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Washout of accumulated testosterone in a watershed

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Abstract

Testosterone is constantly excreted into the environment by both human and animal sources but little is known about how it is transported in the environment. In this study, testosterone was measured in 15 sites in the Upper Jordan Valley after major rain events (238 samplings) for two consecutive rain seasons. The area consists of small farms, cattle pasture, fish ponds with some urban development. One liter samples were extracted on solid phase columns and the eluates measured using specific radioimmunoassay for testosterone and estrogen (estradiol + estrone). The first rain season was the first above average season after a 3-year period of well below average rainfall. It was found in the rain season of 2001/2002, that following a rain sequence of 131 mm/week there was an initial large increase in the concentration of testosterone (maximum 6 ng/l) accompanied by high estrogen (maximum 6 ng/l), which then gradually declined to non-detectable levels (<0.3 ng/l) over a period of 3 months. These peaks originated from runoff from cattle pasture and fish pond effluent. Later peaks consisted only of testosterone that was moderately associated with sulfate ($r^2 = 0.53$, $P < 0.05$) and somewhat associated with total phosphorus ($r^2 = 0.49$, $P < 0.1$) indicating that the origin was leaching from the sulfurous peat soil. In the following rainy season, which had recorded rainfalls, no testosterone peaks above 1 ng/l were seen. We conclude that the testosterone accumulated in the Upper Jordan Valley was washed out in two stages, first as surface runoff from cattle pasture and then as discharge from the soil.

Keywords: Estradiol; Estrone; Ethinylestradiol; Hormones

1. Introduction

Steroid hormones produced by humans and animals are constantly excreted into the environment (see Drewes and Shore, 2001; Lintelmann et al., 2003; Shore and Shemesh, 2003 for reviews). The primary steroid hormones are estrone, estradiol, progesterone, testosterone and cortisol, all of which are lipophilic and poorly soluble in water (log P_{ow} between 3 and 4, Lintelmann et al., 2003). The natural steroids of major concern are estrone and estradiol-17β since they exert their physiological

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effects at lower concentrations than other steroids and can be found in the environment in concentrations above their lowest observable effect level (LOEL) for fish (increased vitellogenin) and plants (increased growth) (10 ng/l) (Shore et al., 1992, 1993; Panter et al., 1998; Routledge et al., 1998). In rivers and soil, estradiol is converted abiotically to estrone, so for in environmental studies estradiol and estrone can be considered together as 'estrogens' (Colucci et al., 2001; Jürgens et al., 2002). Effluent from human sources also contains estriol, a weak estrogen excreted in the urine of pregnant women, and synthetic estrogens such as mestranol and ethinylestradiol (Wenzel et al., 1998). These synthetic compounds are of particular concern as they have LOEL's in the order of 1 ng/l (Lange et al., 2001). Progesterone and testosterone are also excreted in the free active form but at the maximal level found in rivers (approx. 200 ng/l, Kolpin et al., 2002), there are no documented effects in the environment. We have reported that in the Conestoga river valley of the mid-atlantic region of the US, in sampling from 17 streams, four of 10 sites had concentrations of testosterone and estrogen above 1 ng/l. Three of these sites were in areas with heavy use of poultry manure as fertilizer, and one site received effluent from an sewage treatment plant (Shore et al., 1995). Comparison of a stream dominated by forest with a stream dominated by cropland indicated that there was a gradient of estrogen discharge downstream along the stream dominated by cropland fertilized with chicken manure (0.54–1.83 ng/l).

Estrogen and testosterone in the environment are excreted from the same sources in comparable amounts (Shore et al., 1995). The major sources that have been investigated are animal manures and sewage effluent. Studies of fields fertilized with chicken and pig manure, or laboratory studies of soils with exogenous steroid added, indicated that estrogen binds tightly to the soil and does not migrate to the ground water (Shore et al., 1995, 1997). However, estrogen is found in surface runoff and in ground water where the soil is rocky or where the terrain is highly permeable karst (Shore et al., 1993; Peterson et al., 2000). In contrast, testosterone is loosely bound to the soil and is found in both surface and ground water (Shore et al., 1997) as well as in surface soil (Finlay-Moore et al., 2000). Both estrogen and testosterone are rapidly metabolized in wild duck ponds (half life—0.5 h, Shore, unpublished observations) but in irrigation ponds these contaminants may persist for several months (Shore et al., 1993, 1995). In laboratory studies of river water, Jürgens et al. (2002) reported that the half time reduction for estrogen was between 2 and 6 days, while half time reduction for ethinylestradiol was 46 days. However, in field studies, Williams et al. (2003) reported that sewage effluent had little effect on the estradiol and ethinylestradiol content of the river but that estrone persisted for 10 km below the effluent input. In soil, both estrogen and ethinylestradiol absorb rapidly and cannot be extracted after 2 days. However, mineralization of the hormones in the soil may take several months (Colucci and Topp, 2001; Colucci et al., 2001). There is no literature on the degradation rate of testosterone in river water or soil.

The purpose of the present paper was to determine the transport of testosterone in a watershed. The sites were selected based on the concept that subcatchment junctions are very important as network nodes for regulating material flows in a watershed (Vannote et al., 1980). Samples were also tested for estrogen, ethinylestradiol and estriol as well as total phosphorus, ammonia, total suspended solids and sulfate for comparison with testosterone. Ammonia was indicative of agricultural surface runoff and sewage effluent; the total suspended solids indicative of surface runoff and the sulfur indicative of ground water from sulfurous springs (upper valley) or peat soil (lower valley). The hypothesis was that two profiles would be typical of the testosterone concentrations observed: (1) testosterone combined with estrogen would be typical of runoff from cattle pasture or effluent from fish ponds; or (2) testosterone combined with ethinylestradiol, estriol and estrogen would indicate sewage effluent.

2. Materials and methods

2.1. Site description

Fifteen sites in the upper catchment of the Jordan River (UCJR) were sampled after major rain events

(>30 mm) from December 2001 to March 2003 (two rainy seasons – 2001/2002 and 2002/2003). The sampling sites represent all major subcatchments of the Jordan River classified by their drainage area and land use and which were expected to contribute significant loads of nutrients, hormones and other pollutants (Fig. 1). The rainy season in Israel is from November to April, and there is usually no precipitation from May to October. Samples were taken after each rain event of more than 30 mm and during the dry season. Minor rain events early in the rainy season were not sampled as these events mostly moisten the soil and do not result in appreciable runoff (Fig. 2).

Two headwater springs of the Jordan River were also sampled as well as fishpond effluent and seven small canals selected as only draining cattle pasture. The Upper Jordan is formed from three major

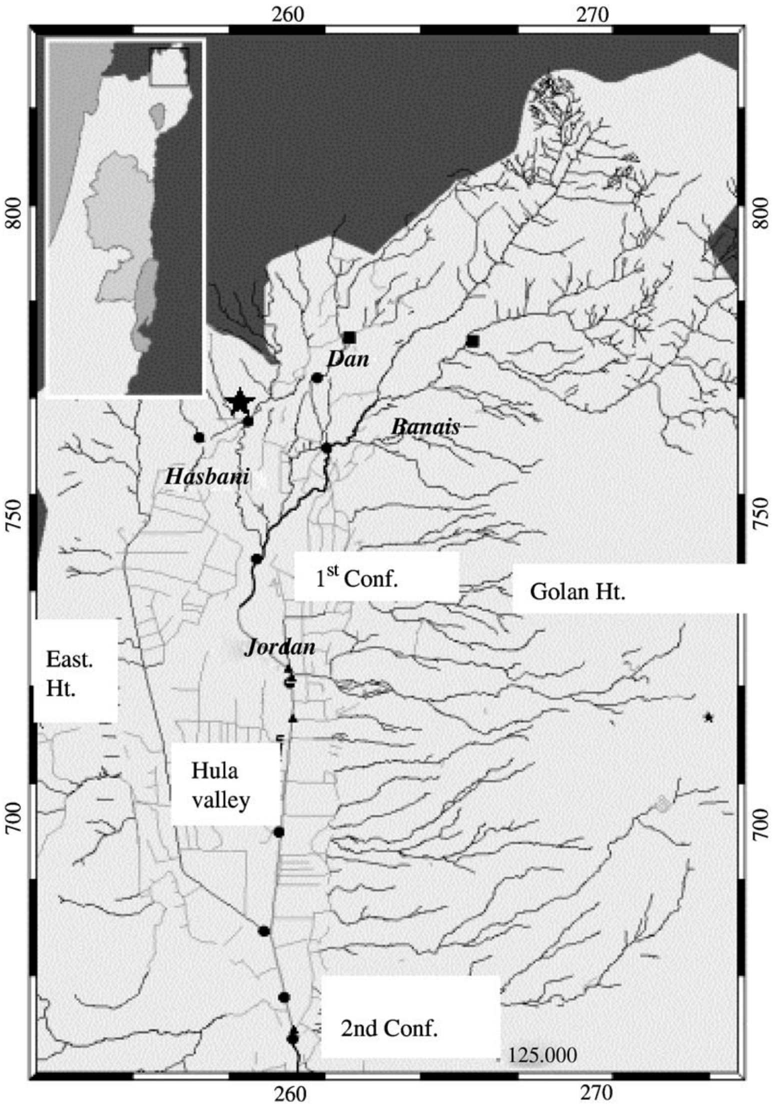

Fig. 1. Upper Jordan watershed and sampling sites. Legend: springs = squares; major tributary and river sites = circles; minor tributaries = triangles; rain monitoring stations = large star – Mayan Baruch, small star – Golan Ht. station.

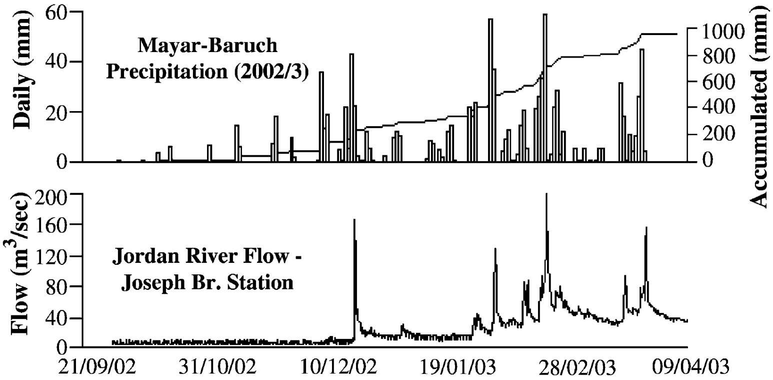

Fig. 2. Relationship between rainfall and flow at confluence #1.

tributaries, the Hasbani, Dan and Banias (three sites) and a smaller tributary (Hyun, one site), which converge at the Joseph Bridge (confluence #1, one site). The river then splits into the major eastern and smaller western canals. On the eastern side, four tributaries or canals were sampled as well as the river itself below the outlet of the tributaries (eight sites) while on the western side, just the Western Canal was sampled near its terminus where most of the water is being diverted away into a holding reservoir and later used for irrigation (one site). The eastern and western portions merge slightly upstream from the Pkak Bridge (confluence #2, one site). The drainage area is characterized by cropland, fish ponds and cattle grazing although there is some urban development on the western side. The flow in the Jordan River originates from the groundwater recharge area of Mount Hermon in the north, runoff with some groundwater flow from the Golan Heights in the east and some runoff and groundwater recharge from the Naftali Mountains in the west. Most of the upper catchment of the Jordan River consists of the Hula Valley in which peat soil is dominant. The discharge from the peat soil in area is characterized by high sulfate (Markel et al., 1998) and phosphorus (Litaor et al., 2003).

The three seasons prior to sampling had below average rainfall (1998–1999 – 551 mm, 1999–2000–813 mm; 2000/2001–515 mm) while the seasons of 2001–2002 (885) and 2002–2003 (1326 mm) were above the 15 year average (871 mm) rainfall (Meirom Golan station).

2.2. Hormone analysis

All the water samples were collected into acid-washed polyethylene 250-ml bottles, and immediately put in a cooler before transporting to the laboratory for further processing.

For hormone analysis, the 1 l samples were acidified on collection with 2 ml of HCl. The samples were then centrifuged at $800 \times g$ for 10 min, 800 ml of the supernatant acidified with 200 ml of 0.1 M sodium acetate, and extracted on C-18 columns. Control samples of tap water, distilled water and spring water were run with each batch. Columns were dried and eluted with 2 ml of methanol. Aliquots of 100 μl were taken for the assays. Testosterone and estrogen (estradiol and estrone) were measured by radioimmunoassay (Shore et al., 1993) with a detection limit of 0.3 ng/l. Commercial Elisa kits were used to measure ethinylestradiol (Ridascreen, r-biopharm, Darmstadt, Germany) and estriol (DRG GmbH, Marburg, Germany) with a detection limit of 0.3 ng/l. The recovery for the hormones was 90% if samples were above 1.0 ng/l. However, below 0.5 ng/l recoveries were less than 50% as determined by LC/MS (Wenzel et al., 1998). If a series of samples had consistently low values, not all of the other hormone analyses were necessarily performed.

2.3. Solute analysis

For solute analysis, samples were filtered through pre-washed cellulose nitrate filters (0.45 μm). For total phosphorus (TP) analysis, water samples were digested in an autoclave at 103.5 kPa and 121 °C with ammonium persulfate in order to convert all the phosphorus into orthophosphate to be determined as TP P (Pt) using the ascorbic acid method (Murphy and Riley, 1962). Ammonia was measured using the Phenate method (Standard Methods 4500-NH_3 F); suspended solids were measured using Standard Methods 2540-D (pp. 2–57). (Standard Methods for the Examination of Water and Wastewater; American Public Health Association 20th Ed.). Sulfate and chloride were analyzed by Dionex-600 ion chromatograph.

2.4. Statistics

For statistical and graphic purposes the non-detectable levels were taken as 0.3 ng/l. Analysis of variance was performed with a significance level of $P<0.05$ and the Student t test used where appropriate. All data are in means ± S.D.

3. Results

3.1. General distribution of the hormones in the various sites

In 19 of 21 samples taken over the 2001/2002 and 2002/2003 rainy seasons, the two headwater springs examined had non-detectable levels of testosterone, estrogen, ethinylestradiol and estradiol. The two exceptions were Banais springs on 21 April, 2002 (2.5 ng/l testosterone and the Dan spring on 12 December, 2001 (1.7 ng/l testosterone). The Banais differed from the other headwater spring in that it had a high content of sulfur (50 mg/l vs. 10 mg/l for the Dan spring).

Between the 2 years, there was no difference in the percentage of all of the sites with detectable (< 0.3 ng/l) testosterone (76%) but there was a rise in the percentage of ethinylestradiol (52–71%). However, the concentration of testosterone in detectable samples (>0.3 ng/l) decreased by some 50% between the 2 years while the level of ethinylestradiol was the same (Table 1). Ethinylestradiol was consistently above 1 ng/l at three sites, two of which were known to receive sewage effluent and one had a high ammonia (>1.2 mg/l) and bacteria count (>50 000 fecal coli/100 ml; courtesy of O. Hadas, Kinneret Limnological Laboratory), probably originating from a leaky sewage holding pond.

There was no correlation ($r<0.2$) with the amount of testosterone, ethinylestradiol, estrogen or estradiol present in the samples with each other. However, in the few sites (5/28) in which estriol was above 1 ng/l, ethinylestradiol was also above 1 ng/l (15/28). Estrogen was present in 30% and 50% and estriol in 13%

Table 1
Comparison of mean testosterone and ethinylestradiol concentrations at 14 sites in the UPJR during two rainy seasons

Site no.	Site name	Testosterone ng/l		Ethinylestradiol ng/l		Comments
		2001/2002	2002/2003	2001/2002	2002/2003	
1	Dan Tributary	1.1	0.5	0.7	1.3	
2	Banais Tributary	0.9	0.5	1.2	1.1	Some raw sewage
3	Hasbani tributary	1.4	0.5	0.4	0.7	
4	Hyun Creek	1.1	2.4	0.5	0.9	
5	Yoseph Bridge	2.0	0.4	0.6	0.5	Confluence of sites 1, 2, 3, 4
6	Kalil Canal	1.3	0.8	0.3	0.8	
7	Yardonin Creek	2.1	0.7	0.8	1.2	
8	Gonen Canal	2.2	0.9	1.6	1.6	High ammonia and fecal coli
9	Bitachon Canal	1.4	0.9	0.5	1.3	
10	Lehavot Bridge	1.2	0.4	0.4	0.7	River below sites 6, 7
11	Green Bridge	1.2	0.5	1.0	0.5	River below site 8
12	Hardale Bridge	1.3	0.5	0.7	0.7	River below site 8
13	Western Canal	2.1	0.8	1.3	1.2	Some treated urban effluent
14	Pkak Bridge	1.5	0.6	0.4	0.8	River below confluence of sites 12, 13 and 9
Mean ± S.D. (no. of samplings) 2001/2002 vs. 2002/2003		1.5 ± 0.4 (125)	0.8 ± 0.5 (109) $P<0.01$	0.7 ± 0.4 (47)	0.9 ± 0.3 (112) NS	

and 17% of the samples in the first and second year, respectively. There was no significant difference in the levels of estrogen (0.8 ± 0.7 vs. 0.5 ± 0.1 ng/l) or estriol (0.8 ± 1.3 vs. 0.7 ± 0.4) between the 2 years.

3.2. Testosterone concentrations at two confluences of the Jordan River

The two confluences representing the upper tributaries and the lower tributaries were sampled more frequently during rain events than the other sites during rain events as well as in the dry season because it facilitates an easy comparison between different sources of contaminants according to land-use. The relationship between rainfall and the sample site at confluence #1 is shown in Fig. 3. In the two confluences, 6–7 pulses of greater than 1 ng/l testosterone were noted in the first rainy season, but none were seen in the subsequent rainy season (Fig. 3).

3.2.1. Coincidence between testosterone pulses in the tributaries of north of origin of the Jordan River and high concentrations of hormones in cattle runoff and fishpond effluent

Four major peaks of testosterone were noted in confluence #1 of the three tributaries of the Jordan River over the period of December 2001–April 2002 (Fig. 4). The initial peaks, particularly in the tributaries, contained both testosterone and estrogen in significant quantities. The initial pulses of testosterone at the confluence contained between 0.5 and 1.0 ng estrogen. At this time, the Hyun and Banais contained between 1.0 and 3.5 ng/l estrogen but the other two tributaries had negligible amounts of estrogen. Since the flow in Hyun was negligible compared to the Banais (0.02 vs. 2.5 m^3/s), the major source of the estrogen was the Banais. The initial peak coincided with an intense rain sequence (151 mm/week).

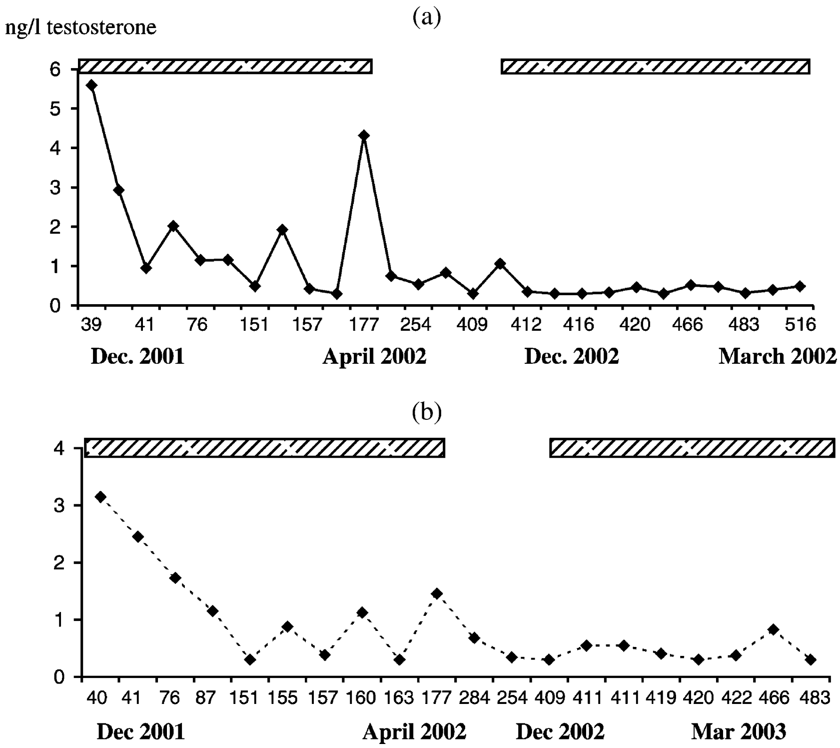

Fig. 3. Testosterone concentrations at the upper (Fig. 2a) and lower confluences (Fig. 2b) of the Jordan River. Samples were taken after each rain event of great than 30 mm/72 h and during the dry season during which there is no precipitation. Hatched bars represent the rainy season. Numbers on the x axis for individual rain events are the days numbered from 26.10.2001, which marked the initial rainfall.

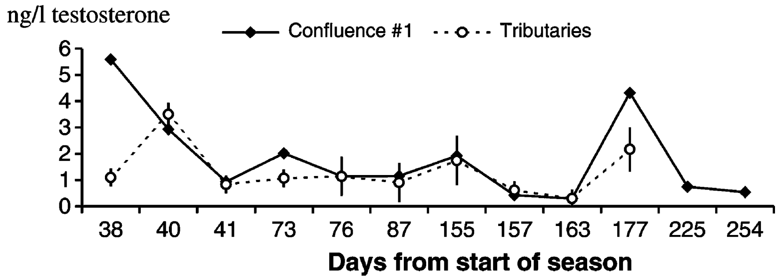

Fig. 4. Testosterone concentrations at the first confluence and in the headwater tributaries of the river. The points for the #1 confluence are single determinations. The points for the tributaries are the means ± S.D. of three or four tributaries The smallest tributary was dry during the dry season (last three points) during which there is no precipitation. Samples were taken after each rain event of greater than 30 mm/72 h and during the dry season during which there is no precipitation.

Two sources emptying into the Banais were identified—cattle pasture and fish ponds. A canal receiving runoff from pasture (4 cows/25 acres) had 1.0–6 ng/l estrogen and a fish pond which also emptied into the Banais had 1.0–5.8 ng/l estrogen during the month of December 2001. Similarly in seven canals draining cattle pasture in the UPJC in December 2001, the level of testosterone was 4.0 ± 1.4 ng/l (range 2–5.6) and for estrogen 2.4 ± 2.0 ng/l (range 1–6). In contrast, estrogen was generally undetectable and below 1 ng/l in all samples taken from 1 January 2002 to May 2003.

3.2.2. Coincidence of testosterone pulses in the tributaries south of the headwaters of the Jordan River and the concentration at the second confluence

Testosterone was measured in five tributaries and canals leading into the Jordan River and the river itself below the headwaters of the river. The tributaries and canals all emptied into the second confluence. The testosterone declined gradually from the start of the rainy season (Fig. 5). There was no correlation with the intensity of rain events and testosterone concentrations (Table 2).

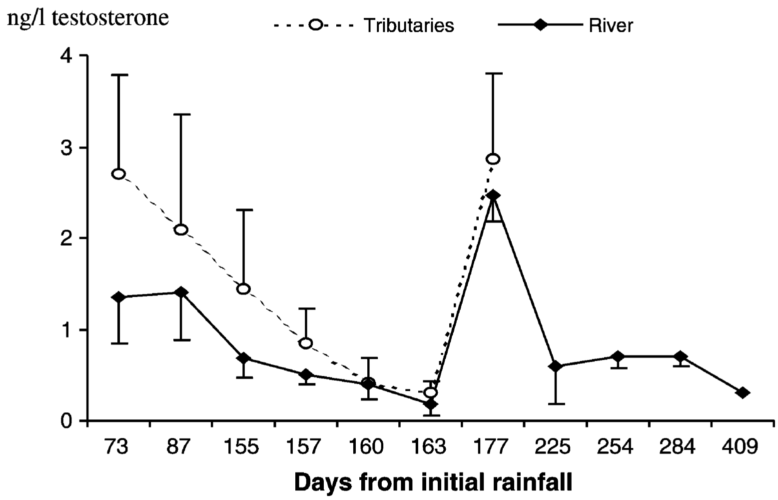

Fig. 5. Testosterone concentrations in the Jordan River until the second confluence. Each point is mean testosterone concentration for four minor tributaries or four points on the river between confluence #1 and #2. The last four points represent the dry season during which there is no precipitation.

Table 2
Estrogen and testosterone concentrations from a canal that received runoff from cattle pasture

Date	Day	Testosterone ng/l	Estrogen ng/l	Rainfall mm (72 h)
12/10/2001	0	Not sampled		28
15/11/2001	20	5.14	6.00	0
05/12/2001	40	2.09	1.01	146
10/01/2002	76	1.85	0.4	65
21/01/2002	87	1.81	0.0	31

The accumulated rain before the first sampling was 52 mm. The day is from the initial rainfall of the hydrological season.

3.2.3. Solute profile of the pulses at confluence #1 and #2

The seven pulses observed differed at the two confluences in their solute profile (Table 3). The first pulses at the first confluence were characterized by high sulfate with low ammonia, indicating ground water or surface water originating from the Banais spring headwater. The later peaks at the first confluence were characterized by high total suspended solids. At the second confluence, ammonia was much higher than at the first confluence showing the effect of runoff and spillage from sewage treatment reservoirs. Sulfate at confluence #1 was significantly lower than that at confluence #2 reflecting the high natural sulfur in the peat soil above confluence #2. There was no overall correlation between the TP, ammonia or sulfate with the testosterone level at the first confluence. At the second confluence there was a good correlation between sulfate ($r^2=0.53$, $P<0.05$) and TP ($r^2=0.49$, $P<0.1$) with testosterone levels while there was no correlation between ammonia with testosterone ($r^2<0.2$). Since the best correlation was between testosterone at the two confluences ($r^2=0.63$, $P<0.05$), the most likely explanation for the testosterone concentrations at the second confluence was dilution followed by discharge from peat soils.

The very high pulse of testosterone and total phosphorus seen on 21 April, 2002 may have been the result of a very focused storm on the western heights where most of the testosterone seems to

Table 3
Solute concentrations of the river on peak testosterone dates

Date	Day	TP	Sulfate mg/l	Testost. ng/l	Ammonia mg/l	Rainfall mm (72 h)	TSS mg/l
Confluence #1							
04/12/2001	39	0.04	29	5.6	0.02	127	12
05/12/2001	40	0.27	37	2.9	0.06	146	98
07/01/2002	73	0.16	16	2.0	0.14	68	860
10/01/2002	76	0.24	21	1.2	0.05	65	167
21/01/2002	87	0.56	24	1.2	0.10	31	197
30/03/2002	155	0.20	13	2.0	0.15	96	69
21/04/2002	177	1.03	14	4.3	0.06	52	828
Mean ± S.D.		0.36 ± 0.34	23 ± 9	2.7 ± 1.7	0.08 ± 0.04		
Confluence #2							
04/12/2001	39	0.31	57	3.2	0.24	127	114
05/12/2001	40	0.30	69	2.5	0.40	146	54
10/01/2002	76	0.58	57	1.7	0.33	65	186
21/01/2002	87	0.41	45	1.2	0.15	31	150
30/03/2002	155	0.37	29	0.8	0.31	96	106
04/04/2002	160	0.19	28	1.1	0.20	48	50
21/04/2002	177	0.45	21	1.5	0.06	52	212
Mean ± S.D.		0.37 ± 0.12	44 ± 18	1.1 ± 0.8	0.24 ± 0.11		
Confl. 1 vs. confl. 2		NS	$P<0.01$	NS	$P<0.01$		

originate. For example, precipitation measured at the Golan station usually was 110 ± 10% of the precipitation measure at the valley station 20 km to the east (Mayan Baruch) while on 21 April, 2002, the ratio was 150%.

4. Discussion

Based on previous observations on fields fertilized with chicken manure in the Conestoga River Valley and observations on sewage effluent (Wenzel et al., 1998), it was expected that testosterone pulses would be accompanied by either an estrogen or ethinylestradiol pulses. Estrogen pulses were observed only in the initial samples taken in December 2001 were runoff from cattle grazed fields and effluent from fish ponds were the main source of the hormones. These initial testosterone pulses were observed to dissipate over the 3-month period in the initial season and no peaks were seen in the subsequent rainy season. The contribution of sewage effluent to hormone load is apparently constant in the two seasons as the amount of ethinylestradiol, which can only come from human sources, was constant in both seasons. Estriol, which was present in less than 17% of the samples, was also unchanged between seasons (Table 1). However, a pattern of testosterone typical of sewage water effluent in which the four hormones are released in comparable amounts was not seen in UPJR other than at three sites known to receive sewage effluent.

We suggest that the slow testosterone decline seen in the initial season was the result of the washout of testosterone from manure accumulated over the previous years, which had below average rainfall. The later peaks of testosterone appear to be due to testosterone, which accumulated in the soil. This is suggested by the absence of an estrogen pulse (estrogen binds tightly to the soil and is not released) and the moderate correlation with sulfate and phosphorus, which was released from peat soils during the same rain events. The absence of such peaks in the subsequent rainy season indicated that the deposition of manure over the summer was not sufficient to cause major pulses of testosterone and estrogen in spite of the record rainfall in the subsequent season. That subsequent rainfall had no further effect on testosterone levels in the river and was either the result of the first year washout or that the efforts of the recently established Kinneret Monitoring Board to enforce in forcing dairy farms to treat their effluents and towns to upgrade sewage facilities, has dramatically reduced the hormonal load entering the watershed.

Finally, the data suggest the hypothesis that there are three patterns of testosterone transport in the environment: (1) testosterone associated with estrone, ethinylestradiol and estriol which is characteristic of sewage effluent; (2) testosterone associated with estrone and estradiol which is characteristic of runoff from cattle pasture and manure fertilized fields; and (3) testosterone alone, characteristic of leaching from soil and baseflow. Further research is needed to substantiate this hypothesis.

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