PURDUE UNIVERSITY

DEPARTMENT OF
ANIMAL SCIENCES

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Alan Blake
Chief Executive Officer
Yorktown Technologies, Inc.

Dear Alan,

This letter is in response to your question as to the possible environmental risks posed by the release of transgenic fluorescent zebra fish into the wild based on published results of Gong et al (2003). From my research on environmental risk assessment of GM fish (see references attached), risk can be pre-determined from the probability of vertical transgene spread, i.e. from inter-mating and natural selection. From basic principle of evolution, I reduced that probability to examination of six major subcomponents, juvenile and adult viability, age at sexual maturity, fecundity, fertility, and mating success. Gong et al (2003) study provided results which allow for evaluation of four of these components: juvenile viability, mating success, fecundity, and fertility.

Regarding the first component, this can be broken down into survival from embryo to 3 day old fry, and from 3 day old fry to adult. The embryo survival rate can be determined by comparison of the observed to expected number of genotypes from crossing hemizygous red with green GM fish. One would expect equal number of each genotype in the next generation. Results do not differ significantly from that expectation. Hence the transgene does not negatively or positively impact embryo survival. Regarding survival from fry to adult, the same chi-square test was applied and no significant difference in survival between genotypes was found.

Mating success, fecundity, and fertility were jointly measured in an experiment where equal numbers of adult GFP males and females were placed in a tank with equal numbers of wild-type males and females. I am assuming the GFP males and females were hemizygous. Eggs were collected for 7–8 consecutive days and the embryos genotyped under a fluorescent microscope. If mating were at random, and fertility and fecundity were equal between genotypes, then one would expect the genotypic frequencies to follow the Hardy-Weinberg law, or square law, because gene frequencies were equal in the sexes, i.e.

$$\text{Expect genotypic frequencies} = \left(\tfrac{1}{4}GFP + \tfrac{3}{4}W\right)^2 = \tfrac{1}{16}\left(GFP/GFP\right) + \tfrac{6}{16}\left(GFP/W\right) + \tfrac{9}{16}\left(W/W\right)$$

or

7/16 transgenic and 9/16 wild type. This result is obtained because the GFP allele is dominant.

This theoretical result differs from that stated by the authors who claim an equal ratio of transgenic to wild type was expected. An equal number of each genotype could only result if

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one genotype of male or female was used, not a mixture of genotypes. In essence they forgot that matings between transgenic x transgenic and wild-type x wild-type could occur. This changes the conclusion of their experiment. In the first experiment the results followed expectation almost exactly with 1158 out of 2656 transgenic while the expected number was 1162 . In their second experiment they observed 2121 out of 5224 to be transgenic while the expected number was 2285. Thus there were significantly ($p<.001$) fewer transgenic fish than expected based on the hypothesis of no difference. Unfortunately which of the components (differential mating success, fecundity, or fertility) produced this effect cannot be determined from the data. Simple counting off eggs from each genotype would have allowed that component to be separated. Regardless, the net effect of these three components on fitness is negative.

The only components not measured by these researchers were age at sexual maturity and adult viability. I would not expect GFP to enhance either of these, but experimental data would be needed for verification.

In conclusion, based on the published results of Gong et al (2003) and with the constructs they used, in the absence of any advantage in age at sexual maturity or adult viability, GFP has a significant net fitness disadvantage, indicating that one would expect natural selection to eliminate the transgene regardless of where it escaped or was released.

Sincerely,

William Muir
Professor Genetics

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