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Research Article

Reproductive health of brown trout inhabiting Swiss rivers with declining fish catch

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Abstract. In recent years, brown trout catches have been declining in many Swiss rivers. One hypothesis is that this declining catch is linked to environmental estrogens, known to have the potential to adversely affect fish reproduction. In order to assess if the reproductive health of brown trout is impaired, we sampled fish at three sites along four rivers with a well documented catch decline. These rivers are affected by inputs of treated sewage effluent. The sampling was conducted during two years; we measured plasma vitellogenin (Vtg) concentrations and surveyed gonadal histology. Analysis of plasma Vtg revealed elevated concentrations (up to 8 µg/mL) in only 10 out of 197 males. Furthermore, there were no site specific patterns to this induction. These results indicate that the exposure to estrogenic compounds is low. Also the incidence of ovarian atresia was low and we found no male intersex fish. In contrast to males, females caught along two rivers had spermatogenic activity in ovarian tissue. However, this intersex condition does not appear to be connected to exposure to environmental estrogens. At one of 12 sites there was a high incidence of gonadal parasites in ovarian tissue, which may affect reproductive output but was not a general problem across sites. In conclusion, the exposure to estrogenic compounds does not appear to significantly affect the reproductive parameters we investigated in Swiss brown trout.

Key words. Trout; endocrine disruptors; temperature stress; vitellogenin; intersex; atresia.

Introduction

Since the 1980ies of the past century several indications caused concern about declining fish populations in Switzerland. Yearly records of anglers indicated an up to 50 % reduced fish catch of primarily brown trout (*Salmo trutta fario*), but also grayling (*Thymallus thymallus*) and the nase (*Chondrostoma nasus*), between 1980 and 1997 (Friedl, 1999). These observations prompted a more detailed study, which revealed a reduced catch in 20 of 26 cantons (Frick et al., 2002). Some of the affected rivers and streams were located in the midlands and the northern part of the country, where several anthropogenic impacts (e.g. degradation of habitat structure or impaired water quality) affect the fish habitat. In order to establish the causes of the catch decline, an interdisciplinary project FISCHNETZ (engl. FISHNET – Project on declining fish catch in Switzerland) was initiated in 1998 (Burkhardt-Holm et al., 2002). By evaluating the catch, fish abundance as well as diverse biotic and abiotic parameters, FISCHNETZ aimed to reveal the causes of the catch decline and to propose measures to ensure the future viability of this ecological and economical important resource.

Within FISCHNETZ various hypotheses were put forward to explain the reduced catch (Burkhardt-Holm et al.,

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2002). Among others, a disturbed reproductive health as a consequence of endocrine disruption has been considered. This hypothesis mostly relies on several reports, particularly from Europe as well as North America, indicating widespread reproductive impairments in feral fish. Effects on reproduction range from an induction of vitellogenin (Vtg, a precursor of yolk protein) in male fish, intersex in gonochoristic fish species, retardation of gonad development or high incidences of oocyte atresia (Jobling and Tyler, 2003a). Although the ecological implications of these effects on fish populations still remain unclear (Arcand-Hoy and Benson, 1998), exposure to estrogenic compounds may affect the long term stability of fish populations (Rolland, 2000). The catch decline observed in Switzerland could be a manifestation of such effects.

Vitellogenin is the major precursor of yolk protein in oviparous vertebrates and has been repeatedly used as an indicator of exposure of fish to estrogens in field and lab studies (Sumpter and Jobling, 1995; Denslow et al., 1999). Vitellogenin is normally expressed in the female liver under estrogenic control, excreted to the blood stream and transported to its target organ – the ovaries. In male fish, base levels of Vtg are usually in the lower ng/mL range (ca. 10–100 ng/mL) and therefore well below background levels (10–1,000 µg/mL) reported for females (Wheeler et al., 2005). However, evidence of elevated Vtg values in feral male fish downstream from sewage treatment works (STW) has been reported for various fish species (Folmar et al., 1996; Jobling et al., 1998). Subsequent surveys provided evidence that certain natural and synthetic compounds, entering the aquatic ecosystem via STW effluents, provoke a vitellogenic response in males (Routledge et al., 1998).

Besides the induction of Vtg, manifold observations of intersex (testicular and ovarian germ cells in one gonad) in geographical vicinity to STWs have been reported. In the United Kingdom, a study by Jobling et al. (1998) revealed intersex in up to 100 % of male roach (*Rutilus rutilus*) living downstream of STWs, compared to 0–4 % in fish of control sites. Intersex has also been reported in flounder (Lye et al., 1997), tilapia (Mousa and Mousa, 1999), barbel (Vigano et al., 2001) gudgeon (van Aerle et al., 2001; Faller et al., 2003), whitefish (Mikaelian et al., 2002), stickleback and European perch (Gercken and Sordyl, 2002), carp (Solé et al., 2003b); white perch (Kavanagh et al., 2004) and catfish (Barnhoorn, et al. 2004) – indicating a widespread issue in feral fish populations worldwide. Although STW effluents carry a large variety of micropollutants besides environmental estrogens (e.g. androgens and anti-androgens), certain effects such as Vtg induction and intersex can be mimicked by estrogens in lab studies (e.g. Gimeno et al., 1996; Gray et al., 1999).

Alongside with the occurrence of Vtg induction and intersex in male roach captured in the proximity of STW effluent discharges, female fish showed an increase of ovarian atresia (Jobling et al., 2002). Atresia is a degenerative process, characterized by nucleolus disintegration, vitelline envelope breakdown and increased follicular cells (Blazer, 2002). Although it is a common physiological event in ovarian development, increased incidence of atresia, particularly in previtellogenic oocytes, has been mainly associated with environmental endocrine disruptors (Janz et al., 1997; Van den Belt et al., 2002), but also environmental stressors like temperature or nutritional deficits (Blazer, 2002).

The study presented here aimed to clarify, whether feral brown trout are suffering from a disturbed reproductive health as a result of exposure to estrogenic compounds. We discuss effects on reproductive health in relation to the declining fish catches in Switzerland. In order to make this link we investigated vitellogenin and gonadal histology in fish from four Swiss rivers that receive treated sewage effluent and have a well documented catch decline.

Materials and methods

Test areas

Passive biomonitoring on brown trout was carried out in four rivers (Venoge, Emme, Necker and Liechtensteiner Binnenkanal (LBK) – for detailed information please refer to Table 1 as well as Burkhardt-Holm and Scheurer, 2007). Each river was sampled at three representative sites. Two sampling sites downstream of STWs (D1 and D2) as well as one head water site (HW). The HW site on the Venoge is affected by a small STW further upstream (Table 1). Most sites are separated by at least one physical barrier that prevents upstream migration but still allows downstream movements of fish. Where sampling sites are not separated by a migration barrier, we selected sites that are reasonably far apart from each other, making upstream migration of brown trout between these sampling sites unlikely (Borsuk et al., 2006).

Venoge. The Venoge represents a mid-sized river located in the western part of the Swiss midland arising from two springs. Until the river finally enters Lake Geneva, the Venoge is draining an overall area of 231 km^2 on a total length of 70 km. While the downstream sites were located on the Venoge, the head water site was situated on a tributary stream (La Veyron). This upper area is geologically characterized by a distinct karst formation. Due to that, in summer La Veyron is occasionally desiccated (Table 1). According to Borsuk et al. (2006), the downstream area of the Venoge is mainly dominated by farmland (58 %), while the La Veyron represents a less agrarian area (40 %) dominated mostly by forests (56 %). A total of seven STWs with a population equivalent (PE) of 1,750 to 50,000 are discharging into the Venoge.

Table 1. Characterisation of the headwater (HW) and downstream sites (D1 and D2) of Venoge, Emme, Necker and LBK (Lichtensteiner Binnenkanal).

	Venoge			Emme			Necker			LBK		
Sampling site	**HW**	**D1**	**D2**	**HW**	**D1**	**D2**	**HW**	**D1**	**D2**	**HW**	**D1**	**D2**
STW	Ballens	Penthaz	Bussigny	–	Ruegsau	Aeflingen	–	Mogelsberg	Necker	–	Balzers	–
Population equivalents [1]	1'750	10'200	21'250	–	26'000	40'000	–	1'500	4'500	–	4'500	–
Distance from STW (km)	6	1	2	–	4	3	–	1	3	–	1	9
EEQ (ng/L) [2]	n.d.	0.2	0.3	0	0.1	0.2	0	0.1	0.1	n.d.	n.d.	n.d.
Q_{347} (m^3/s) [3]	0	0.5	0.6	0.2	4.1	5.3	0.1	0.5	0.6	0.2	0.4	1.0
Q_{median} (m^3/s) [3]	0.2	2.3	2.5	1.1	11.9	14.1	0.3	1.9	2.7	0.2	0.8	1.7
Q_T (L/s) [3]	4	25	34	–	104	176	–	5	10	–	37	–
STW effluent (%) [4,5]	2 [5]	5	6	–	2	3	–	1	2	–	10	4
P_{tot} (mg/L) [6]	0.04	0.08	0.09	0.02	0.05	0.11	0.02	0.06	0.03	0.02	0.03	0.01
NO_3-N (mg/L) [6]	2.39	3.71	3.95	0.40	2.51	3.76	0.52	1.24	1.27	1.00	1.50	0.89
NH_4-N (mg/L) [6]	0.02	0.09	0.16	0.01	0.09	0.31	< 0.01	0.03	0.03	0.08	0.45	0.02
Atrazine (ng/L) [7]	n.d.	n.d.	23–764	n.d.	n.d.	7–728	n.d.	n.d.	BDL-23	n.d.	n.d.	BDL-11
Diazinon (ng/L) [7]	n.d.	n.d.	1–16	n.d.	n.d.	BDL-26	n.d.	n.d.	BDL-5	n.d.	n.d.	BDL-27
T (°C) summer 2002 [8]	10–14	12 –19	12–20	11–23	11–18	9–20	9–16	n.d.	n.d.	10–13	10–15	9–15
T (°C) summer 2003 [8]	12–17	16–24	17–24	11–23	13–22	13–25	9–17	10–24	11–26	11–15	11–18	10–16

STW = sewage treatment works; n.d. = not determined; HW = head water; D1 = downstream 1; D2 = downstream 2; BDL = below detection limit

[1] Population equivalents (PE) were calculated on the basis of biochemical oxygen demand after 5 days (1 PE = 60 g oxygen per 5 days).

[2] 17 beta-estradiol equivalent (EEQ) values were calculated on the basis of Q_{median}. Data kindly provided by Suter et al. (Eawag Dübendorf, unpublished data).

[3] Q_{347} signifies the flow of the river at times of drought; Q_{median} is the median flow rate of the river; Q_T signifies the average dry weather effluent discharge from the STW.

[4] Percent effluent = $Q_T/(Q_T+Q_{347})$.

[5] As a result of karst formation in the upper region of the river Venoge the Q_{347} there is 0 m^3/s. Therefore, we calculated the percent effluent using Q_{median}: percent effluent = $Q_T/(Q_T+Q_{median})$.

[6] Phosphorus and nitrogen values are presented as 80th percentile of monthly measured samples in 2002. Data kindly provided by the cantonal authorities of Waadt, Berne, St.Gallen and the Principality of Liechtenstein.

[7] Pesticides are presented as minimum-maximum values (data from Götz et al., 2003).

[8] Temperature variations during the sampling period (July to August). Temperature data kindly provided by Eva Schager (Eawag Kastanienbaum).

Emme. With a total catchment area of 963 km^2, the Emme represents the largest river of the present survey. Over 80 km long, the Emme is first flowing through a sub alpine region and subsequently through the Swiss midland where it joins the Aare River. The Emme River is considerably influenced by seasonal flow fluctuations which led to intense river management activities in the past centuries (Borsuk et al., 2006). The catchment area is mainly characterized by farmland (35 %) as well as forests (40 %), but also has a residential area (6 %) of partly high density. In total, three large STWs (PE ≤ 40,000) as well as some smaller STWs (PE ≥ 30) are discharging into the river.

Necker. The smallest river of the present study, the Necker, originates from a spring in the prealps and flows into the Thur River nearby Lütisburg. The river is 31 km long and its catchment area corresponds to 123 km^2 (Borsuk et al., 2006). About 38 % of the catchment area is covered by forest, whereas 35 % is used as farmland. Residential areas and public infrastructure are covering approximately 4 % of the examined region. The Necker is influenced by effluents from three STWs with a PE of 1,500 to 4,500.

Liechtensteiner Binnenkanal. The Liechtensteiner Binnenkanal (LBK) is a mid-sized canal with a total length of 29 km. It was engineered in the 1930s for flood protection, but also to drain the Rhine valley of Liechtenstein. Today, the LBK drains roughly 138 km^2 (Borsuk et al., 2006). Near 50 % of the area is covered with forest and only 18 % occupies intensive farmland. At least 10 % is covered by buildings, streets and railroads. Furthermore, almost 10 % of the catchment area is fallow land and therefore not in use. One STW (PE = 4,500) discharges into the LBK.

Table 2. The dates when field sampling took place in four rivers in 2002 and 2003.

Test area	Site	2002	2003
Venoge	**HW**	16.08.2002	B
	D1	21.08.2002	05.08.2003
	D2	21.08.2002	05.08.2003
Emme	**HW**	26.08.2002	23.07.2003
	D1	09.08.2002	24.07.2003
	D2	09.08.2002	A
Necker	**HW**	27.08.2002	26.08.2003
	D1	28.08.2002	27.08.2003
	D2	28.08.2002	27.08.2003
LBK	**HW**	02.08.2002	07.08.2003
	D1	19.08.2002	07.08.2003
	D2	A	05.08.2003

A = no fish was caught at sites LBK D2 in 2002 and Emme D2 in 2003.
B = due to drought no sampling took place at Venoge HW (2003).
HW = head water; D1 = downstream 1; D2 = downstream 2.

Fish sampling and processing

A total number of 424 brown trout were collected by means of electro fishing in summer 2002 and 2003 (Table 2). Upon collection, the fish were anesthetized with MS222 (Sigma Aldrich, Germany) and blood was sampled via the caudal vein by heparinized syringes (Monovette®, Sarstedt, Nürnbrecht, Germany). The plasma samples were centrifuged at 4 °C (Eppendorff, Centrifuge 5415R) at 10,000 g for 5 min, transferred to cryogenic tubes, frozen in liquid nitrogen and stored at –80 °C. After blood sampling the fish was killed by a blow to the head followed by the determination of weight and length. The gonads were removed, their weights recorded and preserved in 10 % buffered formalin. The gonadosomatic index (GSI) and condition factor (CF) was calculated as follows:

$$CF = (\text{weight} \times 100)/\text{length}^3$$
$$GSI = 100 \times \text{gonad weight}/(\text{body weight} - \text{gonad weight})$$

Some scales of each fish were removed and age was determined by cantonal fishery authorities.

Histological analysis

The gonads were processed further for histological analysis (longitudinally sectioned at 3–5 µm; stained with haematoxylin and eosin) as described by Körner et al. (2005). Both, male and female gonads were microscopically examined with a Nikon ECLIPSE E400 (magnifications ranged from 20x to 400x) for histopathological alterations (e.g. intersex, parasites, etc). The percent of atresia was quantified according to Blazer (2002) by counting the number of atretic follicles out of 150 oocytes. To evaluate the stage of male spermatogenesis (Fig. 1a and Fig. 1b), the testes were scored semi-quantitatively according to the criteria described in Blazer (2002). Female gonads were classified according to the most advanced stage of oocytes present (Fig. 1c and Fig. 1d) as described by Blazer (2002).

Vitellogenin analysis

Plasma Vtg was analyzed by means of a competitive brown trout Vtg enzyme linked immunosorbent assay (ELISA). Purified Vtg for coating and standard was isolated as described by Burki et al. (2006). Primary Vtg antibody (Vtg AB) against *Salmo salar* Vtg was raised in rabbit and was kindly provided by Birgitta Norberg (IMR, Austevoll Aquaculture Research Station, Storebø, Norway). Secondary AB (goat-anti-rabbit IgG, horse-radish peroxidase conjugated) was supplied by BIO-RAD, Germany.

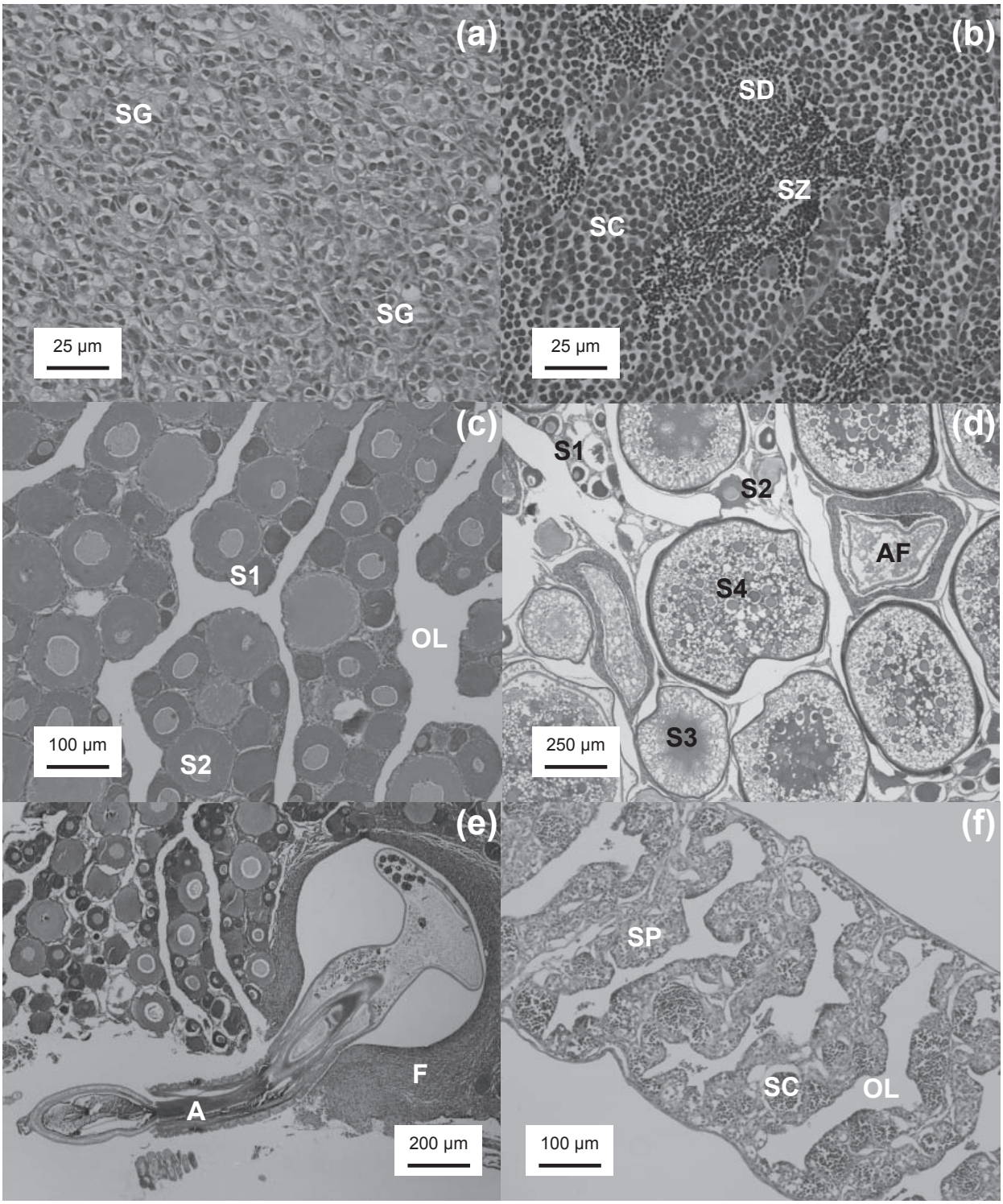

Figure 1. Histological sections of brown trout gonads from Swiss rivers. (a) Immature testis filled with spermatogonia (SG). (b) Mid spermatogenic testis comprising spermatocytes (SC), spermatids (SD) and spermatozoa (SZ). (c) Previtellogenic ovary containing early (S1) and late (S2) perinuclear oocytes as well as the characteristic ovarian lamellae structure (OL). (d) Vitellogenic ovary consisting of early and late perinuclear oocytes (S1 and S2), cortical alveolar stages (S3) as well as vitellogenic oocytes (S4). An atretic follicles is labelled as AF. (e) Acanthocephalan parasite in female ovary (A) as well as pronounced fibrosis (F) in surrounding tissue. (f) Male gonad – filled with spermatogonia (SG) and spermatocytes (SC) showing ovarian lamellae structure (OL). Sections were stained with haematoxylin and eosin.

Ninety-six well plates (Nunc™ F96 Maxisorp Immuno Plate, Nunc, Wiesbaden, Germany) were coated with 15 ng/well Vtg in carbonate buffer (0.05 M sodium carbonate, pH 9.6), sealed and incubated overnight at 4 °C in a humid plastic container. For the standard, bt-Vtg was thawed and diluted in 0.1 M PBS-T (8.1 mM sodium hydrogen phosphate, 2.7 mM potassium dihydrogen phosphate, 1.5 mM potassium chloride, 137 mM sodium chloride, 0.005 % Tween20) to concentrations of 1 µg/mL Vtg to 0.004 µg/mL Vtg. Plasma samples were also diluted in 0.1 M PBS-T. One hundred µL of both, standards and samples, were incubated overnight at 4 °C in sealed non-coated 96-well plates with Vtg-AB (100 µL/well, 1:10,000 in 0.1 M PBS-T). The coated plates were washed three times with 0.05 M PBS-T washing buffer and blocked 1 h at room temperature (RT) with blocking solution (1 % non fat dry milk in PBS-T, w/v). Subsequently the plates were rinsed three times with washing buffer. One hundred µL of standard/AB and sample/AB were transferred to the wells and incubated for 2 h at RT. The plates were rinsed three times with washing buffer, then the secondary AB was added (100 µL/well, 1:1,500 in PBS-T). After incubation for 2 h at RT, plates were rinsed five times with washing buffer and 100 µL substrate solution was added to each well (0.5 mg/mL ortho-phenylene diamine and 0.5 µL /mL 30 % hydrogen peroxide in 0.05 M dibasic sodium phosphate and 0.024 M citrate acid, pH 5.0). The plates were incubated in the dark at RT for at least 30 min. The reaction was stopped by adding 100 µL 0.5 M sulfuric acid to each well. Absorbance was read at 490 nm with a micro plate reader (Spectra Rainbow, Tekka).

The inter assay CV (coefficient of variation) was 9.5 % and the intra assay CV was 11.7 %. The detection limit was 7 ng/mL Vtg. However, due to matrix effects plasma samples had to be diluted at least 1:50. This resulted in a detection limit of 0.35 µg/mL Vtg. For statistical analysis, values below the detection limit were set at 0.35 µg/mL Vtg. According to Vethaak et al. (2002), concentrations of 1 µg/mL Vtg were defined as threshold of Vtg induction in male trout and therefore considered as induced male fish.

Data analysis

Most of the data did not meet statistical requirements for normality and were analyzed by nonparametric techniques. To compare the values between the different sites, the Kruskal-Wallis test was applied. Subsequent multiple comparisons were carried out using the Mann-Whitney-U test (Bonferroni corrected). Significance levels were $p \leq 0.05$. Statistical analysis was carried out using SPSS for Windows (Version 11.0.1).

Results

Despite our efforts to obtain fish of a similar age class (preferred immature fish), this was not always achieved. The age of brown trout sampled in the four test areas ranged from 0+ to 3+ (Table 3). Though, most of the fish were young of the year (0+) or one year (1+) old. The mean CFs in males ranged from 0.91 to 1.22 and in females from 0.90 to 1.04. For almost all areas, the mean CFs were lower at the head water sites compared to the downstream sites (Table 3), though significant differences between sites were restricted to the year 2002 (Table 3). The comparison of male and female CFs revealed higher values in males compared to females at almost all sites (Wilcoxon signed ranks test, $n = 21$; $Z = -3.228$; $p = 0.001$). However, when comparing male and female fish from each site, this trend was only significant at Necker D1 ($p = 0.024$) and LBK D1 ($p = 0.016$) in the year 2003.

Plasma vitellogenin concentrations in male brown trout did not differ between the sampling sites (Table 4). The Vtg concentrations were almost all near the detection limit (DL = 0.35 µg/mL) or lower. In only 10 out of 197 males (~5 %) Vtg values exceeded 1.0 µg/mL; the highest Vtg value (8.3 µg/mL) was found at Necker D1.

In females, the plasma Vtg values ranged from 0.35 to 70 µg/mL. Plasma Vtg concentrations of female fish caught in 2002 at Venoge D1 and D2 were significantly lower compared to Venoge HW (Table 4). In females captured in 2003 at Necker D2 (but not 2002), concentrations of Vtg were significantly lower than in females from the HW site (Table 3). Female Vtg was associated with the stage of ovarian development (Fig. 2a), but not with the age of fish (Fig. 2b).

Large variations in GSI values were noted predominantly in male fish (mean male GSI = 0.05–1.43), while variability in females was relatively low (mean female GSI = 0.16–0.78). In five out of seven samplings (males) as well as in five out of seven cases (females) the mean GSI were highest in the HW region. These differences were significantly different for certain sampling sites (Table 3). However, most of the observed differences were associated with the age of the examined specimen (Table 2 and Table 3; e.g. 1+ fish with a GSI of 0.3 were caught at Necker HW whereas 0+ fish with a GSI < 0.2 were caught at Necker D1 and D2).

The overall sex ratio was arround 1:1, although in five out of 21 sampling events more females were caught – in particular at LBK, but also at one site in the Necker River (Table 4). None of the males showed indications of intersex or other histological alterations in their testes, except for a one year old male fish from Venoge D1. The testes of this fish had a lamellar structure, which is a common histological feature of female gonads (Fig. 1f). The affected gonad did not contain oocytes, however, and was mostly filled with spermatocytes and spermatids. Therefore, it was regarded as a male fish. Most of the males remained in the pre-spermatogenic stage (66 %), whereas 26 % (early-spermatogenic stage) and 8 % (mid-spermatogenic) comprised advanced stages of spermatogenic

Table 3. Age, length, weight and condition factor (CF) of male and female brown trout sampled at Venoge, Emme, Necker and LBK (Liechtensteiner Binnenkanal) in 2002 and 2003. The data are presented as mean ± standard deviation.

Test area	Site	N		Age (year)		Length (cm)		Weight (g)		CF	
		male	female	male	female	male	female	male	female	male	female
2002											
Venoge	**HW**	12	15	1 ± 0	1 ± 0	15.0 ± 1.1	14.5 ± 1.3	33 ± 6	28 ± 7	0.96 ± 0.1	0.94 ± 0.1
	D1	10	15	1 ± 0	1 ± 0	16.2 ± 0.7	15.5 ± 1.4	43 ± 9	38 ± 10	1.00 ± 0.1	1.00 ± 0.1
	D2	16	8	1 ± 0	1 ± 0	15.7 ± 1.4	15.1 ± 1.9	36 ± 8	34 ± 13	0.92 ± 0.1	0.91 ± 0.1
Emme	**HW**	10	13	1 ± 0	1 ± 0	13.6 ± 0.7	13.2 ± 1.0	25 ± 4	22 ± 4	0.96 ± 0.1[1]	0.95 ± 0.1
	D1	12	7	1.4 ± 0.7	1.7 ± 0.5	19.8 ± 1.4	20.2 ± 2.1	81 ± 15	85 ± 27	1.04 ± 0.1[1]	1.00 ± 0.1
	D2	4	2	1.5 ± 0.6	1.5 ± 0.7	18.5 ± 1.7	20.1 ± 2.3	66 ± 20	86 ± 27	1.03 ± 0.1	1.04 ± 0.1
Necker	**HW**	10	10	1 ± 0	1 ± 0	11.0 ± 0.7	10.5 ± 1.1	12 ± 2	11 ± 3	0.91 ± 0.1[2]	0.90 ± 0.1[3]
	D1	12	10	0 ± 0	0 ± 0	10.1 ± 0.5	10.2 ± 0.6	10 ± 2	11 ± 2	0.99 ± 0.1[2]	0.98 ± 0.1[3,4]
	D2	11	19	0 ± 0	0 ± 0	9.7 ± 0.8	10.1 ± 0.6	9 ± 2	10 ± 2	0.94 ± 0.1	0.93 ± 0.1[4]
LBK	**HW**	9	16	1.8 ± 0.7	1.5 ± 0.6	12.9 ± 1.9	12.3 ± 1.7	22 ± 9	20 ± 9	0.99 ± 0.1[5]	1.00 ± 0.1[6]
	D1	9	18	1.9 ± 0.3	2.1 ± 0.6	14.8 ± 1.7	15.2 ± 1.9	36 ± 13	37 ± 12	1.07 ± 0.1[5]	1.03 ± 0.1[6]
	D2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
2003											
Venoge	**HW**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
	D1	11	9	1 ± 0	1 ± 0	14.8 ± 1.7	15.4 ± 1.6	32 ± 10	36 ± 10	0.95 ± 0.1	0.97 ± 0.1
	D2	10	10	1 ± 0	1 ± 0	15.0 ± 2.3	14.2 ± 1.4	32 ± 12	27 ± 9	0.92 ± 0.1	0.91 ± 0.1
Emme	**HW**	8	6	1 ± 0	1 ± 0	13.5 ± 0.8	13.7 ± 0.8	25 ± 4	25 ± 4	0.99 ± 0.1	0.96 ± 0.1
	D1	9	11	1 ± 0	1 ± 0	18.3 ± 1.2	18.7 ± 1.7	64 ± 13	67 ± 17	1.03 ± 0.1	1.01 ± 0.1
	D2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Necker	**HW**	9	10	1.4 ± 0.5	1.3 ± 0.5	14.0 ± 1.9	14.4 ± 1.3	27 ± 9	28 ± 7	0.96 ± 0.1	0.93 ± 0.1
	D1	9	11	0.4 ± 0.5	0.3 ± 0.5	12.6 ± 0.5	12.7 ± 1.5	20 ± 2	21 ± 9	1.01 ± 0.1[7]	0.96 ± 0.1[7]
	D2	7	9	0.7± 0.5	0.6 ± 0.5	14.2 ± 2.2	15.6 ± 3.3	28 ± 12	39 ± 21	0.94 ± 0.1	0.91 ± 0.1
LBK	**HW**	5	14	1.4 ± 0.6	1.4 ± 0.7	13.3 ± 1.3	12.6 ± 1.3	22 ± 6	19 ± 6	0.95 ± 0.1	0.92 ± 0.1
	D1	8	5	1.9 ± 0.4	1.6 ± 0.6	14.8 ± 1.3	15.3 ± 1.0	41 ± 18	35 ± 7	1.22 ± 0.4[8*]	0.96 ± 0.1[8]
	D2	4	11	1.3 ± 0.5	1.3 ± 0.5	12.5 ± 2.3	14.2 ± 1.2	22 ± 11	28 ± 7	1.03 ± 0.1	0.98 ± 0.1

HW = head water; D1 = downstream 1; D2 = downstream 2; n.d. = not determined.
CF (condition factor) = (weight × 100)/length^3.
Values with same super fix are significantly different from each other ($p \leq 0.05$).
* We excluded two points, because of some uncertainty (CF = 1.03 ± 0.1), but the difference between males and females remained significant ($p \leq 0.05$).

Table 4. Gonadosomatic index (GSI), gonadal development and vitellogenin (Vtg) in male and female brown trout sampled at Venoge, Emme, Necker and LBK (Liechtensteiner Binnenkanal) in 2002 and 2003. The data are presented as mean ± standard deviation, except for Vtg. The Vtg values were presented as median and (minimum-maximum).

Test area	Site	N		GSI	testis stage	GSI	oocyte stage	Vtg (µg/mL)		Number of fish below LOD (%)	
		male	female	male		female		male	female	male	female
2002											
Venoge	**HW**	12	15	1.29 ± 1.2[1,2]	1–3	0.30 ± 0.1	2–4	0.35 (0.35–2.52)	1.21 (0.35–62.8)[3,4]	83	13
	D1	10	15	0.09 ± 0.1[1]	1–2	0.23 ± 0.7	1–2	0.35 (0.35–0.53)	0.53 (0.35–2.49)[3]	90	26
	D2	16	8	0.06 ± 0.1[2]	1–2	0.24 ± 0.5	2	0.35 (0.35–1.35)	0.35 (0.35–0.45)[4]	81	63
Emme	**HW**	10	13	1.06 ± 1.9	1–3	0.17 ± 0.1[5]	2	0.35 (0.35–0.52)	0.96 (0.35–0.52)	90	7
	D1	12	7	0.38 ± 0.5	1–3	0.78 ± 0.8[5]	3–4	0.35 (0.35–0.46)	3.27 (0.35–58.41)	67	14
	D2	4	2	0.21 ± 0.2	1–2	0.21 ± 0.1	3	0.35 (0.35–0.35)	0.35 (0.35–0.35)	100	100
Necker	**HW**	10	10	0.26 ± 0.3[6,7]	1–2	0.30 ± 0.1[8,9]	2–3	0.35 (0.35–1.41)	1.38 (0.49–6.33)	70	0
	D1	12	10	0.05 ± 0.1[6]	1	0.14 ± 0.1[8]	2	0.35 (0.35–4.37)	2.13 (0.80–14.82)	58	0
	D2	11	19	0.06 ± 0.0[7]	1	0.13 ± 0.1[9]	2–3	0.35 (0.35–0.48)	1.98 (0.53–5.12)	90	0
LBK	**HW**	9	16	0.29 ± 0.5	1–2	0.39 ± 0.3	2–4	0.35 (0.35–0.98)	1.38 (0.35–70.96)	33	6
	D1	9	18	1.43 ± 1.71	1–3	0.27 ± 0.1	2–3	0.38 (0.35–0.81)	2.13 (0.40–12.27)	33	0
	D2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
2003											
Venoge	**HW**	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
	D1	11	9	0.08 ± 0.1	1	0.22 ± 0.1[10]	2	0.37 (0.35–1.01)	0.69 (0.41–2.06)	46	0
	D2	10	10	0.05 ± 0.1	1	0.30 ± 0.1[10]	2–3	0.50 (0.35–5.00)	0.54 (0.35–1.02)	40	40
Emme	**HW**	8	6	0.33 ± 0.8	1–2	0.16 ± 0.1	2	0.35 (0.35–0.42)	1.65 (0.89–4.47)	50	0
	D1	9	11	0.10 ± 0.1	1–2	0.17 ± 0.1	2–4	0.36 (0.35–1.42)	0.83 (0.38–14.62)	89	0
	D2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.		
Necker	**HW**	9	10	2.07 ± 2.4[11,12]	1–2	0.38 ± 0.1[13,14]	2–4	0.35 (0.35–0.39)	5.53 (0.64–57.29)[15]	78	0
	D1	9	11	0.03 ± 0.1[11]	1	0.22 ± 0.3[13]	2	0.35 (0.35–8.34)	3.46 (2.14–24.34)	88	0
	D2	7	9	0.03 ± 0.1[12]	1	0.16 ± 0.1[14]	2–3	0.35 (0.35–0.61)	1.11 (0.35–4.29)[15]	71	11
LBK	**HW**	5	14	0.50 ± 0.6	1–2	0.32 ± 0.2	2–4	0.35 (0.35–1.93)	1.41 (0.35–4.63)	80	7
	D1	8	5	1.82 ± 1.5	1–2	0.25 ± 0.1	2	0.35 (0.35–1.61)	1.82 (0.35–3.12)	75	20
	D2	4	11	1.84 ± 3.5	1–2	0.26 ± 0.1	2–4	0.35 (0.35–0.35)	1.65 (0.46–5.97)	100	0

HW = head water; D1 = downstream 1; D2 = downstream 2 n.d. = not determined.
GSI (gonadosomatic index) = 100 × gonad weight/(body weight – gonad weight).
LOD = Limit of detection (Vtg = 0.35 µg/mL).
[1–17] Values with same super fix are significantly different ($p \leq 0.05$).

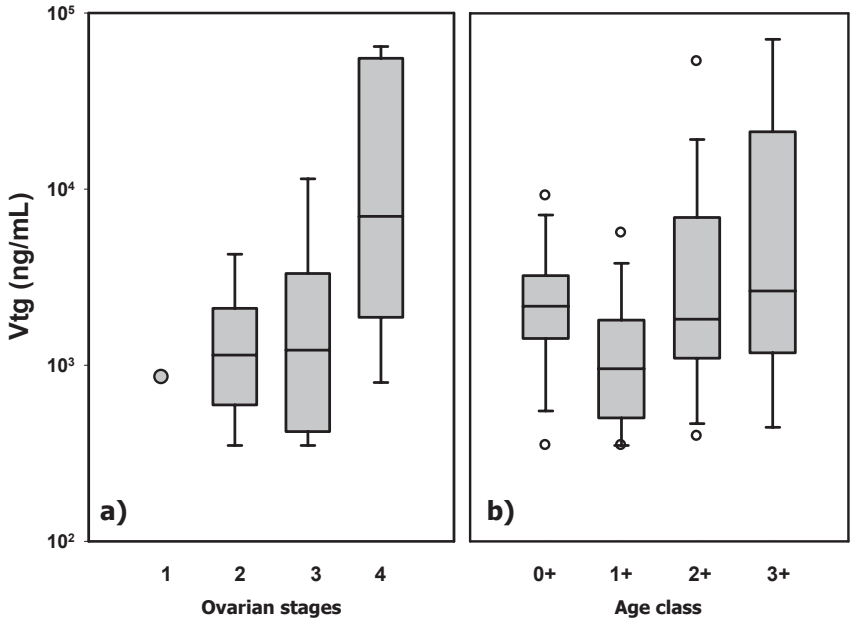

Figure 2. Plasma vitellogenin (Vtg) in dependence on stage of (a) ovarian development (ovarian stage) and (b) age of female brown trout sampled at Venoge, Emme, Necker and LBK (Liechtensteiner Binnenkanal) in 2002 and 2003 (n = 1-182). The data are presented as box plots indicating the median, 75th and 95th percentiles. Gray dot represents one single data point (n = 1) and white circles signify outliers. (1 = early perinuclear stage; 2 = late perinuclear stage; 3 = cortical alveolar stages; 4 = vitellogenic stage).

development. None of the males had testes in late spermatogenic or matured stages.

In contrast to males, we found intersex in females caught at LBK and Venoge in both years. Some ovaries contained several spermatogenic nests, mostly located near the ovarian lamellae. The nests were filled predominately with spermatocytes and spermatids, but also contained some spermatozoa as evidenced by selective staining. The occurrence of intersex (prevalence between 5 and 27 %) was not solely restricted to the downstream sites, but also appeared at one site with no STW effluent input (for details, please see figures and tables in Körner et al., 2005).

Most of the female fish remained at the late perinucleolar stage (80 %), whereas only 12 % (cortical alveolar stage) and 8 % (vitellogenic stage) of the females showed a higher stage of ovarian development. One female at Venoge D1 showed no indications of ovarian development. All her oocytes were at the early perinucleolar stage.

The occurrence of ovarian atresia was relatively low at almost all examined sites and ranged from 0 to 28 %. The highest values of atresia were found in fish from Emme D1 in 2002. Atresia at Emme D1 was significantly elevated compared to Emme HW in 2002 ($p < 0.001$) and 2003 ($p = 0.044$; Fig. 3). This downstream increase of atretic follicles also appeared at the Necker in 2003. Atresia at Necker D1 and D2 was significantly higher than at the corresponding HW site (Fig. 3). The severity of atresia in females caught in 2002 was significantly higher ($p < 0.001$) than in 2003 (Fig. 4), but a clear connection between atresia and stage of ovarian development was absent (Fig. 5).

In ovaries of female brown trout at Venoge D1 we observed high incidences of parasites with an occurrence of 47 % in 2002 and 44 % in 2003. Linked to the occurrence of the parasites, the surrounding tissue showed severe fibrosis (Fig.1e). Females from all other sampling sites as well as all male fish showed no indications of gonadal parasitism. On the basis of several characteristics (e.g. everted proboscis with non-spined collar, spines on the cuticle at the posterior end as well as thick hypodermal layer of felted and cross fibers), the parasites were assigned to the phylum acanthocephalan (Dr. Sarah Poynton, IGB Berlin, Germany, personal communication). A more detailed determination (e.g. genus) would have required the preparation of complete parasites out of ovarian tissue.

Discussion

The present study aimed to assess the hypothesis that certain reproductive parameters in brown trout are impaired and that reduced reproductive health could contribute to the declining fish catch in Swiss rivers. In general, our results do not indicate any significant perturbations of brown trout reproductive health.

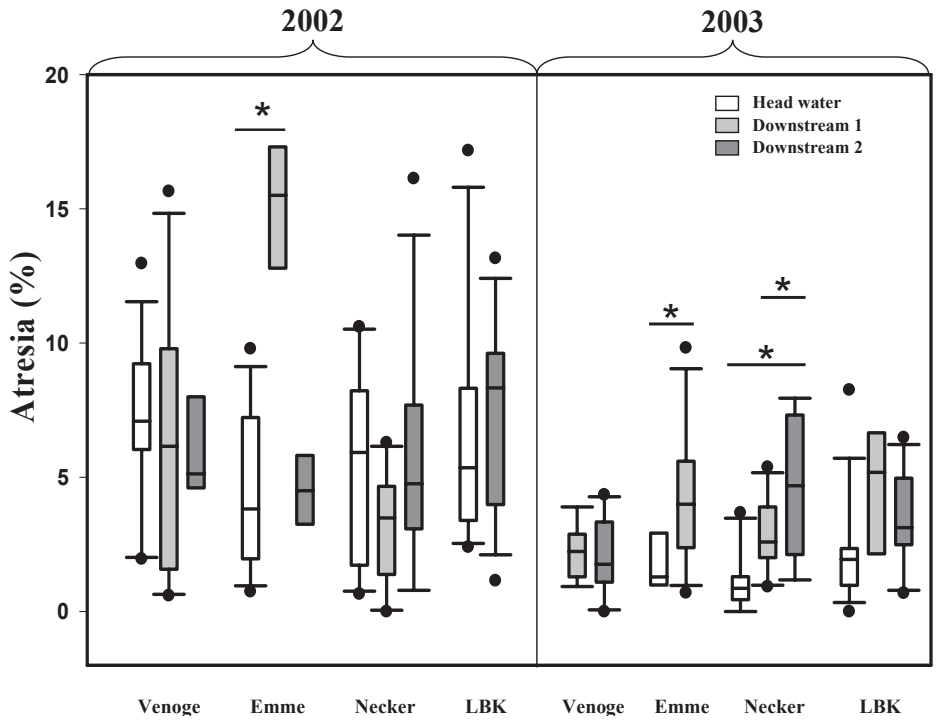

Figure 3. Ovarian atresia in female brown trout sampled at Venoge, Emme, Necker and LBK (Liechtensteiner Binnenkanal) in 2002 and 2003 (n = 2–18). The data are presented as box plots indicating the median, 75th and 95th percentiles. The black dots signify outliers. Asterisks denote significant differences between sampling sites ($p \leq 0.05$).

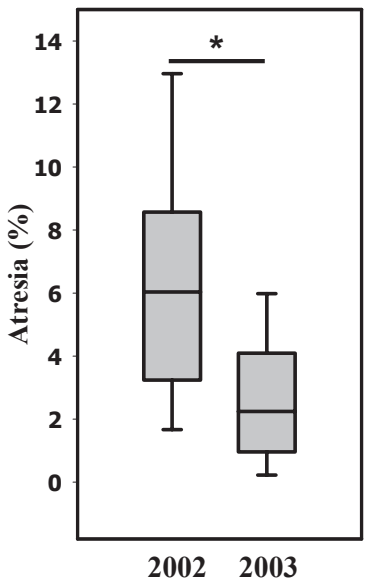

Figure 4. Ovarian atresia in female brown trout sampled in 2002 (n = 126) and 2003 (n = 93). The data are presented as box plots indicating the median, 75th and 95th percentiles. Asterisk denotes a significant difference between groups ($p \leq 0.001$).

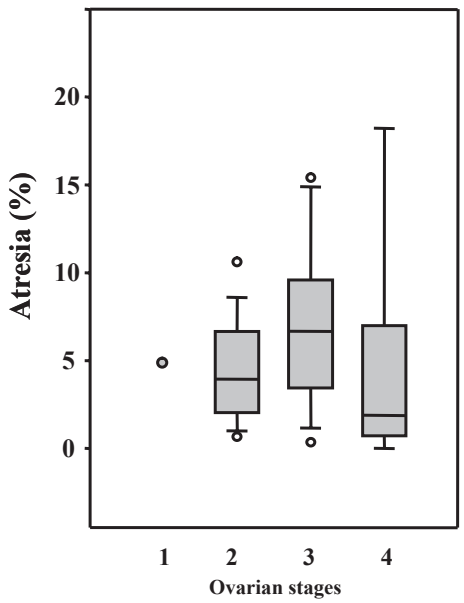

Figure 5. Female atresia in relation to the stage of ovarian development in brown trout sampled at Venoge, Emme, Necker and LBK (Liechtensteiner Binnenkanal) (1 = early perinuclear stage (n = 1); 2 = late perinuclear stage (n = 179); 3 = cortical alveolar stages (n = 25); 4 = vitellogenic stage (n = 17)). The data are presented as box plots indicating the median, 75th and 95th percentiles. Gray dot represents one single data point (n = 1) and white circles signify outliers.

In order to assess a potential exposure of brown trout to estrogenic compounds in STW effluents we measured the estrogen dependent yolk precursor Vtg in plasma of feral brown trout. We only found a few induced males (~5 %) and there was no site specific pattern to this induction. This indicates that the exposure of brown trout to environmental estrogens is low - at least in the four rivers we investigated. It may be that brown trout are not sensitive enough to be an adequate sentinel for environmental estrogens. The sensitivity of fish to certain xenoestrogens varies between different species (Van den Belt et al., 2003; Tyler et al., 2005). However, previous studies on brown trout in Switzerland provided evidence of Vtg induction in males exposed to STW effluents at various hot spots (Wahli et al., 1998; Vermeirssen et al., 2005a, Vermeirssen et al., 2005b). The lack of Vtg in our survey is probably connected with the relatively low effluent load at the investigated downstream sites. In a study by Harries et al. (1999) a significant increase of Vtg in rainbow trout was observed after exposure to at least 25 % effluent. Even under dry weather conditions the effluent percentage at our downstream sampling sites only reaches up to 10 % (LBK), which is quite low. Consequently, the concentrations of steroidal estrogens – thought to be the main contributors to estrogenicity of domestic effluent (Desbrow et al., 1998; Routledge et al., 1998) – in river water are low. This assumption is supported by calculations made by Suter and colleagues (unpublished data, see also Table 1). They modeled the estrogenicity (calEEQs, calculated estradiol equivalents) in three test areas (Venoge, Emme and Necker). The calEEQs only reached values up to 0.3 ng/L at Q_{median} (Table 1) and were in the low range compared to EEQ values measured in Swiss rivers using a recombinant yeast assay (Vermeirssen et al., 2005a; Vermeirssen et al., 2005b). These surveys observed induced Vtg in feral male brown trout at EEQs exceeding ca. 0.8 ng/L.

The female Vtg values observed in the present study appeared to be within the range previously reported for brown trout (Crim and Idler, 1978; Norberg et al., 1989; Burki et al., 2006) and showed a strong association with the stage of ovarian development. However, females from the downstream Venoge (in 2002) and Necker (in 2003) sites revealed significantly lower Vtg concentrations compared to females caught at the HW sites. Reduced female plasma Vtg has recently been used as an indicator of endocrine disruption (Solé et al., 2003a). In that study, female carp collected in the vicinity of STW showed lower Vtg concentrations than fish from upstream and downstream sites as a result of disturbed estrogen homeostasis. Prior to our sampling periods, the temperature in the lower parts of the rivers were considerably higher than in the colder headwaters. Therefore, reduced Vtg at downstream sites in the present study may be linked to this elevated water temperature, which is known to alter the physiology of fish considerably (Wendelaar Bonga, 1997). Previous studies showed that a high summer temperature during natural vitellogenesis of female Atlantic salmon (*Salmo salar*) is associated with a reduction of plasma Vtg (King et al., 2003) - an effect primarily linked to increased plasma cortisol levels (Mommsen et al., 1999; Berg et al., 2004). Ojanguren et al. (2001) reported an upper temperature optimum of brown trout around 20 °C. The river water temperature in the test areas often exceeded 20 °C and in 2003 it reached values up to 25 °C (Hari et al., 2005) – a temperature range known to stimulate a cortisol response in salmonids (Strange et al., 1977). Therefore, the elevated water temperatures during summer probably stressed the fish and may have provoked an inhibition of Vtg synthesis at downstream sites similar to the effects observed by King et al. (2003).

An inhibition of Vtg synthesis may not only influence female fish, but would also affect the assessment of exposure to environmental estrogens using male Vtg as biomarker. A lack of Vtg induction in exposed males could be wrongly interpreted as “not exposed” and therefore lead to an underestimation of the actual exposure. Therefore, subsequent studies are needed to test the influence of temperature stress on Vtg expression in males after exposure to environmental estrogens and finally the applicability of brown trout monitoring studies during the summer months.

The abundant appearance of intersex among gonochoristic fish populations is increasingly ascribed to environmental estrogens entering the aquatic system via STW effluents (Jobling et al., 1998). Although we observed intersex in female brown trout in two of the four rivers, this intersex does not appear to be linked with estrogenic exposure (Körner et al., 2005). This is supported by the absence of Vtg induction in males as well as the low values of atretic follicles in female trout. Additionally, the intersex described in Körner et al. (2005) is completely different from the type commonly observed in estrogen exposed fish. Usually, single or clusters of follicular oocytes randomly distributed within testicular tissue (ovotestis), but also clear zoned ovarian and testicular tissues within one gonad, are reported (Jobling et al., 1998; Nolan et al., 2001; Blazer, 2002). In contrast, Körner et al. (2005) observed small nests filled with all stages of spermatogenesis occupying less than 1 % of the gonads. This finding, with a prevalence up to 27 %, is consistent with field observations in Danish brown trout (Christiansen and Plesner, 2001), but also whitefish (Mikaelian et al., 2002) and pike (Vine et al., 2005), reporting a prevalence between 12 % and 26 %.

The assessment of potential implications of intersex on feral brown trout population remains difficult for several reasons. Although brown trout at LBK showed a female biased population, this situation was not present at the River Venoge. Beyond this, the number of fish (n =

6–30) per sampling date is too low to make reliable statements regarding altered sex ratios. As sex markers of brown trout are still missing, the genetic sex of the affected fish could not be determined. While many laboratory studies established a strong association between certain environmental estrogens and ovotestis (i.e. feminization), relatively little is known about effects of environmental androgenic and anti-estrogenic substances on histological masculinisation of fish. In particularly, with our measurement of Vtg we focused on a possible role of environmental estrogens. However, the fish at most of our sampling sites were obviously exposed to a variety of micropollutants that are carried to the aquatic ecosystem by STW effluents and diffuse sources.

Finally, it is not clear, whether the intersex has a chemical etiology or is a natural phenomenon. The latter does seem to be more likely, because some types of intersex often seen in male fish (i.e. feminization, ovotestis) were not solely restricted to areas in the vicinity of STWs, but also at sites with low or no effluent load (e.g. van Aerle et al., 2001; Faller et al., 2003; Bernet et al., 2004). Additional research will be necessary to provide more details about the natural occurrence of intersex in salmonids (e.g. Kinnison et al., 2000), which are commonly considered as strictly gonochoristic fish species (Woram et al., 2003).

The incidence of ovarian atresia in brown trout was generally low and can be considered as being natural background levels for most of the sites (Billard, 1987; Leino et al., 2005). Highest values were found in females caught at site Emme D1 in 2002 (mean = 15 %). Wood and Van Der Kraak (2001) discussed atresia (and apoptosis respectively) in the context of natural ovarian growth and post ovulatory regression. In our study we did not observe a correlation between ovarian stage and atresia. This may mean that atresia is indicative of stressful conditions for fish inhabiting Emme D1 in 2002. However, the impact of increased atresia on population relevant parameters (e.g. fecundity or egg size) remains vague. For instance, the study by Janz et al. (1997) observed elevated atresia after exposure to bleached kraft mill effluent, but observed no significant reduction in egg fecundity. Ovarian atresia has been associated with different stress factors including starvation, water pollution or unfavorable temperature regimes (Blazer, 2002). Condition factors were all above 0.8, thus little evidence of nutrition deficits was seen. High frequencies of follicular atresia in females inhabiting polluted river water have been reported (Janz et al., 1997; Adams et al., 1999; Jobling et al., 2002). In these studies, atresia was mainly related to certain chemicals like environmental estrogens or pesticides. Indeed, certain environmental estrogens (e.g. nonylphenol or quercetin) as well as pesticides (e.g. atrazine) are known to enhance atresia in fish (Miles-Richardson et al., 1999; Spano et al., 2004; Weber et al., 2002). We did not measure concentrations of estrogens; however, on the basis of low plasma Vtg levels in males, the concentrations of estrogenic contaminants appear to be low. This is the same with respect to the measured pesticides. Although chemical analysis provided evidence of various pesticides (Table 1) in the Emme, the highest concentrations of e.g. atrazine are approximately 100 fold lower than the concentrations used by Spano et al. (2004).

Given the argument that elevated temperatures tend to increase ovarian atresia (Wallace and Selman, 1981; Blazer, 2002), one would expect, that atresia in 2003 would be higher than in 2002 due to the high water temperature. For unknown reasons, atretic oocytes were less abundant in summer 2003. Therefore, higher temperatures in 2003 had apparently no adverse effects on female gonads. This is in contrast to the finding in other fish species like sturgeon or medaka. White sturgeons (*Acipenser transmontanus*) exposed to an increased water temperature showed an increase of follicular atresia (Linares-Casenave et al., 2002). Furthermore, Koger et al. (1999) observed severe incidences of atresia in the medaka (*Oryzias latipes*) after increasing water temperatures.

Gonadal alterations caused by parasites or diseases may provoke significant adverse effects on the reproductive fitness of fish (Barber et al., 2000; Hecker and Karbe, 2005). Previous studies reported a great variety of metazoan parasites, which were found in gonadal tissue of feral fish. The major groups identified in these investigations were trematodes, cestodes as well as nematodes (Blazer, 2002). In contrast, species belonging to the phylum acanthocephala are predominantly parasites inhabiting the gastrointestinal tract of fish (Schäperclaus, 1990; Bakke and Harris, 1998). Therefore, the observation made in the present study describing acanthocephalan parasites in the ovary of feral salmonids is at least something uncommon if not unique. Interestingly, the parasites were only found at one site (Venoge D1). In addition, their prevalence was obviously restricted to females. The reasons for the apparent selection of female trout as well as potential consequences on the female reproductive fitness are not yet assessable. In female Bucchich's goby (*Gobius bucchichi*), however, the abundance of acanthocephalan parasites was negatively correlated with the GSI and parasitism reduces the number of eggs of the host (Sasal et al., 2001). The well studied cestode *Ligula intestinalis* interferes with normal gonadal hormone production of cyprinids resulting in a suppressed development of the gonads (Jobling and Tyler, 2003b). Furthermore, sticklebacks infected with the cestode *Schistocephalus sp.* had smaller gonads and were less reproductively active than uninfected fish (Barber et al., 2000).

The histological evaluation of gonadal development as well as the results of GSI in males and females revealed similar data as previously reported for brown trout (Billard, 1987). While females start their first reproductive cycle as 2+ or 3+ fish, males usually start their first

gametogenesis as 1+ fish (Billard, 1987). This is consistent with the data of the current survey; apart from one 1+ female caught at Venoge HW which showed vitellogenic oocytes and elevated plasma Vtg. As the subjects of our study were predominantly juvenile brown trout, most of the surveyed brown trout were reproductively inactive or just at the beginning of gonadal recrudescence. In addition, particularly in 2002 significant differences of GSI originated mainly from the separated day of sampling.

Condition factors of male and female brown trout in the four test areas were within the range previously reported for feral brown trout in Switzerland (Kobler, 2004; Burki et al., 2006). Interestingly, almost all HW sites showed lower mean CFs than sites downstream of STWs. Weatherly and Gill (1987) proposed that low CFs may be indicators of a low feeding status by reason of food limitation. Indeed, Kobler (2005) also observed higher CF values in fish caught downstream of STWs compared to fish living in the upper river reaches. Data in Table 1 show that the availability of nutrients (nitrogen and phosphor) was generally (and noticeably) higher at the downstream sites. Hence the higher CFs at the downstream sites might be related to higher concentrations of nutrients and possibly an increased food supply. Nevertheless, the mean CF observed in the present survey was well above 0.8 at all sites, indicating no severe lack of food, even at the HW sites. The higher mean CF in males compared to females is probably due to sex dimorphisms (in respect to morphometric parameters) often seen in salmonid species (Reyes-Gavilan et al., 1997; Casselman and Schulte-Hostedde, 2004).

In conclusion, male Vtg and female ovarian atresia data indicate that the effects of estrogenic compounds on brown trout in the examined rivers are relatively minor. Consequently, impaired reproductive health, as a result of exposure to environmental estrogens, does not appear to be a major factor contributing to the marked decline of brown trout catch in the four rivers in our study, though other micropollutants may play a role. In this light, it must also be noted that we did not assess population relevant parameters such as gamete quality or recruitment. In addition, although previous studies documented the occurrence of estrogenic compounds in several Swiss STW effluents (Aerni et al., 2004; Rutishauser et al., 2004), the effects are more likely restricted to a few hot spots (see also Vermeirssen et al., 2005a) which were not investigated here.

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References

Adams, S. M., M. S. Bevelhimer, M. S. Greeley, D. A. Levine and S. J. The, 1999. Ecological risk assessment in a large river-reservoir: 6. Bioindicators of fish population health. Environmental Toxicology and Chemistry **18**: 628–640.

Aerni, H.-R., B. Kobler, B. V. Rutishauser, F. E. Wettstein, R. Fischer, W. Giger, A. Hungerbuhler, M. D. Marazuela, A. Peter, R. Schönenberger, A. C. Vogeli, M. J.-F. Suter and R. I. L. Eggen, 2004. Combined biological and chemical assessment of estrogenic activities in wastewater treatment plant effluents. Analytical and Bioanalytical Chemistry **378**: 688–696.

Arcand-Hoy, L. D. and W. H. Benson, 1998. Fish reproduction: An ecologically relevant indicator of endocrine disruption. Environmental Toxicology and Chemistry **17:** 49–57.

Bakke, T. A. and P. D. Harris, 1998. Diseases and parasites in wild Atlantic salmon (*Salmo salar*) populations. Canadian Journal of Fisheries and Aquatic Sciences **55:** 247–266.

Barber, I., D. Hoare and J. Krause, 2000. Effects of parasites on fish behaviour: a review and evolutionary perspective. Reviews in Fish Biology and Fisheries **10:** 131–165.

Barnhoorn, I. E. J., M. S. Bornman, G. M. Pieterse and J. H. J. van Vuren, 2004. Histological evidence of intersex in feral sharptooth catfish (*Clarias gariepinus*) from an estrogen-polluted water source in Gauteng, South Africa. Environmental Toxicology **19:** 603–608.

Berg, A. H., L. Westerlund and P. E. Olsson, 2004. Regulation of Arctic char (*Salvelinus alpinus)* egg shell proteins and vitellogenin during reproduction and in response to 17 beta-estradiol and cortisol. General and Comparative Endocrinology **135:** 276–285.

Bernet, D., T. Wahli, C. Kueng and H. Segner, 2004. Frequent and unexplained gonadal abnormalities in whitefish (central alpine *Coregonus sp*.) from an alpine oligotrophic lake in Switzerland. Diseases of Aquatic Organisms **61:** 137–148.

Billard, R., 1987. The reproductive cycle of male and female brown trout (*Salmo trutta fario*) – A quantitative study. Reproduction Nutrition Development **27:** 29–44.

Blazer, V. S., 2002. Histopathological assessment of gonadal tissue in wild fishes. Fish Physiology and Biochemistry **26:** 85–101.

Borsuk, M. E., P. Reichert, A. Peter, E. Schager and P. Burkhardt-Holm, 2006. Assessing the decline of brown trout (*Salmo trutta*) in Swiss rivers using a Bayesian probability network. Ecological Modelling **192**: 224–244

Burkhardt-Holm, P., A. Peter and H. Segner, 2002. Decline of fish catch in Switzerland – Project Fishnet: A balance between analysis and synthesis. Aquatic Sciences **64:** 36–54.

Burkhardt-Holm, P. and K. Scheurer, 2007. Application of the weight-of-evidence approach to assess the decline of brown trout (*Salmo trutta*) in Swiss rivers. Aquatic Sciences **69(1):** DOI 10.1007/s00027-006-0841-6

Burki, R., E. L. M. Vermeirssen, O. Körner, C. Joris, P. Burkhardt-Holm and H. Segner, 2006. Assessment of estrogenic exposure in brown trout (*Salmo trutta*) in a Swiss midland river. Environmental Toxicology and Chemistry **25:** 2077–2086.

Casselman, S. J. and A. I. Schulte-Hostedde, 2004. Reproductive roles predict sexual dimorphism in internal and external morphology of lake whitefish, *Coregonus clupeaformis*. Ecology of Freshwater Fish **13:** 217–222.

Christiansen, L. B. and T. Plesner, 2001. Intersex og andre effekter på reproduktionssystemet i skalle og bækørred – relationer til østrogener og østrogenlignende stoffer. Technical report, Arhus Amt, Denmark.

Crim, L. W. and D. R. Idler, 1978. Plasma gonadotropin, estradiol and vitellogenin and gonad phosvitin levels in relation to seasonal reproductive cycles of female brown trout. Annales de Biologie Animale Biochimie Biophysique **18:** 1001–1005.

Denslow, N. D., M. C. Chow, K. J. Kroll and L. Green, 1999. Vitellogenin as a biomarker of exposure for estrogen or estrogen mimics. Ecotoxicology **8:** 385–398.

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

Faller, P., B. Kobler, A. Peter, J. P. Sumpter and P. Burkhardt-Holm, 2003. Stress status of gudgeon (*Gobio gobio*) from rivers in Switzerland with and without input of sewage treatment plant effluent. Environmental Toxicology and Chemistry **22:** 2063–2072.

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

Frick, E., D. Nowak, C. Reust and P. Burkhardt-Holm, 1998. Der Fischrückgang in den schweizerischen Fliessgewässern. Gas Wasser Abwasser **4**: 261–264.

Friedl, C. 1999. Fischfangrückgang in schweizerischen Fliessgewässern. BUWAL, Bern.

Gercken, J. and H. Sordyl, 2002. Intersex in feral marine and freshwater fish from northeastern Germany. Marine Environmental Research **54:** 651–655.

Gimeno, S., A. Gerritsen, T. Bowmer and H. Komen, 1996. Feminization of male carp. Nature **384:** 221–222.

Götz, C., N. Chèvre, H. Singer and S. Müller, 2003. Emme, Necker, Liechtensteiner Binnenkanal, Venoge: Gebietscharakterisierung, Pestizidmessungen, Toxizitätsabschätzung. Fischnetz-Publikation. Swiss Federal Institute of Aquatic Science and Technology, Dübendorf, pp. 23,

Gray, M. A., A. J. Niimi and C. D. Metcalfe, 1999. Factors affecting the development of testis-ova in medaka, *Oryzias latipes*, exposed to octylphenol. Environmental Toxicology and Chemistry **18:** 1835–1842.

Hari, R. E., D. M. Livingstone, R. Siber, P. Burkhardt-Holm and H. Güttinger, 2006. Consequences of climatic change for water temperature and brown trout populations in Alpine rivers and streams. Global Change Biology **12:** 1377–1388.

Harries, J. E., A. Janbakhsh, S. Jobling, P. Matthiessen, J. P. Sumpter and C. R. Tyler, 1999. Estrogenic potency of effluent from two sewage treatment works in the United Kingdom. Environmental Toxicology and Chemistry **18:** 932–937.

Hecker, M. and L. Karbe, 2005. Parasitism in fish – an endocrine modulator of ecological relevance? Aquatic Toxicology **72:** 195–207.

Janz, D. M., M. E. McMaster, K. R. Munkittrick and G. Van Der Kraak, 1997. Elevated ovarian follicular apoptosis and heat shock protein-70 expression in white sucker exposed to bleached kraft pulp mill effluent. Toxicology and Applied Pharmacology **147**: 391–398.

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

Jobling, S., N. Beresford, M. Nolan, T. Rodgers-Gray, G. C. Brighty, J. P. Sumpter and C. R. Tyler, 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. and C. R. Tyler, 2003a. Endocrine disruption in wild freshwater fish. Pure and Applied Chemistry **75:** 2219–2234.

Jobling, S. and C. R. Tyler, 2003b. Endocrine disruption, parasites and pollutants in wild freshwater fish. Parasitology **126:** S103–S108.

Kavanagh, R. J., G. C. Balch, Y. Kiparissis, A. J. Niimi, J. Sherry, C. Tinson and C. D. Metcalfe, 2004. Endocrine disruption and altered gonadal development in white perch (*Morone americana*) from the lower Great Lakes region. Environmental Health Perspectives **112:** 898–902.

King, H. R., N. W. Pankhurst, M. Watts and P. M. Pankhurst, 2003. Effect of elevated summer temperatures on gonadal steroid production, vitellogenesis and egg quality in female Atlantic salmon. Journal of Fish Biology **63:** 153–167.

Kinnison, M. T., M. J. Unwin and F. Jara, 2000. Macroscopic intersexuality in salmonid fishes. New Zealand Journal of Marine and Freshwater Research **34:** 125–134.

Kobler, B., 2004. Effects of treated wastewater on trout: A case study of a Swiss river. Dissertation, Swiss Federal Institute of Technology, Zürich, 99 pp.

Koger, C. S., S. J. Teh and D. E. Hinton, 1999. Variations of light and temperature regimes and resulting effects on reproductive parameters in medaka (*Oryzias latipes*). Biology of Reproduction **61:** 1287–1293.

Körner, O., E. L. M. Vermeirssen and P. Burkhardt-Holm, 2005. Intersex in feral brown trout from Swiss midland rivers. Journal of Fish Biology **67:** 1734–1740.

Leino, R. L., K. M. Jensen and G. T. Ankley, 2005. Gonadal histology and characteristic histopathology associated with endocrine disruption in the adult fathead minnow (*Pimephales promelas*). Environmental Toxicology and Pharmacology **19:** 85–98.

Linares-Casenave, J., J. P. Van Eenennaam and S. I. Doroshov, 2002. Ultrastructural and histological observations on temperature-induced follicular ovarian atresia in the white sturgeon. Journal of Applied Ichthyology **18:** 382–390.

Lye, C. M., C. L. J. Frid, M. E. Gill and D. McCormick, 1997. Abnormalities in the reproductive health of flounder *Platichthys flesus* exposed to effluent from a sewage treatment works. Marine Pollution Bulletin **34:** 34–41.

Mikaelian, I., Y. de Lafontaine, J. C. Harshbarger, L. L. J. Lee and D. Martineau, 2002. Health of lake whitefish (*Coregonus clupeaformis*) with elevated tissue levels of environmental contaminants. Environmental Toxicology and Chemistry **21:** 532–541.

Miles-Richardson, S. R., V. J. Kramer, S. D. Fitzgerald, J. A. Render, B. Yamini, S. J. Barbee and J. P. Giesy, 1999. Effects of waterborne exposure of 17 beta-estradiol on secondary sex characteristics and gonads of fathead minnows (*Pimephales promelas*). Aquatic Toxicology **47:** 129–145.

Mommsen, T. P., M. M. Vijayan and T. W. Moon, 1999. Cortisol in teleosts: dynamics, mechanisms of action, and metabolic regulation. Reviews in Fish Biology and Fisheries **9:** 211–268.

Mousa, S. A. and M. A. Mousa, 1999. Immunocytochemical and histological studies on the hypophyseal-gonadal system in the freshwater Nile tilapia, *Oreochromis niloticus* (L.), during sexual maturation and spawning in different habitats. Journal of Experimental Zoology **284:** 343–354.

Nolan, M., S. Jobling, G. Brighty, J. P. Sumpter and C. R. Tyler, 2001. A histological description of intersexuality in the roach. Journal of Fish Biology **58:** 160–176.

Norberg, B., B. T. Bjornsson, C. L. Brown, U. P. Wichardt, L. J. Deftos and C. Haux, 1989. Changes in plasma vitellogenin, sex steroids, calcitonin and thyroid-hormones related to sexual-maturation in female brown trout (*Salmo trutta*). General and Comparative Endocrinology **75:** 316–326.

Ojanguren, A. F., F. G. Reyes-Gavilan and F. Brana, 2001. Thermal sensitivity of growth, food intake and activity of juvenile brown trout. Journal of Thermal Biology **26:** 165–170.

Reyes-Gavilan, F. G., A. F. Ojanguren and F. Brana, 1997. The ontogenetic development of body segments and sexual dimorphism in brown trout (*Salmo trutta* L). Canadian Journal of Zoology-Revue Canadienne de Zoologie **75:** 651–655.

Rolland, R., 2000. Ecoepidemiology of the effects of pollution on reproduction and survival of early life stages in teleosts. Fish and Fisheries **1:** 41–72.

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

Rutishauser, B. V., M. Pesonen, B. I. Escher, G. E. Ackermann, H.-R. Aerni, M. J.-F. Suter and R. I. L. Eggen, 2004. Comparative analysis of estrogenic activity in sewage treatment plant effluents involving three in vitro assays and chemical analysis of steroids. Environmental Toxicology and Chemistry **23:** 857–864.

Sasal, P., E. Faliex, I. De Buron and S. Morand, 2001. Sex discriminatory effect of the acanthocephalan *Acanthocephaloides propinquus* on a gobiid fish Gobius bucchichii. Parasite-Journal de la Societe Francaise de Parasitologie **8:** 231–236.

Schäperclaus, W., 1990. Fischkrankheiten, Akademie Verlag, Berlin, 1123 pp.

Solé, M., D. Raldua, D. Barceló and C. Porte, 2003a. Long-term exposure effects in vitellogenin, sex hormones, and biotransformation enzymes in female carp in relation to a sewage treatment works. Ecotoxicology And Environmental Safety **56:** 373–380.

Solé, M., D. Raldua, F. Piferrer, D. Barceló and C. Porte, 2003b. Feminization of wild carp, *Cyprinus carpio*, in a polluted environment: plasma steroid hormones, gonadal morphology and xenobiotic metabolizing system. Comparative Biochemistry and Physiology C-Toxicology and Pharmacology **136:** 145–156.

Spano, L., C. R. Tyler, R. van Aerle, P. Devos, S. N. M. Mandiki, F. Silvestre, J. P. Thome and P. Kestemont, 2004. Effects of atrazine on sex steroid dynamics, plasma vitellogenin concentration and gonad development in adult goldfish (*Carassius auratus*). Aquatic Toxicology **66:** 369–379.

Strange, R. J., C. B. Schreck and J. T. Golden, 1977. Corticoid stress responses to handling and temperature in salmonids. Transactions of the American Fisheries Society **106:** 213–218.

Sumpter, J. P. and S. Jobling, 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environmental Health Perspectives **103:** 173–178.

Tyler, C. R., C. Spary, R. Gibson, E. M. Santos, J. Shears and E. M. Hill, 2005. Accounting for differences in estrogenic responses in rainbow trout (*Oncorhynchus mykiss*: Salmonidae) and roach (*Rutilus rutilus*: Cyprinidae) exposed to effluents from wastewater treatment works. Environmental Science and Technology **39:** 2599–2607.

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

Van den Belt, K., P. W. Wester, L. T. M. Van der Ven, R. Verheyen and H. Witters, 2002. Effects of ethynylestradiol on the reproductive physiology in zebrafish (*Danio rerio*): Time dependency and reversibility. Environmental Toxicology and Chemistry **21:** 767–775.

Van den Belt, K., R. Verheyen and H. Witters, 2003. Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens. Ecotoxicology and Environmental Safety **56:** 271–281.

Vermeirssen, E. L. M., O. Körner, R. Schönenberger, M. J.-F. Suter and P. Burkhardt-Holm, 2005a. Characterization of environmental estrogens in river water using a three pronged approach: Active and passive water sampling and the analysis of accumulated estrogens in the bile of caged fish. Environmental Science and Technology **39:** 8191–8189.

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

Vethaak, A. D., J. Lahr, R. V. Kuiper, G. C. M. Grinwis, T. R. Rankouhi, J. P. Giesy and A. Gerritsen, 2002. Estrogenic effects in fish in the Netherlands: Some preliminary results. Toxicology **181:** 147–150.

Vigano, L., A. Arillo, S. Bottero, A. Massari and A. Mandich, 2001. First observation of intersex cyprinids in the Po River (Italy). Science of the Total Environment **269:** 189–194.

Vine, E., J. Shears, R. van Aerle, C. R. Tyler and J. P. Sumpter, 2005. Endocrine (sexual) disruption is not a prominent feature in the pike (*Esox lucius*), a top predator, living in English waters. Environmental Toxicology and Chemistry **24:** 1436–1443.

Wahli, T., W. Meier, H. Segner and P. Burkhardt-Holm, 1998. Immunohistochemical detection of vitellogenin in male brown trout from Swiss rivers. Histochemical Journal **30:** 753–758.

Wallace, R. A. and K. Selman, 1981. Cellular and dynamic aspects of oocyte growth in teleosts. American Zoologist **21:** 325–343.

Weatherly, A. H. and H. S. Gill, 1987. The biology of fish growth. Academic Press, New York, 443 pp.

Weber, L. P., Y. Kiparissis, G. S. Hwang, A. J. Niimi, D. M. Janz and C. D. Metcalfe, 2002. Increased cellular apoptosis after chronic aqueous exposure to nonylphenol and quercetin in adult medaka (*Oryzias latipes*). Comparative Biochemistry and Physiology C-Toxicology and Pharmacology **131:** 51–59.

Wendelaar Bonga, S. E., 1997. The stress response in fish. Physiological Reviews **77:** 591–625.

Wheeler, J. R., S. Gimeno, M. Crane, E. Lopez-Juez and D. Morritt, 2005, Vitellogenin: A review of analytical methods to detect (anti) estrogenic activity in fish. Toxicology Mechanisms And Methods **15:** 293–306.

Wood, A.W. and G. J. Van Der Kraak, 2001. Apoptosis and ovarian function: Novel perspectives from the teleosts. Biology of Reproduction **64**: 264–271

Woram, R. A., K. Gharbi, T. Sakamoto, B. Hoyheim, L. E. Holm, K. Naish, C. McGowan, M. M. Ferguson, R. B. Phillips, J. Stein, R. Guyomard, M. Cairney, J. B. Taggart, R. Powell, W. Davidson and R. G. Danzmann, 2003. Comparative genome analysis of the primary sex-determining locus in salmonid fishes. Genome Research **13:** 272–280.