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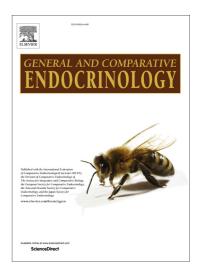
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ACCEPTED MANUSCRIPT

Endocrine disruption of phenanthrene in the protogynous dusky grouper *Epinephelus* marginatus (Serranidae: Perciformes)

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Abstract

The dusky grouper *Epinephelus marginatus* is a protogynous hermaphrodite fish, that maintains high levels of plasma steroids as juveniles, as substrates for sex inversion. These fish are exposed to marine pollution from oil spills during cargo handling. Polycyclic aromatic hydrocarbons (PAHs), such as phenanthrene (Phe), are the main crude oil components and are toxic to fish, acting as endocrine disruptors (ED). This is the first study that investigated impacts of Phe as an ED in *E. marginatus* juveniles. An *in vivo* sublethal exposure (96 h) to Phe was carried out at two concentrations (0.1 mg/L and 1 mg/L); exposure to the vehicle (ethanol; ETOH) was also performed. Plasma levels of 17β-estradiol (E₂), testosterone (T) and 11-ketotestosterone (11-KT) were measured by ELISA. Gonads, liver and spleen were processed for histological analysis. In an *in vitro* bioassay, gonad fragments were incubated with Phe (8.91 mg/L) or ETOH. Steroid levels in the culture media were measured by ELISA. The *in vivo* exposure to Phe triggered an increase of the area of the hepatocytes, increased number of melanomacrophagic centers and hemosiderosis in the spleen; ETOH induced similar effects on spleen. E₂ and T levels did not change in plasma or *in vitro* media. In plasma, ETOH decreased 11-KT levels. Phenanthrene sharply reduced 11-KT levels *in vitro*. Although *in vivo* bioassay

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