

Environmental Impacts of Endocrine Disrupters

- should we be concerned, and
how can we regulate them?

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This presentation

- What is endocrine disruption?
- Endocrine disruption in the aquatic environment
- The regulatory environment
- Development of *in vivo* tests for EDCs
- Hazard and risk assessment of EDCs
- The future

What is endocrine disruption?

A definition (WHO):-

'An endocrine disrupter is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes **adverse** health effects in an **intact organism**, or its progeny, or (sub)populations'

NB: The text shown in red is crucial – endocrine disruption implies adverse effects, and may be suspected on the basis of *in vitro* tests but can only be proven *in vivo*.

What is endocrine disruption?

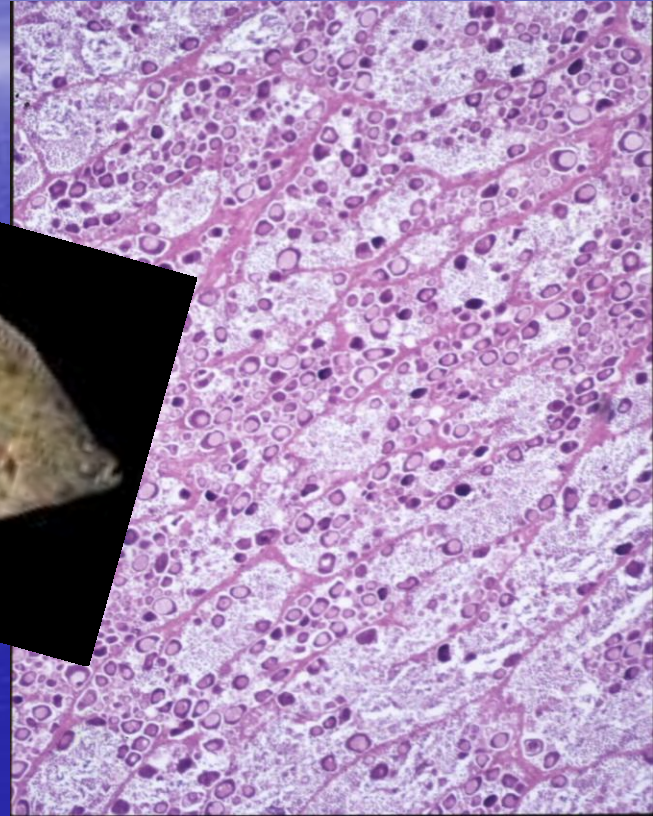
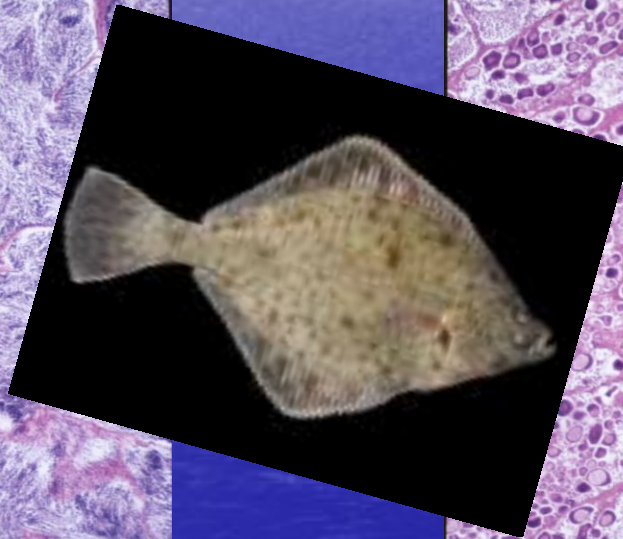
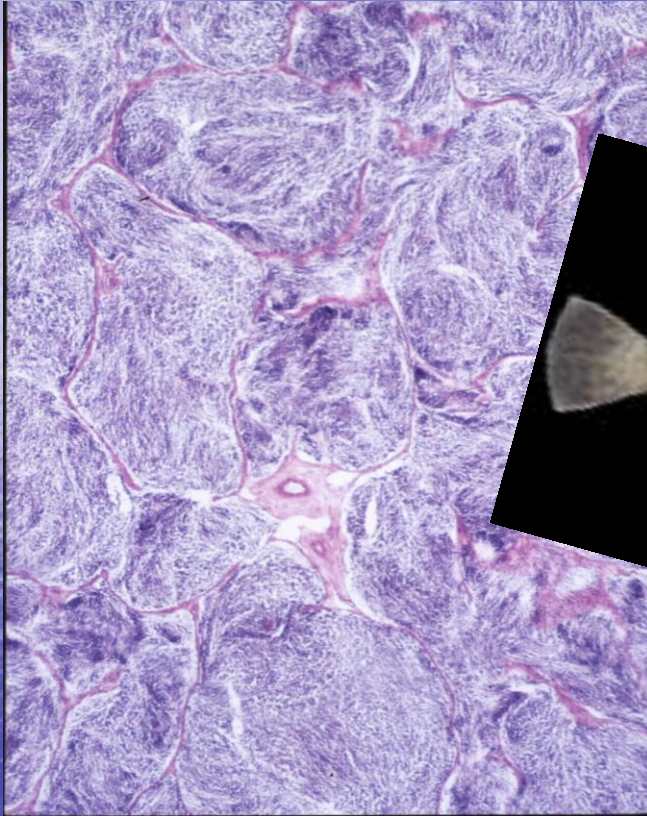
- It's just one more mode of toxic action, but...
- ...It can operate at very low concentrations
- ...Dose-response curves may not be monotonic
- ...Mixtures can produce additive responses at individually negligible concentrations
- ...It has caused serious environmental damage
- ...It causes terror in the hearts of environmentalists, regulators and the chemical industry

Endocrine disruption in the aquatic environment

- Strong evidence for widespread oestrogenic and organotin contamination of rivers and estuaries
- This is causing reproductive impairment of fish and mollusc populations
- The biggest known impacts to date concern tributyltin-based antifouling paints which have devastated some mollusc populations and whole invertebrate communities
- Some evidence exists for other modes of endocrine action (e.g. anti-androgenic effects)

Endocrine disruption in the aquatic environment

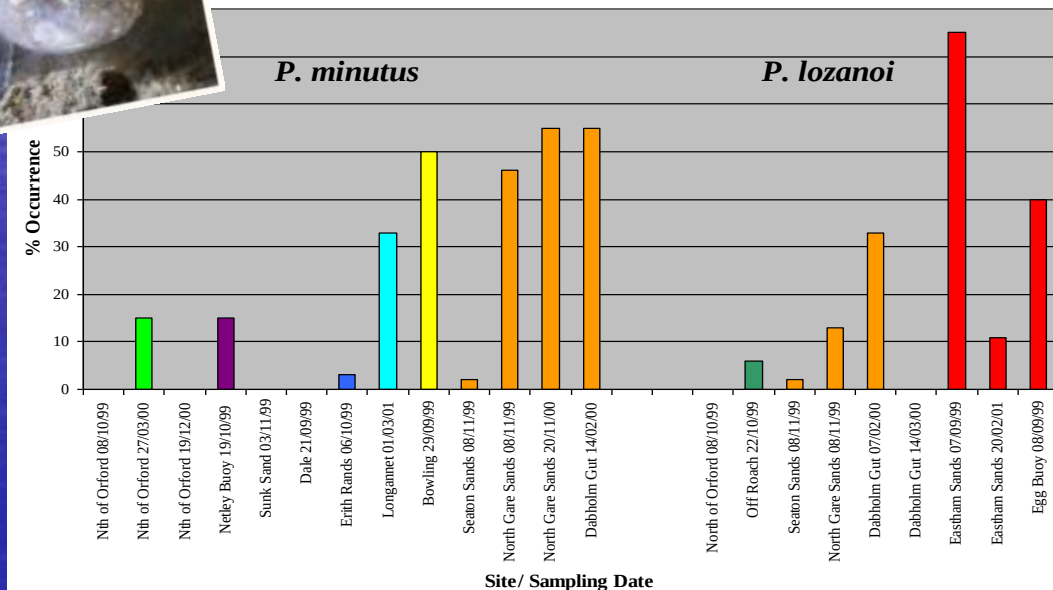
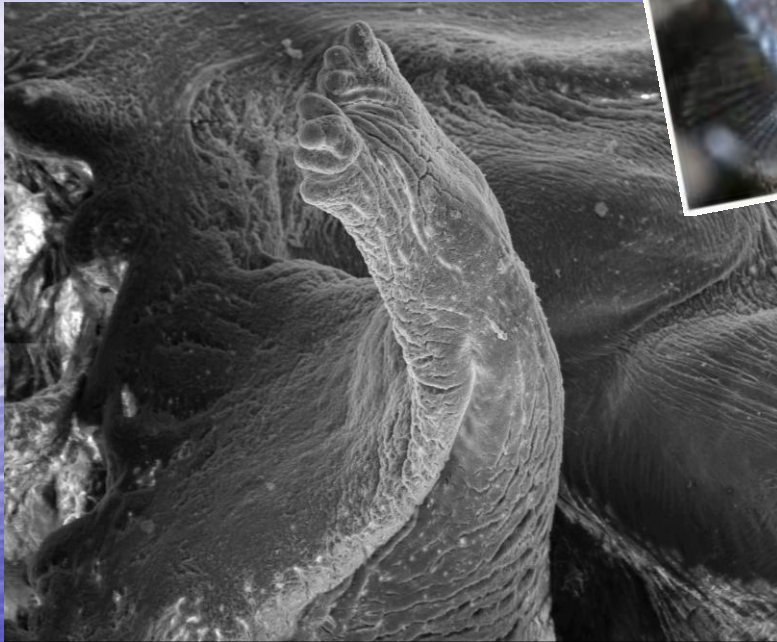
Probable cause of intersex : oestrogens and their mimics in treated sewage



Platichthys flesus ovotestis

Matthiessen *et al.* (1998) *Sci. Ser. Tech. Rep.*, Cefas 107

Endocrine disruption in the aquatic environment



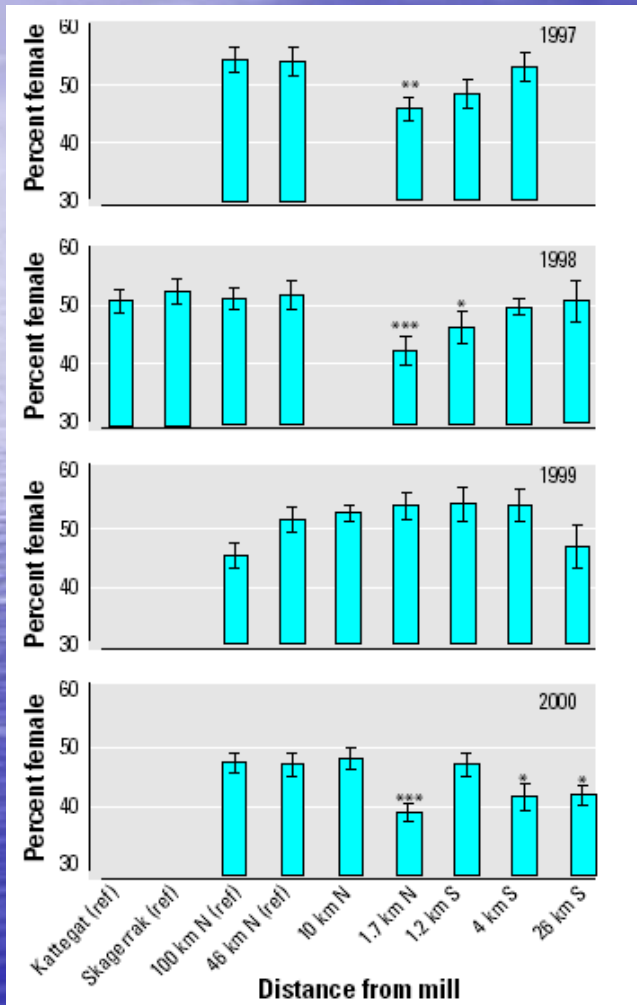
Feminised male urogenital papilla
in *Pomatoschistus minutus*

Occurrence of feminised urogenital papillae
in UK male sand gobies

Probable cause: oestrogens and their mimics in treated sewage

Kirby et al. (2003). *Environmental Toxicology and Chemistry* 22, 239-251.

Endocrine disruption in the aquatic environment



Probable cause: Phytoestrogens in paper pulp mill effluent

Male-biased sex ratio in viviparous blenny *Zoarces viviparus* broods near a Swedish pulp mill



Endocrine disruption in the aquatic environment

Probable cause: oestrogens and their mimics in treated sewage

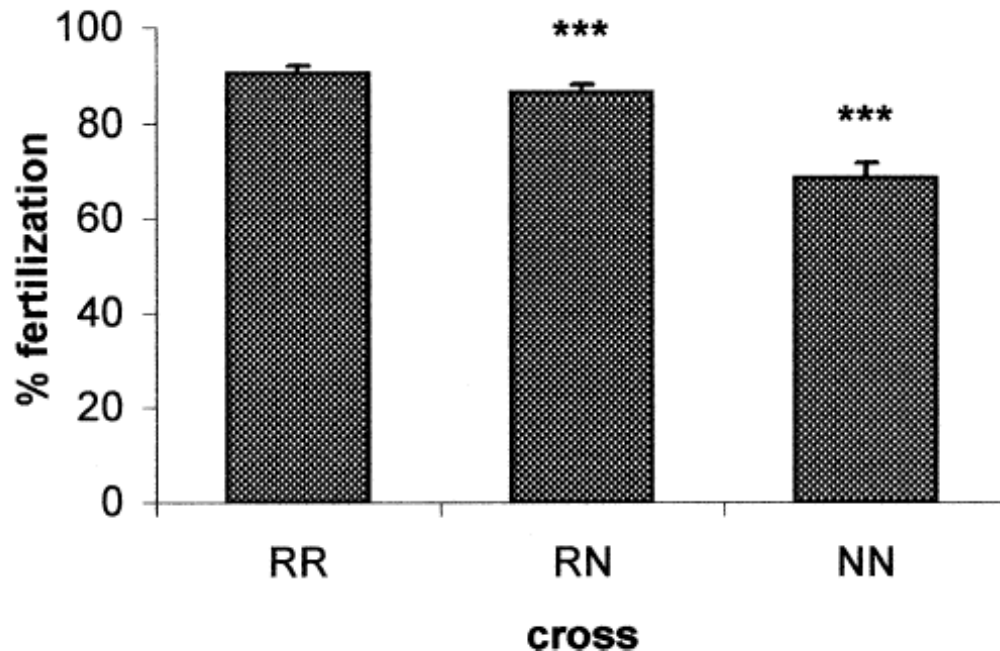


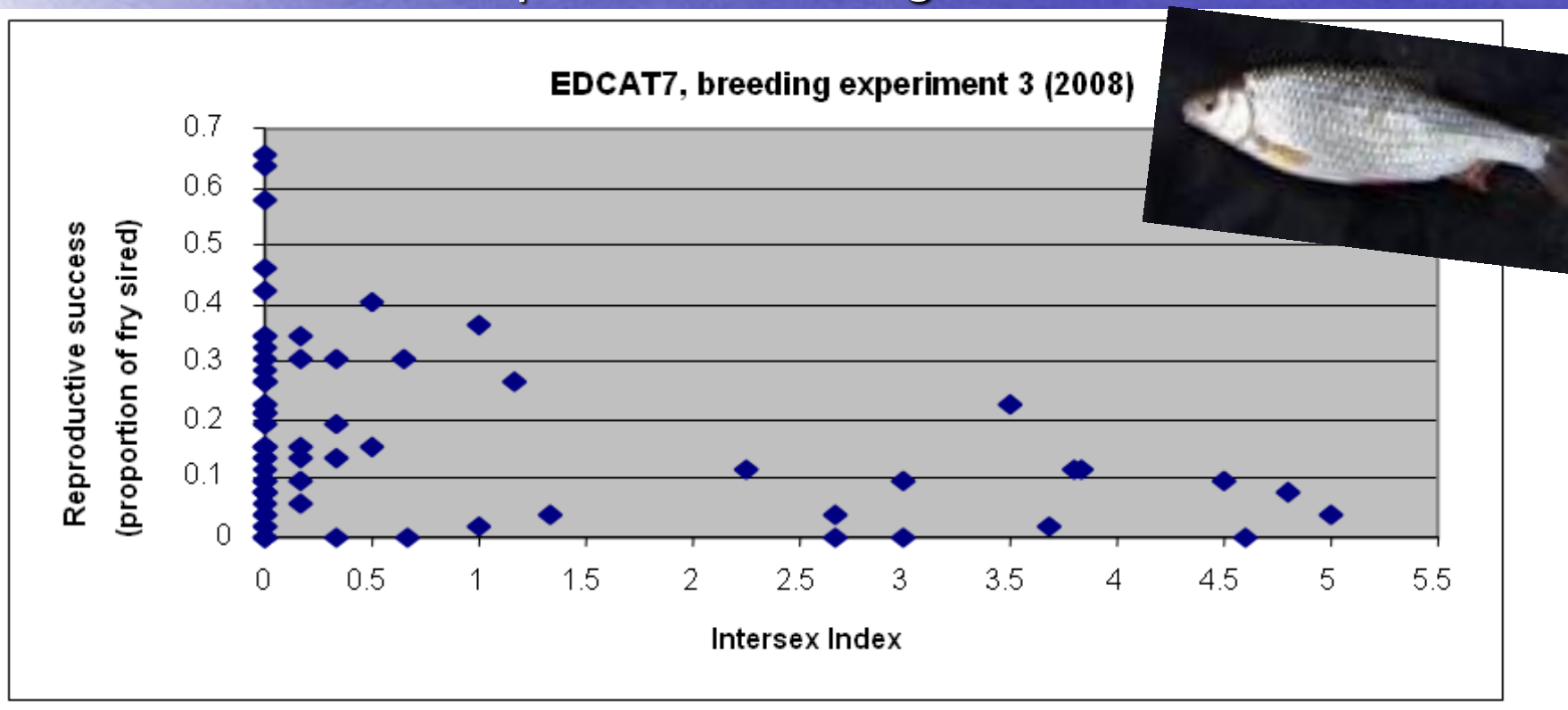
FIG. 5. The reproductive success of reference and River Nene roach in experiment 1. Milt and eggs were collected from reference (R) and River Nene (N) roach and used to establish three crosses: RR (reference males $\times$ reference females), RN (reference males $\times$ River Nene females), and NN (River Nene males $\times$ River Nene females). The percentage of fertilized eggs was determined after 24 h. Error bars represent SEM, and asterisks denote significant differences from the reference cross (RR). *** $P < 0.001$. There were 40–59 crosses in each group.

In vitro fertilisation success in *Rutilus rutilus* from the oestrogen-contaminated River Nene



Endocrine disruption in the aquatic environment

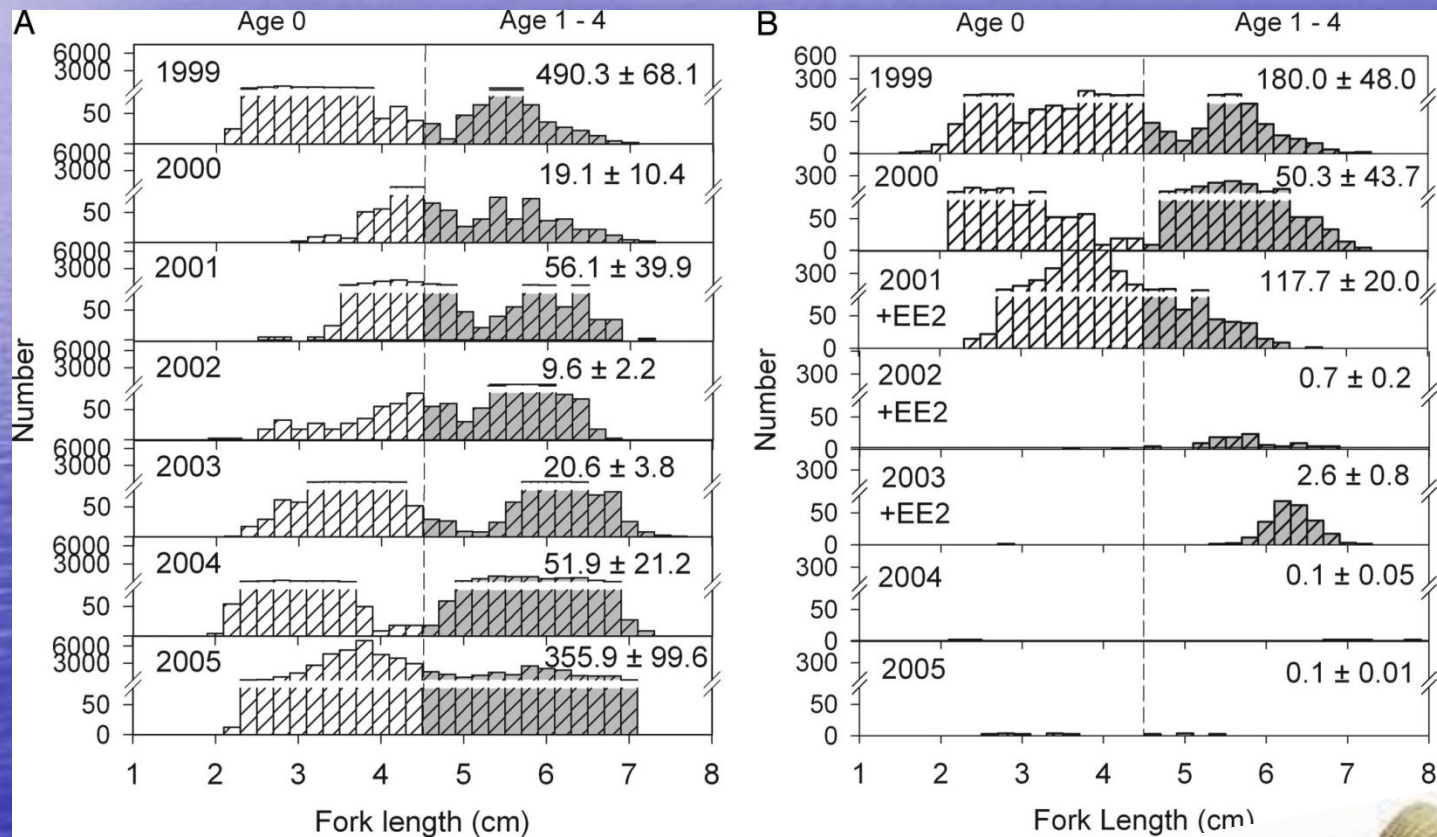
Result of intersex roach breeding experiments – severely intersex males have reduced ability to sire offspring under a competitive breeding scenario



Tyler, Sumpter et al. (2009). In: Final report of the EDCAT project – Endocrine Disruption in Catchments. <http://www.ceh.ac.uk/science/edcat.html>

Endocrine disruption in the aquatic environment

Collapse of a wild fathead minnow population in Canadian Lake 260 after 3 annual treatments with ethynylestradiol at 5-6 ng/l

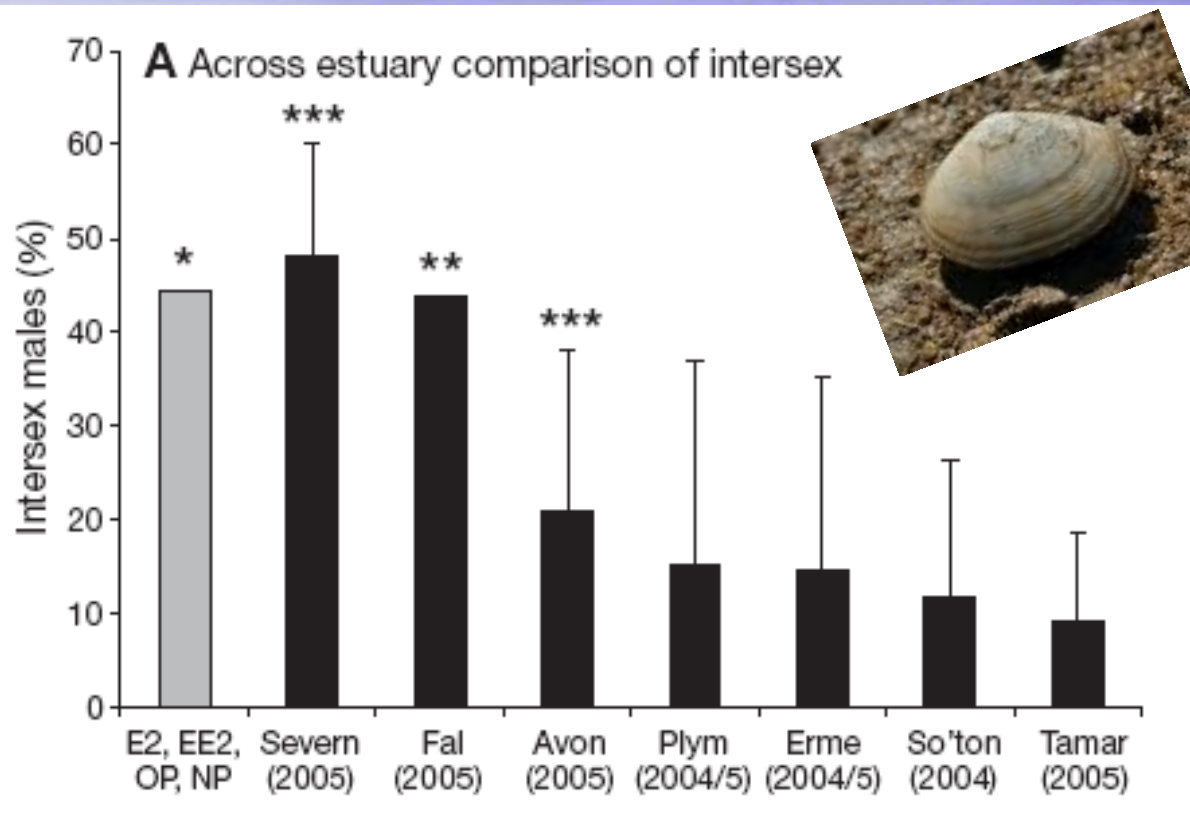


Kidd et al. (2007). PNAS 104, 8897-8901.



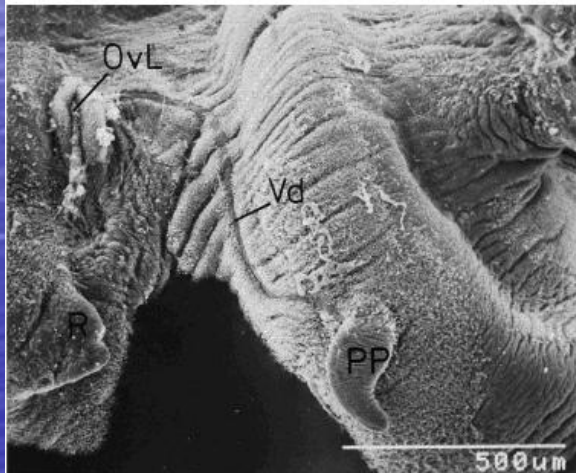
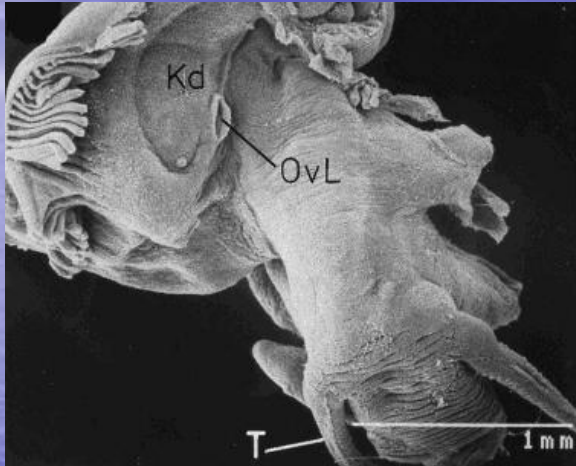
Endocrine disruption in the aquatic environment

Probable cause: oestrogens and their mimics in treated sewage



Incidence of intersex in male *Scrobicularia plana* from UK estuaries, plus intersex induced by exposure to oestrogens (Tamar = reference site)

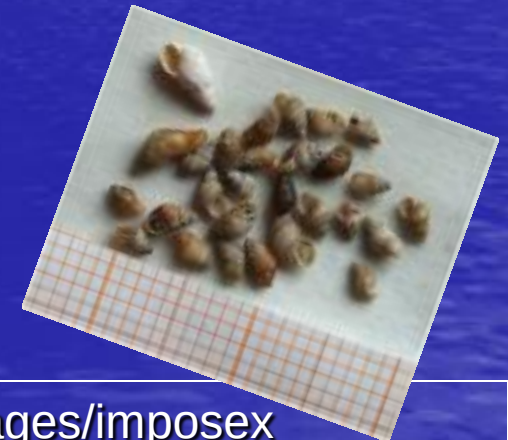
Endocrine disruption in the aquatic environment



Cause: Tributyltin from antifouling paints

Imposex in the mudsnail *Hydrobia ulvae* exposed to tributyltin.

Upper photo: normal female; lower photo: TBT-exposed female showing penis (PP) and blocked oviduct (OvL).



Endocrine disruption in the aquatic environment



Organotin-based antifoulant being scrubbed off a yacht into the Crouch estuary – early 1980s

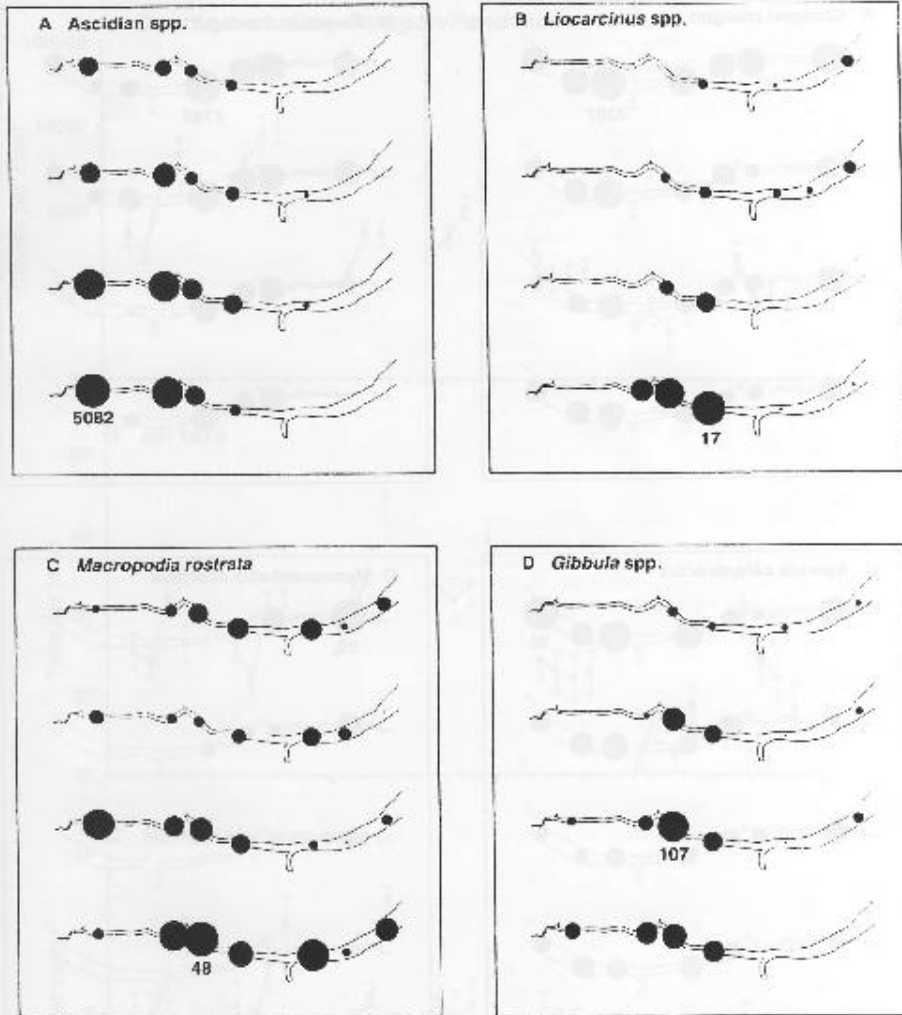
Endocrine disruption in the aquatic environment



A 10-year ecological survey of the Crouch estuary after TBT was banned in 1987 showed that this EDC had caused major ecosystem damage

Endocrine disruption in the aquatic environment

Epibenthic surveys in relation to TBT H.L. Rees et al. 207



Overall, in the 10 years after TBT-use was banned in the Crouch estuary in 1987, the benthic ecosystem returned to near-normal, with total numbers of epi-faunal taxa increasing from 64 to 74 (Rees et al. 2001. *Mar. Pollut. Bull.* 42, 137-144), and in-faunal taxa increasing from 37 to 63 (Waldock et al. 1999. *J. Mar. Biol. Ass. UK* 79, 225-232).

Rees et al. (1999) *J. Mar. Biol. Ass. UK* 79, 209-223

Summary of known impacts

- There is good evidence for the environmental effects of endocrine disrupters in fish and molluscs
- This is probably causing population-level impacts in some fish, and has certainly caused community damage in invertebrates
- Causative factors include natural and synthetic oestrogenic hormones, oestrogen mimicking chemicals, and indirect androgens such as the organotins
- Environmental monitoring programmes are not yet tuned to look for these effects or many of these substances
- Chemical regulations and testing regimes are only just beginning to recognise that these problems need to be tackled at source
- **IN SUMMARY, WE SHOULD BE CONCERNED.** However, not all impacts are being caused by synthetic chemicals

The regulatory environment

- At least 3 jurisdictions (USA, EU, Japan) now require, or shortly will require, information about the endocrine disrupting effects of some chemicals
- The most explicit requirements emanate from the USEPA in their Endocrine Disruptor Screening Program, which is currently requiring screening of 67 suspected EDCs (mainly pesticides)

Suite of Assays in the EDSP Tier-1 Screening Battery

In vitro

Estrogen receptor (ER) binding – rat uterus or recombinant

Estrogen receptor (hER α) transcriptional activation - Human cell line (HeLa-9903)

Androgen receptor (AR) binding – rat prostate

Steroidogenesis – Human cell line (H295R)

Aromatase – Human recombinant

In vivo

Uterotrophic (rat)

Hershberger (rat)

Pubertal female (rat)

Pubertal male (rat)

Amphibian metamorphosis (clawed frog *Xenopus laevis*) (OECD TG 231)

Fish short-term reproduction (fathead minnow *Pimephales promelas*) (TG 229)

The regulatory environment

- The Tier 1 screens are expected to detect (anti)estrogens, (anti)androgens, steroidogenic effects, impacts on the hypothalamo-pituitary-gonadal axis (HPG), and impacts on the hypothalamo-pituitary-thyroid axis (HPT)
- A positive response in the USEPA's Tier 1 screening battery will trigger requirements for definitive Tier 2 *in vivo* testing

Assay	Is an effect detected?			
ER binding	Yes	No	No	No
AR binding	No	Yes	No	No
Steroido- genesis	No	No	Yes	No
Male pubertal	No	Yes	Yes	Yes
Female pubertal	Yes	No	Yes	Yes
Fish screen	Yes	Yes	Yes	Yes
Likely MOA	ER	AR	Steroido- genesis	HPG

The regulatory environment

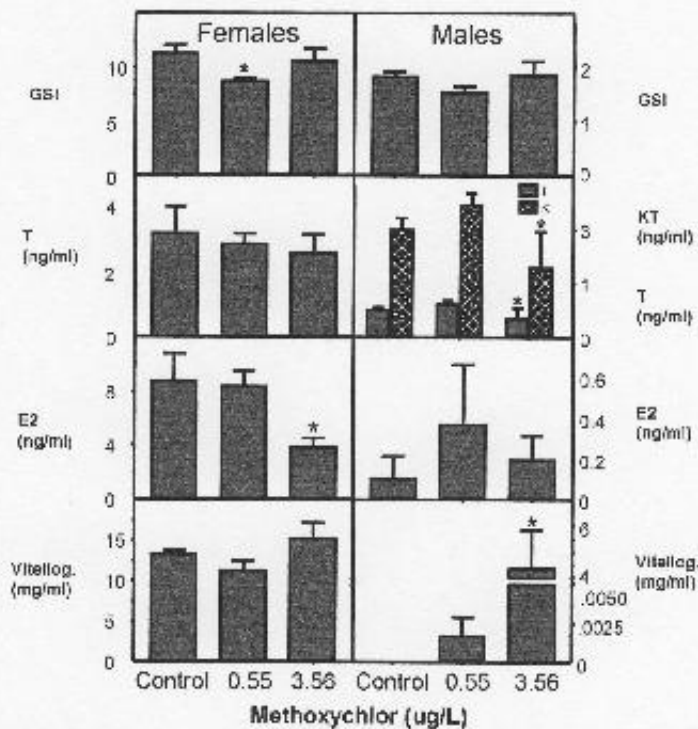
- USEPA screening data on methoxychlor tested in the fish short term reproduction assay with fathead minnow

Biomarkers

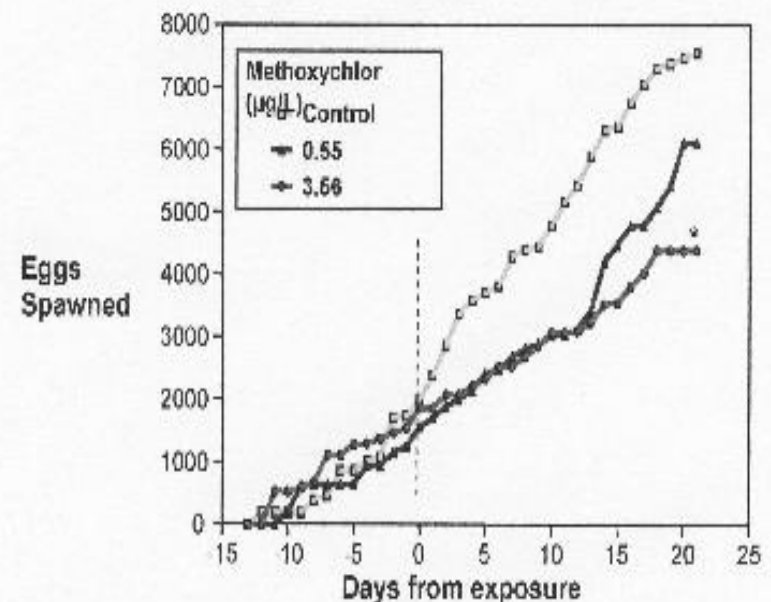


Fecundity

Fish short-term reproduction assay



Fish short-term reproduction assay



The regulatory environment

- The EU and Japan are lagging behind the USA
- In the EU, Regulation EC 1107/2009 on PPPs (which replaces Directive 91/414/EEC) now bans adjuvants and new active substances that cause endocrine disruption in humans or wildlife, and makes existing PPPs with these properties candidates for substitution.
- NB: This is regulation by hazard, not by risk!
- However, the Commission has not yet decided the criteria it will use to identify an EDC (must be done by 2013)
- The EU Biocides Directive will probably have similar requirements
- Under EU REACH, EDCs will be subject to authorisation, but again, the criteria to be used have not yet been clearly defined.
- In Japan and elsewhere, regulations are under development, but none are yet in force.
- In summary, testing for ED properties is, or shortly will be, compulsory for PPPs, biocides and general chemicals in the US and EU

Standardised ecotoxicity tests sensitive to EDCs

- OECD guidelines in print

TG 229 Fish Short Term Reproduction Assay (2009)

TG 230 21-Day Fish Assay (2009)

TG 231 Amphibian Metamorphosis Assay (2009)

- OECD methods in preparation

Androgenised Female Stickleback Screen

Fish Sexual Development Test

Fish Full Lifecycle Test

Medaka (fish) Multi-Generation Test

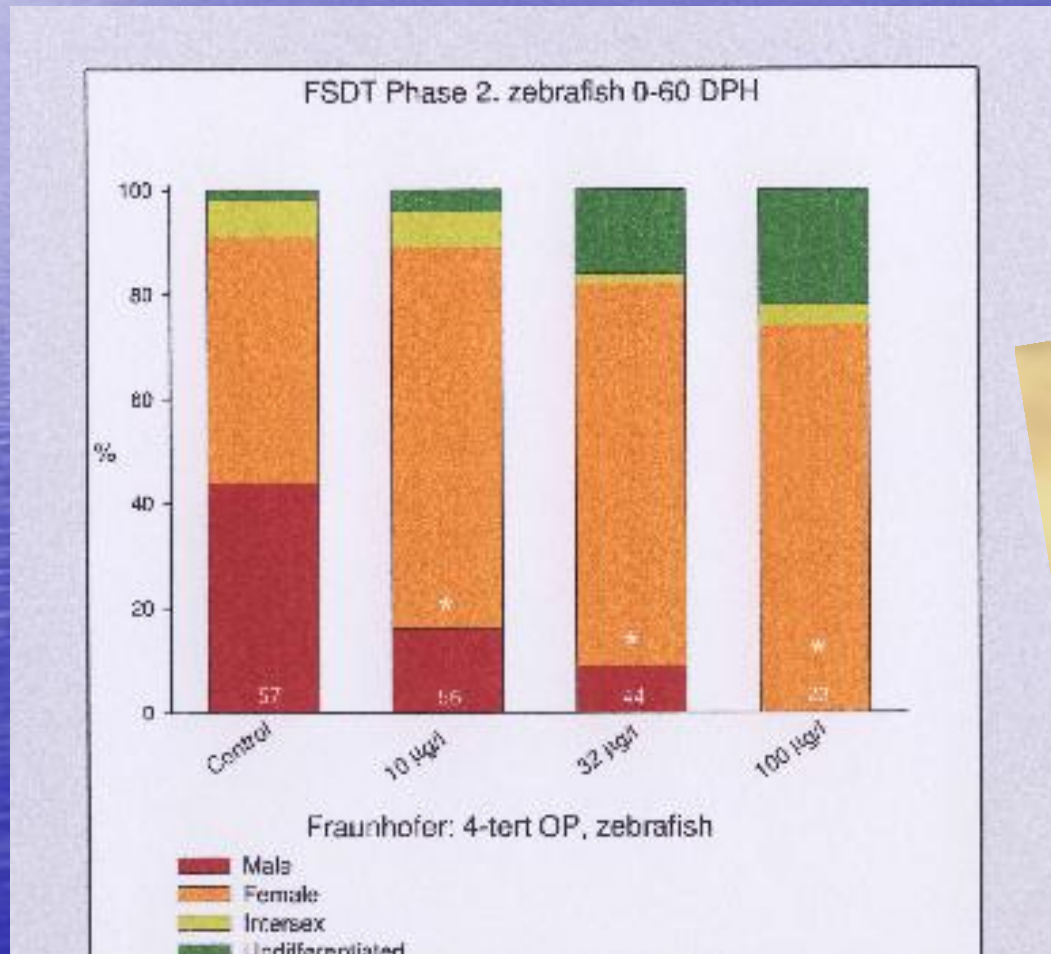
Amphibian Development, Growth and Reproduction Assay

Avian 2-Generation Test

(+ various invertebrate lifecycle tests)

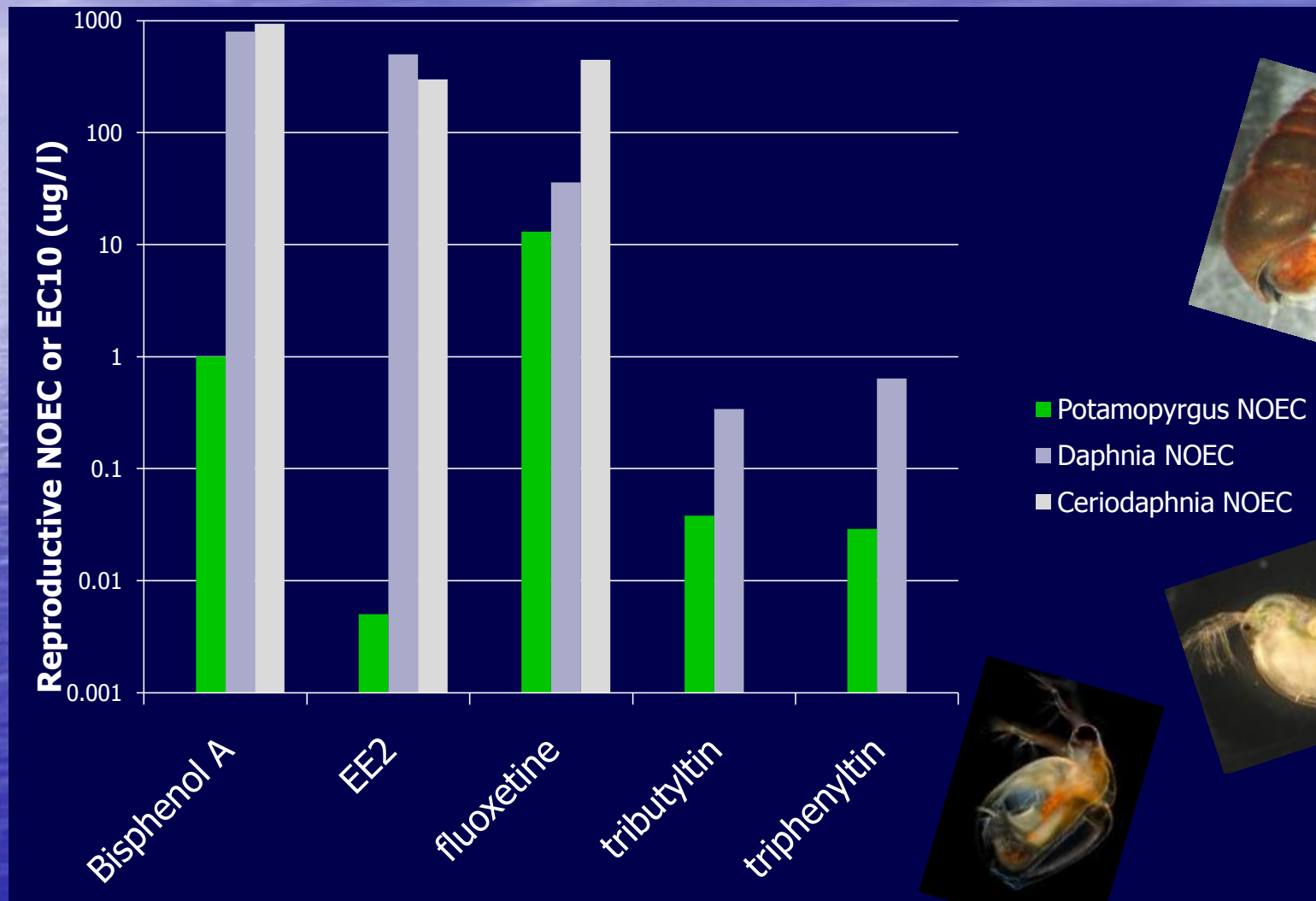
Development of tests for EDCs

OECD validation data for the proposed fish sexual development test – zebrafish exposed to octylphenol – sex ratio endpoint



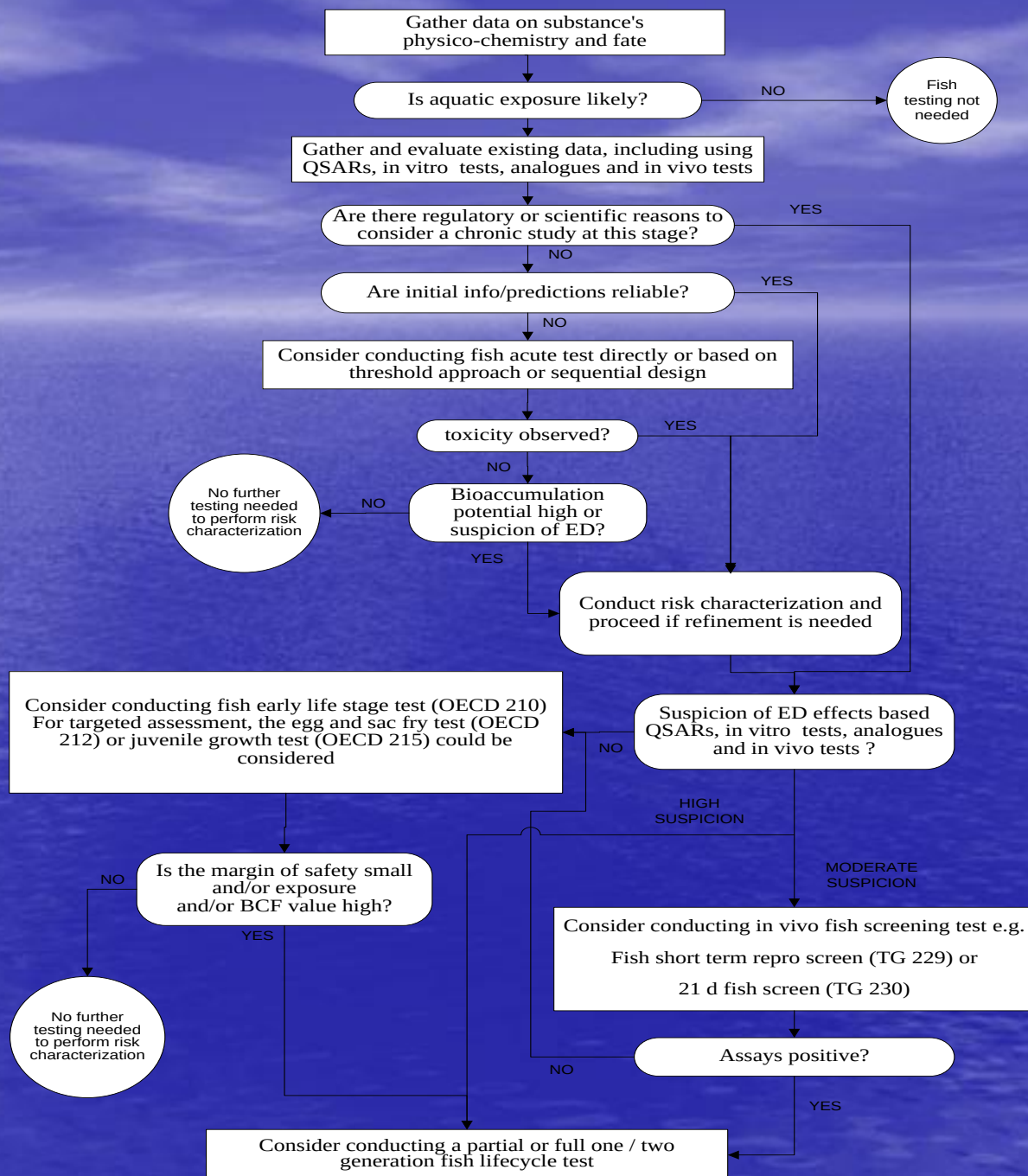
Development of tests for EDCs

Why we need more invertebrate repro tests: e.g. *Daphnia* vs mollusc



Hazard assessment of EDCs

Draft generic OECD chemical hazard assessment scheme for fish, incl. EDCs and other chemicals



Risk assessment of EDCs

- We will soon have an adequate toolbox of standardised *in vivo* ecotoxicity assays for EDCs, both screens and definitive tests, plus agreed hazard testing strategies and guidance on data interpretation
- There is no reason why the environmental risks of EDCs cannot be adequately predicted – resorting to regulation by hazard is a panic reaction
- However, the special features of EDCs will need to be borne in mind e.g. low-concentration effects, mixture effects, non-monotonic dose-response curves, possible multi-generation effects

The Future

- Over the next 3-5 years, many jurisdictions will start requiring the application of *in vitro* and *in vivo* tests designed to detect, and assess the environmental hazards of, endocrine disrupting chemicals
- Over the same timescale, a more or less full suite of EDC-sensitive standardised tests will become available
- An OECD Guidance Document is in preparation which will provide support for regulators with the interpretation of endocrine test data (ecotoxicity and mammalian toxicity)
- The retrograde move away from risk assessment towards regulation by hazard looks set to continue

Thank you for listening 😊

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