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Evidence Project Final Report

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Project identification

1. Defra Project code: SF0255
2. Project title: The impact of diffuse pollution on European eel population
3. Contractor organisation(s): Cefas, Pakefield Road, Lowestoft, Suffolk, NR33 0HT
4. Total Defra project costs (agreed fixed price): £
5. Project: start date 01/04/2009
   end date 31/03/2014

6. It is Defra's intention to publish this form.

   Please confirm your agreement to do so.....**YES** ☒ **NO** ☐

   (a) When preparing Evidence Project Final Reports contractors should bear in mind that Defra intends that they be made public. They should be written in a clear and concise manner and represent a full account of the research project which someone not closely associated with the project can follow.

   Defra recognises that in a small minority of cases there may be information, such as intellectual property or commercially confidential data, used in or generated by the research project, which should not be disclosed. In these cases, such information should be detailed in a separate annex (not to be published) so that the Evidence Project Final Report can be placed in the public domain. Where it is impossible to complete the Final Report without including references to any sensitive or confidential data, the information should be included and section (b) completed. NB: only in exceptional circumstances will Defra expect contractors to give a "No" answer.

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   (b) If you have answered NO, please explain why the Final report should not be released into public domain

Executive Summary

7. The executive summary must not exceed 2 sides in total of A4 and should be understandable to the intelligent non-scientist. It should cover the main objectives, methods and findings of the research, together with any other significant events and options for new work.

The European eel has declined throughout its distribution and recruitment has fallen to less than 5% of the peak levels measured in the late 1970s. As a result the stock is judged to be outside safe biological limits. There is evidence that this decline is the result of a range of factors both anthropogenic, (e.g. exploitation, habitat deterioration, obstructions to migration, pollution, parasites and disease) as well as changing environmental processes (e.g. global warming, changes to oceanic conditions,). In response to this decline, the EU "Eel Recovery" regulation, published in 2007, was implemented. This requires Member States to assess their stocks against target levels based on historic production and implement recovery measures as appropriate. Therefore, to satisfy the EC Eel Recovery Plan, Member States have been tasked with developing Eel Management Plans (UK, EMP 2010) in order to assess national eels stocks and implement measures as necessary to allow for a 40% silver eel biomass escapement compared to pristine conditions. However, the major factors regulating eel populations are still unknown and until there is an understanding of the factors causing the low recruitment of eels, the success of any management plans and conservation measures may be limited. There is growing concern that the quality of the emigrating spawning population of eel may be a major factor forcing the decline of the eel. The quality of the silver eels that undertake the transition from freshwater to begin their oceanic migration may be seriously impaired by pollution, parasites and disease prior to their entry into the marine environment. Previous studies on the Atlantic salmon have indicated that conditions within freshwater and in particular water quality (e.g. diffuse pollution) can affect the ability of migrating fish to adapt both physiologically and behaviourally to the transition between the fresh and marine environments. The European eel, like salmon, is a diadromous fish and having spent between 2 and 20 years in freshwater, must migrate to its marine spawning grounds to successfully reproduce. Therefore, one of the principal objectives of the present research was to investigate the potential impact of freshwater contaminants on specific physiological and morphological parameters that are known biomarkers of the transition from the yellow eel to the migratory silver form and their subsequent survival in the marine environment. In addition, the conditions experienced by the returning juvenile eel within coastal zone and estuarine environment was also studied to understand whether the factors controlling eel recruitment to freshwater were related to contaminant exposure during this phase of the life cycle. During the freshwater stage, juvenile eels inhabit the benthic environment and may remain buried within the sediment, principally during the daylight hours, before emerging at night to forage. Conditions experienced by the eel during this period, and in particular contaminant exposure, are also likely to affect eel population abundance. During this period they may be exposed to sediment-bound

as well as water-borne contaminants, and they may bio-accumulate contaminants that have a negative impact on development and survival. In addition, bioaccumulation of contaminants may occur through the ingestion of contaminated preys that are also resident within the substrate. Therefore, a further objective of the work was to investigate how contaminants taken up by the juvenile eel directly from the sediment or through ingestion of contaminated food could have a potential role in regulating the quality and survival of the European eel.

The overall objective of the research was to investigate the role of specific diffuse aquatic contaminants in regulating populations of European eels. In particular the specific objectives of the research were to: investigate the impact of relevant environmental contaminants on the transition from freshwater to the marine environment and the subsequent adaptation of migrating adult silver eels to saline conditions; investigate the impact of relevant contaminants within coastal and estuarine environments on the transition of juvenile eels from the marine to the freshwater environment; investigate the impact of sediment-derived contaminants on growth and survival in juvenile eels; investigate the impact of freshwater contaminants on the migratory behaviour of silver eels during their initial estuarine/coastal spawning migration; provide advice and recommendations to Policy Divisions on the management of aquatic contaminants in relation to supporting the implementation of the Eel Recovery Plans. The research was based on a general mechanistic approach, integrating behavioural and physiological studies both in the laboratory and in the field. Behavioural studies on adult silver eels were undertaken using an acoustic telemetry system that permitted the movement of tagged fish to be monitored as they migrated from freshwater and into the marine environment. These studies compared the behaviours of groups of fish that had been previously exposed to a contaminant to groups of control eels. Parallel physiological studies were undertaken at the same time in the laboratory to examine the impact of the contaminant on physiological and morphological biomarkers that indicated successful silvering in the eel.

The results of this research indicate that exposure to a range of contaminants within freshwater has very little effect on the physiology or run-timing of silver eels as they migrate out to sea. In a similar way, there was no evidence from the present study that the studied marine contaminants have an effect on the ability of glass eels and elvers to successfully move into the freshwater environment. This is markedly different to earlier studies on Atlantic salmon, which have shown that contaminants have significant effects on the run-timing of smolts, ability to adapt to saltwater and on the olfactory ability to detect the cues considered important in imprinting and used to return to the home river to spawn. Although, the short term exposure of silver eels to contaminant did not affect the silvering process or successful short term migration, the present study did indicate that exposure to certain metals within sea water produced significant genetic damage in the glass eels as measured by the Comet Assay. Damage at the molecular level in many fish can cause serious problems but in the present study it is not clear to what extent the genetic damage is carried through to the whole animal or what the effects might be at the population level. Although, eels are known to bio-accumulate a number of toxins as a result of their high fat content, the present research also indicated no physiological or behavioural effects on juvenile eels as a result of exposure to contaminated sediment or ingested contaminated prey. However, once again the Comet assay carried out on the glass eels did indicate that exposure to metals in the sediment does cause genetic damage although how this is can be assessed at the whole animal and population level is not clear.

There are a wide-range of emerging organic groundwater contaminants (EGCs) which are beginning to be monitored in the UK. These include nano-materials, pharmaceuticals, industrial compounds, personal care products, fragrances, water treatment by products, flame surfactants as well as caffeine and nicotine. Therefore, future targeted studies investigating the role of these EGCs on eels and the impact of these novel compounds on migration and spawning in European eel would be supportive of the required management plans.

Project Report to Defra

8. As a guide this report should be no longer than 20 sides of A4. This report is to provide Defra with details of the outputs of the research project for internal purposes; to meet the terms of the contract; and to allow Defra to publish details of the outputs to meet Environmental Information Regulation or Freedom of Information obligations. This short report to Defra does not preclude contractors from also seeking to publish a full, formal scientific report/paper in an appropriate scientific or other journal/publication. Indeed, Defra actively encourages such publications as part of the contract terms. The report to Defra should include:

- the objectives as set out in the contract;
- the extent to which the objectives set out in the contract have been met;
- details of methods used and the results obtained, including statistical analysis (if appropriate);
- a discussion of the results and their reliability;
- the main implications of the findings;
- possible future work; and
- any action resulting from the research (e.g. IP, Knowledge Exchange).

1. Introduction

The European eel has declined throughout its distribution and recruitment has fallen to less than 5% of the peak levels measured in the late 1970s. As a result the stock is judged to be outside safe biological limits. There is evidence that this decline is the result of a range of factors both anthropogenic, (exploitation, habitat deterioration, obstructions to migration, pollution, parasites and disease) as well as changing environmental processes (e.g. global warming, changes to oceanic conditions,) (ICES, 2002). In response to this decline, the EU "Eel Recovery" regulation, published in 2007, was implemented. This requires Member States to assess their stocks against target levels based on historic production and implement recovery measures as appropriate. Therefore, to satisfy the EC Eel Recovery Plan, Member States have been tasked with developing Eel Management Plans (UK, EMP 2010) in order to assess national eels stocks and implement measures as necessary to allow for a 40% silver eel biomass escapement compared to pristine conditions. However, the major factors regulating eel populations are still unknown and until there is an understanding of the factors causing the low recruitment of eels, the success of any management plans and conservation measures may be limited.

Recommendations by the EIFAC/ ICES Working Group on Eel (EIFAC/ ICES, 2006) included a need to improve understanding of factors within the freshwater environment that may limit eel survival and the quality of the spawning population. However, the present scientific knowledge does not allow the assessment of the effects of these factors on the overall stock. A Workshop on Eel Health, organised by the Defra/Cefas/EA Liaison Group was held in November 2008 which also highlighted the lack of information on the impacts of the freshwater environment on eel and its potential to reduce the survival and reproductive viability of the stock. In particular, the workshop emphasised that very little is known regarding the impact of diffuse pollution on the migratory and reproductive capabilities of eels once they enter the marine environment.

Previous research carried out by Cefas on the effects of diffuse pollution on another diadromous fish species, the Atlantic salmon, has demonstrated that environmental levels of contaminants such as pesticides may have a significant impact on the biology of the fish. For instance, exposure of salmon in freshwater to common herbicides and sheep dip insecticides has been shown to affect pheromonal mediated reproduction (Moore & Waring 1995; Waring & Moore, 1997; Moore & Waring, 1998; Moore & Lower, 2001), embryo survival and development (Lower & Moore, 2003), smolt migration (Moore *et al*., 2007; Moore *et al*., 2008), olfactory imprinting (Lower & Moore 2007) and the ability of the smolts to survive once they have migrated into the marine environment (Waring & Moore, 2004; Moore *et al*. 2003). Overall, this research concluded that conditions in freshwater and in particular diffuse contaminants had a significant effect on both the reproductive capability of adults and the ability of the emigrating smolts to survive during the transition from the freshwater to the marine environment. In particular, the research identified a number of common freshwater contaminants that inhibited the parr-smolt transformation in salmon, the period of development when the juveniles undergo physiological, morphological and behavioural changes, which pre-adapted the fish to a life in the marine environment (McCormick *et al*. 1998).

The European eel, like salmon, is a diadromous fish and having spent between 2 and 20 years in freshwater, must migrate to its marine spawning grounds to successfully reproduce (Tesch, 2003). Eels therefore undergo a physiological and morphological transformation during which they develop from the freshwater yellow eel to the migratory form (Durif et al. 2005), a process that is similar to salmon smoltification and known as 'silvering' (Van Ginneken et al. 2007a,b). The mechanisms involved in the onset of silvering in eels are largely unknown, and only two extensive studies have been undertaken on the morphological and physiological characteristics at the different stages of eel silvering (Durif, 2003: Durif *et al*., 2005). The silvering process is known to be positively related to morphological characteristics such as eye size and internal maturation parameters, such as GSI, vitellogenin and blood-substrates including phospholipids (Van Ginneken *et al*. 2007a). The transformation also occurs in association with hormonal surges of testosterone and estradiol (Van Ginneken *et al*. 2007b). Physiological changes also occur (e.g. salinity tolerance, increased hypo-osmoregulatory ability), which allow the eel to successfully survive within the marine environment.

There is growing concern that the quality of the emigrating spawning population may be a major factor forcing the decline of the eel. The quality of the silver eels that undertake the transition from freshwater to begin their oceanic migration may be seriously impaired by pollution, parasites and disease prior to their entry into the marine environment. Due to specific ecological and physiological traits, eels are particularly sensitive to the bioaccumulation of lipophilic contaminants (Belpaire, 2008). These contaminants may not only impair the long term reproductive viability of the spawners but may also affect the ability of the silver eels to undergo the necessary physiological, behavioural and morphological transformations that are necessary to adapt and survive in saltwater and successfully complete their oceanic spawning migration. As a result of the benthic nature of their ecology and the long periods spent in a wide range of flowing and still waters, the eels may be exposed to a suite of contaminants for extended periods in freshwater. Therefore, a major objective of the present research was to investigate the potential impact of freshwater contaminants on specific physiological and morphological parameters that are known biomarkers of the transition from the yellow eel to the migratory silver form and their subsequent survival in the marine environment.

The adult silver eels are not the only life history stage that must migrate across the saltwater/freshwater boundary. Juvenile eels that return from the oceanic spawning grounds metamorphose once they reach coastal waters and change from the leaf-like leptocephalus to the typically eel-shaped, transparent and colourless juveniles known as glass eels (Tesch, 2003). The glass eels use both passive tidal transport and active migration to move into and through river estuaries where they pigment and begin the elver stage (Tesch, 2003). During this stage, the glass eels and elvers are also exposed to a wide range of contaminants present in both the marine and estuarine environments. Therefore, a further objective of the research was to investigate the potential impact of contaminants commonly found in coastal and estuarine on specific physiological and morphological parameters that are known biomarkers during the transition to the elver stage and the subsequent survival of the juvenile eels in the freshwater environment.

During the freshwater stage, juvenile eels inhabit the benthic environment and may remain buried within the sediment, principally during the daylight hours, before emerging at night to forage. Conditions experienced by the eel during this period, and in particular contaminant exposure, are also likely to affect eel population abundance. During this period they may be exposed to sediment-bound as well as water-borne contaminants, and they may bioaccumulate contaminants that have a negative impact on development and survival. In addition, bioaccumulation of contaminants may occur through the ingestion of contaminated preys that are also resident within the substrate. Therefore, a further objective of the work was to investigate how contaminants taken up by the juvenile eel directly from the sediment or through ingestion of contaminated food could have a potential role in regulating the quality and survival of the European eel.

2. Specific objectives of the research.

The overall objective of the research was to investigate the role of specific diffuse aquatic contaminants in regulating populations of European eels.

In particular the specific objectives of the research were to:

1. Investigate the impact of relevant environmental contaminants on the transition from freshwater to the marine environment and the subsequent adaptation of migrating adult silver eels to saline conditions.
2. Investigate the impact of relevant contaminants within coastal and estuarine environments on the transition of juvenile eels from the marine to the freshwater environment.
3. Investigate the impact of sediment-derived contaminants on growth and survival in juvenile eels.
4. Investigate the impact of freshwater contaminants on the migratory behaviour of silver eels during their initial estuarine/coastal spawning migration.
5. Provide advice and recommendations to Policy Divisions on the management of aquatic contaminants in relation to supporting the implementation of the Eel Recovery Plans.

The research was based on a general mechanistic approach, integrating behavioural and physiological studies both in the laboratory and in the field. Behavioural studies on adult silver eels were undertaken using an acoustic telemetry system that permitted the movement of tagged fish to be monitored as they migrated from freshwater and into the marine environment. These studies compared the behaviours of groups of fish that had been previously exposed to a contaminant to groups of control eels. Parallel physiological studies were undertaken at the same time in the laboratory to examine the impact of the contaminant on physiological and morphological biomarkers that indicated successful silvering in the eel.

The research complements and supports additional research on eel populations that was carried out at Cefas including:

- Defra Research Contract SF0249 – Investigating the influences of habitat on European eel (*Anguilla anguilla*) production in England and Wales.
- EU FP7 Contract - European Eel Investigation and Assessment of their Decline (EELIAD).

Objective 1. Investigate the impact of relevant freshwater contaminants on physiological and morphological parameters associated with the transition from freshwater to the marine environment and the subsequent adaptation of adult silver eels to saline conditions.

Introduction.

Eels undergo a physiological and morphological transformation during which they develop from the freshwater yellow eel to the migratory form a process that is known as 'silvering' and which enables them to survive their subsequent life in the ocean. During this process there is an increase in the size of the eye, changes in fin length, an increase in maturation parameters such as gonadosomatic index (GSI), changes in the hepatosomatic index (HSI), and increases in the fat content. The silvering process also includes histological changes (e.g. salinity tolerance, increased hypo-osmoregulatory ability), which allow the eel to successfully survive within the marine environment. The objective of the work was similar to that previously carried out on salmon smolts, to examine how contaminants could potentially modify and regulate key physiological and morphological parameters associated with silvering process and so effect the ability of migrating eels to survive once they had entered the sea.

General methodology.

Freshwater contaminants.

The specific contaminants used in the experiments were chosen following an extensive literature search, which included recent water quality monitoring studies, to identify potential active contaminants that are both temporally and spatially relevant to the silvering process in the eel. The criteria that were used to identify the most likely contaminants that could impact on adult eels during their transitions between the freshwater and marine environments were similar to those developed for the Atlantic salmon ecotoxicology research. The contaminants chosen were those that occur within the freshwater environment at the time the eel are undergoing a sensitive stage in their life history (e.g. migration and freshwater/saltwater adaptation) and that have a known toxicological impact on the processes controlling the successful transition of the fish between fresh and saltwater. This approach identified the following environmentally relevant contaminants that might affect the biological processes involved in silvering and the transition to the marine environment of the eel: pesticides (fenitrothion, atrazine, chlorpyrifos, chlorotoluron, pendimethalin, flusilazole, metaldehyde and copper oxychloride) and tributyl phosphate.

Fish collection and exposure to contaminants.

Glass eels and elvers where purchased from Glass Eels UK Ltd (Gloucester). The glass eels were caught in the estuary of the River Severn during the spring run (February to April), and the elvers were part of the company's growing-on programme. Glass eels and elvers were supplied in water filled boxes and transported to the Cefas Lowestoft Laboratory. Once in the laboratory they were immediately placed in 100 litre tanks with flow through (1 l/min) seawater (glass eels) (1 l/min) or freshwater (elvers) (1 l/min) and an air supply. Adult eels used at the Lowestoft laboratory were purchased from a commercial fisherman operating in both the River Avon (Hampshire) and the River Stour (Dorset). The eels from the River Avon were caught by fyke nets while the fish from the River Stour were caught using an eel rack at Longham (coordinates 50.7863, -1.90673). Fish were caught while emigrating downstream during the months of October and November. Fish collected over subsequent nights were held in tanks in the river until collection (between 3 and 10 days). Fish were placed in water filled polythene bags in groups of 5 to 10 and oxygenated using battery operated aquarium aerators. Each bag was placed in a dark plastic container to reduce stress levels. Fish were then transported to the Cefas Lowestoft Laboratory and on arrival immediately distributed into tanks (550 l) with a constant flow of dechlorinated tap water and an air supply. Seawater was pumped from wells positioned at a depth of 2 m at the low water mark on the beach adjacent to the Cefas Lowestoft Laboratory (Latitude 52.472°N). Both juvenile and adult fish were kept in a naturally simulated photoperiod (longitude 52°N) and were fed to satiation daily with commercial food pellets of appropriate diameter for their size.

All experiments described were carried out under a Personal Licence (PIL 80/10073) from the Home Office under The Animals (Scientific Procedures) Act 1986. All the work was carried out under an appropriate

Project Licence held by Dr Andrew Moore (PPL 80/2174 and 70/7588). At all times the eels used in the experiments were maintained under carefully controlled conditions that ensured their welfare. Prior to sampling, eels were anaesthetised with 2-phenoxyethanol (2-4 ml/l) dissolved in water. The eels were able to detect the anaesthetic in the bath and would often lift their heads out of the water. Therefore, all procedures with adult eels were undertaken in a suitable sized plastic bag which could be closed at the top without any air left over the water, ensuring that the eel inhaled the anaesthetic. Fish were considered to be fully anaesthetized when there was a total loss of muscle tone and total loss of equilibrium. In eels this was usually achieved within 3 to 6 minutes.

In general, each experiment involved the exposure of randomly distributed groups of eels to up to 3 different concentrations of a contaminant (with duplicates and controls) and was carried out in flow through condition maintaining the desired concentration of the chemical in each of the tanks using a peristaltic pump.

Stock solutions of contaminants were made up measuring amounts of chemicals on a tared weighing boat to the nearest 0.001g (Mettler Toledo AG64) and then dissolved in either distilled water, ethanol or acetone on a magnetic stirrer (Stuart SB162) at room temperature. Dosing in the tanks was achieved either in static or in flow through conditions. For experiments run in static conditions the water inflow to the holding tanks was turned off at the beginning of the experiment and a suitable amount of dissolved testing compound was added in the tank in order to achieve the desired testing concentration in the total tank water volume.
For experiments run in flow through conditions the desired testing concentration was maintained using a peristaltic pump (Watson Marlow 205U) and tubing of 0.88mm bore (orange-orange).

The following calculations were used to determine the amount of stock solution required to reach the testing concentration in the fish tank:

$$S = \left(\frac{Outflow}{Inflow}\right) * F$$

Where:
S = concentration of stock solution (µg/l)
Outflow = flow rate out of the tank (l/min)
Inflow = flow rate into the tank (l/min)
F = final concentration in the tank (testing concentration)
This was derived considering that the final concentration F need to remain constant and therefore the same amount of contaminants that leave the tank must be replaced by a same amount entering

Weight entering = weight leaving

$$Inflow * S = Outflow * F$$

$$S = \left(\frac{Outflow}{Inflow}\right) * F$$

The peristaltic pump was set at 40rpm, which correspond to 1.4ml/min, the dosing solutions were made up in 3 l glass beakers using tank water and were replaced every 24 hours. Mixing of dosing solution entering the tank and the tank water was reached by aeration in the tank.

After the exposure of the eels to the relevant concentrations of the contaminants in freshwater the eels were transferred to full saline conditions for a 72 hour seawater challenge test. This procedure was undertaken to quantify and model whether the contaminant reduced the survival of fish once they had moved from a freshwater to a marine environment.

Physiological and morphological measurements.

In juvenile eels, the wet weight was measured to the nearest 0.01g and the length was measured to the nearest 0.1cm. In adults, weight of fish, liver and gonads were measured to the nearest 0.1g. Liver and gonad weights were used to calculate hepatosomatic (HSI) and gonadosomatic (GSI) indexes respectively. HSI was calculated as 100 x [liver weight (g)/fish weight (g)], and similarly GSI was calculated as 100 x [gonad weight (g)/fish weight (g)]. Fish length was measured on a measuring board to the nearest cm and eye horizontal and vertical diameters were measured with a digital calliper to the nearest 0.01 cm.

Individual length and weight were used to calculate the Condition Factor (CF) as 100{body weight (g)/ [body length (cm)]^3}. Eye diameters were used to calculate the Eye Index (EI) according to Pankhurst (1982).

$$EI = \left[\frac{\left(\frac{A+B}{4}\right)^2 \pi}{L}\right] 100$$

A=horizontal eye diameter
B= vertical eye diameter
L=total fish length
Fat content of adult fish was measured by averaging 4 external readings taken with a Distell fat meter at successive positions along the body of the eel from the head to the tail region. The meter automatically calculated the percentage of fat in relation to the amount of water contained in the measured sample. The instrument calculated the fat percentage using an eel specific calibration relationship (see www.distell.com).

Measurement of gill and kidney Na^+/K^+ ATPase.

Gill samples of juvenile fish were obtained by taking the whole body section posteriors to the head. In adults, one gill arch was removed from the left side of the head, and a kidney sample was taken from the distal portion of the organ. Once collected, gill and kidney samples were placed in a micro-tube with 0.75 ml of SEI buffer (0.3M sucrose, 20 mM Na2EDTA, 0.1mM imidazole) and immediately frozen in liquid nitrogen and later transferred in a -80º freezer until assayed (Zaugg 1982). Prior to protein determination, all the samples were prepared using a Biofuge 15R centrifuge set to the following parameters: temperature - 10°C, time - 8 minutes and speed - 2500 rpm. After centrifugation the supernatant was discarded and the bottom pellet re-suspended in 750 µl of SEI buffer in deoxycholic acid (2.41mM). The tissues were then manually homogenized in a conical glass homogeniser until all filaments or tissues disintegrated. The resulting homogenate was then centrifuged again at 10°C for 8 minutes at 3500 rpm for gill tissues and at 5000 rpm for kidney samples. After centrifugation the supernatant was kept on ice and the pellets discarded to waste. Protein extract of the samples were estimated with a commercial Pierce BCA™ Protein Assay Kit (Perbio Science, UK), consisting of BCA reagent A, BCA reagent B and albumin standard, 2 mg/ml.
The protein content was then used to prepare a solution of sample and SEI buffer containing 1 mg/ml of protein and the enzymatic activity determined.

Gill and kidney Na^+/K^+ ATPase activity was assayed according to the method described by Schrock et al. (1994) with the enzyme activity shown as µmol Pi/mg protein*h. Briefly, 10 µl of the sample solution was added into each well of a 96 well microplate. Then 76 µl of solution A (155.2mM NaCl, 23.02mM MgCl2, 75.12mM KCl, 115.01mM imidazole) or solution B (solution A plus 0.72mM ouabain) is added, and incubated at 37ºC for 10 minutes. The reaction was stopped by adding 200 µl of a 1:1 mixture of 10% trichloric acid and "colour reagent" (9.20mM ammonium molybdate, 0.66 mM sulphuric acid and 0.33mM ion sulphate). After 30 minutes of incubation at room temperature the Pi produced was determined spectrophotometrically at 630 nm using BioRad benchmark microplate reader.

Measurement of blood plasma

Prior to blood sampling, syringes, needles and micro-tubes for blood collection were rinsed with heparin solution (500 units/ml dissolved in 0.9% saline – NaCl dissolved in ultrapure water). Blood was collected from the caudal vein of anaesthetized adult fish using a heparinised 5ml syringe with a 25G needle and then transferred in a 2ml heparinised centrifuge tube. Blood was then centrifuged at 10000 rpm for 3 minutes. After centrifugation the plasma was transferred with a pipette (Gilson) and a clean tip (Gilson) for every sample in aliquotes of 100 µl in 0.5ml labelled micro-tubes and stored in a -20°C freezer until analysis.

Osmolarity was measured using 100 µl aliquots by means of an automatic Osmometer (Löser) displaying the mosm as per Kg of water. The instrument was calibrated according to the manufacturer instruction with deionized water and a standard 300 mosm/kg water (Löser) solution.

Plasma ions: Chloride ions were measured with a Jenway PCLM3 Chloride meter. 20 µl of plasma were used for the measurement of mmol/l of chloride in the sample. The sample was added to 20 ml of a combined acid buffer (Reagecon, Ireland) after calibration with 100mmol/l of chloride standard (Jenway, England). Sodium and potassium were measured with a Sherwood Flame Photometer after diluting the plasma 26 times for potassium and 773 times for sodium.

Glucose was measured with a colorimetric assay. 50 µl of each sample were diluted 10 times with deionized water, then 3 ml of colour reagent (0.12M PBS with 6.8 units/ml Peroxidise enzyme, 6.5 units/ml Glucose Oxidase enzyme and 0.3 mg/l ABTS) was added to the samples and incubated at 37°C for 30 minutes. Absorbance was measured with a spectrophotometer at wavelength of 440 nm. Glucose concentrations were calculated from a standard curve of D-glucose in concentrations ranging from 1.4mM to 0.09mM.

In addition, the tissue samples were assessed for potential disease vectors, which may also exacerbate the effects of the contaminants. All sampled eels were also assessed for parasitic loading (e.g. *Anguillicola crassus*) in order to understand any additional impacts on the survival of the eels.

Experiment 1.1. Impact of fenitrothin on the transition from freshwater to the marine environment.

Fenitrothion (O,O-dimethyl O-4-nitro-m-tolyl phosphorothioate) is a contact organophosphate insecticide that has been widely used since 1959 to control insects in agriculture and for fly, mosquito and cockroach control in public health programmes worldwide. Since 2001, fenitrothion has not been approved for use in the UK as an agricultural plant protection product although it was still permitted to be used as an insecticide for non agricultural purposes (Advisory Committee on Pesticides 2006). In 2007, the Commission of the European Communities agreed on the withdrawal of fenitrothion for use in plant protection products to be implemented later the next year (2007/379/EC), and from this date no authorizations for plant protection products containing fenitrothion can be granted or renewed.
Fenitrothion is not persistent in soil and leaching from agricultural land into water courses is considered not to be significant (WHO 2010). Therefore, there is negligible risk of contamination to groundwater as a result of leaching. However, aquatic organisms (fish and invertebrates) are potentially at risk, especially in the event of overspray from non-agricultural purposes to static and/or running water bodies. Although, fenitrothion is fat-soluble, the rates of biotransformation and the excretion of metabolites largely mitigate bioconcentration in animals, although the high fat content of European eels may make them more susceptible than other freshwater fish species. Typically, the duration of exposure is brief in the aquatic environment because fenitrothion dissipates quite quickly in microbially active natural water systems and it has a half-life of less than one week. Fenitrothion as a compound tends to migrate to the sediment in many water courses (WHO 2010) where it may pose an additional problem to eels, principally during the early life history stages where they are buried within the sediment during daylight hours. Fenitrothion has been measured in a number of water courses in England. Typically, levels are < 0.001 µg l^{-1} but periodically spikes occur with levels as high as 0.1 µg l^{-1} being recorded in the River Avon, Hampshire (Environment Agency Pesticide Monitoring Data 1997-2009).
Fenitrothion is known to be toxic to a number of fish. The 96-hour LC50 for brook trout was shown to be 1.7 mg l^{-1} and 3.8 mg l^{-1} for bluegill sunfish (US EPA, 1987). Takimoto *et al.* (1987) estimated a 96 hour LC50s of 3.5 mg l^{-1} for adult killifish *Oryzias latipes* and 2.6 mg l^{-1} for the mullet *Mugil cephalus*. The LC50s for both fish in freshwater were also similar, at 3.5 and 2.6 mg l^{-1} respectively. In the European eel the 96-hour LC50 is 0.2 mg l^{-1} (Ferrando *et al*. 1991).
Fenitrothion is an organophosphate insecticide reported to be a neurotoxin in fish due to its irreversible inhibitory effect on acetylcholinesterase (AChE). The enzyme AChE degrades the neurotransmitter acetylcholine in cholinergic synapses. The inhibition provokes an accumulation of acetylcholine in synapses with disruption of the nerve function that can lead to a wide range of toxic effects in fish (Kapur *et al*. 1978; Morgan *et al*. 1990; Sebire *et al*. 2009). Fenitrothin is also known to have a wide range of sub-lethal effects on the physiology of the European eel, including energy metabolism (Sancho *et al*. 1997a,1998), inhibition of brain AChE (Sancho *et al*. 1997b) and muscle physiology (Sancho *et al*. 1999). Research has also indicated that fenitrothion may inhibit gill Na^+K^+ATPase activity (Sancho *et al*. 1997c).
During the transition from freshwater to the marine environment there are significant changes to the gill ATPase activity in the gills as well as plasma ion concentrations that allow the fish to successfully osmoregulate in its new environment. Therefore, a laboratory based experiment was undertaken to determine whether environmental levels of fenitrothion had an impact on a range of physiological and morphological parameters associated with the seawater adaptation of wild silver eels and their ability to survive in full strength sea water. The experiment examined three different environmental concentrations of the pesticide: 0.001 µg l^{-1} (Low); 0.01 µg l^{-1} (Medium); 0.05 µg l^{-1} (High).

Methodology
In November 2009, 54 silver eels were obtained from a commercial eel fishermen operating on a trap on the River Stour (Hampshire) during the eel spawning migration. After collection, eel were transported to the

Lowestoft Laboratory, where they were acclimatised for 7 weeks in indoor 550 l freshwater tanks, which were supplied with running de-chlorinated tap water. The water temperature during acclimation and the experiment ranged from 4-11 °C. Fish were kept under naturally simulated photoperiod and were fed daily during acclimation. Most food was not consumed as eels are thought to stop feeding when they metamorphose to the silver stage (van Ginneken *et al*. 2007a).

The eels were then exposed in freshwater to the 3 different concentrations of fenitrothion for a period of two weeks. Exposure was carried out in flow through condition maintaining the desired concentration of fenitrothion in the tanks using a peristaltic pump. Each of the three concentrations was tested in duplicate tanks and 2 additional tanks were used as control. Fish were distributed randomly between the different treatments. At the end of the two weeks exposure, half of the fish were sacrificed and sampled for the physiological biomarkers associated with saltwater adaptation, and the remaining fish were transferred to saltwater for 72 hours to monitor survival. Fish surviving the saltwater challenge test were then sampled for the physiological biomarkers associated with saltwater adaptation. The data were analysed by a 2 way ANOVA using a General Linear Model. To isolate which groups differed from one another this was followed by a Multiple Comparison Procedure (Holm-Sidak Method). The significance level was set at 0.05.

Exposure of eels to fenitrothion in freshwater for two weeks had no effect on any of the measured morphological or physiological biomarkers when compared to the control (Table 1). Similarly, after the fish had been transferred to saltwater for 72 hours the eels showed no significant effects of exposure to any of the three concentrations of fenitrothion when compared to the control (Table 1).

Table 1. Morphological and physiological data from silver European eels exposed to low, medium or high concentrations of fenitrothion in freshwater for 2 weeks and then sampled prior to and after a 72 hours saltwater challenge test.

Freshwater	control		low (0.001ug/l)		medium (0.01ug/l)		high (0.05ug/l)		ANOVA
	(N=7)		(N=6)		(N=7)		(N=6)		
	mean	*sem*	*mean*	*sem*	*mean*	*sem*	*mean*	*sem*	*P value*
length (cm)	58.61	1.8	58.35	2.25	58.47	2.37	59.08	2.27	0.996
weight (g)	411.4	55.02	374.6	43.7	401.83	30.76	397.9	41.29	0.945
condition factor	0.198	0.013	0.184	0.006	0.206	0.021	0.189	0.006	0.275
eye index	9.91	0.53	9.67	0.8	9.16	0.85	9.52	0.41	0.877
fat content (%)	18.26	0.38	19.63	0.65	20.67	0.6	19.45	0.75	0.054
hepatosomatic index	1.601	0.086	1.405	0.093	1.588	0.125	1.484	0.037	0.437
gonadosomatic index	1.678	0.106	1.772	0.121	1.662	0.098	1.853	0.092	0.555
gill Na/K ATPase (umol Pi/hr mg)	14.88	2.49	17.51	2.95	12.91	1.84	15.5	1.35	0.554
osmolarity (mosm/kg water)	333	8.649	368.3	10.2	349.6	8.766	356	6.455	0.058
Cl- (mmol/l)	111.7	5.24	114.67	5.75	118.3	4.602	116.8	4.269	0.793
Na+ (mM)	181.7	5.29	182.27	4.204	189.4	6.262	184.5	3.926	0.696
K+ (mM)	4.234	0.501	4.318	0.496	3.702	0.333	3.511	0.331	0.482
glucose (mmol/l)	2.624	0.361	3.599	0.267	2.948	0.345	3.479	0.307	0.154
Saltwater									
	(N=7)		(N=7)		(N=7)		(N=6)		
	mean	*sem*	*mean*	*sem*	*mean*	*sem*	*mean*	*sem*	*P value*
length (cm)	57.84	4.43	55.6	1.79	56.45	1.9	56.28	2.56	0.954
weight (g)	389.04	75.8	324.4	31.25	326.49	31.2	345.4	46.42	0.773
condition factor	0.181	0.005	0.186	0.007	0.179	0.006	0.188	0.006	0.696
eye index	8.8	0.19	9.59	0.34	9.78	0.42	9.89	0.32	0.104
fat content (%)	18.16	0.94	19.44	0.75	19.2	0.75	18.8	0.8	0.687
hepatosomatic index	1.489	0.091	1.476	0.086	1.607	0.099	1.359	0.12	0.405
gonadosomatic index	1.668	0.063	1.569	0.065	1.615	0.102	1.603	0.044	0.826
gill Na/K ATPase (umol Pi/hr mg)	16.81	2.53	13.45	1.63	14.76	2.16	9.61	1.14	0.094
osmolarity (mosm/kg water)	382.1	13.14	389.9	7.766	400.1	10.42	404.8	14.2	0.518

Cl- (mmol/l)	139.4	3.199	139.4	3.316	140	4.271	147.7	5.719	0.465
Na+ (mM)	191.3	3.066	194.7	2.132	193.7	3.661	196.3	4.889	0.789
K+ (mM)	3.461	0.161	3.528	0.203	3.355	0.297	3.343	0.43	0.25
glucose (mmol/l)	2.598	0.628	2.109	0.147	2.336	0.312	2.4	0.255	0.901

The transfer of the eels from freshwater to saltwater did not have a significant effect on the levels of gill Na^+K^+ATPase activity in either the controls or any of the three treated groups (Figure 1). There was a similar trend in terms of plasma Na^+ and K^+ ions. These results differ from similar studies on the Atlantic salmon where there is an increase in gill activity and plasma sodium and potassium ions (Waring & Moore 2004).

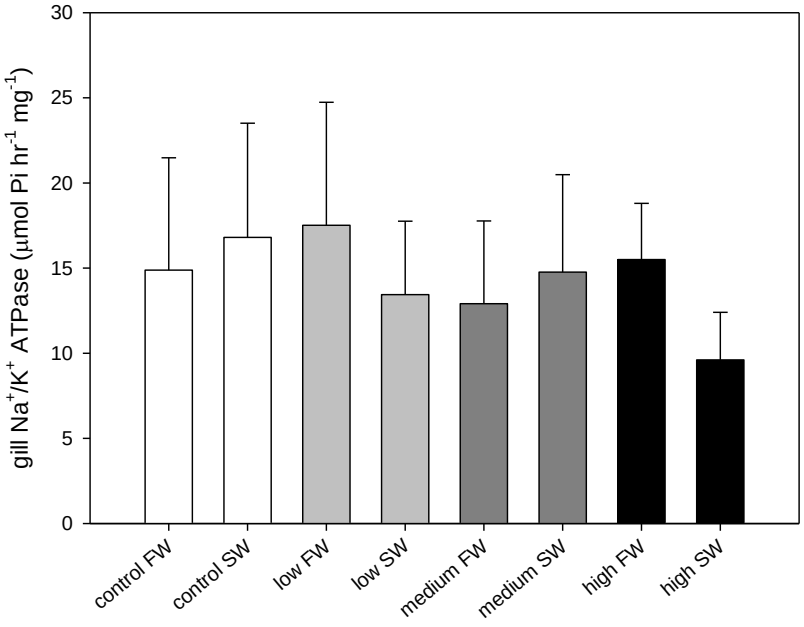


Figure 1. Gill Na^+K^+ATPase activity in eels exposed to concentrations of 0.001 µg l^{-1}(Low), 0.01 µg l^{-1} (Medium) and 0.05 µg l^{-1} (High) fenitrothion in freshwater (FW) and after the saltwater challenge (SW). The data represents mean ± SD of 7 eels per group. * $p<0.05$ between FW and SW groups.

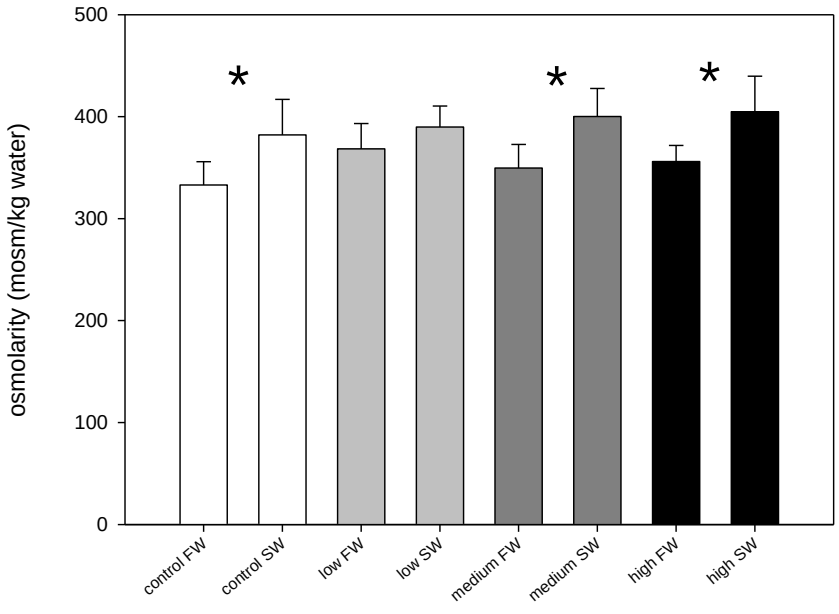


Figure 2. Plasma osmolarity in eels exposed to concentrations of 0.001 µg l^{-1}(Low), 0.01 µg l^{-1} (Medium) and 0.05 µg l^{-1} (High) fenitrothion in freshwater (FW) and after the saltwater challenge (SW). The data represents mean ± SD of 7 eels per group. * $p<0.05$ between FW and SW groups.

The plasma osmolarity showed the expected significant increase in the control groups when the eels were moved from freshwater into saltwater (Figure 2).There were similar increases in the Medium and High exposed groups suggesting that fenitrothion has very little effect on the ability of the eels to osmoregulate in saltwater. A similar trend was shown in the plasma levels of Cl^- ions. The levels significantly increased in the control group and the groups exposed to fenitrothion (Figure 3).

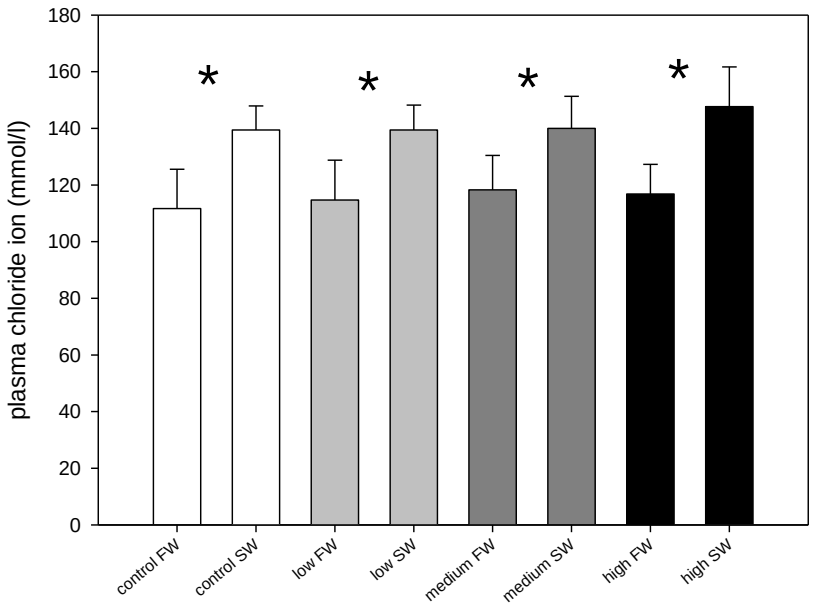


Figure 3. Plasma Cl- ion concentrations in eels exposed to concentrations of 0.001 µg l^{-1}(Low), 0.01 µg l^{-1} (Medium) and 0.05 µg l^{-1} (High) fenitrothion in freshwater (FW) and after the saltwater challenge (SW). The data represents mean ± SD of 7 eels per group. * $p<0.05$ between FW and SW groups.

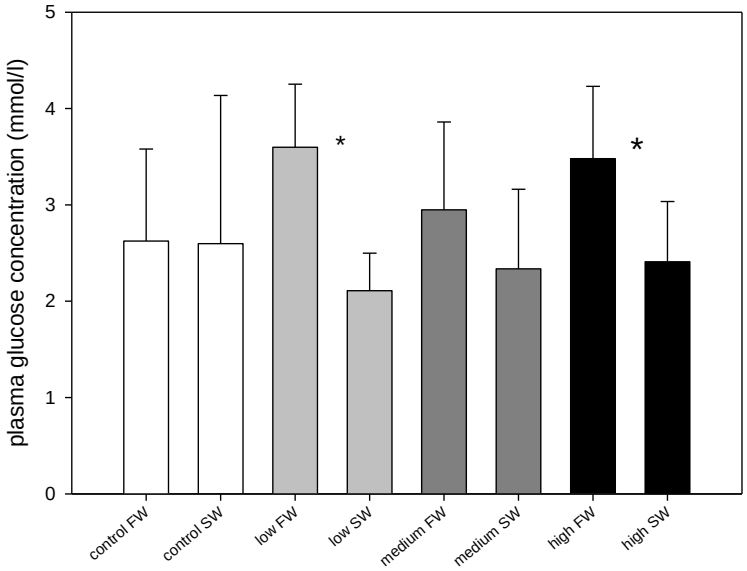


Figure 4. Plasma glucose concentrations in eels exposed to concentrations of 0.001 µg l^{-1}(Low), 0.01 µg l^{-1} (Medium) and 0.05 µg l^{-1} (High) fenitrothion in freshwater (FW) and after the saltwater challenge (SW). The data represents mean ± SD of 7 eels per group. * $p<0.05$ between FW and SW groups.

The glucose levels in the control groups did not change when the eels were moved from freshwater to the saltwater (Figure 4). However, in the Low and High exposed groups there were a significant decline in the

concentrations of plasma glucose after transfer to saltwater. Generally, it would be expected that the glucose levels would decline in eels when moving to saltwater. In a similar study (Privitera et al. 2013), where eels were exposed to TBP (see below), there was a significant decrease in the levels of plasma glucose in the control groups when transferred to saltwater, but no change in the levels of glucose in the group exposed to TBP. Glucose has a major role in the bioenergetics of animals, and carbohydrate metabolism appears to play a major role in the energy supply for osmoregulation. There are considered to be spatial and temporal relationships between the liver and osmoregulatory organs in partitioning the energy supply for ion regulatory mechanisms during changes in salinity (Tseng & Hwang 2008). The decrease in the glucose levels in exposed fish may therefore have been the metabolic cost of osmoregulation by the eels in saltwater. However, it is not clear why there was not a similar decrease in glucose in the control fish, although this may just be due to variation between the samples.

Disease status of experimental fish.

Many countries have started compiling data on the health status of eels in their water bodies. Objectives for these monitoring actions are diverse and there is a large amount of information collected by EU member countries. However, this information is widely scattered over Europe in agencies, institutes or universities. As there is a growing need to collect and report on data on the health status of the eel on international level, in September 2007 the Joint EIFAC/ICES Working Group on Eels established a European Eel Quality Database to collect recent data of contaminants and diseases over the distribution area of the eel. In order to provide information to the database and to determine the general health of the population of eels from the River Stour, a subsample of the experimental fish (5) were screened by the Fish Health Inspectors at Cefas Weymouth. This was considered necessary within the context of the present research in order to establish whether any effects shown by exposure to fenitrothion may have been complicated by the general health of the eels. The screening involved a visual examination of the fish, inspection for known eel parasites and determination of the presence of VHS (viral haemorrhagic septicaemia), IHN (infectious haematopoietic necrosis), IPN (infectious pancreatic necrosis), SVC (spring viraemia of carp) and eel rhabdovirus.
The external screening identified a number of lesions around the caudal peduncle and tail fin, which were considered to be caused by the method of capture. Internally, the only observation was that some fish had fatty deposits surrounding the heart. Overall, there did not appear to be any obvious problems with the general condition of the eels.
Examination of the eels noted the presence of the following parasites.
Anguillicoloides crassus (Nematoda) – present in the swim bladder
Paraquimperia tennerima (Nematoda) - present in the intestine
Acanthocephalus lucii (Acanthocephala) - present in the intestine
Pseudodactylogyrus sp. (Monogenea) – present in the gills.
The virology screening was undertaken using samples of spleen, kidney and brain from each fish and pooled. This was then inoculated onto the following cell lines BF-EP, EP-20, CH-15, FH-15 and FH-20's. The results indicated that the viruses VHS, IHN, IPN, SVC and eel rhabdovirus were not present in any of the eels. A further PCR for eel herpesvirus was also negative.
Overall, the health of the sample of eels from the River Stour was generally good although the presence of the parasite *Anguillicoloides crassus* is of concern. *A. crassus* is not native to British fish species and is classified by the Environment Agency as a Category 2 Parasite. *A. crassus* is considered to be the most aggressive fish parasite to have been introduced anywhere in the world. It was originally discovered in the swim bladder of the Japanese eel, *Anguilla japonica* in south-east Asia, where it is native, but was introduced to Europe in the 1980s when Japanese eels were imported to Europe for aquaculture. Once introduced, *A. crassus* quickly infected adults, elvers and glass eels in wild populations of the European eel. The parasite lives in the swim bladder of eels, where it may do damage and reduce the eel's ability to maintain and adjust buoyancy. Recently, research has shown that infection by the parasite reduces the swimming endurance of eels because the parasite is a 'metabolic burden' i.e. reduces the metabolic efficiency of the eel. Aside from increasing the energy costs of swimming, this stress may also have a long-term effect because eels will be less able to gain and store fat during their time in freshwater. This in turn may affect the ability of the eels to successfully undertake their oceanic migration and produce viable offspring. However, in the context of the present study it is unlikely that the parasite would have interacted with any of the effects shown by the pesticide fenitrothion.
In conclusion, the research indicates that exposure of the European eel to fenitrothion in freshwater for a short period, does not compromise the eel's ability to successfully undergo the silvering process or reduce its ability to osmoregulate as it enters the marine environment. However, the ability to subsequently survive in saltwater was only measured for a short period and the impact of the pesticide on long term survival in

the marine environment is not known. Finally, the present study has not examined other toxic effects that the pesticide may have on the eel such as reducing spawning success and modifying the bioenergetics of oceanic migration.

Experiment 1.2. The impact of tributyl phosphate the morphology and physiology of silver eels during their transition from the freshwater to the marine environment.

Contaminants that modify the silvering process in the European eel may also have subsequent impacts on the migratory behaviour and survival during the transition from freshwater and into the marine environment. One group of ubiquitous chemicals which have previously been shown to have an impact on the smoltification process in Atlantic salmon is flame retardants (Lower & Moore 2007). Organophosphate esters (OPEs) are a class of chemicals also widely used as a flame retardant or plasticiser in a number of commercial products (Campone et al. 2010). One OPE of potential interest, which occurs in surface waters supporting European eel populations, is tributyl phosphate (TBP). Tributyl phosphate is used as a solvent, plasticizer, antifoaming agent, metal extractant and flame retardant. When used in industrial processes as a solvent, extractant or antifoaming agent, it is constantly lost to the air and aquatic environment (WHO Report 1991). Its biodegradation is slow, and its concentration in water is not decreased by standard techniques used in drinking water treatments. Concentrations measured in water range from nanograms to 52 $\mu g \cdot l^{-1}$ (WHO 1991; Marklund et al. 2005). The solubility of TBP in water at ambient temperature is <1 $g \cdot l^{-1}$, and the difference in concentration between water and sediment is ~ 3 orders of magnitude. The 96 hrs LC50 for fish is in the range of 4.2–11.4 $mg \cdot l^{-1}$ (WHO Report 1991), but there is a poor understanding of TBPs mechanistic effects on fish although it is known to directly affect the kidney in rats and mice (Oishi et al. 1982; Laham et al. 1985) an important organ in controlling osmoregulation in fish.

This research examined the impact of freshwater exposure to TBP on migrating wild silver eels. A laboratory-based study was carried out to investigate the morphology and physiological status of groups of eels exposed to TBP to determine whether the contaminant had a direct effect on the silvering process, osmoregulatory capability and survival of the fish once they had moved into saltwater.

Methodology.

In November 2010, 80 silver eels were captured in a modified Wolf trap situated at the Vestbirk hydropower station in the upper part of the River Gudenaa, Denmark (55°58′35.94″N, 9°42′2.53″E), and maintained in a holding pen at the catch site for 0–6 days (Fig. 9, section 4.1). Fish were then brought to the Centre for Vildlaks in Randers, Denmark, where they were distributed between two of four identical indoors tanks (volume of 300 litres) and kept with a continuous freshwater flow (5.4–8.2 °C) and an artificial light-dark cycle of 8h light – 16h dark to represent natural conditions in the area. Fish were not fed, whilst they were left to acclimatise for 1 week as silver eel are considered to cease feeding and show egression of the alimentary tract (Van Ginneken et al. 2007a).

At the end of the acclimatisation week, 40 fish were randomly selected by dip netting and equally distributed over the two remaining tanks (control and exposed) in static oxygenated freshwater. The other 40 fish were used for experiment 4.1 described later on in this report. The treatment consisted of an exposure to 0.5 $\mu g \cdot l^{-1}$ of TBP (Sigma-Aldrich, Gillingham, Dorset, UK) for a period of 5 days. The concentration of 0.5 $\mu g \cdot l^{-1}$ was chosen to best represent the values reported from a limited number of water courses supporting eel populations (For rivers in Japan, Switzerland and Norway see WHO 1991; for rivers in the river Oder catchment see Fries & Püttmann 2003). At the end of the 5-days exposure, 10 fish from each tank were sampled to allow tissue and blood collection for physiological measurements. The remaining fish were left in the tanks, and the freshwater replaced with full strength (35 ‰) saltwater (Red Sea Coral Pro salt, 38.2 g^{-1}). Water temperature during the saltwater challenge was between 5.4 and 6.8 °C. Fish were left in saltwater for 3 days and monitored daily to determine any sign of distressed behaviour (e.g., erratic swimming movements or unresponsiveness) and survival. At the end of the saltwater challenge, all surviving fish were sampled for the same parameters as in the previous 20 fish. A 3-day saltwater challenge test was chosen as previous studies have indicated that a number of the physiological parameters measured in this study undergo significant changes within this period. For instance, plasma Na and Cl levels peak 2–3 days after the introduction of adult eels into saltwater (Bornancin & De Renzis 1972; Kirsch & Mayer-Gostan 1973; Ho & Chan 1980). However, although Na/K ATPase takes longer to reach peak values (e.g., 7 days or more) (Bornancin & De Renzis 1972; Ho & Chan 1980), the increase in activity is initiated immediately after saltwater entry and as such can be used to compare the impact of the contaminant against the appropriate control.

The morphological and physiological biomarkers that were measured to determine the silvering stage of the eel and its ability to osmoregulate within saline conditions have been detailed in the section on fenitrothion.

The results of the saltwater challenge test indicated there were no mortalities in the exposed or control groups whilst maintained in freshwater or when they were introduced to saltwater. Throughout the duration of the experiment, all fish showed no signs of distress or abnormal behaviours. The measured morphological parameters (Table 2) indicated no significant difference [2-way ANOVA, SigmaStat 3.1 (Systat Software, Inc., London, UK)] between control and exposed fish or between fish within each group exposed to the saltwater challenge or not.

Eels in all groups were of similar size; however, their condition factor [CF, calculated as 100 x (weight/length^3)] was significantly different as control eels after saltwater challenge had a higher C.F. than all other groups. This difference is probably due to differences in the fat stores measured as a percentage by means of an ultrasound fat metre (Distell, fish Fatmeter). Fat content was significantly lower in control fish after saltwater challenge. Fish were not fed throughout the experiment, and so, any differences in fat content between the two groups probably existed prior to the exposure to the contaminant.

Results of plasma analyses demonstrated a significantly higher osmolarity for fish in saltwater compared with freshwater (Table 2), which is to be expected in fish moving from a freshwater to a marine environment. Plasma concentrations of potassium and calcium did not differ between the exposed and the control groups either in freshwater or saltwater. However, concentrations of sodium and chloride were affected by exposure to TBP. The concentrations of both ions were lower in the exposed compared with the control groups in freshwater, and there was the expected increase in both ions when the eels were transferred to saltwater. In addition, exposure to TBP did not result in the expected decrease in glucose concentrations when the eels were moved into saltwater as demonstrated by the control group. Eels exposed to TBP did not show any differences in gill Na^+/K^+ ATPase activity in either fresh or saltwater, but there was significant impact of TBP on kidney Na^+/K^+ ATPase activity. There was not the expected decrease in enzyme activity in exposed fish after transfer to saltwater.

Table 2. The effect of TBP on various physiological and morphological parameters in silver eels whilst held in freshwater and after a 3-day period in saltwater. The data represents mean ± S.E. of 10 eels. * $p < 0.05$ compared to the control group.

Morphological and physiological parameter.	Freshwater		Saltwater	
	Mean ± S.E.		Mean ± S.E.	
	Control	Exposed	Control	Exposed
weight (g)	305.5 ± 27.49	289.5 ± 14.0	354.7 ± 21.0	318.6 ± 23.0
length (cm)	55.75 ± 1.44	55.25 ± 1.09	56.35 ± 1.67	57.4 ± 1.21
fin length (mm)	28.03 ± 0.56	27.43 ± 0.78	26.67 ± 0.76	27.26 ± 0.82
eye index	6.71 ± 0.19	7.2 ± 0.28	6.85 ± 0.44	6.7 ± 0.33
G.S.I	1.12 ± 0.08	1.31 ± 0.08	1.26 ± 0.18	1.03 ± 0.08
H.S.I.	1.39 ± 0.07	1.48 ± 0.07	1.58 ± 0.1	1.47 ± 0.1
fat content (%)	23.1 ± 0.39	23.6 ± 0.51	19.7 ± 0.61	22.3 ± 0.61*
C.F.	0.17 ± 0.01	0.17 ± 01	0.2 ± 0.01	0.17 ± 01*
osmolarity (mosm/kg water)	301.7 ± 3.35	293.8 ± 4.29	335.6 ± 4.63	343 ± 3.42
potassium (mmol/l)	3.28 ± 0.24	3 ± 0.27	3.48 ± 0.35	3.47 ± 0.29
sodium (mmol/l)	167.24 ± 2.13	156.47 ± 3.9	186.57 ± 2.7	180.03 ± 3.3
chloride (mmol/l)	105.7 ± 2.83	93.8 ± 2.16	142.3 ± 3.68	127.6 ± 2.01*
glucose (mmol/l)	6.12 ± 0.77	6.25 ± 0.67	3.06 ± 1.55	6.11 ± 0.31*
gill ATPase (μmol Pi/hr/mg)	14.9 ± 1.98	14.96 ± 2.26	17.21 ± 1.98	20.09 ± 2.69
kidney ATPase (μmol Pi/hr/mg)	10.93 ± 0.58	12.92 ± 1.31	8.32 ± 1.22	13.74 ± 1.71*

Although the exposure of eels in freshwater to TBP resulted in certain parameters related to osmoregulation being affected, it did not affect the survival of the fish. Eels were physiologically stressed by exposure to the contaminant as shown by significantly reduced plasma sodium and chloride ion concentrations. Plasma chloride concentrations have been shown to decrease when freshwater fish are stressed (Nomura et al. 2009). There were no differences in the other plasma ions between the two treatment groups in freshwater,

and there were no significant differences in plasma osmolarity. In addition, there were no significant differences in HSI or GSI between the two groups. Esteve et al. (2012), demonstrated that eels that had bio-accumulated a range of metals had a reduced HSI and a lower fitness. The similarity in the HSI (and probably GSI) measured in the present study possibly reflects the low bioaccumulation (World Health Organization 1991) of TBP in fish or the short period of exposure of the eels to the contaminant.

However, when transferred to saltwater, there were additional physiological differences in the eels that had been exposed to TBP, although there were no mortalities in either of the two groups. Generally, fish in freshwater need to eliminate excess water gained osmotically via their kidneys but conserve ions, and the reverse is true in saltwater (Rankin 2009; Tang et al. 2012). The expected decrease in kidney Na^+/K^+ ATPase activity was evident in the control group, but levels in the exposed group were similar to those measured in freshwater. These effects may help explain the significant declines in plasma sodium in exposed eels, but not those in chloride, nor the lack of differences in potassium or calcium. However, there was no impact of TBP exposure on gill Na^+/K^+ ATPase as seen in eels after exposure to the heavy metal cadmium (Lionetto et al. 1998) nor was there the expected increase in gill Na^+/K^+ ATPase activity in the control group after transfer to saltwater (Tang et al. 2012). TBP is readily assimilated by fish, but after metabolic transformation in the liver, hydroxylated butyl moieties are eliminated via the kidneys (Sasaki et al. 1982; World Health Organization 1991). The activation of the metabolic pathways involved in TBP degradation, transport and elimination could help explain why plasma glucose was elevated in exposed eels and why TBP exposure affected kidney but not gill Na^+/K^+ ATPase and affected plasma sodium and chloride but not calcium and potassium. The plasma levels of sodium and chloride ions increased when the eels were transferred to saltwater (Bornancin & De Renzis 1972) and were similar to those recorded by Kirsch (1972). In eels, plasma level of sodium normally peaks after introduction in saltwater but decreases back to freshwater levels rapidly and within seven days (Ho & Chan 1980). Plasma chloride concentration also increases after entry into saltwater and remains high (Kirsch & Mayer-Gostan 1973). Although there was an increase in both ions in the exposed group, the levels were still significantly lower than in the controls, again suggesting physiological stress as a result of contaminant exposure.

There was a significant decrease in the levels of plasma glucose in the control group when transferred to saltwater, but no change in the levels of glucose in the group exposed to TBP. Glucose has a major role in the bioenergetics of animals, and carbohydrate metabolism appears to play a major role in the energy supply for osmoregulation. There are considered to be spatial and temporal relationships between the liver and osmoregulatory organs in partitioning the energy supply for ion regulatory mechanisms during changes in salinity (Tseng & Hwang 2008). The decrease in the glucose levels in control fish may therefore have been the metabolic cost of osmoregulation by the eels in saltwater. However, as described above it is not clear why there was not a similar decrease in glucose in those fish exposed to TBP, although the high levels of glucose may be related to the stress of exposure to the contaminant. In suboptimum or stressful conditions (e.g., exposure to poor water quality/pollution), the chromaffin cells in fish release catecholamine hormones, adrenaline and noradrenaline towards blood circulation (Reid et al.1998). Those stress hormones, in conjunction with cortisol, mobilise and elevate glucose production in fish through glucogenesis and glycogenolysis pathways (Iwama et al. 1999) to cope with the energy demand produced by the stressor for the 'fight-or-flight' reaction. This glucose production is mostly mediated by the action of cortisol which stimulates liver gluconeogenesis and also halts peripheral sugar uptake (Wedemeyer et al. 1990). However, cortisol has also been identified as a seawater-adapting hormone in a large number of teleost species (McCormick 2001) and to be implicated in osmoregulation, regulating Na^+, K^+-ATPase activities which are prime determinants of osmoregulatory capacity (Mancera & McCormick 2007). In eels, there is a transitory increase in plasma levels of cortisol on transfer to saltwater (Forrest et al. 1973). The increased levels of glucose in the exposed fish may therefore represent the additional glucose produced by an increase in stress related cortisol which is not utilised metabolically for osmoregulation.

However, the physiological changes observed in the eels after exposure to TBP did not appear to have had a significant impact on the short-term migration patterns observed within the River Gudenaa and fjord which are described in experiment 4.1. This is in contrast to similar studies on Atlantic salmon smolts which also undergo a physiological transformation during the transition from the fresh to marine environment but have been shown to be sensitive to exposure to freshwater contaminants (Waring & Moore 2004; Lower & Moore 2007). Although certain contaminants can affect the physiological processes involved in salmonid smoltification, where there is a period to allow recovery from the exposure to the chemical, the subsequent migratory behaviour of the fish may not be significantly affected (Moore et al. 2008). Therefore, the period between exposure in freshwater and entry into the sea may be critical in terms of whether migration and survival in the marine environment are compromised. Contaminants that occur within estuaries and which

the eels are exposed to immediately prior to saltwater entry may be more of a concern than those occurring in areas of the freshwater environment where there is a significant period between exposure and the migration of the eels into the sea (Moore et al. 2008).

The results of this study suggest that exposure to the contaminant in freshwater does modify the physiological processes involved in osmoregulation once the eels have migrated into seawater. In terms of the life cycle of the eel, the freshwater and marine environments cannot be considered in isolation, and the conditions experienced by the eels in rivers and lakes may have a direct impact on their subsequent physiology and survival in the marine environment (Waring & Moore 2004). In the present study, the eels were exposed to a short contaminant exposure period. However, the freshwater stage of the eel may last for a number of years (Tesch 2003) and exposure to single and suites of contaminants for longer periods may have a more significant impact on salt water survival. Further, it is not known how the physiological perturbations observed in the present study may affect the extensive marine migration of the eel or in terms of its subsequent reproductive success. Eels are known to spend long periods residing within sediments in rivers and lakes and further studies on the potential effects resulting from contaminated sediments on eel physiology and migratory behaviour is therefore required to examine the long-term impacts of contaminant exposure on marine survival in the European eel.

Experiment 1.3. The impact of a pesticide mixture on the morphology, physiology and metabolism of silver eels during their transition from the freshwater to the marine environment.

A similar integrated physiological and behavioural study was carried out to investigate the impact of a mixture of pesticides on the physiology and the downstream migratory behaviour (see experiment 4.2) of silver eels during their transition from the freshwater to the marine environment. However, in this study the impact of the pesticide mixture on eel metabolism was also investigated. There is very little information on the potential metabolic cost of exposure to contaminants in the European eel. The detrimental effects of pollution on fitness and fecundity have been suggested by a number of other studies to be factors causing the decline of the eel. Any contaminant that results in a high metabolic cost to the eel as a result of reduced lipid levels or excess energy requirements to excrete or store these compounds may reduce the migratory and/or spawning success in eels. Therefore, a laboratory based study was undertaken to examine the metabolic cost of exposure to pesticides as determined by changes to the individual standard metabolic rate (SMR) and routine metabolic rate (RMR) of eels.

In November 2012, 16 eels were obtained from the River Avon. The fish were kept in a holding pen at the catch site for 6 days and then brought to the aquarium facilities at the Cefas, Lowestoft Laboratory and tagged with PIT tags for individual recognition purposes. Once tagged, eight fish were placed in each of two identical tanks (volume of 550 liters) and kept with continuous freshwater flow (water temperature varying between 6.0 and 11 °C) in automated naturally simulated photoperiod. Fish were left to acclimatize for two weeks. After acclimatization, one fish each day was assigned to either a control or an exposed group and exposed for 5 days to static freshwater or to the pesticide mixture described in Table 3 respectively.

Table 3. Mixture of pesticides.

Group	Compound	Concentration
herbicide	pendimethalin	0.8 µg/l
herbicide	chlorotoluron	0.5 µg/l
fungicide	flusilazole	0.01µg/l
fungicide	copper oxyxhloride	1 µg/l
molluschicide	metaldehyde	0.2 µg/l
insecticide	chlorpyrifos	0.05 µg/l

The pesticides were chosen as they are commonly used in agriculture in England and are detected in rivers mainly during the winter months when the silver eels are migrating out to the marine environment. The concentrations selected represented environmental levels that are routinely monitored by the Environment Agency.

At the end of the 5 days exposure each fish was removed from the experimental tank and transferred to a respirometer chamber filled with full strength seawater. Metabolic rate was indirectly determined by measuring oxygen consumption (MO_2) (Clarke & Johnston, 1999) in the swim tunnel respirometer. Oxygen measurements were made using the intermittent flow respirometer described by Wright et al (2014). Briefly, the respirometer (swim chamber section of 25 × 25 × 87 cm) was submerged in an outer tank, which

measured 232 × 95 × 70 cm having a total water capacity of 187 l (Loligo Systems, ApS). The outer tank served as a source of aerated water used for flushing (flush pump, Eheim, 20 l min^{-1}) the respirometer (swim chamber) after each 'closed' measuring phase. Water quality in the outer tank was maintained by providing an inflow (10 l min^{-1}) of fresh ambient seawater. The water in the outer tank was kept fully aerated. Each swim trial was broken down into 'measurement', 'flush' and 'wait' phases. During the measurement phase, the oxygen tension of the water in the swim chamber was recorded using a galvanic oxygen electrode, while the swim chamber was completely closed from the outer tank. MO_2 was calculated from the rate of decrease in oxygen tension. Subsequently, the swim chamber was flushed with aerated seawater from the outer tank to replenish oxygen levels (flush phase), and then a 'wait' phase enabled the oxygen levels to stabilise before the next measurement phase. During swim trials, chamber flushing and the recirculation valve were controlled through an interface (DAQPAC- G1X, Loligo Systems) connected to a PC running AutoResp™ software (Version 1.6, Loligo Systems). Oxygen tension within the swim chamber was measured using a mini-DO galvanic cell oxygen probe suspended into the water current of the respirometer, which was connected to the DAQ interface, and oxygen saturation data was calculated using Auto - Resp™. To avoid effects of temperature or feeding activity, the respirometer was run with ambient seawater, which was the same as used in acclimation tanks and experimental tanks for the fish. Eels were not fed during the 5 days exposure period nor while in the respirometer. Water speed in the respirometer was low (0.23 m sec^{-1}), the "measurement" phase was 2500 seconds long, the "flush" phase was 399 seconds and the "wait" time was of 1 second. Fish were transferred individually to the respirometer, and only one fish was tested each day. Fish were moved in the chamber between 10:00 and 12:00 each morning and measure of oxygen consumption started immediately and continued for 24 hours. When a fish is transferred to a new environment, such as a respirometer, an oxygen debt due to anaerobic exercise during handling and the unfamiliar surroundings results in an initial elevation of MO_2. This may last several hours and during this period a gradual decrease in MO_2 can be observed. Following this period, MO_2 stabilizes with a clear lower limit. This lower limit is the Standard Metabolic Rate (SMR) of the fish, while higher measurements are due to random activity and can be considered Routine Metabolic Rates (RMR) (Schurmann & Steffenson, 1997). SMR for each fish was calculated as the mean of the six lowest measurements of *M*O2, as described by Schurmann and Steffenson (1997), and the RMR was calculated by averaging all the MO_2 recorded after the initial settling period. Oxygen consumption was calculated from the rate of decline in oxygen tension, the volume of the swim chamber and the solubility of oxygen in seawater at the experimental temperature (Schurmann & Steffensen 1997, Lee et al. 2003). Values for MO_2 in mg O_2 kg^{-1} h^{-1} were therefore recorded every 42 minutes (the duration of the flush cycles). MO_2 values were then converted from milligrams of O_2 $kilogram^{-1}$ $hour^{-1}$ to micromoles of O_2 $kilogram^{-1}$ $hour^{-1}$ before further processing. Subsequently, the individual standard metabolic rate (SMR) and routine metabolic rate (RMR) were calculated for each eel. After the 24 hours in the respirometer fish were removed and sampled for blood and tissue to allow for physiological measurements. The morphological and physiological biomarkers that were assessed have been previously detailed in the section on fenitrothin.

There were no differences in either the SMR or the RMR of eels exposed to the mixture of pesticides when compared to the control group. In addition, the results of the experiment indicated that there were no negative effects of exposure to the mixture on eel morphological or physiological parameters. The results are similar to the previous studies on fenitrothion and TBP.

Table 4. The effect of a pesticide mixture on various physiological and morphological parameters in silver eels exposed in freshwater and then transferred in a respirometer chamber with full strength seawater for 24 hours. The data represents mean ± S.E. of 8 eels per group.

Morphological and physiological parameters	Control	Group	Exposed	Group	t-test
	mean	± sem	mean	± sem	(Significance at > 0.05)
weight (g)	180.72	29.6	161.4	29.68	0.65
length (cm)	43.69	2.31	42.65	2.37	0.76
Eye Index	5.87	0.58	6.98	0.63	0.21
fat %	19.07	1.41	21.71	1.5	0.22
CF	0.2	0.007	0.2	0.008	0.44
HSI	1.39	0.1	1.4	0.07	0.94
GSI	0.54	0.25	0.47	0.21	0.83
gill ATPase	7.62	1.12	5.44	1.06	0.18
kidney ATPase	7.52	1.04	6.78	0.5	0.54
SMR	16.2	1.48	20.05	2.83	0.26

RMR	20.42	1.78	23.74	4.03	0.48
osmolarity	390.87	7.96	386.87	11.85	0.78
chloride ions	145.81	3.46	144.19	6.91	0.84
sodium ions	328.09	47.48	357.53	14.13	0.57
potassium ions	5.1	0.2	4.12	0.48	0.09
glucose (absorbance)	0.74	0.07	0.73	0.07	0.91

In conclusion, exposure of silver European eels to this specific mixture of pesticides for a short period did not appear to affect their physiological abilities to adapt to the marine environment. Further, the mixture of pesticides did not appear to carry a metabolic cost to the eels at least in the short term.

Additional study not included in the original objectives.

The impact of the pharmaceutical fluoxetine on the physiology and metabolism of saltwater adapted silver eels.

In January 2013, a group of 16 saltwater adapted silver eels became available from a batch originally collected in the River Stour (Dorset) the previous year. As a result, a further study was undertaken to investigate the effect of short term exposure to a common pharmaceutical on the physiology and metabolic rate of eels within sea water. The compound chosen for the study was fluoxetine, which is routinely monitored in both river and estuarine environments. Fluoxetine is the active ingredient of antidepressant drugs (e.g. Prozac) and is a selective serotonin reuptake inhibitor (SSRI). The concentration tested was 0.1µg l^{-1} which is similar to concentrations measured in waters worldwide (Guler & Ford, 2010).
The eels were maintained in one 700 litre tank with a continuous seawater flow under an automated naturally simulated photoperiod. For the experiment, one fish every day was attributed to either a control group or an exposed group (5 days to 0.1µg/l of fluoxetine in static saltwater). At the end of the 5 days exposure each fish was removed from the experimental tank and transferred to a respirometer chamber filled with clean seawater. Each fish was left in the respirometer for 24 hours and the oxygen consumption was measured and logged to allow the calculation of individual standard metabolic rate (SMR) and routine metabolic rate (RMR). After the 24 hours in the respirometer fish were removed, measured (length, weight, organs weight, fat content) and sampled for blood and tissue to allow physiological measurements (plasma ions, osmolarity, gill and kidney ATPase). The results are summarized in Table 5.

Table 5. Metabolic activity of silver eels exposed to fluoxetine in saltwater

	Control	Group	Exposed	Group	t-test
	mean	± sem	mean	± sem	(Significance at > 0.05)
weight (g)	342.7	21.77	358.25	32.47	0.7
length (cm)	55.55	1.19	55.85	1.27	0.87
CF	0.2	0.005	0.2	0.007	0.77
Eye Index	6.05	0.62	7.31	0.58	0.16
fat %	17.47	1.4	16.92	1.75	0.81
HSI	1.2	0.07	1.08	0.04	0.18
GSI	0.96	0.18	1.06	0.16	0.68
gill ATPase	13.44	1.44	11.26	1.57	0.32
kidney ATPase	5.35	0.32	7.25	1.13	0.14
SMR	11.23	0.94	10.35	0.51	0.43
RMR	14.76	1.05	12.27	0.8	0.08
osmolarity	370.37	10.42	362.37	7.11	0.54
chloride	151.62	2.63	146.88	2.84	0.24
sodium	281.82	39.68	290.23	10.9	0.84
potassium	6.39	0.65	6.9	0.44	0.53
glucose	0.116	0.025	0.147	0.228	0.35

There were no significant differences in either the SMR or the RMR of eels exposed to fluoxetine when compared to the control group. In addition, the results of the experiment indicated that there were no

negative effects of fluoxetine on eel morphological or physiological parameters. The results of the physiology study are similar to the previous studies on fenitrothion, a mixture of pesticides and TBP.

Fluoxetine is one of the most commonly detected pharmaceuticals in wastewater and bioaccumulates in wild-caught fish, especially in brain, liver and muscle tissues. Previous studies indicated that it is pharmacologically active in fish species exerting anorexigenic effects. Waterborne fluoxetine has also been shown to regulate food intake and energy metabolism. Carp exposed for a period of 28 days to environmental levels of fluoxetine showed a significant decrease in food intake, weight gain and levels of circulating glucose levels (Mennigen *et al*. 2010). The authors examined the potential mechanisms and investigated gene expression of feeding neuropeptides in the neuroendocrine brain of goldfish as well as gene expression and enzymatic activity of glycolytic and gluconeogenetic enzymes in liver and muscle tissues. They were able to confirm changes in brain gene expression patterns in line with potential anorexigenic effects in the hypothalamus, with increased expression in corticotropin-releasing factor (CRF) and decreased expression of neuropeptide Y (NPY). With respect to glucose metabolism, liver gene expression of the gluconeogenic enzyme fructose-1,6-bisphosphatase decreased and muscle hexokinase activity increased in fish exposed to fluoxetine.

In the present study, there appeared to be no effect on the glucose levels in the eel and although there was a lower RMR in the exposed group compared to the control group it was not significantly different. However, the eels in the present study were not exposed for as long a period as those in the work by Mennigen *et al.* (2010). It is possible that a longer exposure to the antidepressant, particularly during the freshwater stage may have more significant effects on feeding behaviour (not addressed in the present study) and subsequent, metabolism and bioenergetics of migrating eels. As stated previously any effects on the quality of emigrating silver eels has been suggested to reduce both migratory and reproductive success.

Experiment 1.4. The impact of the pesticide fenitrothion on silver eel olfaction.

Olfaction or the sense of smell plays a major role throughout the life history of most fish being intimately involved in feeding, reproduction, prey/predator detection and homing. The European eel is also known to have a very sensitive sense of smell and is able to detect a range of substances (Huertas *et al*. 2010). In particular, the sense of smell is considered to be the principal sensory modality that is used by the eel to detect prey and in general feeding. It is very likely that the sense of smell may also be involved in the synchronisation of spawning. In previous studies, a range of contaminants have been shown to compromise the sense of smell of Atlantic salmon and inhibit the ability of male adult salmon to detect the pheromones released by the female which synchronise spawning physiology and behaviour (Moore & Waring, 1995; Waring & Moore, 1997; Moore & Waring, 1998; Moore & Lower, 2001). It is possible that contaminants may also affect the sense of smell in the eel and if this is the case there may be a deleterious effect on feeding, growth, fat deposition, metabolic activity and subsequently successful spawning migration.

Laboratory studies were carried out to assess the effect of fenitrothion on eel olfaction and in particular the ability to detect amino acids and bile acids. Fenitrothion was selected as it is an OP insecticide similar to diazinon which is commonly used as the active ingredient in dips to control parasites on sheep and has previously been shown to inhibit the sense of smell in salmon (Moore & Waring 1995). In November 2009, silver eels were obtained from the River Stour (Hampshire) and maintained at the Cefas, Lowestoft Laboratory. The eels were exposed to an environmental concentration of fenitrothion (0.05 μg l^{-1}) or a control (no fenitrothion) for 3 weeks. At the end of the 3 weeks, the olfactory responses of each eel to three odorants were measured using an electrophysiological technique (electro-olfactogram: EOG) (Moore & Waring, 1995; Waring & Moore, 1997; Moore & Waring, 1998). EOG recording measures trans-epithelial voltage gradients from the surface of the olfactory epithelium (olfactory receptors) and is considered to reflect multi-unit cell activity. The three odorants tested were glutamine (10^{-3}M), bile from gall bladders of eels and ecdysone (10^{-5}M). At the end of each recording the eels were sacrificed and morphological and physiological parameters were measured.

After exposure to a 0.05 μg l^{-1} concentration of fenitrothion, the electrophysiological responses recorded from the olfactory epithelium of the eels to all three odorants were similar to the responses recorded from the control fish (Table 6). The physiological and morphological measurements taken from all fish after completion of the EOG recordings are also shown in Table 6.

The results suggest that the pesticide has no effect on the olfactory ability of the eel. However, the eel was shown to be sensitive to the compound ecdysone. Ecdysone is a steroidal prohormone of the major insect moulting hormone 20-hydroxyecdysone. It is also produced by crustacean during moulting and growth. This is the very first evidence that a fish is able to detect via the sense of smell this hormone and may be the major mechanism as to how eels detect prey during the freshwater stage of their life cycle.

Table 6. EOG experiment with silver eels exposed to 0.05 µg/l of fenitrothion for 3 weeks.

Parameters	Control			Exposed			t-test
	mean	sem	N	mean	sem	N	P value
weight (g)	379.8	26.5	16	380.3	26.7	15	0.991
length (cm)	59.26	1.215	16	59.560	1.337	15	0.868
condition factor	0.179	0.005	16	0.176	0.004	15	0.724
eye index	7.700	0.315	16	7.596	0.223	15	0.789
fat content (%)	19.225	0.626	16	19.000	0.550	15	0.789
hepatosomatic index	1.374	0.065	15	1.260	0.050	14	0.181
gonadosomatic index	1.867	0.064	16	1.878	0.063	15	0.906
gill Na/Na ATPase (umol Pi/hr mg)	11.362	1.381	16	9.845	1.169	15	0.409
haematocrit (%)	28.139	2.510	9	28.615	1.082	13	0.865
osmolarity (mosm/kg water)	292.286	8.289	14	294.467	8.750	15	0.858
Cl^- ((mM)	83.786	3.337	14	79.200	3.626	15	0.360
Na^+ (mM)	168.250	4.083	14	163.539	2.783	15	0.350
K^+ (mM)	3.197	0.195	14	3.701	0.303	15	0.175
glucose (mmol/l)	2.651	0.260	14	2.193	0.222	12	0.193
EOG - 10^{-3}M glutamine (mV)	1.714	0.300	14	1.964	0.349	13	0.592
EOG - 10^{-1}M glutamine (mV)	4.873	0.513	15	4.938	0.755	15	0.944
EOG - bile (mV)	2.124	0.196	15	2.244	0.338	14	0.762
EOG - 10^{-5}M ecdysone (mV)	2.169	0.399	7	3.546	0.864	7	0.184

Experiment 1.5.The impact of the pesticide atrazine on yellow eel olfaction.

Similar laboratory studies were carried out to assess the effect of the pesticide atrazine on olfactory function in eels. Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine) is a water-soluble pre- and post-emergence herbicide for the control of annual and perennial grass and annual broad-leaved weeds. Atrazine is known to have high mobility through soil and is a known contaminant of aquatic ecosystems in England and Wales. In 1992 and 1993, atrazine was one of the five pesticides most frequently present in both ground and surface water at levels in excess of the Maximum Admissible Concentration (MAC) of 0.1 µg l^{-1} imposed by the Water Act 1991. In addition, analyses of UK surface waters demonstrated levels exceeding the proposed Environmental Quality Standard (EQS) of 2.0 µg l^{-1} based on the annual combined average of atrazine and simazine. In 1993, the use of atrazine was banned on non-cropped land and as a result there has been a decline in its detection in UK surface waters. In 2004 a total ban on the use of atrazine by implemented by the EU. However, atrazine was included in the present study as it is a good example of a triazine pesticide and previous studies have indicated that it inhibits the sense of smell in salmon (Moore & Waring 1998).

In 2011, 10 non-migratory yellow eels were collected from the River Avon (Hampshire) and brought to the Cefas, Lowestoft facilities. Five fish were placed in each of two identical tanks with flow-through freshwater. One tank served as control and the other was spiked with atrazine in order to maintain a concentration of 1 µg/l of atrazine for a period of 2 weeks. During these 2 weeks, food consumption in each tank was monitored daily to assess potential effects of exposure on feeding behaviour. At the end of the exposure period each fish was assessed for the olfactory response (EOG) to a known odorant, glutamine at a 10^{-1}M concentration. At the end of each experiment the eels were sacrificed and morphological and physiological measurements were obtained.

The pesticide atrazine had no significant effect on the olfactory response to glutamine. However, there was a significant effect of atrazine on the eel gill Na/K ATPase (p=0.048; Table 7) and a slightly higher GSI in exposed fish compared to the control (p=0.125) (Table 7).

Table 7. Olfactory, physiological and morphological recordings from adult eels exposed to a concentration of 1 μg l^{-1} of atrazine for two weeks.

Parameter	control (N=5)		exposed (N=5)		t-test
	mean	sem	mean	sem	P value
weight (g)	405.9	62.2	338.3	51.1	0.493
length (cm)	62.54	3.066	60.220	2.963	0.603
condition factor	0.16	0.009	0.151	0.009	0.560
eye index	7.23	1.034	6.498	0.567	0.611
fat content (%)	24.90	1.366	22.180	4.368	0.563
hepatosomatic index	1.07	0.068	1.350	0.241	0.342
gonadosomatic index	1.08	0.205	1.247	0.074	0.125
gill Na/K ATPase (μmol Pi/hr mg)	7.10	0.807	8.638	1.871	0.535
kidney Na/K ATPase (μmol Pi/hr mg)	15.37	1.506	9.532	2.171	0.048
EOG to 10^{-1}M glutamine (mV)	6.66	1.126	5.538	2.446	0.700

Objective 2. Investigate the impact of relevant contaminants within coastal and estuarine environments on the transition of juvenile eels from the marine to the freshwater environment.

Introduction.

The upstream migration of juvenile eels from coastal waters and into the freshwater zone occurs mainly between March and October and its commencement appears to be mainly dependent upon water temperature. During this period the elvers may be exposed to a wide range of diffuse and point source contaminants derived from a number of sources which may have a deleterious impact on the initiation of migration and successful transition from saline to freshwater conditions. This is therefore another potential bottleneck in the survival of the eel which could regulate eel populations in England.

In a similar way to the studies on emigrating silver eels, laboratory-based studies were undertaken to examine how exposure to environmental levels of identified contaminants may affect the key physiological processes involved in freshwater adaptation (e.g. plasma ion concentrations, gill and intestinal ATPase activity, regulation of thyroid hormones) and the subsequent survival of the eels in freshwater. In addition, laboratory-based behavioural studies were undertaken to determine whether the relevant contaminants had the potential to modify migratory behaviour and inhibit or delay the movement of the juveniles into freshwater.

Experiment 2.1. Impact of tributyl phosphate on transition of glass eels from the marine to the freshwater environment.

Glass eels were caught by commercial netsmen in the lower reach of the Severn estuary and transferred to the Cefas, Lowestoft facilities. In April 2010, ~500 glass eels were collected from Glass Eel UK and transported to Cefas Lowestoft laboratory as described in paragraph 2.1. Once in the laboratory, eels were distributed equally between six identical 63 liters glass tanks (85 eels in each tank) and supplied with brackish water (average salinity 18 ppm) at ambient temperature (ranging between 10 and 12°C). Each tank was enriched with hiding features and three sides and the top were blacked out to create an environment more suitable for glass eels. Fish were left to acclimatize for 10 days. At the end of acclimation three of the six tanks were exposed in flow through conditions to 0.5 μg/l of TBP while the other three tanks were used as control.

At the end of the 2 week exposure period half of the eels were sampled for parameters associated with the transition to freshwater and the other half transferred to freshwater. There was no difference in mortality between control and exposed fish and no difference was observed between treatments in the levels of gill Na/K ATPase activity (Figure 5), but fish did differ in their condition factor (Figure 6). The observed difference in condition factor could indicate a differential allocation of resources of the fish held in water of different quality. Due to the small size of the individuals, it was not possible to collect blood samples for plasma ion analysis.

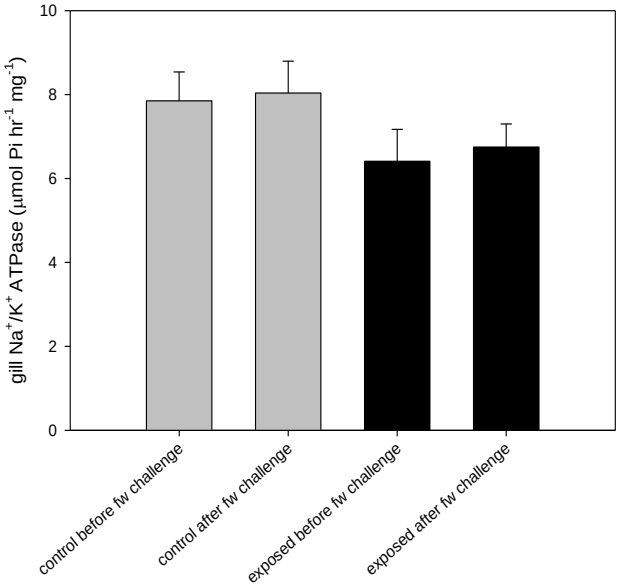
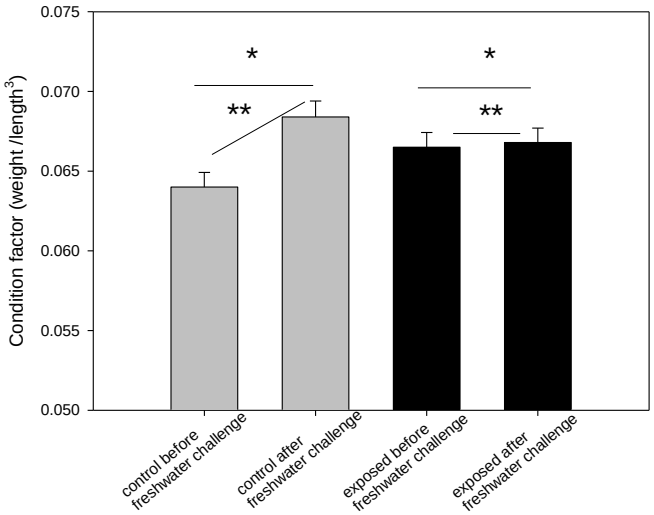


Figure 5. Na^+K^+ gill ATPase levels in glass eels exposed to tributyl phosphate



* effect of freshwater challenge P=0.013

** interaction between treatment and freshwater challenge P=0.028

Figure 6. Condition factor of glass eels exposed to tributyl phosphate.

Experiment 2.2. The impact of metals on the transition of glass eels from the marine to freshwater environment – damage to eel DNA.

In April 2013 approximately 500 glass eels were collected from the River Severn estuary and brought to the Cefas, Lowestoft Laboratory. Fish were kept in ambient seawater in a 500 litre tank in simulated natural photoperiod until the beginning of the experiment. Before the start of the experiment 30 glass eels were transferred into each of 22 glass aquaria (50 litre) with running seawater. At the start of the experiment the fish were kept in static seawater and treated with one of the metals listed in Table 8.

Table 8.Treatments used for water borne exposure of glass eels.

Treatment	Concentration	Treatment	Concentration
Copper low (Cu low)	2 µg/l	Copper high (Cu high)	10 µg/l
Lead low (Pb low)	1 µg/l	Lead high (Pb high)	5 µg/l
Zinc low (Zn low)	5 µg/l	Zinc high (Zn high)	20 µg/l
Chromium low (Cr low)	1 µg/l	Chromium (Cr high)	4 µg/l
Mixture low (Mix low)	2 µg/l (Cu), 1 µg/l (Pb), 5 µg/l (Zn), 1 µg/l (Cr)	Mixture high (Mix high)	10 µg/l(Cu), 5 µg/l (Pb), 20 µg/l (Zn), 4 µg/l (Cr)
Control	none		

The metals were chosen because they represent major contaminants present in the Severn estuary and Bristol Channel where large numbers of the glass eels enter English inland waters. The low and high concentrations were chosen to represent historic values (high concentrations) measured during 1970's and more recent values (low concentration) as a result of the decreasing pollution in the Bristol Channel (Jonas & Millward, 2010; Harper, 1991; Apte et al, 1990; Owens, 1984; Abdullah & Royle, 1974).

Glass eels were kept in the experimental tanks for 2 weeks. Behavioural observations were taken daily to look for changes in activity level between the different treatments. After the 2 weeks exposure 10 fish were sampled from each tank for morphological and physiological measurements. In addition a blood sample was taken from those fish for DNA investigation. The blood sample was used to carry out a Comet assay, which is a well established assay used to estimate DNA damage incurred by an individual after exposure to a stressor or a contaminant.

The remaining 20 fish in each tank were transferred to clean freshwater and given a 72 hours freshwater challenge. After 72 hours the fish were sampled for morphological and physiological parameters. The results of the morphological and physiological measurements are summarized in Table 9 and the results of Comet Assay are shown in Figure 7.

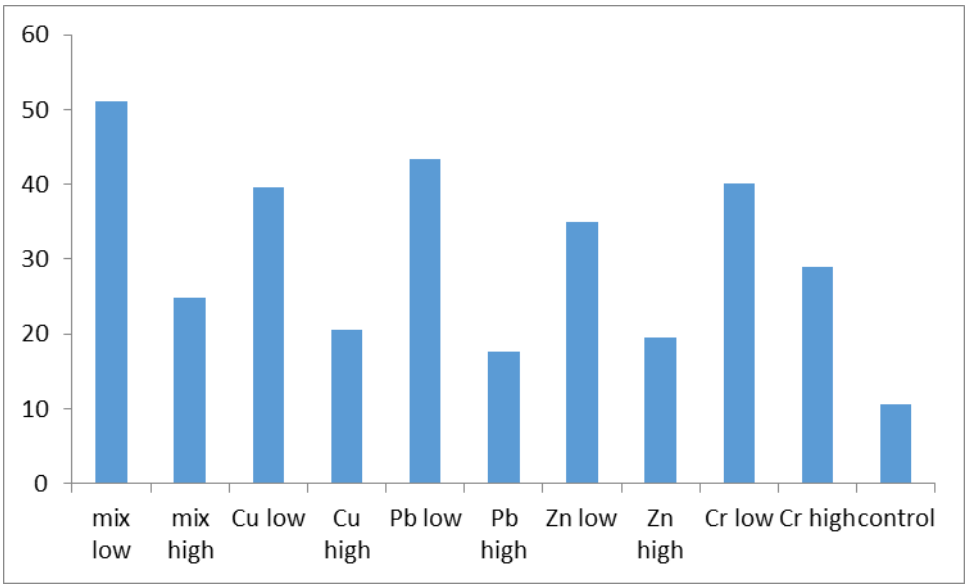


Figure 7. Comet Assay results of glass eels exposed to metals in seawater for 2 weeks. The graph indicates the mean tail intensity which represents the level of DNA damage sustained by the fish. Results are given for the 10 fish in each treatment tanks. The duplicates for each treatment have been pooled.

Table 9. Morphological and physiological data from eels exposed to metals in seawater and then transferred to clean freshwater.

before freshwater challenge								
treatment	weight (g)		length (mm)		Condition Factor		ATPase (μmol Pi/hr mg)	
	mean	sem	mean	sem	mean	sem	mean	sem
control	0.16	0.012	68.34	0.74	0.051	0.003	5.89	2.69
Cu low	0.13	0.011	66.25	0.94	0.043	0.003	9.72	1.53
Cu high	0.11	0.007	66.11	0.53	0.036	0.003	7.23	2.21
Pb low	0.15	0.010	67.96	0.79	0.045	0.002	9.87	0.93
Pb high	0.13	0.008	67.01	0.82	0.044	0.002	12.71	1.42
Zn low	0.12	0.010	66.82	0.77	0.040	0.003	8.38	1.72
Zn high	0.14	0.012	67.28	0.77	0.044	0.003	10.69	0.99
Cr low	0.20	0.051	68.65	0.67	0.060	0.015	9.29	2.16
Cr high	0.13	0.008	66.50	0.63	0.044	0.002	9.68	1.51
mix low	0.13	0.009	66.91	0.39	0.044	0.003	10.99	2.08
mix high	0.16	0.012	69.43	0.79	0.047	0.002	7.47	1.14
after freshwater challenge								
treatment	weight (g)		length (mm)		Condition Factor		ATPase (μmol Pi/hr mg)	
	mean	sem	mean	sem	mean	sem	mean	sem
control	0.12	0.007	66.93	0.77	0.039	0.002	11.18	1.21
Cu low	0.13	0.007	67.23	0.51	0.044	0.002	6.97	1.71
Cu high	0.13	0.010	67.37	0.76	0.040	0.002	8.44	2.02
Pb low	0.13	0.008	67.78	0.59	0.040	0.003	9.59	0.91
Pb high	0.13	0.008	68.05	0.61	0.040	0.002	7.59	1.59
Zn low	0.13	0.008	67.59	0.53	0.042	0.002	8.01	2.57
Zn high	0.12	0.007	67.28	0.51	0.040	0.002	11.54	3.45
Cr low	0.13	0.007	67.09	0.42	0.044	0.002	8.04	1.40
Cr high	0.15	0.007	68.52	0.52	0.045	0.002	7.98	0.87
mix low	0.13	0.006	66.28	0.63	0.045	0.001	4.86	2.69
mix high	0.14	0.006	66.62	0.61	0.046	0.002	6.73	1.56

The results of the experiment indicated that there were no differences in any of the measured morphological, physiological or behavioural parameters between the various metal treatments. However, the Comet assay indicated that the mean tail moment (i.e. DNA damage) was significantly higher in exposed eels compared to the control eels (Figure 7). All the eels exposed to the low concentrations of the metals demonstrated significant damage to the DNA (Multiple comparison against control, Dunn's method $P<0.05$). However, it was only the mixture and Cr groups that showed a significant damage to the DNA at the high concentrations.

The exact mechanisms for how the metals caused damage to the DNA are not known. DNA damage can be induced: through apoptosis or necrosis; through the interaction with oxygen radicals or other reactive intermediates; or as a consequence of exclusion repair enzymes. The metals could be acting as a chemical stressor that induces oxidative stress as has been shown for water borne selenium in juvenile rainbow trout (Miller et al. 2007). Although there was damage to the DNA of the eels it is not known what the long term effect of this would be to the viability and survival of the eels.

Objective 3. Investigate the impact of sediment-derived contaminants on growth and survival of juvenile eels.

Introduction

Previous research carried out at Cefas as part of a Defra funded project SF0240 –*Pesticides in salmonid*

spawning gravels, indicated that environmental levels of contaminants bound to sediment as well as the water borne contaminants circulating within the gravels, may directly affect the subsequent survival of the intragravel stages of salmonids. During their initial residence in freshwater juvenile eels are often buried for long periods within the river sediment during the day and as a result will be directly exposed to sediment borne as well as water borne contaminants. Further, many of the eels major prey items are also benthic in nature and would also reside within sediments. As a result, the ingestion of contaminated prey may also provide another potential route for uptake and bioaccumulation of toxicants. Therefore, it is considered that the early life history of the eel may also be a sensitive period where exposure to contaminants may influence growth and survival and subsequently contribute to the decline of the eel population.
The present work built on the previous studies on salmon undertaken in the project SF0240. The objective was to identify potential toxic compounds commonly occurring in river sediments and examined their impact on juvenile eels. Laboratory-based studies exposed groups of juvenile eels, within artificial substrate tanks, to environmentally relevant concentrations of key contaminants bound to sediment and examined the impact of these compounds with respect to development and survival. Additional groups of eels were also fed food items spiked with known concentrations of contaminants and their survival subsequently monitored. Tissue samples from each group were analysed to determine the potential impact on DNA integrity and gene expression and assess the potential impact on eels in freshwater.

Experiment 3.1. Impact of sediment derived HBCD on growth and survival of juvenile eels.

Brominated flame retardants (BRF) are substances used in the manufacture of a wide range of materials such as plastics and textiles and are very prevalent in freshwater, estuarine and marine environments. The majority of flame retardants contain brominated organic compounds, making them persistent and lipophilic with the ability to bio-accumulate. A European Risk Assessment has concluded that HBCD has a high bioaccumulation potential and is found in increasing levels in the environment and biota. Brominated flame retardants are similar in chemical structure to the thyroid hormones and have been shown to disrupt this endocrine system in many animals. In salmon, thyroid hormones play a vital role in smoltification and migratory behaviour and any modification of thyroid hormone concentrations (thyroxine T_4 and tri-iodothyronine T_3) are likely to significantly alter physiology and behaviour and may reduce the survival of smolts in the sea.

In May 2011 70 glass eels were collected from the River Tees and transported to Cefas Lowestoft Laboratory where they were kept in a large 500 l tank to acclimatise. The tank was supplied with constant ambient freshwater (temperature range 15-16 °C), an airline and a naturally simulated photoperiod (15:9 hours of day:night). Fish were fed daily (Monday to Friday) with commercial Artemia. In June the fish were equally distributed over 4 glass tanks (16 fish per tank with volume of 63 litres) containing each 5 kg of fine gravel and supplied with running freshwater at ambient temperature (16-19.5 °C). The eels were left to acclimatize for 10 days and then half of the fish from each tank (7 fish per tank as one fish had died in each of 2 experimental tanks) were collected, measured and cryo-preserved for subsequent analysis. The remaining animals were presented with weekly addition of clean or spiked clay (Newplast™ clay modelling clay - Newclay Products Ltd, Newton Abbot, UK) for a period of three months. Each of the treatment was done in duplicate tanks. Prior to use, the clay was combusted in an oven at 450ºC for five hours to remove any organics (Crooks, 2011). It was then mechanically ground with a pestle and mortar to achieve the desired sediment particle size i.e., fines. Toxicity information supplied by Newclay Products Ltd and measured under BS EN 71-3, indicated undetectable levels of common metals (e.g., mercury, lead, cadmium, copper, arsenic) in the modelling clay. To prepare the clay for the exposure experiment, for each tank, 10g of fine clay was measured and spiked with 5 ml of a solution of HBCD (for exposed tanks) dissolved in acetone at a concentration of 500µg/ml in order to achieve the desired concentration of 500 µg/kg in the tank containing 5 kg of sediment. The clay for the control tanks was spiked with 5ml of acetone. All spiked clay was left for at least 5 hours in an exhaust ventilation unit to completely dry before being added to the tanks. The concentration of the BFR added on a weekly basis was chosen to mimic potential run off from grounds alongside natural watercourses. The concentration of HBCD was consistent with value described in published literature and equal to 500 µg/kg of sediment. At the end of this period, eels were sampled, anaesthetized then measured for length and weight and cryo-preserved for further analysis.

The results of the study indicated there were no differences in either survival or the measured morphological parameters between control and exposed fish. The eels' behaviour during the 3 months exposure was recorded on tape and initial analysis indicated that there were no differences in the measured behaviours (swimming behaviour, burying behaviour, foraging behaviour).

Experiment 3.2. Impact of sediment and food derived metals on growth and survival of juvenile eels.

In June 2013, 500 juvenile eels were obtained from the eel growing programme of "Glass eel UK" – Gloucester, and transferred to the Lowestoft Laboratory. Fish were kept in a large 500 litre tank with flow through freshwater until the experiment started later in the year. The aim of this experiment was to expose juvenile eels to contaminated sediment and/or food and measure the potential impact on their growth, survival, DNA integrity and gene expression. The eels were exposed to the same metals as in the previous study at levels found in sediment and invertebrates in the Bristol Channel (Langston et al, 2010; Duquesne et al, 2006, Allen & Rae, 1986). The concentrations tested are summarized in Table 10.

Table 10. Concentrations of metals used in sediment and food exposure experiment.

Treatment	Sediment (µg/g)	Food (µg/g)
control	None	None
Cu low	40	40
Cu high	80	80
Pb low	80	1.5
Pb high	120	3
Zn low	150	150
Zn high	300	300
Cr low	25	0.2
Cr high	50	2.5
Mix low	Cu 40; Pb 80; Zn 150; Cr 25	Cu 40; Pb 1.5; Zn 150; Cr 0.2
Mix high	Cu 80; Pb 120; Zn 300; Cr 50	Cu 80; Pb 3; Zn 300; Cr 2.5

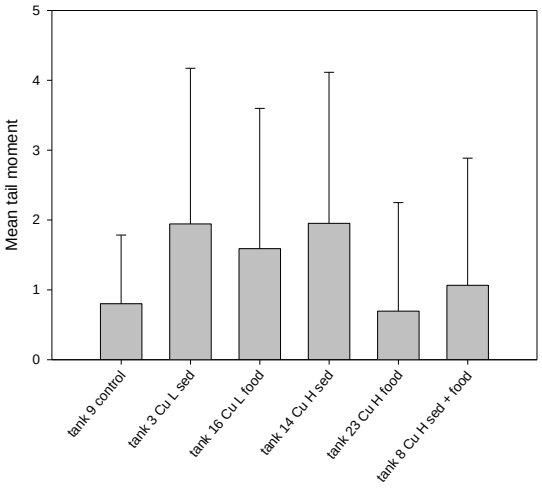
At the beginning of the experiment, 31 identical glass aquaria (50 litres) were provided with 2 kg of artificial sediment (a mixture of pebbles and soil commercially available for use in ponds). Each aquarium was a different treatment where the metal tested would be present in the sediment or in the food or in both. Tanks were filled with 50 litres of fresh water and 10 to 20 eels were placed in each tank. Tanks were kept in static water conditions, and eels were fed daily with commercial fish pellets of appropriate size. Prior to feeding, the pellets had been spiked with either tank water or the relevant metal at the concentration for the treatment. The experiment lasted for 5 weeks at the end of which all fish in each tank were sampled for length and weight, a blood sample taken for Comet Assay and the liver collected for gene expression determination. Table 11 reports the morphological data taken at the end of the exposure experiment and the calculated weight increase from the beginning of the experiment. Results of the Comet assay are shown in Figure 8.

Table 11. Morphological data from elvers exposed to metals in either sediment, food or both.

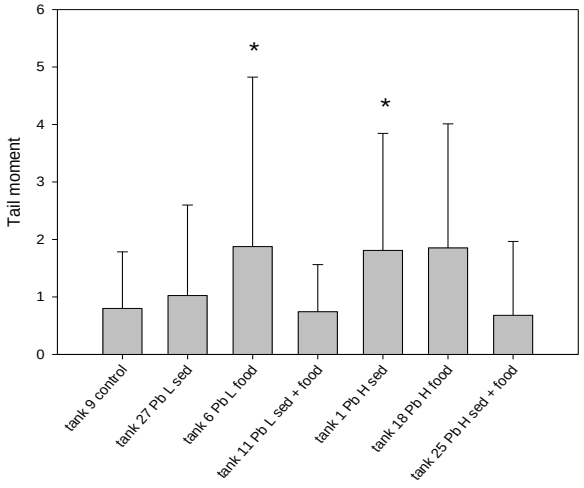
			final weight		final length		CF		% weight
metal	Conc.	medium	mean	±sem	mean	±sem	mean	±sem	increase
control			1.74	0.31	103.65	5.05	0.132	0.004	17.42
Cu	low	sed	1.95	0.39	106.80	5.93	0.125	0.005	23.54
Cu	low	food	2.08	0.39	108.06	5.72	0.137	0.004	29.82
Cu	low	sed + food	0.93	0.05	89.80	1.91	0.128	0.005	37.63
Cu	high	sed	1.61	0.31	102.38	5.66	0.127	0.008	23.83
Cu	high	food	1.95	0.52	108.79	7.34	0.125	0.005	0.15
Cu	high	sed + food	1.71	0.28	104.25	5.88	0.127	0.007	27.05
Pb	low	sed	1.90	0.37	107.71	6.75	0.127	0.004	32.57
Pb	low	food	1.77	0.34	101.95	5.68	0.138	0.004	25.07
Pb	low	sed + food	1.59	0.26	99.74	4.52	0.136	0.007	23.54
Pb	high	sed	1.74	0.26	104.77	4.77	0.130	0.003	22.68
Pb	high	food	1.66	0.27	101.90	5.02	0.134	0.003	18.52
Pb	high	sed + food	2.53	0.51	114.07	8.59	0.139	0.003	35.13

Zn	low	sed	1.98	0.37	107.32	6.33	0.134	0.003	20.26
Zn	low	food	1.57	0.23	101.19	4.26	0.133	0.003	22.12
Zn	low	sed + food	2.62	0.78	111.63	9.32	0.128	0.005	28.38
Zn	high	sed	1.82	0.25	104.94	4.54	0.142	0.003	33.78
Zn	high	food	0.93	0.11	88.78	2.49	0.128	0.006	41.94
Zn	high	sed + food	1.55	0.27	99.23	5.18	0.123	0.006	18.18
Cr	low	sed	2.04	0.34	108.72	5.43	0.135	0.003	34.47
Cr	low	food	1.79	0.32	104.63	5.95	0.129	0.006	27.05
Cr	low	sed + food	1.49	0.27	100.15	5.04	0.121	0.004	8.87
Cr	high	food	2.01	0.43	107.26	6.54	0.128	0.004	21.71
Cr	high	sed + food	2.22	0.41	112.73	7.14	0.128	0.006	34.8
mix	low	sed	1.69	0.25	103.80	5.18	0.135	0.003	34.91
mix	low	food	1.74	0.29	104.32	4.47	0.127	0.006	22.94
mix	low	sed + food	1.87	0.41	105.74	5.80	0.130	0.007	21.76
mix	high	sed	1.86	0.34	104.70	5.76	0.133	0.004	25.46
mix	high	food	2.00	0.55	106.92	7.40	0.129	0.007	28.57
mix	high	sed + food	2.44	0.51	113.94	7.36	0.129	0.004	21.17

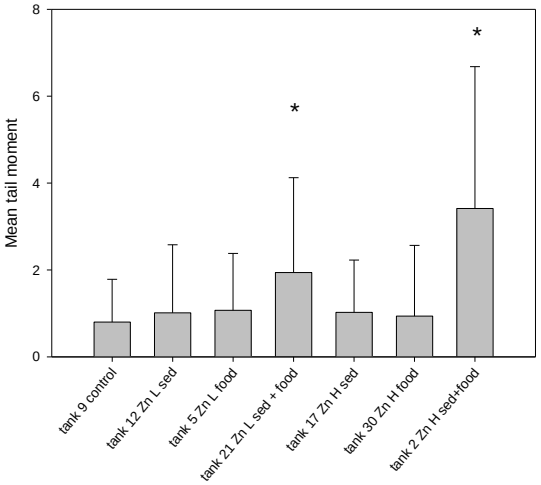
Copper



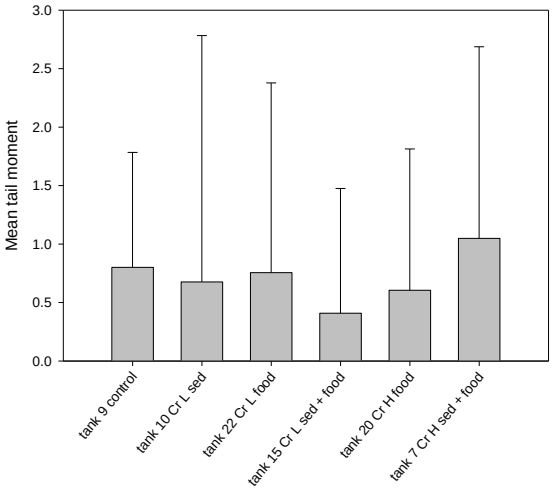
Lead



Zinc



Chromium



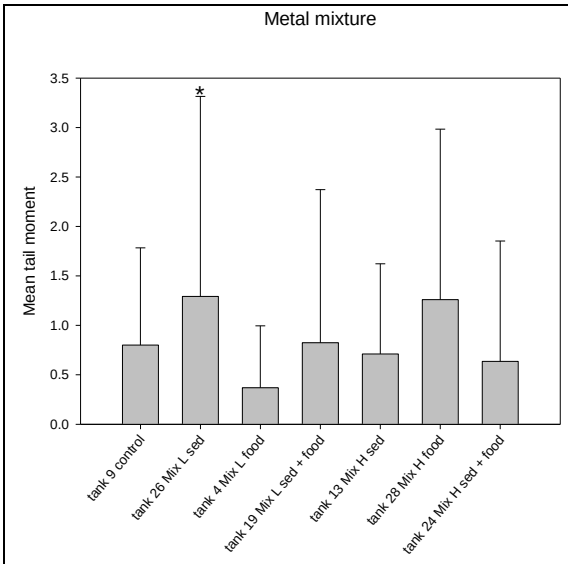


Figure 8. Juvenile eel DNA damage (Comet Assay) in response to metal exposure via sediment and or food. The bars represent mean tail moment and the standard deviation. * indicate a statistically significant difference versus control (Multiple comparison, Dunn's method, $P<0.05$)

Exposure to the metals in either the sediment or food had no significant effect on the measured morphological parameters in the glass eels. However, there was significant damage to the DNA of the eels when exposed to the metals zinc and lead both in the sediment and food (Figure 8).
The liver taken from each eel was used to extract RNA and quantify the corresponding level of gene expression of two genes linked with detoxification (CYP450) and metal detoxification (Metallothionin). Gene expression was determined using quantitative real time PCR.
The results of the study indicated no differential gene expression between any of the various treatments. Therefore, exposure to metals had no effect on the eels and did not change the expression of the measured genes.

Objective 4. Investigate the impact of freshwater contaminants on the migratory behaviour of silver eels during their initial estuarine/coastal spawning migration.

Experiment 4.1.Migratory behaviour of silver eels exposed to TBP.

This research examined the impact of freshwater exposure to TBP on migrating wild silver eels during their subsequent emigration from freshwater and into the coastal zone. The study used an integrated physiological and behavioural approach and was undertaken in collaboration with DTUAqua (Denmark). European eels were tagged with miniature coded acoustic transmitters and exposed in the laboratory to an environmental concentration of TBP (Fries & Püttmann 2003). Their subsequent movements were monitored as they migrated through a freshwater river and fjord and into the marine environment, using strategically positioned acoustic receivers.

Forty silver eels were captured by modified Wolf nets as described for Experiment 1.2 and brought to the Centre for Vildlaks in Randers Denmark. At the end of the acclimatisation week, 40 fish were randomly selected by dip netting and tagged intraperitoneally with VC9 acoustic tags (Vemco, Inc., Bedford, Nova Scotia, Canada). Fish were anaesthetized with 2-phenoxyethanol (2 $ml \cdot l^{-1}$), a small incision was made on the upper abdomen and the tag inserted in the body cavity. Two sutures were used to close the incision, and a powder mixture of Orahesive and amoxicillin was applied to help the healing process and prevent infection. Tagged eels were then divided between the two remaining tanks (control and exposed) in static oxygenated freshwater. Fish were allowed to recover from surgery for 2 days, before the fish were exposed to the treatment. The treatment consisted of an exposure to TBP at a concentration of 0.5 $\mu g \cdot l^{-1}$ (Sigma-Aldrich, Gillingham, Dorset, UK) for a period of 5 days exactly as done for Experiment 1.2. The duration of the experiment was short as the fish used in this experiment were at the end of the normal migration period for this river system (K. Aarestrup, pers. comm.), and as indicated in a previous study (Aarestrup et al. 2010), eels can migrate downstream rapidly and some take as little as 3 days to enter the Kattegat from the first acoustic listening station (Figure 9). On the 19th November, at the end of the 5-days exposure period, all tagged fish were transported and released at Tange hydropower station (Figure 9), on the River Gudenaa,

Denmark (coordinates 56° 21′ 18.34° N; 9° 36′ 14.76° E). The River Gudenaa (mean annual discharge of 32 $m^3 \cdot s^{-1}$) is the major freshwater source to the narrow Randers Fjord (Figure 9). The Randers Fjord (30 km long) is principally divided into two parts: a narrow inner section and a wider outer section, which exits into the Kattegat (Aarestrup et al. 2010). The salinity varies with water discharge in the River Gudenaa, but the fjord can generally be characterised as brackish, and salinity increases with depth and with increasing distance from the head of tide. Eight VR2 acoustic receivers (Vemco, Inc.) were deployed at four sites (Acoustic Listening Stations) in the river and fjord and left until May 2011 (Aarestrup et al. 2010) except for one receiver at site 4 (see Figure 9) that was lost during the winter before its stored data could be retrieved. The receivers recorded and stored the code and time (to the nearest second) of passage for individual fish within their range. Two receivers were positioned at each site, separated by a few hundred metres in a longitudinal direction, to confirm detection and swimming direction. A previous study has indicated that detection ranges of each receiver varied between 130 and 400 m during range tests, the width of the river or fjord adjacent to the receivers varied between 50 and 240 m (Aarestrup et al. 2010), and therefore all fish should have been recorded at each of the four sites. The distances from the release site to each of the four receiver sites were 22, 37, 49.2 and 65.6 km. The movement of the eels as they were initially detected by the acoustic receivers was analysed using circular statistical methods (Batschelet 1981). The data sets were specifically tested to show whether the movement of the eels was random with respect to time of day and state of tide using the Raleigh test (*r* value) (Batschelet 1981). High water at the estuary mouth has been used as a reference time for all tidal cycles.

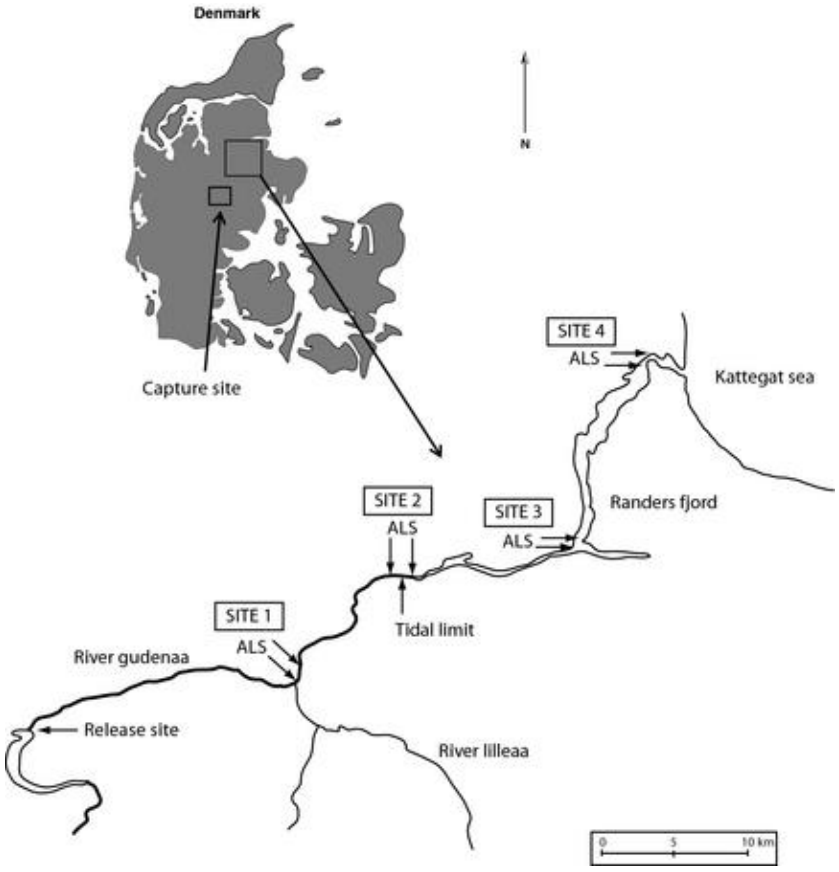


Figure 9. Capture and release sites for tagged silver eels and position of the eight pairs of acoustic receivers (ALS) at sites 1–4 along the River Gudenaa and Randers Fjord.

Eels were assigned randomly to control and exposed group. Fish length (control 64.05 ± 0.9 cm; exposed 63.6 ± 1.07 cm, mean ± SEM – *t*-test $P = 0.777$) and fish weight (control 465.05 ± 25.59 g; exposed 481.9 ± 24.93 g, mean ± SEM – *t*-test $P = 0.646$) did not differ significantly between the two groups.

Data obtained by the acoustic receivers indicated that there were no differences in the migration success of control and exposed fish throughout the study area or in their diurnal or tidal patterns of migration. The eels migrated downstream predominantly at night and moved through the estuary at all states of the tide. Only five tagged eels were detected by the most seaward receiver (two exposed and three control eels), and the low detection rate may be the result of the loss of one of the two receivers located at the exit into the Kattegat Sea. There were no significant differences between the two groups in terms of the mean times taken to reach the receivers at each of the four receiver locations. The mean time taken for the two groups to

reach the first receiver position were: control 6.54 ± 3:26 h; exposed 12.54 ± 23:59 h (mean ± SD – *t*-test $P = 0.275$). Migration rates from the point of release to the exit into the Kattegat Sea were also not significantly different between the two groups, and the mean time for all the fish (data pooled) to reach the most seaward receiver was 138 ± 32:41 h (mean ± SD). One fish was recorded passing by ALS 1 and 3 but not by ALS 2 suggesting that potentially more eels may have migrated through the system without being detected by any listening stations.

Overall, the results of the study indicated that exposure to TBP for a short period had no significant impact on the migratory behaviour or migratory success of eels moving through the lower section of the River Gudenaa and Randers Fjord. However, only 12% of the tagged eels were detected at the receiver site closest to the exit into the Kattegat Sea. In a study in the River Meuse, Belgium (Verbiest et al. 2012), 15% of the tagged eels that migrated within freshwater entered the North Sea. The suggested high mortality was considered to be the result of hydroelectric power stations, exploitation or predation although a number of the fish also remained within freshwater and did not migrate further. A previous study in Randers Fjord demonstrated that 40% of eels were detected migrating through the study area (Aarestrup et al. 2010) and that the likely cause of the low detection rate of tagged fish was related to fishing mortality within the fjord. Whilst fishing pressure has probably played an important role in the low detection rate observed in this study, it is also worth noting that there are other possible causes for the low detection rates of tagged eels other than fishing mortality. The procedures used in the capture and tagging of the eels may have an impact on their subsequent behaviour and migration through the study area (Jepsen et al. 2002). Fish are known to expel tags that have been surgically implanted into the body cavity (Moore et al. 1990), although Winter et al. (2005) studied tagging effects in European silver eels and recorded no transmitter expulsion or mortality related to handling or tagging of the fish. A recent study on eels in the River Stour in southern England (2011) using similar tagging methods reported in this paper indicated that the effects of tag attachment on subsequent migratory behaviour were negligible (A. Moore & L. Privitera, unpublished data). Tags may also have failed due to technical problems and as such migrating eels would not be detected as they moved through the study area. In addition, changes in salinity, swimming speed and the levels of sediment within the water column can all affect the detection rates of acoustic tags, and so, it is possible that some eels may not have been detected by all receivers. In the present study, it should be noted that one of the two receivers at the exit of the fjord was lost at some point during the study and reduced the detection rates of migrating eels. It is possible that fish were also not detected because they remained close to the release site after tagging and either did not migrate or only migrated later after the study period. Although the eels were trapped during what is considered to be the main migration period for the population in the River Gudenaa, the eye index of the eels, an indicator of silvering in eels, was relatively low compared with other studies (Durif et al. 2005; Van Ginneken et al. 2007a); however, the GSI measured in this experiment is in line with values typical of silver eels (Van Ginneken et al. 2007a). Eels that were not totally preadapted to saline conditions may not have migrated immediately into the sea but resided for a longer period in freshwater (as reported by Feunteun et al. 2000). Migration through the estuary in the present study was also predominantly nocturnal as previously described by Aarestrup et al. (2010) although the seaward migration of the eels in the present study appeared to be more rapid. The time taken for the eels to reach the most seaward receivers in the fjord from the point of release was 138 ± 32:41 h (mean ± SD) compared with 742 ± 375 h (mean ± SD) as reported by Aarestrup et al. (2010). The differences in migration rates could be attributed to possible differences in the migratory behaviour of the eels as a result of differences in release date (November vs. September–October), development (silver eels in the previous study by Aarestrup et al. 2010 were heavier than the eels used in the present study), handling and tagging or differences in the environmental conditions within the river during the two studies.

Experiment 4.2. Migratory behaviour of silver eels exposed to a pesticide mixture.

Forty eels were trapped on the River Avon, southern England and transported to the Cefas Laboratory at Lowestoft. The eels were equally distributed into 4 identical tanks (volume of 550 liters) and maintained with a continuous freshwater flow (water temperature varying between 4.0 and 10 °C) and an automated naturally simulated photoperiod. The eels were left to acclimatise for 2 weeks. At the end of the acclimatisation period the fish were surgically implanted with acoustic tags as described above and left undisturbed to recover for 7 days. At the end of recovery, the tagged eels were assigned to either a control or an exposed group. The exposed group was maintained for 5 days in a static water system containing the pesticide mixture shown in Table 3. At the end of the exposure period the eels were transported back to the River Avon and released close to where they were trapped 4.5 km above the head of tide. Their subsequent movements were monitored using 10 strategically placed VEMCO VR2W acoustic receivers located between the release point and the exit of Christchurch Harbour (Figure 10). The movement of the eels as

they were initially detected by the acoustic receivers was analysed using circular statistical methods (Batschelet 1981). The data sets were tested to show whether the movement of the eels was random with respect to time of day and state of tide using the Raleigh test (*r* value) (Batschelet 1981). High water at Christchurch Harbour has been used as a reference time for all tidal cycles.

Figure 10. Map showing the positions of the 10 VR2W acoustic receivers within the River Avon and Christchurch harbour. The arrow indicates where the tagged eels were released.

The migration of the tagged eels was nocturnal and there were no recorded movements during the day. The first recorded movement of the eels was 1-2 hours after dusk and migratory behaviour ceased 1-2 hours before daylight. The mean times of day that the two groups were detected are shown in Table 10. There was no difference in the mean time of day that the two groups were detected at each receiver. At a number of the receivers the mean times of the two groups were within 1 hour of each other. Movement through the estuary was predominantly on an ebbing tide with the eels detected moving out into the marine environment just after high water. The number of exposed eels detected moving out into the marine environment was low but this could simply be the result of a poor detection rate by the most seaward positioned receiver. These results are very similar to those in the study undertaken in Denmark. Exposure to the pesticide mixture did not appear to affect the behaviour of the eels either in freshwater or during the migration through the estuary.

Table 10. The downstream movements of eels detected by the receivers located throughout The River Avon and Christchurch Harbour in relation to the tidal cycle and time of day. The mean value for the tidal cycle is calculated as hours from the previous High Water. The mean times at which the eels were recorded passing the acoustic receivers have been calculated from the mean vectors (Batschelet, 1981). The value n is the total number of eels moving past the respective receiver.

	Control			Exposed		
Receiver	Tidal Cycle mean time after High Water (hr)	Time of day	N	Tidal Cycle mean time after High Water (hr)	Time of day	N
1	FW	20:16	18	FW	19:05	19
2	FW	21:45	15	FW	21:38	13
3	FW	23:02	19	FW	21:24	19
5	02:23	22:24	18	00:44	19:39	19
6	Random	21:14	18	Random	20:52	19
7	Random	22:01	12	Random	22:07	15
8	Random	21:08	9	Random	21:32	7
9	Random	22:35	14	Random	22:03	10
10	00:37	22:11	6	01:03	20:51	3

Objective 5. Provide advice and recommendations to Policy Divisions on the management of aquatic contaminants in relation to supporting the implementation of the Eel Recovery Plans.

The research has highlighted that short-term exposure of eels to a range of freshwater and estuarine contaminants does not have a significant effect on the ability of juvenile and adult eels to migrate successfully between the freshwater and marine environments. Further, that the short-term exposure to a range of environmentally relevant concentrations of contaminants within sediments do not have a detrimental impact on the survival of yellow eels whilst resident in freshwater. However, eels are resident in freshwater for a number of years and the long-term impact of contaminants was not investigated and therefore cannot be discounted. The eel is also known to bio-accumulate a number of chemicals as a result of their high body fat content. It is recommended that the potential effect of contaminants on the marine spawning migration together with the potential impact on reproductive ability of eels is subject to further investigation. In addition, eels are exposed to a suite of different chemicals within both the estuarine and freshwater environments and so the combination of different contaminants may be an important factor in the survival of the eel. It is recommended that the additive and synergistic effects of contaminants on eels be further investigated. The present work has focused on the impact of water quality of the freshwater and estuarine environments. The marine environment has not been studied but should be considered when implementing the Eel Recovery Plans. In particular, changes in oceanic conditions (sea surface temperatures and ocean currents) together with the role of oceanic acidification on the survival of the early stages of eel are important considerations when determining the relative proportions of silver eels escaping from river catchments.

Discussion and Conclusions.

The overall objective of the research was to examine the potential impact of contaminants on what are considered to be sensitive life history stages of the European eel and determine whether exposure to contaminants located in freshwater, estuaries and coastal zones may be one of the reasons contributing to the decline in the species. Following on from successful work on Atlantic salmon, relating to smolt migration (Moore *et al*., 2007; Moore *et al*., 2008), olfactory imprinting (Lower & Moore 2007) and the ability of the smolts to survive once they have migrated into the marine environment (Waring & Moore, 2004; Moore *et al*. 2003), the work focused on the impact of freshwater contaminants on the ability of emigrating adult silver eels to physiologically and behaviourally adapt for the transition to the open sea. The previous work on salmon indicated that the freshwater and marine environments could not be considered in

isolation and the conditions experienced in freshwater had a significant impact on the behaviour and survival of the fish once they had entered the sea. In addition, it has been hypothesised for the salmon that conditions in the marine and estuarine environments may also affect the ability of the returning salmon to adapt to freshwater and subsequently impact upon their migratory and spawning success. Therefore, the present work also examined the impact of contaminants regularly monitored within the marine environment on the migration of the juvenile eels when they move from the open sea into the freshwater environment. Eels were also considered to be potentially sensitive to contaminant exposure during the freshwater phase, when they adopt a benthic life-style and are potentially exposed to contaminated sediments and contaminated prey. The results of this research indicate that exposure to a range of contaminants within freshwater has very little effect on the physiology or run-timing of silver eels as they migrate out to sea. In a similar way, there was no evidence from the present study that the studied marine contaminants have an effect on the ability of glass eels and elvers to successfully move into the freshwater environment. Overall, the results are different to earlier studies on Atlantic salmon, which have shown that a range of contaminants such as simazine and the brominated flame retardant hexabromocyclododecane modify the run-timing of smolts, reduce their ability to adapt to saltwater and inhibit the olfactory system's ability to detect the cues considered important in imprinting and used to return to the home river to spawn. However, the only exception in the present study was the pesticide atrazine which did reduce the gill Na/K ATPase activity in a similar way to previously reported for the Atlantic salmon smolt. Whether this physiological affect had a long term impact on the ability of the silver eel to survive in the marine environment is not known but does require further investigation.

Although, the short term exposure of silver eels to contaminant did not affect the silvering process or successful short term migration, the present study did indicate that exposure to certain metals within sea water produced significant genetic damage in the glass eels as measured by the Comet Assay. Damage at the molecular level in many fish can cause serious problems but in the present study it is not clear to what extent the genetic damage is carried through to the whole animal or what the effects might be at the population level. Although, eels are known to bioaccumulate a number of toxins as a result of their high fat content, the present research also indicated no physiological or behavioural effects on juvenile eels as a result of exposure to contaminated sediment or ingested contaminated prey. However, once again the Comet assay carried out on the glass eels did indicate that exposure to metals in the sediment does cause genetic damage although how this is can be assessed at the whole animal and population level is not clear.
The lack of any effect on the eels in the present work may reflect the short duration of exposure that was experienced during the experiments. Although the studies reflected the short term exposure immediately prior to the transition from the fresh to the marine environment, eels are normally present in freshwater for periods in excess of 5 years and so long term exposure may be an additional issue in relation to the successful transition from fresh to salt water. Bioaccumulation of toxins is a concern and many studies have indicated that eels are particularly prone to the uptake of lipophilic contaminants such as polychlorinated biphenyls, organochlorine pesticides and brominated flame retardants. In addition to these contaminants, there are also a wide-range of emerging organic groundwater contaminants (EGCs) which are beginning to be monitored in the UK. These include nano-materials, pharmaceuticals, industrial compounds, personal care products, fragrances, water treatment by products, flame surfactants as well as caffeine and nicotine. Therefore, future targeted studies investigating the role of these EGCs on eels and the impact of changes to the metabolism in eels are warranted.

Although the current research indicated that exposure to key contaminants had no effect on the short term migration of silver eels, it is possible that exposure to contaminants could have a greater affect on the ability of silver eels to undertake their marine migratory and their spawning viability. However, conducting experimental trials on these phases of the life-cycle are extremely difficult. Other potential causes of the decline in the European eel that need further investigation is the impact of oceanic acidification on the survival of leptocephali and their prey, and changes in oceanic conditions (temperature and currents) which may change and move the returning juveniles away from their historic distribution within Northern Europe.

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References to published material

9. This section should be used to record links (hypertext links where possible) or references to other published material generated by, or relating to this project.

Peer reviewed publications

Privitera, L., Aarestrup, K., Moore. A. 2014. Impact of a short-term exposure to tributyl phosphate on morphology, physiology and migratory behaviour of European eels during the transition from freshwater to the marine environment.

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Conference Abstracts

Privitera, L., Aarestrup, K., Moore. A. Impact of a short-term exposure to tributyl phosphate on morphology, physiology and migratory behaviour of European eels during the transition from freshwater to the marine environment. (Oral communication)

Ecology and Conservation of Freshwater Fish. 28th May-2nd June 2012 Vila Nova de Cerveira, Portugal

Privitera L., Bean, T., Lyons, B. Moore, A. Effects of short term metal exposure on glass eel DNA integrity and freshwater adaptation. (Oral communication)

Eel Genome Symposium. 16-17 January 2014 Leiden, The Netherlands.

Privitera, L. & **Moore, A**. Effects of silver eel exposure to a pesticide mixture on their physiology and downstream migration. (Oral communication)
IFM tagging and telemetry workshop. 22-23 July 2014 Leeds, United Kingdom