Environmental Pollution 158 (2010) 3279–3284

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Plasma vitellogenin in male teleost fish from 43 rivers worldwide is correlated with upstream human population size

Jean-Pierre W. Desforges [a], Brendan D.L. Peachey [a], Pauline M. Sanderson [a], Paul A. White [b], Jules M. Blais [a,*]

[a] *Department of Biology, University of Ottawa, 30 Marie Curie Street, K1N 6N5, Ottawa, Ontario, Canada*
[b] *Environmental Health Sciences and Research Bureau, Health Canada, Ottawa, Ontario, Canada*

Concentrations of vitellogenin in riverine teleost fish were related to population size.

A R T I C L E I N F O

Article history:
Received 1 March 2010
Received in revised form
9 July 2010
Accepted 16 July 2010

Keywords:
Vitellogenin
Wastewater treatment plant
Fish species
EDCs
Endocrine disrupting chemicals
Fish

A B S T R A C T

It has been previously demonstrated that vitellogenin (VTG) – a precursor egg yolk protein – is produced in male fish exposed to estrogenic compounds in wastewater treatment plant (WWTP) effluent. However, little attention has been given to examine whether any patterns of male VTG production exists across fish species on a global scale. We hypothesized that a composite measure of human population size over river discharge would best explain variations of protein levels in male fish. We compiled VTG data in 13 fish species from 43 rivers receiving municipal WWTP effluent on 3 continents. We found that human population size explained 28% of the variation in male VTG concentrations, whereas population/flow rate failed to significantly correlate with VTG. We suggest this result may be explained by the low solubility of estrogenic compounds, resulting in localized contamination near WWTP outfalls, rather than dilution by river water.

1. Introduction

Hormone-mimicking xenobiotic chemicals released into natural ecosystems are a potential risk for human and ecosystem health, and have sparked global scientific interest for several decades (Folmar et al., 1996). These chemicals come in a wide variety of forms, and have the capacity to interact with hormone receptors within cells at environmentally relevant concentrations (Hayes et al., 2003). Several researchers have discovered measurable concentrations of numerous EDCs in surface waters, sediments, groundwater, and drinking water around the world (Campbell et al., 2006).

Estrogenic EDCs represent a sub-class of compounds that can be divided into two groups: natural hormonal estrogens, and synthetic chemicals that have the ability to mimic or induce estrogen-like responses in exposed organisms (Campbell et al., 2006). The predominant origin of these compounds in aquatic environments has been identified as Wastewater Treatment Plant (WWTP) effluent; however, agricultural run-off and industrial wastewater contribute significantly as well (Atkinson, 2008). Natural steroidal estrogens of significance in WWTP effluents include Estrone (E1), 17β-Estradiol (E2), and Estriol (E3). Synthetic estrogens, or xenoestrogens, derive from agriculture, industrial plastics, and pharmaceuticals. Notable chemicals in this category include: ethynylestradiol (EE2), a synthetic steroidal estrogen found in oral contraceptive metabolites, alkylphenolic compounds, which originate through the degradation of non-ionic detergents, bisphenol A (BPA), a monomer used primarily in the manufacturing of plastics and resins, in addition to a variety of pesticides (Atkinson, 2008; Folmar et al., 2001). All of these compounds have varying degrees of estrogenic potency, depending on their affinities for estrogen receptors (de Voogt and van Hattum, 2003). Estrogen Equivalent Factors (EEF) are used to express the estrogenicity of compounds in wastewater effluent relative to natural estradiol. E1, E2, and EE2 have been identified as the principal sources of estrogenicity in wastewater effluent, with EEF values of 0.5, 1, and >1.5 respectively (Campbell et al., 2006; Desbrow et al., 1998).

Several biomarkers can be used to assess estrogen exposure and estrogenic effects in fish; one of these is male production of vitellogenin (VTG), an egg yolk precursor protein, which is naturally synthesized in the liver of oviparous vertebrates. In normally functioning female fish, endogenous estrogens, such as E2, regulate the expression of genes that control protein synthesis. Thus, E2 is

* Corresponding author.
E-mail address: jules.blais@uottawa.ca (J.M. Blais).

0269-7491/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.envpol.2010.07.017

secreted into the bloodstream, and via the estrogen-receptor pathway activates the production of VTG (Burki et al., 2006). Under normal circumstances, estrogen levels in male organisms are insufficient to initiate VTG production; however, production of the protein can be induced by exposure to environmental estrogenic compounds (Bjorkblom et al., 2008). As such, blood plasma VTG concentration in males can provide a convenient indicator of environmental estrogens. Moreover, VTG synthesis occurs in a dose dependent fashion, which implies that its relative concentration in males provides an indication of the amount of estrogen to which they were exposed (Purdom et al., 1994; Legler et al., 2002; Thorpe et al., 2008). Indeed, many studies have reported elevated VTG concentrations in male fish from rivers across the United States and Europe (Purdom et al., 1994; Folmar et al., 1996, 2001; Jobling et al., 1996; Vermeirssen et al., 2005). For example, Jobling et al. (2006) examined the occurrence of vitellogenin and intersex frequency in male *Roach* at 45 sites in 39 rivers across the United Kingdom as a function of steroid estrogen presence in effluent. The present study builds on these investigations to determine whether VTG production in male fish inhabiting a wide range of rivers throughout the world is empirically linked to anthropogenic wastewater loading.

The primary objective of the study was to determine whether global patterns of VTG production in male fish are quantitatively related to anthropogenic loading. More specifically, this study, which is a meta-analysis of data for 13 fish species from 43 rivers on 3 continents, examined the relationship between the magnitude of the populations served by wastewater treatment facilities and serum VTG concentrations in male teleost fish inhabiting downstream regions. We hypothesized that there is a positive correlation between the size of the population served by WWTPs and the serum VTG level in male fish collected downstream from the effluent discharge sites. In addition, we use the ratio of WWTP population size to river discharge (Q) to provide a discharge-corrected effluent load value for rivers. This correction to effluent load should account for dilution, and therefore, variations in EDC response (Jobling et al., 1998).

2. Materials and methods

We conducted a topical search of peer reviewed articles using on-line electronic databases (e.g., SCOPUS, Web of Science, JSTOR, PubMed). Keywords were selected to target publications containing quantitative information on serum VTG levels in wild or caged teleost fishes. In order for a publication to be included in our meta-analysis, it was necessary for it to include the following information: river name, location and discharge rate (e.g., Q in m^3/sec), location of sampling and/or name and size of the population served by the upstream WWTP. In some cases it was possible to acquire the requisite information (e.g., WWTP details) using additional on-line resources (Municipal websites, Google maps, etc.).

Although a river's EDC content may derive from multiple sources, we limited the analysis to WWTPs receiving predominantly domestic wastes. In a few instances the precise size of the population served by a WWTP was not available, and population equivalence (PE) was used to estimate the size of the population served. PE assumes that each individual 'person equivalent' contributes 60 g to the five-day BOD (biochemical oxygen demand) of a municipal wastewater. Thus, BOD values can be employed to provide PE, an estimate of the true population size (Keller et al., 2006). River discharge rates were gathered directly from the studies, or, as with other parameters, average annual discharge values were acquired from on-line resources or other published articles.

Prior to analysis, all values were $\log_{10}$ transformed to equalize the variance across the range of observations, and meet the assumptions of ordinary least squares linear regression and analysis of variance. Least squares linear regressions to examine the relationships between anthropogenic loading and serum VTG concentrations were performed using Microsoft Excel 2007. Regression and correlation coefficients, as well as analysis of variance results, were employed to examine the magnitude, direction and statistical significance of the relationships. As this study included results from several fish species, analysis of covariance was used to examine the effect of species and population, as well as the population–species interaction, on VTG concentration. ANCOVA was performed using SAS 9.2 (SAS Institute Inc., NC, USA). The residual error term for all analyses was assumed to be independent and normally distributed with mean zero and variance of σ^2. This was verified by visual examination of a normal probability plot.

3. Results

The meta-analysis was conducted on data from 23 publications, and included 56 observations of VTG concentration in 13 fish species from 43 rivers on 3 continents. The dataset includes values from 13 species encompassing 6 separate families, with the Percidae, Salmonidae and Cyprinidae being most frequently observed (Table 1). Due to data limitations, the analysis was not restricted to any specific fish taxa. The dataset includes values from rivers in North America, Europe, and Asia. Table 1 provides a summary of the collected data, including river name and location, average VTG serum concentration in μg/mL, and the size of the population served by the upstream WWTP. Average VTG concentrations in male fish ranged between 0.1 μg/ml in brown trout from small streams in Denmark to 52,000 μg/ml in rainbow trout from the river Aire in the United Kingdom (Table 1). Similarly, the size of the populations served by the WWTPs ranged from 500 to 1,800,000 people. River and effluent discharge rates varied by several orders of magnitude.

Of the 56 observations of VTG concentrations, values for WWTP effluent discharge were only available for 20 sites. Initial analyses showed that VTG concentrations are significantly correlated with WWTP effluent discharge rate (Fig. 1, F ratio $= 7.8$, $p = 0.012$, $r^2 = 0.30$). Additional analysis showed a stronger relationship between serum VTG concentration and the size of the population served by the upstream WWTP. The results obtained, which are illustrated in Fig. 2, showed a statistically significant positive relationship that explains 28% of the variation in VTG concentration ($n = 56$, $r^2 = 0.28$, F ratio $= 21.5$, $p < 0.0001$).

Using 19 observations where data were available, it was found that serum VTG concentration did not correlate significantly with water estrogen concentrations, measured as estrogen equivalents (EEQ) ($n = 19$, $r^2 = 0.11$, F ratio $= 2.11$, $p = 0.16$). Similarly, water EEQ was not correlated with river discharge rates ($n = 18$, $r^2 = 0.14$, F ratio $= 2.69$, $p = 0.12$). As the distance from the WWTP as well as the ratio of effluent discharge to river discharge is presumed to influence estrogenic activity in streams, analyses were done to examine these variables on VTG concentrations. Firstly, no general trend was observed between fish sampling distance from WWTPs and VTG concentration ($n = 35$, $r^2 = 0.038$, F ratio $= 1.32$, $p = 0.26$), presumably because most sites were under 5 km from the effluent discharge location. Likewise, VTG concentrations were not significantly correlated to effluent loading, measured as the ratio of effluent flow rate to river flow rate ($n = 20$, $r^2 = 0.16$, F ratio $= 3.6$, $p = 0.074$).

Since the magnitude of the downstream exposure to EDCs released from WWTPs may depend on the amount of discharge dilution, subsequent analyses investigated the relationship between serum VTG concentration and the ratio of population served to river discharge in m^3/s (i.e., discharge-corrected population). The ratio of WWTP population size and river discharge should provide a superior measure of wastewater effluent concentration and downstream exposure to substances released in municipal wastewaters. Jobling et al. (1998) proposed a similar measure to account for effluent dilution in river water, and as such, allow for better comparisons between sites. Interestingly, our analysis revealed no significant relationship between the discharge-corrected measure of population and serum VTG in male fish ($n = 56$, $r^2 = 0.055$, F Ratio $= 3.16$, $p = 0.08$).

Analysis of covariance was employed to simultaneously examine the effect of species and WWTP population on VTG concentration. Although the collected data include values for 13

Table 1
Sources of data included in the meta-analysis examining the effect of anthropogenic loading on serum VTG in male teleost fish collected downstream from WWTPs.

Source	Country	River	Fish Species	Population served by WWTP ($\times 10^5$)	River flow rate (m^3s^{-1})	Sampling distance from WWTP (km)	Mean VTG (μg/ml)	# of Fish examined
Aerni et al., 2007	Switzerland	Glatt	*Oncorhynchus mykiss*	0.88	0.52	<1	130.0	28
	Switzerland	Rontal	*Oncorhynchus mykiss*	0.27	0.09	<1	10.0	11
	Switzerland	Sureatal	*Oncorhynchus mykiss*	0.38	0.17	<1	500.0	14
	France	Confidential	*Oncorhynchus mykiss*	0.30	0.08	<1	500.0	29
	France	Confidential	*Oncorhynchus mykiss*	0.29	0.07	<1	500.0	29
Allen et al., 1999	England	Mersey	*Platichthys flesus*	13.65	66	3.5	1000.0	65
Bjerregaard et al., 2006	Denmark	Giber Brook	*Salmo trutta*	0.053	0.65	NA	0.4	58
	Denmark	Knubbro Brook	*Salmo trutta*	0.033	0.08	NA	0.4	82
	Denmark	Voel Brook	*Salmo trutta*	0.01	0.06	NA	4.0	61
	Denmark	Hoed Brook	*Salmo trutta*	0.006	0.14	NA	0.1	52
	Denmark	Orum Brook	*Salmo trutta*	0.011	0.31	NA	0.1	45
	Denmark	Usserod Brook	*Salmo trutta*	0.30	0.78	NA	1.5	38
	Denmark	Badrekaer Ditch	*Salmo trutta*	0.008	0.10	NA	0.2	27
	Denmark	Hojen Brook	*Salmo trutta*	0.005	0.17	NA	0.5	17
Burki et al., 2006	Switzerland	Luetzelmurg	*Salmo trutta*	0.12	0.70	1	0.2	41[a]
Folmar et al., 1996	USA	Mississippi River side channel	*Cyprinus carpio*	18.00	129.41	<1	1113.0	10
Folmar et al., 2001	USA	Mississippi River side channel	*Stizostedion vitreum*	18.00	229.14	5–32	790.0	10
Giesy et al., 2003	USA	Battle Creek	*Carassius auratus*	0.082	17.94	NA	0.1	10[a]
	USA	Grand	*Carassius auratus*	0.053	11.98	NA	0.1	10[a]
	USA	Shiawassee	*Carassius auratus*	0.15	9.71	NA	0.2	10[a]
	USA	Red Cedar	*Carassius auratus*	0.034	4.67	NA	1.7	10[a]
Harries et al., 1997	England	GreatRiverStour	*Oncorhynchus mykiss*	0.89	2.21	0.1	10.0	20[a]
	England	Arun	*Oncorhynchus mykiss*	0.70	2.10	13	11.0	20[a]
	England	Chelmer	*Oncorhynchus mykiss*	0.12	0.85	1	8.0	20[a]
	England	Stour	*Oncorhynchus mykiss*	0.012	0.14	8	9.0	20[a]
	England	Aire	*Oncorhynchus mykiss*	1.12	35.80	2	52000.0	20[a]
Hecker et al., 2002	Germany	Elbe	*Abramis brama*	20.00	788	NA	1.0	26
	Germany	Elbe	*Abramis brama*	8.25	788	NA	12.0	15
Hemming et al., 2001	USA	Lewisville	*Pimephales promelas*	1.10	4.20	1	25.4	20[a]
Hinck et al., 2006	USA	Columbia	*Micropterus salmoides*	0.55	7790	NA	700	10
Hinck et al., 2007	USA	Green	*Micropterus salmoides*	0.10	137	NA	280	9
Jeffries et al., 2008	Canada	Bow	*Rhinichthys cataractae*	10.00	91.10	59	650.0	10
	Canada	Oldman	*Rhinichthys cataractae*	0.73	81.80	41	11.0	10
Jobling et al., 2002	England	Nene	*Rutilus rutilus*	0.22	6.00	3	76.0	111
	England	Aire	*Rutilus rutilus*	6.75	35.72	15	9.3	95
Lavado et al., 2004	Spain	Ebro	*Cyprinus carpio*	4.60	340.00	8	300.0	6
Nichols et al., 1999	USA	Grand	*Pimephales promelas*	0.053	14.70	NA	1.1	35[a]
	USA	Shiawassee	*Pimephales promelas*	0.15	9.71	NA	5.9	35[a]
	USA	Grand	*Pimephales promelas*	6.14	23.02	NA	0.9	35[a]
	USA	Red Cedar	*Pimephales promelas*	0.034	4.67	NA	3.2	35[a]
Solé et al., 2002	Spain	Anoia	*Cyprinus carpio*	1.80	2.00	23	60.0	30
	Spain	Cardener	*Cyprinus carpio*	2.00	2.70	4	22.0	30
Solé et al., 2003	Spain	Anoia	*Cyprinus carpio*	1.80	2.00	23	2180	21
Tarrant et al., 2008	Ireland	Liffey	*Salmo trutta*	0.68	13.80	2.5	260.0	57
Vajda et al., 2008	USA	Boulder Creek	*Catostomus commersoni*	1.36	0.21	0.2	200.0	64
Van Aerle et al., 2001	England	Aire	*Gobio gobio*	6.75	35.72	10	8.0	71
	England	Aire	*Gobio gobio*	8.47	35.72	10	13.0	21
Vermeirssen et al., 2005	Switzerland	Frenke	*Salmo trutta*	0.12	0.09	0.5	0.3	4
	Switzerland	Glatt	*Salmo trutta*	0.50	0.45	0.5	1.1	5
	Switzerland	Glatt	*Salmo trutta*	0.48	1.67	0.5	0.3	2
	Switzerland	Luetzelmurg	*Salmo trutta*	0.14	0.13	0.5	0.5	3
	Switzerland	Schuss	*Salmo trutta*	0.08	0.13	0.5	1.1	5
	Switzerland	Sissle	*Salmo trutta*	0.09	0.12	0.5	66.8	3
	Switzerland	Winkelbach	*Salmo trutta*	0.066	0.05	0.5	9.7	4
	Switzerland	Wyna	*Salmo trutta*	0.08	0.41	0.5	4.0	1
Vethaak et al., 2002	Netherland	Dommel	*Abramis brama*	0.55	6.00	NA	15000	23

[a] Caged fish were used in experiment.

species, the dataset contained 5 or more observations for only 4 species (i.e., *Onchorynchus mykiss*, *Salmo trutta*, *Cyprinus carpio*, *Pimephales promelas*). The results, which are summarized in Table 2, reveal significant effects of species and population, but no significant interaction between the two variables (i.e., slopes are homogeneous). The ANCOVA results ($N = 38$, $r^2 = 0.59$, F ratio $= 11.7$, $p < 0.0001$) yielded the model shown below. Coefficients accompanied by the same letter are not significantly different at $p < 0.05$.

$$\text{Log VTG}(\mu g/mL) = (0.53 \pm 0.24 * \text{Log Population}) \times \begin{bmatrix} -1.96(\text{Salmo}) & \text{A} \\ -1.83(\text{Pimephales}) & \text{A} \\ -0.54(\text{Cyprinus}) & \text{B} \\ -0.39(\text{Onchorynchus}) & \text{B} \end{bmatrix}$$

Fig. 3 illustrates the ANCOVA result, showing the effect of fish species and population on VTG.

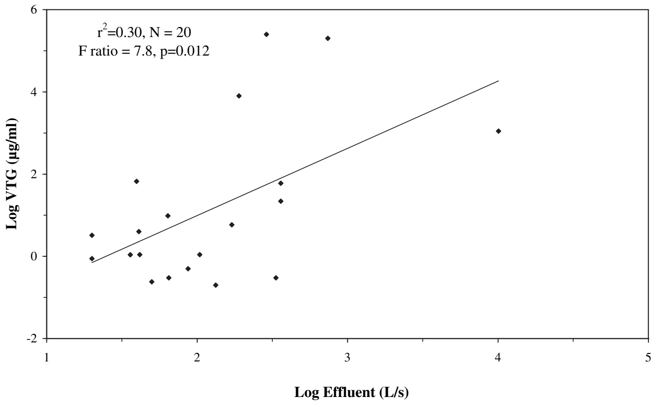

Fig. 1. Average vitellogenin concentrations (µg/ml) in male teleost fish in rivers receiving WWTP effluent as a function of effluent discharge rate (*L/s*) ($n = 20$). VTG concentrations were Log10 transformed and effluent discharge was multiplied by 1000, then Log10 transformed.

4. Discussion

In their survey of multiple river systems in the United Kingdom, Jobling et al. (1998) found highly significant relationships between the concentration of effluent in rivers, expressed as adjusted PE, and both intersex frequency and plasma VTG level. Our expanded analysis across numerous species and river systems revealed that the estrogenic activity in rivers, expressed as serum VTG in male fish, is significantly correlated to population size, but not discharge-corrected population size. Contrary to the Jobling et al. study, our findings reveal that the inclusion of an effluent dilution effect of river discharge rates significantly obscures the relationship between VTG concentrations in fish and the population served by the upstream WWTP. Our results also showed that general water parameters, such as the effluent to river discharge ratios, river EEQs, and river flow rates, did not better correlate with serum VTG concentrations than human population size.

In order to better understand why population alone was more indicative of exposure than discharge-corrected population, or adjusted PE, and other water parameters, it is necessary to consider contaminant transport and fate. Estrogenic activity downstream from WWTP effluent discharge sites has been shown to decline due to the physical processes of dilution, chemical degradation, and sorption to particulate matter (Holthaus et al., 2002). In our study, river discharge significantly hindered the VTG-population relationship, suggesting that the magnitude of the river's discharge does not strongly affect exposure of fish to EDCs. Similarly, our results indicated that river EEQ was not significantly correlated with serum VTG. In general, estrogenic EDCs are relatively hydrophobic and tend to adsorb to sediment rather than remaining in solution (Lei et al., 2009). Specifically, natural and synthetic estrogens, as well as BPA and alkylphenols, have log K_{oc} values ranging from 2.5 to 6.6 (Campbell et al., 2006). Robinson et al. (2009) reported higher concentrations of BPA, EE2 and E2 in sediment than in surface waters in Halifax Harbour. BPA, for example, reached 2.6 ng/L in seawater and 9.5 ng/g in sediments, and E2 concentrations were almost doubled in sediment compared to seawater (Robinson et al., 2009). Likewise, Peck et al. (2004) found that estrogenic activity in sediments of streams and rivers in the United Kingdom are 532- to 748-fold higher than that of the overlying water. This indicates that exposure by fish to many of these compounds may derive from sediment or benthos rather than via water, and this seems to conform with the results of our meta-analysis.

The low solubility of these compounds further supports the notion that they will not remain in solution, and will be associated with suspended and deposited particulate material (Campbell et al., 2006). Their high log K_{oc} values indicate that these compounds will preferentially associate with organic material in aquatic environments, such as suspended particulate matter, which tend to aggregate and settle onto surface sediment (Robinson et al., 2009). Williams et al. (1999) estimated the likely distribution of E1, E2, and EE2 in three U.K. rivers using the EXAMS model and found that in two of the three rivers, the bed sediment accounted for over 50% of E1 and EE2 load, with slightly lower values for E2. The proportion of the estrogens associated with the sediment of the third lake was much lower because it was assigned lower sediment distribution coefficients and had greater water depth (Williams et al., 1999). Similarly, Staples et al. (1998) modeled the fate of BPA using a Level 1 Fugacity Model, and concluded that about 50% of the residual BPA would tend to bind to aquatic sediments or soils.

Fig. 2 reveals a cluster of values with extremely high VTG concentrations for population values in the 500–15,000 µg/ml range. These outliers tended to be observations downstream from regions with significant industrial and/or agricultural loading. The Osberstown WWTP in the Liffey River, for instance, is also located downstream from a contraceptive pill manufacturer. Similarly, the WWTP in River Aire receives significant influent from textile industries. We tried to restrict the analysis to areas that received wastewater predominantly from municipal WWTPs; however, in several instances, industrial wastewater input into the municipal WWTPs was significant, and this input may have contributed to the total EDC load. Indeed, removal of values where specific industrial activity was cited to significantly contribute to the WWTP influent improved the strength of the relationship between mean serum VTG and population size ($n = 51$, $r^2 = 0.32$, $F = 23.3$, $p < 0.0001$). These results support the assertion that industrial inputs can also affect VTG production in male fish. Nevertheless, it should be noted that although the volumetric proportion of WWTP effluents for large municipalities that are of industrial origin can be as high as 60%, most are below 25% (White and Rasmussen, 1998).

The analysis of covariance revealed that fish species also has a significant effect on observed VTG concentration ($p < 0.0001$). As illustrated in Fig. 3 and shown in the predictive model, *Cyprinus carpio* (carp) and *Oncorhynchus mykiss* (rainbow trout) had significantly higher plasma VTG responses than *Pimephales promelas* (fathead minnow) and *Salmo trutta* (Brown trout). Both carp and trout are bottom-dwelling omnivores that feed predominantly on benthic invertebrates (DFO, 2010). This observation supports the hypothesis that the major source of EDCs to aquatic fish is from the benthic environment; however, other species, such as *Pimephales*

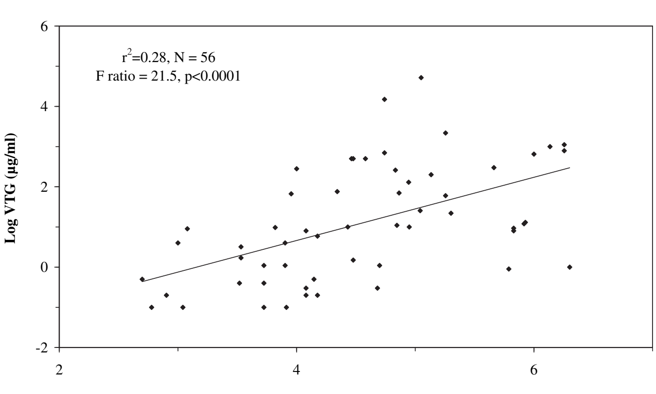

Fig. 2. The relationship between mean serum vitellogenin concentration (µg/ml) in male teleost fish and the size of the human population served by the upstream WWTP. Data were Log10 transformed.

Table 2
Analysis of covariance of plasma vitellogenin concentration in male teleost fish. Analyses restricted to *Onchorynchus mykiss*, *Salmo trutta*, *Cyprinus carpio*, and *Pimephales promelas*.

Source of Variance	Adjusted SS	df	MS	F
Species	36.64	3	12.21	13.94***
Log Pop	4.22	1	4.22	4.82*
Species*Log Pop	1.51	3	0.50	0.55^{NS}
Error	28.92	33	0.88	

***$p < 0.001$.
*$p < 0.05$.
^{NS}Not significant at $p < 0.05$.

promelas, also feed on benthic organisms but yield VTG responses that are significantly lower than those observed for Carp and Rainbow Trout. Thus, feeding and habitat preference alone cannot explain the noteworthy differences in species illustrated in Fig. 3.

Estrogen receptor density and affinity, as well as receptor sensitivity to EDCs, have been proposed as important factors in explaining differences in species specific VTG responses (Routledge et al., 1998; Latonnelle et al., 2002; Palace et al., 2009). In a study of 4 wild fish species exposed to similar EE2 levels in a whole lake experiment, Palace et al. (2009) found significant differences in VTG induction in each species. They proposed that the varying VTG responses were related in part to species differences in the density of hepatic estrogen receptors. Latonnelle et al. (2002) confirmed that differences in estrogen receptor density exist between species, showing that sturgeon required less estradiol to saturate estrogen binding sites on hepatic nuclear extracts compared to trout. Furthermore, based on EC50 values for vitellogenesis, Rankouhi et al. (2004) observed that carp and rainbow trout were much more sensitive to E2 than bream, indicating interspecies differences in E2 binding affinity for the estrogen receptor or interspecies differences in metabolic rates. These findings suggest that *Cyrprinus* and *Oncorhynchus* are physiologically more sensitive to EDCs, and thus exhibit much higher VTG responses for a given population size. The ANCOVA results are consistent with this assertion.

There are numerous additional factors that are potentially contributing to the unexplained variance in serum VTG concentration. First, VTG kinetics and the transient nature of estrogen exposure with wild populations of fish are two factors that may be influencing the results of this study. Hemmer et al. (2002) found that maximum ceiling concentrations of VTG can be reached by low exposures to estrogenic compounds, and that these concentrations

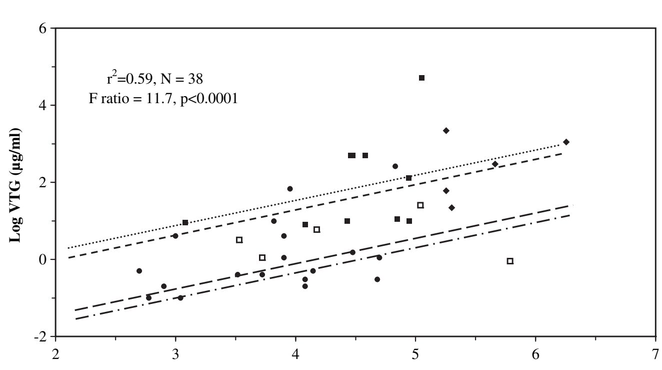

Fig. 3. Relationships between mean serum vitellogenin concentration (μg/ml) and human population served by the upstream WWTP in 4 species of male teleost fish. The trend lines represent predicted VTG concentrations for each fish species based on the ANCOVA model. From top to bottom, the trend lines represent: *Oncorhynchus mykiss* (■), *Cyprinus carpio* (◆), *Pimephales promelas* (□), and *Salmo Trutta* (●). Data were Log10 transformed.

can be sustained for prolonged depuration periods of up to several weeks. The long half life of plasma VTG combined with the migratory nature of fish in streams and the large geographical range of certain fish species results in difficulty to establish a clear temporal relationship between sampling location and VTG concentrations (Hyndman et al., 2010). The type of wastewater treatment system is another factor that influences VTG as it can affect the estrogen loading of effluent entering nearby rivers and streams (Campbell et al., 2006). Braga et al. (2005) compared the effectiveness of a primary and tertiary sewage treatment plant for removal of natural and synthetic estrogens from influent and found that the advanced tertiary plant removed between 70 and 90% more EDCs than the primary plant. Duong et al. (2009) suggested that sediment composition and size might also influence the bioavailability of estrogenic chemicals to fish. Other factors that could contribute to the magnitude of serum VTG include the magnitude and types of industrial contributions, the chemical composition of the EDCs, differences in VTG measurement techniques, and the method employed for effluent disinfection. Despite these sources of variability, this study has successfully demonstrated that population size alone accounts for a defined fraction of the variation in serum VTG concentration in male teleost fish worldwide.

This study has highlighted the need for researchers to evaluate and publish data not only on VTG and other biomarkers, but to also include the many other parameters that come into play (i.e., river and effluent discharge rates, EEQ, population size, water quality, etc.). The inclusion of these parameters in future studies will facilitate the development of a more comprehensive model for the prediction of estrogenic effects in fish from rivers downstream of WWTPs.

Acknowledgements

This research was supported by a Natural Sciences and Engineering Research Council (Canada) Discovery Grant to JMB and an NSERC USRA to JPWD.

References

Aerni, H., Kobler, B., Rutishauser, B.V., Wettstein, F.E., Fischer, R., Giger, W., 2007. Combined biological and chemical assessment of estrogenic activities in wastewater treatment plant effluents. Analytical and Bioanalytical Chemistry 378, 688–696.

Allen, Y., Matthiessen, P., Scott, A.P., Haworth, S., Feist, S., Thain, J.E., 1999. The extent of oestrogenic contamination in the UK estuarine and marine environments – further surveys of flounder. Science of the Total Environment 233, 5–20.

Atkinson, S., 2008. The Persistence of Steroidal Estrogens in the Aquatic Environment. M. Sc. Thesis, University of Ottawa, Ottawa, Ontario, Canada.

Bjerregaard, L.B., Madsen, A.H., Korsgaard, B., Bjerregaard, P., 2006. Gonad histology and vitellogenin concentrations in brown trout (*Salmo trutta*) from danish streams impacted by sewage effluent. Ecotoxicology 15, 315–327.

Bjorkblom, C., Salste, L., Katsiadaki, I., Wiklund, T., Kronberg, L., 2008. Detection of estrogenic activity in municipal wastewater effluent using primary cell cultures from three-spined stickleback and chemical analysis. Chemosphere 73, 1064–1070.

Braga, O., Smythe, G.A., Schafer, A.I., Feitz, A.J., 2005. Steroid estrogens in primary and tertiary wastewater treatment plants. Water Science & Technology 52, 273–278.

Burki, R., Vermeirssen, E.L.M., Korner, O., Joris, C., Burkhardt-Holm, P., Segner, H., 2006. Assessment of estrogenic exposure in brown trout (*Salmo trutta*) in a Swiss midland river: integrated analysis of passive samplers, wild and caged fish, and vitellogenin mRNA and protein. Environmental Toxicology & Chemistry 25, 2077–2086.

Campbell, C., Borglin, S., Green, B., Grayson, A., Wozei, E., Stringfellow, W., 2006. Biologically directed environmental monitoring, fate, and transport of estrogenic endocrine disrupting compounds in water: a review. Chemosphere 65, 1265–1280.

Department of Fisheries and Oceans (DFO), 2010. Retrieved Feb 11, 2010. <http://www.dfo-mpo.gc.ca/science/publications/uww-msm/index-eng.asp>.

Desbrow, C., Routledge, E.J., Brighty, G.C., Sumpter, J.P., Waldock, M., 1998. Identification of estrogenic chemicals in STW effluent. 1. Chemical fractionation and in vitro biological screening. Environmental Science & Technology 32, 1549–1558.

de Voogt, P., van Hattum, B., 2003. Critical factors in exposure modeling of endocrine active substances. Applied Chemistry 75, 1933–1948.

Duong, C.N., Schlenk, D., Chang, N.I., Kim, S.D., 2009. The effect of particle size on the bioavailability of estrogenic chemicals from sediments. Chemosphere 76, 395–401.

Folmar, L., Denslow, N., Rao, V., Chow, M., Crain, A., Enblom, J., Marcino, J., Guillette, L., 1996. Vitellogenin induction and reduced serum testosterone concentrations in feral male carp (*Cyprinus carpio*) captured near a major metropolitan sewage treatment plant. Environmental Science & Technology 104, 1096–1101.

Folmar, L.C., Denslow, N.D., Kroll, K., Orlando, E.F., Enblom, J., Marcino, J., Metcalfe, C., Guillette Jr., L.J., 2001. Altered serum sex steroids and vitellogenin induction in walleye (*Stizostedion vitreum*) collected near a metropolitan sewage treatment plant. Archives of Environmental Contamination and toxicology 40, 392–398.

Giesy, J.P., Snyder, E.M., Nichols, K.M., Snyder, S.A., Villalobos, S.A., Jones, P.D., 2003. Examination of reproductive endpoints in goldfish (*Carassius auratus*) exposed in situ to municipal sewage treatment plant effluent discharges in michigan, USA. Environmental Toxicology & Chemistry 22, 2416–2431.

Harries, J.E., Sheahan, D.A., Jobling, S., Matthiessen, P., Neall, P., Sumpter, J.P., Tylor, T., Zaman, N., 1997. Estrogenic activity in five United Kingdom rivers detected by measurement of vitellogenesis in caged male trout. Environmental Toxicology & Chemistry 16, 534–542.

Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C., Vonk, A., 2003. Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (Rana pipiens): laboratory and field evidence. Environmental Health Perspectives 111, 568–575.

Hecker, M., Tyler, C.R., Hoffmann, M., Maddix, S., Karbe, L., 2002. Plasma biomarkers in fish provide evidence for endocrine modulation in the Elbe River, Germany. Environmental Science & Technology 36, 2311–2321.

Hemmer, M.J., Bowman, C.J., Hemmer, B.L., Friedman, S.D., Marcovich, D., Kroll, K.J., Denslow, N.D., 2002. Vitellogenin mRNA regulation and plasma clearance in male sheepshead minnows, (*Cyprinodon variegatus*) after cessation of exposure to 17β-estradiol and p-nonylphenol. Aquatic Toxicology 58, 99–112.

Hemming, J.M., Waller, W.T., Ammann, L.P., 2001. Assessment of wastewater effluent estrogenicity in north central texas using the vitellogenin biomarker in male fathead minnows (*Pimephales promelas rafinesque*). Texas Journal of Science 53, 335–344.

Hinck, J.E., Schmitt, C.J., Blazer, V.S., Denslow, N.D., Bartish, T.M., Anderson, P.J., Coyle, J.J., Dethloff, G.M., Tillitt, D.E., 2006. Environmental contaminants and biomarker responses in fish from the Columbia river and its tributaries: spatial and temporal trends. Science of the Total Environment 336, 549–578.

Hinck, J.E., Blazer, V.S., Denslow, N.D., Echols, K.R., Gross, T.S., May, T.W., Anderson, P.J., Coyle, J.J., Tillit, D.E., 2007. Chemical contaminants, health indicators, and reproductive biomarker responses in fish from the Colorado river and its tributaries. Science of the Total Environment 378, 376–402.

Holthaus, K., Johnson, A., Jurgens, M., Williams, R., 2002. The potential for estradiol and ethynylestradiol to sorb to suspended and bed sediments in some English rivers. Environmental Toxicology & Chemistry 21, 2526–2535.

Hyndman, K.M., Biales, A., Bartell, S.E., Schoenfuss, H.L., 2010. Assessing the effects of exposure timing on biomarker expression using 17β-estradiol. Aquatic Toxicology 96, 264–272.

Jeffries, K.M., Nelson, E.R., Jackson, L.J., Habibi, H.R., 2008. Basin-wide impacts of compounds with estrogen-like activity on longnose dace (*Rhinichtys cataractae*) in two prairie rivers of Albreta, Canada. Environmental Toxicology & Chemistry 27, 2042–2052.

Jobling, S., Sheahan, D., Osborne, J.A., Matthiessen, P., Sumpter, J.P., 1996. Inhibition of testicular growth in rainbow trout (*Oncorhynchus mykiss*) exposed to estrogenic alkylphenolic chemicals. Environmental Toxicology & Chemistry 15, 194–202.

Jobling, S., Nolan, M., Tyler, C., Brighty, G., Sumpter, J., 1998. Widespread sexual disruption in wild fish. Environmental Science & Technology 32, 2498–2506.

Jobling, S., Beresford, N., Nolan, M., Rodgers-Gray, T., Brighty, G.C., Sumpter, J.P., 2002. Altered sexual maturation and gamete production in wild roach (*Rutilus rutilus*) living in rivers that receive treated sewage effluents. Biology of Reproduction 66, 272–281.

Jobling, S., Williams, R., Johnson, A., Taylor, A., Gross-Sorokin, M., Nonal, M., Tyler, C., van Aerle, R., Santos, E., Brighty, G., 2006. Predicted exposures to steroid estrogens in U.K. rivers correlate with widespread sexual disruption in wild fish populations. Environmental Health Perspectives 114, 32–39.

Keller, V., Fox, K., Rees, H., Young, A., 2006. Estimating population served by sewage treatment works from readily available GIS data. Science of the Total Environment 360, 319–327.

Latonnelle, K., Fostier, A., Le Menn, F., Bennetau-Pelissero, C., 2002. Binding affinities of hepatic nuclear estrogen receptors for phytoestrogens in rainbow trout (*Oncorhynchus mykiss*) and Siberian sturgeon (*Acipenser baeri*). General & Comparative Endocrinology 129, 69–79.

Lavado, R., Thibaut, R., Ralduća, D., Martićn, R., Porte, C., 2004. First evidence of endocrine disruption in feral carp from the ebro river. Toxicology & Applied Pharmacology 196, 247–257.

Legler, J., Jonas, A., Lahr, J., Vethaak, A.D., Brouwer, A., Murk, A.J., 2002. Biological measurement of estrogenic activity in urine and bile conjugates with the in vitro ER-CALUX reporter gene assay. Environmental Toxicology & Chemistry 21, 473–479.

Lei, B., Huang, S., Zhou, Y., Wang, D., Wang, Z., 2009. Levels of six estrogens in water and sediment from three rivers in Tianjin area, China. Chemosphere 76, 36–42.

Nichols, K.M., Miles-Richardson, S.R., Snyder, E.M., Giesy, J.P., 1999. Effects of exposure to municipal wastewater in situ on the reproductive physiology of the fathead minnow (*Pimephales promelas*). Environmental Toxicology & Chemistry 18, 2001–2012.

Palace, V., Evans, R., Wautier, K., Mills, K., Blanchfield, P., Park, B., Baron, C., Kidd, K., 2009. Interspecies differences in biochemical, histopathological, and population responses in four wild fish species exposed to ethynylestradiol added to a whole lake. Canadian Journal of Fisheries & Aquatic Science 66, 1920–1935.

Peck, M., Gibson, R., Kortenkamp, A., Hill, E., 2004. Sediments are major sinks of steroidal estrogens in two United Kingdom rivers. Environmental Toxicology & Chemistry 23, 945–952.

Purdom, C.E., Hardiman, P.A., Bye, V.J., Eno, N.C., Tyler, C.R., Sumpter, J.P., 1994. Estrogenic effects of effluents from sewage treatment works. Chemical Ecology 8, 275–285.

Rankouhi, T.R., Sanderson, J., van Holsteijn, I., van Leeuwen, C., Vethaak, A., van den Berg, M., 2004. Effects of natural and synthetic estrogens and various environmental contaminants on vitellogenesis in fish primary hepatocytes: comparison of bream (*Abramis brama*) and carp (*Cyprinus carpio*. Toxicological Sciences 81, 90–102.

Robinson, B., Hui, J., Soo, E., Hellou, J., 2009. Estrogenic compounds in seawater and sediment from Halifax harbor, Nova Scotia, Canada. Environmental Toxicology & Chemistry 28, 18–25.

Routledge, E., Sheahan, D., Desbrow, C., Brighty, G., Waldock, M., Sumpter, J., 1998. Identification of estrogenic chemicals in STW effluent. 2. In Vivo responses in trout and roach. Environmental Science & Technology 32, 1559–1565.

Solé, M., Raldua, D., Piferrer, F., Barcelo, D., Porte, C., 2003. Feminization ofwild carp, Cyprinus carpio, in a polluted environment: plasma steroid hormones, gonadal morphology and xenobiotic metabolizing system. Comparative Biochemistry and Physiology Part C 126, 145–156.

Solé, M., Barceló, D., Porte, C., 2002. Seasonal variation of plasmatic and hepatic vitellogenin and EROD activity in carp, C*yprinus carpio*, in relation to sewage treatment plants. Aquatic Toxicology 60, 233–248.

Staples, C., Dorn, P., Klecka, G., O'Block, S., Harris, L., 1998. A review of the environmental fate, effects, and exposures of bisphenol a. Chemosphere 36, 2149–2179.

Tarrant, H., Mousakitis, G., Wylde, S., Tattersall, N., Lyons, A., Maloney, M., 2008. Raised plasma vitellogenin in male wild brown trout (*Salmo trutta*) near a wastewater treatment plant in Ireland. Environmental Toxicology & Chemistry 27, 1773–1779.

Thorpe, K.L., Benstead, R., Eccles, P., Maack, G., Williams, T., Tyler, C.R., 2008. A practicable laboratory flow-through exposure system for assessing the health effects of effluents in fish. Aquatic Toxicology 88, 164–172.

Vajda, A.M., Barber, L.B., Gray, J.L., Lopez, E.M., Woodling, J.D., Norris, D.O., 2008. Reproductive disruption in fish downstream from an estrogenic wastewater effluent. Environmental Science & Technology 42, 3407–3414.

Van Aerle, R., Nolan, M., Jobling, S., Christiansen, L.B., Sumpter, J.P., Tyler, C.R., 2001. Sexual disruption in a second species of wild cyprinid fish (the gudgeon, *Gobio gobio*) in United Kingdom freshwaters. Environmental Toxicology & Chemistry 20, 2841–2847.

Vermeirssen, E., Burki, R., Joris, C., Peter, A., Segner, H., Suter, M., Burkhardt-Holm, P., 2005. Characterization of the estrogenicity of swiss midland rivers using a recombinant yeast bioassay and plasma vitellogenin concentrations in feral male brown trout. Environmental Toxicology & Chemistry 24, 2226–2233.

Vethaak, A.D., Larh, J., Kuiper, R.V., Grinwis, J.C.M., Rankouhi, T.R., Giesy, J.P., Gerritsen, A., 2002. Estrogenic effects in fish in The Netherlands: some preliminary results. Toxicology 181–182, 147–150.

White, P.A., Rasmussen, J.B., 1998. The genotoxic hazards of domestic waste in surface waters. Mutation Research – Reviews in Mutation Research 410, 223–236.

Williams, R., Jurgens, M., Johnson, A., 1999. Initial predictions of the concentrations and distribution of 17β-oestradiol, oestrone and ethinyl oestradiol in 3 English rivers. Water Ressources 33, 1663–1671.