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Rangeland Grazing as a Source of Steroid Hormones to Surface Waters

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Cattle and other livestock excrete endogenous steroid hormones, including estrogens, androgens, and progestins; therefore, allowing grazing livestock direct access to surface waters can result in the release of steroids in agricultural watersheds. Elevated concentrations of steroids are problematic because low concentrations of certain steroids can affect fish reproduction. To assess the occurrence and transport of steroids arising from grazing cattle, gas chromatography−tandem mass spectrometry (GC/MS/MS) was used to quantify a suite of estrogens, androgens, and progestins in small creeks impacted by rangeland grazing. Steroids were detected in 86% of samples from rangeland creeks where cattle had direct access to the water, with concentrations as high as 44 ng/L observed shortly after rain events at the beginning of the winter wet season. Estrogens were present at concentrations above the predicted no-effect concentrations for fish in 10−20% of the samples, and androstenedione was detected at concentrations higher than response thresholds for pheromonal communication in fish. The results suggest that, in certain cases, measures such as stream fencing in rangeland areas to limit direct discharge of animal wastes to surface waters or better manure management practices might be merited to protect ecosystem health.

Introduction

Research conducted over the last two decades has shown that the reproduction and development of fish are affected by low concentrations of dissolved steroid hormones. The attention of the scientific community first focused on this phenomenon after reports implicated estrogenic steroids in municipal wastewater effluent as the cause of feminization of fish (*1*−*3*). More recent studies have documented the masculinization of fish after exposure to androgens in pulp and paper mill effluent (*4*, *5*) and runoff from confined animal feeding operations (*6*). Although exposure of fish to progestins has not been shown to cause observable morphological changes; progestins, along with certain androgens, are important pheromones in many fish species (*7*−*9*). Because fish biochemistry and behavior are altered by pheromonal steroids at extremely low concentrations (*7*−*9*), pheromonal communication and reproductive behavior may be susceptible to disruption near anthropogenic sources of steroids (*10*).

Relative to point sources, diffuse sources of steroids related to agriculture, such as rangeland grazing or runoff from manure-treated fields, have received much less attention despite the large areas of land used for rangeland grazing. For example, rangeland grazing is the predominant land use for over 32% of the land in the United States (*11*), and in many rangeland areas, cattle are allowed direct access to ponds, rivers, and creeks for drinking water. Although the potential impact of allowing livestock to access surface waters has been recognized as part of a strategy to control water-borne pathogens and stream erosion, the potential for this practice to contribute steroids to surface waters has received only limited attention (*12*, *13*). Given that cattle excrete 0.045−105 mg/day of steroids per animal (*14*, *15*), the daily steroid excretion from a single animal can potentially elevate steroid concentrations by 1 ng/L in as much as 105,000 m^3 of uncontaminated water.

Most studies of steroid occurrence in watersheds with animal agriculture have focused on estrogenic steroids derived from land application of animal manures or discharges from waste storage lagoons (*16*−*19*). However, livestock excrete androgens and progestins at rates comparable to or greater than rates for estrogens (*14*), and the available data indicate that these steroids also occur in watersheds with animal agriculture (*20*−*22*). Recent studies examining receiving waters in grazing rangelands suggest that estrogen contamination may be widespread, with direct discharges of animal wastes to receiving waters serving as a significant source of contamination (*12*, *13*). Additional research is needed to assess the temporal and spatial variability of steroid occurrence and the role of storms in mobilizing steroids in watersheds with grazing livestock.

To investigate the contribution of rangeland grazing areas to steroids in surface waters, samples were collected and analyzed from sites representative of cattle-grazing rangelands in the western United States. Concentrations of steroids detected during wet and dry seasons were compared with threshold values for biological effects to assess the potential impacts of grazing on sensitive fish species. To evaluate the effects of livestock density and rangeland management practices on water quality, the loading of steroids to surface waters was estimated from measured concentrations and estimates of stream discharge.

Materials and Methods

Materials. All reagents were purchased from Fisher Scientific (Pittsburgh, PA) at the highest possible purity. Steroids, also at the highest possible purity, and heptafluorobutyric anhydride (purity >98%), were purchased from Sigma-Aldrich (St. Louis, MO). Deuterated steroids, which were used as surrogate standards, were purchased from CDN Isotopes (Quebec, Canada). Reverse osmosis water was produced using a Nanopure II system (Barnstead, Dubuque, IA).

Sample Sites. Between April 2005 and March 2006, 30 sites were sampled in Stanislaus, Marin, and Sonoma counties in central California (Figure 1). The sampling locations and frequencies were chosen to evaluate the effects of precipitation, streamflow, density of animals, and creek accessibility on concentrations of steroids. These locations are representative of the small headwater creeks (discharge 0.01−9.7 m^3/s, average width 1−12 m) that characterize many watersheds where cattle grazing is the predominant land use. This area of California has a Mediterranean climate, with a dry season typically lasting from April to October. Approximately 60% of the creeks sampled were ephemeral and only contained water between October and July. Samples

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FIGURE 1. Grazing rangeland study locations in California.

were collected at all locations with flowing water during both dry and wet seasons.

The Stanislaus county sites were all located in the Dry Creek watershed in California's Central Valley. This watershed is approximately 540 km^2 and contains over 390 km of tributaries. The maximum elevation of the watershed is 384 m and the average annual rainfall is approximately 32 cm. The basin is primarily low−middle relief, characterized by grasslands, oak woodland, and oak chaparral vegetation types. Although the number of cattle in the Dry Creek watershed is unknown, 284,000 cattle were present in Stanislaus county (2004 data), and the Dry Creek watershed covers 14% of the land area in Stanislaus county. Cattle were present throughout the watershed with estimated densities between 0 and 20 per hectare, with higher densities observed sporadically. Samples were collected from the Stanislaus county sites on April 22, May 16, August 31, and December 29, 2005, and on March 9, 2006.

The Marin/Sonoma county sites were located in five contiguous coastal watersheds with a mixture of rangeland grazing, dairy farms, and crop agriculture land uses. The watersheds encompassing this study area are approximately 845 km^2 in area, with at least 310 km of tributaries upstream of the sampling locations. Relative to the Stanislaus county sites, the Marin/Sonoma county sites are wetter (average rainfall approximately 63 cm/yr), hillier (maximum elevation 601 m), and more densely vegetated with coastal grasslands, oak woodlands, and mixed deciduous−conifer forest types. There were 35,500 cattle in Marin county (2145 km^2) and 80,000 cattle in Sonoma county (4580 km^2) in 2004, and cattle grazing densities were similar to those observed in the Stanislaus sites. Samples were collected from the Marin/Sonoma county sites on June 23, September 8, November 8, and December 2, 2005.

Chemical Analysis. All samples were collected in 12-L fluorinated Nalgene (Rochester, NY) containers. After pressure filtration, the steroids were extracted using 90 mm C-18 solid-phase extraction discs followed by derivatization and gas chromatography−tandem mass spectrometry as described previously (Supporting Information, Table S1, *10*, *20*). The method was modified to include 17α-estradiol, which eluted 1.2 min prior to 17β-estradiol. The daughter ions used for quantification of the 17α-estradiol are identical to those used for 17β-estradiol. Also, d_5-17β-estradiol and d_4-estrone were used as surrogate standards in addition to mesterolone.

Quality assurance and quality control consisted of one reverse osmosis water blank, one duplicate sample, and one matrix recovery sample amended with a mixture of all steroid analytes at concentrations of 0.5−5.5 ng/L per sample event. No steroids were observed in the blank samples, and the coefficient of variation for the duplicate samples was less than 15%. Recovery of the matrix spikes typically ranged from 72 to 160%, with the exception of medroxyprogesterone, where one spike exhibited an unexpectedly high recovery of 261%. In general, the highest matrix spike recoveries were observed for steroids that did not have a deuterated surrogate. For the two analytes that had deuterated surrogates (i.e., 17β-estradiol and estrone), the spike recoveries ranged from 72 to 106%. Due to a problem with the solvent wash step used for cleanup of organic matter, the recovery for estriol, the most polar of the analytes, was unexpectedly low; therefore, estriol was not reported. Method detection limits ranged from 0.1 to 0.2 ng/L, and were analyte dependent (Table S1).

To evaluate the occurrence of steroids in cattle manure leachate, on August 31, November 8, and December 2, 2005, 500 g (wet weight) of fresh cattle manure was collected in the field and placed in a container with 10 L of Nanopure water at 4 °C. After 1 day, 1 L of this water was sampled, filtered, and analyzed for steroid hormones. A second sample from this container was collected and analyzed 1 week later.

Streamflow was measured on March 9 (Stanislaus county sites) and March 20, 2006 (Marin/Sonoma county sites) using a Flowmate model 2000 (Marsh-McBirney, Frederick, MD) electronic flowmeter. Stream discharge was estimated for all sample sites and dates by adjusting the measured stream discharges to archived data from the Dry Creek gauging station (DCM station; CA Dept. of Water Resources) for the Stanislaus county sites and the Lagunitas Creek station (station 11460400; U.S. Geological Survey) for the Marin/Sonoma county sites.

Results

All of the steroid analytes were detected in one or more of the 88 water samples (see Table S2 for complete data). Estrone was detected more frequently than the other steroids, with detectable concentrations in 78% of the samples at concentrations as high as 38 ng/L (Table 1). The estrogen 17α-estradiol was present in 31% of the samples at concentrations up to 25 ng/L, while 17β-estradiol was present in 18% of the samples at concentrations up to 1.7 ng/L. The androgens testosterone and androstenedione were detected less frequently than the estrogens, with detectable concentrations in 11% and 18% of the samples, respectively. Testosterone concentrations never exceeded 2.3 ng/L, whereas androstenedione was detected at concentrations up to 44 ng/L. Progesterone was present in 5% of samples; however, when detected, the concentrations of progesterone were generally higher than those of the other steroids. In three of the four samples in which progesterone was detected, the occurrence of progesterone coincided with the detection of androstenedione. Medroxyprogesterone was only detected in one sample near an urbanized area at a concentration below the limit of quantification.

The maximum concentrations detected for each of the steroids occurred during the wet season (November−March), with the exception of medroxyprogesterone, which was only detected in May. The highest concentrations of 17α-estradiol, estrone, and progesterone occurred in the Marin/Sonoma county watersheds immediately after the first major storm (i.e., >2 cm of precipitation) of the wet season, which occurred on November 6−7, 2005 (Figure 2). This storm was the first storm with enough rainfall for the ephemeral creeks to begin

TABLE 1. Occurrence and Maximum Concentration of Steroid Hormones in Surface Waters on Grazing Rangelands, near Dairy Farms, and in Municipal Wastewater Effluent

receiving water	17α-estradiol	17β-estradiol	estrone	testosterone	androstenedione	medroxy-progesterone	progesterone
Grazing Rangeland (Surface Water)							
percent occurrence [%]; $N = 89$	31	18	78	11	18	1	5
max. concentration [ng/L]	25	1.7	38	4.3	44	0.4	27
Dairy Farms (Surface Water)[a]							
percent occurrence [%]; $N = 32$	NA[c]	6	38	25	0	12	0
max. concentration [ng/L]	NA	0.7	17	1.9	< 0.3	1.0	< 0.4
Municipal WWTP (2°, 3° Effluent)[b]							
percent occurrence [%]; $N = 95-144$	NA	32	37	21	11	14	NA
max. concentration [ng/L]	NA	7.8	19.5	8.0	4.5	14.9	NA

[a] Data from ref *10*. [b] Data from ref *20* and unpublished data. [c] NA = not analyzed.

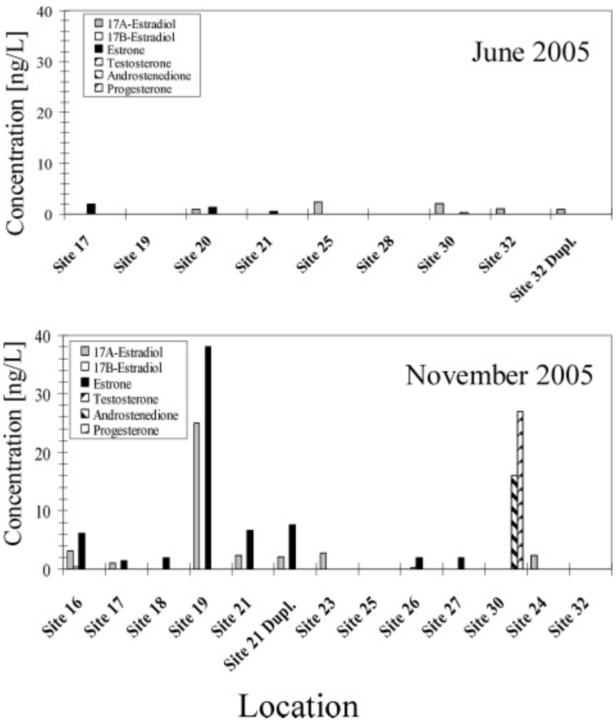

FIGURE 2. Steroid hormone concentrations in the Marin/Sonoma county sampling locations in June 2005 and after the first winter rainstorm in November. Locations are arranged in order of increasing streamflow, from left (lowest flow) to right (highest flow).

flowing, suggesting that a pulse of steroids occurred as winter rains leached steroids from cattle wastes deposited near the creeks. During the dry season, steroid concentrations were below 3 ng/L in all Marin/Sonoma county samples, with the exception of progesterone which was detected at 22 ng/L in one sample. After the first storm, 17α-estradiol, estrone, androstenedione, and progesterone were all detected at several sites in the Marin/Sonoma county watersheds at concentrations above 10 ng/L. Elevated concentrations of steroids also were detected in the Marin/Sonoma county sites after the second major winter storm in December 2005.

At the Stanislaus county sites (Figure 3), concentrations of steroids were similar before and after the first major winter storm on December 26−27, 2005. The differences between the two study areas may be due to the lower rainfall (15−20 mm) of this storm compared to the first storm at the Marin/Sonoma county sites (20−25 mm), to differing intensities of rainfall during the storms, to differing hill slopes, or to other geographical attributes of the study locations.

To investigate the contribution of cattle manure as a source of the steroids detected in the surface water samples, steroids in leachate from three cattle manure samples were quantified (Table S2). Although the data are limited by the small number of samples and a lack of information regarding the physiological status of the cattle that produced the manure, the steroids 17β-estradiol, estrone, testosterone, androstenedione, and progesterone were all detected in leachate samples. Estrone was present in all leachate samples, while the androgens detected in two of the three manure samples suggests that these particular samples were from male cattle.

In an attempt to correlate steroid concentrations to other water quality parameters, nitrate, total coliforms, and *E. coli* were measured in the samples (Table S2). Nitrate concentrations ranged from <0.1 to 70.8 mg/L (as $N-NO_3$). Total coliforms ranged from 480/100 mL to $>2.4 \times 10^6$/100 mL, while *E. coli* ranged from 0/100 mL to $>2.4 \times 10^6$/100 mL. Concentrations of *E. coli* were generally higher during the wet season, with the highest concentrations measured in wet season samples from the Marin/Sonoma county watersheds (Figure S1).

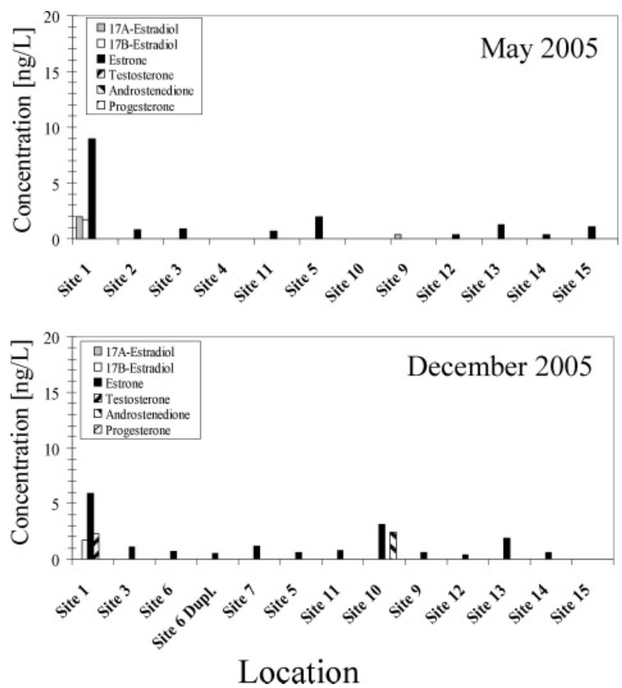

FIGURE 3. Steroid hormone concentrations in the Stanislaus county sampling locations in May 2005, and after the first winter rainstorm in December. Locations are arranged in order of increasing streamflow, from left (lowest flow) to right (highest flow). Locations 1−13 are headwater tributaries where grazing is the predominant land use, while locations 14−15 are downstream locations with little grazing.

Discussion

The concentrations of nitrate and fecal coliforms observed in the creeks in these grazing rangelands are similar to concentrations reported for other surface waters impacted by animal agriculture (*23*, *24*), indicating that animal wastes may be contaminating the creeks. Because animal wastes contain significant concentrations of steroids (*14*), it is not surprising that steroids also were present in the creeks. In these watersheds, cattle often have direct access to surface waters to drink; therefore, it is likely that animal wastes are introduced directly into these surface waters. The frequent detection of 17α-estradiol implicates cattle as the main source of the steroids, as this steroid is predominantly excreted by cattle and not by other livestock or humans (*15*). Additionally, the occurrence of steroids in the surface waters agrees with the data from the manure leachate, also implicating cattle as the main source of steroids. The exception was 17α-estradiol, which has been detected in cattle wastes (*25*) and was observed frequently in the watershed, but was not detected in the manure leachate samples. The most likely explanation for this observation is that 17α-estradiol is predominantly excreted by cattle in urine (*13*).

In the majority of the samples, the concentrations of estrogens were below levels that result in effects in fish. However, in some samples, concentrations of estrogens were high enough to potentially affect fish or other aquatic organisms. While concentrations of the most potent endogenous estrogen, 17β-estradiol, were generally low, with only two detections above 1 ng/L, 17α-estradiol and estrone were present at concentrations up to 25 and 38 ng/L respectively, and estrone was present in most samples. To assess the potential effects of these estrogens on fish, the estrogen concentrations were converted to 17β-estradiol equivalents and compared to predicted no-effect concentrations (PNECs; *26*, *27*). PNECs represent a concentration below which biological effects (i.e., vitellogenesis, morphological changes) have not been observed for sensitive species, while also accounting for the differing potencies of the estrogens (*26*, *27*). 17β-Estradiol equivalents were calculated using a potency factor of 1.0 for 17β-estradiol and 0.2 for estrone (*28*). The potency factor for 17α-estradiol is uncertain, with Khanal et al. (*28*) reporting a potency factor of 1−2 and Legler et al. (*29*) reporting a potency factor of 0.05. Assuming that 17α-estradiol is substantially less potent than 17β-estradiol (potency factor = 0.05), 9 of the 88 samples contained more than the 1 ng/L 17β-estradiol equivalent PNEC (*26*, *27*). By season, 6 samples exceeded PNECs during the dry season and 3 wet season samples exceeded PNECs. If a potency factor of 1.0 is used (*28*), the number of samples exceeding PNECs increases to 20; with 9 of these occurring during the dry season and 11 occurring during the wet season. The prevalence of samples exceeding PNECs in this study indicates that the estrogenic steroids originating from cattle wastes pose a potential risk to aquatic organisms in grazing rangeland surface waters.

In addition to the elevated concentrations of estrogens, relatively high concentrations of androstenedione and progesterone were observed in some samples (Table S2). In particular, these steroids were present at concentrations above 10 ng/L in samples from Site 30 during both the November and December sampling. Androstenedione is a known fish pheromone (*9*). The maximum concentrations of androstenedione detected in this study are above levels at which responsive fish detect this pheromone and are comparable to or higher than concentrations that would be expected to alter biochemistry and behavior in receptive fish (*9*, and personal communication, Peter W. Sorensen, University of Minnesota). The effects of androstenedione at these concentrations might include the inhibition of responses to other fish reproductive pheromones (*9*), potentially altering the fertility and reproductive success in fish species employing pheromonal communication.

The detection of progesterone in these rangeland surface waters was somewhat unexpected, as Schwarzenberger et al. (*30*) suggested that the rapid metabolic transformations of progesterone would decrease its concentrations very quickly. However, progesterone may be more stable than expected as evidenced by its previous detections in surface waters (*31*) and in municipal wastewater effluent (*32*). Additionally, progesterone, along with androstenedione, has been detected in pulp and paper mill effluent by Jenkins et al. (*33*), who hypothesized its formation from microbial transformations of phytosterols. Because androstenedione and progesterone were detected in leachate from cow manure, it is likely that the source of these steroids in the rangeland surface waters was animal wastes; however, phytosterol transformations cannot be ruled out as a source. Consistent with the findings of Jenkins et al. (*34*), it is possible that microbial transformations of phytosterols produce androstenedione and progesterone in the intestinal tract of livestock, raising the possibility that animal wastes could contain two separate pools of steroids: one due to excretion of endogenous steroids and one due to microbial transformations of phytosterols. Finally, because cattle excrete 5−30 times more progestins than either estrogens or androgens (*14*), it is likely that progestins other than progesterone (e.g., 17-hydroxyprogesterone, pregnanediol, pregnanetriol) also are present in animal wastes and contaminated surface waters.

The data indicate that certain locations within these watersheds more frequently exhibit elevated steroid hormone concentrations. For example, Site 1 in the Stanislaus county watershed consistently had some of the highest concentrations of steroids detected. Estrogen concentrations at Site 1 exceeded PNECs on three of the four sampling occasions (assuming 17α-estradiol potency factor = 0.05), while only one other sample from the Stanislaus county watershed exceeded PNECs. Although the land uses adjacent to Site 1 closely resembled the surrounding sampling locations, it also had one of the lowest streamflows of any location in this study, suggesting a relationship between flow and concentration. At Site 30 in the Marin/Sonoma county watersheds, androstenedione and progesterone concentrations exceeded 15 ng/L in both of the wet weather samples. Although more data are needed to determine the temporal variability in steroid concentrations, these results suggest that certain locations might have elevated concentrations of steroids that persist over time periods of a month or more. The persistence and timing of elevated steroid concentrations is a significant issue, as recent studies have demonstrated that exposing fish to low concentration mixtures of estrogenic chemicals, including steroids, resulted in reduced fecundity after periods of less than one week (*35*).

Although steroid concentrations were usually low, the maximum concentrations detected in receiving waters in grazing rangelands were comparable to, or in some cases, higher than, concentrations that were observed in surface waters near dairy operations (*20*) and in municipal wastewater effluent (*10*, *31*, *36*, *37*; Table 1). This suggests that fish in rangeland receiving waters might exhibit some of the symptoms associated with endocrine disruption as have been observed in fish downstream of municipal wastewater effluents. Observation of biological responses to steroids in agricultural watersheds is likely to be complicated by spatial and temporal variability in steroid concentrations. In rangeland receiving waters, steroid concentrations should vary with livestock density, behavior patterns, creek access, weather, streamflow, and geography. Additional research is needed to assess the relative importance of these variables and to identify biological effects.

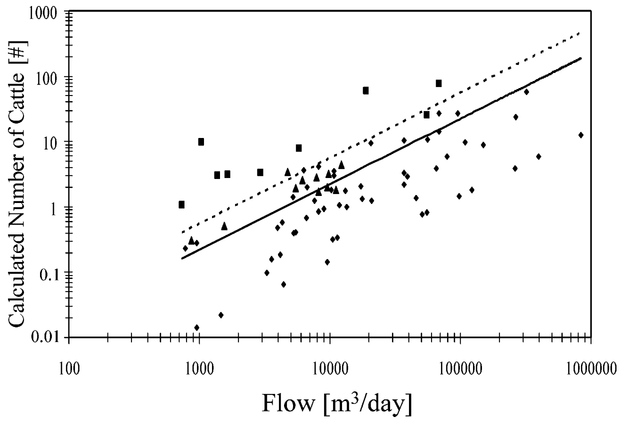

FIGURE 4. Minimum number of cows needed to account for all of the estrogens measured at each field site as a function of streamflow: (■) represent sites > PNEC if 17α-estradiol potency = 0.05 17β-estradiol potency; (▲) represent sites > PNEC if 17α-estradiol potency = 1.0 17β-estradiol potency; (◆) represent sites < PNEC. The dashed line plots the theoretical dilution capacity for each stream if 17α-estradiol potency = 0.05 17β-estradiol potency, while the solid line plots the theoretical dilution capacity if 17α-estradiol potency = 1.0 17β-estradiol potency.

Anecdotal field observations indicate that in some cases the detection of 17α-estradiol might be correlated with the presence of cattle near or in the creek upstream of the sampling location at the time sampling occurred. For example, some of the highest concentrations of 17α-estradiol measured in this study were at Site 1 (April 2005), Site 13 (August 2005), and Site 16 (November 2005). On these dates, cattle were observed near or in the creek 100−400 m upstream of the sampling location during sampling. Biotransformation reactions produce estrone from 17β-estradiol over time scales of 0.2−9 days in rivers (*28*), and 17α-estradiol is likely to react at similar time scales. Therefore, elevated concentrations of 17α-estradiol, and also 17β-estradiol, might be evidence that animal wastes were recently released to surface waters.

In the Stanislaus watershed, steroid concentrations were generally higher in headwater reaches where grazing is the predominant land use (sites 1−13) relative to downstream reaches where farming and residential land uses dominate (sites 14 and 15, see Figure 3 and Table S2). Headwater reaches contained steroids at concentrations up to 14 ng/L, while downstream reaches had no concentrations above 2 ng/L. Although the differences between headwaters and downstream locations are not statistically different due to small sample size, organisms in headwaters probably are exposed to higher steroid concentrations than those living downstream where agricultural steroid inputs are diluted with uncontaminated water from other sources.

The mass of steroids discharged from these watersheds was estimated using the streamflow measurements and data from nearby stream gauging stations. In these watersheds, the maximum mass discharges for estrogens were 800 mg/day, for androgens 3000 mg/day, and for progestins 1400 mg/day. The mass discharges can be used to estimate the minimum number of cows needed to account for the steroids measured in these samples (Figure 4 and Table S3) using previously published excretion data (*14*, *15*, *25*). For the estrogens, the data were used to determine the dilution capacity at each location, which is an estimate of the number of cattle that could discharge all of their wastes into the receiving waters before the total estrogen load exceeds the PNEC. The data in Figure 4 indicate that in small creeks (flow of ~1000 m^3/day, or ~0.012 m^3/s), one animal can elevate estrogen concentrations above PNEC values, while PNECs are exceeded in the largest creeks only by the wastes of 100 cattle or more. Therefore, if cattle are allowed direct access to receiving waters, each animal needs at least 1800−4500 m^3/day (range due to different values for 17α-estradiol potency) of streamflow to maintain estrogen concentrations below PNECs. For comparison, the estimated dilution capacity for nitrate is 250−300 m^3/day, assuming a PNEC of 1 mg NO_3−N/L for sensitive organisms (*23*, *38*, *39*). This estimate assumes that all nitrogen excreted by cattle is quickly oxidized to nitrate, and all excreted nitrogen is deposited in, or transported to, the receiving water. By this metric, 68% of the samples exceeded nitrate PNECs, although it is unclear whether the nitrate is entirely derived from animal wastes and not fertilizer or atmospheric deposition. These dilution estimates indicate that a larger volume of dilution water is needed for estrogens than for nitrate.

In cases where the density of cattle discharging to a watershed exceed the dilution capacity, rangeland management practices that limit the direct access of animals to the water could be used to reduce steroid concentrations below PNECs. Limiting the access of cattle to streams is likely to be effective because only a fraction of steroids produced by an animal will enter the water if they are deposited away from stream banks.

In the case of androstenedione and progesterone, our estimates suggest that some of the Marin/Sonoma county sites discharge steroid concentrations equivalent to hundreds or even thousands of cattle. The large number of cattle needed to explain these steroid concentrations suggests that large masses of dispersed steroids have been accumulating in the watershed during the dry season and are being transported during the first storm. Alternatively, it may indicate that steroids are being transported from concentrated sources of animal wastes, such as leaking animal waste lagoons or leachate from manure processing areas.

One of the objectives of this study was to correlate steroid occurrence and concentration to easily measured surrogate parameters that might be indicative of animal waste contamination. For this reason, nitrate and fecal coliforms were measured along with the steroids. The measured nitrate concentrations were comparable to those reported for receiving waters in agricultural areas contaminated with animal wastes (*23*, *39*), and the coliform concentrations in the rangeland creeks were similar to concentrations downstream of animal agriculture operations and pastures (*24*). Despite the fact that the highest concentrations of steroids and fecal coliforms occurred in the Marin/Sonoma county watersheds during the wet season, no direct correlation was observed between these parameters (data not shown). These data suggest that elevated fecal coliforms or nitrate might be useful in identifying impacted watersheds, but will not necessarily indicate the presence of elevated concentrations of steroids in a specific location.

The data from this study indicate that non-point sources of steroids such as grazing cattle can, in certain instances, elevate steroid concentrations in receiving waters to levels of concern for aquatic organisms. In the majority of the samples, steroid concentrations were not above threshold concentrations, suggesting that rangeland practices that allow cattle direct access to surface waters do not impact ecosystem health when there is adequate dilution of the receiving water. However, in approximately 10−20% of the rangeland samples, steroid concentrations exceeded PNECs for the feminization of fish, indicating that allowing cattle direct access to surface waters may impact the health of aquatic organisms in the receiving waters. Additionally, the detection of endogenous steroids in these rangeland watersheds likely indicates that natural and synthetic steroids administered to livestock for pharmaceutical purposes could be released through similar mechanisms. None of the common synthetic steroid hormones were analyzed during this study. One period where special concern might be warranted is immediately after heavy rains when wastes that have accumulated in the watershed are flushed from the system. These pulses of steroids may correspond to periods when certain species are vulnerable. For example, in some of the Marin/Sonoma county sites, endangered Coho salmon (*Onchorhynchus kisutch*) enter the watersheds to spawn soon after the first winter rains. Coincidentally, this is also the time period when the highest concentrations of steroids were detected. More research is needed to assess the temporal and spatial variation of steroid concentration during these periods, as well as the effect of anthropogenic sources of pheromonal steroids such as androstenedione on aquatic organisms.

Acknowledgments

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Supporting Information Available

Detailed description of the analytical methods, a table of the analytes and related instrument parameters, the complete data set, and a table of the mass discharges of the androgens and progesterone. This information is available free of charge via the Internet at http://pubs.acs.org.

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