Sex-specific gene expression in early life stage fathead minnows (*Pimephales promelas*) throughout development and after exposure to synthetic hormones

Jessica Leet, Jon Amberg, Allen Olmstead, Gary Ankley, Linda S. Lee, Maria Sepúlveda

There is evidence that exposure to endocrine disrupting chemicals (EDCs) during early life stages can alter sex differentiation in fishes. Fathead minnows (Pimephales promelas) are commonly used as a model fish species in endocrine disruption studies. However, limited knowledge exists on molecular pathways associated with sex differentiation in early life stages of this species. Changes in the expression of genes important in sex differentiation could be useful in evaluating the effects of EDCs on gonadal development and sex differentiation. Until this point no study has characterized the sex-specific baseline expression of genes in developing fathead minnows. Using a sex-linked DNA marker to verify gender, we evaluated the expression of genes involved in gonad development and sex differentiation (dmrt1, cyp19a, cyp17, star, esr1, ar) in developing fathead minnows (10 - 45 days post hatch; dph) to establish this baseline data. Expression of all genes remained relatively constant in males except for *cyp17*, which increased to almost 20 fold by 45 dph. Gene expression patterns in females varied more than those in the males, but generally expression increased 10 to 20 fold by 45 dph. Since these genes have been linked with sex differentiation, we hypothesize expression of these genes will be altered in a sex-specific manner when fathead minnows are exposed to androgens, estrogens, or mixtures of EDCs during this same life-stage. Preliminary results show a significant increase in cyp19a expression in males exposed to 1.5 ng/L 17 α -ethinylestradiol (a potent synthetic estrogen) between 10 and 20 dph, but no significant increase in expression was observed in females. Evaluation of these molecular markers in combination with gender identification is being used in developing tools to efficiently evaluate sex-specific responses in early life stage fish exposed to EDCs. These tools will help us better understand the mechanisms regulating sex differentiation in fathead minnows and how EDCs may alter these processes.