Evaluation of Endocrine-Disrupting Chemical Effects Across Multiple Levels of Biological Organization: Integration of Physiology, Behavior, and Population Dynamics in Fishes

Project Scope

Evidence suggests that a variety of endocrine disrupting chemicals (EDCs), such as some heavy metals, pesticides, and polychlorinated biphenyls (PCBs), can impair vertebrate reproduction. The overall aim of this research was to estimate the impacts of several representative EDCs on Atlantic croaker (*Micropogonias undulatus*) populations in marine environments using a suite of reproductive and larval response inputs into an integrated population model.

The main objectives of this research were to:

- Determine the effects of three representative EDCs (i.e., methylmercury, Aroclor 1254, and nonylphenol) on measures of gonadal growth and production;
- Investigate the impacts of the EDCs on gamete maturation, fertilization success, and larval survival;
- Assess the parental transfer of the EDCs to gametes and offspring;
- Determine the effects of parental exposure to the EDCs on several larval behaviors (i.e., level of activity, swimming ability, and response to vibratory and visual stimuli) which indicate the ability of larvae to successfully forage for food and to avoid predators;
- Determine the influence of parental exposure to the EDCs on larval metabolism, growth, and development; and
- Develop a suite of predictive computer models for scaling individual-level effects of EDCs to predictions of fish population responses.

Grant Title and Principal Investigator

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Key Findings

- After exposure of adult Atlantic croaker to representative EDCs, impairment of reproductive function was observed at all reproductive life-history stages examined, including gamete production, gamete maturation, spawning, and early larva survival.
- All of the reproductive and larval stages examined are sensitive to exposure to EDCs; thus, effects at multiple life-history stages need to be taken into account in assessments and modeling of EDC effects on fish populations.
- Methylmercury exposure can impair three critical reproduction stages: gonadal growth, gamete maturation, and fertilization/early egg and larval survival.
- Larval exposure to Aroclor 1254 and methylmercury had negative impacts on many survival parameters, including swimming ability, maximum burst speed, and response to visual stimuli (indicating impaired predator response).
- Three linked population models were developed to simulate population-level effects of low-dose EDC exposure using the measures of reproductive success and larval survival skills measured for EDCs in this study.

Project Period: October 1997 to September 1999.

Project Results and Implications

Effects on Reproductive Function

Aroclor. Groups of Atlantic croaker were fed 0 (controls), 0.20, or 1 mg Aroclor 1245 per kg body weight per day (mg/kg-day) in the diet for two months during the beginning of ovarian and testicular recrudescence (i.e., period of gonadal development and maturation generally following a period of regression). The highest dose of PCB caused significant reductions in gonadal growth in females (23.7 percent, $P<0.01$) and males (26.9 percent, $P<0.004$). The low and high doses of PCB caused 12.5 percent and 25 percent decreases, respectively, in the production of fully mature oocytes in the females, although this was only significant for the high-dose group ($P<0.03$). Decreases in testicular testosterone production in males occurred in the low-dose group, and estrogen production decreased in females treated with both PCB doses.

Relevance to ORD's Multi-Year Research Plan

This project contributes to the second long-term goal of the ORD's MYP: to determine the extent of the impact of endocrine disruptors on humans, wildlife, and the environment.

Impacts of EDCs on Atlantic croaker populations were estimated using a suite of reproductive, physiological, and larval behavioral responses as inputs into an integrated population model. Laboratory studies were conducted to assess the potential impacts of EDC exposure on adult croaker reproductive success. After exposure of adult Atlantic croaker to three EDCs (Aroclor 1254, methylmercury, and nonylphenol), adverse effects were observed at all life-history and reproductive stages examined, including gamete production, gamete maturation, spawning, and early larva survival. Laboratory studies were also conducted to assess the parental transfer of EDCs to gametes and offspring and to determine the effects of parental exposure to the EDCs on several larval behaviors. Larval exposure to Aroclor 1254 and methylmercury had negative impacts on many survival parameters, including swimming ability, maximum burst speed, and response to visual stimuli. Using laboratory findings as inputs, three linked models were developed as a tool for predicting potential population effects on Atlantic croaker associated with EDC exposure.

PCB treatments also produced dose-related impairment of final gamete maturation. Oocyte maturation was decreased from 80 percent in controls to 48 percent at the low dose and 11 percent at the high dose. Ovulation was also significantly decreased at the high PCB concentration. Sperm motility showed a dose-dependent decline after the PCB treatments from 77 percent motile sperm in controls to 54 percent at the low dose and 43 percent at the high dose. Several measures of reproductive success, including embryo survival 12 hours after fertilization (control, low-dose, and high-dose groups: 97, 86, and 66 percent survival, respectively) and hatching success (control, low-dose, and high-dose groups: 76, 61, and 43 percent hatching, respectively) also exhibited dose-dependent reductions. Early survival of the yolk-sac larvae that hatched was reduced in the PCB-treated groups. At 24 hours, the control, low-dose, and high-dose survival rates were 87, 43, and 57 percent, respectively. By 48 hours, the control, low-dose, and high-dose survival rates were 80, 15, and 27 percent, respectively, with both treatment groups significantly different from controls but not from each other. Overall, survival through the entire period from fertilization to 48 hours post hatching was reduced 81 percent in the high-dose group compared with controls.

Exposure of croaker to the PCBs during gonadal recrudescence resulted in a significantly greater concentration of PCBs in the gonads of the females than in the males (females: low dose 5.1 ppm, high dose 34.5 ppm; males: low dose 0.24 ppm, high dose 1.1 ppm. PCB concentrations in spawned eggs ranged from 0.8 ppm to 1.6 ppm.

This portion of the study provides evidence that PCBs in the diet of adult croaker can impair three critical stages of the reproductive cycle: gonadal growth, gamete maturation, and fertilization/early egg and larval survival.

Methylmercury**.** Groups of male and female Atlantic croakers were exposed for 6 weeks to one of three concentrations of methylmercury in the diet (target doses of 0, 0.05, or 0.10 mg/kg bw-day), resulting in muscle concentrations of approximately 0 mg/kg tissue (not detected), 0.5 mg/kg tissue, and 4.0 mg/kg tissue methylmercury, respectively.

Methylmercury treatment resulted in a dose-dependent elevation of plasma testosterone levels in females (low dose: 2.4 fold increase, $P<0.1$; high dose: 4.2 fold increase $P<0.0001$), but did not significantly alter androgen levels in males. Plasma estradiol levels and ovarian growth (as assessed by the gonadosomatic index (GSI)) also were elevated significantly in females exposed to the higher methylmercury dose. Ovarian tissues extracted from females in this higher dose group exhibited significantly increased steroidogenesis when incubated *in vitro*. Interestingly, increases in ovarian steroidogenesis were not accompanied by elevations in basal or luteinizing-hormone-releasing-hormone-(LHRH)-stimulated gonadotropin secretion *in vivo*. In contrast, the gonadotropin response to LHRH was attenuated in the high-dose group. The results suggest that methylmercury at both doses exerts direct effects on teleost ovaries to stimulate androgen production. At the higher dose, methylmercury appeared to exert some estrogen-related effects (e.g., increased circulating estradiol levels, increased ovarian growth).

Both methylmercury treatments significantly impaired final gamete maturation. Sperm motility showed a dose-dependent decline after methylmercury treatments, from 86 percent motile sperm in controls to 62 and 58 percent in the low- and high-dose methylmercury groups respectively ($P < 0.05$). Several measures indicated impaired reproduction after treatment of adults with methylmercury, including reduced hatching success (controls 88.7 percent, low dose 63.2 percent, high dose 71.8 percent, although reductions are not statistically significant). Hatching success in both methylmercury treatment groups was significantly reduced compared with controls. Survival 12 hours after hatching also was decreased (controls 48.1 percent, low dose 27.9 percent, high dose 41.1 percent; low dose significantly different from controls, $P<0.05$). The mean accumulation of methylmercury per egg was 0.04 ng and 0.4 ng in the low- and high-dose groups, respectively.

These studies show that dietary methylmercury can induce androgen production by ovaries and can impair three critical stages of the croaker reproductive cycle: gonadal growth, gamete maturation, and fertilization/hatching and early egg and larval survival. However, dose-related effects of methylmercury were not observed with all the reproductive measures (e.g., hatching success and 12-hr post-hatching survival).

Nonylphenol. Croaker were exposed to three doses of the estrogenic chemical nonylphenol (0, 0.1, or 0.2 mg/kg bw-day) for six weeks during the period of gonadal recrudescence. At the highest dose, ovarian growth was suppressed 39 percent and testicular growth was decreased 21 percent compared to controls. Gonadal production of estradiol in females was significantly decreased (45-52 percent) compared to controls in both nonylphenol-treatment groups, whereas androgen production in males was only decreased (25 percent) in the lower dose group. The decrease in estrogen secretion in females was accompanied by a decline in the production of fully grown oocytes. However, estrogen receptor levels were increased 1.5- and 2.25-fold in the livers of females exposed to low and high doses of nonylphenol, respectively, and this was associated with 364 and 114 percent increases, respectively, in the plasma levels of vitellogenin, the yolk precursor. Nonylphenol did not change ovarian growth and decreased oocyte growth, presumably because endogenous estradiol levels had declined. Thus, nonylphenol produced a variety of estrogen-related responses, including reduced gonadal production of estradiol and large increases in the levels of the hepatic estrogen receptor and vitellogenin production, but without an increase in ovarian and oocyte growth. Nonylphenol treatment also resulted in a dramatic decrease in the subsequent maturation of the oocytes in response to gonadotropin treatment. The number of oocytes completing maturation, as assessed by germinal vesicle breakdown, was 42-43 percent that of controls in both nonylphenol treatment groups. In males, as observed previously in other studies, nonylphenol induced vitellogenin synthesis, but the induction was modest (less than ten-fold) compared to results in some other species. These long-term nonylphenol treatments also produced dose-related decreases in measures of reproductive success; for the control, low-dose, and high-dose groups, hatching success was 77, 60, and 38 percent, respectively, while 24-hr post-hatch survival was 75, 50, and 36 percent, respectively. Thus, at the highest dose, the percentage of fertilized eggs that hatched and survived at least 24 hrs after hatching was around 50 percent that of control fish. Overall, the results with nonylphenol show that this chemical disrupts multiple stages of the reproductive cycle in both males and females in ways consistent with its classification as an estrogenic EDC.

Larval Effects

***Aroclor*.** Two groups of adult Atlantic croaker (8 males and 16 females/group) were administered doses of 0 (control) or 0.4 (dosed) mg Aroclor 1254/kg bw-day in the diet for two weeks during the final stages of gonadal recrudescence. After the two-week exposure stopped, two female fish were removed from the control and dosed groups, were injected with 20 µg/kg bw of LHRH analogue to induce spawning, and were placed in spawning tanks with two spermiating males from the same treatment group. This process was repeated 3 to 12 days apart using two female and two male fish from each group, for a total of five separate spawns per group. Eggs were collected within eight hours of fertilization. For each spawn, some eggs were analyzed for Aroclor 1254 concentrations, while the remaining eggs were allowed to hatch. Two behavioral assays, potential foraging rate (routine swimming speed and activity) and response to a startle (transient vibratory) stimulus, were performed on days 5 (complete yolk absorption), 9 (complete oil globule absorption), and 13 (larva wholly dependent on external food sources) post-hatching. In addition, total length was measured at intervals to determine growth rates.

Fertilized eggs collected from control and dosed adults immediately after spawning contained 0 and 0.66 µg Aroclor 1254/g egg (mg/kg egg), respectively. Growth rate (increase in total length) of dosed larvae was significantly lower than that of control larvae between 2 and 13 days post-hatching, with dosed larvae showing a 4-day delay in attaining the same size as control larvae. Routine swimming speed and activity were similar for control and dosed larvae on days 5, 9, and 13 post-hatching. There was a significant dose × age interaction in the responses of the control and dosed larvae to a vibratory stimulus. The percentage of control larvae responding to the stimulus, and their average and maximum burst speeds, increased with age. In contrast, no such age-related response was found in the dosed larvae. These results indicate that environmentally realistic body burdens of Aroclor 1254 transfer to the eggs and larvae, reducing their growth rates and impairing their startle responses, possibly making the larvae more susceptible to predation.

Methylmercury. Three groups of adult Atlantic croaker (8 males and 16 females/group) were fed a methylmercury-contaminated diet at three different levels (0, 0.05, or 0.1 ppm in the diet daily) for one month. After exposure stopped, the same paradigm was followed as for Aroclor 1254. Two male and two female fish were induced to spawn at 3- to 12-day intervals, methylmercury levels in eggs from part of each spawn were measured, and the remainder were allowed to hatch. Behavioral performance of control and exposed larvae was measured at four developmental stages: end of yolk absorption (“yolk”); end of oil absorption (“oil”); four days after oil absorption (“oil+4”); and 11 days after oil absorption (“oil+11”). The first two stages were chosen to help identify the primary source of methylmercury in the larvae, and the latter two stages were chosen to help determine whether effects were temporary, and therefore likely to be physiological adaptations, or persistent, and therefore possibly indicating permanent developmental effects. Behaviors analyzed included swimming speed and activity and response to startle (visual and vibratory) stimuli, and total length was measured at intervals to determine growth rates.

Maternally-transferred methylmercury induced a range of stage- and dose-dependent effects. Methylmercury levels in the eggs varied among spawns from 0.0004 (control) to 4.6 ng/g (0.0046 mg/kg). Since the feeding rate of each adult croaker varied, methylmercury concentrations in the eggs varied among spawns within a nominal treatment group. The mean methylmercury concentrations in eggs from two successful spawns for the low-dose group were 0.294 and 0.639 ng/g, and the mean methylmercury concentrations in eggs for three successful spawns for the high-dose group were 0.567, 3.874, and 4.574 ng/g. As a result, it was appropriate to assess the effects of methylmercury exposure on the larvae by regressing measures of larval effect against the concentrations of methylmercury in eggs from the same spawn. Concentrations of methylmercury in the three successful spawns of control eggs were below the detection limit (0.001 ng/g), so a value of 0.0004 was assumed for each to allow a logarithmic transformation of the data in a regression analysis.

There was no significant correlation between larval growth and methylmercury exposure in adults. The effect of maternally-derived methylmercury exposure was assessed on routine larval behavioral traits (rate of travel, active swimming speed, and level of activity). For some developmental stages, rate of travel for larvae correlated with methylmercury concentrations in eggs from the same spawn. An inverse correlation between rate of travel and methylmercury concentrations in eggs was statistically significant for the yolk and oil+4 developmental stages. For oil-stage larvae, active swimming speed was negatively correlated with methylmercury concentrations in eggs. There was also a significant negative correlation between measured activity levels of larvae at the yolk and oil+4 stages of development and methylmercury concentrations in eggs from the same spawn.

For the visual startle assay, stage-specific regression analysis showed that responsiveness increased with methylmercury concentration in eggs from the same spawn in the oil+4 stage of development. No other significant relationship with methylmercury concentration in eggs was observed for any of the other variables computed from the visual startle assay. Methylmercury concentration in the eggs did not significantly affect larval response distance or speed of the response, or average and maximum burst speeds. Larvae from all treatment and spawn groups reacted at a similar distance from the stimulus source, and response duration was comparable at all stages.

For the vibratory startle assay, oil+4 was the only stage for which response duration significantly increased with increasing methylmercury concentration in eggs. Response speed decreased with increasing methylmercury in the eggs for the yolk and oil+11 stages. Response distance and responsiveness of larvae to the vibratory stimulus were not significantly affected by methylmercury concentrations in the eggs.

Most of the effects occurred in early development, disappearing in later stages (i.e., by the oil+11 stage) indicating a physiological rather than developmental effect of methylmercury.

Fish Population Modeling

Three linked fish population models were completed: Statistical Model; Individual-Based Model; and Matrix Projection Population Model. The Statistical Model relates the swimming speed and behavioral responses of the fish larvae exposed to EDCs to the probability of escaping a real fish predator. The Individual-Based model tracks 10,000 individual larvae of a cohort through their daily growth and mortality. It predicts daily growth rate from encounters with zooplankton and predicts daily mortality from encounters and capture by individual jellyfish predators (sea nettle and ctenophore) and fish predators. The 10,000 initial larvae are configured with swimming speeds and probabilities of escaping predator attacks characteristic of the control larvae or the contaminant-exposed larvae. It then predicts ocean larval stage growth and mortality for control and contaminant-exposed larvae. The Matrix Projection Population Model simulates 100-years of fish population abundances and age-structures, and is quite detailed to permit realistic simulation of life-stage-specific effects of contaminants. It allows for multiple-time steps (e.g., eggs are daily; adults are annual), multiple spatial regions, one or two spawning cohorts within each year, density-dependence, and stochastic variation. One hundred-year simulations are performed under baseline conditions and assumed contaminant exposure.

The three linked models were developed and illustrated using PCB effects on the Atlantic croaker. Performance of control and PCB-exposed larval fish were evaluated using the Statistical Model. Results from the Statistical Model were input into the Individual-Based Model, which determined stage duration and mortality rate for ocean larvae under control and PCB-exposure conditions. Larval stage durations and mortality rates were converted into values of G (probability of surviving and becoming an estuary larva) or P (probability of surviving and staying an ocean larva). G and P values were used by the Matrix Projection Model to predict long-term population abundance under control and PCB-exposure conditions.

The Matrix Projection Model was configured to simulate croaker population dynamics for 100 years. Six life stages that comprise the first year of life were represented: egg, yolk-sac larva, ocean larva, estuarine larva, early juvenile, and late juvenile. The first year of life was separated into two regions (Chesapeake

Bay in Virginia and estuaries of North Carolina) and two spawning cohorts (fall and spring). As much as possible, field data from Virginia and North Carolina long-term monitoring of young of the year croaker were used to determine baseline growth and mortality rates of each of the six life stages. Finally, additional factors including density-dependent survival of the late juvenile stage and inter-annual variation in mortality rates of eggs and yolk-sac larvae were incorporated into the model. The culmination of the three linked models gave 100-year simulations of croaker population abundances under baseline conditions and assuming croakers were exposed to low-dose PCB concentrations.

For illustrative purposes, exposure was assumed to occur in North Carolina estuaries. The matrix simulation of PCB effects resulted in the following: higher egg mortality; lower spawning fecundity; smaller G and P values of ocean larva due to slower swim speed and increased capture by predators; PCBs in female croaker are eliminated after first spawning. The matrix simulation resulted in total population effects as illustrated in Figure 1.

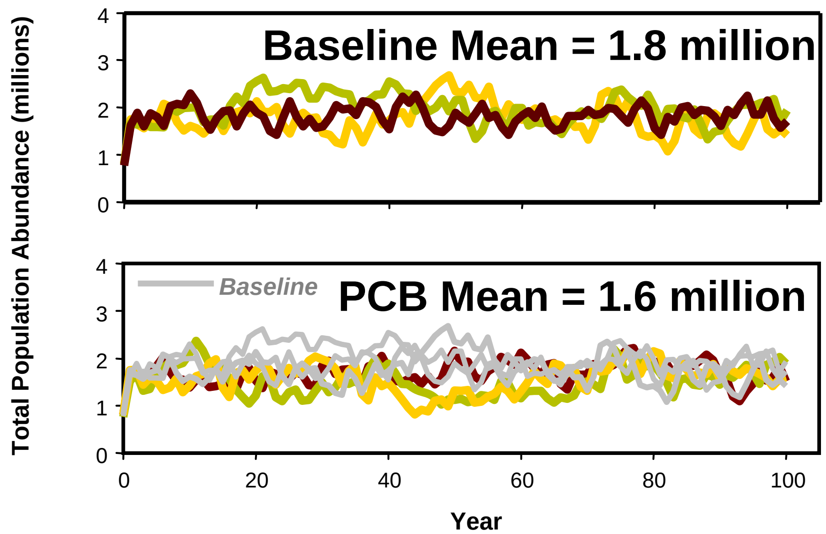

Figure 1. Matrix model simulations of croaker population abundance for 100 years under baseline conditions and PCB exposure with effects on first-time spawning. Three replicate simulations are shown for each condition.

It is important to note that the predicted effects associated with PCB exposure shown by the models are not meant to represent what actually happened to croakers. Rather, the results demonstrate that the approach of coupled laboratory and linked models can be used to predict population effects of EDC exposure. The models can be used by resource protection agencies to predict effects on reproductive success, recruitment, and population abundance of fish from changes in early warning indicators of endocrine disruption, including reduced fecundity, increased egg mortality, and sub-lethal behavioral responses.

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NCER Project Abstract and Reports:
http://cfpub2.epa.gov/ncer_abstracts/index.cfm/fuseaction/display.abstractDetail/abstract/450/report/0