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The long shadow of our chemical past – High DDT concentrations in fish near a former agrochemicals factory in England

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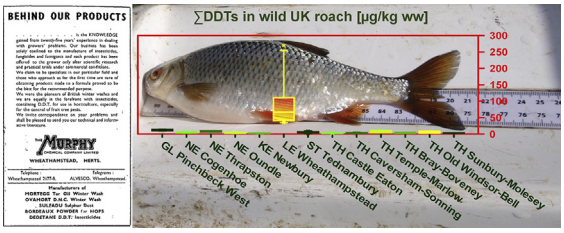
HIGHLIGHTS

- Roach (*Rutilus rutilus*) from 13 UK river sites were analysed for pesticides.
- Fish from one site had much higher ∑DDT content (DDT + DDE + DDD) than others.
- The explanation was found in a former pesticide factory nearby.
- A review found some similar hot-spots in recent European ∑DDT data in fish.
- Some fish contained levels of DDT harmful to them or their predators.

GRAPHICAL ABSTRACT



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ABSTRACT

A total of 81 roach (*Rutilus rutilus*) collected from 13 southern English river sites between 2007 and 2012, were analysed for organochlorine pesticides, PCBs, PBDEs and some metals. Unexpectedly high concentrations of the banned insecticide DDT and its degradation products DDE and DDD (∑DDTs) were found in the 10 fish from the river Lee (or Lea) which averaged 88 ± 70 (standard deviation) µg/kg ww, almost 20 times higher than the average for the remaining sites (4.8 ± 3.1 µg/kg). All fish from that site exceeded the Canadian Tissue Residue Guideline (environmental quality standard) of 14 µg/kg ∑DDTs. Concentrations of the insecticides chlordane and lindane as well as copper, which is often used as a fungicide, were also elevated in fish from the Lee, though not as much as those of DDTs. A likely explanation for these observations was found in a nearby former pesticide factory, which had stopped production about three decades earlier.

An extensive review of recent literature data on DDT in wild European fish found that, while levels are now generally low, there were several other hotspots with ∑DDTs levels that may still be of concern.

Abbreviations: DCM, dichloromethane; DDT, dichlorodiphenyltrichloroethane; DDE, dichlorodiphenyldichloroethylene (degradation product of DDT); DDD, dichlorodiphenyldichloroethanes, also known as TDE, tetrachlorodiphenylethane (degradation product of DDT); d.s., downstream; EQS, environmental quality standard; HCB, Hexachlorobenzene; PCB, polychlorinated biphenyls (industrial insulating fluids); PBDE, polybrominated diphenyl ethers (flame retardants); POPs, persistent organic pollutants; u.s., upstream; ww, wet weight; ±, denotes standard deviation (based on a sample).

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1. Introduction

Much of the concern about chemical hazards in the aquatic environment in recent years has focused on pharmaceuticals and personal care products (PPCPs) such as endocrine active compounds and more recently nanoparticles, but as their name implies, persistent organic pollutants (POPs) have the potential to cause harm many decades after their production and use was stopped. We continue to have concerns over PCBs and more recently PBDEs, but some of the older POPs produced just after World War II, such as the insecticide DDT, now receive considerably less attention.

1.1. Project background

There are few recent data on levels or trends of legacy chemicals in UK freshwater fish and whether they pose a threat to fish populations and/or their predators. To address this knowledge gap, we have an ongoing project, the National Fish Tissue Archive (see Jürgens et al., 2013 and http://www.ceh.ac.uk/our-science/projects/national-fish-tissue-archive), which was started in 2007 and in which fish samples (mainly roach, *Rutilus rutilus*) are regularly collected from a number of river sites in England and stored frozen for future analyses. While the archive was set up primarily for the purpose of monitoring chemical pollution, other applications -for example genetics of the fish and/or their associated microorganisms-are also possible. Comparing stored samples with more recent ones will allow the detection of temporal trends, while a spread of sampling sites goes some way of covering spatial differences. Sub-samples of a proportion of the fish collected have already been analysed for a number of persistent chemicals, including organochlorine pesticides, PCBs, PBDEs, and a suite of metals. The following discussion focuses on DDT, because surprisingly high levels of ∑DDT (DDT and its main degradation products DDE and DDD) were found in fish from one site (see results section), but some of the other results are also briefly mentioned to help put the data into context. The ∑DDT concentrations were also compared to DDT data from other recent European fish studies (Tables 2 and 3) and effect concentrations from eco-toxicology studies (Table 1).

1.2. History and sources of DDT

DDT was first synthesized in 1873 and it was developed as an insecticide in 1939 (Mellanby, 1992). Its high efficiency, speed of action, relatively low toxicity to humans, low cost and persistence in the environment appeared to make it perfect both for combatting insect-borne diseases and protecting crops and it earned its inventor Paul Müller the Nobel Prize for medicine and physiology in 1948 (Mellanby, 1992). Due to limited availability, it was mainly used for disease control during the 2nd World War, but its use in agriculture increased dramatically in the 1950s and 60s. Enthusiasm for DDT waned in the 1960s when it was linked to a large decline in raptor populations across several continents and other negative effects on non-target species (Mellanby, 1992). In the UK its use was restricted from the early 1970s (Hansard Report, 1969) and agricultural uses were banned throughout the European Union in 1981 (EEC, 1978). In 2001 DDT was included in ANNEX B in the Stockholm convention of POPs to be eliminated or restricted worldwide. This allows only limited production and use for disease vector control, mainly to combat the spread of malaria (UNEP, 2001).

Another source for DDT and related compounds is the more recently used pesticide dicofol, which was produced from DDT by replacing one of the protons with an OH-group (see Figs. S1 and S2 in supplementary information). This small modification decreases stability, reducing half-life in soil to approximately 1 month compared to about 10 years for DDT (Aronson et al., 2006) or >20 years for its degradation product DDE (Mackay et al., 2006). If the conversion from DDT to dicofol is incomplete, some DDT may remain in the product. Impurities in dicofol have been identified as a recent source of DDT and related compounds mainly in Asia (Ricking and Schwarzbauer, 2012). In the European Union, the use of dicofol containing >0.1% residues of DDT and related compounds or <78% *pp'*dicofol has been banned since 1991 (European Union, 1990) and all dicofol use was banned in 2010 (European Union, 2008). Therefore, environmental DDT contamination from dicofol use – at least after 1991 – should be negligible in Europe. Due to the use of DDT as an intermediate, local DDT contamination related to dicofol production has however been significant in some cases (de la Cal et al., 2008). The Stockholm convention (UNEP, 2001) originally made provisions for the use of DDT in dicofol production, but by 2009 none of the 180 signatories (179 states + the European Union) had applied to produce or use DDT for this purpose and that option was automatically withdrawn.

1.3. Congeners of DDT and its degradation products

Technical DDT (the mixture typically used) consists of about 85% *pp'*DDT, the active insecticidal ingredient, and about 15% *op'*DDT with trace amounts of DDEs and DDDs (ATSDR, 2002) (see Fig. S1 for molecular structures and degradation pathways). Under aerobic conditions, DDT mainly degrades to DDE and under anaerobic conditions to DDD, which are both more stable than the parent compound (Ricking and Schwarzbauer, 2012). During these transformations the *pp'* or *op'* configuration is maintained, but differences in water-solubility, vapour pressure and stability between the *pp'* and o*p'* congeners (see Table S2) tend to lead to shifts towards higher *pp'*/*op'* ratios from DDT to DDD and DDE (Ricking and Schwarzbauer, 2012). The compound typically found in the highest concentrations in the (aerobic) environment is therefore *pp'*DDE. Environmental concentrations are often reported as the sum of the six *pp'* and o*p'* congeners of DDT, DDE and DDD, which are collectively known as DDTs (∑DDTs).

1.4. DDT in the aquatic environment

DDT has rarely been detected in water, especially after the restrictions and bans on use took effect. In England and Wales the Environment Agency has been monitoring aquatic pollutants since the 1970s, but DDTs (sum of *pp'*DDT, *pp'*DDE, *pp'*DDD) were only detected in 2% of over 60,000 river water samples according to a publicly available dataset for 1974–2013 (Environment Agency, 2014). While water concentrations of POPs reduce fairly quickly after they are restricted or banned, concentrations in sediments can persist for decades and contamination stored in deep sediments can re-emerge in the food chain, especially when these sediments are disturbed. This was observed in Germany, where DDT concentrations in eels increased sharply following dredging activities a decade after a nearby factory had stopped processing DDT (Heinisch et al., 2007) and recent increases of DDT in zooplankton and fish related to a long closed DDT factory were also observed in Italy (Bettinetti et al., 2012). In fish tissue, the ∑DDTs concentrations are usually even higher than in sediments – a review found fish-sediment accumulation factors for the main congener *pp'*DDE to be typically between 30 and 75 on a dry weight basis (van der Oost et al., 2003) and a more recent study (Niewiadowska et al., 2014) measured factors of 2–59 in roach and 5 to 125 in bream. Therefore, fish are very suitable for monitoring DDTs and other persistent pollutants in the aquatic environment.

Table 1
Tissue-burden based risks of DDT and its degradation products to fish and their predators (more details are given in Table S3).

Species	Effect	Fish tissue conc. (whole body ∑DDTs) [μg/kg]	Reference
Observed LOECs for fish exposed in early life			
Japanese medakaa	intersex and altered gene expression	58 (∑*op*'DDTs)	Sun et al. (2016)
Atlantic croaker	behaviour	70 in eggs	Faulk et al. (1999)
Japanese medaka[a]	intersex, increased plasma estradiol, altered gene expression	271 (*pp*'DDE)	Sun et al. (2016)
9 Studies, 7 species	survival	890 -2400	Beckvar et al. (2005)
coho salmon	behaviour	1100	Beckvar et al. (2005)
Summer flounder, rainbow trout	increased vitellogenin and physiological changes	≤60,000[b]	Mills et al. (2001)
		≤135,000[b]	Donohoe and Curtis (1996)
Observed LOECs for fish exposed as adults			
6 Species	survival	290 -1130	Beckvar et al. (2005)
Goldfish	behaviour	1650	Beckvar et al. (2005)
Brook trout	reproduction	7600	Beckvar et al. (2005)
Brook trout	growth	11,200	Beckvar et al. (2005)
Tilapia	osmoregulation	ca. 20,000	Mills et al. (2001)
Published estimated safe tissue concentrations for fish themselves			
Adult fish	provisional due to insufficient sub-lethal data	600	Beckvar et al. (2005)
Fish early life stages	provisional due to insufficient sub-lethal data	700	Beckvar et al. (2005)
Published estimated safe tissue concentrations for consumers of fish			
Birds	based on egg shell thinning in ducks	14	Canadian Council of Ministers of the Environment (1999)
Kingfisher	based on egg shell thinning in pelicans (US EPA, 1995)	20	Lazorchak et al. (2003)
Humans	risk based threshold for cancer (from 4 fish meals/month)	69	Stahl et al. (2009)
Osprey	dietary effects concentration	90–190 (*pp*'DDE)	Hinck et al. (2009a)
Mammals	based on effects on rats and food intake rate for mink	94	Canadian Council of Ministers of the Environment (1999)
Bald eagle	dietary effects concentration	130 (*pp*'DDE)	Hinck et al. (2009a)
Pelicans	reduced fledgling rate when fish conc. were still 0.15 μg/g	<150 (LOEC)	US EPA (1995)
Bald eagle	no effects hazard concentration	270	Hinck et al. (2009b)
Mink	based on effects on rats and food intake rate for mink	360	Lazorchak et al. (2003)
Otter	based on effects on rats and food intake rate for otter	490	Lazorchak et al. (2003)
Humans	food standard for meat (none exists for fish)	1000	European Union (2005)

[a] Transgenic medaka in which oocytes in male testes are easier to detect, but sensitivity should be equal to the wild-type. Data is for males.
[b] Total dose injected.

1.5. Toxic effects and safe levels of DDT in fish

DDT has a number of toxicological effects on fish themselves as well as on their predators. In the 1960s, studies found that accumulation of DDT up the food chain had resulted in egg-shell thinning in birds to a point where the eggs frequently broke (Ratcliffe, 1967). In fish DDTs have been linked to various effects on fertility and development (Baatrup and Junge, 2001; Bayley et al., 2002; Okoumassoun et al., 2002; Uchida et al., 2010) due to their structural similarity to steroid hormones, and osmoregulation (Janicki and Kinter, 1971; Kinter et al., 1972; Riou et al., 2012) or thyroid function (Brar et al., 2010) may also be affected.

Beckvar et al. (2005) reviewed available toxicological data (reproduced in Table 1 and Table S3 along with some more recent data) and estimated that a maximal body burden of 600–700 μg/kg wet weight (ww) would likely be a protective ∑DDTs concentration for the fish themselves, but declared this value provisional due to the scarcity of data, especially on non-lethal endpoints. A number of other studies estimated safe levels for human and wildlife consumers of fish (Table 1). Of those only the Canadian Tissue Residue Guideline (i.e. environmental quality standard, biota EQS) of 14 μg/kg ∑DDTs, which is based on the risk to fish-eating birds (Canadian Council of Ministers of the Environment, 1999) has a legal status with regards to the environment. The European Union does not have a biota EQS for DDTs, but there is one for surface water, which is 25 ng/l for ∑DDTs and 10 ng/l for *pp*'DDT (European Union, 2013).

2. Materials and methods

The current study examined the presence of DDT and some other pollutants in roach (*Rutilus rutilus*) from a number of sites in the Thames and Anglian regions of England. Roach were chosen because they are relatively common throughout the UK and not much sought after by anglers. Although they can migrate several km if suitable spawning areas are not available nearby, the range is much smaller than that of truly migratory species (Baade and Fredrich, 1998). In the rivers sampled, their movement is further restricted by obstacles, such as locks and weirs and the separation of populations within the Thames Catchment was confirmed by analysis of population-genetic structure using DNA microsatellites (Fig. S4 and Hamilton et al., 2014).

2.1. Fish collection

Roach were caught by Environment Agency staff in connection with regular fish population surveys using electrofishing techniques. They were killed with an overdose of the anaesthetic 2-phenoxyethanol (ca. 4 ml in a 10 l bucket) packed in plastic bags (polyethylene/polyacryl or fluoro-ethylene-propylene) and then frozen on site using a liquid nitrogen-cooled dry shipper. On return to the laboratory the frozen fish were transferred to a −80 °C freezer. For the current study a total of 81 roach (9–21 cm, estimated about 2–8 years old) were collected between 2007 and 2012

Table 2
Concentrations of DDT and its metabolites in fish from confirmed or suspected **industrially contaminated** sites[a]. Data for some commonly monitored freshwater or brackish water fish species caught in the last 20 years (1995–2014). Eels, which often have higher POP concentrations than other species because of their high fat content and association with sediments, have already been reviewed for *pp'*DDE in Jürgens et al. (2015). Agricultural use of DDT ceased in the 1970s or 1980s in all the countries mentioned in this table.

Country	n sites	∑n samples	Sites	Year(s)	Sample[b]	DDTs	Site averages [μg/kg]	Overall average [μg/kg]	Overall range [μg/kg]	Ref.	Comments
Barbel (cyprinid, bottom feeder)											
Italy	1	4	Po River d.s. of river Lambro	1995	m	Σ2 DDTs[c]	174[d,e]	n.a.	n.a.	Viganó et al. (2000)	Lambro is very influenced by chemical industry
Spain	2	2	Cinca river just d.s. of operating dicofol factory and 30 km d.s.	1999	m	Σ6 DDTs[f]	ca. 8000, ca. 2000	n.a.	1188–10,431	de la Cal et al. (2008)	
Spain	2	11	Cinca river just d.s. of operating dicofol factory and 30 km ds.	2002	m	Σ6 DDTs[f]	997, 562	n.a.	211-2098	de la Cal et al. (2008)	decreased since 1999 (see above)
Spain	1	1 pooled	Cinca river just d.s. of operating dicofol factory.	2003	m	Σ6 DDTs[f]	616[e]	n.a.	n.a.	Lacorte et al. (2006)	slight further decrease since 2002
Spain	1	5	lower Jarama river, Iberia	2006–09	m	Σ3 DDTs[g]	20	n.a.	8.2–51	Nicola et al. (2014)	High industrial and urban impact. DDT/DDE >1 → recent contamination.
Bream (cyprinid, omnivorous, bottom feeder)											
Czech republic	5	26	Elbe river	2008/09	m	Σ6 DDTs[f]	14–405	156	6.4–791	Hrádková et al. (2012)	Elbe river has numerous industries
Czech republic	3	3 pooled	2 reservoirs, 1 river Elbe site	2006–10	m	Σ6 DDTs[f]	97–263	178	n.a.	Cerveny et al. (2014)	Elbe river has numerous industries
Germany	2	10	Elbe tributaries Saale and Mulde	2009–13[h]	m	Σ2 DDTs[c]	151, 173	162	61–249	Umweltbundesamt (2016)	1995–2015 Saale possible upwards trend, Mulde no trend
Germany	5	25	Elbe river	2009–13[h]	m	Σ2 DDTs[c]	72–125	95	53–178	Umweltbundesamt (2016)	1993–2013, slight downwards trends, but not clear. Elbe river has numerous industries
Poland	1	15	river Vistula (Wisła) near Cracow	2011/12	m	Σ4 DDTs[i]	654	n.a.	179-1921	Niewiadowska et al. (2014)	
Roach (cyprinid, omnivorous)											
Southern England	1	10	Lee at Wheathampstead	2011	w	Σ6 DDTs[f]	88	n.a.	35–270	current study	just d.s. former DDT factory, which closed in 1982
Czech republic	2	6	Elbe at Usti nad Labem and Decin	2008	m	Σ6 DDTs[f]	10, 24	17	6.7–26	Hrádková et al. (2012)	included in polluted list, because values for bream were high at these sites
Italy	1	4 times	Lake Maggiore	2009	m	Σ3 DDTs[g]	ca. 35[j]	n.a.	n.a.	Bettinetti et al. (2012)	DDT factory closed 1996, but new release of DDT in 2009 was likely, showing first in zooplankton and then in fish
Italy	1	4 times	Lake Maggiore	2010	m	Σ3 DDTs[g]	ca. 85[j]	n.a.	n.a.	Bettinetti et al. (2012)	
Poland	1	17	river Vistula (Wisła) near Cracow	2011/12	m	Σ4 DDTs[i]	121	n.a.	28–414	Niewiadowska et al. (2014)	
Shad (clupeid (belonging to the herring family), pelagic, omnivorous)											
Italy	1	5 times	Lake Maggiore	2002–04	m	Σ2 DDTs[k]	ca.90-190[j]	125	n.a.	Bettinetti et al. (2006)	Influenced by DDT factory which closed in 1996
Italy	1	3 times	Lake Maggiore	2008	m	Σ3 DDTs[g]	28–56[d]	43[d]	n.a.	Bettinetti et al. (2010)	Influenced by DDT factory which closed in 1996
Italy	1	4 times	Lake Maggiore	2009	m	Σ3 DDTs[g]	n.a.	ca. 55[j]	n.a.	Bettinetti et al. (2012)	Influenced by DDT factory which closed in 1996
Italy	1	4 times	Lake Maggiore	2010	m	Σ3 DDTs[g]	n.a.	ca. 80[j]	n.a.	Bettinetti et al. (2012)	Influenced by DDT factory which closed in 1996, new release likely in 2009, which showed first in zooplankton and then in fish
Italy	1	5-6 per year	Lake Como, Como branch	2005–09	m	Σ3 DDTs[g]	54–120[d]	84[d]	n.a.	Bettinetti et al. (2008;2016)	In Zebramussels lw DDT conc. increased >100-fold between 2003 and 2005 in lake Como and lake Iseo, but not lake Maggiore. In Lake Como fish, the highest values were also in 2005. Melting glaciers were suggested as secondary DDT source.
Italy	1	5-6 per year	Lake Como, Lecco branch	2007–08	m	Σ3 DDTs[g]	79, 98[d]	98[d]	n.a.	Bettinetti et al. (2016)	
	1	5 or 6	Lake Iseo	2007	m	Σ3 DDTs[g]	71[d]	n.a.	n.a.	Bettinetti et al. (2008)	
Perch (piscivorous)											
Italy	1	5 times	Lake Maggiore	2002–04	m	Σ3 DDTs[g]	ca.14-250[j]	93[j]	n.a.	Bettinetti et al. (2006)	Influenced by DDT factory which closed in 1996

n.a. – not available.
[a] Where possible sites were assigned "contaminated" or "background" status based on the information in the paper, but sometimes the choice was based on the reported concentrations.
[b] w: whole body, m: muscle, the footnotes describe any data transformations performed where concentrations were not presented on a wet weight basis.

[c] *pp*'DDE + *pp*'DDD. In the current study this provided on average 85% of ∑DDTs at the contaminated site and 92% at the other sites.
[d] Converted from lipid weight (lw) to ww using (average) lipid contents given in the reference.
[e] Converted from dw to ww, assuming 26% dry matter, which is the value for a EU "standard" fish (European Union, 2014) and also approximately the average for the roach in this study.
[f] *op*'DDT, *pp*'DDT, *op*'DDE, *pp*'DDE, *op*'DDD, *pp*'DDD (∑DDTs).
[g] All 3 *pp*'congeners. In the current study this provided on average 90% of ∑DDTs at the contaminated site and 93% at the other sites.
[h] One composite sample per year and site. The last five available years were selected.
[i] All 3 *pp*'congeners + *op*'DDT. In the current study this provided on average 98% of ∑DDTs at the contaminated site and 94% at the other sites.
[j] Converted from lw to ww using the EU "standard" fish lipid content of 5% (European Union, 2014) to estimate a fresh weight based concentration.
[k] *pp*'DDE + *pp*'DDT. In the current study this provided on average 82% of ∑DDTs at the contaminated site and 75% at the other sites.

from several sites along the Rivers Glen, Nene and Thames as well as three Thames tributaries, the Kennet, Lee (also commonly spelled "Lea") and Stort (Fig. 1). The sites were chosen from Environment Agency fish population monitoring sites. They varied in impact from treated sewage between 1% of the flow on average in the Glen and 43% in the Stort (Table S1) but were otherwise thought to represent fairly typical levels of chemical inputs for their area, rather than deliberately choosing known pollution hotspots. Descriptions of the rivers Lee and its tributary Stort are found in Snook and Whitehead (2004) and of the other sampled rivers in Jürgens et al. (2013) and detailed catchment information is in Table S1. The catchments above most sampling sites were dominated by agricultural land except for the site on the river Lee where the town of Luton with its suburbs occupies a large proportion of the area (12% urban and 35% suburban or rural developments).

2.2. Chemical analysis

For all samples from 2008 onwards the whole frozen fish were ground into a powder without defrosting them using a cryogrinder (SPEX SamplePrep 6850). The resulting frozen fish powder was divided into pre-cooled 20 ml glass scintillation vials and stored at −80 °C until use. For the nine roach collected in 2007 the process was slightly different, in that the liver and gall bladder as well as a blood sample were removed before freezing and the remaining carcass was divided dorsally with one half (or part of it for very large individuals) being used for POPs analysis. The other half was cryo-ground and used for metals analysis. For POPs analysis ca. 5 g of the cryo-ground homogenate was dried by mixing with 30 g sodium-sulphate (or in the case of the 2007 fish, the sample was ground with sodium-sulphate in a pestle and mortar). After addition of recovery standards (^{13}C PCB mix: 28, 52, 101, 138, 153, 180 and PBDE mix 51, 128, 190), DDTs and other persistent organic pollutants were extracted overnight with dichloromethane (DCM) in a Soxhlet apparatus. Lipid content was determined gravimetrically from a subsample of the Soxhlet extract and the rest of the DCM was evaporated in a vacuum rotary evaporator and replaced with 10 ml hexane, which was reduced to about 1 ml. This was added to a glass column with 11 g acidified silica (200 ml silica baked at 450 °C and acidified with 25 ml concentrated sulfuric acid) and eluted with hexane as a first clean up step, which removes the fats. Then the sample was passed through a gel permeation chromatography column with 50:50 hexane:DCM and only the fraction from 17 to 51 ml collected as second clean up step to remove molecules outside the size range of interest. The solvent was then again replaced with hexane and the sample added to 25 μl internal standards (PCB 30, ^{13}C-PCB141, ^{13}C-PCB208, BDE69, BDE181) in dodecane, before evaporating the hexane, so that the whole sample was contained in the 25 μl dodecane. The extracts were analysed by gas chromatography − mass spectrometry, single ion monitoring using a 50 m Varian CP-SIL8 CB Pesticide column (Varian-Chrompack, Middelburg, The Netherlands) with electron impact + ionisation. Standards and blanks were run along with the samples. The instrument blank contained only solvent and procedural blanks went through the whole extraction and clean-up procedure without the addition of fish homogenate (i.e. extracting only sodium sulphate). The instrument limit of detection (LOD), defined as the lowest observable standard was between 1 and 6.25 pg/μl for the analysed chemicals, which is equivalent to 5–31 ng/kg for a 5 g sample. To measure metal concentrations 1–2.5 g wet weight was weighed into a PTFE vial, digested in a microwave digester (MARSXpress, CEM) with 10 mL ultrapure nitric acid (Baker, Ultrex II), then made up with ultrapure water (>18 MΩ/cm) to 25 mL before analyzing using a Perkin Elmer Elan DRC II inductively coupled plasma mass spectrometer (ICPMS) instrument. Certified reference materials (DORM-3 and additionally DOLT-4 for later batches, both from National Research Council, Canada) were analysed alongside each batch and for copper measured at 83–116% of the published values. The LOQ was about 4 μg/kg ww for a 2 g sample. The current paper is focused mainly on pesticides, not metals, so the only metal included in the results and discussion is copper, because it is frequently used as a fungicide. Mercury is discussed in Jürgens et al. (2013) and all results can be found in Jürgens (2015).

2.3. Genetic analysis

To investigate the origin of roach used for analytical chemistry from the Lee, Kennet, Stort, and the Thames at Castle Eaton, all fish from these sites were genotyped using 14 DNA microsatellites as described by Hamilton et al. (2014). Fifty roach were also genotyped from Calverton Fish farm, which is the source of fish used for restocking by the England and Wales Environment Agency. Together with University of Exeter's existing dataset (Hamilton et al., 2014), genotypes from 1858 roach from 33 different river stretches in England were available. The whole dataset was analysed using the Bayesian clustering program STRUCTURE v2.3.3 which assigns individuals to probable genetically differentiated clusters (*K*) using a Bayesian approach (Pritchard et al., 2000). In STRUCTURE, analyses were conducted using the standard model and a "*locprior*" model and used 125,000 repetitions (burn-in = 25,000 iterations). The number of clusters *K* was set to 16 which was previously shown to differentiate roach from many of the river stretches (Hamilton et al., 2014).

3. Results and discussion

3.1. High levels of DDT and some other chemicals in fish from the Lee at Wheathampstead

All the chemicals in this study (∑DDTs, chlordane (α+γ), lindane (γ-HCH), HCB, copper, ∑7 PCBs, ∑6 PBDEs) were detectable in all fish with statistically significant differences (ANOVA, $\alpha = 0.05$) between at least some of the sampling sites (see Tables S4–S10 for details of the statistical calculations). Of particular note were the ∑DDTs concentrations in fish from the Wheathampstead site, which were very significantly higher ($p < 0.001$, Table S4) than at any of the other sites (Figs. 2a and 3). The average ∑DDTs concentration in roach from that site was 88 ± 70 μg/kg ww, compared to the overall average of 4.8 ± 3.1 μg/

Table 3
Concentrations of DDT and its metabolites in fish from **"background"** sites[a]. Data for some commonly monitored freshwater or brackish water fish species caught in the last 20 years (1995–2014). Eels, which often have higher POP concentrations than other species because of their high fat content and association with sediments, have already been reviewed for *pp*'DDE in Jürgens et al. (2015). Agricultural use of DDT ceased in the 1970s or 1980s in all the countries mentioned in this table.

Country	n sites	∑n samples	Sites	Year(s)	Sample[b]	DDTs	Site averages [μg/kg]	Overall average [μg/kg]	Overall range [μg/kg]	Ref.	Comments
Barbel (cyprinid, bottom feeder)											
UK	1	1	River Don	2008	m	*pp*'DDE[c]	10	n.a.	n.a.	Rose et al. (2015)	
Greece	2	2	River Nestos	2004	m	Σ3 DDTs[d]	0.25, 0.47	n.a.	n.a.	Christoforidis et al. (2008)	
Italy	1	4	Po River	1995	m	Σ2 DDTs[e]	50[f,g]	n.a.	n.a.	Viganó et al. (2000)	
Macedonia	3	11 (?)	Lakes Ohrid, Prespa, Dorjan	ca. 2005?	m	Σ3 DDTs[d]	8.8, 13, 16	n.a.	ca. 9-17	(Veljanoska-Sarafiloska et al., 2011, 2012; 2013)	
Spain	5	5 pooled	Ebro river basin	2003	w	Σ6 DDTs[h]	<LOD-19[g]	8.7[g]	n.a.	Lacorte et al. (2006)	
Spain	2	12	Cinca river u.s. of dicofol factory	2002	m	Σ6 DDTs[h]	51, 11	24	8–64	de la Cal et al. (2008)	
Spain	1	1	Cinca river immediately upstream of dicofol factory	1999	m	Σ6 DDTs[h]	n.a.	n.a.	29–104	quoted in de la Cal et al. (2008)	
Spain	2	10	upper + middle Jarama river	2006–09	m	Σ3 DDTs[d]	0.92, 2.7	1.8	0.24–3.66	Nicola et al. (2014)	DDT/DDE <0.5–> old contamination
Bream (cyprinid, omnivorous, bottom feeder)											
UK	3	3	River Don, 2 ponds	2008	m	*pp*'DDE[c]	<5, <5, 10	n.a.	n.a.	Rose et al. (2015)	
Czech republic	1	2	River Oder (Odra) at Ostrava	2008/09	m	Σ6 DDTs[h]	24	24	23–25	Hrádková et al. (2012)	
Czech republic	24	24 pooled	various rivers and reservoirs	2006–10	m	Σ6 DDTs[h]	1–65	17	n.a.	Cerveny et al. (2014)	
Germany	2	10	River Saar	2009–13[i]	m	Σ2 DDTs[e]	18,24	21	14–30	Umweltbundesamt (2016)	one site clear decrease 1994–2013, 63->19
Germany	1	3	Lake Belau (clean control site)	2009–13[i]	m	Σ2 DDTs[e]	2.2	n.a.	1.4–3.8	Umweltbundesamt (2016)	no clear trend 1997–2013
Germany	4	18	River Rhine	2009–13[i]	m	Σ2 DDTs[e]	11–39	28	8–55	Umweltbundesamt (2016)	no clear trend 1997–2013
Germany	3	15	River Danube	2009–13[i]	m	Σ2 DDTs[e]	10–13	12	4.4–20	Umweltbundesamt (2016)	no clear trend 2004–2013
Italy	1	2	River Po	2014?	liver	Σ6 DDTs[h]	27[f,g]	n.a.	15,39	Viganó et al. (2015)	3 further species were mostly lower
Moldova	1	3	River Dniester near Dubassari	2001	m	Σ6 DDTs[h]	19	n.a.	n.a.	Sapozhnikova et al. (2005)	
Poland	8	74	various sites	2011/12	m	Σ4 DDTs[j]	11–65	37	3.8–295	Niewiadowska et al. (2014)	
Poland	1	5 (?)	Szczecin lagoon	1995	m	Σ3 DDTs[d]	65	n.a.	n.a.	Ciereszko and Witczak (2002)	
Romania	2	4	Enisala and Caraorman, Danube Delta	2001	m	Σ5 DDTs[k]	5.9, 32[f]	19[f]	n.a.	Covaci et al. (2006)	
Roach (omnivorous)											
Southern England	12	71	Thames catchment, Glen, Nene	2007–12	w	Σ6 DDTs[h]	1.8–12	4.8	0.6–14.3	current study	
UK	2	2	2 ponds	2008	m	*pp*'DDE[c]	<5, 2			Rose et al. (2015)	
Bulgaria	2	2 pooled	Lake Burgas, Lake Mandra	2014	m	Σ3 DDTs[d]	2.31, 2.59	2.45	–	Georgieva et al. (2015)	
Moldova	2	6	river Dniester near Dubassari Moldova	2001	m	Σ6 DDTs[h]	8,19	14	n.a.	Sapozhnikova et al. (2005)	
Poland	1	5 (?)	Szczecin lagoon	1995	m	Σ3 DDTs[d]	23.7	n.a.	n.a.	Ciereszko and Witczak (2002)	
Poland	5	25	Oder delta	2003	m	Σ3 DDTs[d]	n.a.	9.1	n.a.	Tomza-Marciniak and Witczak (2009)	
Poland	9	52	various sites	2011/12	m	Σ4 DDTs[j]	4.5–44	15	3.0–61	Niewiadowska et al. (2014)	
Romania	1	2	Enisala, Danube Delta	2001	m	Σ5 DDTs[k]	11.4[f]	n.a.	n.a.	Covaci et al. (2006)	
Spain	1	1 pooled	river Zadorra (Ebro river basin)	2003	w	Σ6 DDTs[h]	17[g]	n.a.	n.a.	Lacorte et al. (2006)	
Shad (clupeid (belonging to the herring family), pelagic, omnivorous)											
France	2, 2 times	69	Vilaine estuary	2004–05	m	Σ3 DDTs[d]	2.0–4.7[g]	8.7[g]	n.a.	Bocquene and Abarnou (2013)	
Perch (piscivorous)											
UK	5	5	3 ponds, river Don, Thames	2008	m	*pp*'DDE[c]	<5	<5	n.a.	Rose et al. (2015)	
Bulgaria	1	1 pooled	Lake Mandra	2014	m	Σ3 DDTs[d]	1.81	n.a.	n.a.	Georgieva et al. (2015)	
Latvia	9	9 pooled	6 lakes, 2 rivers	1995	m	*pp*'DDE[c]	0.6–5.9[f]	2.0[f]	n.a.	(Valters et al., 1998)	
Latvia	4	43	4 lakes	1996–97	m	Σ2 DDTs[e]	0.5–5.9[f]	2.3[f]	n.a.	(Valters et al., 1999a)	

Table 3 (*continued*)

Country	n sites	∑n samples	Sites	Year(s)	Sample[b]	DDTs	Site averages [μg/kg]	Overall average [μg/kg]	Overall range [μg/kg]	Ref.	Comments
Latvia	6	60	rivers Daugava and Lielupe	1997	m	Σ3 DDTs[d]	1.0–2.8[f]	1.8[f]	n.a.	(Valters et al., 1999b)	
Moldova	2	5	river Dniester near Dubassari	2001	m	Σ6 DDTs[h]	6,10	8	n.a.	Sapozhnikova et al. (2005)	
Romania	1	2	Caraorman, Danube Delta	2001	m	Σ5 DDTs[f]	10[f]	n.a.	n.a.	Covaci et al. (2006)	

n.a. – not available.

[a] Where possible sites were assigned "contaminated" or "background" status based on the information in the paper, but sometimes the choice was based on the reported concentrations.

[b] w: whole body, m: muscle, the footnotes describe any data transformations performed where concentrations were not presented on a wet weight basis.

[c] In the current study *pp'*DDE provided on average 75% of ∑DDTs.

[d] All 3 *pp'*congeners. In the current study this provided on average 90% of ∑DDTs at the contaminated site and 93% at the other sites.

[e] *pp'*DDE + *pp'*DDD. In the current study this provided on average 85% of ∑DDTs at the contaminated site and 92% at the other sites.

[f] Converted from lipid weight (lw) to ww using (average) lipid contents given in the reference.

[g] Converted from dw to ww, assuming 26% dry matter, which is the value for a EU "standard" fish (European Union, 2014) and also approximately the average for the roach in this study.

[h] *op'*DDT, *pp'*DDT, *op'*DDE, *pp'*DDE, *op'*DDD, *pp'*DDD (∑DDTs).

[i] One composite sample per year and site. The last five available years were selected.

[j] All 3 *pp'* congeners + *op'*DDT. In the current study this provided on average 98% of ∑DDTs at the contaminated site and 94% at the other sites.

[k] ∑DDTs except *op'*DDT. In the current study this provided on average 92% of ∑DDTs at the contaminated site and 99% at the other sites.

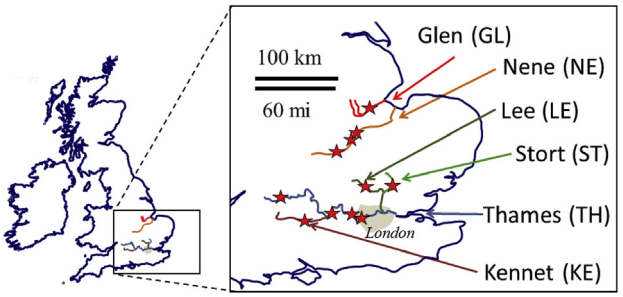

Fig. 1. Map of the UK, showing fish sampling sites (outline ©Daniel Dalet/d-maps.com).

kg ww for all other sites. The roach from Wheathampstead also had unusually high lipid contents (8.8%–13.6%, average 11.6%, compared to 1.4%–7.9%, average 4.7% in the remaining roach), but that alone is insufficient to explain the almost 20 fold difference in DDT concentrations compared to the remaining fish.

The ∑DDTs concentrations at the other sites were more similar, but the two most upstream sites in the Thames catchment (Newbury on the Kennet and Castle Eaton on the Thames) had the lowest concentrations (1.8 ± 0.5 and 2.4 ± 0.9 μg/kg ww), each significantly ($\alpha < 0.05$) lower than seven of the 12 other sites. The highest concentrations, apart from Wheathampstead, were found in the four roach measured from the river Glen (12.4 ± 1.9 μg/kg), and the difference was significant compared to six of the 12 other sites (Table S4 and Fig. 3).

Some, but not all, of the other chemical concentrations measured were also elevated in Wheathampstead fish, though not as much as for ∑DDTs (Fig. 3): For the insecticide chlordane, Wheathampstead came second to the Sunbury to Molesey reach on the river Thames, both being significantly higher than nine of 11 other sites analysed. The insecticide lindane (γ-HCH) was analysed with the same method as for DDT, which for γ-HCH is only semi-quantitative. Extracts of fish from other sites had previously been analysed with a separate GCMS method for HCH (Jürgens, 2015; Jürgens et al., 2015) but the results are not easily comparable, so semi-quantitative data from only five sites are shown here. The γ-HCH concentrations in Wheathampstead were highest and significantly different from all four others (Table S6). By contrast, neither the fungicide HCB nor PCBs concentrations were elevated in Wheathampstead roach, being significantly different to only 1/11 or 3/12 other sites respectively (Tables S7 and S9). Although the Wheathampstead values for PBDE flame retardants were among the highest measured, the difference was only significant compared to half of the other 12 sites (Table S10), but copper concentrations were also significantly higher at Wheathampstead than at most (10/12) of the other sites (Table S8). In summary: compared to others in the study, the fish from Wheathampstead had strongly elevated ∑DDT levels and slightly elevated levels of the insecticides chlordane and lindane and also of copper, which may have been used as a fungicide, while the site did not particularly stand out for the fungicide HCB, PCBs, or PBDEs.

3.2. A likely source of the contamination

An investigation into potential sources of DDT and other pesticides in Wheathampstead roach revealed evidence of DDT production at Murphy Chemical Company in an advertisement (reproduced in Fig. S3) published in a 1940s book on pesticides (Massee, 1946), where they stated "[…] we are equally in the forefront with insecticides containing D.D.T. […]" and the company's address was given as "Wheathampstead, Herts". The Murphy Chemical Company was founded when Murphy and Son Brewery Supplies acquired the Wheathampstead site in 1928 and branched out into agricultural chemicals. The agro-chemicals arm "Murphy Chemical Company Ltd" in Wheathampstead was sold to Glaxo in 1956 (http://www.murphyandson.co.uk/heritage) and then changed hands a few more times (Dalgety, Dow, Fisons). Both production and development of pesticides took place in Wheathampstead between about 1931 and 1982 and test beds were located close to the river Lee. In 1967 a major fish kill was caused by a spill of the pesticide Mecarbam (not measured in this study) at Wheathampstead (Environment Agency, 2010). Since the factory closed, an attempt has been made to clean up the considerable pesticide contamination of the ground by removing contaminated soil and cleaning ground water by treating it on site in reed beds which were completed in 1998 (http://www.oceans-esu.com/case-studies/) and appeared to be still operational when the lead author revisited the area in summer 2016. Comparisons of historic and recent aerial photos revealed the approximate extent of the area formerly occupied by the company, which has been redeveloped

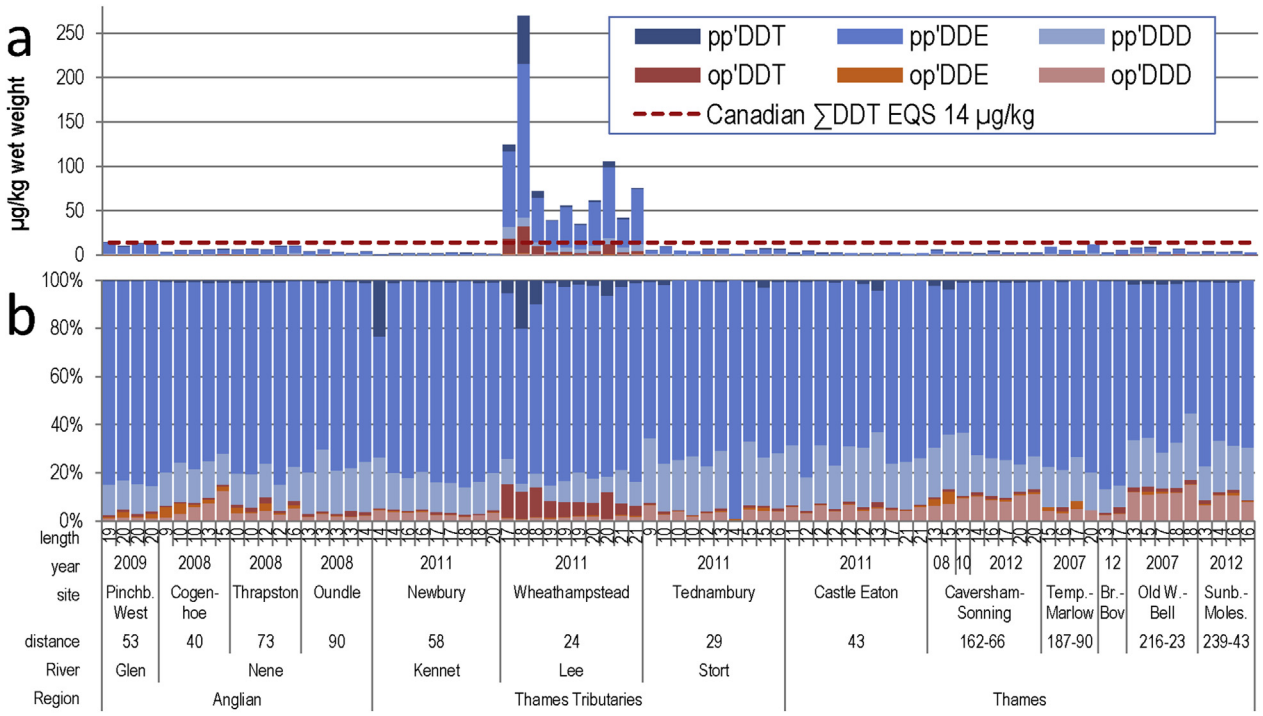

Fig. 2. Concentration (a) and relative contribution of congeners (b) of DDT and its degradation and by-products DDE and DDD in roach. Individuals at each site are ordered by year and length (cm). Sites on each river are ordered by distance from the source (river-km). Br.-Bov: Bray-Boveney 203–209 km. The Canadian Tissue Residue Guideline for the protection of wildlife consumers (Canadian Council of Ministers of the Environment, 1999) is also shown (there is currently no equivalent EU guideline).

with domestic and commercial properties (Fig. 4). The site appears to have stretched from the riverbank to the northern edge of town, bordering the river for several hundred meters immediately upstream of the fish-sampling site. Without knowing about the history of the site, we had collected fish right next to the former factory with its associated test fields. This is likely to explain not only the very elevated DDT concentrations, but also the relatively high concentrations of the pesticides chlordane and lindane, and of copper. Copper is mentioned in the advertisement (Fig. S3) under the name "Bordeaux Powder", a mixture of copper (II) sulphate ($CuSO_4$) and slaked lime ($Ca(OH)_2$), which is commonly used as a fungicide, so its elevated levels are likely to be also related to the former pesticide factory. By contrast, HCB, which was banned from agricultural use in the European Union in the early 1980s along with chlordane and DDT (EEC, 1978), does not appear to have been released in Wheathampstead to a significant degree and the relatively high PBDE contamination there is likely to be a result of the original reason the site was chosen, namely its relatively high sewage effluent content of the river flow (Schreder and La Guardia, 2014; Jürgens, 2015).

Previous analysis of the roach population genetic structure revealed the population at Wheathampstead to be genetically distinct from populations sampled about 5 km upstream and 5 km downstream, and migration into and out of the Wheathampstead population to be minimal (Hamilton et al., 2014). Bayesian clustering analysis of the DNA microsatellite genotype dataset in the programme STRUCTURE (Pritchard et al., 2000) showed that the Wheathampstead roach used for chemical analysis in this study had high genetic similarity to roach sampled previously from the same river stretch, but were distinct from those from the Lee tributary Stort (Fig. S4). Importantly, they were also distinct from roach from Calverton Fish Farm, so are not recently restocked fish. This suggests that these fish had been confined to this river stretch for their entire lives, helping to explain the high concentrations of pesticides in this population. Roach from the Stort and the Kennet also showed high genetic similarity to fish previously sampled from the same river stretches (Fig. S4).

3.3. Congener distribution

At all sites, the ∑DDTs concentration was dominated by *pp'*DDE, the main aerobic degradation product of *pp'*DDT. However, at Wheathampstead roach had an unusual congener distribution with relatively high levels of *op'*DDT, and lower proportions of both *op'*DDD and *pp'*DDD, than other fish in the study (Fig. 2b). In general, a higher DDT/DDE ratio reflects more recent contamination, while a high *op'*/*pp'* ratio may indicate that DDT contamination originated from impurities in dicofol (Ricking and Schwarzbauer, 2012). The higher proportion of untransformed DDT may therefore point to a relatively recent contamination, despite the fact that production ceased at the factory in 1982. The lower proportion of both DDD congeners in Wheathampstead compared to other sites would indicate that the transformation processes occurring there were mainly aerobic, hence favouring DDE, while at other sites anaerobic processes in sediments may have had more influence. There is, however, also a possibility that differing congener distributions between Wheathampstead and other sites resulted from the use of different formulations. Murphy's factory had a development department for improving pesticides, so it is possible that some formulations or varieties of formulations that were not or not yet on the market were tested on the fields close to the river Lee. In the case of DDT, this seems, however, unlikely because its constitution varied little between manufacturers.

There were no obvious differences between *op'* and *pp'* ratios between the different sites for any of the DDT family, and the overall distribution showed a strong dominance of *pp'* congeners which points to contamination from DDT itself rather than from impurities in dicofol (Ricking and Schwarzbauer, 2012). A shift

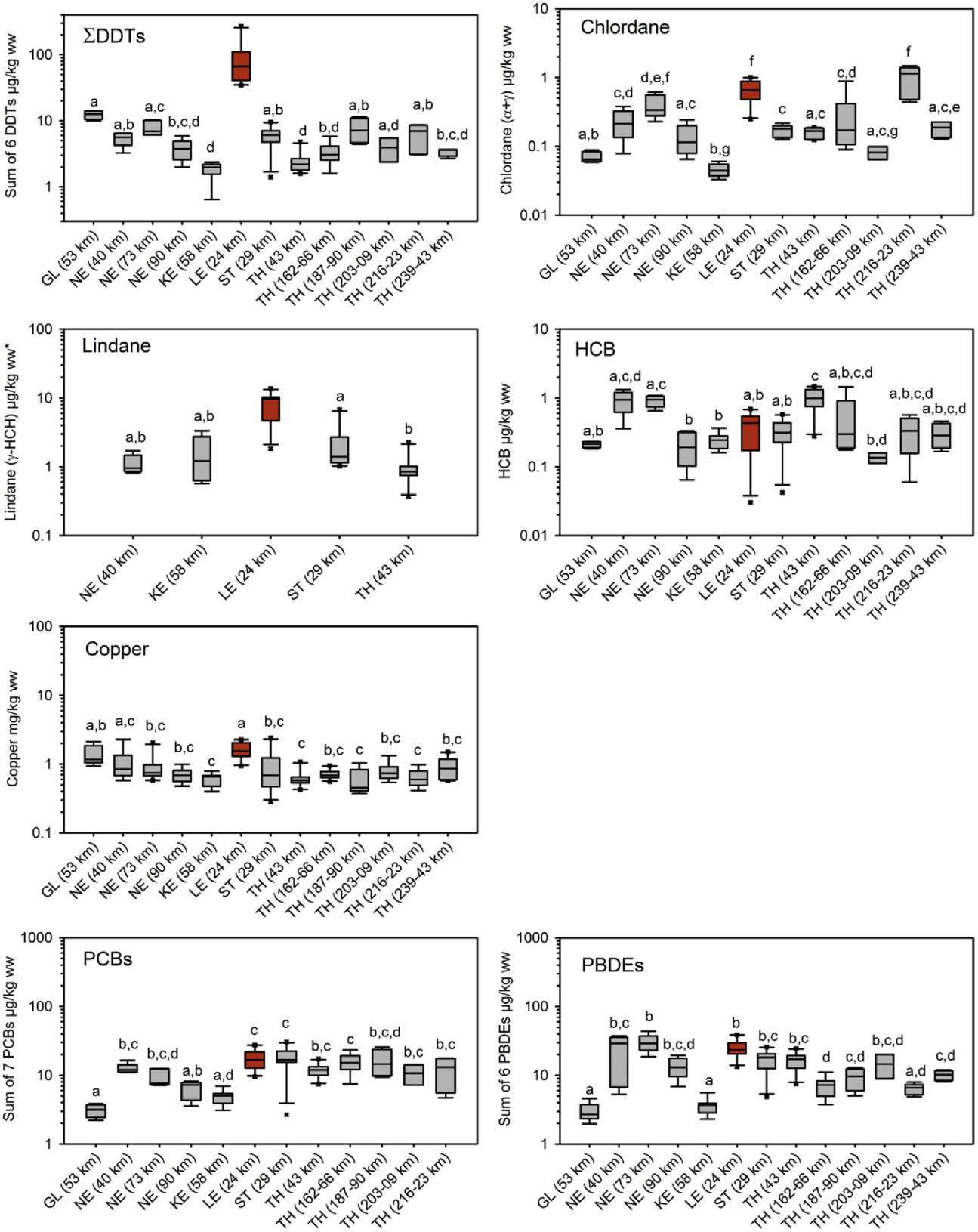

Fig. 3. ∑DDT concentrations in roach (details in Fig. 2) compared to the insecticides chlordane and lindane (γ–HCH), the fungicide HCB, copper, PCBs (∑ indicator congeners 28, 52, 101, 118, 138, 153, 180) and PBDE flame retardants (∑ indicator congeners 28, 47, 99, 100, 153, 154). Fish were caught between 2007 and 2012, river codes are given in Fig. 1, the distance in brackets refers to the distance of the sampling site from the source of the river. Boxplots show, 25-, 50-, and 75-%iles with 10- and 90-%ile whiskers. Wheathampstead on the Lee has been coloured in. * Lindane concentrations are semi-quantitative because they were analysed with the standard GC/MS method, which is less accurate for HCHs than the method used in Jürgens et al. (2015). Groups that do not share a common letter are significantly different from each other at $\alpha = 0.5$ (details in Tables S4–S10).

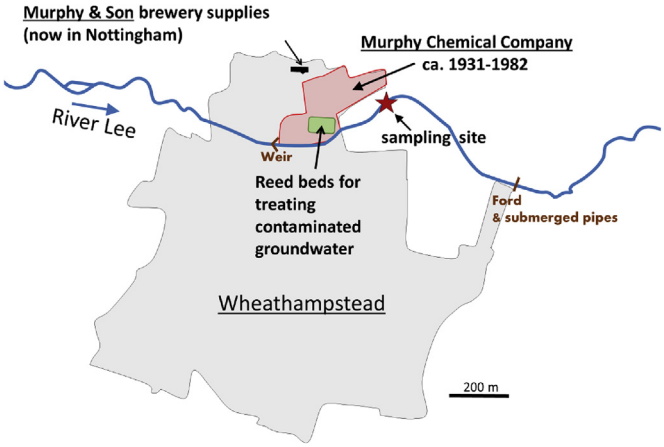

Fig. 4. Schematic map of Wheathampstead, with the fish sampling site, obstacles to fish movement, and the approximate extent of the area formerly occupied by the Murphy Chemical Company.

towards higher *pp'/op'* ratios for the transformation products was however visible (Fig. 2b). Since the *pp'* or *op'* configuration remains during transformation of DDT to DDE or DDD, one would expect the ratio to be about 85% *pp'* and 15% *op'* as in the parent compound (ATSDR, 2002). However, for the *pp'* congeners *pp'*DDE was the largest contributor with *pp'*DDD playing a minor role and the untransformed *pp'*DDT being almost absent, whereas for the *op'* congeners, DDD was generally more prominent than DDE and *op'*DDT occurred at similar concentrations to *pp'*DDT despite the very different starting ratio. This confirms that the fate of *op'* and *pp'* DDT congeners in the environment differs, reflecting their different physico-chemical properties (Ricking and Schwarzbauer, 2012) (Table S2).

3.4. DDT contamination in other recent European freshwater fish studies

The results from the current study were compared to ∑DDTs contamination of commonly monitored fish species collected at freshwater sites or lagoons in Europe between 1995 and 2014, i.e. well after agricultural use of DDT was banned in the EU in 1981 (Table 2 lists values from known or suspected polluted sites, while "background" values are found in Table 3). Concentrations in our roach from sites other than Wheathampstead were fairly typical for recent European data from "background" sites (Table 3), probably reflecting the legacy of historic agricultural use of DDT and possibly dicofol. However, in a number of places high concentrations of DDTs in fish were observed long after its use and production ceased (Table 2). Some areas of high contamination were linked to (former) factories producing DDT (Bettinetti et al., 2006, 2010, 2012; Hrádková et al., 2012) or dicofol (Lacorte et al., 2006; de la Cal et al., 2008). In other cases DDT hotspots were reported without identifying a source (Niewiadowska et al., 2014) or as in the case of the river Elbe, the legacy of several large industrial areas (presumably including pesticide manufacturing) were considered responsible for elevated DDT levels in fish along large parts of its length through two countries (Hrádková et al., 2012; Umweltbundesamt, 2016).

In a few locations, ∑DDT contamination of fish was monitored over several years. It might be expected that the concentrations in fish would decline following the end of production and use of DDT. However, this was not always observed. For example, in Germany DDE + DDD were monitored in bream for 10–21 years at 17 sites (Umweltbundesamt, 2016). Of these only one site on the river Saar clearly followed the expected exponential decline with $R^2 > 0.5$ ($R^2 = 0.86$, $p < 0.001$), the concentrations at five more locations showed a less clear but significant ($\alpha < 0.05$) downward trend and at four sites a significant increase was observed. In the USA, concentrations in largemouth bass caught near a former DDT factory that had closed in 1963, remained at very high levels (average about 9 mg/kg ∑DDTs) for three decades (Hinck et al., 2009a) and at some locations in Germany (Heinisch et al., 2005) and Italy (Bettinetti et al., 2012) ∑DDTs concentrations in fish and zooplankton increased many years after a nearby factory had stopped processing it. This suggests a continuing (secondary) source of DDTs such as contaminated soils or sediments.

3.5. Implications

The review of recent European data (Tables 2 and 3) showed that while the DDT levels in fish influenced only by diffuse pollution from its agricultural use are now low, some fish from industrially impacted sites had body burdens associated with negative effects on the fish themselves or their predators (Table 1). This shows that even decades after its ban this chemical still has the potential to cause harm. All ten roach from Wheathampstead, but only one of the 71 others in the current study exceeded the Canadian EQS for the protection of fish-eating birds of 14 μg/kg. The concentrations in some of them were higher than reported effect concentrations in early life stages of fish and approaching reported effect concentrations for adults (see Table 1), while whole-life exposure or multigenerational effects are largely unknown. However, there was no evidence of actual harm to the roach in the current study as the population of the contaminated site appeared to be in good condition with larger and fatter individuals than were caught at other sites. Furthermore there is genetic evidence for a population, which is self-sustaining rather than consisting of recent immigrants and which has recovered from a population bottleneck – perhaps due to the 1967 fish kill (Hamilton et al., 2014). However health effects on individuals do not necessarily result in population level effects (Hamilton et al., 2015) and we cannot rule out adaptation that enables fish to tolerate the harmful effects of exposure. Whether fish-eating birds near this and other contaminated sites are adversely affected is not known, but is at least a possibility judging from the effect levels reported in Table 1.

The unexpected high ∑DDTs concentrations found in some fish in this study can serve as an example how monitoring chemicals in fish tissue can be useful to spot previously unknown problems. A spike in a temporal or spatial series would indicate that something unusual happened, which warrants further investigation. In this case, a former pesticide factory was identified as the likely cause of the pollution in fish.

Acknowledgements

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.chemosphere.2016.07.078.

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