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Adaptive host choice and avoidance of superparasitism in the spawning decisions of bitterling (*Rhodeus sericeus*)

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Abstract Choice of a site for oviposition can have fitness consequences. We investigated the consequences of female oviposition decisions for offspring survival using the bitterling, *Rhodeus sericeus*, a freshwater fish that spawns inside living unionid mussels. A field survey of nine bitterling populations in the Czech Republic revealed a significantly lower rate of release of juvenile bitterling from *Anodonta cygnea* compared to three other mussel species. A field experiment demonstrated that female bitterling show highly significant preferences for spawning in *A. anatina*, *Unio pictorum*, and *U. tumidus*. Within a species, female bitterling avoided mussels containing high numbers of bitterling embryos. Mortality rates of bitterling embryos in mussels were strongly density dependent and the strength of density dependence varied significantly among mussel species. Female preferences for mussels matched survival rates of embryos within mussels and females distributed their eggs among mussels such that embryo mortalities conformed to the predictions of an ideal free distribution model. Thus, female oviposition choice is adaptive and minimizes individual embryo mortality.

Key words Oviposition · Density dependence · Freshwater mussel · Ideal free distribution model

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Introduction

Among egg-laying species, oviposition choice can have significant fitness consequences. Sites for oviposition may vary in quality, or may already contain the eggs of other individuals, of the same or another species. In addition, individuals must choose whether to deposit all their eggs in one site or distribute them among several sites. These decisions may affect the survival and fitness of offspring. The fitness consequences of oviposition decisions have been studied for parasitoids (reviewed by Godfray 1994), and granivorous insects (e.g., Smith and Lessells 1985). However, the fitness consequences of oviposition decisions and how fitness changes with competitor density is less well studied in vertebrates. Understanding the fitness consequences of oviposition choice allows prediction of density-dependent breeding output, and thereby the population consequences of oviposition decisions.

Here we investigate the fitness consequences of oviposition decisions using the bitterling, *Rhodeus sericeus*, a freshwater fish that uses the gills of unionid freshwater mussels as a spawning substrate. Male bitterling defend territories around mussels, to which they attract females to spawn. Female bitterling develop long ovipositors which they use to place their eggs into the gills of a mussel through the its exhalant siphon. Males fertilize the eggs by releasing sperm over the inhalant siphon of the mussel, such that water filtered by the mussel carries sperm to the eggs. Female bitterling can spawn in more than one mussel and lay 50–100 clutches during a breeding season. Mussels can contain multiple clutches (Wiepkema 1961; Reynolds et al. 1997), thus spawning by bitterling is often analogous to superparasitism in parasitoids, whereby a clutch of eggs is deposited on a host that has already been parasitized by a member of the same species (Godfray 1994). Embryonic development of the eggs is completed inside the mussel gill and lasts from 3–6 weeks (Reynolds et al. 1997; Aldridge 1999). At least four mussel species (*Anodonta anatina*, *A. cygnea*, *Unio pictorum*, *U. tumidus*) are used by bitter-

ling as spawning hosts (Reynolds et al. 1997). In Europe, spawning by bitterling typically occurs from April to June.

There is some evidence that female bitterling may be selective about which mussel species they use, and these results are consistent across studies. After dissecting the gills of mussels, Balon (1962) concluded that bitterling showed a preference for spawning in mussels of the genus *Unio*. while avoiding *Anodonta* spp. In an experimental comparison of four mussel species, Reynolds et al. (1997) demonstrated that *U. pictorum* released significantly more juvenile bitterling than *A. cygnea*. Aldridge (1997) dissected 161 mussels belonging to four species and showed that *U. pictorum* contained more embryos than the other three species. In a study of the spawning preferences of rose bitterling (*Rhodeus* spp.), Kondo et al. (1984) dissected the gills of 5,703 unionid mussels belonging to eight species and showed significant differences in the frequencies of bitterling embryos contained in among the mussel species. Hence, some degree of spawning host selectivity by female bitterling may occur.

However, if mussels have evolved host defenses, for example by ejecting or killing bitterling embryos, then these data from dissections (i.e., Balon 1962; Kondo et al. 1984; Aldridge 1997) or releases of juvenile bitterling from mussels (i.e., Reynolds et al. 1997) will not demonstrate unambiguously whether bitterling make choices among mussel species as spawning hosts, since these methods do not accommodate the possibility that embryos are lost from mussels. Mussels are known to eject bitterling embryos prematurely (Kondo et al. 1987) and ejections can increase under certain environmental conditions (Reynolds and Guillame 1998).

In this study we conducted a field survey to estimate the rate of juvenile bitterling release from four species of mussel among nine bitterling populations, to investigate whether there are differences among bitterling populations in host mussel use. We also conducted a field experiment to test whether bitterling differentiate among mussel species in which to spawn, and whether they adjust their spawning behavior as a result of superparasitism. We linked these results to field and laboratory experiments to investigate whether mortality rates of embryos within mussels varied with the level of superparasitism and whether this varied among mussel species. Finally, we tested whether oviposition decisions by bitterling in relation to host quality and superparasitism conform to a pattern expected from an ideal free distribution model of embryo survival among mussels.

Methods

Study sites

Fieldwork for this study was conducted in the southeast of the Czech Republic, at the center of the natural range of the bitterling in Europe (Lever 1997). Field sites comprised nine artificial lakes created between 25–30 years ago and situated along a 40-km stretch of the Rivers Morava and Dyje (Jurajda 1995). Bitterling and unionid mussels were present in all the lakes. We used one lake with good visibility (Secchi disc reading of 1.0–1.3 m) for behavioral experiments.

Bitterling release from mussels

The rate of release of juvenile bitterling from mussels was estimated in the years 1995–1997 from May to July by enclosing mussels in mesh bags (mesh size of 0.5×0.5 mm) measuring approximately 15×20 cm and sealed with a Velcro strip. The bags permitted mussels to filter water normally but retained any juvenile bitterling that were released. After being sealed in a bag, mussels were placed back in the substrate in the exact location from which they had been taken. Bags were checked after 24 h and the numbers of juvenile bitterling that had been released were recorded. Mussels were collected by hand by a diver and were selected as they were encountered. To avoid sampling the same mussel repeatedly within lakes within years, mussels were collected from different areas of each lake on each sampling occasion within years. The same mussels may have been sampled more than once among years. On each sampling occasion, up to 110 mussels were sampled.

Mussel choice experiment

The aim of this experiment was to test whether bitterling discriminated among mussel species as spawning hosts, and whether the presence of embryos already in a mussel influenced this choice. Thus, we designed a choice experiment with two factors; mussel species (*A. anatina*, *A. cygnea*, *U. pictorum*, and *U. tumidus*), and embryo abundance ('high embryos' and 'low embryos').

We collected 70 mussels of each of *A. anatina*, *A. cygnea*, *U. pictorum*, and *U. tumidus* in mid April 1998, before the onset of the bitterling spawning season, from a lake that was not used in our study but that contained a population of bitterling, and transported them to the lake chosen for the behavioral experiment. The lake chosen for observations contained only one species of mussel, *A. anatina*. At the end of the experiment, all mussels that were not native to the lake were removed.

Mussels were randomly assigned to two groups of 140 mussels, with 35 mussels of each species in each group. Each group of mussels was placed in six plastic baskets, half filled with a substrate of sand. Three of the baskets were covered with netting that prevented bitterling from spawning in them, but allowed normal feeding and ventilation by the mussels. The remaining mussels were exposed to allow bitterling to spawn in them. The baskets were placed close to the lake edge, approximately 3 m from the bank and in approximately 70 cm of water. Those mussels exposed to spawning by bitterling were termed the 'high embryos' group, those from which bitterling had been prevented from spawning were termed the 'low embryos' group.

Behavioral tests began 6 days after transferring the mussels to the test lake. All choice tests were conducted at three locations or arenas, spaced approximately 30 m apart around the lake margin at a distance from the shore of 2–3 m and in a water depth of approximately 40–60 cm. At each test arena we placed eight mussels in a circle in a predetermined random order, each in a separate 8 cm depth×11 cm diameter flower pot with a sand substrate. The eight mussels comprised two of each mussel species, one belonging to the 'high embryos' group and one belonging to the 'low embryos' group.

After placing the mussels in position, a snorkeller observed the mussels from a distance of 1.0–1.5 m. Pilot studies had shown that male bitterling would continue to defend territories around mussels and females would spawn in the presence of a snorkeller. The mussel into which females spawned was recorded. Following a spawning, the location of each of the mussels under test was rearranged according to the next random pattern. Following a second spawning, all the mussels in the arena were replaced. A subsample

of mussels from the 'low embryos' and 'high embryos' groups were dissected to quantify the number of embryos in the mussels' gills. Data were recorded by two snorkellers at different arenas.

To avoid pseudoreplicating female choice, we recorded distinguishing features, such as lost scales or the presence of external parasites, particularly *Lernaea cyprinacea*, of spawning female bitterling at each test arena. Females spent relatively little time at the test arenas and it was sometimes impossible to record individual distinguishing features. However, females for which we did record distinguishing features rarely returned to spawn again in our test arena during the experiment and large shoals of females continually visited the test arenas. Consequently, we believe our choice results have little pseudoreplication.

During the course of the experiment we measured the angle from horizontal (in degrees) of the exhalant siphon of every mussel in each choice test following every spawning. We also recorded at random intervals during each choice test whether each of the eight mussels in the test arena had its exhalant siphon open. Siphon angle and duration open may play a role in bitterling mussel choice.

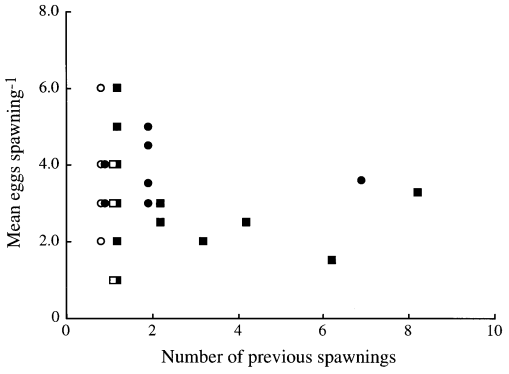

Fig. 1 Mean rate of spawning by female bitterling in four species of mussel in relation to the number of spawnings already deposited in them. *Anodonta anatina* (*filled circles*), *A. cygnea* (*open circles*), *Unio pictorum* (*open squares*), *U. tumidus* (*filled squares*)

Mortalities of bitterling embryos in mussels

Estimates of mortality rates of embryonic bitterling during development in mussels were obtained in two ways. First, a laboratory experiment was conducted in which the number of eggs spawned into mussels was systematically varied. A group of 24 mussels of each of four species was randomly assigned to six treatments of 1, 2, 4, 6, 10, and 14 spawnings. Mussels were exposed to one of six groups of spawning bitterling, comprising eight females and three males. Once the prescribed number of spawnings had been achieved, the mussel was isolated in the base of a 2-l plastic bottle with a sand substrate. The walls of the bottle had been replaced with 1-mm mesh netting. The netting allowed the mussel to filter water but retained any juvenile bitterling it released. Mussels were randomly assigned to nine 80-l aquaria from which 12 l of water was exchanged daily with water from a garden pond. Water exchange was intended to remove toxic metabolites from the aquarium water and also supplied mussels with phytoplankton to feed upon. Mussels were stocked in aquaria at a maximum density of eight mussels per aquarium and were retained under these conditions for 40 days. The number of juvenile bitterling released by each isolated mussel was recorded daily.

The results of this experiment would be confounded if bitterling vary the number of eggs spawned in relation to mussel species or embryo density. Therefore, we used data from laboratory and field pilot studies, in which the number of spawnings completed by bitterling was recorded followed by immediate dissection of the mussel, to investigate whether there were any differences in the number of eggs spawned by bitterling among mussel species or in relation to the number of embryos already in a mussel. We found that female bitterling deposited a mean of 2.9 (SE 0.18, n=43) eggs per spawning, that there was no relationship between the number of eggs already in a mussel and mean number of eggs spawned (r^2=0.02, F=1.48, df=1,21, P=0.237), and that mean number of eggs released did not vary significantly among mussel species (Kruskal-Wallis, H=6.96, df=3, P=0.073; Fig. 1).

The second method used to estimate mortality rates of bitterling embryos in mussels was through analysis of the age structure of the embryonic stages of bitterling within mussels under natural conditions. By estimating the age of embryonic stages, the mortality rate of bitterling embryos in mussels was calculated under the assumption of constant spawning and mortality rate, since numbers surviving will decline exponentially with age. These assumptions are tested in the Results section. A line of best fit through the descending arm of the logarithm of numbers of embryos surviving successive stages will produce a linear relationship, with a slope numerically equal to the instantaneous mortality rate (Ricker 1975). To make estimates of mortalities using this method, samples of mussels belonging to each of the four mussel species were collected during mid-April 1998 and placed in individual flower pots around the margin of a lake containing bitterling in mid May for 15 days. Sample sizes were *A. anatina* 45, *A. cygnea* 17, *U. pictorum* 20, and *U. tumidus* 24. After the mussels were recovered, the gills were dissected and preserved in ethanol. All bitterling embryos were removed from the gills under a binocular microscope within 2 weeks of collection and the embryos staged using Suzuki and Hibiya (1984).

Results

Bitterling release from mussels

A total of 865 juvenile bitterling were collected from 855 mussels in nine lakes (Fig. 2). Data were only used for lakes in which all four species of mussel occurred. There was a significant difference among mussel species within populations in the rate of bitterling release (Friedman test, S=14.18, df=3, P=0.003) and a significant difference among populations within species (Friedman test, S=21.31, df=8, P=0.007). The lowest rate of release of embryos was from *A. cygnea*. The ranking of mussel release rates of bitterling among oxbows showed a consistent pattern, with either *A. anatina* and *U. pictorum* the highest ranked in eight of the nine study lakes and *U. tumidus* the highest ranked in one lake. *A. cygnea* was the lowest ranked in all lakes. Because mussel species composition varied among populations and sample composition varied with time, we also tested for a difference in release rates from the four species of mussel among populations and among sampling dates. There was a significant difference in release rates of the four species among lakes (Kruskal-Wallis, H=9.09, df=3, P=0.028) and dates (Kruskal-Wallis, H=19.42, df=3, P <0.001).

Mussel choice experiment

The rate of spawning of bitterling in the field experiment was recorded over a 7-day period. As expected, the number of bitterling embryos in the gills of mussels belonging to the 'high embryos' group was significantly higher

Table 1 Data from mussel choice experiment for mean (SE in parentheses) number of bitterling embryos, angle of mussel exhalant siphon from horizontal, and exhalant siphon opening frequency in treatment groups

Embryo density	Mussel species	Mean embryos per mussel	*n*	Mean mussel angle (°)	*n*	Mean frequency of siphon open (h^{-1})	*n*
Low embryos	*Anodonta anatina*	5.8 (2.33)	14	5.4 (0.87)	14	11.4 (1.77)	66
	A. cygnea	0.4 (0.29)	14	4.7 (0.79)	14	9.7 (1.15)	64
	Unio pictorum	10.4 (2.55)	15	6.1 (0.88)	15	10.5 (1.09)	66
	U. tumidus	4.9 (1.25)	14	7.0 (1.12)	14	10.5 (1.12)	66
High embryos	*A. anatina*	95.1 (13.07)	15	4.2 (0.97)	15	12.1 (1.58)	64
	A. cygnea	31.7 (8.62)	16	4.8 (0.75)	16	8.7 (1.14)	59
	U. pictorum	82.9 (11.26)	15	6.5 (1.07)	15	10.5 (1.11)	66
	U. tumidus	75.7 (10.57)	15	5.8 (0.96)	15	10.5 (1.12)	64

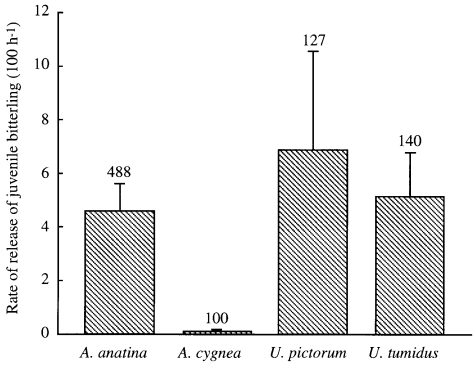

Fig. 2 The mean (+1 SE) rate of release of juvenile bitterling from four species of mussel from nine populations. Values above bars refer to replicates

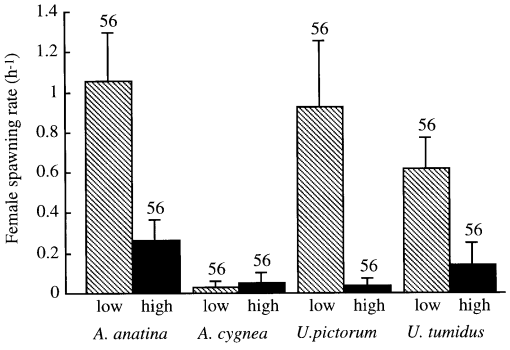

Fig. 3 The mean (+1 SE) rate of spawning by female bitterling into four species of mussel belonging to the 'low embryos' and 'high embryos' treatments. Values above bars refer to replicates

than the 'low embryos' group (unpaired *t*-test, t=13.05, df=112, P <0.001; Table 1). Thus, although there were some embryos in the 'low embryos' group, received from spawnings that had occurred before the mussels were collected, there was a pronounced difference in embryo numbers in the gills of mussels belonging to the two groups. The mean rate of spawning was significantly lower into mussels belonging to the 'high embryos' group in comparison with 'low embryos', irrespective of species (ANOVA, F=21.13, df=3,48, P <0.001; Fig. 3). There was also a significant difference in the rate of spawning into different species of mussel irrespective of fullness (ANOVA, F=5.04, df=3,48, P=0.004). The mean rate of spawning was highest in *A. anatina* and lowest in *A. cygnea* (Fig. 3). Fisher's pairwise comparison showed a significant difference in the rate of spawning by female bitterling in *A. anatina*, *U. pictorum*, and *U. tumidus* in comparison with *A. cygnea* (all P-values <0.05). There was no significant difference in the spawning rate of bitterling into the four mussel species among mussels in the 'high embryos' group (ANOVA, F=1.77, df=3,24, P=0.181; Fig. 3).

There were no significant differences in the angle from horizontal of the exhalant siphons of different mussel species during the experiment (ANOVA, F=0.75, df=7,507, P=0.634; Table 1), nor in the frequency at which the exhalant siphons of mussels in each treatment remained open during the experiment (Kruskal-Wallis, H=5.37, df=7, P=0.615, Table 1).

Mortalities of bitterling embryos in mussels

Experimental analyses of embryo mortalities were not completed for *A. anatina* and *A. cygnea* and estimates of embryo mortalities for these species are based solely on analysis of the age structure of embryos in field-collected mussels (Fig. 4). However, estimates of embryo mortalities for *U. pictorum* and *U. tumidus* using experimental and field methods show considerable overlap (Fig. 4c,d). Consequently, we used both experimental and field methods for *U. pictorum* and *U. tumidus*, but only field methods for *A. anatina* and *A. cygnea.* An example of an estimate of mortality from embryo age structure is shown in Fig. 5. Mortality estimates using the age structure of the embryonic stages were made under the assumption of constant spawning and mortality rate. Both these assumptions were met. Spawning rates for bitterling in the mussel choice experiment, conducted during the period in which the mussels for estimating mortality rate were exposed, did not differ significantly among days (Kruskal-Wallis, H=4.92, df=6, P=0.555), and all estimates of mortality rate by linear regression were significant (P<0.05).

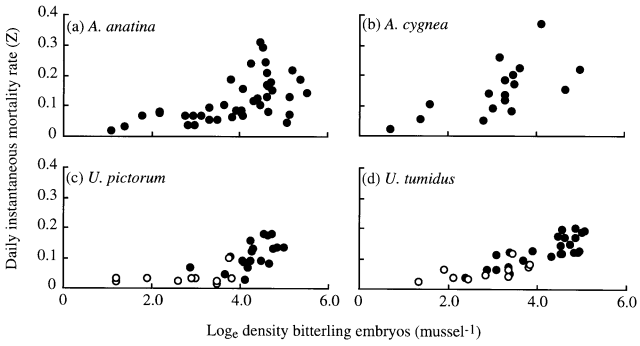

Fig. 4a–d Density-dependent mortality of bitterling embryos inside their mussel hosts. Mortality rates (*Z*) were estimated from an experimental analysis of mortalities (*open circles*) and analysis of the age structure of embryos in mussels (*filled circles*) (Z=–log$_e$ proportion of embryos surviving each day of incubation)

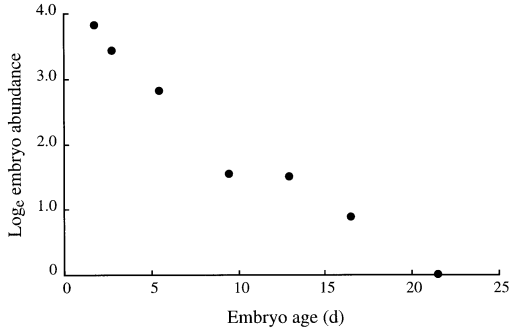

Fig. 5 Estimate of embryo instantaneous mortality rate from a linear regression of log$_e$ abundance against embryo age. The regression takes the form: log$_e$ abundance=3.87–0.186 age (days) (r^2=0.96, P<0.001)

Table 2 Linear regressions of daily instantaneous mortality rates (*Z*) (where Z=–log$_e$ proportion of embryos surviving each day of incubation) of bitterling embryos during incubation in mussels against log$_e$ embryo density in four species of mussel

Mussel species	Model	r^2	P
A. anatina	y=0.034x–0.014	0.46	<0.001
A. cygnea	y=0.054x–0.021	0.44	0.004
U. pictorum	y=0.035x–0.049	0.53	<0.001
U. tumidus	y=0.044x–0.062	0.70	<0.001

The survival of embryonic bitterling in their host mussel was significantly negatively density dependent for all species (Fig. 4, Table 2). The strength of the relationship between mortality rate and embryo density differed significantly among the four mussel species (ANOVA, F=5.23, df=3,118, P=0.003). The strength of density dependence was highest for *A. cygnea* (Fig. 4b) and lowest for *A. anatina* (Fig. 4a).

Predicted mortality rates among mussels

We compared the abundance of embryos in mussels that had been exposed to natural spawning by bitterling in the analysis of the age structure of embryos. There was a significant difference in the mean number of bitterling embryos in the four mussel species (Kruskal-Wallis, H=22.99, df=3, P<0.001; Table 3). Using the relationships between mortality rate and embryo density we estimated the mean mortality rates of embryos among the four mussel species and found there no significant difference (Kruskal-Wallis, H=6.69, df=3, P=0.083; Table 3).

Table 3 Mean embryo density (SE in parentheses) in four species of mussel exposed to natural spawning by bitterling (Kruskal-Wallis, H=22.99, df=3, P<0.001), and estimated mean (SE in parentheses) instantaneous mortality rate (*Z*) of bitterling embryos over the period of incubation in mussels (Kruskal-Wallis, H=6.69, df=3, P=0.083)

Mussel species	Mean embryo density	Mortality rate (*Z*)
A. anatina	117.8 (11.65)	1.9 (0.19)
A. cygnea	36.7 (9.05)	1.9 (0.24)
U. pictorum	81.6 (7.95)	1.3 (0.21)
U. tumidus	82.5 (9.78)	1.5 (0.14)

Discussion

Our field studies demonstrate that female bitterling discriminate in two ways with respect to where they spawn their eggs: they discriminate among host species, and within species they discriminate relative to the degree of superparasitism. There are clear adaptive explanations for these behavioral choices; mortality of embryos in mussels is density dependent, so embryo survival is much higher in mussels with few embryos, and density dependence is significantly stronger in *A. cygnea* than in the other three species of mussel tested in this study.

Female bitterling did not appear to discriminate among the four species of mussels after they had been naturally filled with eggs (Fig. 3). Our density-dependent experiments show that mussel ‘quality’ declines as the mussels are filled with embryos. Therefore, if bitterling are allowed to fill mussels with embryos, the ‘quality’ of mussels belonging to different species, measured by embryo survival, may be reduced to a common level, as expected from an ideal free distribution model (Fretwell and Lucas 1970). This result is supported by the comparison of estimated embryo mortality rates in mussels filled under natural conditions, which showed no significant difference (Table 3).

Releases of embryos from mussels broadly reflected behavioral choices. Bitterling avoided spawning in *A. cygnea* (Fig. 3) and relatively few *A. cygnea* released juvenile bitterling (Fig. 2). However, embryo mortalities within mussels occur at different rates among species (Fig. 4); thus, the release of juvenile bitterling from mussels is not an accurate reflection of spawning choice in this species.

Empirical studies conducted on fishes have demonstrated that in some nest-building species, females prefer

to mate with males whose nests already contain eggs rather than males with empty nests. Females may make these choices because the number of eggs in the male's nest may reflect his quality, courtship rate or, alternatively, may serve to dilute the risk of egg cannibalism or egg predation (reviewed by Dugatkin and FitzGerald 1997). In contrast, bitterling avoid using the same spawning sites as other females, presumably because this will tend to reduce embryo survival.

Male bitterling may also be choosy about where spawning occurs and may be able to influence females in their choice of host mussel. However, male choice of spawning host may depend on the risk of sperm competition with other males. Consequently, male host choice may be selected to maximize paternity rather than embryo survival, leading to a male-female conflict in host choice (C. Smith and A. Douglas, unpublished data).

In parasitoids, superparasitism reduces the probability of successful development of an embryo, though the developing parasitoid already present in the host appears to suffer no disadvantage (Godfray 1994). In addition, the probability of survival of eggs deposited by superparasitizing females declines with increasing time after initial parasitism (Visser 1993). Thus, both order and timing of egg deposition strongly affect subsequent embryo survival. Whether bitterling embryos in mussel gills similarly obtain a competitive advantage if they are spawned first is unclear. Kraak et al. (1997) describe a technique for staining fish eggs that might enable mortality rates in relation to order of spawning to be investigated.

Host preference by bitterling may be relative rather than absolute. In lakes where only *A. cygnea* was present, bitterling readily used this species as a spawning host (unpublished data). Thus, bitterling may choose mussels on the basis of the quality of those available, and host acceptance may change with experience. Further investigations of host choice should explore the effect of experience on choice. Other variables that may influence choice include predation risk and encounter rate with hosts.

The results of this study raise two further questions. First, what are the proximate cues used by female bitterling to discriminate among hosts prior to spawning? Second, what is the mechanism for density-dependent mortality of embryos in mussels?

Discrimination by bitterling among mussel species as spawning hosts may arise because some mussel species are more accessible than others. We measured the angle of the exhalant siphon of each mussel following every spawning in our choice test and found that they were not different (Table 1). Thus, our results do not support the suggestion of Aldridge (1997), who observed fewer bitterling embryos in dissected *A. cygnea* and attributed the result to *A. cygnea* orientating themselves in the substrate such that females are unable to insert their ovipositors into the exhalant siphon. We further demonstrated that the proportion of time spent by mussels with their exhalant siphons open could also not account for the observed spawning pattern.

We can also exclude the possibility that the observed differences were due to host specialization by bitterling on species of mussel in which they were themselves incubated as embryos, an hypothesis that has been suggested for the cuckoo, *Cuculus canorum*,(Davies and Brooke 1989a, 1989b). The population of bitterling used for this experiment was isolated and had been exposed to only one species of mussel, *A. anatina*, for approximately 30 generations. Nevertheless, females spawned as frequently in *U. pictorum*, a species of mussel to which no bitterling in the population had previously been exposed, as readily as in *A. anatina*, the natal mussel of every bitterling in the population. We discount the possibility that *A. cygnea* was absent from this lake because conditions were unsuitable for this species; consequently, it was avoided by bitterling as a spawning host on the basis of three observations. First, both *U. pictorum* and *U. tumidus* were also absent from the lake but were readily used by bitterling. Second, there were no mortalities among any experimental mussels during the study and all mussels showed apparently normal behavior with respect to the time they spent with their exhalant siphons open. Third, our data on bitterling releases from mussels in lakes where *A. cygnea* did occur also indicate that *A. cygnea* are avoided by bitterling.

Possible cues for oviposition behavior may include the rate of water flow from the exhalant siphon of the mussel and/or the oxygen content of the exhalant water. Filtration and oxygen depletion rate may vary among mussel species, and the presence of bitterling embryos in the gills of mussels may also reduce the capacity of the mussel to filter water and could directly remove oxygen from water passing over the mussel gill. This hypothesis would also account for the second question raised by this study, that of the mechanism for density-dependent survival of embryos. Availability of oxygen in the mussel may limit the survival and development of bitterling embryos, and embryos may compete with conspecifics for oxygen within the mussel gills.

In conclusion, our study demonstrates adaptive host choice in the bitterling at two levels: in response to host species and to the level of superparasitism of mussels by conspecifics. There is evidence that bitterling distribute eggs among mussels in accordance with an ideal free distribution model, which has the effect of minimizing embryo mortality. One possibility is that the proximate cue for oviposition choice by bitterling may be the oxygen content of the water flowing from the exhalant siphon of a mussel.

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