Troubled Waters

The impact of water-polluting chemicals on fish populations

Decades of research have proved that potent synthetic hormones released into the world's water supplies have adverse effects on aquatic life. Oestrogens in sewage effluents, notably ethinyloestradiol (EE2) – an active ingredient in the contraceptive pill – are known to trigger the development of female characteristics in male fish, which decreases fertility and increases the risk of population-level harm. The potential impact on human health remains unclear.

Rising concerns over EE2 pollution of waterways have sparked a fiercely contested regulatory debate in Europe. The European Commission, in the face of opposition from the water and pharmaceutical industries, and many EU member states, has stopped short of imposing a precedent-setting limit on concentrations of EE2 in rivers. It would have cost the UK tens of billions of pounds to meet these targets through upgrades in wastewater treatment. Instead it has placed EE2, along with anti-inflammatory drug diclofenac, which has been shown to cause declines and even localised extinctions of certain vulture populations in Asia, on a watchlist and delayed further regulatory decisions until after 2017. Critics argue there should be a more open public debate on the issue.

With the price of action so high, regulators are demanding cast-iron evidence that endocrine-disrupting chemicals (EDCs) such as EE2 cause damaging falls in fish population levels and more data on the costs of eliminating them from wastewater. Academics from the University of Exeter and Brunel University, policymakers, water industry figures and representatives from environmental organisations recently gathered at the Environment Agency (EA) offices in Leeds to discuss the latest available scientific data and explore new ways to tackle this form of water pollution.

Kicking off the event, Martin Christmas, the EA's Area Environment Manager for North Yorkshire, said that after delivering a "step change" in the UK's water quality over the last 45 years, other issues, such as intersex fish, are coming to the fore. He said: "If we can't get fish to breed in the right places we will be constantly under pressure to manage fish stocks in an unsustainable way. We need more guidance and help to understand key issues like intersex fish and the implications this has for the UK's water supply."

The Story So Far

Scientists raised the notion that the contraceptive pill might cause environmental damage in the 1970s, says Susan Jobling, professor in ecotoxicology and Head of Brunel University's Institute for the Environment.

Since wastewater treatment plants were not designed to remove micropollutants, including pharmaceuticals, it was considered likely that synthetic oestrogens, of which EE2 is the most potent, contained within the pill were entering surface water supplies.

In 1978 Thames Water scientists led by Roger Sweeting found eggs developing in the testes of five out of 26 male roach in the River Lea. Reacting to the findings, he said: "It was amazing to see macroscopically hermaphrodite fish that were both male and female all at the same time."

Concern for public health prompted further analysis by Thames Water. Jobling, who has studied the effects of environmental contaminants on wildlife and humans for over 20 years, said: "The findings were quite shocking." They showed that giving female rats the samples to drink for a year harmed their reproductive system. But the issue never came into public view.

"Since further samples taken part way through the drinking water treatment process were found to have no effects on the rats, the Department of Health discounted the possibility of any risk to human consumers. The studies were never published," she said.

Leap forward 15 years and the risks of EE2 contamination no longer lurked beneath the surface. A seminal 1998 paper by Jobling and colleague John Sumpter, and Charles Tyler, now professor of environmental biology at the University of Exeter, revealed that 100% of a sample of wild male roach in effluent-contaminated river stretches displayed female characteristics.

Jobling said: "By 2003, after millions had been spent on research around Europe, scientists and industry agreed there was extensive evidence of wildlife being adversely impacted by exposure to EDCs, and that EE2 was playing a leading role."

A year later the EA drew up a risk management strategy for steroid oestrogens. It recommended end-of-pipe treatment of effluent by water companies. Jobling said: "This placed the responsibility for risk management on the water industry and ultimately the cost of treatment on the tax-paying public."

The water industry, in collaboration with the EA, moved to quantify the cost of removing oestrogens from UK rivers. It found that a novel approach called granular activated carbon (GAC) was effective at removing EE2 from effluents but the costs were huge.

The cost of setting up GAC for a sewage plant serving a town of 250,000 people, like Swindon, was estimated at €8m. UK government estimates put the total cost of implementing GAC at 1,360 sewage plants at between €32bn and €37bn.

Jobling said: "It confirmed what everyone had long suspected: EE2 is potent and hard to get rid of." In January 2012, the EC proposed a revised list of 'priority substances' for the Water Framework Directive. EE2 was on the shortlist, raising the real possibility of regulation that would require enforcement by 2021.

Six months later however, following determined lobbying by the water and pharmaceutical industries, the EC chose instead to put EE2 on a 'watchlist', for review after 2017, and called for more evidence of steroid oestrogens causing actual declines in fish populations.

EE2 and the feminisation of fish in UK rivers – a timeline

1978 First evidence of harm to fish living in contaminated water in the UK.

1983 Evidence of harm to rats drinking contaminated river water.

1988 Proof that oestrogenic effluents in UK rivers were widespread.

1995-2000 Trials showed intersex in wild roach was widespread and especially prevalent downstream from large sewage treatment works.

1998 Research found that widespread sexual disruption in fish does result from exposure to oestrogenic chemicals like EE2 present in UK rivers.

2004 The UK government accepted that EDCs such as EE2 posed a significant risk to aquatic life.

2007 The EA drafted an Environmental Quality Standard – a target concentration of EE2 that could be used for regulatory compliance.

2013 The European Commission places EE2 on a watchlist, suspending a decision on regulation until after 2017.

Latest Research

Breeding discontent

Studies that exposed fish to sewage effluents for three years prove effluents cause sexual disruption and feminisation in wild roach.

Research by the University of Exeter's Dr Anke Lange, postdoctoral researcher, and Professor Charles Tyler found evidence that indicated male fish exposed to effluents struggled to reproduce in a competitive breeding environment.

Funded by NERC, Defra and the EA, the researchers exposed the roach to three scenarios: clean water, 50% effluent and 100% effluent. Analysis in the first two years revealed that one fifth of males exposed to the 50% effluent displayed female characteristics and in the 100% effluent one half of males were found to have either feminised ducts or testes that contained female eggs.

The academics devised a competitive breeding experiment in which nine colonies of fish in four combinations of control and effluent-exposed fish were allowed to breed naturally. They found effluent-exposed fish were unable to breed in the absence of control fish and 100% of effluent-exposed fish were phenotypic females.

Further tests showed EE2 contributes significantly to sexual disruption in wild roach. Lange and Tyler found continued exposure to high levels of EE2 (4ng/L) induced complete feminisation of a fish population just two years from fertilisation. The intersex condition persisted after the male roach were transferred to clean water for 400 days.

Lange said: "Effluents have significant effects on male fish – and there is no reason to suggest that wild roach are a special case. The key question now is whether the affected males are contributing. If not, this has serious consequences for fish populations."

The population question

The impact of oestrogens on individual fish is clear. But future EU regulation is dependent on evidence that EDCs like EE2 are damaging to fish at *population* level. Research at Exeter and Brunel set out to determine whether effluents threaten the long-term viability of roach populations.

Dr Patrick Hamilton, postdoctoral research fellow at Exeter, and Dr Catherine Harries, postdoctoral research fellow at Brunel, took wild fish from the Rivers Bourne and Arun to the EA's Calverton Fish Farm to breed. They sampled the fry four days after hatching and used DNA profiles to determine their parents. Results showed a 76% decrease in reproductive performance for fish on an intersex index of 5 (0 being male and 7 being female).

Research in Canada had found that the population of one fish species in one of the country's main experimental lakes had collapsed after exposure to EE2. To investigate whether roach populations in the most oestrogenic UK rivers were self-sustaining, Liz Nicol, PhD student at Brunel, and Hamilton, with the help of the EA, collected fish from 32 locations and extracted DNA from their fins or scales.

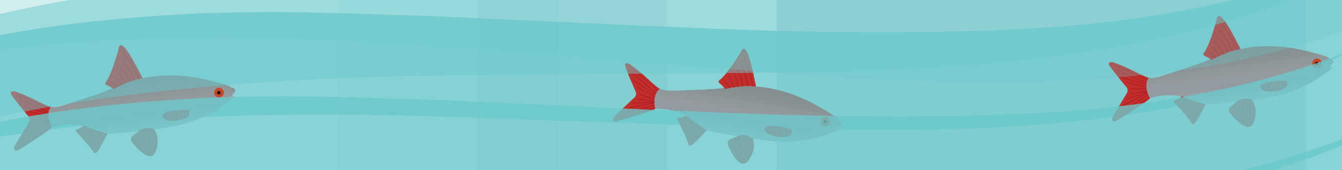
Hamilton said: "Our findings suggested that there were no statistically major effects of the effluent on the size of the fish breeding populations, but that it was still possible for a 60% reduction in effective population size to occur at the most impacted sites because of the variation in the data."

Further analysis of fish from the River Lea, which experiences 30-70% effluent, revealed that populations downstream are not maintained by migration from the unpolluted stretch upstream. The effective population size is similar in both polluted and unpolluted sections, indicating the effluent-exposed populations are self-sustaining.

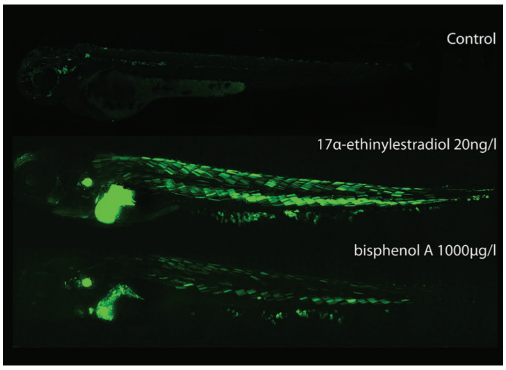
The research has moved on to explore whether roach are able to adapt to the harmful effects of oestrogen exposure, with results expected in 2016. Hamilton said: "If we find that fish can adapt then the long-term risk to populations will be greatly reduced. We're currently exposing fish to EE2 in our aquarium at Exeter and we will study the fish when they are older to understand if those from the more polluted sites are better able to cope with the effects of oestrogen."

Earning its stripes

A fish that heralds from the River



Ganges could hold the key to unravelling the true extent of the health effects of EDCs. The zebrafish represents one of the largest families of freshwater fish worldwide. Professor Tyler believes it offers scientists a new model for developing a better understanding of how and where EDCs work in the body.



He said: "The zebrafish when modified with some clever genetics - it glows green in target tissues affected by chemicals - can be used as a highly sensitive tool to assess the interaction of environmental oestrogens in real time. It allows us to target our analyses of potentially damaging health effects more intelligently. And as it develops very quickly, we can investigate changes over a much shorter time period compared with the roach."

Since every cell in the body is thought to have an oestrogen receptor, the potential health effects from exposure to chemicals that mimic oestrogens go further than reproductive disruption. John Moreman, a PhD researcher in Tyler's lab, and Dr Tetsu Kudoh, a developmental biologist at Exeter, used their zebrafish model to study the effects of bisphenol A (BPA), a weak oestrogen found in plastics and resins used in food and beverage containers. They found bisphenol A was affecting specific regions of the heart.

Tyler said: "Furthermore, we found that a metabolic product of BPA in mammals, called MBP, is up to a thousand times more potent than BPA in the zebrafish. BPA can be detected in most human urine and blood samples and has associated human health risks."

Exposure of the zebrafish to EE2 caused a change in breeding dynamics. As in roach populations, a breeding hierarchy exists in zebrafish populations, with dominant males and females that sire proportionally more offspring. Tyler found that EDCs can alter that dominance structure and change the breeding outcomes.

Tyler said: "Molecular technologies are playing a greater role in unravelling how EDCs affect physiological functions. The zebrafish models can be used in chemical and drug screening, potentially reducing the number of animals used in testing. They can also identify specific oestrogenic activity within effluents."

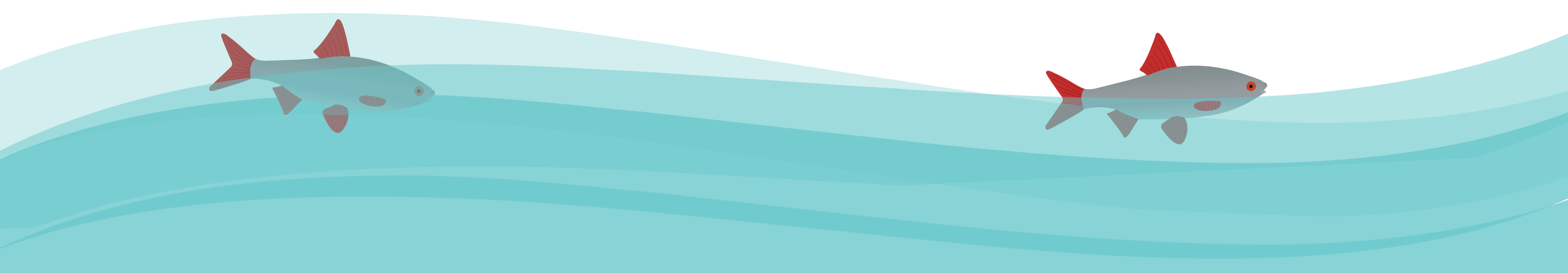
Climate of risk

Effects of endocrine disruption are likely to be magnified under climate change, according to research carried out at NERC's Centre for Ecology and Hydrology. Dr Virginie Keller has modelled future concentrations of steroid oestrogens in rivers in England and Wales under varying climate change scenarios to assess the risk of intersex in fish in 2050.

Keller and her team used three climate change scenarios as defined by the 2009 UK Climate Projections: the wettest, the driest and the average. They employed a hydrological model to estimate flows at unmonitored sites, which was adapted to include water quality, and they drew on data on sewage plant locations and the populations they serve.

Analysis showed that the percentage of the total length of rivers in England and Wales at risk from EDCs rose from 38% to 43-45% in 2050, depending on the climate change scenario. Stretches of river classified as high risk increased from 1% to 3-4%. In densely populated areas the effects of climate change, particularly in the driest scenario, were greater. While at-risk areas rose from 67% to 70-71%, high-risk areas climbed from 3% to 5-8%.

Keller said: "These findings allow us to identify the river areas across England and Wales that should be targeted for risk assessment. And they pinpoint the sewage treatment works that are failing the Environmental Quality Standard, making it possible to introduce changes in sewage treatment. We can also apply our methodology to the study of other chemicals."



Green chemistry: a silver bullet?

With the cost of eradicating EDCs from the UK's rivers estimated at £30bn, we must explore the potentially game-changing role that green chemistry could play in tackling oestrogens, and other pollutants, in the environment.

This is the argument of Dr Alice Baynes, postdoctoral research fellow at Brunel University, who highlighted the recent development of TAML® catalysts by Carnegie Mellon University in the United States. They can rapidly and efficiently purify water of many chemicals, including pharmaceuticals and EDCs.

As Baynes put it: "If there's enough evidence to require new EU regulation that limits concentrations of EDCs then we'll have to start cleaning up our rivers."

The UK faces an uphill struggle. According to 2007 data from Eurostat, 50% of the UK's wastewater is still subject to 'secondary' treatment (e.g. biological filters), which only removes 50-90% of oestrogens.

The other half is subject to 'tertiary' treatment (e.g. sand filtration), which can remove 90-99% of oestrogens. This is in stark contrast to the Netherlands where 95% of wastewater goes through the tertiary treatment process already.

Over a six-month period at two sewage works Baynes and Dr Amy Filby, then an associate research fellow at Exeter, evaluated the comparable effectiveness of three types of tertiary wastewater treatment technologies in removing oestrogen: the use of Ozonation (O3), Granular Activated Carbon (GAC) and Chlorine Dioxide (ClO2).

Biological analysis of a study on the fathead minnow by Filby revealed that GAC and ClO2 were very effective (98% and 96% respectively) in removing oestrogens but O3 treatment fell short (74%).

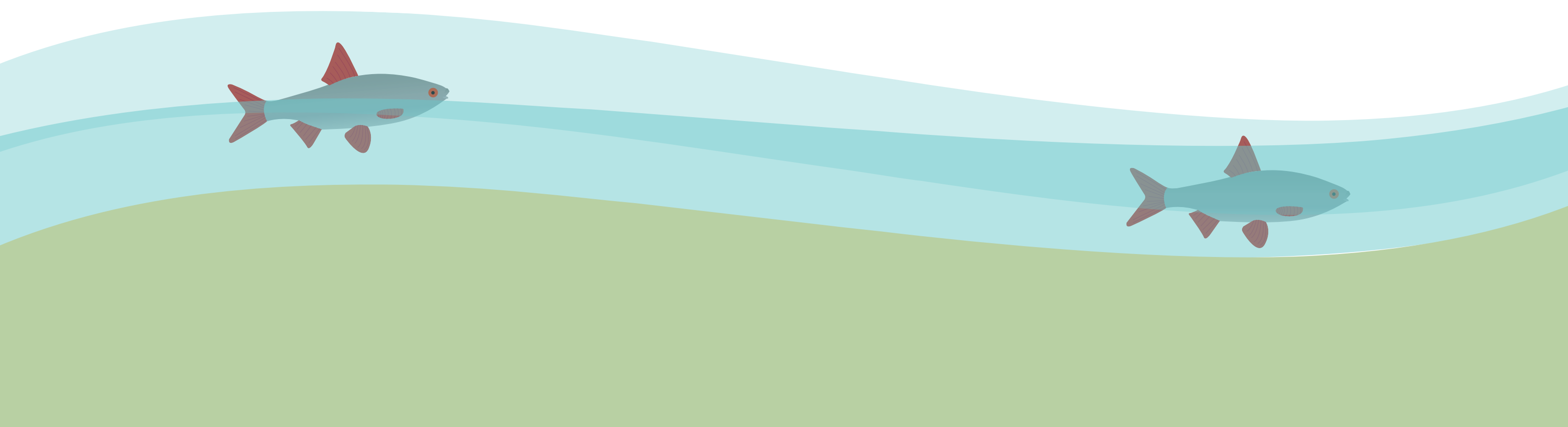
But a further study that exposed male roach to treated wastewater for six months gave rise to what Baynes describes as "very surprising" results.

Baynes explained: "ClO2 was found to significantly induce intersex in males. As expected, GAC was the most effective but is by far the most expensive. The much cheaper process of sand filtration was also extremely effective at removing oestrogens, but not down to proposed environmental quality standard levels.

"However the cost of upgrading 1,400 wastewater treatment works in England and Wales to a GAC system would total more than £30bn. Furthermore, the carbon footprint of the GAC process is significant."

Could TAML® then be the answer? A lab experiment at Brunel, led by Baynes, exposed fathead minnows to EE2-dosed water for 21 days. It showed TAML® succeeded in significantly reducing EE2 concentrations.

Baynes said: "These initial results are very promising. We now need to test its performance on a much wider scale. Given the costs associated with GAC, green chemistry solutions could be more cost effective and sustainable – the operating costs and energy usage of a TAML plant are estimated at 3-5 times and 5-10 times lower than a comparable Ozone plant."



Up for debate: the way forward

Evidence challenge

The EU chose not to place EE2 on the priority substances list because of an "insufficient evidence base" and a number of obstacles block a change to the status quo, according to EA research scientist Katie Sumner.

Questioning whether measurable declines in fish populations could be unequivocally linked to EDCs, she said: "If we take action on one chemical then do we go through others one by one? Or can we analyse a group of chemicals together?"

Responding to Tyler's comment that the EA has been reluctant to invest in tackling EDCs, Sumner said: "It's difficult to get something prioritised when there are so many other problems which you know how to address, for which you can measure the benefits and which don't cost that much. It is very difficult to attract the financial support required to tackle these sub-lethal issues."

Unrealistic expectations?

It is unlikely that scientists could ever deliver the degree of certainty the government wants over the dangers posed by EDCs in order to justify the costs in tackling them, contended Susan Jobling. She said: "The EA identified EDCs as a major problem in 2004 but the issue has been lost in the reeds."

Sumner said that unlike EE2 no other substance on the EU's priority list has needed to demonstrate a population-level impact. She speculated whether further scientific evidence that endocrine disruption is widespread across Europe would be enough to regulate on EE2.

Only three substance classes in the world – tributyltin (used in anti-fouling boat paint), selected organochlorine (e.g. pesticides) and diclofenac (a pharmaceutical anti-inflammatory) – have been shown to reduce population levels, said Tyler. "The level of evidence we are being asked for is almost insurmountable. If we have sufficient weight of evidence then we have to move this forward otherwise we won't do it for anything."

Setting a precedent

Any decision on EE2 will shape the regulatory response to chemical pollution of the environment for the foreseeable future, Jobling warned. She said: "Whatever we do with EE2, it sets a precedent. Lobbying from the pharmaceutical industry, water industry and governments is preventing action."

Baynes added: "Advanced wastewater treatment would not only remove EE2. There are many other pollutants – EE2 is the tip of the iceberg. Humans would benefit from cleaner drinking water too."

Next steps

TAML could hold the key but it remains unproven on a large scale. Jobling said: "If TAML can remove substances already on the priority list we'll have more chance of persuading regulators to act on EE2. Now we need funding for research into whether TAML is capable of removing all these chemicals from our rivers."

Attempts to introduce regulation of EE2 rely on public support. But, as Baynes explained, people have limited understanding of how pharmaceuticals end up in water supplies. Tyler said: "In the UK around 50% of pharmaceuticals are not taken and much of this is just chucked away – straight into the water supply. In Germany legislation ensures pharmaceuticals are disposed of more responsibly."

Getting the public onside is crucial, said Steve Axford, Vice-President of the Institute of Fisheries Management, especially if the cost of sewage treatment upgrades falls on the taxpayer. He said: "The water industry is in a difficult position. They are held accountable by the public, who want to know why their water bills are going up. We must engage the public to explain the issue of EDC pollution in our rivers."

2 Mers Seas Zeeën INTERREG IV A "Investing in your future" Crossborder cooperation programme 2007-2013 Part-financed by the European Union (European Regional Development Fund)

TC2N Trans Channel Neuroscience Network

ifm Institute of Fisheries Management

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To find out more about combined research at the University of Exeter and Brunel University into the environmental impact of endocrine disrupting chemicals, or to explore opportunities for collaboration, please email Professor Susan Jobling susan.jobling@brunel.ac.uk or call 01895 266284, or Professor Charles Tyler c.r.tyler@ex.ac.uk; 01392 264450.