



Abnormal production of vitellogenin in marine fish from urban embayments in Puget Sound, Washington, USA.

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Vitellogenin (Vtg) is a yolk protein produced in the liver of oviparous animals in response to estrogens. Its synthesis is normally observed only in sexually mature females with developing eggs, however males can synthesize Vtg when exposed to exogenous estradiol or to substances that mimic estrogens. Thus, the abnormal production of Vtg in male animals can be used as a biomarker for exposure to environmental estrogens. The objective of this study was to assess whether estrogenic compounds are present in the waters or sediments of Puget Sound at levels high enough to induce vitellogenesis in benthic marine fish. Plasma of adult male English sole collected from urban and non-urban embayments throughout Puget Sound was analyzed for the presence of Vtg utilizing a quantitative ELISA for this species. Among the stations sampled were areas with bottom sediments known to have elevated levels of contaminants with suspected estrogenic activity, such as certain DDTs and PCB congeners. Significant levels of Vtg were detected in fish from several urban sites, with one site near a combined sewage overflow (CSO) exhibiting the greatest percentage (>50%) of fish affected. These findings suggest that estrogenic substances may be affecting the health of marine fish in Puget Sound.

INTRODUCTION

Environmental chemicals, both synthetic and natural, that disrupt normal endocrine function in humans and wildlife generate their effects through various mechanisms. Adverse effects can be generated either through direct interaction of endocrine disrupting compounds (EDCs) with steroid receptors to block or imitate the action of the steroids, or by indirect modifications of steroid and receptor levels through alterations of their synthesis, distribution or metabolism.

Xenoestrogens, compounds that mimic the female hormone estrogen, are one group of EDCs that have received an enormous amount of attention because they can interfere with estrogen responses crucial for normal development and reproduction. As a result, exposure to these compounds could potentially cause adverse effects in fish at the individual and population level (Ankley et al., 1998).

To address these concerns we have developed an enzyme-linked immunoassay (ELISA) for plasmatic vitellogenin (Vtg) in English sole (Lomax et al. 1998) to assess the estrogenic potential of compounds present in the aquatic environment. Vitellogenin is a sex specific protein synthesized in the liver of oviparous animals in response to circulating estrogens. Although vitellogenesis normally occurs only in sexually mature females, it can be induced in males by exposure to estrogens or to substances that mimic estrogen. Thus, its abnormal production in males can be used as a biomarker for exposure to xeno-estrogens. Although uncommon, several cases of Vtg production in wild male fish (both marine and freshwater) have been documented. Two notable examples are rainbow trout in Great Britain (Purdom et al. 1994; Harries et al. 1996) and flounder in Japan (Hashimoto et al. 2000).

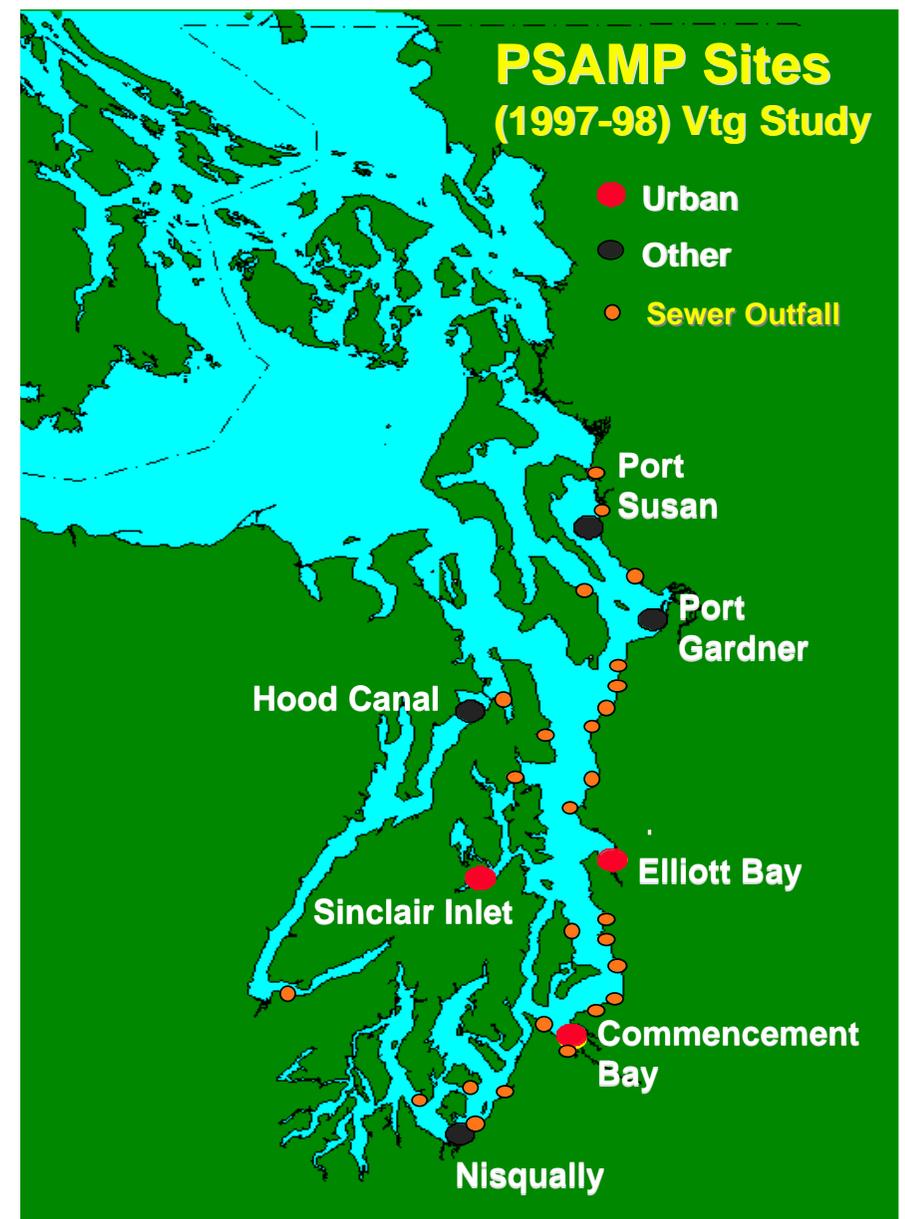
English sole is a primary sentinel species in marine environmental monitoring programs in the United States and Canada. Beginning in 1997 we have been collecting blood plasma from male English sole as part of the Puget Sound Ambient Monitoring Program (PSAMP). Our objective in this pilot study was to discover if Vtg induction is occurring in male English sole at various sites in Puget Sound. What we discovered was surprising, and suggests that we should be concerned about estrogenic substances in some areas of Puget Sound.

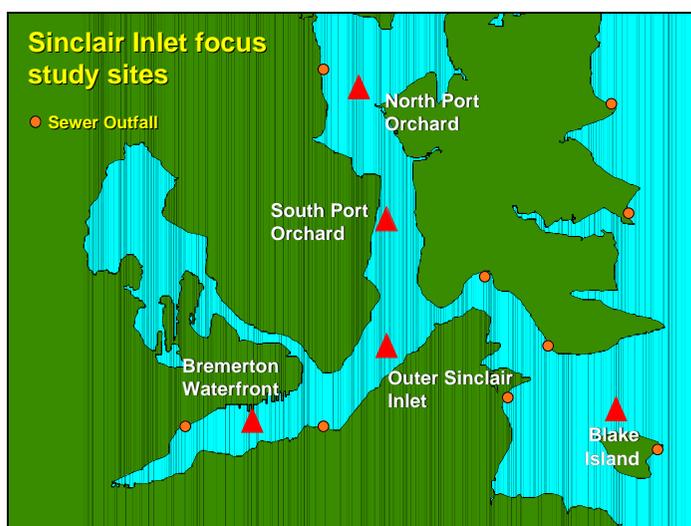
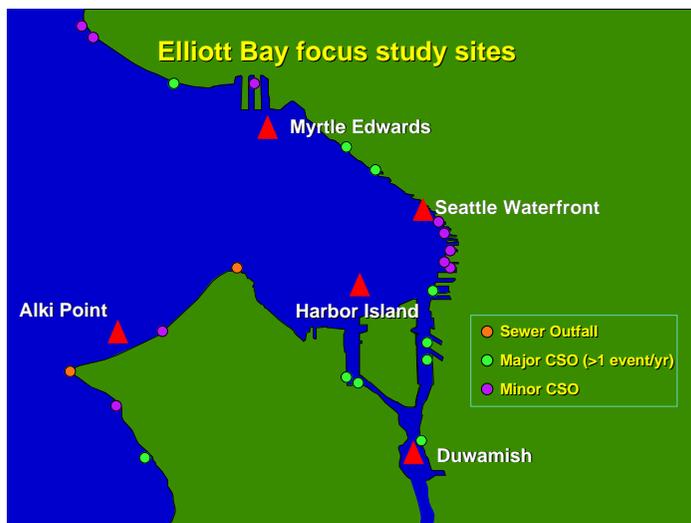
METHODS

Blood was withdrawn from the caudal vein of adult male English sole using heparinized syringes. After centrifugation, plasma was collected and treated with the protease inhibitor phenyl methyl sulfonyl sulfate (PMSF, 1mM in plasma).

Plasma of English sole between 3-15 years of age (determined by otolith) was assayed by ELISA. The number of samples analyzed from each station ranged from a low of 8 at Harbor Island to a high of 17 at the Sinclair Inlet site. Most of the sites had samples sizes of 11-14 fish.

Levels of contaminant exposure in fish were determined by analysis of PCB and DDT concentrations in muscle tissue and bile FACs (fluorescent aromatic compounds).





SUMMARY OF RESULTS

Blood plasma of male English sole from 15 sites in Puget Sound was analyzed for the presence of Vtg. Fish sampled from many urban sites had quantifiable levels of Vtg, concentrations ranged between 2-17ug Vtg per ml of plasma.

The station at Myrtle Edwards showed the highest percentage (8 of 12) of fish with Vtg. This station is in close proximity to 3 major CSOs, which are potential sources of environmental estrogens. Two other stations (Commencement Bay and Harbor Island) with more than 30% of the animals exhibiting Vtg are located in highly industrialized areas with CSOs and sewage treatment plant (STP) outfalls nearby.

The presence of Vtg was also discovered in fish from two non-urban stations, Port Susan and Blake Island. Vtg was not detected at the other non-urban stations in Hood Canal and Nisqually.

Muscle PCB and DDT concentrations and Bile FACs results show that English sole from urban stations near Seattle, Tacoma, Bremerton and Everett have the highest exposure to contaminants. However, there does not appear to be a correlation between exposure to these chemicals and Vtg induction in male fish.

Measures such as fecal coliform, coprostanol and caffeine to better assess the influence of sewage discharges at these stations were not performed. In addition, other chemicals with known estrogenic activity such as

alkylphenols were not measured in sediments or in fish. However, plans are being developed to collect these data in the future.

CONCLUSIONS

Our discovery of male English sole with Vtg indicates that we should be concerned about the presence of estrogen-like substances in Puget Sound waterways, particularly near urban and industrial centers.

The stations with the highest percentage of fish affected are usually near CSO and/or STP discharges. A CSO is where a combination of stormwater and untreated sewage overflows from pipes directly into surface water during periods of heavy rainfall. A major CSO is where this occurs more than once per year.

The Myrtle Edwards station had the highest percentage of fish exhibiting Vtg production and is near several major CSOs. In addition, the prevailing northerly current in Elliott Bay would likely move contaminants closer to the site.

The discovery of vitellogenic fish from Port Susan was a surprise. However, in addition to two small STP discharges in the area, the Port Susan area is known to have failing septic systems and also receives agricultural sources of pollutants. Port Susan is also a relatively confined embayment with minimal currents that do not readily disperse pollutants from the area.

The results of this pilot study show that vitellogenesis has been induced in wild male English sole in Puget Sound. Additional sample analyses are being conducted to determine the extent and severity of the problem.

ACKNOWLEDGEMENTS

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