantism (Gerard and Theron, 1997; Theron et al., 1998; Sorensen and Minchella, 2001). It would also be of interest to study same-sized snails that differed in age. Age variation alone could prove sufficient to explain both intensity and prevalence.

These results, if confirmed, have implications for *T. ocellata* transmission. Same-aged snails in these experiments developed 6-mm differences in size in 12–17 wk (almost certainly faster than in the field; though in the field they might have lived longer). Given the slope of the size-intensity relationship, this translates into a 10-fold difference in daily cercariae output. These schistosomes use lymnaeid hosts that live a long time and under diverse conditions, such that same-aged snails in different sites differ greatly in size (Lam and Calow, 1989a; Ward et al., 1997). If age-related and size-related effects synergize to enhance the contribution of large-old infected snails to the size of the cercariae population, then foci of snail-to-bird transmission would be predicted in areas that foster snail growth.

ACKNOWLEDGMENTS

S. E. Roberts provided invaluable assistance in both field and laboratory. The staff and facilities at FLBS made the project possible, and advice on snail maintenance and infection was kindly provided by W. O. Granath and F. A. Lewis. M. Mackinnon generously shared her analytical expertise. Finally, the comments of C. D. Harvell, E. V. Iyengar, E. J. Pearce, A. G. Power, D. J. Shaw, and 2 anonymous referees improved the manuscript enormously. A.L.G. was a predoctoral fellow of the Howard Hughes Medical Institute.

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