The Use of Quantitative PCR to Determine the Impact of Zygosity (Hemizygotes vs. Homozygotes) on the Fitness and Impact in the Wild of Transgenic Fluorescent Zebrafish

Pollak, Adrianna

Danio rerio are tropical transgenic zebrafish that contain a mutant strain, making them glow when their muscles fluoresce (Zebrafish, 2010). The question was: Can Quantitative Polymerase Chain Reaction (QPCR) be used to determine transgenic zebrafish zygosity (hemizygotes vs. homozygotes) and relate it to fitness? Hemizygous red transgenic zebrafish (GloRFP/Glo-) were naturally bred. The twenty-two offspring were sedated. Their length (centimeters) and mass (grams) were measured; a tissue sample of the caudal fin was obtained. Each tissue sample was prepared for amplification, and QPCR analysis was done. The dilution equation determined the volume of DNA, added to Milli-Q (MQ) water, to obtain a final concentration of fifty nanograms per microliter. To calculate QPCR threshold cycle (Ct), the velocity, (rate of change in fluorescence per number of QPCR cycles) was subtracted from the control primers to determine the second derivative max [threshold cycle (Ct)]. Next, zebrafish were sorted by zygosity in order to differentiate hemizygous from homozygous red, and wild type grey fish, to relate zygosity to fitness measurements. The original hypothesis was supported; QPCR can be used on transgenic fluorescent zebrafish to determine zygosity, relating zygosity to fitness. A fitness advantage was noted for hemizygous RFP GloFish when compared to the homozygous fish with twice the relative quantity of the inserted transgene, and wild fish, ANOVA $p<.046$. It appears that extra burden of biosynthesizing the fluorescent protein is only manifested in the homozygous fish. It is difficult to know if the hemizygote’s advantage would be duplicated in a non-native wild environment.

Awards Won: