Vitellogenin content in European eel (*Anguilla anguilla*) in Flanders, Belgium

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''Capsule'': *European eel,* Anguilla anguilla*, did not show enhanced plasma VTG concentrations, despite a very high internal pollution load.*

Abstract

As part of a large-scale monitoring program of bioaccumulating contaminants in the European eel (*Anguilla anguilla*) in Flanders (Belgium), we investigated potential effects of xenoestrogens in these fish. The present paper describes the results of the plasma vitellogenin (VTG) content, measured in 142 eels sampled at 20 different locations, in relation to the internal pollution levels. To validate the blood VTG assays, a small number of eels (n=8) was exposed to 10 mg ethinylestradiol/l (EE2) for 9 days. In this experiment, VTG was detected as a protein with a molecular weight of 214 kDa and confirmed by Western blotting. Compared with the solvent controls, significantly higher concentrations of VTG were measured in EE2 exposed eel. However, the VTG content was relatively low compared with other fish species exposed to high concentrations of estrogens. The plasma VTG content of eels from the field study was very low, despite a very high internal load of endocrine disrupters. These results, together with previously published studies, suggest that immature yellow European eel might not be the best sentinel species to study the effects of estrogenic compounds on VTG levels of wild fish populations.

Keywords: Alkali labile phosphate; Calcium; Endocrine disruption; Organochlorine pesticides; PCB

1. Introduction

There is growing evidence that anthropogenic xenobiotics can affect the endocrine status of wildlife (Damstra et al., 2002). Although precise mechanisms of action are poorly understood and the causative chemicals are not always known, extensive evidence is available that sewage treatment effluents can disturb endocrine function in fish (Harries et al., 1999; McArdle et al., 2000; Damstra et al., 2002). Flanders (Belgium) is a very densely populated area in Western Europe with about 440 inhabitants/km^2. About 57% of the household sewage water is treated (De Cooman et al., 2002). From this, it may be suggested that compounds with estrogenic activity can occur in these surface waters in relatively high concentrations. Organochlorine pesticides for instance are detected at concentrations exceeding the environmental standards in 42% of the routinely monitored sampling stations (De Cooman et al., 2002). Despite the concerns, little is known about the incidence of endocrine disrupters and their effects in fish of Flemish surface waters. Eel (*Anguilla anguilla*) has proven to be a good indicator organism for measuring chlorobiphenyls and persistent organochlorine compounds (de Boer and Hagel, 1994; de Boer and Brinkman, 1994). Through carnivorous feeding behaviour—predating on insect larvae, worms, crustaceans, snails, mussels and fish—eel bioaccumulates numerous chemical residues (Tesch, 1977). Eels are widespread and can be found in most aquatic habitats. Furthermore, eels are present throughout the year, the species is not very sensitive to (handling) stress (Livingstone et al., 2000) and European eel is quite resistant to various forms of water pollution. In the yellow eel phase, eels are sedentary and normally do not migrate (Tesch,

1977). Measurements of residues in the tissues of the yellow eel phase therefore reflect the quality of their environment, at least with respect to organochlorines (Belpaire et al., 1999). Many persistent organic micropollutants have an extremely low solubility in water and are consequently not easy to measure in water. Hence, measurements of bioaccumulation in the eel for pollution monitoring has been initiated in several countries (e.g. Desjardins et al., 1983; Castonguay et al., 1989; de Boer and Brinkman, 1994; de Boer and Hagel, 1994; Hodson et al., 1994; Knights, 1997). Also in Flanders a monitoring network has been developed and implemented (Belpaire et al., 1999; Goemans et al., 2003). However, attempts to use eels for measuring the biological effects of pollution are scarce. Considering the high pollution pressure on eels in some locations, one might expect endocrine effects, like VTG induction, in these eel populations. Contaminants like PCB's, organochlorine pesticides and heavy metals can be found in considerable concentrations in eels living in Flemish waters (Goemans et al., 2003). Although a number of these compounds interact with the endocrine system, specific endocrine disrupters (e.g. synthetic or natural hormones, bisphenol A, alkylphenols. . .) can be less persistent, bioaccumulative and lipophilic than those aromatic hydrocarbons (Yamamoto et al., 2003).

A. anguilla is an undifferentiated gonochoristic fish, i.e. its gonad development occurs through an ambisexual stage in which both male and female germ cells are present (Grandi et al., 2000). In natural circumstances, European eel only starts reproducing after 6–12 years of juvenile growth in continental waters. Investigations in natural European waters show that the 'continental' age ranges from 8 to 12 years in females and from 6 to 9 years in males (Tesch, 1977). At the start of their reproductive migration towards the Sargasso Sea (central north Atlantic Ocean), European eels are still immature. The lack of sexual maturation (vitellogenesis in females) is due to a deficiency in the production of pituitary gonadotropin. The circulating gonadal steroid levels are low and plasma VTG concentrations range from undetectable to 10 mg/ml (Peyon et al., 1997, Luizi et al., 1997). To date, adult mature European eels have never been caught, so gonadal steroid and VTG levels during natural reproduction are still unknown (Peyon et al., 1997). VTG induction in fish has been widely used to detect exposure to xenoestrogenic compounds. Under natural conditions, VTG is only produced by mature female fish as a yolk precursor. When male or juvenile fish are exposed to (xeno-)estrogens, they can also produce this protein (Copeland et al., 1986; Allner et al., 1999; Tyler et al., 1998). Therefore, VTG induction is considered to be a good biomarker of exposure to compounds with estrogenic properties. It has been demonstrated that treatment with 17b-estradiol (E2) can also induce VTG synthesis in *Anguilla* sp. (Peters et al., 2001).

To investigate the use of European eel as a sentinel species to monitor pollution in surface waters, and to determine the extent of possible endocrine disruption in Flemish surface waters, 650 eels from various localities with varying bioaccumulation profiles were collected in the field and the plasma VTG concentration was determined in 142 of the specimens. In this paper we discuss the results of length, weight and VTG measurements in these 142 eels. Further, the results of a laboratory exposure to waterborne EE2 to validate the VTG assays are discussed.

2. Material and methods

2.1. Chemicals

All chemicals were purchased from Sigma-Aldrich (Belgium), except where indicated differently.

The synthetic estrogen 17a-ethinylestradiol (98% pure, 17a-ethinyl-1,3,5[10]-estratriene-3,17b-diol 3-cyclopentyl ether) was dissolved in ethanol (96%, Merck-Eurolab, Belgium).

2.2. Eel exposure

Eels for the laboratory experiments were purchased from a local aquaculturist (Borremans, Belgium) and acclimated in aerated 200-l tanks with carbon filtered tap water (pH 7.8, hardness 350 mg $CaCO_3$/l, temperature 18.0 ± 1.5 °C, 14/10 light/dark cycle) for 10 days. The same water was used in the experiments. Eight eels were exposed in a 200-l aquarium to 10 mg EE2/l for 9 days. EE2 was dissolved in EtOH and the final EtOH concentration was 0.01%. As a control, eight fish were kept in a 200-l aquarium in 0.01% EtOH. The fish had a length of 50 ± 8 cm and a weight of 178 ± 47 g. After exposure, the fish were anaesthetized with 2-phenoxyethanol and blood was sampled with heparine-rinsed syringes. After addition of 25 ml aprotinin/ml blood, the blood was centrifuged (3500g, 4 °C, 10 min), plasma was shock-frozen in liquid nitrogen and stored at -80 °C. The condition factor (Cf) was calculated as total weight (g)/total length^3 (cm).

2.3. Sampling

In total, 650 eels with varying bioaccumulation profiles were sampled at 160 different locations. Blood of 142 eels (from 20 locations) was sampled for VTG analysis (Fig. 1, Table 1). The eels were captured by electrofishing or fike-fishing between May and November 2000. The selected eels were kept alive in freshwater tanks until processing (0–6 days after capture). Length

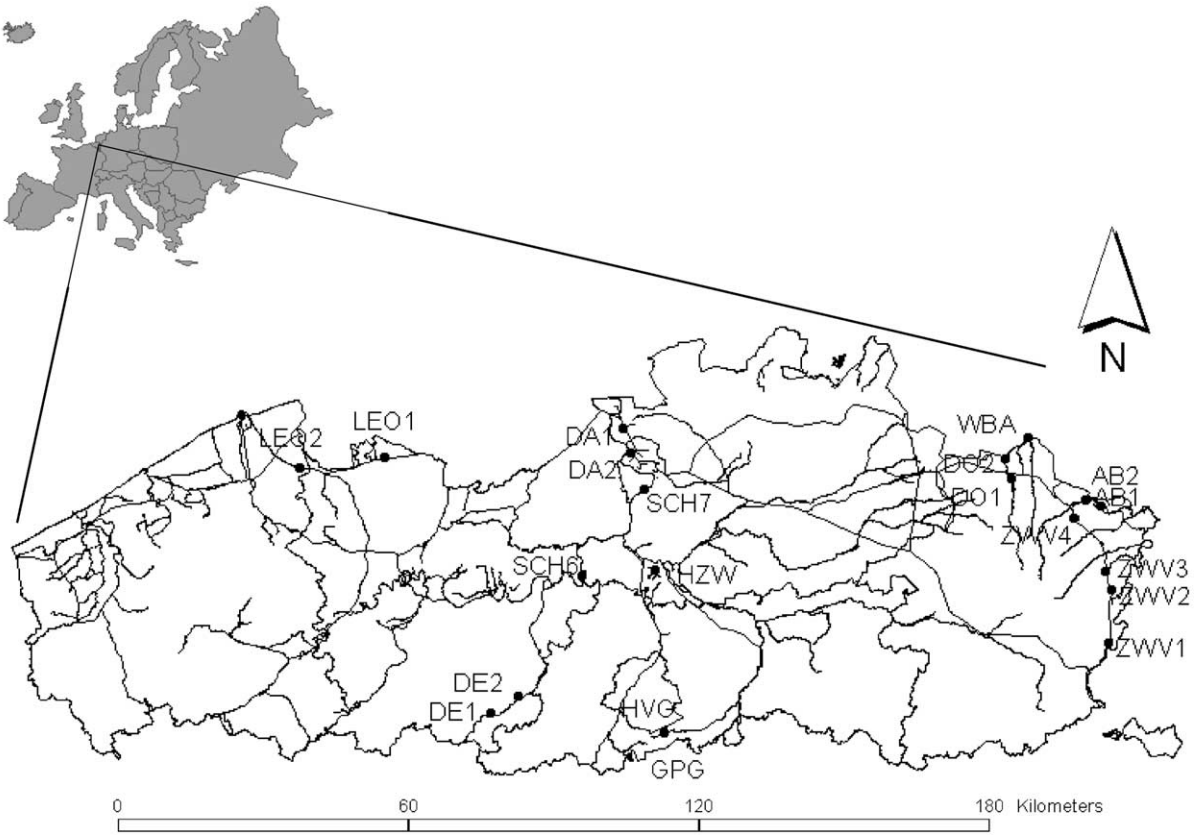

Fig. 1. Location of the different sampling sites (the inset top left is Europe).

Table 1
Summary of sampling sites and date of collection, mean length (± standard deviation) of the eels and organochlorine and PCB load

Code	Location	Water type	Date of collection	Organochlorines class[a]	PCBs class[b]	Mean length (cm)
AB1	Abeek, Bocholt	River	24/05/2000	2	3	45±5
AB2	Abeek, Kinrooi	River	24/05/2000	2	3	40±4
DA1	Docks of Antwerp	Canal	03/10/2000	2	4	48±9
DA2	Docks of Antwerp	Canal	03/10/2000	2	4	51±10
DE1	Dender, Geraardsbergen	River	13/10/2000	3	3	32±6
DE2	Dender, Ninove	River	16/10/2000	3	3	56±11
DO1	Dommel, Overpelt	River	23/05/2000	2	2	41±8
DO2	Dommel, Neerpelt	River	23/05/2000	2	2	45±8
GPG	Lake Ganzepoot, Hoeilaart	Closed water body	05/05/2000	1	2	48±6
HVG	Fishing pond, Hoeilaart	Closed water body	05/05/2000	1	2	47±5
HZW	Lake Hazewinkel, Willebroek	Closed water body	10/05/2000	2	2	36±6
LEO1	Canal Leopold, Sint Laureins	Canal	03/10/2000	2	1	41±4
LEO2	Canal Leopold, Damme	Canal	03/10/2000	2	1	33±4
SCH6	Scheldt, Hamme	River	10/10/2000	3	3	52±19
SCH7	Scheldt, Antwerp	River	10/10/2000	2	4	38±4
WBA	Warmbeek, Hamont-Achel	River	23/05/2000	2	3	39±3
ZWV1	Zuid-Willemsvaart, Maasmechelen	Canal	09/05/2000	2	4	36±3
ZWV2	Zuid-Willemsvaart, Dilsen-Stokkem	Canal	09/05/2000	2	4	42±9
ZWV3	Zuid-Willemsvaart, Dilsen-Stokkem	Canal	09/05/2000	2	4	39±5
ZWV4	Zuid-Willemsvaart, Bree	Canal	09/05/2000	2	4	42±5

[a] 1: <375 ng/g, 2: 375–950 ng/g, 3: 950–2400 ng/g, and 4: >2400 ng/g lipid weight (sum of the concentrations of hexachlorobenzene, endrin, dieldrin, a-hexachlorohexane, g-hexachlorohexane, DDT and its metabolites).

[b] 1: <475 ng/g, 2: 475–1192.5 ng/g, 3: 1192.5–2995.5 ng/g, and 4: >2995.5 ng/g lipid weight (sum of the concentrations of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180).

and weight were determined and blood samples of the fish were taken and treated as in the exposure experiment. Plasma VTG was determined with protein electrophoresis. Plasma alkali labile phosphate (ALP) and Ca measurements were performed on a selected number of fish (n=20).

2.4. Protein measurement, blood protein electrophoresis and Western blotting

Denaturing sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS–PAGE) was performed as described in Versonnen et al. (2003). In short, the protein concentration of the samples was determined according to Bradford (1976) and plasma samples corresponding to 10 mg of protein were loaded on the gel. Denaturing protein electrophoresis was performed with Bio Rad Protean II xi Cell electrophoresis equipment (Bio Rad, Belgium). Protein electrophoresis was performed according to Laemmli (1970). Per sample two replicates were loaded. Gels were run with a constant voltage of 200 V and stained with Coommassie blue dye which was obtained by dissolving 1.2 g of Coommassie Brilliant Blue R-250 (ICN Biomedicals, Belgium) in 500 ml of methanol and adding 200 ml of acetic acid (Merck-Eurolab, Belgium). Destaining was performed with a 40% methanol 10% acetic acid solution in water.

For the Western blotting, plasma samples were separated by SDS–PAGE. A prestained broad range molecular weight standard was used (Bio-Rad, Belgium). The proteins were subsequently transferred onto nitrocellulose membranes (Bio-Rad, Belgium) with a Mini Trans-Blot Electrophoretic Transfer Cell (1 h at 100 V). The primary antibody (ND-3G2 monoclonal mouse anti-striped bass VTG) was purchased from Biosense (Norway). The blotted membranes were shaken in 5% low-fat powdered milk phosphate buffered saline (PBS) for 1 h, prior to incubating them with the primary antibody for 1 h, under continuous agitation and at room temperature. The primary antibody was diluted 1/3000 in 5% low-fat powdered milk PBS. After incubation, the membranes were washed twice in PBST (1 ml Tween/l PBS) and once in PBS. The secondary antibody (anti-mouse IgG alkaline phosphatase conjugated) was diluted 1/1000 in low-fat powdered milk PBS and membranes were incubated under continuous agitation and at room temperature for 1 h, after which they were washed twice with PBST and once with PBS. Coloration was performed with Sigma fast BCIP/NBT tablets for 20 min.

Protein gels and Western blot gels were scanned with a GelDoc 2000 system and analysed with Quantity One[1] software (Bio-Rad, Belgium). The relative VTG content was calculated as the percentage of protein(s) with a weight of 214 kDa, relative to the total protein content. The results of each sample are the mean of at least two determinations.

2.5. Plasma calcium and ALP determination

Due to the high calcium and ALP concentrations present in the VTG protein, plasma Ca and ALP concentrations can be used as indirect measures of the plasma VTG concentration (Verslycke et al., 2002). Therefore, the plasma Ca and ALP concentrations were determined in the eels from the laboratory experiments and in 20 eels from the field sampling, as described in detail elsewhere (Verslycke et al., 2002). Briefly, plasma Ca was measured by atomic absorption spectrophotometry (SpectraAA-100, Varian) in 1/10 dilutions in 1% HNO_3. Plasma ALP concentrations were determined through a colorimetric measurement of acidified phosphomolibdate complexes using a commercially available kit (Sigma-Aldrich, Belgium). The Ca and ALP concentration in each plasma sample were measured at least twice and the results are the mean of these measurements.

2.6. Statistical analysis

Statistical analysis was performed with Statistica[1] software (Statsoft Inc., USA): all data were tested for homogeneity and normality with Levene's test and Kolmogorov–Smirnov's test, respectively. If these assumptions were met, one way analysis of variance (ANOVA) followed by Dunnett's test was performed. Mann–Whitney U tests were performed when the homogeneity and normality assumptions were not met. The differences described were statistically significant at $P < 0.05$. Non-parametric Spearman tests were used in the correlation analyses (R^2 and P-level).

3. Results and discussion

3.1. Laboratory exposure

All fish survived the experiment. No significant differences in length, weight and condition factor were detected between the control and EE2-exposed fish (Table 2). The plasma protein content of exposed fish was significantly different from that of the controls, and this is most probably due to the production of VTG in exposed eels. This is similar to findings with other fish species (e.g. rainbow trout) where exposure to environmentally relevant EE2 concentrations resulted in elevated plasma protein concentrations (e.g. Verslycke et al., 2002; Bon et al., 1997). Plasma protein concentrations in control fish (55 ± 10 mg/ml) correspond well with the concentrations obtained by Luizi et al. (1997), who measured 50–60 mg/ml in the plasma of untreated eels. An average concentration of 128 ± 9 mg Ca/ml was measured in plasma of control fish. These Ca concentrations are similar to those reported by Suzuki et al.

(1999), who measured a plasma Ca concentration of 105 ± 5 mg/ml in Japanese eel (*Anguilla japonica*). Plasma ALP concentrations in the controls (37 ± 4 mg/ml) were similar to those found in mud eel (*Amphipnous cuchia*, Srivastav et al., 1998), rainbow trout (*Oncorhynchus mykiss*, Verslycke et al., 2002) and in crucian carp (*Carassius carassius*, Tinsley, 1985) but up to 25 times higher than those measured in European eel by Luizi et al. (1997). It is not clear what caused this discrepancy.

A differentially induced protein with a molecular weight of 214 kDa was detected in the EE2-exposed fish. Only background concentrations (less than 2%) of proteins with this molecular weight were measured in unexposed eels (Table 2, Fig. 2a). Monoclonal mouse anti-striped bass (*Morone saxatilis*) VTG antibodies cross-reacted with this protein in the exposed fish, but not in unexposed fish during western blotting of the plasma samples (Fig. 2b). Peters et al. (2001) and Livingstone et al. (2000) detected a plasma protein of 211 kDa with protein electrophoresis in E2-injected European eel, which also cross-reacted with antibodies against VTG of striped bass. Komatsu and Hayashi (1998) detected VTG in Japanese eel (*A. japonica*) as a protein with a molecular weight of 196 kDa. Burzawa-Gerard and Dumas-Vidal (1991) and Hara et al. (1980), however, reported molecular weights of 340 and 350 kDa, respectively. We assume for several reasons that the detected 214 kDa protein is VTG: (1) the protein has a similar weight as the VTGs observed in recent studies with *A. anguilla* (Peters et al., 2001) and *A. japonica* (Komatsu and Hayashi, 1998); (2) the protein cross-reacted with antibodies against VTG of striped bass; (3) the 214 kDa protein is induced by EE2 and only very

Table 2
Weight, length, condition factor (Cf), plasma protein content and plasma vitellogenin content (VTG, expressed as mg ALP/ml, mg Ca/ml or % of total protein content determined with protein electrophoresis) of eel exposed to 10 mg ethinylestradiol/l (EE2) compared with controls (n=8, values in parentheses are standard deviations on the mean)

Treatment	Weight (g)	Length (cm)	Cf (g/cm^3)	Plasma protein (mg/ml)	Plasma VTG content		
					ALP (mg/ml)	Ca (mg/ml)	%
Solvent control	157 (31)	49 (4)	0.0013 (0.0001)	55 (10)	37 (4)	128 (9)	1.6 (1.9)
EE2	161 (36)	50 (4)	0.0013 (0.0001)	*75*[a] (*19*)	*762*[a] (*145*)	*636*[a] (*183*)	*28.3*[a] (*10.6*)

[a] Significantly different from the control, $P < 0.05$.

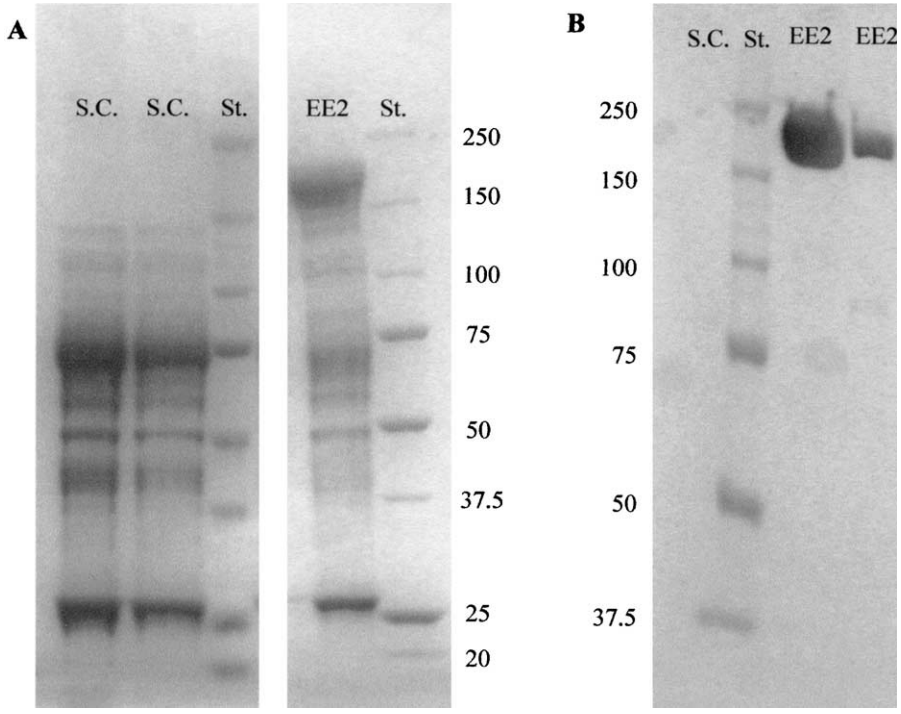

Fig. 2. (A) A typical gel after protein electrophoresis of plasma of ethinylestradiol-exposed eel (EE2) and solvent control eel (S.C.) and (B) a typical Western blot gel. Values indicate the molecular weight of the protein standard (St.), given in kilodaltons.

low to undetectable levels circulate in sexually immature control fish. A drawback of using protein electrophoresis is that proteins with similar molecular weights as VTG, might erroneously be quantified as VTG. This is confirmed by the Western blotting of the untreated eels, showing no reaction products in the controls, while the VTG content measured with protein electrophoresis was 1.6%.

Significantly elevated VTG-concentrations (as determined by Ca, ALP and relative plasma VTG content) were detected in EE2-exposed fish (Table 2). Although the eels were exposed to a high concentration of EE2 (10 mg/l) for 9 days, the relative VTG content was relatively low (28.3 ± 10.6%, measured with protein electrophoresis). Studies with other fish species showed that VTG concentrations in EE2- or E2-exposed fish can account for 40% or more of the total plasma proteins (Allner et al., 1999; Versonnen et al., 2003). According to Allner et al. (1999), a 10 day exposure of *Leuciscus idus* to 50 ng EE2/l led to an increase in plasma VTG content (measured with protein electrophoresis) from 0.07 to 32% of the total protein content. Exposure of rainbow trout to 4 ng EE2/l for 7 days increased VTG levels from 0.15 to 10%. Previous studies at our laboratory (Versonnen et al., 2003) showed that relative VTG levels (measured with protein electrophoresis) increased to up to more than 40% in adult zebrafish exposed to 10, 50 or 100 ng EE2/l. De Vlaming et al. (1980) even measured concentrations of up to 80% in estradiol-exposed goldfish (*Carassius auratus*). These data may suggest that immature eels are relatively insensitive for the effects of xenoestrogens on vitellogenesis, compared with other species: eels exposed to 10 mg EE2/l in the present research had a relative blood VTG concentration of 28%. Although nominal concentrations (10 mg EE2/l) were used in this research, one can assume that the actual concentration in the exposure tank will be far exceeding 4–100 ng EE2/l (used by Allner et al., 1999; Versonnen et al., 2003). The fact that eel might be relatively insensitive to waterborne endocrine disrupters is also confirmed by Burzawa-Gerard and Dumas-Vidal (1991) and Luizi et al. (1997) who found that high doses of (injected) E2 (at least 5 x 0.5 mg/kg during 12 days) were needed to induce VTG production in immature eels. Peters et al. (2001) and Livingstone et al. (2000) induced VTG in eel by intraperitoneal injection with high doses of E2 (4x10 mg/kg during 4 weeks and 2x5 mg/kg during 6 days, respectively). Plasma VTG levels were 260 000–750 000 times higher in exposed fish (up to 50 mg VTG/ml), compared to the controls after 4 weeks of exposure (Peters et al., 2001).

When comparing the different techniques for measuring VTG used in our study, we found that all techniques were capable of detecting enhanced VTG concentrations, but the highest induction factor (exposed/control fish) was obtained with the ALP assay. EE2-exposed fish had a 21 times higher ALP concentration than unexposed fish. Protein electrophoresis and Ca measurements showed an induction factor of 17.5 and 4.5, respectively. However, the coefficient of variation of 4 consecutive measurements of the same plasma samples (six samples were measured four times) is less than 6% for protein electrophoresis and Ca measurement, and 22% for ALP. A more thorough comparison of these techniques can be found in Verslycke et al. (2002). Overall, the data show that the three techniques are suited for measuring VTG.

3.2. Field samples

A map of the sampling sites is shown in Fig. 1. The length of the eels at the different sampling points is given in Table 1. Length and weight are strongly correlated (R^2=0.92, data not shown) and a similar pattern is found for the weight at the different sampling points (data not shown). All fish had a length between 25 and 68 cm and a weight between 21 and 618 g.

The pollutant load, measured in fish tissue is discussed in detail elsewhere (Goemans et al., 2003). Internal PCB and organochlorine pesticide concentrations are represented in classes in Table 1. For the PCBs, the total indicator PCB concentration (sum of concentrations of PCB 28, PCB 52, PCB 101, PCB 118, PCB 138, PCB 153, PCB 180 on lipid basis) is given, divided in four classes. For the organochlorine pesticides, the sum of the concentrations of hexachlorobenzene, endrin, dieldrin, a-hexachlorohexane, g-hexachlorohexane, DDT and its metabolites are given, classified in four classes (Goemans et al., 2003). The total indicator PCB concentrations ranged from 274 ± 176 ng/g in station LEO1 to 14400 ± 9700 ng/g in station ZWV3, the sum of organochlorine pesticides ranged from 286 ± 114 ng/g in station HVG to 2370 ± 440 ng/g in station DE1. These data indicate that a wide range of pollutants are present at sometimes extremely high concentrations in eel tissue (Goemans et al., 2003). Nevertheless, none of the eels collected in the field had increased plasma VTG levels, measured with all three methods: protein electrophoresis (Fig. 3), and for 20 eels ALP or Ca (data not shown). The overall mean VTG concentration in the samples was 0.9 ± 0.5%. The highest relative plasma VTG concentration was 2.45%. No correlations were found between VTG content and weight, length, Cf, fat content, contaminants (PCBs, organochlorine pesticides, metals) or date of sampling. Our results are in agreement with findings of Livingstone et al. (2000) and Peters et al. (2001). These authors did not detect any differences in plasma VTG content of eel sampled at different locations in the UK during different seasons. Only limited data exist on the endocrine status of wild maturing eels, because to date, no adult sexually mature eels have been caught (Peyon et al., 1997, Lokman et al.,

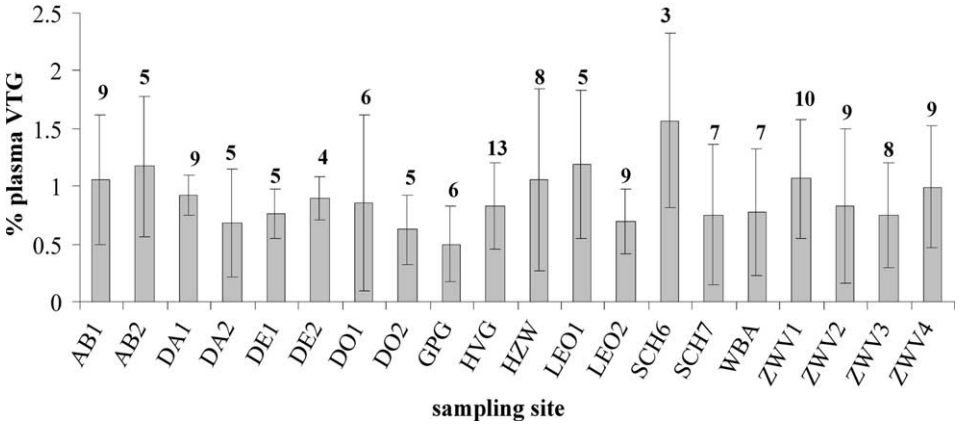

Fig. 3. Relative plasma vitellogenin (VTG) content of the eels sampled at different locations in Flanders, represented as mean±standard deviation. The number of fish is given for each sampling site.

1998). However, since eel is a gonochoristic undifferentiated fish, one might expect that it can be influenced by xenoestrogens (Peters et al., 2001). Although high internal levels of pollutants were measured, no evidence for effects of estrogenic compounds was detected in eels caught in Flemish surface waters. Therefore, the present research provides a number of hints that continental European eel is rather insensitive to the effects of xenoestrogens. This is confirmed by our laboratory results and the studies of others mentioned above. Internal concentrations of a number of potent endocrine disrupters like natural and synthetic hormones were, however, not measured in the present research. Moreover, it must be emphasized that European eel only comes to full sexual maturation when spawning in the Sargasso Sea. Furthermore, eel stops feeding at the start of the migration (Tesch, 1997). It is therefore possible that effects of pollutants (e.g. endocrine disrupters) become apparent during the starvation period while migrating or during the spawning itself. Sexually mature eels, however, have not been caught to date.

Few reports are available on endocrine disruption in Belgian surface waters. Witters et al. (2001) measured the estrogenic activity in 16 Flemish rivers, effluents of municipal wastewater treatment plants and reservoirs for drinking water with a yeast estrogenic screen. The highest estrogenic potency (up to 81 ng/l E2-equivalents) was detected in rivers. It was suggested that the potencies detected in these rivers could adversely influence resident fish populations. Further, studies on field collected roach (*Rutilus rutilus*), tench (*Tinca tinca*) and rudd (*Scardinius erythrophtalmus*) performed by our laboratory have revealed that intersex and elevated plasma VTG concentrations occur in fish sampled in highly polluted areas in Flanders (Versonnen et al., in preparation, Van Campenhout et al., 2002).

The determination of VTG in plasma of sexually immature eels caught in surface waters did not confirm these findings, although very high concentrations of (possible) endocrine disrupters were sometimes present in their tissues. This high pollutant load in eel indicates that a wide range of pollutants and possible endocrine disrupters occur in Flemish surface waters and can be accumulated in fish.

4. Conclusions

Measuring internal concentrations of pollutants in eel has proven to be very useful as a monitoring tool for the quality of surface waters as it gives additional information which can be used in an ecological risk assessment. Recently, the very high pollutant loads detected in European eels of Flanders have lead to a catch and release obligation for anglers.

The present study and the results of previous studies do not prove that—despite the high exposure to and uptake of pollutants—European yellow eel under natural conditions are sensitive to the effects of (xeno-)estrogens, as measured by the VTG induction. Although European eel is a useful species for measuring pollutants, we did not find any indications for estrogenic effects to occur in natural freshwater eel populations in Flanders.

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