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Dairy Wastewater, Aquaculture, and Spawning Fish as Sources of Steroid Hormones in the Aquatic Environment

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A suite of androgens, estrogens, and progestins were measured in samples from dairy farms, aquaculture facilities, and surface waters with actively spawning fish using gas chromatography−tandem mass spectrometry (GC/MS/MS) to assess the potential importance of these sources of steroid hormones to surface waters. In a dairy waste lagoon, the endogenous estrogens 17β-estradiol and estrone and the androgens testosterone and androstenedione were detected at concentrations as high as 650 ng/L. Samples from nearby groundwater monitoring wells demonstrated removal of steroid hormones in the subsurface. Samples from nearby surface waters and tile drains likely impacted by animal wastes demonstrated the sporadic presence of the steroids 17β-estradiol, estrone, testosterone, and medroxyprogesterone, usually at concentrations near or below 1 ng/L. The endogenous steroids estrone, testosterone, and androstenedione were detected in the raceways and effluents of three fish hatcheries at concentrations near 1 ng/L. Similar concentrations were detected in a river containing spawning adult Chinook salmon. These results indicate that dairy wastewater, aquaculture effluents, and even spawning fish can lead to detectable concentrations of steroid hormones in surface waters and that the concentrations of these compounds exhibit considerable temporal and spatial variation.

Introduction

It has been established that steroid hormones in municipal wastewater effluent can affect fish (*1*−*3*). Most notably, estrogens such as 17β-estradiol, estrone, and ethinyl estradiol have been implicated in feminization of male fish at concentrations as low as 1 ng/L (*1*−*4*). Androgens also have been linked to reproductive abnormalities in fish at similarly low concentrations (*5*−*7*), and the presence of these and other hormonally active agents in the aquatic environment is an issue of general concern (*8*). Furthermore, numerous estrogens, androgens, and progestins act as reproductive pheromones in fish at nanograms per liter concentrations (*9*−*12*), and it is possible that anthropogenic discharges of steroid hormones at these concentrations interfere with pheromonal signaling in fish and adversely affect reproduction in sensitive species (*13*).

Most research to date has focused on the role of municipal wastewater treatment plants as the source of steroid hormones despite the fact that other sources of steroid hormones may be as or more important in certain watersheds. Although the possibility that animal agriculture could act as a strong source of steroid hormones to the aquatic environment was raised decades ago (*14*, *15*), with continued research in the past decade (*16*−*18*), the limited scope of the research and the lack of analytical sophistication of the analytical methods employed make it difficult to assess the importance of these sources. Steroid hormones and their metabolites are excreted by all vertebrates, and there may be situations in which sources unrelated to domestic sewage could contribute to the loading of steroid hormones to surface waters. If these sources are significant as compared to municipal wastewater effluents, efforts to predict concentrations of steroid hormones in surface waters may be complicated, and additional measures may be needed to control steroid hormones in the aquatic environment.

Intensive animal agriculture operations represent one potentially important source of steroid hormones to the aquatic environment where a critical lack of research regarding the occurrence, fate, and transport of these compounds exists. Concentrations of natural and synthetic steroids in agricultural wastes and manure are high enough that relatively small discharges could result in elevated concentrations in surface waters (*14*−*24*). Although excretion rates of estrogens, androgens, and progestins from cattle, swine, sheep, and poultry have been estimated (*18*, *25*, *26*), the importance of confined animal operations as a source of steroids is unclear because modern agricultural practices are designed to curtail routine discharges of untreated wastes. Discharges attributable to overland flow, land application, or groundwater infiltration of treated waste are not commonly quantified. Furthermore, the few studies that have reported steroid hormones in surface waters subjected to agricultural inputs (*16*−*23*, *27*, *28*) have been limited in scope or have been questioned due to the potential for artifacts caused by matrix interferences (*26*, *29*).

Aquaculture represents another rapidly growing intensive agricultural operation that could serve as a source of steroid hormones to surface waters. For example, in excess of 3.2×10^8 kg of fish was raised in aquaculture operations in the United States in 1999 (U.S. Joint Subcommittee on Aquaculture, www.ag.ansc.purdue.edu/aquanic/jsa). Fish excrete free and conjugated steroids (*9*, *10*, *30*), and the rudimentary wastewater treatment practices employed by most aquaculture facilities are unlikely to remove steroid hormones. If excretion rates of steroids from fish are comparable to excretion rates for livestock or humans after normalization to the mass of the animal, the steroid discharge from a typical aquaculture operation (i.e., 50 000−200 000 kg of fish) might resemble the steroid production of a cattle herd of several hundred animals or a wastewater treatment plant serving several thousand people.

Steroid hormones also are released in surface waters by fish, especially before and during periods of reproduction (*9*, *10*). Many small and medium size rivers along the west coast of North America currently have or have had historical returns of tens of thousands of adult spawning salmon during certain periods of the year. During low flow conditions, concentrations of steroid hormones due solely to the presence of the spawning salmon could reach levels detected in municipal

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wastewater effluent if excretion rates of steroids by these fish are again comparable to excretion rates of steroid hormones in livestock. Such releases of steroid hormones, which are natural parts of the reproductive cycles of certain species of fish, are not necessarily problematic for aquatic organisms, but they could complicate efforts to assess contributions and effects of steroid hormones from agriculture and wastewater discharges.

To investigate the contributions of steroid hormones to surface waters from dairy farms, aquaculture operations, and spawning fish, samples were collected and analyzed from sites in California's Central Valley. To assess the potential importance of dairy facilities as a steroid hormone source, dairy waste lagoon water and groundwater samples were collected from two dairy farms where dairy wastewaters are known to affect shallow groundwater quality. To assess the relative importance of aquaculture and spawning fish as sources of steroid hormones, samples were collected from three aquaculture facilities and a river where spawning salmon were present. Another objective of this study was to determine whether the attenuation (i.e., sorption to soil organic matter and mineral surfaces, abiotic transformation reactions, and biotransformation) of steroid hormones in groundwater would limit the transport of steroid hormones in groundwater to nearby receiving waters. The attenuation of steroid hormones in the subsurface was evaluated by comparing results from wells located downgradient of dairy farming operations with other water quality parameters at the study sites.

Material and Methods

Sampling Sites. All samples were collected in the northeastern San Joaquin Valley of central California. This region is characterized by extensive agricultural operations located in low-relief basins underlain by shallow, alluvial aquifers. The most widespread and common confined animal feeding operations in this region are family owned dairy farms with an average herd size of nearly 1000 animal units (*31*). The farmland associated with the dairies in the region is irrigated with surface water. At these farms, the water table level is maintained through an extensive system of tile drains and drainage wells; as the water table rises, shallow groundwater is pumped to the surface and discharged to a series of irrigation canals. The irrigation canals eventually discharge into the Merced, Tuolumne, and San Joaquin Rivers.

Groundwater samples from the shallow portion of the regional unconfined aquifer and samples of liquid animal waste from dairy waste lagoons were collected at several locations at two dairy farms. At these sites, located on the topographically flat Central Valley floor, the sandy to loamy sand soils are subject to high percolation rates and overlie a shallow regional groundwater table at 2−5 m depth. Shallow groundwater monitoring wells used for this study are screened from the water table to depths of 7−10 m and represent a mix of groundwater ages ranging from several days (shallowest groundwater entering the well screen) to 1−2 yr (deepest groundwater entering the well screen) (*31*). The shallow groundwater obtained in the monitoring wells at these dairies originates from percolation of excess irrigation and manure water applied to fields on or adjacent to the dairies, from corrals, and from infiltration of water from unlined dairy waste lagoons (*31*). Routine operations at these dairies have resulted in shallow groundwater nitrate levels above 10 mg/L throughout the site (*31*). In shallow groundwater immediately downgradient of the dairy waste lagoons, altered redox conditions and ammonia concentrations in excess of 3 mg/L have been observed (*31*).

As is common for dairies throughout this region, wastes from the animal housing areas (freestalls) and wash water from the milking barn are flushed, via flushing lanes, to large waste lagoons after separation of solid wastes, which are dewatered, processed, and collected as soil amendment for surrounding fields. Liquid supernatant from the dewatering process is collected and pumped to the dairy waste lagoons. In the system tested, the total solids concentration in the waste lagoon typically ranges from 3 to 6%. Controlled land application of the liquid wastes, used as fertilizer and diluted with irrigation water, occurs at frequent intervals during the summer cropping season on fields adjacent to the waste lagoons.

To assess the occurrence of steroid hormones in shallow groundwater, samples were collected from 13 groundwater monitoring wells. Wells were grouped depending on the source of the monitored groundwater: three "lagoon wells" are located immediately downgradient from dairy waste lagoons, four "corral wells" are located within the feedlot area of the dairy farm, and five "field wells" are located immediately downgradient from fields that regularly receive manure applications. One well located outside the zone of influence of a dairy ("upgradient well") and a domestic well located at the site that pumps groundwater from a depth below 25 m ("deep aquifer well") also were sampled. Groundwater samples were collected immediately prior to the irrigation season (approximately June−August) and 1 month after the end of the irrigation season. Diluted dairy wastewater is generally applied to forage fields 4−6 times during the irrigation season. The total amount of dairy wastewater applied during the irrigation season is managed to meet crop nutrient management requirements, mostly with respect to nitrogen (approximately 250−300 kg of N/ha per growing season). The samples from the dairy waste lagoon itself were collected near the discharge point of the wastewater influent pipe where the inflowing water caused partial mixing of the waste lagoon. In addition to steroid hormones, all groundwater samples were analyzed for pH, electric conductivity, nitrate, and ammonia (*31*).

Surface water sampling sites were chosen to represent the areas most likely to be impacted by agricultural operations. Samples were collected at sites upstream and downstream of dairy farms and irrigation canal discharge points, near tile drain pump discharges, and in irrigation canals that discharge to surface waters. Although the rivers and irrigation canals (which receive mostly surface water) may receive some municipal wastewater effluent from upstream communities, the contribution of steroid hormones from municipal wastewater treatment plants is believed to be negligible given the absence of major urban areas upstream and the relatively large flows of these surface waters, which exceed the volume of sewage produced by the population in this area by several orders of magnitude.

Samples also were collected at three State of California Department of Fish and Game fish hatcheries. The Nimbus Hatchery and Mokelumne River Hatchery are hatcheries that primarily raise juvenile Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead trout (*Salmo gairdneri*) to replenish stocks of these anadromous fish in California rivers. The American River Hatchery raises Rainbow trout (*Oncorhynchus mykiss*) for sport fishing. Samples were collected from the influent to the hatcheries, at the end of the raceway pens holding fish, and in the effluent waste streams, which either discharge directly to the Mokelumne River (Mokelumne River Hatchery), or to a small settling pond that discharges to the American River (Nimbus and American River Hatcheries) after approximately 25 m of bank infiltration through a high-permeability, alluvial sand−gravel−cobble berm.

Finally, samples were collected from the fish ladder at the Nimbus Hatchery and from a gravel bar occupied by actively spawning adult Chinook salmon in the American River. The sample from the fish ladder at the Nimbus Hatchery was

TABLE 1. Gas Chromatography—Tandem Mass Spectrometry (GC/MS/MS) Analytical Conditions and Method Detection Limits

compound	retention time (min)	parent ion	product ion (MS/MS only)	collision energy (eV)	LOD[a] (ng/L)	LOQ[b] (ng/L)
mesterolone[c]	18.90	414 (SIM)[d]				
hexachlorobenzene	6.68	284 (SIM)				
testosterone	14.15	681	451, 466, 665	1.10	0.1	0.3
17β-estradiol	14.52	664	451	1.20	0.1	0.3
estriol	14.74	876	449, 662, 663	1.15	0.14	0.4
androstenedione	15.69	482	253, 268, 467	1.00	0.1	0.3
estrone	16.00	467	422, 448	1.00	0.14	0.4
medroxyprogesterone	19.29	479	383	1.15	0.14	0.4
progesterone	20.57	511	425, 477, 495	1.25	0.2	0.6

[a] LOD, limit of detection. [b] LOQ, limit of quantification. [c] The trivial names and systematic nomenclature for these steroids are as follows: mesterolone (17β-hydroxy-1α-methyl-5α-androstan-3-one), testosterone (4-androstene-17β-ol-3-one), 17β-estradiol (1,3,5(10)-estratrien-3,17β-diol), estriol (1,3,5(10)-estratrien-3,16α,17β-triol), androstenedione (4-androstene-3,17-dione), estrone (1,3,5(10)-estratrien-3-ol-17-one), medroxyprogesterone (17α-hydroxy-6α-methyl-4-pregnene-3,20-dione), progesterone (4-pregnene-3,20-dione). [d] SIM, single-ion monitoring.

collected immediately below holding pens that contained approximately 1000 adult Chinook salmon (personal communication, Robert Burks, California Department of Fish and Game) held as sources of eggs and milt for hatchery operations. The fish ladder and the two hatcheries share the same water source, which is bottom-draw water from the Nimbus Reservoir on the American River.

Sample Collection. All samples were collected in 12-L fluorinated Nalgene (Rochester, NY) containers. The typical sampling protocol for surface waters consisted of collecting a grab sample in the 12-L container, immediately placing the sample in a cooler with ice, and transporting the samples to the laboratory. Groundwater samples were obtained after purging at least three well volumes using either a stainless steel submersible pump or a peristaltic pump. Samples were stored at 5 °C for no more than 24 h after collection, at which time the samples were extracted.

Surface water samples were collected during the low-flow summer conditions of August 1 and September 11, 2003, and near the end of a moderate/heavy rain event on February 2−3, 2004, during which time approximately 25−30 mm of precipitation fell after several weeks of dry weather. Groundwater samples were collected on May 29 and September 24, 2003. Samples from the California Department of Fish and Game fish hatcheries, the fish ladder at the Nimbus Hatchery, and the active salmon spawning site on the American River were collected on November 21, 2003. The November sampling date coincided with the peak of the fall Chinook salmon spawning run in the American River when spawning sites were occupied by actively spawning adult salmon.

Chemical Analysis. Steroid hormones were extracted using C-18 solid-phase extraction disks followed by derivatization and gas chromatography−tandem mass spectrometry (GC/MS/MS) as described previously (*13*). Briefly, suspended particles were removed by pressure-filtering 4-L aliquots of sample through 90-mm AP 40 glass fiber filters (Fisher Scientific, Pittsburgh, PA). For the dairy waste lagoon samples, 1-L samples were centrifuged at 2000 rpm for 20 min to reduce solids prior to pressure filtration of the supernatant. The surrogate standard mesterolone was then spiked into the filtered samples at a concentration of 100 ng/L. Steroid hormones were pressure-extracted from the filtrate using preconditioned 90-mm Empore (3-M, Minneapolis, MN) C-18 solid-phase extraction disks. To remove polar organic matter from the extraction disks, the C-18 disks were washed twice with 25 mL of a 70:30 water:methanol solution prior to elution of the steroid analytes with 20 mL of a 10:90 water:methanol solution. The composition of the water:methanol wash and elution solutions were modified from the original method due to the addition of the steroids estriol and progesterone to the analytical method. The eluent was dried under vacuum, resuspended in methanol, and transferred to flasks. After again drying under vacuum, the extract was resuspended in 200 μL of HPLC-grade acetonitrile and derivatized with 50 μL of heptafluorobutyric anhydride, sealed, and placed in a 55 °C oven for 1.5 h. The extracts were then cooled and evaporated under a gentle stream of nitrogen prior to resuspension in 100 μL of isooctane that contained hexachlorobenzene (400 μg/L) as an internal standard.

The previously published GC/MS/MS analytical method (*13*) was modified to include analysis for estriol and progesterone in addition to 17β-estradiol, estrone, testosterone, androstenedione, and medroxyprogesterone. Relevant analytical conditions, GC/MS/MS instrument parameters, and method detection limits for the steroid hormones are summarized in Table 1. Quality assurance and quality control consisted of at least one distilled water blank, one duplicate sample, and one matrix recovery sample spike of 10 ng/L of the steroid analytes per 10 samples (*13*). Recovery of the matrix spikes ranged from 56 to 85%, was consistent between analytes, and was correlated with the recovery of the surrogate standard mesterolone. Duplicate samples agreed within 15%, and steroids were never detected in distilled water blanks. Steroid hormones also have not been detected in background samples collected from sites with no significant hormone sources. Method detection limits ranged from 0.1 to 0.2 ng/L and were analyte dependent (*13*).

Results

Dairy Waste Lagoon, Groundwater Monitoring Wells, and Tile Drains. In the dairy waste lagoon water, concentrations of steroids ranged from below detection limit to 650 ng/L (Figure 1). Steroid concentrations in the waste lagoon varied considerably between the two sampling dates. Measured concentrations of estrone, the only steroid detected in both sets of samples, varied by an order of magnitude between the two sampling trips. Three of the six steroid hormones analyzed were detected in the dairy waste lagoon water during May and 5 of the seven steroids were detected in September (progesterone was not analyzed in May). The observed variability in the waste lagoon samples is consistent with prior research that has shown large variations in nutrient and salt content within the waste lagoon (*32*). Although the variability in concentration cannot be accounted for completely, it is evident that the composition of the waste being discharged to the groundwater varies considerably over time.

Steroid hormones were detected in 7 of the 26 shallow groundwater samples collected during the two sampling rounds. When detected, concentrations of steroids were considerably lower than those in the waste lagoon samples. Steroid hormones were detected in three wells located immediately adjacent to and hydraulically downgradient of the dairy waste lagoons (lagoon wells 11−13). In the three

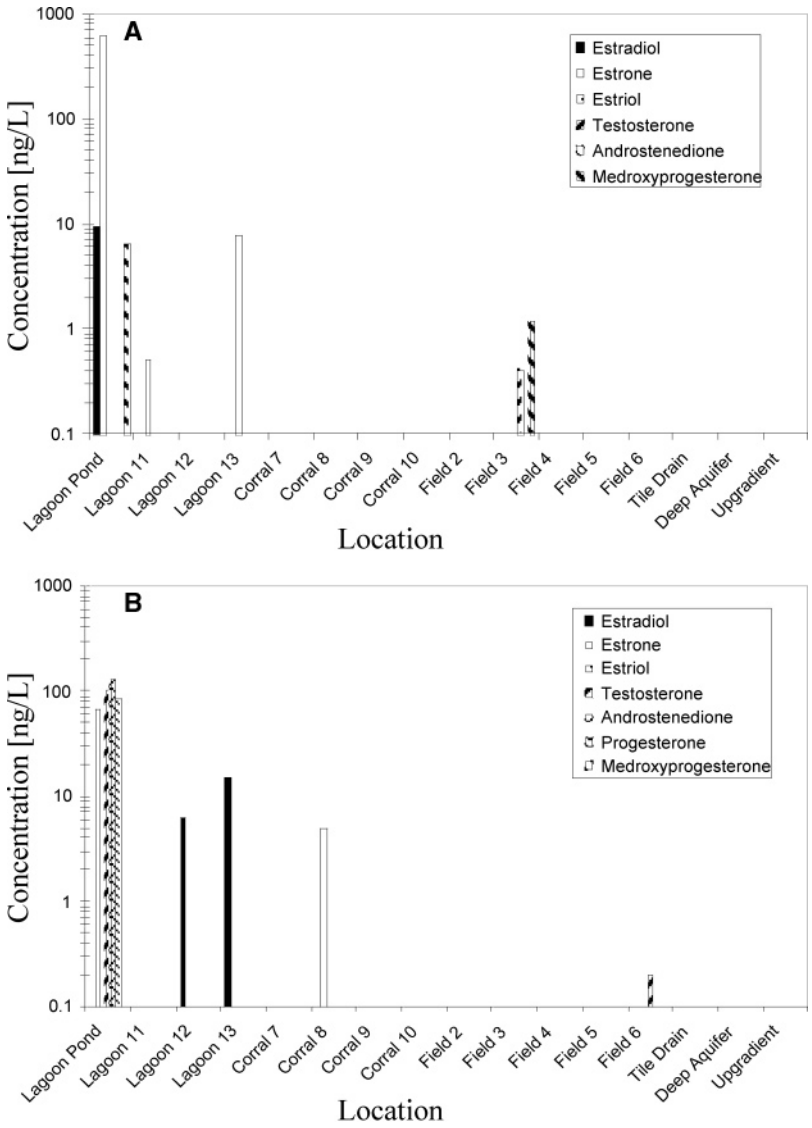

FIGURE 1. Steroid hormones in dairy monitoring wells, May 2003 (A) and September 2003 (B).

corral wells, the only steroid detected was estrone in one of the September samples. Steroid hormones also were detected in two of the five field wells. In these samples, testosterone and medroxyprogesterone were detected in the field 3 well in May, and testosterone was detected in the field 6 well in September. The steroids estriol, androstenedione, and progesterone were not detected in any of the groundwater samples. No steroids were detected in the upgradient control well, the deep aquifer well, or in samples from the tile drain system at the study site. Prior studies have determined that samples from the tile drain system closely resemble a composite average of the shallow groundwater throughout the dairy (*31*).

Steroid hormone concentrations in groundwater samples exhibited large temporal and spatial variability. While 6 of the 13 monitoring wells had detectable levels of one steroid in either the May or the September sampling round, there was no consistency among detections in either space, time, or with respect to the specific compound detected. Significant spatial and temporal variations also have been observed in other water quality parameters at the site, although not of this magnitude. For example, salinity and nitrate often vary by a factor of 2−4 over a 6-month period in the same well as compared with order of magnitude variations in steroid concentrations.

In the six samples collected from the tile drain groundwater discharge, steroids were not detected at levels above the limit of quantification (Table 2; Table 4 in Supporting Information). One of the six samples indicated the presence of testosterone, and another indicated the presence of testosterone and medroxyprogesterone at detectable levels but below the limit of quantification.

Surface Water Samples. A total of 26 samples was analyzed from six locations in three rivers and from nine locations in six irrigation canals (Table 2; Table 4 in Supporting Information). Not all locations were sampled during all three sampling trips. During the February 2004 sampling trip, three irrigation canals sampled previously were dry, and access to the San Joaquin River sampling site was prevented due to high water. Steroid hormones in the surface water samples were detected sporadically, and no correlation was evident between concentration and sampling location. Among the samples in which steroids were detected, 25% contained 1 steroid, 13% contained 2 steroids, 3% contained 3 steroids, and 3% contained 4 steroids. Estrone was the most frequently detected steroid, with a maximum concentration of 17 ng/L observed in a sample from a drainage canal after the storm in February 2004. The highest concentration of testosterone (1.9 ng/L) detected was observed in an irrigation canal in August 2003. 17β-Estradiol and medroxyprogesterone were

TABLE 2. Summary of Steroid Hormone Concentrations in Surface Waters and Tile Drains in an Agricultural Region[a]

		testosterone		estrone		17β-estradiol		medroxyprogesterone		
location	*N*	%[b]	max (ng/L)[c]	%	max (ng/L)	%	max (ng/L)	%	max (ng/L)	nitrate range (mg/L)
rivers	11	18	0.6	45	0.9	9	0.6	18	<0.4	3.1−42
irrigation canals	15	27	1.9	47	17	7	0.7	7	1.0	3.1−140
tile drains	6	33	<0.3	0	<0.1	0	<0.1	17	<0.4	9.5−350

[a] The steroid hormones androstenedione, estriol, and progesterone were also analyzed in these samples; however, they were not detected in any of the samples and are not included in the table. [b] The percentage of samples in which steroids were detected. [c] Maximum concentration at which steroid hormone was detected. Values indicated with "<" either correspond to the limit of quantification (for cases in which the highest concentration detected was between the limit of detection and the limit of quantification) or the limit of detection (for cases in which the steroid was never detected).

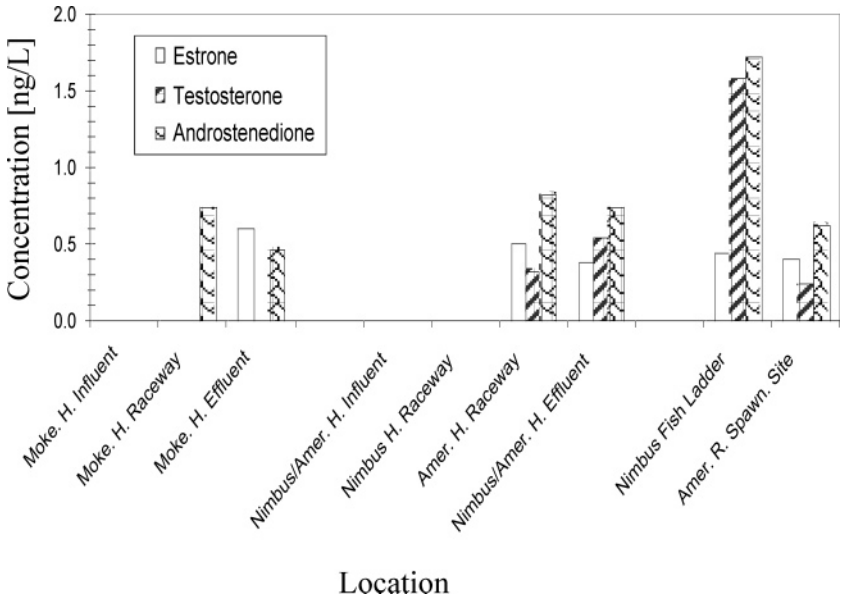

FIGURE 2. Steroid concentrations at fish hatcheries and in a river with spawning salmon.

detected less frequently than estrone and testosterone in the surface water samples always at or below 1 ng/L. The steroids estriol, androstenedione, and progesterone were not detected in any surface water sample. Nitrate was present in all 29 of the samples in which it was analyzed at concentrations between 3 and 350 mg/L, and steroid detections were not correlated with nitrate concentrations ($R^2 < 0.26$).

Aquaculture Sites. Estrone, testosterone, and androstenedione were detected in effluent samples from the hatcheries but not in any of the influent samples (Figure 2). At the Mokelumne River Hatchery, androstenedione was detected at 0.7 ng/L in the effluent of a raceway pen containing 9,150 kg of 25−30 cm rainbow trout. In the hatchery effluent, which was discharged directly to the Mokelumne River, androstenedione and estrone were detected at similar concentrations. The influent for the Nimbus Hatchery also serves as the influent for the American River Hatchery and the fish ladder at the Nimbus Hatchery. No steroids were detected in this influent sample (labeled Nimbus/American Hatcheries influent" in Figure 2). Steroids also were not detected in the effluent of a raceway pen containing 5280 kg of juvenile steelhead trout at the Nimbus Hatchery. However, in the effluent of a raceway pen at the American River Hatchery that contained 14 100 kg of 30−35 cm rainbow trout, estrone, testosterone, and androstenedione were detected. The effluents from the raceways at the Nimbus Hatchery and the American Hatchery are combined and discharged to a small settling pond with a short hydraulic retention time (i.e., < 8 h). Each hatchery contributes approximately equal volumes of effluent to this settling pond. In the sample from this combined effluent (labeled Nimbus/American Hatcheries effluent" in Figure 2), collected at the discharge point to the settling pond, estrone, testosterone, and androstenedione were detected at similar concentrations to those observed in the raceway effluent sample from the American River Hatchery.

Spawning Fish. In the fish ladder sample, taken below approximately 1000 adult salmon in holding pens, testosterone, androstenedione, and estrone were detected (Figure 2). The same three steroids also were detected in a sample collected in a spawning area on the American River where numerous adult salmon engaged in typical spawning behaviors (redd building, aggression, courtship) were observed.

Discussion

The concentrations of steroid hormones observed in effluents from aquaculture facilities and in surface waters in an intensive agricultural region are comparable to those typically observed in municipal wastewater effluents (*13*, *33*−*35*). The three endogenous steroids detected in the surface waters near dairies (i.e., 17β-estradiol, estrone, and testosterone) have been observed previously in manure (*18*, *23*, *25*, *26*, *36*), and the synthetic progestin detected in these samples, medroxyprogesterone, is used in human and veterinary medicine as an estrus regulator and has been suggested as a means of synchronizing estrus in dairy cattle (*37*). The endogenous steroids detected in aquaculture effluents and in rivers with spawning salmon (i.e., estrone, testosterone, and androstenedione) are present in the blood plasma of fish (*38*) and are excreted via urine or bile (*10*, *30*) or across the gills (*30*). These results suggest that sources of steroid hormones other than municipal wastewater effluent may

need to be considered when efforts are made to predict or control concentrations of steroid hormones in surface waters. In terms of the potential contributions to endocrine disruption, municipal wastewater effluent that contains similar concentrations of 17β-estradiol and estrone to those detected here may be even more potent because domestic sewage also contains potent synthetic estrogens such as ethinyl estradiol. Additionally, the presence of steroid hormones in receiving waters near these sources raises the possibility that other compounds related to these activities such as veterinary pharmaceuticals, antibiotics, or growth promoters used in animal agriculture also could be present in the receiving waters.

The relatively high concentrations of steroid hormones observed in the dairy waste lagoon are consistent with previous studies (*18*, *23*, *25*−*27*). Concentrations of steroids hormones in dairy waste lagoons can be several orders of magnitude higher than the nanograms per liter levels at which feminization of fish or pheromonal responses have been observed (*1*, *4*, *10*). However, data collected from groundwater wells impacted by dairy waste lagoons and land applications of dairy wastes indicate that steroids are strongly adsorbed or degraded when wastewater is infiltrated into the soil. For example, samples from the dairy waste lagoon contained steroids at concentrations as high as 650 ng/L, but only one steroid, 17β-estradiol at 6.3 ng/L, was detected in a monitoring well located less than 15 m downgradient from the waste lagoon (i.e., lagoon well 12). Evidence of strong attenuation of steroid hormones in the waste lagoon also was obtained from two other wells (i.e., lagoon wells 11 and 13) located immediately adjacent to and downgradient of dairy waste lagoons.

The absence of steroid hormones in most of the field and corral wells, the sporadic nature of the steroid detections, and the lack of quantifiable levels of steroids in the tile drain samples indicates that steroid hormones are strongly adsorbed or degraded over distances of 10−100 m in the subsurface when dairy wastewaters are infiltrated to groundwater. The sporadic detections of steroid hormones in several of the groundwater wells may indicate some transport along preferential flow paths in the subsurface, which is consistent with previous studies on transport of moderately hydrophobic pesticides transport in the subsurface (*39*, *40*). It is important to recall that the size of the recharge (source) area contributing to each groundwater sample is significantly different between the shallow monitoring wells and the tile drain discharge: the shallow monitoring wells observe groundwater from a relatively small recharge area (approximately 0.1−0.5 ha or less), whereas the tile drain discharges reflect the integrated shallow groundwater quality across an area of one to several square kilometers (*31*). The integrated area of a tile drain can include several dairy operations and their surrounding fields that receive applications of liquid or solid manure. These results indicate that groundwater discharged from tile drains is not a major source of steroid hormones to surface waters in this area.

The observed attenuation of steroid hormones is of significance beyond the study area: the soil and geologic site conditions at the dairy farms sampled represent a highly vulnerable groundwater region that is not very conducive to steroid attenuation. The low organic matter content of the sandy soils and their high hydraulic conductivity at these dairies provide relatively low potential for retardation relative to many other soils and groundwater sediments. Also, concurrent nitrate, ammonia, oxygen, and redox potential measurements (data not shown) indicate that oxygen was depleted in most of the shallow groundwater at this site, particularly in the vicinity of the dairy waste lagoons, and that anaerobic biotransformation of steroid hormones is much slower than aerobic biotransformation (*41*). Average travel times of infiltrating water to the monitoring wells at the site are estimated to be on the order of weeks to months (*31*). The observation of significant attenuation of steroid hormones during this period agrees well with laboratory studies that have shown rapid removal of steroids via sorption to soils and sediments with half-lives for steroid dissipation on the order of hours to days in the subsurface (*16*, *42*−*45*). Thus, even under relatively vulnerable conditions (anaerobic conditions, low organic matter, sandy soils) steroid hormones are sorbed or degraded over relatively short distances with no apparent impact on shallow tile drain discharges. However, in other regions, shallow soils with significant fracturing or macroporosity (e.g., clayey soils, till) overlying sandy to gravelly or highly fractured aquifers with rapid flow rates may provide less attenuation than afforded under the conditions encountered here (*46*, *47*).

Overland flow, rather than groundwater discharge (e.g., via tile drains) of agricultural wastes may contribute to the steroid hormones detected in surface waters, especially during rain events, as evidenced by the higher concentrations of steroids observed in the irrigation canals and rivers as compared to the tile drains (e.g., estrone was detected in approximately half of the canal and river samples but was never detected in tile drain samples). Field studies of pesticide transport in agricultural soils have demonstrated that overland flow after rainfall events can release substantially greater amounts of strongly adsorbing pesticides from agricultural fields relative to infiltration to groundwater, even though the total volume of infiltrating water often is greater than the overland flow volume (*39*, *40*). Further research is needed to assess the potential importance of overland flow and other agricultural practices as sources of steroid hormones in surface waters.

Aquaculture operations also can serve as sources of the steroids testosterone, androstenedione, and estrone with concentrations ranging from 0.1 to 0.8 ng/L in hatchery effluents. Although the concentrations in aquaculture wastes are relatively low, they are potentially significant because, unlike land-based confined animal operations that are prohibited from discharging wastes directly to receiving waters, aquaculture effluents are often discharged to receiving waters with little or no treatment. Typically, aquaculture operations such as hatcheries rely on high flow rates of water through the facilities to dilute wastes, and discharges from these facilities can account for a significant fraction of water in some streams.

Quantitative comparisons between measured steroid hormone concentrations and fish excretion rates are not available. However, data from aquaculture facilities can be used to estimate bulk excretion rates using the water flow rates and estimates of the mass of fish present (Table 3). For this calculation, it was assumed that the mass of each adult Chinook salmon was 10 kg, all samples were well mixed, and the steroid hormone concentrations in the water samples represented the average concentration discharged by the fish. The calculated excretion rates for fish fall into the same range as those reported for livestock, as summarized by Hanselman et al. (*26*). This comparison suggests that hatchery fish excrete comparable amounts of steroids as livestock once excretion rates are normalized to animal mass. For example, normalized excretion rates for estrogens from nonpregnant/nonlaying livestock (dairy cattle, sows, and poultry) range from 100 to 1400 μg/day (*26*), while the calculated excretion rates for estrone estimated for hatchery fish ranged from 260 to 1300 μg/day. Excretion rates for the androgens testosterone and androstenedione from these fish were found to be comparable to the estrogen excretion rates (170−1000 μg/day).

Endogenous steroid hormone production rates from natural aggregations of adult Chinook salmon at a salmon

TABLE 3. Calculated Bulk Steroid Excretion Rates for Fish in Fish Hatcheries, a Fish Ladder, and a Spawning Site in a River

location	mass fish[a] (kg)	calculated excretion rates[b] testosterone (μg/day)	androstenedione (μg/day)	estrone (μg/day)
Mokelumne Hatchery raceway	9 200	na[c]	600	na
Mokelumne Hatchery effluent	55 000	na	1 000	1 300
Nimbus Hatchery raceway	5 300	na	na	na
American Hatchery raceway	14 000	170	430	270
Nimbus/American Hatcheries effluent	180 000	350	490	260
Nimbus fish ladder	10 000 (est)	7 400	8 100	2 100
American River	260 000 (est)	5 300	14 000	8 100

[a] The mass of fish in the fish hatcheries and the holding pens at the fish ladder is calculated from estimates provided by hatchery personnel. The mass of fish in the American River is based upon the estimated return of Chinook salmon to the river (26 000; personal communication, Robert Burks, California Department of Fish and Game), although some of these fish were certainly below the sampling point. [b] Calculated excretion rates are normalized per 1000 kg live animal mass. [c] na, not applicable.

spawning area in the American River were similar to those estimated for fish hatcheries and livestock facilities. Using the same assumptions employed for the hatchery estimates, the estimated excretion rates from adult salmon that were close to or actively spawning were about an order of magnitude higher than the excretion rates estimated for juvenile and adult fish in the fish hatcheries. These results suggest that large aggregations of spawning fish and fish hatcheries may result in concentrations of steroid hormones in surface waters comparable to concentrations from sources such as municipal wastewater effluent.

The results from this study suggest the possibility that, in certain cases, steroid hormones detected in surface waters could be attributable to sources other than municipal wastewater. Aquaculture operations and spawning fish can result in concentrations of androstenedione, testosterone, and estrone comparable to those detected in municipal wastewater effluent. Although the transport pathway by which the steroid hormones are reaching receiving waters is unclear, surface water samples collected in dairy farming areas with no obvious significant sources of municipal wastewater sometimes contain 17β-estradiol, estrone, testosterone, and medroxyprogesterone at concentrations comparable to those observed in municipal wastewater effluent. While inconclusive, these results suggest that overland flow from dairy farming operations or from fields treated with dairy wastes may be significant sources of steroid hormones, given the prevailing dairy management practices of the northeastern San Joaquin Valley. This finding is noteworthy because feminization of male Chinook salmon has been observed in this area and the cause is unknown (*48*).

Our findings provide alternative explanations for steroid hormone detections in surface waters in which wastewater effluent does not account for a large fraction of the overall flow. Furthermore, the detection of similar concentrations of steroid hormones in anthropogenic sources and in water where fish are spawning supports the hypothesis that human activities could disrupt the chemical communication that plays an important role in fish behavior and reproduction (*13*). Additional research is needed to assess the spatial and temporal variation in steroid hormones in surface waters and the potential for these compounds to affect the behavior and reproduction of fish that use these compounds for chemical communication. Further study is also needed to better understand the various pathways through which steroids in agricultural wastes could reach surface waters.

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Supporting Information Available

Table 4. This material is available free of charge via the Internet at http://pubs.acs.org.

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