Prelude to Intersex in Fish

Identifying a Sensitive Period for Feminization

Field studies have shown a high occurrence of intersex (the presence of both male and female characteristics) and ovotestis (the presence of eggs in the testis) in wild populations of a fish known as roach (*Rutilus rutilus*) in rivers in the United Kingdom that are downstream from wastewater treatment plants. Furthermore, studies have demonstrated that intersex males are less fertile, which may have population-level effects. However, to date, scientists have been unable to induce intersex in male fish with controlled exposures to wastewater effluents. A study conducted at The University of Exeter now shows that the sensitive period for feminization of the reproductive duct—in which the male testis forms an ovary-like cavity—may occur earlier than previously thought, and raises new questions about the conditions that lead to actual germ cell disruption [***EHP*** **113:1299–1307**].

Many questions persist about the causes of and the most vulnerable life stages for various types of sexual effects induced by estrogenic chemicals in wastewater effluent. In this study, the researchers collected two different U.K. wastewater effluents and exposed wild roach at two life stages: during early life and development of the gonads (from fertilization up to 300 days post-hatch) and as adults producing germ cells following annual spawning. These adults included one group of fish that had been raised in clean water and another that had hatched and grown to maturity in the wild.

Both effluents induced synthesis of vitellogenin (an estrogen-dependent yolk precursor and biomarker of estrogen exposure) at both life stages, with the extent of this induction correlating with the steroid estrogen content of the effluent. Previous studies have demonstrated that feminization of the sperm duct to form an ovary-like cavity occurs when exposure to effluent comes during the time of sexual differentiation, which in roach occurs from 50 to 150 days post-hatch. This study showed alteration of the sperm duct with an exposure earlier in life, from fertilization to 60 days post-hatch, before any signs of sexual development appear. The alteration, furthermore, was permanent, persisting even after 240 days' maintenance in clean water after exposure.

However, no ovotestis was observed in any of the juvenile fish. There was also no evidence of ovotestis in post-spawning adult male roach raised in a clean environment and subsequently exposed to effluent. There was evidence that the wild males had previously been exposed to estrogenic stimuli, as some of males had ovotestis when the study began. The severity of this condition increased slightly during the study period, but the increase occurred across both exposed and control fish and thus appeared unrelated to the study effluent exposure.

The authors suggest possible explanations that need further study—one is that ovotestis is induced only by effluents with greater levels of estrogenic chemicals than those used in the study. The researchers evaluated the effluents for content of two chemicals previously implicated in causing intersex—steroidal estrogens and alkylphenols—and found that these levels were similar to concentrations reported in wastewater effluents in the United Kingdom and worldwide. They emphasize that chemical content and interactions ideally should be taken into account when trying to determine the conditions that lead to sexual effects.

The results of these studies raise the possibility that ovotestis may be a result either of longevity of exposure or of programming in early life that manifests itself as fish mature sexually. Previous findings from the authors support this idea by showing that the severity of intersex increases with age. The authors are further exploring these possibilities now with a laboratory study of roach that includes an environmentally relevant estrogen exposure of two years' duration. **–Angela Spivey**

Shift in Sex Ratio

Male Numbers Sink in Great Lakes Community

Sex ratio—the proportion of male to female live births—can be an important indicator of the reproductive health of a population, whether animal or human. This figure is typically fairly constant. For example, the worldwide human sex ratio ranges from 102 to 108 male births for every 100 female births; in other words, male babies make up about 50.4–51.9% of live births worldwide. Now, however, investigators have documented a significant skewing of the human sex ratio in a population located in a heavily polluted Great Lakes area [***EHP*** **113:1295–1298**].

In response to concerns about a shifting sex ratio among members of the Aamjiwnaang First Nation community near Sarnia, Ontario, a team of Canadian researchers examined birth records for the group from the years 1984–2003 as part of a broader community-based investigation. The researchers discovered that, as community members had suspected, there had been a significant and precipitous shift in the sex ratio.

Chemical culprit? The Sarnia–Lambton area in Ontario is home to Chemical Valley as well as the Aamjiwnaang First Nation community, which has experienced a significant skewing of the ratio of male to female babies born in recent years, leading some to question whether environmental exposures may be to blame.

Dennis MacDonald/Alamy

The expected sex ratio in Canada is 51.2% male babies to 48.8%

female babies. For the period 1984–1992, that ratio held fairly constant among this community. In the period 1993–2003, however, male babies made up only 41.2% of live births. The five-year period from 1999 to 2003 showed an even sharper decline, with male babies making up 34.8% of live births. According to the researchers, although there is normal variation in sex ratio within populations, the deviation in this case appears to be outside the normal range.

Although there is as yet no direct evidence linking this human sex ratio decline to environmental exposures, the circumstantial evidence suggests there may be a connection. The Chippewas of the Aamjiwnaang reserve reside within the St. Clair River Area of Concern, situated immediately adjacent to several large petrochemical, polymer, and chemical industrial plants. The area is one of Canada's largest concentrations of industry. Prior soil and sediment assessment has shown that the reserve land is heavily contaminated with pollutants such as polychlorinated biphenyls, polyaromatic hydrocarbons, hexachlorobenzene, mirex, a variety of potentially toxic metals, volatile organic compounds, phthalates, and dioxins; many of these are known or suspected endocrine disruptors.

As the investigators point out, past studies have documented reproductive outcomes in wildlife populations within the same region, including reduced hatching success, altered sexual development, and changes in sex ratios. Scientific suspicion has long been focused on environmental endocrine disruptor exposures as the root cause of these effects.

The authors acknowledge that there are many other potential factors that could influence the declining sex ratio they describe. But the combination of close proximity to industrial facilities emitting known endocrine-disrupting chemicals and the documented adverse reproductive outcomes in wildlife populations in the region leads them to conclude that further investigations are warranted into the types and routes of chemical exposures—via air, water, food, soil, and sediment—for this population. A community health survey designed to explore health concerns among residents of the reserve is in progress, including information on potential covariates that may influence the sex ratio, such as parental age or smoking. **–Ernie Hood**

Lead in Cocoa Products

Where Does Contamination Come From?

Manufactured cocoa products frequently have higher lead concentrations than other foods, even though cocoa beans, the main ingredient, have some of the lowest reported lead levels for any natural food. In 2001 the Codex Alimentarius Commission, an international body based in Rome, proposed reducing the maximum permissible level of lead in cocoa products by half, to 100 nanograms per gram (ng/g) for cocoa butter and 1,000 ng/g for cocoa powder. At a March 2002 meeting in West Africa, where most of the world's cocoa supply originates, producers agreed that to reduce lead in their products, they needed research to identify the source of contamination. Now a U.S.–Nigerian research team has uncovered some of the first clues about where the lead is coming from [*EHP* **113:1344–1348**].

Lead contamination of candies has been recognized as a problem since 1820, when a British study found the poison widespread in London confectionary products. In recent years, documented lead content in candy has ranged from a mean concentration of 21 ng/g in milk chocolate bars in an Australian study to an average of 1,920 ng/g in chocolates seen in research in India. In Nigeria, a 1999 study found an average of 310 ng/g lead in cocoa powders. (For comparison, the mean U.S. lead concentration for apples is 20 ng/g, 200 ng/g for dry table wine, and 100 ng/g for canned pineapple.) Lead is known to cause anemia, muscle weakness, and brain damage, with children particularly susceptible to effects.

PhotoEuphoria/Stockphoto

Searching for the golden ticket. Cocoa beans are naturally low in lead, but cocoa products frequently are not. Now researchers are following new clues to identify the source of the contamination.

In the current study, the researchers studied the lead isotopic compositions of cocoa beans and shells from six farms in Nigeria's top three producing states to determine if soil or farm sources might be the cause of lead contamination. The team took bean and sediment samples and homogenized them to make composites for soil, beans, and cocoa bean shells for each farm. They analyzed lead concentrations using high-resolution inductively coupled plasma mass spectrometry to make preliminary isotopic measurements, followed by thermal ionization mass spectrometry measurements.

The lead concentrations for cocoa beans ranged from less than 0.103 to 1.78 ng/g, averaging 0.512 ng/g—among the lowest lead concentrations reported for any food. The average concentrations found in the cocoa bean shells, however, was about 320-fold higher (160 ng/g). Soils showed a range of isotopic compositions overlapping those of the shells.

The cocoa bean shells all had an extremely similar isotopic composition, indicating a singular source of contamination, perhaps leaded gasoline. The authors conclude that although the soil may have caused a small degree of the contamination, the narrower range of isotopic composition in the shells suggested the more singular source of contamination was the true culprit. According to the paper, cocoa bean shells are known to be very effective at removing lead from solutions. So, although they provide excellent protection of the bean inside, the shells may also serve to contaminate the cocoa beans during fermentation or drying.

Eating for two, thoughtfully. Despite the threat posed by high mercury levels in certain types of fish, new findings suggest a healthy prenatal diet most likely should include some low-mercury seafood.

Digital Vision

The team also compared the cocoa beans with finished cocoa products and found much higher lead concentrations and greater variability in the isotopic composition among the finished products. They therefore deduced that most of the contamination occurred after the cocoa left the farm stage.

The researchers conclude that while cocoa bean shells may be one source of lead, most contamination occurs during shipping or processing of the beans and in manufacturing. Further research on those stages of the process will help to isolate the source. **–David A. Taylor**

Moms and Mercury

Fine-Tuning Fish Consumption During Pregnancy

Due to ongoing concerns that high mercury intake via fish can cause adverse neurologic effects in the developing fetus, the U.S. Food and Drug Administration now recommends that expectant mothers should limit their consumption of fish to two or fewer meals per week. But pregnant women shouldn't throw the baby out with the bathwater. A new study by a group of Harvard researchers suggests that this advice, which could result in many pregnant women eliminating fish from their diets altogether, may be denying some babies substantial neurocognitive benefits gained from important nutrients found in fish, such as n-3 polyunsaturated fatty acids [*EHP* **113:1376–1380**].

The scientists sought to determine whether fish consumption during pregnancy is harmful or beneficial to fetal brain development. To do this, they examined associations of maternal fish consumption during pregnancy, maternal hair mercury levels (a sensitive marker of organic mercury body burden) at delivery, and infant cognition at age 6 months. Study subjects were 135 mother–infant pairs who participated in Project Viva, a prospective pregnancy and child health cohort study in eastern Massachusetts.

The mothers completed questionnaires about fish consumption during their second trimester. That period of time was used to best coordinate temporally with the mercury exposure reflected in maternal hair samples, which were taken at delivery. The questions concerned how much and what categories of fish (canned tuna, dark meat, light meat, shellfish) the women ate.

Mothers consumed an average of 1.2 servings of combined fish categories per week. Their mean hair mercury level was 0.55 part per million (ppm), with 10% of the samples higher than 1.2 ppm, the current U.S. reference dose. Fish consumption was directly correlated with hair mercury levels.

Infant cognition was assessed using a test called visual recognition memory (VRM). In the VRM test, which has been shown to correlate with later IQ, the child is first shown two identical photographs of an infant's face, side by side, at a standardized distance. Then, one of the photos is replaced with a photo of another infant's face. By tracking the percentage of time the baby looks at each photo, a novelty preference score is derived, reflecting the infant's ability to encode a stimulus into memory, to recognize that stimulus, and to look preferentially at a novel stimulus.

Mean VRM score among the children was 59.8, with a range of 10.9–92.5. After accounting for characteristics such as maternal age and education level, higher fish intake was found to be associated with higher infant cognition, especially after adjusting for mercury levels, which had a dose-dependent negative impact on the infants' cognition. For each additional weekly serving of fish, the infants' VRM score was 4.0 points higher. Conversely, the researchers found that an increase of 1 ppm in hair mercury was associated with a decrement in VRM score of 7.5 points. The babies with the highest cognition scores were from mothers who had eaten more than two weekly fish servings but had mercury levels of 1.2 ppm or less.

Although the results may seem contradictory, the authors suggest that the most cognitive benefit is derived by mothers eating fish types with the combination of relatively little mercury and high amounts of beneficial nutrients. However, since the study assessed maternal fish consumption of four broad categories, there is no information presented on associations with specific types of fish. The researchers say that future studies could incorporate more detailed dietary information to help pregnant women make informed decisions about which fish meals are better or worse for their children's cognition.

Ultimately, the message behind these findings is that pregnant women should continue to eat fish, but should try to choose varieties known to be low in mercury and high in nutrients, such as canned light tuna and sardines. Finding the most appropriate balance between risk and benefit may be challenging in this situation, but given the strong associations found in the current study, making the right decisions about which fish to eat during pregnancy, and how often, may be even more important than previously suspected. **–Ernie Hood**