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Does exposure to domestic wastewater effluent (including steroid estrogens) harm fish populations in the UK?

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Highlights

ABSTRACT

Historic fisheries data collected from locations across the UK over several years were compared with predicted estrogen exposure derived from the resident human population. This estrogen exposure could be viewed as a proxy for general sewage (wastewater) exposure. With the assistance of the Environment Agency in the UK, fisheries abundance data for *Rutilis rutilis* (roach), *Alburnus alburnus* (bleak), *Leuciscus leuciscus* (dace) and *Perca fluviatilis* (perch) from 38 separate sites collected over 7 to 17 year periods were retrieved. From these data the average density (fish/m^2/yr) were compared against average and peak predicted estrogen (wastewater) exposure for these sites. Estrogen concentrations were predicted using the LF2000-WQX model. No correlation between estrogen/wastewater exposure and fish density could be found for any of the species. Year on year temporal changes in roach population abundance at 3 sites on the middle River Thames and 4 sites on the Great Ouse were compared against estrogen exposure over the preceding year. In this case the estrogen prediction was calculated based on the upstream human population providing the estrogen load and the daily flow value allowing concentration to be estimated over time. At none of the sites on these rivers were temporal declines in abundance associated with preceding estrogen (effluent) exposure. The results indicate that, over the past decade, wastewater and estrogen exposure has not led to a catastrophic decline in these four species of cyprinid fish.

Key Words: Wastewater, estrogens, roach, cyprinid fish, population

1. Introduction

For thousands of years man's activities have disturbed the river environment. The river can be exploited as a food, drinking water and irrigation resource, used as a highway for goods transport, a generator of energy, and a conduit for our waste products. Rivers are also feared as a source of flooding, so they may be excavated to ensure they act as efficient drains. Many of these human activities have had damaging impacts on the river as a habitat for fish. The fish that live in our rivers are at, or near, the top of a complex food web. Unfortunately, the abundance of fish in rivers have not been consistently recorded through history, but it would appear that serious declines in some major rivers in the UK occurred from the 1930s to 1950s. Inadequate treatment of sewage and industrial waste led to the disappearance of fish in the lower reaches of big rivers like the Trent (Mann, 1989), Mersey (Jones, 2006) and Thames rivers (Wheeler, 1979). Fortunately, an increasing appreciation of the amenity value of rivers, legislation, industrial decline, and more investment in water treatment has largely eliminated the problem of gross organic pollution, at least in the UK, with the exception of occasional combined sewer overflows. However, it has been increasingly recognised that as individuals we now consume many more pharmaceuticals and personal care products (PPCPs) than ever before. Sewage treatment plants (STPs) were never designed to remove all of such micropollutants. Could it be that we are now harming our river environment and fish through this insidious 'invisible' pollution (Daughton and Ternes, 1999)?

When we examine the tissue of freshwater wild fish, we can certainly find many hydrophobic pollutants present (Jurgens *et al.*, 2015), but what evidence do we have that chemicals can harm fish individuals and populations? There are, of course, examples of extreme one-off pollution events with industrial, oil and farm waste killing fish (Giger, 2009; Kubach *et al.*, 2011; Kennedy *et al.*, 2012; Eros *et al.*, 2015). But our concern here is with chronic pollution. The strongest evidence seems to be related to metals. Soil acidification thanks to 'acid rain' from coal combustion led to the release of the toxic monomeric forms of Al into upland streams and lakes, leading to fish kills in the 70s and 80s (Henriksen *et al.*, 1984).   Freshwaters with high metal concentrations associated with mine waste or heavy industry have also had a recorded impact on fish populations (Filipek *et al.*, 1987).

Thus, there are examples of fish kills due to exposure to acutely toxic chemicals at pollution hot-spots. But what of the chemicals routinely discharged in domestic sewage effluent? The chronic sub-lethal phenomena of endocrine disruption, associated with sewage effluent, has had and continues to have a major influence on our thinking regarding PPCPs. There is overwhelming evidence that a ubiquitous component of sewage effluent has led to endocrine disruption effects in resident wild roach (*Rutilis rutilis*) (Jobling *et al.*, 1998; Jobling *et al.*, 2006). The most likely agents being the natural and synthetic steroid estrogens excreted by humans (Desbrow *et al.*, 1998). Similarly, there is evidence that increasing exposure to wastewater effluent elevates the level of the stress hormone cortisol in fish, at least in stickleback (Pottinger *et al.*, 2016). Recently, a disastrous decline in Asian vultures has been strongly linked to the non-steroidal anti-inflammatory agent diclofenac (Oaks *et al.*, 2004). Given that diclofenac is a common constituent of sewage effluent, this has now risen as a concern for fish in rivers too (Schwaiger *et al.*, 2004; Cuklev *et al.*, 2011). So now both the steroid estrogens and diclofenac have been identified by the European Union as requiring special monitoring, with a view to control at a later stage (COM(2011)876). It is also recognised that freshwater fish will be exposed to a wide range of pharmaceuticals and this chronic exposure is a concern (Fent *et al.*, 2006). Given the fear and uncertainty over this chronic exposure to PPCPs, there are increasing arguments that an end of pipe solution at STPs will be needed to protect aquatic wildlife (Eggen *et al.*, 2014; Oehlmann *et al.*, 2014; Stamm *et al.*, 2015). But is this fear justified? We know that if the synthetic estrogen ethinylestradiol reaches a high enough level some fish populations will collapse (Kidd *et al.*, 2007). It can be presumed that our consumption of PPCPs has been growing steadily since the 1970s (Richardson and Ternes, 2014), so it would seem a reasonable question to ask how fish populations have fared since then? Rather surprisingly, examining responses in the abundance of wildlife populations to chemical or estrogen exposure has not been a frequently asked question in the aquatic environment (Mills and Chichester, 2005; Johnson and Sumpter, 2016). In contrast, such approaches are seen as central in the terrestrial environment, such as with neonicotinoid pesticides and bees (Woodcock *et al.*, 2016).

Unfortunately, until recently there has been little systematic collection of data on fish populations in rivers. However, some species that were relatively common in many UK lowland rivers have declined or disappeared, was this due to chemicals or estrogens even? These include the migrating salmonids (*Salmo salar* and *Salmo trutta)* and Barbel (*Barbus barbus*) but these declines are most closely linked with habitats becoming unsuitable (Johnson and Sumpter, 2014). We are sadly aware that there has been a decline in eel numbers in many parts of the world. But the evidence suggests that the eel decline, which started in the early 1980s, occurred in a period of reduced chemical challenge (Jurgens *et al.*, 2015). Eel populations appeared to have done better in the much more polluted post-war period. There are, however, quite a lot of encouraging information on cyprinid fish, such as bream (*Abramis brama*), whose average length for 5 year olds increased from 1966 to 1976 in the Dutch Rhine (Slooff and Dezwart, 1983) and whose condition steadily improved in several major German rivers from 1992 to 2014 (Teubner *et al.*, 2015). Data appear to show that UK cyprinid populations have been recovering since reaching a low-point in the 1950-1970s period (Mann, 1989; Robinson *et al.*, 2003). However, although encouraging, the limited information available is too coarse and not sufficiently focused to address whether the chemicals routinely present in domestic sewage effluent are harming wildlife populations.

To begin addressing the question in a more systematic way, we compared routine fish population monitoring data collected in the UK by the Environment Agency of England and Wales with predicted wastewater effluent exposure. This study tested the following hypotheses:

- Any fish population (average density) will be severely harmed by average exposure to domestic wastewater
- Any roach population will be severely harmed by temporal increases in domestic wastewater exposure

It should be pointed out the intention of this study was not to identify the most important environmental factors that stimulate fish population abundance and aid recruitment in UK rivers. The complex interactions of flow, temperature, habitat, disease, and position of the Gulf Stream in the North Atlantic, amongst others, are all likely to be playing a role together. Nor will simple population data, such as we use here, reveal sub-lethal impacts that could hamper fish performance and well-being. The aim was to see whether it was possible to rule out sewage and estrogen exposure as having a consistent and seriously damaging impact on fish populations.

2. Materials and methods

2.1. Fisheries monitoring data

The fisheries data were collected for the National Fisheries Monitoring Programme by the Environment Agency of England and Wales. Only sites where the electro-fishing method was used for counting were examined. The method involves a boom boot applying a 50 Hz pulsed DC current to the water. Downstream runs may be up to 2 km between dividing locks or be of shorter duration, such as around islands or weir pools (Table 1). The sampling runs were mainly carried out in close proximity to the river margins, as the method is somewhat ineffective at depths greater than 1.5 m. The electric current stuns the fish, which on floating to the surface are collected, identified, counted, and their fork length recorded before being returned to the water. For the data examined in this study, fish down to 21 mm in length were recorded. The fish counts were recorded and can be normalised to the survey area. This sampling method is not suitable for counting bream, which are most numerous in the deeper mid-channel. Smaller species such as bullhead (*Cottus govio*), stone loach (*Noemecheilus barbatulus*), minnow (*Phoxinus phoxinus*) and stickleback (*Gasterosteus aculeatus*) were noted only as presence/absence. The method is semi-quantitative, but most importantly it was carried out in the same way, at the same time, and in the same locations for 10 years or more. Thus, a site on the middle stretch of the Thames might always be sampled in July. For logistical reasons not all river sites were sampled in the same month. So for one site this may be a regular sampling date in May and for another it might be October. Occasionally the fisheries team might have to delay sampling if river conditions were very adverse. The fish recorded with the greatest regularity and the highest numbers were roach, bleak (*Alburnus alburnus*), dace (*Leuciscus leuciscus*) and perch (*Perca fluviatalis*).

A central assumption behind this study is that fish counted at a particular location are ‘native’ to that area and remain exposed to sewage effluents in their local area throughout their lives. The fish that were examined in this study are non-migratory and so would be presumed to be born and die in the same river and indeed many authors refer to fish having a ‘home range’. However, fish will move naturally depending on their life stage, such as movement to spawning grounds, and depending on the time of day, as they change from foraging to avoiding predators (Baade and Fredrich, 1998; Reichard and Jurajda, 2007; Nunn *et al.*, 2010). Movement may also be forced due to high flow events or man-made habitat degradation (Bruylants *et al.*, 1986; Lucas, 2000). Movement can be artificially restricted by rivers being controlled by locks and weirs, such as occurs on the Thames. But much of the available information suggests that adult roach largely remain local to a small area, perhaps with a range of only 70 to 400 m along a river (Williams, 1965; Baade and Fredrich, 1998; Penczak, 2006) and more recently it has been revealed that roach can have considerable, and stable, genetic diversity within a river network (Hamilton *et al.*, 2014), supporting a view of distinct populations. Similarly, genetically distinct populations of perch have been identified across distances of only a few km in Sweden (Bergek and Bjorklund, 2009), with each fish having a range of up to 225 m (Williams, 1965; Penczak, 2006). The dace would appear to range between 1 and 3 km (Clough and Beaumont, 1998; Penczak, 2006). The movement and range of bleak is unclear from the literature. In summary, whilst there is not complete consensus on the degree of cyprinid movement, there is evidence that the majority of roach, dace and perch adults would reside within 3 km of the sampling point, with many remaining within 500 m. Assuming fish sampling re-occurs at the same location, month and time of day, it is probable that any fluctuations in population size observed over time would not be due to the vagaries of fish migration.

However, it must be admitted that different river sites may be more or less amenable to electro-fishing, and different teams of people are responsible in different regions. Thus, the effort that one team puts into electro-fishing in one region may be different from a different team in a different region. To reduce some of these sampling anomalies, comparisons against estrogen (effluent) exposure was only made within a single river/region, rather than between them. In an attempt to normalise the results within a river, average fish density rather than fish numbers was used. Thus, a comparison of fish density from these locations against sewage effluent exposure remains crude and only serious population failure would be likely to be discernible. To further reduce sampling anomalies in the second study, trends at single sites over time were followed. It was presumed that the same team returning to the same site each year would provide consistency.

2.2. Calculating effluent and steroid estrogen exposure

In this study steroid estrogen exposure was used as a proxy for sewage effluent/wastewater exposure. The two are intimately linked as the estrogen concentration in the prediction used here is a function of the local human population and dilution. The most potent steroid estrogens in sewage effluent are estradiol (E2), estrone (E1) and ethinylestradiol (EE2), their combined estrogenic impact can be calculated as an overall estradiol equivalent (EEQ). Thus, high predicted estrogen exposure would represent a high sewage effluent exposure. At any point in the river network of England and Wales it is possible to estimate the steroid estrogen exposure using the LF2000-WQX model. The LF2000-WQX model was originally designed to estimate river flows at ungauged sites and intended for the development of catchment and regional water resource assessments (Holmes *et al.*, 2005). By the incorporation of an estrogen predictive model (Johnson and Williams, 2004), it was further developed to predict estrogen concentrations throughout the 357 catchments of England and Wales (10,313 individual river reaches comprising 21,452 km and run using a 40 year climate dataset) which contains physical and spatial data for over 2000 STPs serving over 29 million people (Williams *et al.*, 2009). The model output is moderated by dilution and in-stream degradation for the estrogens.

This approach to predict estrogen exposure has been tested against measured concentrations and found to predict overall estrogen exposure in sewage effluent and receiving waters to an acceptable degree of accuracy for the UK (well within one order of magnitude) (Jobling *et al.*, 2006; Huo and Hickey, 2007; Balaam *et al.*, 2010; Williams *et al.*, 2012). The Environmental Agency of England and Wales (Agency, 2008) recommend that the overall EEQ should be calculated as follows, based on their relative potencies:

$$[EEQ] = \frac{[EE_2]}{0.1} + \frac{[E_2]}{1} + \frac{[E_1]}{3}$$

Table 1.

Site location (national grid reference), record duration, length and area fished.

Catchments	Sites	National grid reference	Start & length of records (years)	Length of river fished (m)	Area of river fished (m^2)
River Thames	Boulters Weir Stream	SU9040082700	1995-2014 (16)	990	12,000
	Boveney Main	SU9454777812	1995-2014 (17)	2,100	126,000
	Bray-Boveney, Upper Main Channel	SU9109879702	1995-2014 (17)	1700	85,000
	Bray Weir Pool	SU9096979720	2000-2014 (15)	130	6,500
	Cliveden Island	SU9086883984	1998-2014 (16)	170	3,400
	Odney Weir Stream, Cookham.	SU9050085500	2002-2014 (12)	600	24,400
	Marlow-Cookham Upper Main Channel	SU8730086500	1995-2014 (16)	2000	140,000
	Molesey - Thames Ditton Island, Upper Main Channel	TQ1600067700	1995-2014 (13)	1600	148,200
	Molesey Weir Pool	TQ1492768955	1995-2014 (15)	400	20,000
	Ham Loop	SU9980075400	1995-2014 (16)	2300	103,500

Catchments	Sites	National grid reference	Start & length of records (years)	Length of river fished (m)	Area of river fished (m^2)
	Penton Hook to Chertsey (Laleham Main)	TQ0485069221	1995-2014 (16)	2800	168,000
	Desborough Cut	TQ0788065972	1995-2014 (16)	1,990	40,000
	Sunbury Weirpool	TQ1047468091	1995-2014 (13)	500	16,500
	Caversham-sonning (Margin)	SU7378574196	2001-2013 (13)	4,230	190,350
	Cleeve-Goring (Margin)	SU5970081300	2001-2013 (13)	1,000	40,000
	Hambleden-Hurley (margin)	SU7985983648	2001-2013 (13)	1,000	294,500
	Shiplake-marsh (Margin)	SU7776980072	2002-2013 (12)	4,800	240,000
	Whitchurch to Mapledurham (Margin)	SU6550877460	2001-2013 (13)	3,670	183,500
River Great Ouse	Wolverton Mill	SP7911941157	2003-2011 (9)	120	1485
	Newport Pagnell	SP8820044100	2003-2011 (9)	155	2,945
	Clifton Reynes	SP8960050700	2003-2011 (9)	121	1,996
	Turvey	SP9370052600	2003-2011 (9)	95	1,615
	Oakley	TL0120052900	2003-2011 (9)	140	2,490
River Calder	Brighouse Industrial Estate	SE1688421974	1999-2008 (7)	300	7,500
	Chantry Bridge	SE3398320073	2002-2012 (10)	200	8,000
	Cornmill Weir	SE1688321973	1999-2010 (8)	400	8,800
	Dewsbury	SE2404020932	2004-2012 (7)	250	6,750
River Aire	Castleford	SE4280026000	2001-2009 (8)	300	15,000
	Chappel Haddlesey	SE5760023300	2002-2010 (8)	400	16,000
	Thwaite Weir	SE3270031300	2002-2007 (6)	400	6,600
	Kirkstall	SE2640035000	2001-2007 (7)	800	16,000
River Avon	Chippenham	ST9193172909	2003-2014 (9)	90	1,215
	Christian Malford	ST9575078900	1999-2014 (10)	100	1,450
	Great Somerford	ST9675083280	2002-2014 (11)	77	546
	Lacock	ST9230068030	2003-2014 (9)	70	840

2.3. Comparing fish abundance with temporal changes in sewage effluent (estrogen) exposure

Given the dynamic nature of many rivers, the exposure, which is a feature of dilution, can vary dramatically over the course of a year and between years (Johnson, 2010). Thus, if chemicals in effluent, such as estrogens, are problematic for fish populations it might be expected that years with high exposure could be identified by a subsequent reduction in abundance. The most numerous fish in these lowland UK rivers are roach, and because we have much information on their sensitivity to estrogens, this part of the study focused on the roach.

The next question is what are the ages of the roach which have been sampled each year? During the electro-fishing process the lengths of fish were recorded. However, fish growth rates are variable, so length is not an absolute guidance for age. But a review of UK data suggests roach up to 115 mm would be considered within the normal range of fish being up to 2 years of age (Britton, 2007). By this measure, for the period of 2002 to 2013, on average 44-48% of roach at the Great Ouse sites and 33% to 42% of roach at the Middle Thames sites were up to 2 years of age (Table S1). Therefore, the conditions of the preceding 12 months could be seen as being highly influential to the development of a substantial proportion of the roach population present. Thus, in this analysis we are tracking changes in the fish population at the same site over several years with respect to their estrogen (effluent) exposure over the preceding year.

The estrogen model (Williams *et al.*, 2009) predicts an effluent loading of 3.49 µg EEQ per capita per day. Once the daily flow (m^3/s), taken from the nearest automatic flow gauging station (Table S2) and total upstream population served by STPs is identified, so the daily EEQ concentration as ng/L (calculated here as $\mu g/m^3$, which is equivalent to ng/L) of a site can be calculated by:

EEQ (d) = (3.49 * P) / (F* 86,400),

Where EEQ (d) is daily EEQ concentration (ng/L)

P is total upstream population,

F is daily flow (m^3/s),

86,400 is the total number of seconds in a day.

So in this case the abundance of roach for a particular time point, say 12th July 2010, was compared with the average or peak estrogen (EEQ) predicted for the period 12th July 2009 to 12th July 2010, or for those in the windows of April-June 2009, July 2009. Comparisons were also made with average of peak flow of the preceding year. The comparisons were made by standard linear regression.

3. Results and discussion

3.1. Fish Density compared to estrogen (effluent) exposure

Depending on the site, the fisheries monitoring records start from 1995 to 2004, and thus the average density of fish per site were calculated based on a minimum of 7 to a maximum of 17 years of fisheries data

(Table 1). The predicted mean EEQ exposure at these sites ranged between 0.6 ng/L and 3.2 ng/L, a five-fold difference (Table 2). If we were to assume water use of 200 L per capita per day, then this would represent a wastewater content of 3 to 18% in the river. Whilst the 90%ile exposure the EEQ exposure ranged from 1.2 ng/L to 6.4 ng/L, which would indicate a wastewater content of 7 to 37%. What might we expect from such expected estrogen exposure? Based on a field study, at EEQ values over 1.6 ng/L between 20% of fish would be expected to have oocytes in testes and 15% feminised reproductive ducts. This rises to 30% and 20% respectively at EEQ values over 16 ng/L (Jobling *et al.*, 2006). So there is some dose dependency. Thus, many of the monitoring sites in this study would be expected to lead to detectable endocrine disruption. What might this mean for fish reproduction? In a breeding experiment with moderately to severely intersex 'male' roach it was found that reproductive success declined (Harris *et al.*, 2011).

Over this reporting period no relationship can be found between average roach, bleak, perch or dace density within any of the rivers and the mean or 90%ile EEQ (general effluent) exposure over a 7 to 17 year time period (Table 3). In particular, no significant damage to the population (very low population density) was associated with wastewater/estrogen exposure. However, there is a suspicion that wastewater effluent in the Great Ouse has a unique component that is negatively affecting roach and perch density although this was not significant (Table 3).

Table 2

Predicted estrogen exposure using the LF2000-WQX model (sewage effluent exposure proxy) compared to average fish density at each of the monitoring locations over the recording period (7-17 years)

Fish monitoring locations	Mean EEQ (ng/L)	90%ile EEQ (ng/L)	Roach density (fish/m^2)	Bleak density (fish/m^2)	Perch density (fish/m^2)	Dace density (fish/m^2)
R. Thames						
Boulters Weir Stream	1.9	3.3	0.00072	0.0017	0.00027	0.00099
Boveney Main	2	3.5	0.0005	0.00025	0.00016	0.0003
Bray Boveney upper main	1.9	3.3	0.00278	0.00164	0.00242	0.00406
Bray Weir pool	1.9	3.3	0.0798	0.0557	0.0113	0.0272
Cliveden Island	1.9	3.3	0.0215	0.0068	0.00247	0.00067
Odney Weir stream	1.9	3.3	0.00695	0.0284	0.00113	0.00074
Marlow Cookham	1.8	3.1	0.00867	0.00341	0.00301	0.00168
Molesey Thames Ditton	1.9	3.4	0.00017	0.00002	0.00015	0.00006
Molesey Weir pool	1.9	3.4	0.0534	0.0365	0.0186	0.00888
Ham Loop	2.2	3.7	0.00459	0.0348	0.00205	0.00154
Penton Hook	2.7	3.8	0.01012	0.00215	0.00418	0.00077
Desborough Cut	2.1	3.5	0.003	0.00095	0.00632	0.00625
Sunbury Weir pool	1.9	3.4	0.1596	0.0432	0.0106	0.029
Caversham	1.3	2.4	0.00156	0.00013	0.00008	0.0001
Cleeve Goring	1.7	3.1	0.00238	0.00091	0.0001	0.00008
Hambledon	1.7	3.1	0.00043	0.00005	0.00005	0.00008

Fish monitoring locations	Mean EEQ (ng/L)	90%ile EEQ (ng/L)	Roach density (fish/m²)	Bleak density (fish/m²)	Perch density (fish/m²)	Dace density (fish/m²)
Shiplake Marsh	1.8	3.1	0.00062	0.00019	0.00004	0.00005
Whitchurch Mapledurham	1.5	2.8	0.00092	0.00015	0.00006	0.00009
R. Great Ouse						
Wolverton Mill	0.9	2	0.1139	0.00088	0.0327	0.00565
Newport Pagnell	1.5	3	0.0398	0.0307	0.01162	0.03362
Clifton Reynes	2.7	5.1	0.05968	0.01891	0.00831	0.0139
Turvey	2.6	5	0.0599	0.02888	0.005	0.0184
Oakley	2.3	4.5	0.0425	0.0139	0.00384	0.02282
R. Aire & Calder						
Brighouse	1.4	2.9	0.00597	0	0.00196	0.0002
Chantry Bridge	2.6	4.5	0.0366	0.0003	0.00215	0.00288
Cornmill Weir	1.4	2.9	0.00246	0	0.00104	0.00017
Dewsbury	2	3.8	0.01338	0	0.00025	0.00216
Castleford	3.2	6	0.02813	0.00197	0.0232	0.00096
Chappel Haddlesey	3.1	5.5	0.00314	0.00009	0.00161	0
Thwaite Weir	2.3	4.6	0.00277	0	0.0008	0.00072
Kirkstall	2.3	4.7	0.00068	0	0	0.0013
R. Avon						
Chippenham	1	2.1	0.26	0.0426	0.023	0.0315
Christian Malford	0.8	1.7	0.11327	0.0136	0.01147	0.01314
Gt Somerford	0.6	1.2	0.0327	0.00892	0.01004	0.03946
Lacock	1.3	2.9	0.1113	0.00676	0.00703	0.0487

Table 3

Attempted linear correlation expressed as R^2 values and trend (positive or negative) between the density of the different fish species within a particular river and estrogen (sewage effluent) exposure

River	Estrogen exposure	Roach density	Bleak density	Perch density	Dace density
R. Thames	Mean EEQ	0.0033	0.0252	0.0487	0.0049
	90%ile EEQ	0.0274	0.0867	0.0934	0.0301
R. Gt. Ouse	Mean EEQ	0.3495 (-ve)	0.2402 (+ve)	0.7564 (-ve)	0.0203
	90%ile EEQ	0.3442 (-ve)	0.299 (+ve)	0.7585 (-ve)	0.0186
R. Aire & Calder	Mean EEQ	0.1928	0	0.298 (+ve)	0.0304
	90%ile EEQ	0.1132	0	0.3392 (+ve)	0.0105
R. Avon	Mean EEQ	0.184	0.0057	0.0029	0.207 (+ve)
	90%ile EEQ	0.0635	0.0007	0.0107	0.2064 (+ve)

Note no correlation was significant at P 0.05 level

3.2. Comparing roach abundance with the preceding 12 months of estrogen (effluent) exposure

As an example, variation in the size of a roach population for Caversham-Sonning on the R. Thames can be seen in Figure 1. It will be noted that roach numbers recorded here varied by up to 18-fold over the period 2001-

2013. When comparing the rises and falls in the roach population over the period of 2001 to 2013 for the sites on the middle Thames, there appeared to be a relatively weak positive relationship with sewage effluent exposure of the preceding year, particularly for the period of April to June of the previous year (Table 4) and a weak negative one with flow (although this was not significant). For the Great Ouse a positive relationship with sewage effluent exposure cannot be clearly seen, although a strongly significant negative one with flow at some sites was apparent (Table 5). But these positive or negative relationships between the roach and estrogens/wastewater or flow cannot be attributed with certainty. Other variables may be playing a role. However, there does not appear to be a consistent pattern of seriously negative impacts of estrogens/wastewater from the previous year on roach numbers.

It has been argued that successful fish recruitment is related to the environmental conditions in the first few weeks after hatching of the eggs. Negative correlations have been seen with river flow, where too much water flushes the juveniles out of the river (Mann and Bass, 1997; Nunn *et al.*, 2007), and positive correlations with temperature (juveniles grow faster and stronger and so become better foragers and better able to maintain themselves against the current) (Mann, 1997; Beardsley and Britton, 2012). Roach are recorded as the most common of our lowland fish and it has been noted that around a month after hatching the diet of juveniles is dominated by grazing on biofilms (Mann, 1973; Mann *et al.*, 1997). It could be hypothesised that in periods of low flow in late spring and summer, elevated dissolved organic concentrations would stimulate these biofilms, which are a useful food source in a critical period of development for the juvenile roach. The observation of fish populations on occasions appearing to prosper in situations of lower water quality associated with wastewater has been noted before (Mills and Chichester, 2005; Liu *et al.*, 2015).

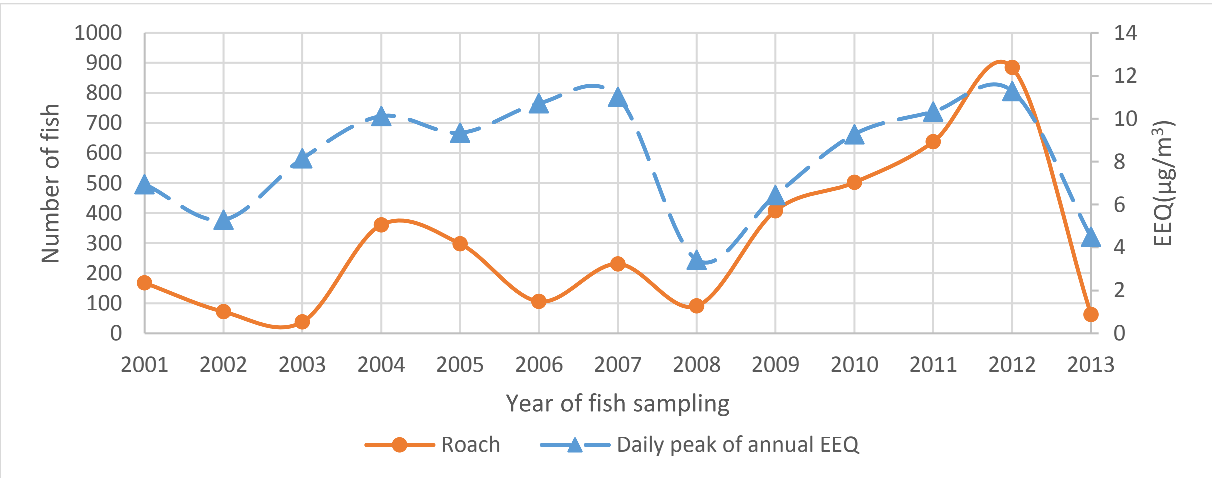

Fig. 1. Comparison between number of roach monitored each July and the maximum estrogen concentration (EEQ) predicted to have occurred over the preceding year at the Caversham Sonning site on the River Thames from 2001 to 2013

Table 4

R^2 value (standard linear regression) for correlations between numbers of roach and environmental variables for the Middle River Thames over 14 years of monitoring (2001-2013)

Environmental variables	Caversham-Sonning(R^2)	trend if any	Shiplake-Marsh (R^2)	trend if any	Whitchurch-Mapledurham (R^2)	trend if any
Average EEQ of preceding April-June	0.5*	+ve	0.61*	+ve	0.28	+ve
Peak EEQ of preceding April-June	0.34*	+ve	0.34*	+ve	0.32	+ve
Average EEQ of preceding July	0.4*	+ve	0.21	+ve	0.3	+ve
Peak EEQ of preceding July	0.33*	+ve	0.19		0.28	+ve
Average flow of preceding year	0.29	-ve	0.13		0.11	
Peak flow of preceding year	0.20	-ve	0.03		0.05	
Average EEQ of preceding year	0.44*	+ve	0.34*	+ve	0.25	+ve
Peak EEQ of preceding year	0.33*	+ve	0.14		0.21	+ve

* R^2 values shown with an asterisk are significant at P 0.05 level

Table 5

R^2 value (standard linear regression) for correlations between numbers of roach and environmental variables at River Great Ouse over 9 years of monitoring (2003-2011)

Environmental variables	Clifton-Reynes（R^2）	trend if any	Newport-Pagnell （R^2）	trend if any	Oakley（R^2）	trend if any	Turvey（R^2）	trend if any
Average EEQ of preceding April-June	0.09		0.05		0.06		0.08	
Peak EEQ of preceding April-June	0.2	+ve	0.06		0		0	
Average EEQ of preceding July	0.14		0.1		0		0	
Peak EEQ of preceding July	0.02		0.02		0.01		0.02	
Average flow of preceding year	0.60*	-ve	0.27	-ve	0.06		0.16	
Peak flow of preceding year	0.58*	-ve	0.09		0.10		0.29	-ve
Average EEQ of preceding year	0.37	+ve	0.17		0.03		0.09	
Peak EEQ of preceding year	0.30	+ve	0.08		0.04		0.08	

* R^2 values shown with an asterisk mean significant at P 0.05 level

4. Conclusions

At 38 sites across England (UK), the density of roach, bleak, dace and perch populations over a period of 7 to 17 years, starting from the early 2000 period, were not obviously linked to estrogen (sewage effluent) exposure. Hence it is possible to conclude that wastewater was not a clearly damaging factor on fish density. As a test case, the temporal rises and falls of roach populations in the middle Thames and Great

Ouse were compared over several years with the preceding 12 months of sewage effluent exposure, and again no severe negative relationships found. Thus, returning to the original hypotheses:

- Any fish population (density) will be severely harmed by average exposure to domestic wastewater
- The roach population will be severely harmed by temporal increases in domestic wastewater exposure

These hypotheses appear to have been falsified, at least as far as the sites, fish species and time periods examined here. However, this does not mean that there are no problems associated with chemical contaminants in effluent. Chemicals in wastewater may be harming other animal groups, such as invertebrates, or other fish species, perhaps at other sites and at other time periods. Nor can we say that the chemicals in sewage effluent are benign for fish health, although it does appear from this limited study that they are not severely damaging population abundance. This type of analysis has many limitations, yet the picture that emerges from these preliminary studies is that exposure to wastewater effluent in the recent past, with all its estrogens, PPCPs and complex mixtures of chemicals, has not been catastrophic for populations of cyprinid fish in the same way that TBT from boats was for mollusc populations (Langston *et al.*, 1990). It must be admitted that only having consistent monitoring records back to the late 1990s and occasional records back to the 1970s we cannot say whether fish numbers or densities should be much higher than they are now.

We would encourage other scientists around the world to search for more data, sites and fish species, and utilise perhaps more suitable analytical techniques, to assess whether routine chemicals in sewage effluent are harmful to fish populations. The information gathered here may be seen as encouraging and perhaps reflects a greater resilience in these wild fish populations then some might have expected (Reid *et al.*, 2016).

Whilst there does seem to be increasing enthusiasm to examine, assess and perhaps in the future even regulate sewage treatment plants based on toxic or harmful effects detected by a suite of bioassays (Busch *et al.*, 2016; Schroeder *et al.*, 2016), the link with whole organisms and populations remains unclear (Power and McCarty, 1997; Mills and Chichester, 2005). We would argue that knowledge of the trends in wildlife populations with respect to chemical exposure is actually the most critical factor and so long-term wildlife monitoring should be vigorously supported and maintained.

Acknowledgements

The authors are grateful for support from the CEH Science budget provided by NERC and fisheries data provided by the Environment Agency of England and Wales. The authors are grateful for helpful advice from Francois Edwards and John Sumpter

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Table. S1 Sites and years where fish length was measured giving percentage of roach in the up to 2 year age classes (0-115 mm length).

Gt. Ouse - Newport Pagnell			
year	All roach	0-115 mm	0-115 mm fish as a %
1991	242	121	50
1994	197	64	32.5
2001	83	28	33.7
2011	150	100	66.7
2014	156	91	58.3
Average	165.6		48.2

Gt Ouse - Clifton Reynes			
Year	All roach	0-115 mm	0-115 mm fish as a %
1991	793	583	73.5
1994	250	118	47.2
1997	10	1	10.0
2011	125	79	63.2
2014	38	11	28.9
Average	243.2		44.6

Thames - Caversham			
Year	All roach	0-115 mm	0-115 mm fish as a %
2001	168	130	77
2002	72	12	15
2003	38	15	37
2004	361	238	63
2005	298	68	23
2006	106	6	6
2007	231	67	29
2008	91	33	36
2009	408	77	19
2010	507	198	39
2011	637	306	48
2012	884	150	17
2013	63	16	25
Average	297		33.4

Thames – Shiplake Marsh			
Year	All roach	0-115 mm	0-115 mm fish as a %
2002	45	13	28.9
2003	80	52	65.0

2004	177	104	58.8
2005	66	18	27.3
2006	33	8	24.2
2007	47	15	31.9
2008	43	4	9.3
2009	147	66	44.9
2010	152	108	71.1
2011	148	50	33.8
2012	764	373	48.8
2013	97	40	41.2
Average	150		40.4

Thames – Whitchurch Mapledurham			
Year	All roach	0-115 mm	0-115 mm fish as a %
2001	110	46	41.8
2002	57	7	12.3
2003	57	6	10.5
2004	54	27	50.0
2005	76	20	26.3
2006	131	30	22.9
2007	181	109	60.2
2008	34	15	44.1
2009	227	58	25.6
2010	113	79	69.9
2011	441	254	57.6
2012	529	228	43.1
2013	177	135	76.3
Average	168		41.6

Table S2 Relationship between sampling sites on the Thames and Great Ouse and closest flow gauging site

Catchmants	Sites	National grid reference	Upstream human population	Flow gauging site	Distance to flow gauging site (miles)
River Thames	Caversham-sonning	SU 73785 74196	991811	Reading 39130 flow station	1.23
	Shiplake-Marsh	SU 77769 80072	1892531		5.24
	Whitchurch to Mapledurham	SU 65508 77460	991811		4.43
River Great Ouse	Clifton Reynes	SP 89600 50700	395879	Bedford 33002 flow station	9.89
	Newport Pagnell	SP 88200 44100	76182		11.24
	Oakley	TL 01200 52900	413672		3.4
	Turvey	SP 93700 52600	402748		7.57