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Genotyping Sex in Fathead Minnows, *Pimephales promelas*, for Use in Endocrine Disruption Assays.

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Endocrine disrupting compounds have been shown to completely sex reverse both male and female individuals in amphibian, avian, fish, invertebrate, and reptile species. In many cases these sex-reversed individuals are morphologically indistinguishable from normal individuals. Detection of low-level sex reversal often requires large numbers of organisms to achieve the necessary statistical power, especially in those species with genetic sex determination and homomorphic sex chromosomes (such as amphibians and many fish). We developed a genotyping method utilizing amplified fragment length polymorphisms (AFLP) in the fathead minnow, *Pimephales promelas*, for incorporation into endocrine disruptor screening assays that examine the effects of chemicals on gonad differentiation. In total 10 sex-linked AFLP's were identified, isolated, and sequenced. No recombination events were observed with any sex-linked AFLP in the animals examined (n=112). A PCR method was then developed that determined the presence of one sex-linked polymorphism for utilization in environmental toxicological assays. These markers were not capable of genotyping sex in minnows collected from outside sources limiting their usefulness to controlled laboratory settings. Analysis of additional spawns from our in-house culture indicate that fathead minnows utilize a XY sex determination strategy and confirm that these markers can be used to genotype sex given the right genetic makeup of breeders. The increased statistical power gained from incorporating sex genotypes compared to analyzing changes in sex ratios was demonstrated using Monte Carlo simulations. These simulations model 1000 experiments in which sex reversals occur in one treatment at frequencies between 2.5-25%. Increased statistical power with sex genotyping was observed in all scenarios modeled.

Impact Statement:

Fathead minnows are used as a model fish species for the characterization of the endocrine-disrupting potential of environmental contaminants. This research describes the development of a PCR method that can determine the genetic sex in this species. This method, when incorporated into endocrine disruptor testing, enables the detection of sex reversals in exposed, developing fish. The increase in statistical power when utilizing this method over conventional methods of comparing sex ratios is demonstrated through Monte Carlo simulations.