Fish & estrogenic endocrine disrupting chemicals

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Atrazine

RESTRICTED USE PESTICIDE
(GROUND AND SURFACE WATER CONCERNS)
FOR RETAIL SALE TO AND USE ONLY BY CERTIFIED APPLICATORS OR PERSONS UNDER THEIR DIRECT SUPERVISION, AND ONLY FOR THOSE USES COVERED BY THE CERTIFIED APPLICATOR'S CERTIFICATION.
THIS PRODUCT IS A RESTRICTED-USE HERBICIDE DUE TO GROUND AND SURFACE WATER CONCERNS. USERS MUST READ AND FOLLOW ALL PRECAUTIONARY STATEMENTS AND INSTRUCTIONS FOR USE IN ORDER TO MINIMIZE POTENTIAL FOR ATRAZINE TO REACH GROUND AND SURFACE WATER.

- Use is highly regulated in Vermont. All products are state or federally restricted for use only by certified applicators.
- Only allowed for agricultural uses.
- *All* atrazine product labels have additional environmental restrictions for ground & surface water protection. (*e.g.,* cannot be used with 200' of lake or reservoir)
- In addition to product label restrictions, the VT RAPs have field buffer requirements (to water) to prevent soil erosion and runoff.

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- Atrazine half-life in soil (approx. 150d); relatively stable in water (does not break down)
- Bioconcentration in aquatic organisms is low to moderate, therefore not likely to accumulate
- On the first list of pesticides to be reviewed for endocrine disruption by US EPA
- Weight-of-evidence report was released by the US EPA in June 2015 based on required endocrine disruption studies.

The Endocrine Disruption Studies Process by the US EPA

EPA integrates and interprets all of the endocrine disruptor-related hazard data in consideration with other available hazard information:

- performs a *hazard assessment* to identify potential health effects that may occur from different types of chemical exposure;
- conducts an *exposure assessment* by looking at the amount of chemical to which wildlife or humans are likely to be exposed; and
- performs the *risk assessment* through which EPA integrates the information about the potential harm of a chemical with the likelihood that someone or something will be exposed.

Based on the endocrine results from the Tier 1 assessment, additional studies for atrazine were not recommended because it was unlikely to impact the US EPA's human or ecological risk assessment.

Endocrine disrupting chemicals are ubiquitous in our environment

To name a few…

- DDT
- PCBs
- Dioxins
- Plasticizers (*e.g.,* bisphenol-a)
- Flame retardants
- Phytoestrogens*
- Some pesticides (*e.g.,* Atrazine, TFM)
- Pharmaceuticals (synthetic estrogen)
- Personal care products

Plant Sources of Phytoestrogens

Common Food Sources

Often Includes Seeds & Oils

Alfalfa
Anise
Apples
Barley
Beans
Clover
Coffee
Carrots
Fennel
Flax
Ginseng
Hops
Lentils
Licorice
Mint
Nuts
Oats
Pomegranates
Rice
Sesame
Soybeans
Wheat
Yams

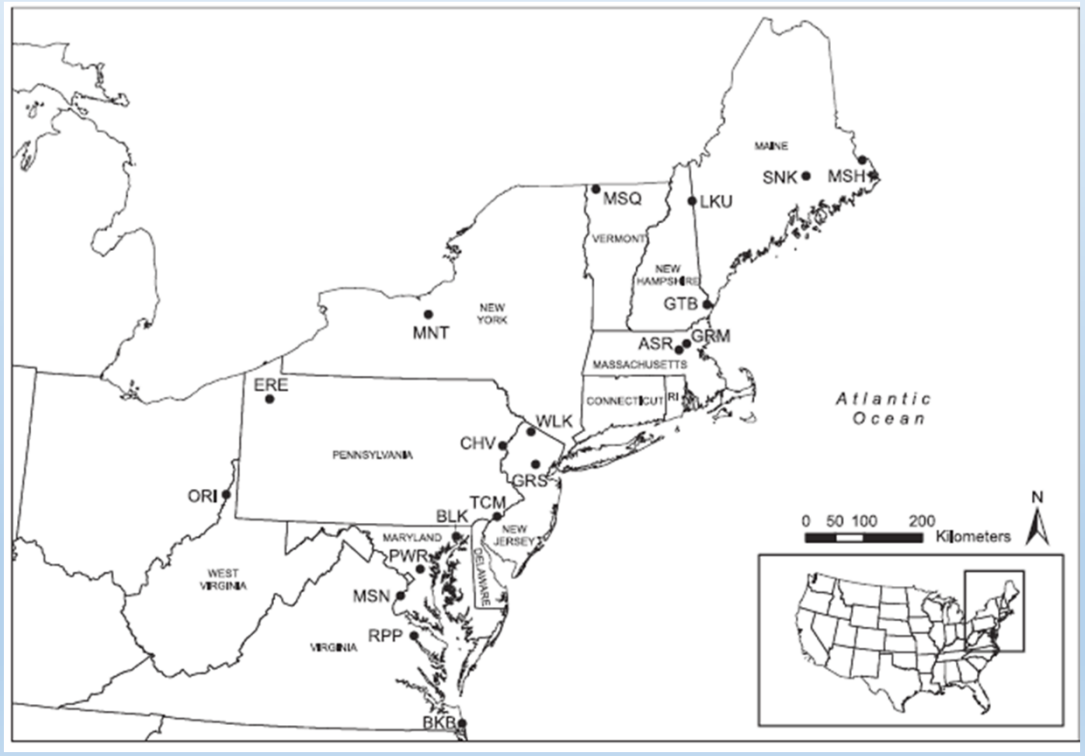
Sources that have been associated with EEDCs in surface waters

- Wastewater treatment facilities (permitted discharges)
- Pulp & paper mills
- Agriculture land use
- Other polluting activities by humans

  (spills, unpermitted discharges, septic systems)

THE STUDY

A reconnaissance study: a few samples from a lot of different places in 2008->2010



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Evidence of estrogenic endocrine disruption in smallmouth and largemouth bass inhabiting Northeast U.S. national wildlife refuge waters: A reconnaissance study

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ABSTRACT

Intersex as the manifestation of testicular oocytes (TO) in male gonochoristic fishes has been used as an indicator of estrogenic exposure. Here we evaluated largemouth bass (*Micropterus salmoides*) or smallmouth bass (*Micropterus dolomieu*) form 19 National Wildlife Refuges (NWRs) in the Northeast U.S. inhabiting waters on or near NWR lands for evidence of estrogenic endocrine disruption. Waterbodies sampled included rivers, lakes, impoundments, ponds, and reservoirs. Here we focus on evidence of endocrine disruption in male bass evidenced by gonad histopathology including intersex or abnormal plasma vitellogenin (Vtg) concentrations. During the fall seasons of 2008–2010, we collected male smallmouth bass ($n=118$) from 12 sites and largemouth bass ($n=173$) from 27 sites. Intersex in male smallmouth bass was observed at all sites and ranged from 60% to 100%; in male largemouth bass the range was 0–100%. Estrogenicity, as measured using a bioluminescent yeast reporter, was detected above the probable no effects concentration (0.73 ng/L) in ambient water samples from 79% of the NWR sites. Additionally, the presence of androgen receptor and glucocorticoid receptor ligands were noted as measured via novel nuclear receptor translocation assays. Mean plasma Vtg was elevated (> 0.2 mg/ml) in male smallmouth bass at four sites and in male largemouth bass at one site. This is the first reconnaissance survey of this scope conducted on US National Wildlife Refuges. The baseline data collected here provide a necessary benchmark for future monitoring and justify more comprehensive NWR-specific studies.

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1. Introduction

Since the early 1980s, endocrine disruption in humans, fish, and wildlife has been recognized as a global environmental concern (Bernanke and Kohler, 2009; Hotchkiss et al., 2008; Kortenkamp, 2007; Lathers, 2002; Rhomberg et al., 2012). Endocrine disrupting compounds (EDCs) are collectively grouped into the category of emerging contaminants (ECs) or chemicals of emerging concern (CEC). They run the gamut of natural and synthetic chemicals, but also include biogenic plant and animal hormones. To date, estrogenic endocrine disrupting chemicals (EEDCs) have received considerable attention due to the perceived risk they pose to vertebrate reproduction and better established biomarkers. In aquatic ecosystems, two dominant sources of EEDCs are agricultural production such as animal feeding operations (AFOs) and crop fields applied with manures and herbicides (Battaglin et al., 2009; Blazer et al., 2012; Ciparis et al., 2012; Gall et al., 2011; Orlando et al., 2003) and wastewater treatment plant (WWTP) effluents (Kusk et al., 2011; Sarmah et al., 2006; Vajda et al., 2008). As a result, aquatic vertebrates including fish can potentially be

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Looked at:

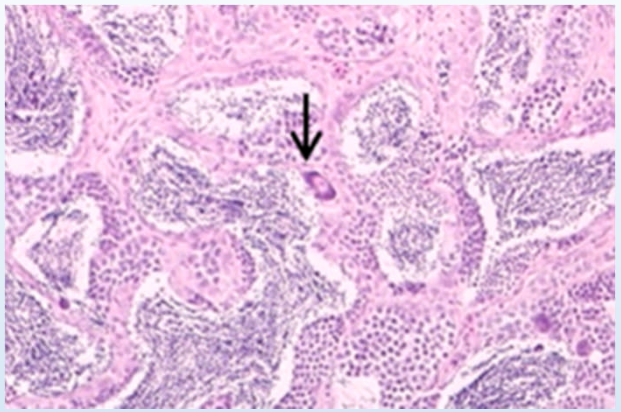
- Fish for biomarkers of exposure to estrogenic endocrine disrupting chemicals (EEDCs).
  - Intersex: Presence (Yes/No) and Severity (Scale of 1-4)
  - Vitellogenin: protein in blood plasma, created in liver and moved to plasma-associated with immunity and egg yolk precursor
  - GSI: (weight of gonads/weight of body weight) x 100
  - Other factors, age, length
- "Estrogenicity" in wastewater effluent samples. Estrogenic activity was detected in 100% of WWTP effluents.

When possible, attempted to pair impacted and non-impacted sites. i.e., one downstream from a WWTP, one upstream with a dam in between

Did *not* look at:

- Water samples were *not* tested for specific EEDCs.
- No chemical analyses.
- No correlation to atrazine.
- No in-depth analysis of land uses near wildlife refuges.

Intersex in fish



Taken from *Iwanowicz et al 2016*

- Presence of cells (oocytes) in the testes
- Results from endocrine disrupting chemicals
  - Also occurs naturally in about 10-14% of fish or SMB? (*Iwanowicz et al 2016*)
- Other causes of intersex in fish (*Schwarz et al 2006*)
  - Senescence
  - Genetic abnormalities
  - Radiation
  - Temperature changes
  - Hybridization

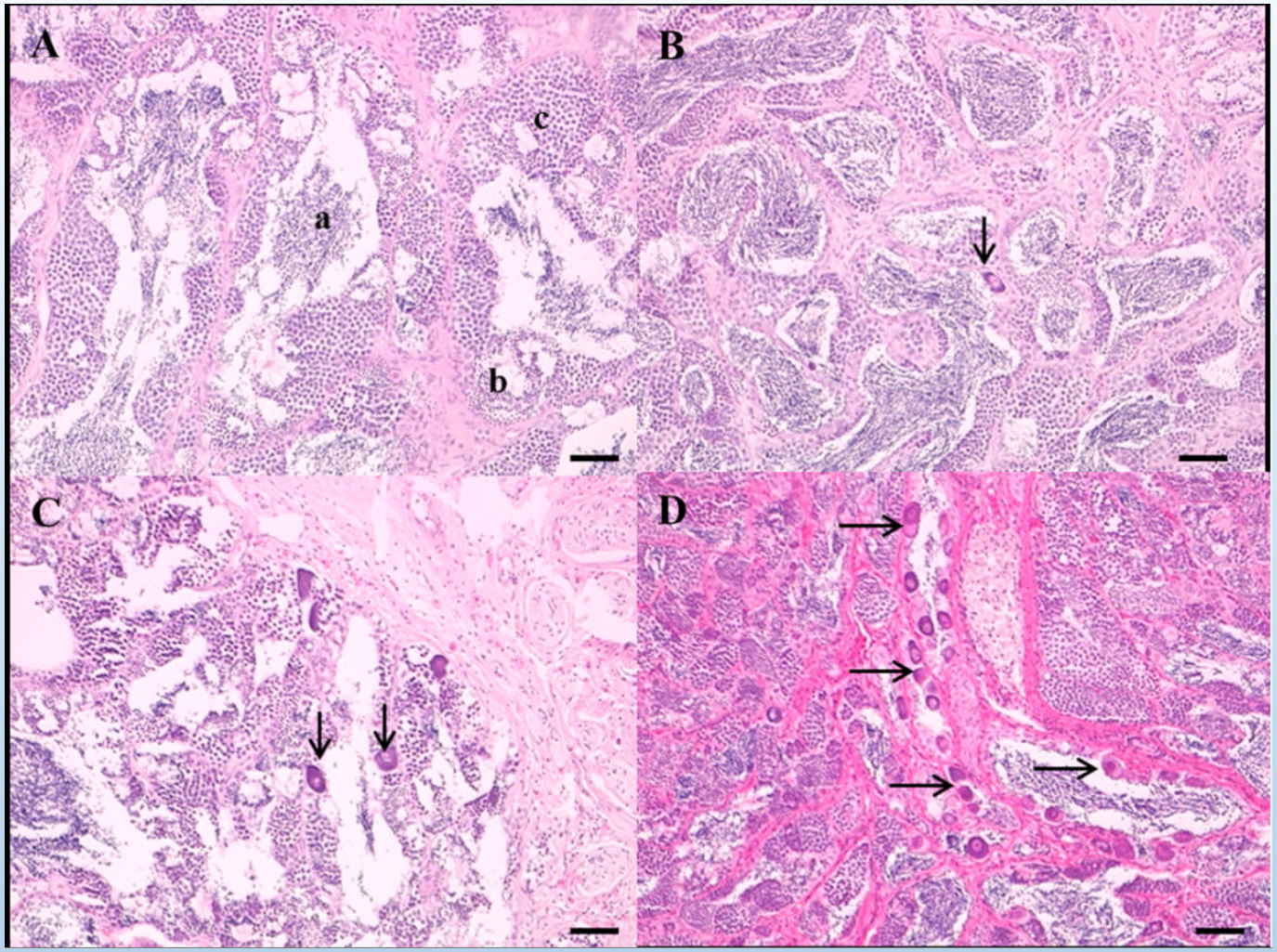
Intersex Severity (ratings: 1-4)

(1) Single oocyte per field of view (B)

(2) Multifocal, more than one oocyte per field of view, but oocytes not closely associated (C)

(3) Cluster groups(2–5) of oocytes closely associated with each other And

(4) Zonal, multiple clusters or more than five closely associated oocytes

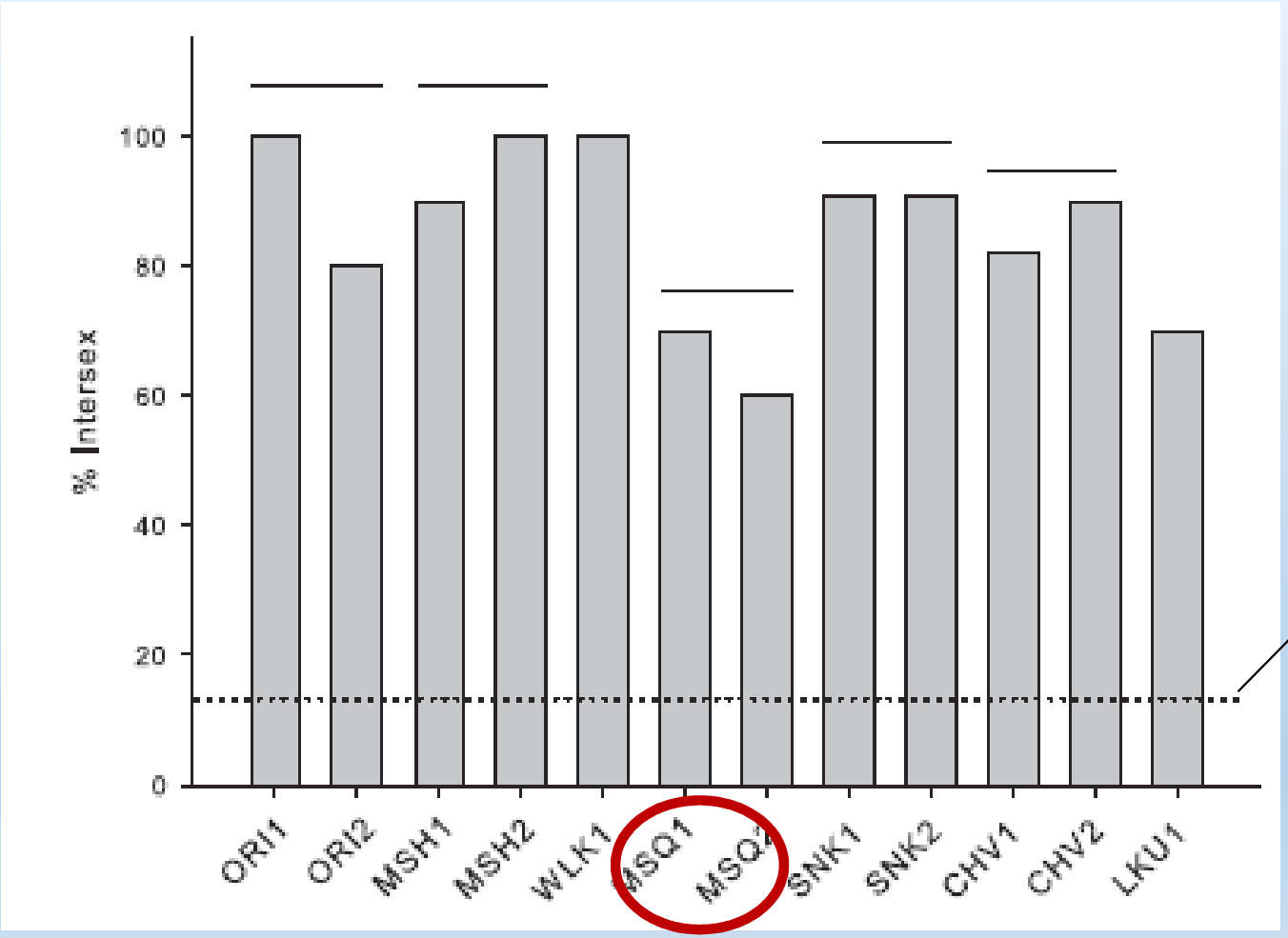


Taken from *Iwanowicz et al 2016*


MSQ3
Highgate Springs
Highgate
West Swanton
Highgate
MSQ2
WWTF
Swanton
MSQ1
Missisquoi River
Study Sites
Maquam
© 2016 Google
Imagery Date: 5/13/2015 lat 44.940325° lon -73.108022° elev

BL1

Results: Smallmouth Bass & Percent Intersex



Expected natural level of intersex in male SMB

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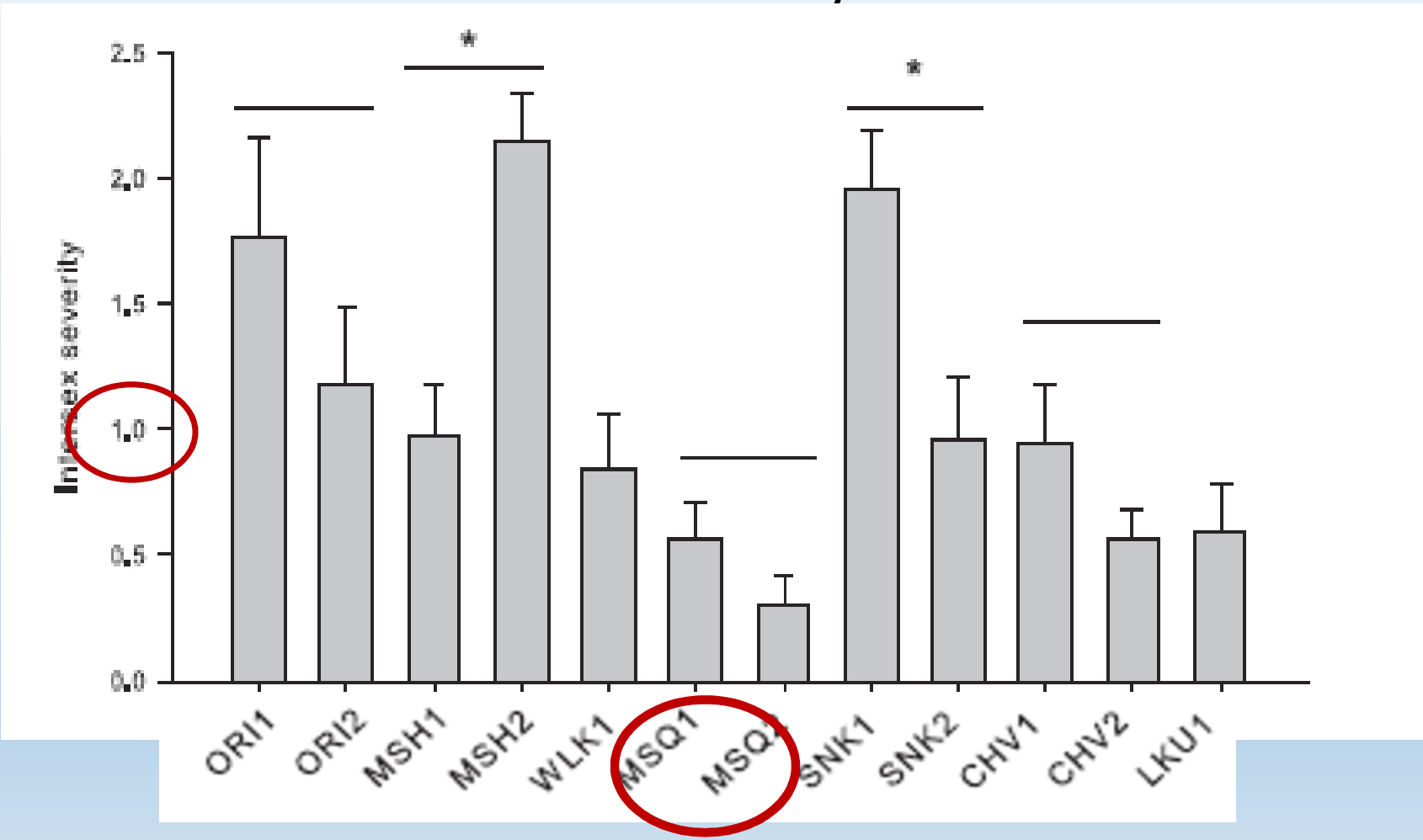
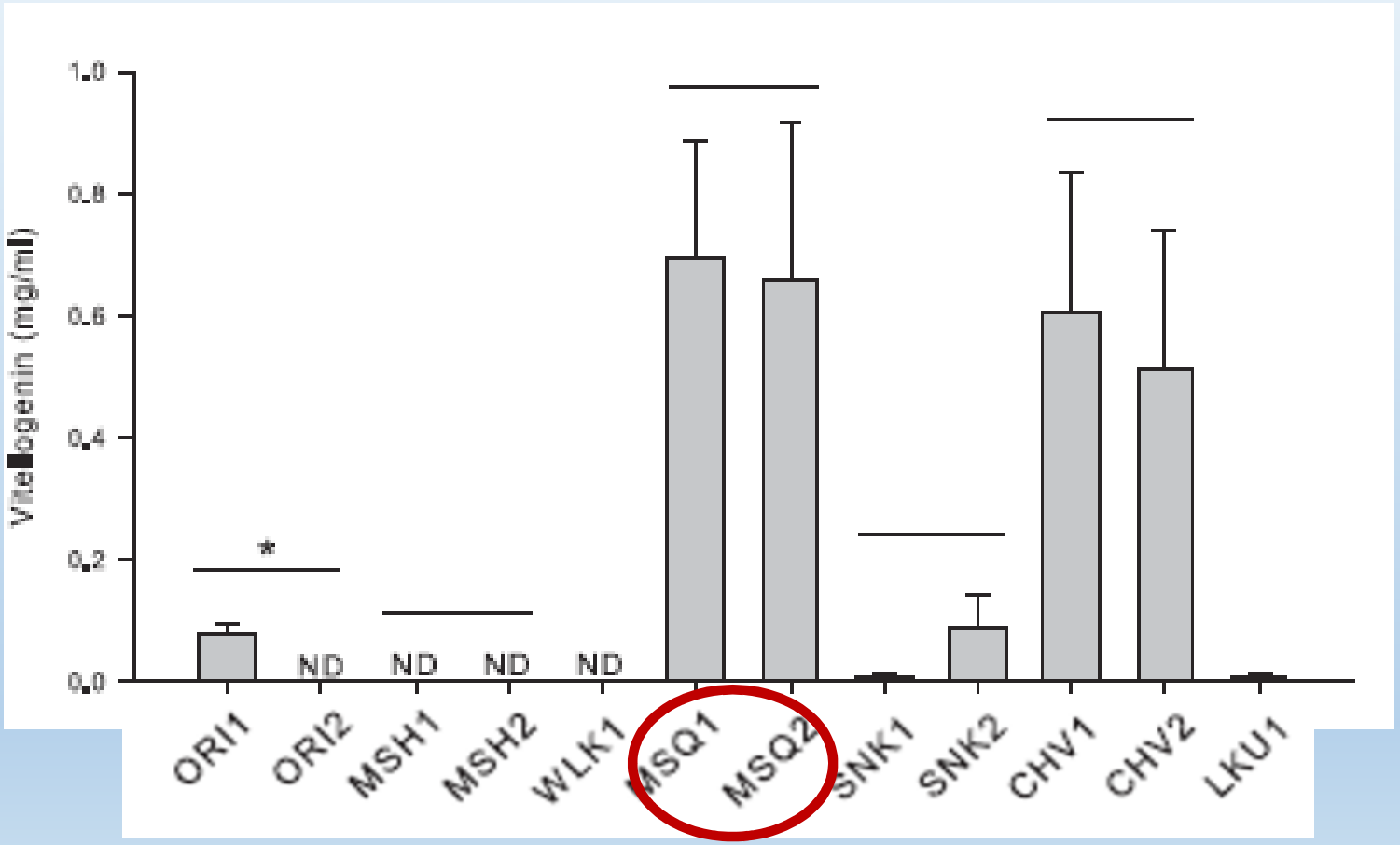
Boccuzzo, Linda, 2/2/2016

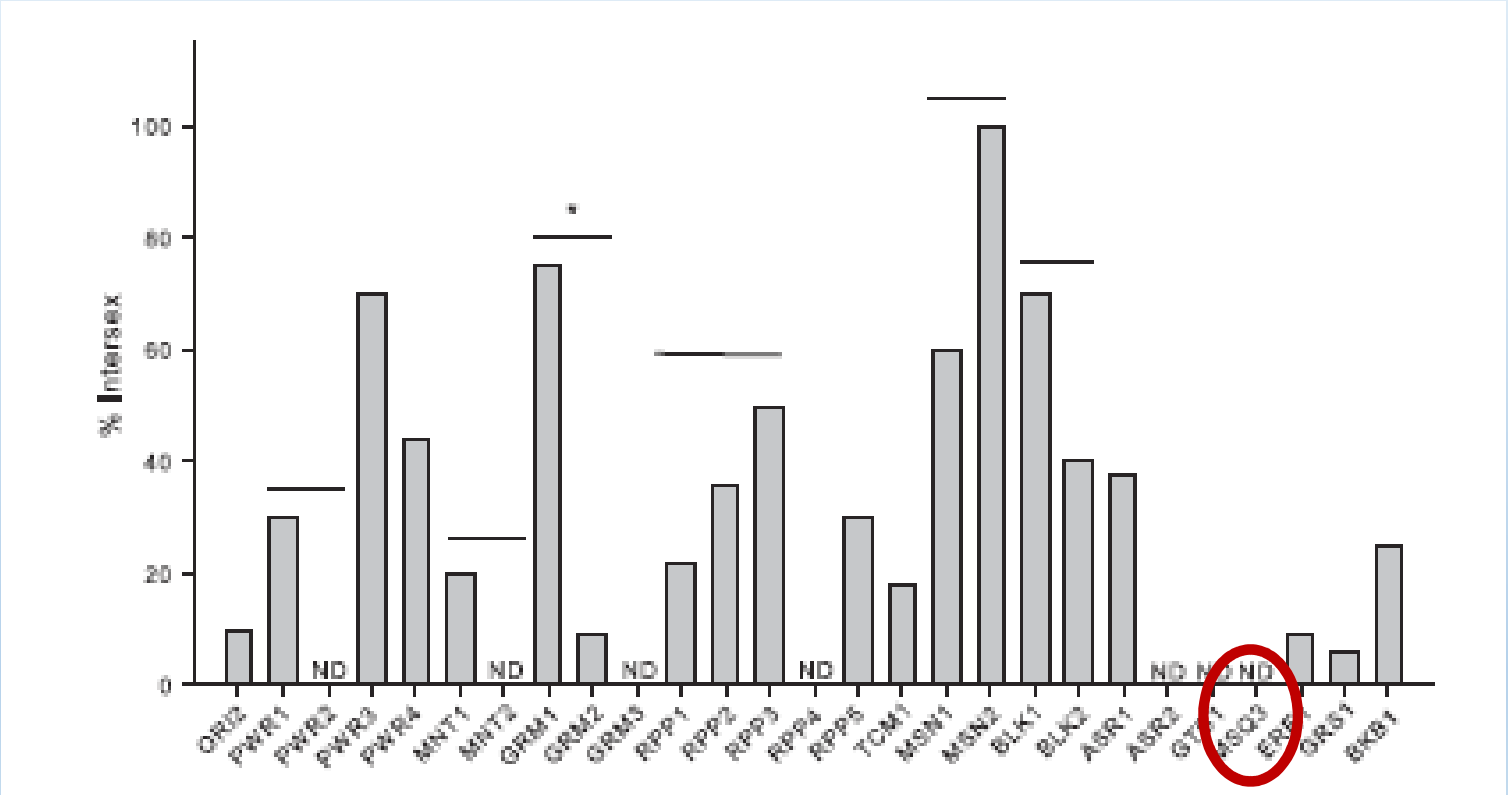
Results: Intersex Severity in Smallmouth Bass



Results: Vitellogenin in Smallmouth Bass Plasma



Results: % Intersex in Largemouth Bass



Summary Results: Missisquoi River Study Sites
(VT Specific Data)

Site Name	Site Description	% Intersex	Intersex Severity	Vitellogenin mg/mL	Species
MSQ1	Missisquoi River 5km Upstream Swanton Dam Swanton-Highgate	72 %	0.3	0.7 +/- 0.20	SMB
MSQ2	Missisquoi River 6km Downstream Swanton Dam Swanton-Refuge	65 %	0.6	0.65 +/- 0.15	SMB
MSQ3	Gander/Goose Bay @ Lake Champlain	ND	ND	ND	LMB

So what?

- Throughout study SMB demonstrated higher levels of intersex. Could be related to habitat, species sensitivity or something else…
- Largemouth bass collected from other surface waters in the northeast exhibited biomarkers of exposure to EEDCs. Largemouth bass from Lake Champlain exhibited no biomarkers.
- All smallmouth bass collected from surface waters in the northeast exhibited biomarkers of exposure to EEDCs.
- Of all northeast sites, Vermont smallmouth bass had the lowest intersex prevalence and severity of all fish in study. Other sites in the study were selected because they had no AFO, WWTP or paper mills or other industrial impacts.
- Vermont smallmouth bass had elevated levels of a protein in blood plasma, noticeably more than other sites. This protein is associated with exposure to these EEDCs. The protein may have other sources (microbes), and may even provide a protective effect against intersex occurrence. A research question at this point.

So what?

- The paper is looking at *effects* associated with an entire class of chemicals-EEDCs. Atrazine, is only one of any number of natural and human-made EEDCs.
- No chemical tests were done to determine EEDC type/quantity, could have been any of them.
- There are & have been known point source contributors of EEDCs into Lake Champlain & Missisquoi river to associate *all estrogenic* activity to atrazine is invalid.
- No conclusions about agricultural land use and intersex can be made from this study. In fact, at some sites they selected/sampled because they had no known impact of a potential source (AFO, WWTP, paper mills or urban land) there was actually more evidence of intersex than at its paired “impacted” site. The authors note this in the paper.
- It is important to note that other areas where researchers have conducted studies have entirely different ag systems then VT.

TFM-Lampricide treatments

Environmental fate and effects of the lampricide TFM

Hubert, T. D., 2003, Environmental fate and effects of the lampricide TFM: a review: Journal of Great Lakes Research, v. 29, p. 456-474.

Abstract

Use of 3-trifluoromethyl-4-nitrophenol (TFM) is limited geographically to the Great Lakes basin where it is the principal agent used in control of the sea lamprey (*Petromyzon marinus*). It is clear from available data that TFM has effects on the environment, but the effects reported are transient. Individual organisms and aquatic communities return to pretreatment conditions after lampricide treatments have concluded. TFM is not persistent, is detoxified, and presents minimal long-term toxicological risk. TFM is relatively nontoxic to mammals. Treatment levels do not pose a threat to wildlife. However, TFM is an estrogen agonist and additional testing to define the nature and magnitude of this effect will likely be required. Because stream treatments are done on 3 to 5 year cycles, and exposures are limited to approximately 12 h, minimal risk to aquatic organisms is expected.

Hepatic mixed function oxygenase activity and vitellogenin induction in fish following a treatment of the lampricide 3-trifluoromethyl-4-nitrophenol (TFM)

L M Hewitt, K R Munkittrick, G J Van Der Kraak, I M Scott, L P Schleen, M R Servos

Published on the web 12 April 2011.

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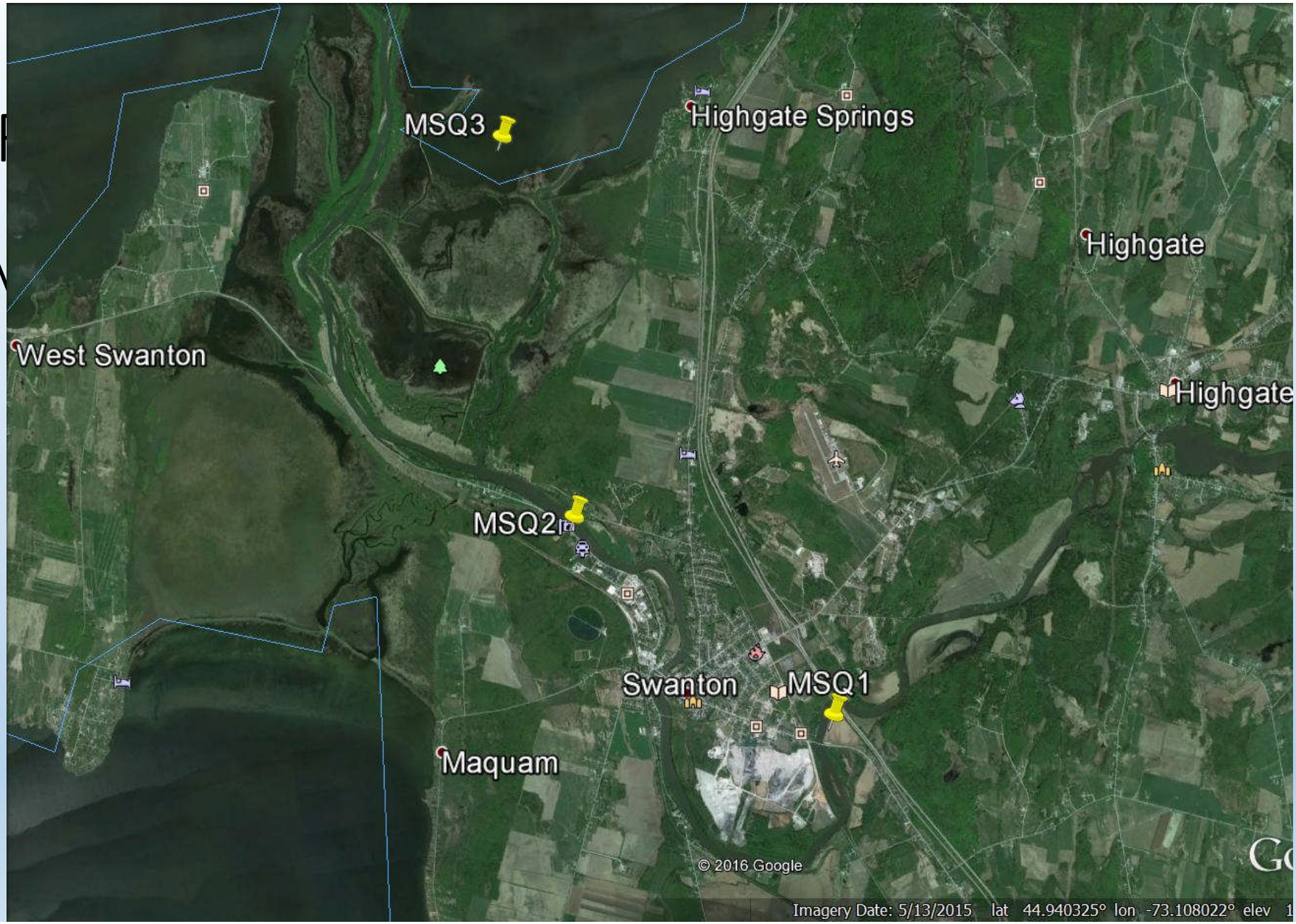
MSQ3
Highgate Springs
Highgate
West Swanton
Highgate
MSQ2
Swanton
MSQ1
Maquam
© 2016 Google
Imagery Date: 5/13/2015 lat 44.940325° lon -73.108022° elev

Missisquoi treated with TFM November 2008

Chemical	Location	Quantity (gal.)	Pounds Active
TFM-HP			
#070506A	Swanton Dam	159.4	510
#070512A	"	860	2,752
#070513A	"	290	928
#070506A	Trib MT2	0.6	2
Total:		**1,310**	**4,192**

Raw Water after TFM treatment in Missisquoi