# Fear Factor: Effects of the Anti-Diabetic Drug Metformin on the Production and Response to an Anti-Predator Alarm Signal in Fathead Minnows

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Pharmaceutical pollution of aquatic ecosystems, especially by endocrine disruptors – a class of chemical that have been shown to alter normal hormone function, is becoming a worldwide issue of growing concern. Pharmaceuticals and their metabolites enter surface waters throughout the world mostly through wastewater treatment effluent discharged from facilities lacking the ability to remove trace amounts of pharmaceuticals. One such pharmaceutical with endocrine disrupting behavior is Metformin, a commonly prescribed anti-diabetic medication that has been shown to induce intersex gonads in male juvenile fathead minnows at a concentration found in treated wastewater effluent (40 μg/L). The current study aims to determine the effects of metformin exposure on epidermal club cell investment in fathead minnows. Epidermal club cells release a compound when damaged, such as during a predator attack, that triggers an anti-predator response in nearby minnows. During mating season, male fathead minnows lose the ability to produce these club cells, and thus the alarm cue, potentially due to increased testosterone production. If a hormone such as testosterone can regulate club cells investment, an endocrine disruptor such as metformin may be able to alter that regulation. For this study, male and female fathead minnows, both in their mating cycle and in the juvenile phase were exposed to a concentration of metformin shown to cause endocrine disrupting effects in previous studies (40 μg/L) and also a higher dose (100 μg/L). During this exposure period secondary sex characteristics were recorded to judge metformin effects on sexual development. At the end of the 30-day period the density of club cells in skin samples was modeled by examining anti-predator behavior displayed by minnows exposed to skin extracts from the tested fish. Changes in club cell investment could cause harm to minnow communities due to changes in the chemical signaling function of club cells associated with triggering predator avoidance behavior, and additionally decreasing male fathead minnow sexual fitness.