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Differentiated egg size of the cannibalistic salamander *Hynobius retardatus*

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Abstract Larvae of the salamander, *Hynobius retardatus*, are carnivorous, and even though there are two morphs, a typical morph and a broad-headed or “cannibal” morph, both of them are cannibalistic. They also sometimes eat other large prey, for example larvae of the frog, *Rana pirica*. In natural habitats, the use of both conspecific and *R. pirica* larvae as food may contribute more strongly to high survival and substantially to fitness when larval densities are higher, because early-stage *H. retardatus* larvae sometimes experience scarcity of their typical prey. In cannibalistic oviparous amphibians, larger individuals that developed from larger eggs can more efficiently catch and consume larger prey and thus their survival maybe better than that of smaller individuals developed from smaller eggs. Populations might therefore diverge with respect to egg size in response to variation in the density of conspecific and *R. pirica* larvae in natural ponds, with eggs being larger when larval density is higher. I examined how variance in hatchling size correlated with the incidence of cannibalism, and whether increasing larval density in natural ponds correlated with increasing egg size. Variance in initial larval body size facilitated cannibalism, and egg size increased as larval density in the ponds increased. In ponds with high larval density, where cannibalism and large prey consumption is a critical factor in offspring fitness, the production of fewer clutches with larger eggs, and thus of fewer and larger offspring, results in greater maternal fitness. Variation among the mean egg size in populations is likely to represent a shift in optimum egg size across larval density gradients.

Key words: cannibalism ▪ clutch size ▪ egg size ▪ maternal effect ▪ salamander

Introduction

Embryo mass and growth rate, especially in animals that lack parental care, are important fitness-related traits because they potentially affect juvenile survival and recruitment (Mousseau and Fox 1998). Egg size can, moreover, directly affect juvenile survival and recruitment as a result of its effect on embryo propagule mass and growth (Bernardo 1996; Sinervo and Doughty 1996; Fox et al. 1997; Eium and Fleming 1999). Larger eggs are therefore thought to confer a fitness advantage to the offspring (McGinley et al. 1987) although sometimes smaller eggs are better (Parichy and Kaplan 1995). Egg size is, in part, determined genetically (Berven 1982; Azevedo et al. 1997; Shaw and Byers 1998; Wade 1998), suggesting it can be affected by natural selection, causing subsequent variation in egg size among species or among populations within species (Shaw and Byers 1998; Wade 1998). Patterns in egg size are thought to reflect adaptive processes, especially if selection acts on offspring after hatching (Mousseau and Fox 1998).

In amphibians, variation in egg size among species or among populations within species has been commonly observed (Kaplan 1998). Both biotic (e.g. competition, predation, and resource availability) and abiotic (e.g. desiccation, temperature, and oxygen availability) environmental conditions may cause differentiation in egg size or offspring size among populations (Seymour and Bradford 1995; Kaplan 1998; Laugen et al. 2002; Thumm and Mahony 2005). Interspecific variation in egg size is therefore likely to evolve in response to variability in aquatic environmental conditions where offspring live (Crump 1981; Berven 1982; Kaplan and Cooper 1984), although variation in egg size may also result in part from differences in environmental effects correlated with female body size (Kaplan 1998) or in the maternal environment during egg maturation.

Larval–larval cannibalism is well documented in amphibians (Crump 1992) and, in particular, is extremely common in salamanders (Crump 1992). The larvae of the salamander, *Hynobius retardatus*, are carnivorous, and even though there are two larval morphs, a typical morph and a broad-headed or “cannibal” morph, both are cannibalistic (Wakahara 1997). They also sometimes eat other large prey, for example larvae of the frog, *Rana pirica* (Michimae and Wakahara 2002a). Because cannibalism can greatly enhance an individual’s growth or rate of development (Michimae and Wakahara 2002a), it can increase the disparity in size among larvae throughout the larval period (Wakahara 1997). The presence of *R. pirica* larvae can also contribute to the size variance that induces cannibalism, irrespective of salamander’s morph. Variation in the larval size distribution (i.e. size disparities) stimulates and facilitates the potential for cannibalism in *Ambystoma tigrinum* (Maret and Collins 1994), and, similarly, asynchronous hatching and variation in initial egg size may facilitate the potential for cannibalism in *A. opacum* (Kaplan 1980; Scott 1990).

The advantages to larvae and juveniles hatched from large eggs have been demonstrated for a wide range of amphibian taxa (Kaplan 1998). In cannibalistic oviparous amphibians relatively larger individuals that developed from larger eggs can more efficiently catch and consume relatively larger prey, for example conspecific larvae, and may thus have more chance of survival than individuals developed from smaller eggs. Michimae (2006) reported that carnivorous *H. retardatus* larvae live in ponds with different larval densities of conspecifics and of *R. pirica* larvae, which are detritus feeders. In natural habitats, utilization of conspecifics and *R. pirica* larvae as food may contribute to high survival and, substantially, to fitness when larval densities are higher, because *H. retardatus* larvae sometimes experience a scarcity of their typical prey (macrobenthos such as Oligochaeta and Chironomidae) during the early larval stage in ponds created by melting snow. Cannibalism accelerates metamorphosis,

however; as a result, cannibals are much smaller at metamorphosis than larvae fed typical prey only (Michimae and Wakahara 2002a). Thus, although cannibalism can be adaptive if it increases survival during the larval life stage, there is a trade-off associated with cannibalism (accelerated development results in smaller size at metamorphosis), which can affect traits expressed later in life, resulting in negative fitness consequences and, therefore, compromising reproductive fitness (Goater 1994; Scott 1994; Altwegg and Reyer 2003).

Here I hypothesize that populations can be expected to diverge in respect of egg size in response to variation in larval densities, with larger eggs being produced when larval density is higher. To test this hypothesis, I examined:

- 1 whether the presence of larger larvae hatched from larger eggs promotes cannibalism, that is, whether large variation in initial larval body size triggers frequent cannibalism; and
- 2 whether increasing larval density in natural habitats correlates with increasing egg size.

Methods

Study populations

Larval densities in each larval habitat had previously been investigated in four ponds, located in Erimo, Konuma, Nopporo, and Tomaru, from 2002 to 2004. The mean larval density in each of four additional ponds, located in Atsuta, Kamitobetsu, Okusawa, and Toyoha, was determined in a two-year field survey conducted during the breeding seasons of 2003 and 2004 (Michimae 2006). All study sites are located in Hokkaido, Japan. The number of *H. retardatus* egg clutches in each pond and the number of eggs in

each collected clutch were counted to estimate the conspecific larval density in each habitat. The annual density of conspecific larvae in each pond was estimated by multiplying the mean clutch size by the estimated density of clutches in the pond. The number of clutches of *R. pirica* eggs in the eight *H. retardatus* habitats was also counted to estimate the larval densities of *R. pirica* in each habitat. The annual density of *R. pirica* larvae in each pond was estimated by multiplying the assumed value of 800 eggs per clutch by the estimated density of clutches in the pond. The mean annual density of conspecific and *R. pirica* larvae in each pond was calculated by dividing the sum of the annual total larval densities by the number of years for which clutches were collected.

To analyze the possible effect of larval density on egg size (described below), I used the following mean larval densities (individuals/m²): Erimo (1393.5), Konuma (13.533), Nopporo (131.0), Tomaru (3799.943), Atsuta (2709.3), Kamitobetsu (423.15), Okusawa (155.62), and Toyoha (392.835). The detailed abiotic and biotic features (surface area, depth, density of clutches, and larval densities of *H. retardatus* and *R. pirica*) and geographic locations of the ponds have been described by Michimae (2006).

In 2003, fertilized clutches of *H. retardatus* were collected during the breeding season (early April to late May) from each of the eight ponds: 30 clutches each from Erimo, Konuma, Nopporo, and Tomaru, and nine clutches each from Atsuta, Kamitobetsu, Okusawa, and Toyoha.

Egg size, clutch size, and initial body size

The clutches from each population of *H. retardatus* were numbered, and the number of eggs in all clutches was counted. Five randomly selected eggs from each clutch were dejellied, and their diameters

were measured to the nearest 0.01 mm under a dissecting microscope. The eggs were measured at the blastula developmental stage or earlier to minimize the effect of increasing egg size with further development. The volume of each egg was estimated by use of the formula egg volume = $(4/3) \times 3.14 \times r^3$, where r is the egg radius. The mean egg size of each clutch was then calculated (sum of five egg volumes/5).

Each clutch from each population of *H. retardatus* was placed, until hatching, in a stock tank filled with 1.6 L dechlorinated tap water at room temperature (20–21 °C). When the eggs hatched, five larvae from all clutches of four populations, Erimo, Konuma, Nopporo, and Tomaru, were immediately selected and measured for total body length to the nearest 0.05 mm using calipers, and the mean body size of each clutch was calculated. For each clutch, the variance in initial body size were also recorded.

Rearing experiment

The induction experiment was performed with twelve clutches of *H. retardatus* from each of four ponds, Erimo, Konuma, Nopporo, and Tomaru. Each collected clutch was placed, until hatching, in a stock tank filled with 1.6 l dechlorinated tap water at room temperature (20°–21°C). When the collected *H. retardatus* clutches hatched, ten selected larvae (five of which were used to measure body size, as described above) from each of the 48 were assigned to a tank (8 cm×8 cm×8 cm deep) filled with 0.3 l dechlorinated tap water. Thus, each tank contained ten larvae from the same clutch. The experimental larvae were fed every other day by offering frozen Chironomidae from 0900 to 1200 hours. The larvae were always given enough food to eat within 3 h, and any food remaining in their tanks was removed after the feeding period. The rearing water was also exchanged every other day during the experiments.

All experiments were conducted in the laboratory at room temperature and with a natural light-dark schedule. Every morning during the experimental period (2 weeks), I recorded the tank where cannibalism had occurred because the goal of this experiment was to determine whether differences in initial larval body size promoted cannibalism.

Data analysis

To test whether the relationship between egg size and clutch size differed among populations, I performed analysis of covariance (ANCOVA) with log-transformed clutch size as covariate, log-transformed egg size as a dependent variable, and population as a factor. Next, I regressed log-transformed clutch size against log-transformed egg size of either the pooled data for the eight populations or of the separate data for each population, depending on the result from the previous ANCOVA, and calculated slopes of the resulting linear equations. Finally, I analyzed the effect of mean larval density on mean egg size in each pond by regression analysis.

Similarly, to test whether the relationship between egg size and larval body size differed among the four populations, I performed ANCOVA of larval body size, with egg size as covariate and population as a factor. I then regressed larval body size against egg size of either the pooled data for the four populations or of the separate data for each population, depending on the result from the previous ANCOVA, and calculated the slopes of the resulting linear equations.

I used a multiple logistic regression model to look for a possible association between the response variable (presence of cannibalism in each clutch) and two independent variables, variance in initial larval body size as a fixed factor and population as a random factor. The final model was determined by

comparing the deviance (likelihood ratio test statistic) to evaluate the fit of the models, which consisted of the different combinations of and interactions among the independent variables. To determine the effects of each independent variable on the presence of cannibalism in the final model, the odds ratios were then compared among treatment groups (categorical) or for units (continuous) by the Wald χ^2 test.

Although I originally prepared 12 egg clutches from each population for the rearing experiment and the statistical analysis, one clutch from the Tomaru population was removed from the statistical analysis because the tank was lost as the result of an accident in the laboratory.

Results

ANCOVA revealed no significant interaction between population and egg size (Table 1), indicating that the relationship between egg size and clutch size did not differ among populations. The population factor was therefore pooled. The slope of the linear regression equation was significant (Fig. 1a; slope=-0.463, $r^2=0.440$, $F_{1,155} = 120.756$, $P<0.0001$), and the slope was also significantly larger than -1 ($t_5=37.4$, $t_{0.001}[155]=3.291$, $P<0.001$). The mean larval density of the pond to which a female belonged significantly affected the size of the eggs that she produced (Fig. 1b; slope = 0.002, $r^2 = 0.506$, $F_{1,7} = 6.139$, $p = 0.048$), with larger eggs produced in ponds with higher larval density (Fig. 1b).

No significant interaction between population and larval body size was found by ANCOVA (Table 2), indicating that the relationship between egg size and larval body size (slope) did not differ among populations. The slope (0.184) of the best-fit regression line of larval body size against egg size in the eight pooled populations was significant (Fig. 2; $r^2=0.706$, $F_{1,118}=283.223$, $P<0.0001$).

The most parsimonious logistic regression for the presence of cannibalism was acquired by stepwise

reduction of the number of explanatory terms from the full model and by comparison of the deviance between the models with more explanatory terms and those with fewer (Table 3). The final model included only variance in initial larval body size (Table 3). The relationship between the variance and the presence of cannibalism in the tank is plotted in Fig. 3. The probability of the presence of cannibalism in the tank increased as the variance in initial larval body size increased, as indicated by the positive logistic regression (constant: regression coefficient=3.338, Wald $\chi^2=9.63$, $P=0.0019$; variance in initial body size: regression coefficient=-6.871, Wald $\chi^2=9.32$, $P=0.0023$; Fig. 3).

Discussion

In the gape-limited cannibalistic predator *H. retardatus* (Ohdachi 1994) larger body size can begin to extend an individual's cannibalistic advantage from the initial larval stages, as indicated by the positive relationship between variance and the presence of cannibalism in a tank (Fig. 3). Thus, larvae that hatch from larger eggs can grow at a proportionally faster rate than larvae that hatch from smaller eggs. To utilize other larvae more efficiently as a potential prey item, larvae must be larger at birth. Wakahara (1997) reported that size-dependent cannibalism results in an increasing disparity in size throughout the larval period and causes cannibalistic larvae to have a higher rate of successful metamorphosis. Similar examples of this phenomenon have been found in *A. opacum*, where the potential for size-dependent cannibalism may be greatly increased by variation in initial egg size (Kaplan 1980). These results suggest that production of larger eggs may result in higher maternal fitness, depending on specific circumstances described below, because larvae from small eggs are suitable prey items for larger larvae.

An egg size-egg number trade-off explains the egg size and clutch size variances in the eight

populations of *H. retardatus*. The negative slope of the regression line (-0.463), significantly larger than -1 , indicates that clutches with a large mean egg size have a larger total egg mass than those with a small mean egg size. In this study, whether egg size depended on female size or age was not examined. It is possible that the observed differentiation in egg size among populations is, in part, attributable to nongenetic environmental effects, for example those related to female body size or age (Kaplan 1998). Genetic variation enabling selection on the basis of egg size at the population level has been shown by several studies, however (Shaw and Byers 1998; Wade 1998), suggesting that egg size is likely to evolve in response to selection imposed by the offspring's environment (Crump 1981; Berven 1982; Kaplan and Cooper 1984). In other words, for selection to act on egg size, there must be differences between fitness among offspring derived from eggs of different size.

The strategies of producing clutches of fewer large eggs or clutches of more numerous smaller eggs should result in different maternal fitness consequences in different larval-density environments. In ponds with high larval density, in which cannibalism is frequent, that is, when larval-larval cannibalism becomes a critical factor in offspring fitness, the production of clutches of fewer and larger eggs, and thus fewer and larger offspring, may result in higher maternal fitness. It is probable that females used larger amounts of resources to produce larger eggs in accordance with the increased larval density in the environment. Newly hatched *H. retardatus* larvae, which inhabit ponds created by melting snow, sometimes experience a scarcity of their typical prey during the early larval stage, which usually occurs during early spring. In the absence of typical prey it is particularly adaptive for newly hatched larvae to consume frequently encountered large prey such as conspecific or *R. pirica* larvae (Ohdachi 1994; Nishihara 1996; Michimae and Wakahara 2001, 2002a, b). Maternal investment strongly affects the size of eggs or offspring and the potential for cannibalism in the larval period; this results in offspring fitness

and thus maternal fitness.

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Figure legends

Fig. 1. a Relationship between log-transformed egg size and clutch size for individual females of *Hynobius retardatus* in eight populations, each of which occupies a different point along the environmental continuum in respect of the densities of conspecific and *R. pirica* larvae; $r^2=0.440$, $F_{1,155}=120.756$, $P<0.0001$. Comparison of the slope with -1 : $t_s=37.4$, $t_{0.001}[155]=3.291$, $P<0.001$. **b** The relationship between mean egg size (\pm SD) and larval density in the eight populations; $r^2=0.506$, $F_{1,7}=6.139$, $P=0.048$. *Open circles*, Erimo; *open squares*, Konuma; *open triangles*, Tomaru; *open diamonds*, Nopporo; *filled circles*, Okusawa; *filled squares*, Atsuta; *filled triangles*, Kamitobetsu; *filled diamonds*, Toyoha

Fig. 2. Relationship between egg size and larval body size for the four populations, Erimo, Konuma, Tomaru, and Nopporo; $r^2=0.706$, $F_{1,118}=283.223$, $P<0.0001$. *Open circles*, Erimo; *open squares*, Konuma; *open triangles*, Tomaru; *open diamonds*, Nopporo

Fig. 3. Relationship between variance in initial larval body size and the presence of cannibalism in a tank; constant: regression coefficient=3.338, Wald $\chi^2=9.63$, $P=0.0019$; variance in initial body size: regression coefficient=-6.871, Wald $\chi^2=9.32$, $P=0.0023$. *Open circles*, Erimo; *open squares*, Konuma; *open triangles*, Tomaru; *open diamonds*, Nopporo

Figure 1

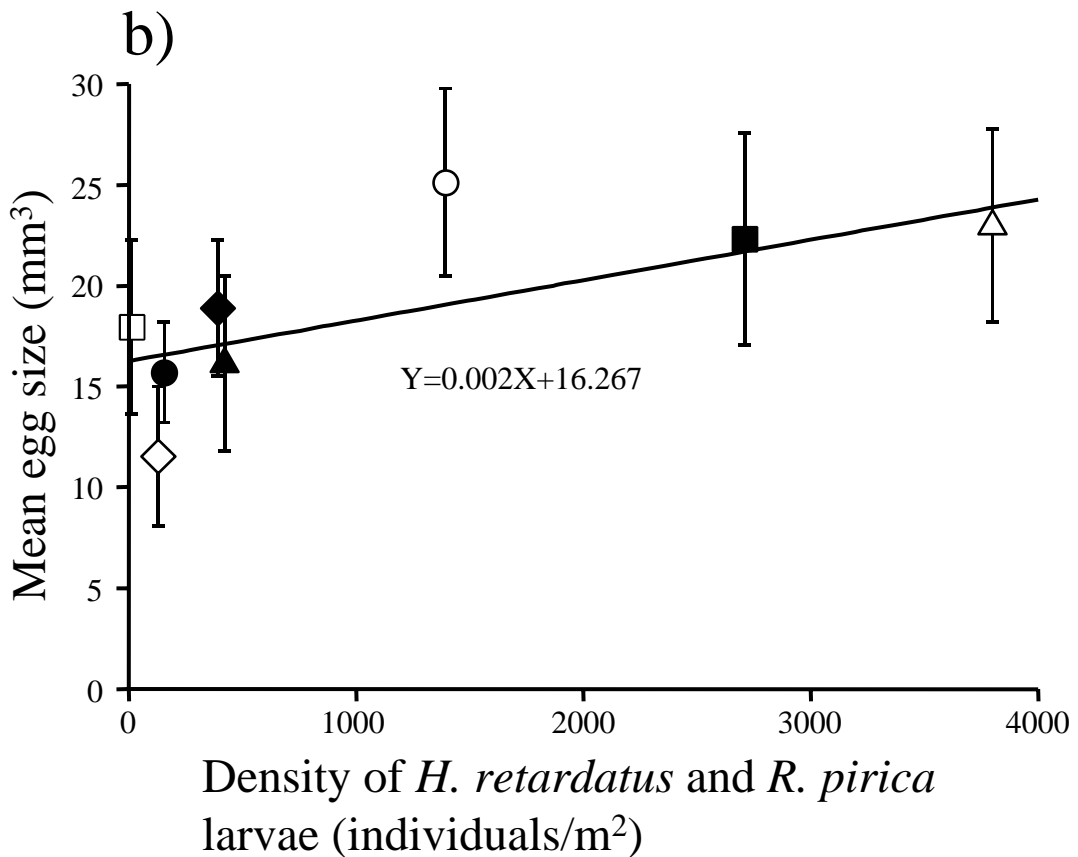
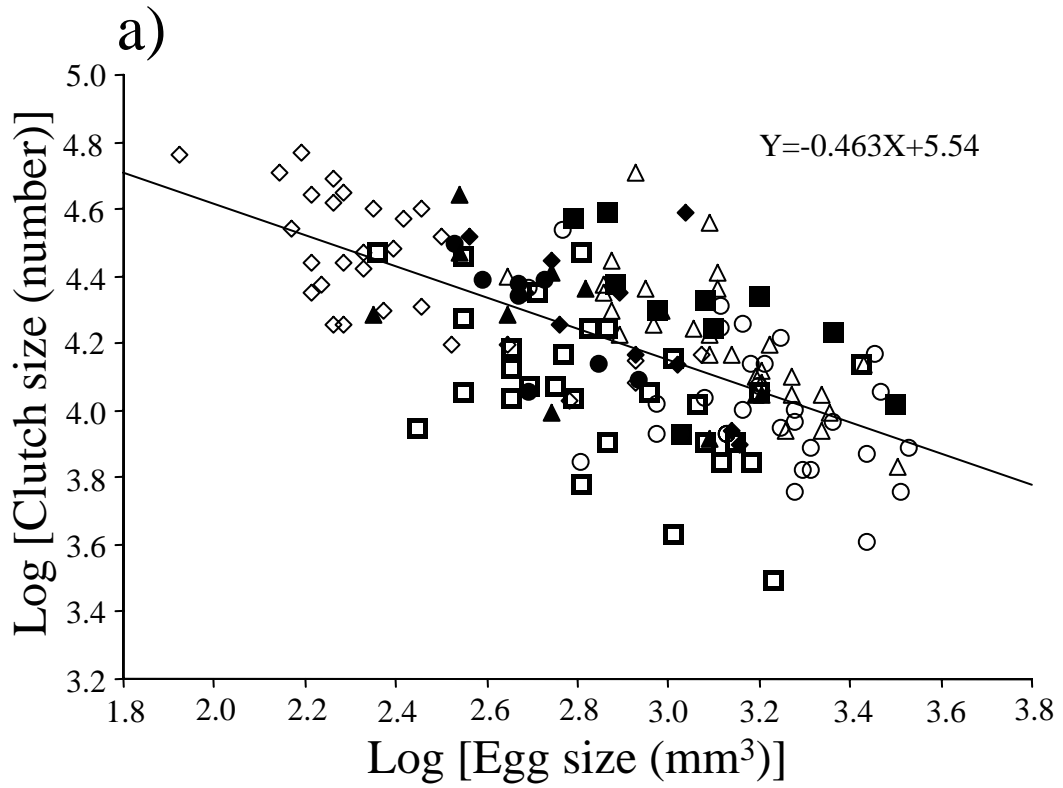


Figure 2

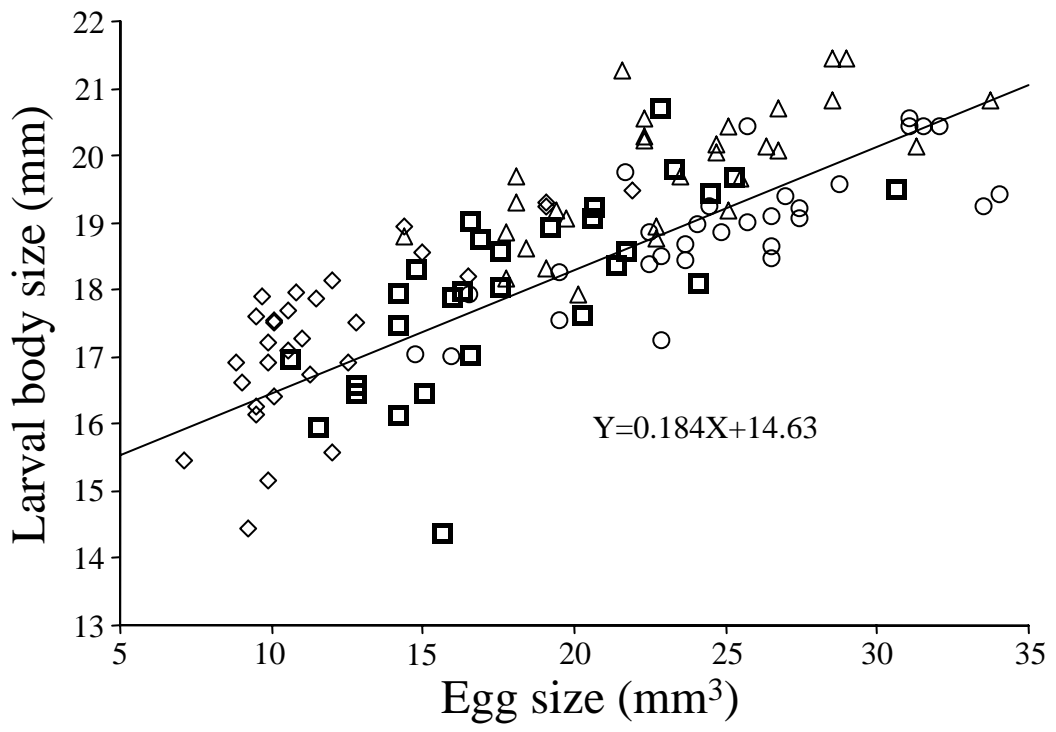


Figure 3

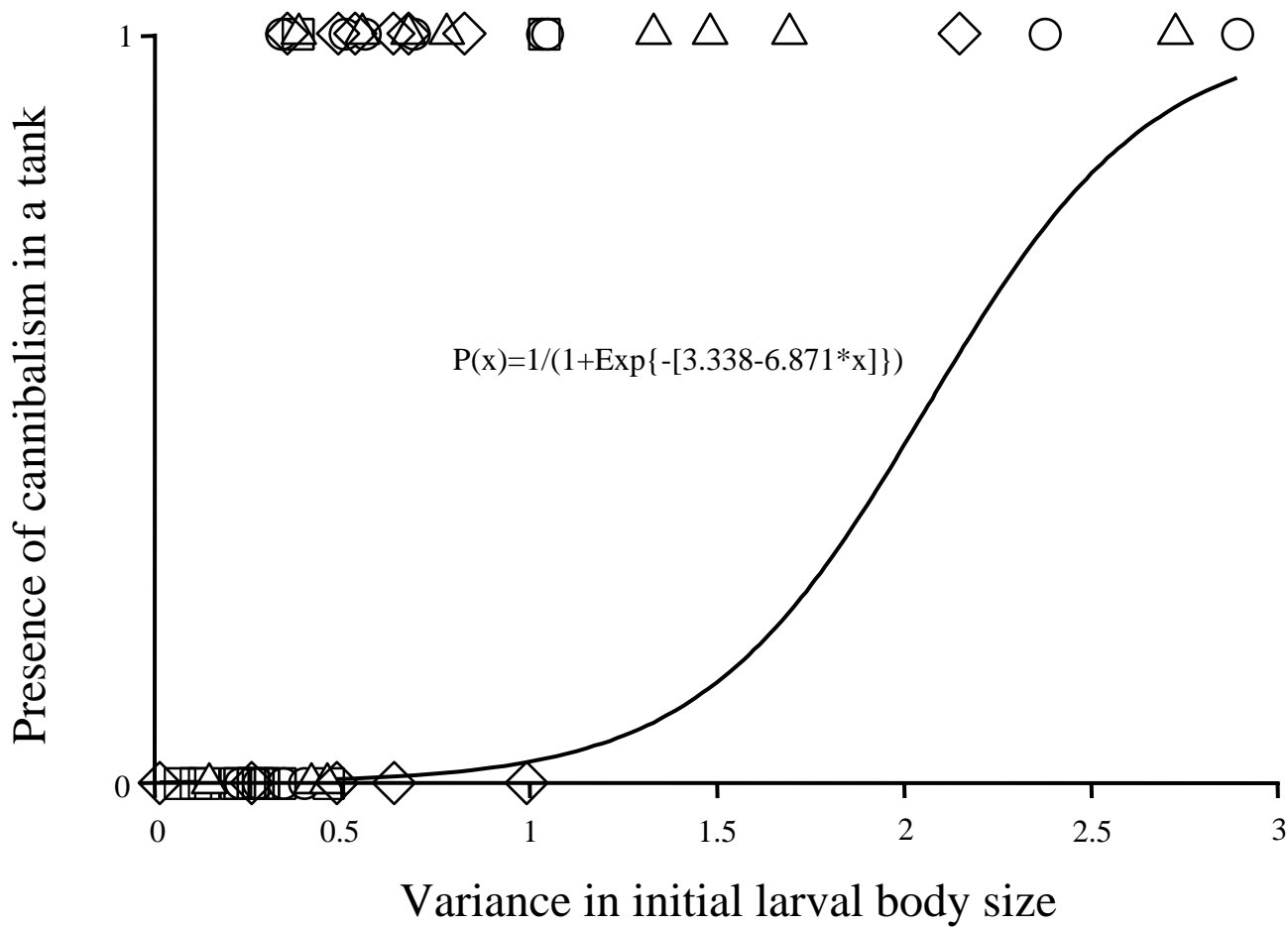


Table 1. Results from ANCOVA of the effect of population on clutch size, with egg size as covariate.

Source	MS	d.f.	<i>F</i>	<i>P</i>
Population	0.025	7	0.947	0.4723
Egg size	1.980	1	75.657	<0.0001
Population × Egg size	0.017	7	0.646	0.7175
Error	0.026	140		

Table 2. Results from ANCOVA of the effect of population on larval body size, with egg size as covariate.

Source	MS	d.f.	<i>F</i>	<i>P</i>
Population	1.234	3	2.004	0.1175
Egg size	79.858	1	129.695	<0.0001
Population × Egg size	1.257	3	0.646	0.1121
Error	0.616	112		

Table 3. Comparisons of multiple logistic regression models, which consisted of the different combinations of and interactions between the independent variable (V variance in initial body size, P population). Constant+V was selected as the final model.

Model	Log-Likelihood	d.f.	variable evaluated	Deviance (d.f.)	<i>P</i>
Constant+V+P+V × P	15.3935	7			
Constant+V+P	18.2351	4	V × P	5.6832 (3)	NS
Constant+V	19.0205	1	P	1.578 (1)	NS
Constant+P	27.6407	3	V	17.2404 (2)	<0.0005