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Early warning signs of endocrine disruption in adult fish from the ingestion of polyethylene with and without sorbed chemical pollutants from the marine environment

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HIGHLIGHTS

- We saw down-regulation of Chg H in males exposed to marine plastic.
- We saw down-regulation of Vtg I, Chg H and ERα in females exposed to plastic.
- We saw abnormal proliferation of germ cells in a male exposed to marine plastic.
- Our results suggest that the ingestion of plastic may alter endocrine system function.

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ABSTRACT

Plastic debris is associated with several chemical pollutants known to disrupt the functioning of the endocrine system. To determine if the exposure to plastic debris and associated chemicals promotes endocrine-disrupting effects in fish, we conducted a chronic two-month dietary exposure using Japanese medaka (*Oryzias latipes*) and environmentally relevant concentrations of microplastic (<1 mm) and associated chemicals. We exposed fish to three treatments: a no-plastic (i.e. negative control), virgin-plastic (i.e. virgin polyethylene pre-production pellets) and marine-plastic treatment (i.e. polyethylene pellets deployed in San Diego Bay, CA for 3 months). Altered gene expression was observed in male fish exposed to the marine-plastic treatment, whereas altered gene expression was observed in female fish exposed to both the marine- and virgin-plastic treatment. Significant down-regulation of choriogenin (Chg H) gene expression was observed in males and significant down-regulation of vitellogenin (Vtg I), Chg H and the estrogen receptor (ERα) gene expression was observed in females. In addition, histological observation revealed abnormal proliferation of germ cells in one male fish from the marine-plastic treatment. Overall, our study suggests that the ingestion of plastic debris at environmentally relevant concentrations may alter endocrine system function in adult fish and warrants further research.

1. Introduction

Case studies regarding endocrine disruption have proliferated since the release of Rachel Carson's *Silent Spring* in 1962. The endocrine system is critical to organisms as it plays a role in reproduction, development, and immune-system function (Colborn et al., 1993; Crisp et al., 1998). In the past, endocrine-disruption was not addressed when assessing the hazards associated with synthetic chemicals, and as a consequence chemicals once considered benign have become ubiquitous as environmental contaminants (Colborn, 1994).

Similarly, hazards associated with plastic in aquatic habitats were also likely not addressed when assessing hazards associated with plastic products and plastic debris is now ubiquitous in the environment (NOAA, 2011). Plastic debris is a multiple stressor in aquatic habitats as a consequence of the large mixture of chemical contaminants associated with it (Rochman, 2013). Smaller plastic debris (<1 mm), often termed microplastics, is associated with large concentrations of chemicals, including >78% of priority pollutants (Rochman et al., 2013a), introduced during the manufacturing process (Lithner et al., 2011) and sorbed from surrounding environmental media (Ogata et al., 2009). Several of these plastic-associated chemicals have been linked to endocrine disrupting effects. Styrene (Iguchi et al., 2006), a monomer of several plastic types including polystyrene, rubber and acrylonitrile–butadiene–styrene, and bisphenol-A (BPA; vom Saal and Hughes, 2005), a monomer of polycarbonate, can disrupt endocrine-system function. Furthermore, there is evidence that UV-stabilizers, phthalates and nonylphenol, additives to plastic, are estrogenic and/or antiandrogenic (Harris et al., 1997; Fent et al., 2014). Moreover, chemicals historically known to promote adverse affects to the functioning of the

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endocrine system, including organochlorine pesticides, heavy metals and petroleum hydrocarbons (Fry, 1995; Crisp et al., 1998), are found sorbed to plastic debris globally (Hirai et al., 2011; Holmes et al., 2012). As such, plastic debris may be associated with a mixture of endocrine-disrupting chemicals and hypotheses regarding risks from exposure of such a mixture to wildlife should be tested.

The ingestion of plastic debris, documented in marine mammals (Tarpley and Marwitz, 1993), sea turtles (Lazar and Gracan, 2011), seabirds (Spear et al., 1995), fish (Davison and Asch, 2011) and invertebrates (Murray and Cowie, 2011), may introduce a "cocktail" of endocrine-disrupting chemicals upon ingestion (Besseling et al., 2012; Browne et al., 2013; Rochman et al., 2013b). In a recent study, we found significantly greater concentrations of several polybrominated diphenyl ethers (PBDEs), the polychlorinated biphenyl congener PCB#28 and the polycyclic aromatic hydrocarbon (PAH) chrysene in fish, Japanese medaka (*Oryzias latipes*), exposed to polyethylene that had been deployed in the marine environment compared to fish exposed to a virgin-polyethylene and a control treatment (Rochman et al., 2013b). PCBs (Crisp et al., 1998), PBDEs (Legler and Brouwer, 2003) and PAHs (Crisp et al., 1998) can all affect the functioning of the endocrine system.

Here, the Japanese medaka from the chronic dietary exposure described above (Rochman et al., 2013b) were used to measure effects related to endocrine disruption. Fish are useful as sensitive indicators of endocrine disrupting chemicals in aquatic habitats, as exposure can result in changes in gonadal growth, gonadal degeneration, sex-specific gene protein induction and the occurrence of intersex (Zhao et al., 2014). Furthermore, plastic debris is detected in the gut contents of several fish species (Hoss and Settle, 1990), including from subtropical gyres (Davison and Asch, 2011) and estuaries (Possatto et al., 2011).

Our objective was to measure the potential for plastic debris to disrupt endocrine function in fish. We used polyethylene because it is the most common plastic debris found in aquatic habitats (Andrady, 2011) and sorbs greater concentrations of organic pollutants than the other common plastics (Rochman et al., 2013c). We hypothesized that we would observe changes in the expression of genes mediated by the estrogen receptor in the liver, including estrogen receptor alpha (ERα), vitellogenin I (Vtg I) and choriogenin H (Chg H). Chemicals that can bind to the estrogen receptor, or antagonize binding of endogenous estrogen can influence transcription of estrogen-dependent genes (Henry et al., 2009; Klinge, 2000) and ultimately affect the function of the reproductive system (Klinge, 2000). Vtg and Chg are transformed into the egg yolk (Crisp et al., 1998) and egg envelope proteins (Murata et al., 1997) respectively in the gonads. Thus, they are good biomarkers of hormonal control in relation to oogenesis (Murata et al., 1997) and are suggested to act as indicators of exposure to estrogenic or anti-estrogenic substances in aquatic environments (Arukwe and Goksoyr, 2003). It is also important to understand responses at different levels of organization, as responses at the lower levels (e.g. molecular, tissue) can be an early-warning predictor of impacts at higher levels (e.g. organism, population) of organization (Hagger et al., 2005; Ankley et al., 2010). As such, we measured and expected to observe responses at the tissue level, using histology, to try and confirm phenotypic changes and/or diagnosis in the gonads. Because fish fed the polyethylene that had been deployed in the marine environment were exposed to a complex mixture that includes estrogenic (e.g. some PCB (Yum et al., 2010) and PBDE (Legler and Brouwer, 2003) congeners) and anti-estrogenic (e.g. PAHs (Goksoyr, 2006) and some PCB (Thomas, 1989) and PBDE (Legler and Brouwer, 2003) congeners) compounds, it is difficult to hypothesize directional changes in the levels of gene expression and any particular changes in histology.

2. Materials and methods

2.1. Test species

Fish from a culture of Japanese medaka (*O. latipes*), maintained in the Aquatic Health Program at UC Davis, were used for this study. Care, maintenance, handling, and sampling followed protocols approved by the UC-Davis Animal Care and Use Committee. Japanese medaka are a good model organism to look for endocrine disruption (Arcand-Hoy and Benson, 1998; Patyna et al., 1999; Koger et al., 2000) as the mechanisms of germ cell development, sex determination and differentiation and reproduction are well known (Grim et al., 2007).

2.2. Diet preparation

The American Chemistry Council donated the virgin polyethylene pre-production pellets. To prepare the marine-plastic treatment, virgin polyethylene pellets (3 mm diameter) were deployed from docks in San Diego Bay, CA for three months (see Rochman et al., 2013c for details). Marine- and virgin-plastic pellets were ground to <0.5 mm, a size range that occurs commonly in the marine environment (Goldstein et al., 2012), using a conical burr grinder for conventional use. The control diet contained 62 g vitamin free casein, 30 g wheat gluten, 54.4 g dextrin, 8 g egg albumin, 10.4 g soy lecithin, 4 g vitamin premix, 6 g mineral premix, 4 g corn oil, 10 g cod liver oil and 7.2 g celufil. Vitamin and mineral mixes were purchased from ICN (Biomedical, Inc., Irvine, CA) and all other ingredients from U.S. Biochemical Corporation (Cleveland, OH). Diets containing plastic (10% plastic by weight) were prepared by substituting 20 g of dextrin with polyethylene. Fish from all treatments were exposed to organic pollutants via the cod liver oil in the control diet (see Supplementary Table 1 for concentrations of targeted PCBs, PAHs and PBDEs in treatment diets and Rochman et al., 2013b for further information). As such, the only difference among the control treatment and the two plastic treatments was the addition of polyethylene with or without sorbed chemicals from San Diego Bay, CA.

2.3. Experimental design

We initiated a 2-month dietary exposure consisting of three treatments: a control (no polyethylene), a virgin-plastic (polyethylene virgin pre-production pellets) and a marine-plastic treatment (polyethylene deployed in San Diego Bay). By using plastics deployed in the marine environment, we were able to establish environmentally relevant concentrations of contaminants on polyethylene (see Fig. S1 for concentrations of targeted PAHs, PCBs and PBDEs on deployed polyethylene and Rochman et al., 2013b for further detail regarding the experimental design, information regarding chemical analyses, and/or data regarding chemical contamination in the plastic, diet and fish).

Adult medaka (7 month old) were randomly placed into nine 38 L tanks (71 fish per tank) on a 16-hour light-cycle. Water flow-rate and temperature were 720 mL/min and 22–25 °C respectively. Water quality (pH: 7.8 ± 0.2, ammonium and nitrite: not detectable, nitrate: 7.9 ± 1 ppm, water hardness: 120 mg L^{-1} $CaCO_3$, electrical conductivity: 400 UMHOS, and alkalinity: 100 mg/$CaCO_3$) was monitored weekly. After one-month acclimation, three tanks were randomly assigned to each treatment ($n = 3$). During the exposure, fish were fed 2% body-weight per day divided into two portions. To assign portions each week, 20 fish per tank were weighed weekly and average body-weight per tank assessed. Medaka were exposed to diets sprinkled at the top of each tank. Plastic in diets dissociated at the surface and thus fish were exposed to plastic similar to the way they are in the wild (i.e. floating in the water column). As such, this translates to 8 ng of plastic per mL of water in the tank. Maximum concentrations reported in the North Pacific Subtropical Gyre are 300 ng/mL (Goldstein et al., 2012), and thus the concentrations of plastic used in this experiment may be considered environmentally relevant. Water-flow to the tanks was stopped during 30-minute feedings to prevent plastic contamination in the recirculating system. Afterward, waste and 30% of the water were siphoned from each tank and floating plastic removed by net. Tanks were cleaned weekly. To prevent cross-contamination, activated charcoal filters were used and changed twice per month. PCBs, PBDEs or PAHs were not detected above a detection limit of 1 μg/L in