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Age at Sexual Maturity of Male *Xiphophorus nigrensis* in Nature

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Male *Xiphophorus nigrensis* drastically reduce growth when they reach sexual maturity and exhibit a genetically determined polymorphism in size. We determined that the growth increments on the otoliths in *X. nigrensis* indicate true age in days for fish that have not reached sexual maturity. After maturity, these rings provide an estimate of the age at which sexual maturity was reached. Age at sexual maturation was significantly different across geneotypically determined size classes for field caught animals. On average, males from the smallest size class matured 46.6 d sooner than did males from the largest size class. The variance in age at sexual maturity was much greater for laboratory-reared individuals than for individuals reared in either semi-natural conditions or from the field. This is consistent with the hypothesis that the environment can influence the age at which sexual maturity is reached, and suggests that for life history studies of poeciliid fishes age at sexual maturation needs to be measured in the field. This study also demonstrates that otoliths provide an accurate estimate of age at sexual maturity but not of true age of mature males.

MEASURING the costs and benefits of life history strategies is an important component of life history studies. Determining the age of individuals is often necessary for a full evaluation of the costs and benefits, because many variables are age specific. In this study we determine age at sexual maturation in nature for males of the swordtail *Xiphophorus nigrensis*. Males of this species exhibit a genetically determined polymorphism in life history strategy that involves both size at sexual maturation and mating behavior. Some males mature at a large body size and court females, while others remain small and use an alternative sneak-chase behavior to copulate with females (Ryan and Causey, 1989). Larger males have greater mating success due, in part, to female preference for large size and courtship (Ryan and Wagner, 1987; Ryan, 1989; Ryan et al., unpubl.). Estimates of age at sexual maturity will make it possible to determine if small males compensate for their mating disadvantage by reaching sexual maturity sooner than large males. Early maturation would benefit small males through either a decreased generation time, an increased probability of reaching sexual maturity, or a longer reproductive lifespan.

Studies of the evolution of life history strategies usually rely on the assumption of a genetic basis to the observed differences (Lloyd, 1977). *Xiphophorus nigrensis* is a particularly good organism for life history analysis, because a genetic basis for within population variation in life history strategy has been demonstrated. Males exhibit significant variation in body size, most of which can be explained by allelic variation at the pituitary (P) locus on the Y chromosome (Kallman, 1984; Ryan and Wagner, 1987). In *X. nigrensis*, as in many species of *Xiphophorus*, male growth declines drastically at the completion of sexual maturity (Kallman, 1983, 1984). Males possess one of three alleles (Y-s small, Y-I intermediate, Y-L large) that determines their size at sexual maturity (Kallman, 1984). There is only one P factor on the X chromosomes and it codes for sexual maturity at a small size; therefore all females mature at a small size. Males with the Y-s allele use sneak-chase behavior exclusively, males with the Y-I allele use sneak-chase and courtship, while males with the Y-L allele almost always court females (Ryan and Causey, 1989).

Males with the Y-s allele reach sexual maturity much sooner than males with the Y-I and Y-L alleles in laboratory studies (Kallman, 1983). However, it is not known how accurately laboratory results reflect field conditions. In the congeneric *X. variatus*, it was shown that age at sexual maturation, but not size, varied with environmental conditions (Borowsky, 1987). Therefore, to further compare the strategies of the three male phenotypes, age at sexual maturity needs to be measured for individuals from the field. In many organisms, field estimates of

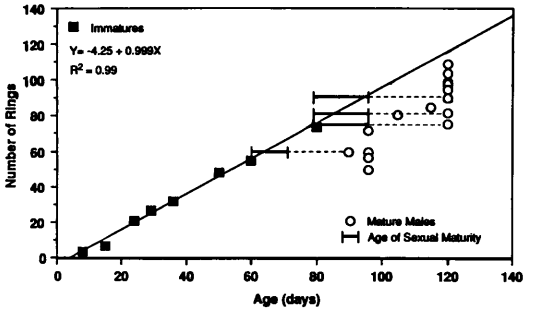

Fig. 1. Calibration of otolith growth increments to true age for immatures and mature males. Age at sexual maturity is shown for four of the males sampled after they were mature.

age are often difficult to obtain. However, Pannela (1971) discovered that growth increments are deposited on the otoliths of some fish with 24 h periodicity. Since then, the technique of counting growth increments on otoliths has been used to estimate age in many teleost fish, as reviewed by Campana and Neilson (1985). While most studies assume that growth increments are formed daily, the rate of formation may vary and should be validated before used in age determination (Campana and Neilson, 1985).

There is little consensus as to the fate of daily rings in fish with determinant growth; that is, if the rings are reliable indicators of true age or of age when growth ceased. In this study, we first examined the formation of growth increments in *X. nigrensis* to determine the relation between ring number and age. We then compared age estimates from field data to laboratory and semi-natural conditions to determine if laboratory results reflect field results. Finally, we examined differences in age at sexual maturity for the three size classes, and discuss the possible role of early maturation in maintaining males with the Y-s allele in this population.

Methods

Five males from each of the three size classes were collected on each of three collecting trips (June, July, and Oct.) from the head waters of the Rio Choy, San Luis Potosi, Mexico. The SL of all specimens was measured, and the head preserved in 95% ethanol. Thirty females were collected from the same site and transported to Austin, Texas, where they were placed in breeding traps in the laboratory.

Two cohorts (born on the same day) of 25 offspring born to these females were placed in two 7500 liter outdoor tanks at the Brackenridge Field Station within 24 h of birth. Two offspring from each cohort were preserved immediately to determine the number of growth increments produced before birth. Tanks were lined with netting to aid in sampling. In addition, each tank was outfitted with an overflow drain and a continuous flow of water. Surface water temperatures of the two tanks were similar and ranged from 28–33 C.

An individual was sampled every 5–10 d from one cohort, while the other cohort was allowed to grow to sexual maturity. Before a specimen was preserved, it was examined for evidence of sexual maturity as denoted by differentiation of the gonopodium (Kallman and Schreibman, 1973) or presence of a brood spot. The SL of all specimens was measured, and the head preserved in 95% ethanol.

By repeatedly inspecting males in the second tank and keeping track of size and color markings, we determined the age at which four males reached sexual maturity. Males in the second tank were not sampled until several weeks after the last male reached sexual maturity. The age at which the four males reached sexual maturity was compared to the number of rings counted on their otoliths.

Otoliths from all specimens were removed and mounted on slides in microscope immersion oil. Growth increments were counted on the asterisci, the most transparent of the three otoliths for this species, with phase contrast under a 100× oil immersion lens and 10× eyepiece (1000×). The labels on the slides were covered to insure a blind count. Each otolith was counted 3–5 times and the ring counts averaged for the final score (mean SD = 2.4, n = 55).

The number of rings counted on the otoliths for fish collected in the field and fish reared in the outdoor tanks were compared to the age at sexual maturity for fish reared in the laboratory. In the laboratory, each brood (5–10 fish) was raised in an 11 liter aquarium. Fish were fed live brine shrimp three times each day and Tetramin fish food and liver paste once each day. Age at sexual maturity was determined by differentiation of the gonopodium.

Results

Otoliths from the offspring preserved at birth had an average of 5 growth increments (n = 4, SD = 0.82). Therefore, five rings were sub-

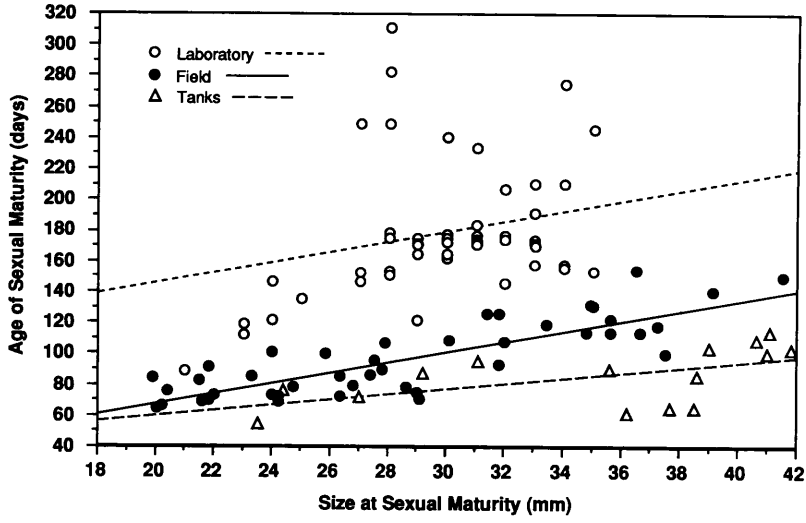

Fig. 2. Age at sexual maturity compared to male size for three different environments.

tracted from the ring counts of all fish preserved at later dates. There was a strong correlation between the number of rings counted and the true age of the individual ($r^2 = 0.98$, df = 6, $P < 0.001$; Fig. 1) for fish sampled before sexual maturity. The slope of unity (0.999) demonstrates that one ring equals one day. The number of rings for sexually mature individuals, however, was always less than the animal's age (Fig. 1). When age at sexual maturity was known (within 7–10 d), the number of rings counted was within the same range (Fig. 1). Therefore, ring counts are accurate estimates of true age up until the time of sexual maturity. Once a male is sexually mature, the ring count provides an estimate of age at which sexual maturity was reached.

We compared the age and size at sexual maturity for fish from three different environments: males reared in the laboratory, males reared in the outdoor tanks, and males collected from the field (Fig. 2). Age at sexual maturity was positively correlated with size in all three data sets (Laboratory, $r^2 = 0.06$, df = 1, 60, $P = 0.05$; Field, $r^2 = 0.69$, df = 1, 40, $P < 0.001$; Tanks, $r^2 = 0.32$, df = 1, 13, $P = 0.03$). While the slopes of the three regressions were not significantly different ($F = 0.54$; df = 2, 113; $P = 0.58$), covariate analysis showed that there was a significant difference in age at sexual maturity across environments ($F = 105$; df = 2, 115; $P < 0.0001$). Also, variance in age at sexual maturity was significantly greater in the laboratory than the field ($F = 1.85$; df = 61, 41; $P < 0.05$), but did not differ significantly between the outdoor tanks and the field ($F = 1.27$; df = 41, 14; $P > 0.25$).

We compared age at sexual maturity for the three size classes of males collected in the field. Males in the small size class (<26.0 mm) reached sexual maturity in an average of 77.6 d (n = 15, SD = 10.1), intermediate males (25.0–31.0) in 90.4 d (n = 14, SD = 15.2) and large males (>31.0) in 124.2 d (n = 13, SD = 16.3). The variance in age at sexual maturity was not significantly different among size classes (Bartlett test for homogeneity, $\chi^2 = 3.1$, df = 2, $P = 0.21$), but the age at sexual maturity was significantly different among size classes ($F = 40.3$; df = 2, 39; $P < 0.001$). Therefore, because the phenotypic size classes are good estimates of male genotype, the age at sexual maturity is significantly different among genotypes.

Discussion

The growth increments on the otoliths in *X. nigrensis* indicate true age in days prior to sexual maturity. After maturity, when males drastically decrease growth, fish do not add daily rings. In sexually mature individuals, therefore, rings are more representative of the age at which sexual maturity was reached. If fish continue to add rings at a slower rate after sexual maturity, rings would not provide the exact age at which

sexual maturity was reached. However, our data show that even if this were true, rings provide an estimate of age at sexual maturity. Despite the widespread use of poeciliids in life history studies and the use of otoliths to estimate age in many species, there is almost a total lack of information on the relationship of age and otolith ring number in any poeciliid fish (Snelson, 1989).

In several species of fishes, growth increments are useful in estimating the age of mature males (Pannela, 1971). We have shown for *X. nigrensis,* however, that growth increments are not deposited daily after sexual maturity, and therefore cannot be used to estimate true age for mature individuals. This study suggests that otoliths will probably not provide the necessary information to estimate age of sexually mature males in poeciliids with determinant growth.

The greater variance in age at sexual maturity in the laboratory, as compared to the field or outdoor tanks, demonstrates that the laboratory environment, often considered a more homogeneous environment, can produce more heterogeneous results. In contrast, the variance in age at sexual maturity in the semi-natural conditions of the outdoor tanks is statistically indistinguishable from nature. Natural conditions may be more heterogeneous in those factors influencing growth rate, but fish are able to seek out the more favorable microhabitats in nature. In the laboratory, fish are constrained to the environment as provided. While it is possible to provide a more homogenous laboratory environment than we did in this study, it would be difficult to determine if the environment provided matched the environment a male would have sought in nature. Therefore, the difference in the laboratory and field results suggests that laboratory data not be used to estimate age for life history studies. In addition, the immense variation in age under different environmental conditions is consistent with Borowsky's (1987) demonstration that in *X. variatus* size, not age, is fixed by the P locus.

Otolith data provided the necessary information to compare the age of sexual maturity across the three genotypic size classes for a natural population. The strong correlation between size and age at sexual maturity in the field data demonstrates that small males reach sexual maturity sooner than large males. A correlation between age and size at sexual maturity has previously been demonstrated for laboratory-reared *X. nigrensis* (Kallman, 1983), but this is the first time that this correlation has been measured for a natural population.

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