A Source of Sexual Dysfunction: Estrogen Output from Wastewater Treatment Plants to the San Francisco Estuary

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ABSTRACT

Given the incidence of estrogenic activity in the San Francisco Estuary (SFE) and corresponding declines in open-water fish species since 2001, a key initial goal in reducing future exposure to estrogenic compounds is to identify the primary routes they take into the ecosystem. Anthropogenic estrogenic-endocrine disrupting chemicals (e-EDCs) typically enter the environment through wastewater treatment plants (WWTPs) and their associated effluent discharge points. Estimates for the daily outputs of the four most potent estrogenic compounds, estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethynylestradiol (EE2), are calculated over the course of the 2018 water year along with the corresponding estrogenic potency the combined effluent possesses upon discharge. Average discharge concentrations for E1, E2, E3, and EE2 are 1.643 ng/L, 0.561 ng/L, 0.537 ng/L, and 0.0330 ng/L respectively. Combined estrogenic activity is calculated in estradiol equivalents (EEQs [ng/L]) and ranges from 18.699 EEQs to 31.388 EEQs with an average value of 25.236 EEQs. Results indicate that with the exception of the American Canyon and Las Gallinas WWTPs, all other WWTPs discharge at EEQ values greater than the 0.30 EEQ threshold associated with long-term exposure risk to aquatic organisms. This suggests that effluent discharge points into the SFE are likely candidates for being contributing sources of the sexual dysfunction observed in some open-water fish species in the estuary. The persistent exposure of organisms in the SFE to sub-lethal yet deleterious concentrations of estrogenic compounds suggests that additional attention and precaution should be paid to these increasingly prevalent and ubiquitous aquatic pollutants.

Keywords

estrogenic endocrine-disrupting chemical, Estradiol Equivalent, natural estrogen, wastewater treatment plant, effluent

INTRODUCTION

With contemporary advances in the sensitivity of environmental quality assessment techniques, anthropogenic compounds can now be identified in concentrations too diffuse to have been previously detected by traditional analysis techniques like gas and liquid chromatography as well as mass spectroscopy (Noguera-Oviedo et al. 2016). Liquid chromatography instruments coupled with quadruple ion trap mass spectroscopy have been demonstrated to be able to detect compound concentrations in the nanogram per liter (ng/L) range as early as 1978 (Yost and Enke 1978), however it was not until 2001 that it was demonstrated that this technology could be used in tandem to high-resolution mass spectroscopy to accurately identify unknown compounds in water (Pastorova et al. 2001). The pollution of surface water ecosystems in particular has attracted international attention due to their economic, public health, ecosystem service, and recreational values (Ternes 1998, Stumpf 1999, Ellis 2006, Andreozzi et al. 2013, Grabicova et al. 2017). However, despite increased attention being placed on the identification and quantification of environmental contaminants, relatively little is known in regard to how trace amounts of many anthropogenic pollutants and their metabolites interact with and within aquatic ecosystems.

Concerns regarding the chronic exposure of aquatic ecosystems to trace chemical compounds largely stems from how little we truly know about them. The ecotoxicological effects of many compounds is simply unknown (Fent et al. 2005, Tambosi et al. 2010). Among these compounds, particular emphasis has been previously placed on assessing the ecological impact of endocrine-disrupting chemicals (EDCs). Endocrine-disrupting chemicals are substances that either mimic or interfere with the operation of biosynthesized hormones such as estrogen, androgen, and anti-androgen within an organism (Marcus 2009, Swan 2009). EDCs are considered toxic compounds due to their adverse effects on both individual organism functioning as well as reproductive health (IPCS 2002, Fuhrman et al. 2015). EDCs related in some manner to the operations of estrogen and other estrogen-like hormones are referred to as estrogenic EDCs (e-EDCs). These compounds are known to leach from most commercially available plastic products, even those advertised as being bisphenol A (BPA) free (Yang et al. 2011, Bittner et al. 2014) and are classified by the World Health Organization to be both endocrine disruptors as well as group 1 carcinogens (WHO 2005) . e-EDCs are ubiquitously present in aquatic environments worldwide and the chronic exposure to low-level concentrations of e-ECDs is common for both aquatic biota and humans alike (Kolpin et al. 2002, Snyder and Benotti 2010). The major sources of naturally produced estrogens are livestock manure as well as human urine and feces (Shore and Shemesh 2003). The four anthropogenic e-EDCs of greatest environmental concern are the steroid estrogens estrone (E1), 17β-estradiol (E2), estriol (E3), and 17α-ethynylestradiol (EE2) (Campbell et al. 2006, Vandenberg et al. 2013, Fuhrman et al. 2015). These compounds often account for approximately 90% of estrogenic activity in surface water ecosystems (Windsor et al. 2018). E1, E2, and E3 are naturally synthesized mammalian estrogens, and EE2 is a synthetic hormone primarily used as the active ingredient in oral contraceptives, the most prescribed drug in the world (Briciu et al. 2009). Concentrations of EE2 and E1 as low as 0.5 ng/L and 25 ng/L respectively have been shown to initiate the production of vitellogenin in fish populations (Routledge et al. 1998). The production of vitellogenin is often used as a chemical indicator of feminization in male fish (Silva et al. 2012). Additional studies have identified intersex fish (Hinck et al. 2009) and amphibian (Lambert et al. 2015) populations in the United States as well sex ratio imbalances in fish populations in the United Kingdom (Jobling et al. 2006) and the SFE (Spearow et al. 2011) as a result of ambient estrogen exposure. E2 and EE2 ability to bioaccumulate via predation presents cascading risks to species of higher trophic levels (Hibberd et al. 2009, Magi et al. 2010). Consequences of e-EDC exposure to humans include reproductive system abnormalities, decreased sperm count, and the increased incidence of testicular and breast cancer (Martínez et al. 2011, Pereira et al. 2011).

The primary route through which anthropogenic e-EDCs entire the environment is through wastewater treatment plants (WWTPs) (Ternes 1998, Tambosi et al. 2010, Fuhrman et al. 2015). Thusly, substantial research has gone into the identification and study of e-EDCs upstream, downstream, and within sewage treatment plants (Sumpter and Jobling 1993, Purdom et al. 1993, Oulton et al. 2010, Liao et al. 2014). In attempting to access the potential risks an e-EDC or combination of e-EDCs may present to any given region or population, one of the metrics of paramount importance is the concentration in which the chemicals are present. EDCs are known to be hormonally active in concentrations as low as being on the scale of parts per billion or parts per trillion by weight in the human body and manifest their presence physiologically in radically different manners as their concentrations increase (Hecker and Hollert 2011, Campbell et al. 2006). The predicted-no-effect concentration (PNEC) for E1, E2, and EE2 are as low as 6, 2, and 0.1 ng/L respectively (Caldwell et al. 2012). In the United States, the US Food and Drug Administration only requires environmental risk assessments for contaminants predicted to be present in aquatic environments at concentrations greater than one microgram per liter (1 μg/L) (FDA-CDER 1998), concentrations that are three orders of magnitude greater than those commonly reported in the scientific literature for e-EDCs in surface water ecosystems. Even for those compounds that do possess Federal Drug and Food Administration (FDA) water quality standards, substance toxicities are evaluated on an individual basis rather than considering their potential toxicities when present together in a solution (Stackelberg et al. 2004). This further complicates both the study of how these compounds may behave in synergistic or antagonistic manners when concurrently present in an aquatic system. Comparable shortcomings in terms of consistent dilute chemical monitoring exist in European Union due to environmental risk assessments of human medicinal products only being necessary for commercially advertised products as of 2005 (EMEA 2005).

There is considerable debate regarding whether or not dose-response curves for many EDCs are linear or parabolic (Vandenberg et al. 2013). Such parabolic dose-curves are known as Non-Monotonic Dose Response Curves (NMDRCs), and they are employed by endocrinologists to depict the physiological activity of a hormone in low and high concentrations but not intermediary ones (Vandenberg et al. 2013). NMDRCs have been identified for at least 70 EDCs of varying chemical classes (Vandenberg et al. 2012), but should this trend be a more common characteristic of EDCs than is currently understood, precise quantifications of EDC concentrations may be essential to any future risk assessment of these chemicals. Granted both the warranted concern that e-EDCs place on public health as well as our current shortcomings in terms of their identification, mechanism of action, and dose-response, models that provide reliable estimates of e-EDC discharge from WWTPs present themselves as vital tools in both predicting and preemptively addressing the public health concerns these chemicals may ultimately present.

e-EDC modeling

Directly measuring estrogen concentrations from WWTP effluent is a costly and time-intensive process. The time, labor, and funds employed to ensure accurate readings are highly inefficient and impractical for providing consistent and accurate quantifications of e-EDCs leaving WWTPs (Umali et al. 2012). In developing countries, the lack of funding, expertise, and available labor to conduct direct measurements of e-EDC concentrations makes this approach for monitoring and risk assessment practically impossible (Dotan et al. 2017). The cumulative prevalence of anthropogenic e-EDCs is expected to increase in concurrence with the logistic growth of the human population worldwide (Fleming et al. 2016). Predictive technologies unhindered by time, labor, and funding constraints have presented themselves as the most practical risk assessment tools for monitoring estrogen concentrations in WWTP effluent.

e-EDC concentration models

To assess the risk of e-EDC discharge, one option is to take a mechanistic approach towards modeling e-EDC output from WWTPs. By employing regional demographic data, known e-EDC excretion rates from contributing demographic populations, and WWTP operational parameters, researchers have estimated the concentrations of E1, E2, and EE2 in WWTP effluent (Johnson et al. 2000, Johnson and Williams 2004, Atkinson et al. 2012, Umali et al. 2012, Fleming et al. 2016). Johnson et al. (2000) first developed a model estimating E1, E2, and EE2 output from WWTPs based upon estimates of the total load of e-EDCs excreted via urine from a contributing population. Additional refinements to this model were implemented to incorporate the fecal excretion of e-EDCs and the natural degradation of e-EDCs in sewer transit (Johnson and Williams 2004, Fleming et al. 2016). Further calibration then addressed the conjugation of e-EDCs in wastewater that had previous resulted in consistent overestimations of E1 outputs (Liu et al. 2015). The drawback of this modeling approach lies in it requiring extensive information regarding the operational parameters of WWTPs, contemporary and accurate census data, and accurate estimations of natural degradation and conjugation rates in sewer transit (Liu et al. 2015, Ting et al. 2017). Accurate census data in particular is a major limiting factor to this modeling approach since it requires accurate representations of female age distributions as well as estimates of the number of pregnant, menopausal, and ovulating women serviced by the WWTPs to produce reliable and accurate results. However, due to this being among the only modeling approaches capable of producing relatively accurate results for EE2 effluent concentrations, I plan on using this approach to model EE2 output.

An alternative and simplified approach to estimating e-EDC concentrations in untreated wastewater draws on the positive correlation that exists between the biochemical oxygen demand (BOD) of untreated wastewater and influent e-EDC load (Drewes et al. 2005, Dotan et al. 2017).

That is to say, as influent BOD values increase, one would expect to find a corresponding increase in E1, E2, and E3 concentrations (Dotan et al. 2017). This suggests that influent BOD measurements can be used as proxy measures to estimate e-EDC influent concentrations. Although this model offers little insight into the mechanism that may or may not be responsible for the observed correlation, the strength of the direct correlation of BOD with E1, E2, and E3 concentrations ($r^2 = 0.84$, $r^2 = 0.80$, and $r^2 = 0.89$ respectively) suggests that it can yield reliable estimations of their concentrations in untreated wastewater (Drewes et al. 2005). In tandem to data regarding WWTP removal efficiency for these compounds, this approach can be used to estimate e-EDC effluent concentrations. The key benefits of this approach over more mechanistic approaches lie in its computational simplicity and its reliance on data that is far more available in the relevant literature than demographics (Ting et al. 2017). Additionally, this approach appears to provide more accurate estimations of E1, E2, and E3 concentrations than the demographic approach; EE2 concentrations appear to be much more loosely correlated to wastewater BOD (Dotan et al. 2017). In light of the greater accuracy provided by the correlative approach to modeling E1, E2, and E3 concentrations relative to the mechanistic approach, as well as the greater abundance of BOD data relative to accurate census data, I will rely on the correlative model developed by Drewes et al. (2005) to estimate E1, E2, and E3 effluent concentrations.

Estrogenic activity assessment

The concentration added (CA) and estradiol equivalent concentration (EEQ) frameworks have been employed to assess estrogenic activity as well as evaluate the toxicological risk posed by extant estrogens (Ting et al. 2017). CA models are among the most commonly employed assessment tools for chemical mixture toxicity research and assume that each compound of the mixture contributes a toxic effect on the environment relative to its own dose-response curve and potency (USEPA 2000). The EEQ model simplifies the CA model by summing the concentrations of each individual component after weighting their estrogenic activity relative to that of E2 (Ting et al. 2017).

Study site

The San Francisco Bay Estuary is the largest estuary system on the western coast of the United States, spans over 500 square miles, and provides critical habitat, breeding, and foraging grounds to thousands of species, including many that are currently threatened or endangered (SFBCDC 2015). From the nine counties that occupy lands adjacent to the bay, 30 WWTPs release effluent into the bay or bay-bound receiving waters. Being home to thousands of species, a provider of important ecological services like carbon sequestration, nutrient cycling, and flood protection, as well as containing one of the nation's most economically vital shipping harbors, the SFE was identified by residents and the international community alike as a "Wetland of International Importance in 2012 (SFBCDC 2015). Due to its ecological, economic, and recreational utilities, the degradation and pollution of the SFE waters is a topic that consistently attracts political and academic attention.

Estrogenic activity in particular has been reported in the San Francisco Bay with EEQ values as high as 242 ng/L, far above estimated biological no-effect-thresholds (Lavado et al. 2009, Caldwell et al. 2012). Pelagic fish species, such as the striped bass (*Morone saxatilis)*, have experience precipitous declines in population since 2001, and studies suggest that e-EDCs may be contributing factors resulting in observed reproductive dysfunction (Spearow et al. 2011). Estrogen concentrations found in the SFE are comparable to those observed in a subestuary of the Chesapeake Bay, the Back River, where the estrogenic activity was primarily attributed to wastewater (Schlenk et al. 2012, Loyo-Rosales et al. 2010). I have selected this study site in order to assess the potential impact estrogenic WWTP effluent discharge may have on the observed sexual dysfunction of fish within the SFE. To explore this topic, I address the following sub-questions: (1) What quantities of E1, E2, E3, and EE2 are discharged to the San Francisco estuary system?; (2) Do trends in estrogen output vary spatially and/or temporally?; and (3) Do output concentrations of estrogens exceed presumed safety thresholds?

METHODS

E1, E2, and E3 estimates

To estimate the total output of E1, E2, and E3 into the SFE, I produced estimates of effluent output from each of the 30 WWTPs discharging into the bay using the BOD model first developed by Drewes et al. (2005) and later refined by Dotan et al. (2017). I collected daily influent BOD and flow values as well as daily effluent flows from the California Integrated Water Quality System Project's Electronic Self-Monitoring Reports program for the 2018 water year (October 1, 2017 – September 30, 2018). I then input these values into the estimation equations developed and statistically validated for accuracy by Dotan et al. (2017). The equations for raw wastewater estrogen concentrations are as follows:

$$\text{Estrone (E1) [ng/L]} = 10^{0.9916 \text{ X } \log(\text{BOD load [kg/day]}) - 0.5.5568} / \text{Q} \quad (1)$$

$$17\beta - \text{estradiol (E2) [ng/L]} = 10^{1.019 \text{ X } \log(\text{BOD load [kg/day]}) -1.3016} / \text{Q} \quad (2)$$

$$\text{Estriol (E3) [ng/L]} = 10^{1.1934 \text{ X } \log(\text{BOD load [kg/day]}) -1.1615} / \text{Q} \quad (3)$$

Where Q is the daily influent flow [m^3 / day].

Using natural estrogen (NE) removal efficiencies from WWTPs utilizing secondary treatment processes, I estimated NE effluent concentrations for each of the 30 WWTPs discharging into the bay using median removal efficiencies sourced from Schaider et al. (2017). I completed such calculations using the following equation:

$$\text{Estrogen Influent Concentration [ng/L] X Removal Efficiency [\%] = Estrogen Effluent Concentration [ng/L]} \quad (4)$$

EE2 estimate

I determined EE2 output into the SFE in accordance to the methods used by Johnson and Williams (2004). I acquired census data for each city serviced by their representative WWTP via the United States Census Bureau. I used estimates of city populations for the year of 2017 to determine the population size and sex distributions for each population contributing to their corresponding treatment plant. Since only women taking oral contraceptives are relevant contributors of EE2 to the wastewater system, I took the population of women in the age range of 15 to 44 years old from census data to be considered as potential contributors. I then multiplied these population values by the average percentage of US women between the ages of 15 to 44 years old that are taking oral contraceptives as reported by the CDC. I conducted these calculations as follows:

$$P_{G,i} = P_i * G_i \tag{5}$$

Where $P_{G,i}$ is the number of women ages 15 to 44 taking oral contraceptives within the population being serviced by WWTP i, P_i is the percentage of women taking oral contraceptives for the county WWTP i services, and G_i is the number of women between the ages of 15 to 44 serviced by WWTP i.

I then multiplied these values by the mean per capita EE2 net excretion rates as published by Johnson and Williams (2004). Altogether, I calculated EE2 influent load using the following equation:

$$\text{EE2 Influent Concentration [µg/L]} = \frac{\sum_{i=0}^{n}[P_{G,i}(U_{E,i}+F_{E,i})^{i}]}{Q} \tag{6}$$

Where $U_{E,i}$ [µg/L] is the per capita estrogen excretion rate in urine, $F_{E,i}$ [µg/L] is the per capita estrogen excretion rate in feces, and Q [L/day] is the WWTP average daily flow rate. I used a median removal efficiency of 52%, as provided by Schaider et al. (2017), to determine EE2 effluent concentration estimates. I calculated EE2 effluent concentration estimates using Eq. (4).

Estrogenic activity assessment

I derived the estrogenic activity of WWTP effluent using the EEQ model. Adjusting for the relative potency of each estrogen present in the effluent water sample, I computed the estrogenic activity of the sample as follows:

$$C_{mix} = \sum_{i=1}^{n} C_i \left[\frac{(EC_{50,E2})}{(EC_{50_i})}\right] \quad (7)$$

Where C_{mix} is the sum of estrogen concentrations in a given sample, C_i is the concentration of compound i in the mixture, and $\frac{(EC_{50,E2})}{(EC_{50_i})}$ is the estrogen equivalency factor (EEF) of compound i relative to 17β – estradiol. I obtained EEF values for E1, E2, E3, and EE2 from Vega-Morales et al. (2013). These values are summarized in table 1.

Table 1. EEF Values Derived Using Recombinant Yeast Assay: Data sourced from Morales et al. (2013)

Compound	EEF
E1	0.11
E2	1.00
E3	0.11
EE2	1.25

I evaluated short and long term risk to marine life near effluent discharge points against study safe concentrations of Estrogenic Equivalents (EEQs –SSEs) as derived by Jarošová et al. (2013); short term exposure risk and long term exposure risk EEQ values are determined to be 1.40 EEQs and 0.30 EEQs respectively. EEQs-SSEs are defined as the concentration of EEQs for each of the four contributing chemicals for which no individual chemical exceeds its corresponding PNEC and are calculated as follows:

$$EEQ\text{-}SSE_{Ei} = EEF_{Ei} \text{ X } PNEC_{Ei} / (P_{Ei\text{-}Max} / 100\%) \quad (8)$$

Where EEQ-SSE_{Ei} is the EEQ value considered safe regarding each steroid estrogen, EEF_{Ei} is the estrogenic equivalency factor of estrogen i, $PNEC_{Ei}$ is the Predicted-No-Effect Concentration of estrogen i, and $P_{Ei\text{-}Max}$ is the maximal percentage of cumulative EEQ (cEEQ) for estrogen i. Long-term and short-term exposure risks are delineated as greater than 60 days of exposure and less than 60 days of exposure respectively and are based upon calculated *in vitro* PNECs for these corresponding time periods in all four compounds.

RESULTS

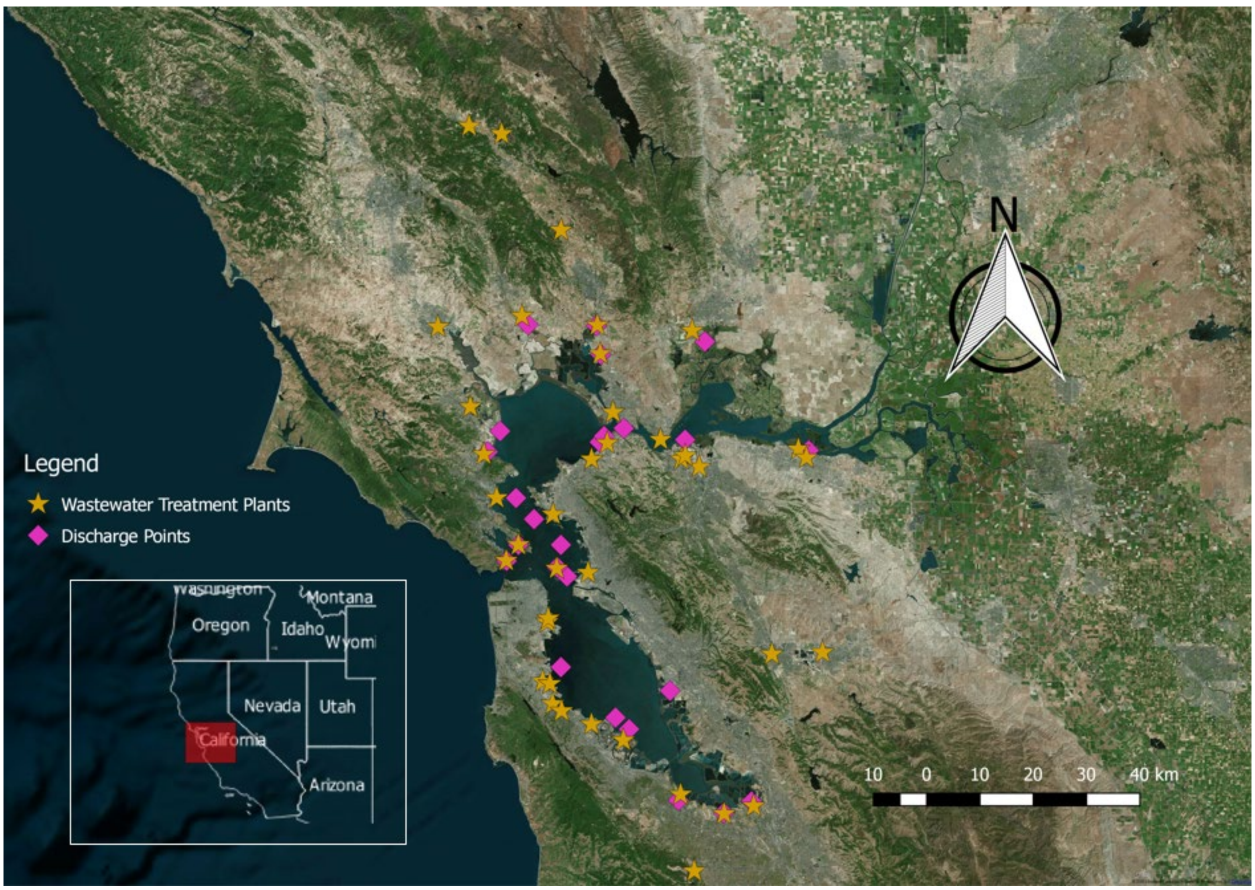

Figure 1. Map of San Francisco Bay Estuary System: Labeled are the locations of wastewater treatment plants discharging into the SFE, their corresponding effluent discharge points, and the location of wetlands.

E1, E2, and E3 estimates

Among the 30 plants and 25 effluent discharge locations modelled, E1 effluent concentrations ranged from 0 ng/L to 10.528 ng/L, E2 effluent values ranged from 0 ng/L to 2.909 ng/L, and E3 effluent values ranged from 0 ng/L to 3.080 ng/L. Average discharge concentrations for E1, E2, and E3 were 1.643 ng/L, 0.561 ng/L, and 0.537 ng/L respectively. The greatest average daily contributors of NEs to the SFE over the course of the 2018 water year were the North Bay System Unit (NBSU), East Bay Discharger Authority (EDBA), and West County Agency WWTPs. In contrast, the Las Gallinas, American Canyon, and Sausalito WWTPs were the least significant average daily NE contributors to the SFE. Both the NBSU and EDBA discharged E1 and E2 at concentrations above their respective PNEC values of 6 ng/L and 2 ng/L. No plant discharged E3 in concentrations near its PNEC of 60 ng/L; the greatest average daily contributor of E3 was the NBSU at 3.080 ng/L. Average daily discharge data for NEs is summarized in table 2.

Table 2. Natural Estrogen Discharge Summary

Plant #	Wastewater Treatment Plant Name	Average Daily Flow (MGD)	Average Daily E1 contribution (ng/L)	Average Daily E2 contribution (ng/L)	Average Daily E3 contribution (ng/L)
1	American Canyon	1.138	0.983	0.320	0.198
2	Benicia	1.999	1.724	0.581	0.422
3	Central Contra Costa County	34.797	1.114	0.399	0.442
4	Central Marin	8.986	1.695	0.592	0.557
5	Delta Diablo	8.850	1.954	0.682	0.694
6	EBDA	70.301	8.320 *	2.909 *	2.783
7	EBMUD	52.252	1.599	0.584	0.740
8	Fairfield Suisun	13.143	1.426	0.498	0.475
9	Las Gallinas **	1.364	0.583	0.194	0.137
10	Marin CSD 5	0.586	0.998	0.319	0.319
11	Napa **	4.327	0.691	0.691	0.691
12	NBSU	79.702	10.528 *	2.746 *	3.080
13	Novato & Ignacio	4.097	1.693	0.578	0.453
14	Palo Alto	19.300	1.542	0.547	0.570
15	Rodeo	0.575	1.154	0.369	0.199
16	San Jose & Santa Clara	87.396	1.675	0.625	0.901
17	San Mateo	5.836	1.434	0.496	0.442
18	SASM	2.334	1.578	0.530	0.324
19	Sausalito	1.079	1.054	0.344	0.211
20	Sunnyvale	10.304	1.040	0.362	0.336
21	SVC	2.217	1.054	0.344	0.211
22	SVCW	13.959	1.040	0.362	0.336
23	Treasure Island	0.314	0.351	0.351	0.351
24	Vallejo	9.283	1.326	0.459	0.409
25	West County Agency	10.402	2.720	0.942	0.826

* = Output concentration is above Predicted-No-Effect Concentration as reported by Caldwell et al. (2012)
** = Plant diverts effluent flow to water recycling facility from 5/1/2017 – 10/31/2018

EE2 estimates

Among the 30 plants and 25 effluent discharge locations modelled, EE2 effluent discharge concentrations ranged from 0 ng/L to 1.34 ng/L with an average discharge concentration of 0.0330 ng/L. The greatest average daily contributors of EE2 to the SFE over the course of the 2018 water year were the NBSU, Rodeo, and Sewerage Agency of South Marin (SASM) WWTPs. In contrast, the Las Gallinas, Napa, and Sausalito WWTPs were the least significant average daily EE2 contributors to the SFE. Only the NBSU discharged EE2 at concentrations above its PNEC values of 0.1 ng/L. No other plant discharged above this threshold concentration. Average daily discharge data for EE2 is summarized in Table 3.

Table 3. Synthetic Estrogen (EE2) Discharge Summary

Plant #	Wastewater Treatment Plant Name	Average Daily Flow (MGD)	Average Daily EE2 contribution (ng/L)
1	American Canyon	1.138	0.0302
2	Benicia	1.999	0.0237
3	Central Contra Costa County	34.797	0.0231
4	Central Marin	8.986	0.0156
5	Delta Diablo	8.850	0.0393
6	EBDA	70.301	0.0242
7	EBMUD	52.252	0.0231
8	Fairfield Suisun	13.143	0.0172
9	Las Gallinas **	1.364	0.0080
10	Marin CSD 5	0.586	0.0185
11	Napa **	4.327	0.0087
12	NBSU	79.702	0.2833 *
13	Novato & Ignacio	4.097	0.0175
14	Palo Alto	19.300	0.0123
15	Rodeo	0.575	0.0829
16	San Jose & Santa Clara	87.396	0.0293
17	San Mateo	5.836	0.0333
18	SASM	2.334	0.0611
19	Sausalito	1.079	0.0120
20	Sunnyvale	10.304	0.0266
21	SVC	2.217	0.0586
22	SVCW	13.959	0.0260
23	Treasure Island	0.314	0.0128
24	Vallejo	9.283	0.0235
25	West County Agency	10.402	0.0456

* = Output concentration is above Predicted-No-Effect Concentration as reported by Caldwell et al. (2012)
** = Plant diverts effluent flow to water recycling facility from 5/1/2017 – 10/31/2018

Estrogenic activity

Upon adjusting effluent concentrations to EEQ values, the daily cEEQ summed across all 25 discharge points for the 2018 water year ranged from 18.699 EEQs [ng/L] to 31.388 EEQs [ng/L] with an average value of 25.236 EEQs [ng/L]. Individual daily average discharge EEQ values for each WWTP are summarized in table 4. In order of descending magnitude, E2, E1, E3, and EE2 contributed to daily cEEQ values. Average daily contributions for E2, E1, E3, and EE2 were 16.820 EEQs [ng/L], 5.421 EEQs [ng/L], 1.772 EEQs [ng/L], and 1.224 EEQs [ng/L] respectively. Daily contribution trends for both NEs, EE2, and cEEQ values are depicted in figures 2-4.

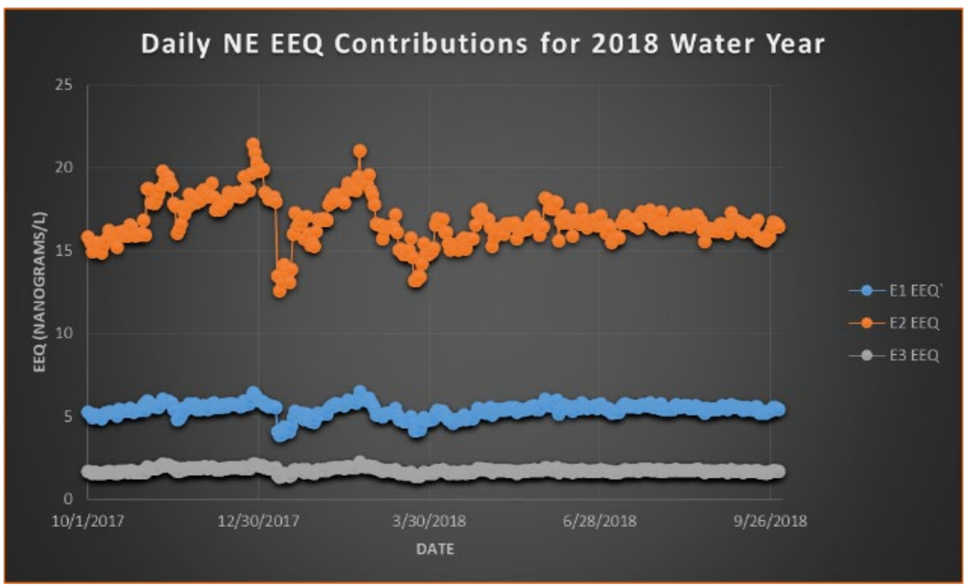

Figure 2. Daily NE EEQ Contributions for 2018 water year

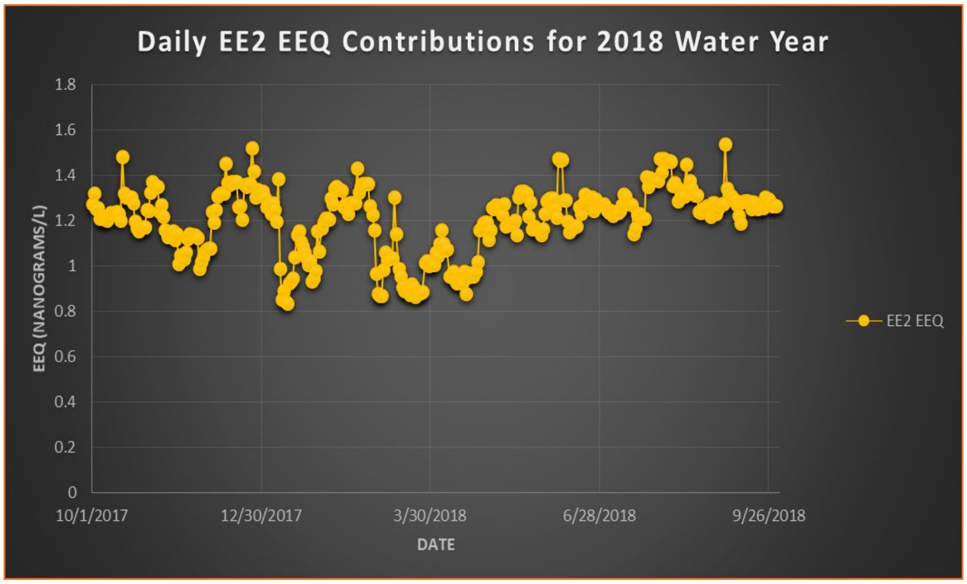

Figure 3. Daily EE2 EEQ Contributions for 2018 water year

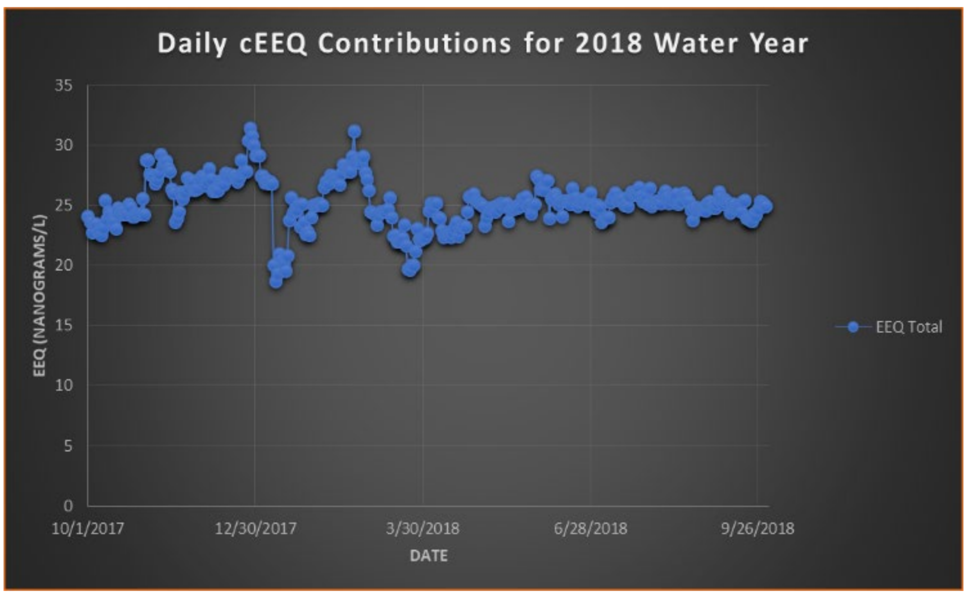

Figure 4. Daily cEEQ Contributions for 2018 water year

Table 4. Average Daily EEQ Contribution

Plant #	Wastewater Treatment Plant Name	Average Daily EEQ (ng/L)
1	American Canyon	0.259
2	Benicia	0.563
3	Central Contra Costa County	0.576
4	Central Marin	0.819
5	Delta Diablo	0.965
6	EBDA	3.447
7	EBMUD	0.634
8	Fairfield Suisun	0.692
9	Las Gallinas **	0.283
10	Marin CSD 5	0.450
11	Napa **	0.944
12	NBSU	4.957
13	Novato & Ignacio	0.769
14	Palo Alto	0.774
15	Rodeo	0.473
16	San Jose & Santa Clara	0.972
17	San Mateo	0.681
18	SASM	0.682
19	Sausalito	0.438
20	Sunnyvale	0.578
21	SVC	0.514
22	SVCW	0.502
23	Treasure Island	0.481
24	Vallejo	0.629
25	West County Agency	1.389

** = Plant diverts effluent flow to water recycling facility from 5/1/2017 – 10/31/2018

Short-term and long-term exposure risk

Comparing average daily EEQ values from all 25 discharge points, only two plants, the NBSU and EDBA WWTPs discharged at EEQ values greater than the 1.40 EEQ [ng/L] threshold associated with short term exposure risk to marine organisms. These values were 4.597 EEQ [ng/L] and 3.447 EEQ [ng/L] respectively. All other plants discharged below the short-term exposure risk threshold with an average discharge value of 0.869 EEQ [ng/L]. The West County Agency discharged at an average value of 1.389 EEQ [ng/L], which distinguished it as a notable outlier from the other plants as well as a likely candidate for short-term exposure risk due to its average discharge being so near the established threshold value. Discharge points associated with short-term exposure risk are represented in figure 5.

In comparison to short-term exposure risk, far more plants discharged above the EEQ threshold associated with long-term exposure risk to marine organisms. With the exception of the American Canyon and Las Gallinas WWTPs, which discharged at average daily EEQ values of 0.259 EEQs [ng/L] and 0.283 EEQs [ng/L], all other WWTPs discharged at EEQ values greater than the 0.30 EEQ [ng/L] threshold. The average discharge value for these plants was 0.921 EEQs [ng/L]. Comparable to that of the West County Agency WWTP in regards to short-term exposure risk, the American Canyon and Las Gallinas WWTPs discharged at values so near the established long-term exposure risk threshold that they remain strong candidates for potential risk to marine organisms. Discharge points associated with long-term exposure risk are represented in figure 6.

Figure 5. Short-Term Exposure Risk Spatial Distribution

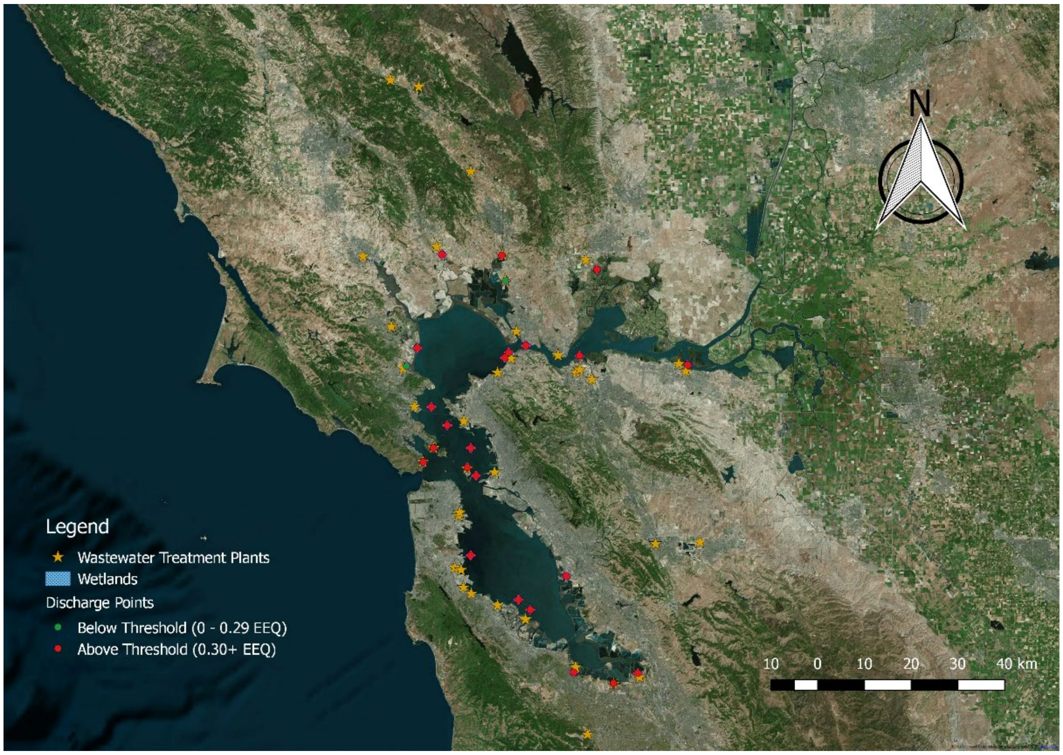

Figure 6. Long-Term Exposure Risk Spatial Distribution

DISCUSSION

Estimated outputs of both natural and synthetic estrogens into the SFE are well below those typically found in the primary literature. Despite this discrepancy, output values seem to correspond to *in vitro* sample results and are present in significant enough concentrations to present a consistent, sub-lethal downward pressure on overall ecosystem health. The long-term exposure EEQ-SSE threshold was consistently exceeded in WWTP output across the SFE. Even if it is the case that recent declines in fish populations within the estuary system are not a direct result of estrogen exposure from WWTP discharge, WWTP discharge points are primary candidates for being increasingly potent sources of species-specific sexual dysfunction and the subsequent decline in overall ecosystem health over time.

E1, E2, and E3 estimates

Estimated effluent concentrations for E1, E2, and E3 are consistently below those published in the primary literature. Average values for E1, E2, and E3 as reported by Jarošová et al. (2013) reported median effluent concentrations from 112 WWTPs worldwide as 7.0 ng/L, 1.7 ng/L, and 1.4 ng/L respectively. Specifically looking at *In vitro* measurements taken from receiving waters downstream of WWTPs utilizing similar treatment regimens to those used in the San Francisco Bay area have resulted in estimates for E1, E2, and E3 discharge concentrations in the range of 3 - 100 ng/L, 1 - 54 ng/L, and 0 - 280 ng/L (Drewes et al. 2005, Lavado et al. 2009, Atkinson et al. 2012, Schaider et al. 2017). Of the three NEs, the average discharge concentration of 00.537 ng/L for E3 was the only value that fell within this observed ranges. This suggests that the model is underestimating the true discharge concentrations of NEs from WWTPs to the SFE.

One potential explanation for why the model may be underestimating true discharge concentrations could be due to the plants in the bay area exhibiting greater daily effluent flow values and subsequently diluting the NE concentration in effluent. Atkinson et al. (2012) analyzed the discharge concentrations of NEs for two WWTPs in Canada, the city of Ottawa WWTP and the Cornwall WWTP. The Ottawa WWTP services approximately 786,130 individuals with an average daily flow of 111.48 MGD, and the Cornwall WWTP services approximately 45,965 individuals with an average daily flow of 4.31 MGD. Receiving waters downstream of the Ottawa plant were determined to possess average discharge concentrations ranging from 35.0 - 311 ng/L for E1, 0 – 66.9 ng/L for E2, and 0 – 5.7 ng/L for E3. Receiving waters downstream of the Cornwall plant were determined to possess average discharge concentrations ranging from 13.1 – 29.3 ng/L for E1 and 1.0 – 9.8 ng/L for E3; E2 values were below detection limits for E2 at this site (Atkinson et al. 2012). In comparison, the EBDA services approximately 800,000 individuals with an average daily discharge of 70.301 MGD. The Novato and Ignacio WWTP services approximately 52,000 individuals with an average daily discharge of 4.097 MGD. Despite possessing comparable service populations and daily discharge rates, both the EDBA and Novato and Ignacio WWTPs are estimated to discharge at drastically lower effluent concentrations than reported downstream of their Canadian counterparts. This comparison suggests that underestimation of effluent discharge is not likely to be a result of effluent dilution.

This being said, observed effluent concentrations possess high geographic and temporal variability due to the complex influence of seasonal precipitation patterns, water usage patterns, and plant treatment regimes. The plants discharging into the SFE for instance demonstrate a clear pattern of increased effluent discharge variability between the months of October, 2017 and March, 2018 (see figure 7). In accordance the region's Mediterranean climate, these are the wet months in which the vast majority, if not all, of annual precipitation falls. With increased precipitation, WWTPs must increase overall discharge to accommodate for the increased water load on the watershed and prevent wastewater overflows (see figure**s** 7 & 8). This results in the dilution of extant solutes within the plants' water flows and subsequently results in decreased effluent pollutant concentrations. Corresponding dips in the estimated discharge of E1, E2, and E3 can be directly correlated to the months in which rainfall was greatest in the region (Figures 2 & 8). This same pattern of inverse proportionally between rainfall and pollutant discharge concentrations has been recorded for other WWTPs and their corresponding receiving waters worldwide (Mohagheghian et al. 2014).

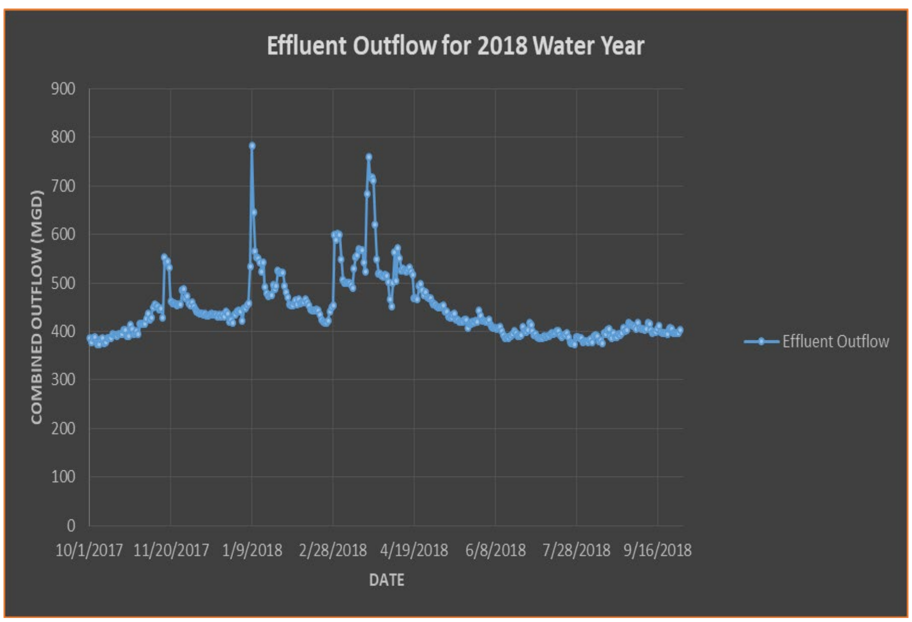

Figure 7. Daily effluent flows for 2018 water year

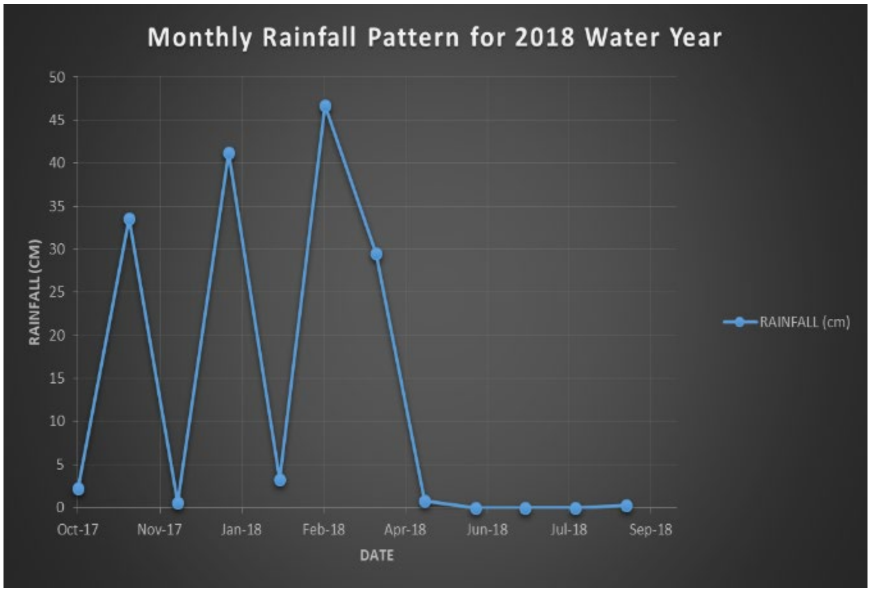

Figure 8. Monthly rainfall for 2018 water year

EE2 estimates

Like with E1, E2, and E3, the model consistently predicted EE2 discharge concentrations to be well below the values typically reported in the primary literature. Average values for EE2 effluent concentrations derived from 112 WWTPs worldwide by Jarošová et al. (2013) reported median EE2 effluent concentrations of 0.6 ng/L. *In vitro* measurements taken from receiving waters downstream of WWTPs under similar treatment regimens to those used in the San Francisco Bay area have resulted in estimates for EE2 discharge concentrations in the range of 0.7 – 9.8 ng/L with an average concentration of 0.96 ng/L (Drewes et al. 2005, Atkinson et al. 2012, Schaider et al. 2017). Again, this suggests that the model is underestimating the true discharge value of the plants to the SFE.

This underestimation could be a result of inaccurate census data and the unavailability of regional data on oral contraceptive use. Since the model for estimating EE2 is based upon the population size of women between the ages of 15 and 44 as well as regional estimates of the percentage of women taking oral contraceptives, it is very difficult to obtain accurate data. The most recent US census was conducted in 2010, and all population estimates used for this model were based upon estimates derived from this data. Inaccuracies in these estimates have a cascading effect of decreasing the accuracy of EE2 output. It is unlikely that this had a significant influence on the model's underestimations since estimates were on average 25 times lesser than values reported in the scientific literature. A potentially more influential factor impacting the underestimation of the model could be due to underestimates of the number of women contributing to EE2 discharge by taking oral contraceptives. Oral contraceptives are the most widely prescribed medication in the world, however due to patient confidentially policies in the United States, it is virtually impossible to discern accurate values for the number of women taking them in any given region of the country (Briciu et al. 2009). More accurate data regarding size of the contributing population for EE2 discharge may potentially increase estimated output values.

Interestingly, EE2 output did not demonstrate the same annual variation as seem with the NEs. Although there is greater variation from the average total EE2 EEQ contributions for the between the months of October, 2017 and March, 2018 (2.20%) in comparison to the months of April, 2018 and September, 2018 (1.23%), the influence of annual precipitation patterns and effluent outflow values seems to be less impactful on the resulting values of the EE2 model.

Estrogenic activity

Despite the model consistently underestimating the output of the NEs as well as EE2, cEEQ do seem to correspond to *in vitro* samples taken from the SFE. Samples taken from the SFE are summarized in table 4 and figure 9. Comparable EEQ values have also been documented in the primary literature. Vajda et al. (2008) reported mean EEQ concentrations ranging from 3.4 – 11 ng/L between the years of 2003 and 2005, and Johnson and Chen (2017) reported EEQ values of 0.6 – 3.2 ng/L across 35 sites downriver of WWTPs in the UK.

Table 4. *in vitro* SF Bay EEQ Values

Source	Location	Latitude	Longitude	cEEQ (ng/L)
Schlenk et al. 2012				
	Napa River	38.0551	-122.15439	0.9
	Carquinez Strait	38.02229	-122.09018	25.65
	Grizzley Bay	38.06504	-122.02463	1.05
Lavado et al. 2009	Napa River	38.18631	-122.16682	0.00

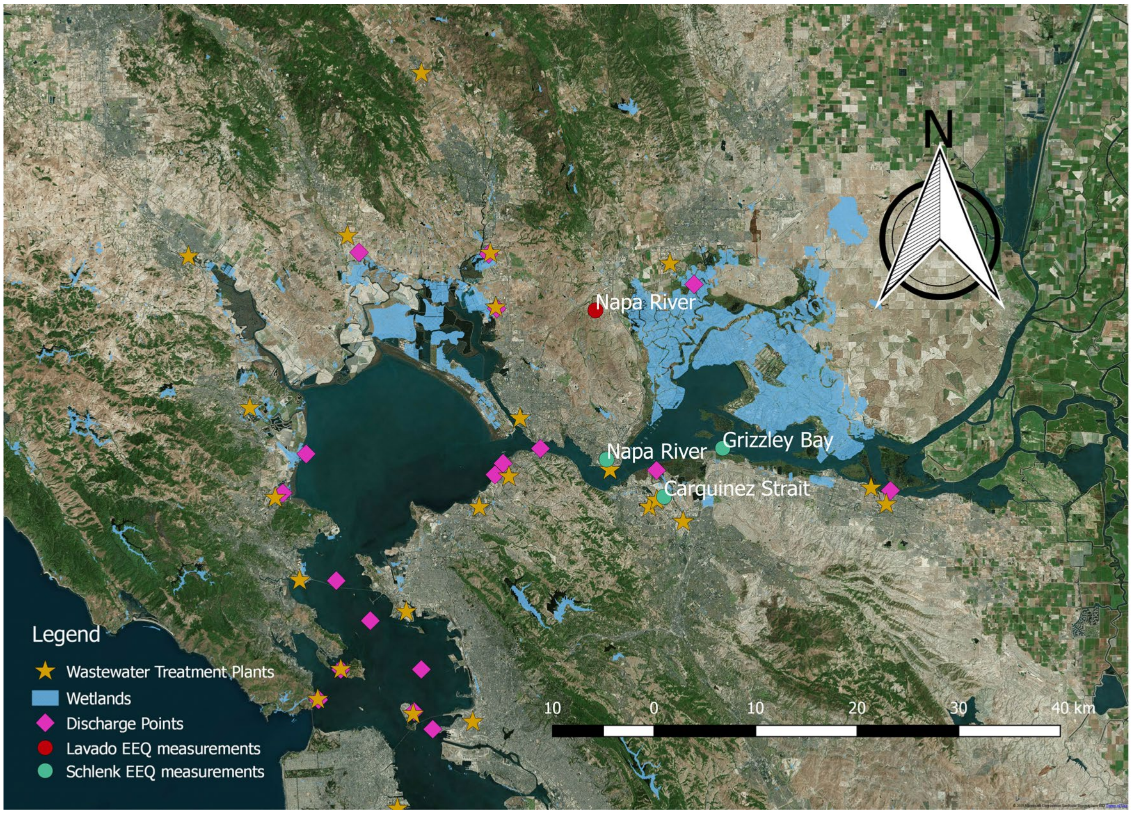

Figure 9. *in vitro* Sample Sites

Of the available data taken from samples in the SFE, it can be seen that the sample taken upriver from the treatment plants on the Napa River exhibit no estrogenic activity, whereas the sample site nearer to discharge points exhibits a cEEQ value of 0.9 ng/L. This is very near the average daily discharge values of the four adjacent WWTPs: The Fairfield Suisun, American Canyon, Vallejo, and Central Contra Costa County WWTPs which contribute effluent with an estimated EEQ values of 0.69 ng/L, 0.41 ng/L, 0.63 ng/L, 0.58 ng/L respectively. Given the rapid degradation of constituent estrogens such as E3 and to a lesser extent E1 (Jarošová et al. 2013) and combined effect of these mixing of effluent derived water from each plant, it is not unreasonable to assume that these plants produced the observed estrogenic activity recorded by Schlenk et al. (2012). A similar logic can be applied to the sample collected from Grizzley Bay. The relatively high cEEQ value recorded in the Carquinez Strait is less adequately explained by the introduction of estrogenic effluent, however given that this sample was collected within a wetland system where waters are more stagnant in comparison to the flowing waters of the Napa River and the significant mixing that occurs within Grizzley Bay, it may be the case that long-lived estrogenic compounds such as E2 and EE2 accumulate in this system and incur a greater estrogenic effect on local water samples. The potential for both E2 and to a greater extent EE2 to persist within aquatic systems is well documented in the primary literature (Campbell et al. 2006, Jarošová et al. 2013, Chen et al. 2018, Matthiessen 2018), and given the continual introduction of estrogenic compounds via WWTPs year-round from adjacent plants, the observed levels of estrogenic activity may represent an accumulation of these compounds at a rate greater than their natural degradation and dilution to large water bodies such as Suisun Bay. No other *in vitro* samples could be found in the primary literature to expand on this analysis within different regions of the SFE.

Exposure risk

Only two WWTPs discharge at EEQs greater than the 1.40 ng/L EEQ-SSE value associated with short-term risk to marine organisms. These plants are the EBDA and NBSU WTTPs and discharge with average daily EEQ values of 3.447 ng/L and 4.597 ng/L respectively. Granted that these concentrations are on the order of two to three times greater than the short-term risk threshold, these plants in particular represent a significant risk to marine biota. For instance, EE2 was added three times per week over the course of three years to an experimental lake in Canada (Lake 260) with mean EE2 concentrations ranging from 4.8 – 6.1 ng/L, comparable values to the EEQ concentrations discharged by these plants on a daily basis. During this time period, and over the following four years, the population of fathead minnows (*Pimephales promelas*) was documented. After only seven weeks, Vitellogenin levels in male fathead minnows rose from 0.5 to 2,000 – 12,000 µg/g wet weight; this is direct evidence of an estrogenic impact on this population in a short-term period (<60d). By year three, four out of nine males were shown to possess ovetestis, and by year four, all reproduction had ceased and the population crashed (Park and Kidd 2005). Although this study was conducted in an isolated system, it demonstrates the potential for e-EDC exposure to induce short-term feminization in some fish species. It is important to note, that during this same time period, the populations of three other species did not crash, although two did gradually decline. These results in tandem to the predicted output concentrations give further credence to the decline of the striped bass population since the year 2001 in the SFE being the result of e-EDC exposure as suggested by Spearow et al. (2011).

In contrast to short-term exposure risk, all but two WWTPs are predicted to be discharging with average daily EEQ values greater than the long-term risk EEQ-SSE of 0.30 ng/L. This is far more concerning than the threat of short-term exposure due to the fact that nearly all discharge points can be identified as threats to the estuary's aquatic life. Additionally, the two plants that did not reach the long-term exposure risk threshold had average daily discharge EEQ concentrations so near the threshold that they should still be considered likely candidates for long-term exposure risk. Persistent exposure to effluent flows have been correlated to the increased incidence of fish feminization worldwide. For instance, roach (*Rutilus rutilus*) exposed to treated sewage in five UK rivers were demonstrated to possess intersex male populations between 40 and 100% of the total male population (Jobling et al. 1998); although no corresponding estrogen concentrations were collected during this sampling period, a later study found a positive correlation between the incidence of intersex male roach populations and living downstream of WWTP discharge (Jobling et al. 2006). Male intersex fish populations are known to be correlated to decreased milt production by volume and decreased sperm motility, which places a negative pressure on reproductive success (Matthiessen 2018). Given that nearly all of the treatment plants discharging into the SFE are discharging average effluent concentrations above the short-term EEQ-SSE, a variety of fish species that occupy these waters likely face increased pressures on reproductive success due to e-EDC exposure.

When considering both short-term and long-term exposure risk as determined by the aforementioned EEQ-SSEs, it is crucially important to recall that the effluent output values used are suspected to be underestimates of true discharge concentrations. This indicates that it is highly likely that the long-term exposure risk EEQ-SSE is being exceeded at most if not all discharge points, and it cannot be ruled out that the EBDA and NBSU WWTPs are the only culprits for exceeding the short-term exposure risk EEQ-SSE. Native open-water species such as the delta smelt (*Hypomesus transpacificus*), longfin smelt (*Spirinchus thaleichthys*), green sturgeon (*Acipenser medirostris*), Sacramento splittail (*Pogonichthys macrolepidotus*), Central Valley steelhead trout (*Oncorhynchus mykiss*), and Pacific lamprey (*Entosphenus tridentatus*) have all exhibited precipitous declines in population since 2002; implicated in these declines are water diversions for water storage projects as well as exposure to environmental pollutants, including estrogenic compounds (Miller 2019). All of these species rely on the SFE for breeding, forage, and shelter over the course of any given year, and all inhabit estuary waterways for timespans greater than 60 days at a time per year. This places them at threat for potentially dangerous long-term exposure to e-EDCs and associated reproductive dysfunctions. As noted by Park and Kidd (2005), not all fish species exhibit population declines as a result of e-EDC exposure, and it is currently not understood how to preemptively identify which species are the most susceptible to adverse effects upon short-term nor long-term exposures. Given this uncertainty, the assumption should be made that a species is susceptible until proven otherwise.

Limitations and future directions

The key limitation ascribed to this modeling approach lies with the general lack of scientific research regarding e-EDCs from the production, transport, and exposure perspectives. Relatively more research has been conducted regarding the production and excretion of endogenous and exogenous estrogens in comparison to the mechanisms that regulate the transport, degradation, and toxicity of e-EDCs in aquatic environments (Johnson and Williams 2004, Ma et al. 2015, Matthiessen 2018). A great deal more of site-specific *in vitro* data is necessary to develop more accurate models with greater predictive power on e-EDC output from treatment plants to surrounding aquatic ecosystems (Dotan et al. 2017). Given that the accurate identification of both NEs and EE2 on the ng/L level in water samples has only been feasible since 2001, a greater wealth of data will be produced as this topic continues to be studied. Additional limitations to this modeling approach exist in the lack of accurate census data and oral contraceptive use data. These data could greatly increase the accuracy of estimates regarding EE2 input and output concentrations in WWTPs since average excretion rates are fairly well defined and understood (Johnson and Williams 2004).

Further directions that can be made in this line of research apart from the refinement of output models include species-specific toxicity evaluations at differing EEQ concentrations. As previously mentioned, not all species display the same degree of reproductive dysfunction as a result of e-EDC exposure at any given concentration. Species-specific data regarding susceptibility to the adverse effects of e-EDC exposure could be used to not only identify species that at the greatest risk but also establish indicator species whose level of reproductive dysfunction can be used as a proxy measure for the relative health of their surrounding environment in the context of estrogen exposure. Additional considerations regarding the impact of environmental e-EDC exposure should be explored in regards to its inter- and intra-specific interactions within aquatic communities and food webs to gain a greater appreciation for the residence time and cumulative impact these compounds impart on aquatic ecosystems.

Broader implications

The severity of e-EDC exposure is a difficult metric to quantify in part due to differences in species vulnerability. As the largest estuary system in the Western Americas, the SFE provides crucially important habitat to many fish species, particularly during breeding, larval, and pupal stages of development. During these initial developmental periods, organisms are particularly susceptible to environmental pollutants and stressors, such as e-EDCs, and exposure during these formative stages can impart adverse effects on individuals that span their own lifetime as well as into subsequent generations (Head, 2014). To add further complexity to this issue, it is suggested that the toxicity of e-EDC exposure should be evaluated along non-monotonic dose response curves such that adverse effects are realized at very high or very low doses but not intermediary ones (Vandenberg 2012). Given the variation in susceptibility to adverse effects of exposure that exists between species, this may help explain why only some fish species demonstrate sexual dysfunction and a subsequent decline in population numbers as exhibited in the SFE. With a shift in environmentally relevant levels of e-EDC exposure within the system, it could be hypothesized that the assemblage of fish species struggling to reproduce may alter from what is currently seen.

Another factor that adds complexity to evaluating the environmental impact of e-EDC exposure stems from the unknown effects e-EDCs exhibit when acting in concert to other environmental stressors and pollutants within the water column. Studies that assess the toxicity of e-EDC exposure typically assess their impacts under the exposure to individual compounds rather than mixtures. Any given sample of wastewater effluent taken from any plant across the globe is almost certainly bound to contain additional pollutants along with e-EDCs. Because of this, it is important to consider that the effects of e-EDCs acting in concert with other chemicals operating through different metabolic pathways to effect fish potentially possess additive adverse effects not properly accounted for in current risk assessments. Although E1, E2, E3, and EE2 are generally regarded to account for approximately 90% of the estrogenic activity in WTTP receiving waters, the potential for synergistic action and subsequent amplifications in toxicity cannot be discounted (Jarasova et al. 2014, Windsor et al. 2018).

Apart from concurrent pollutant exposure, the importance of accounting for environmental factors such as water temperature and biotic interactions has recently been realized when attempting to assess the impact of e-EDC exposure on broader temporal and ecosystem scales (Windsor et al. 2018). For instance, *Menidia beryllina,* an estuarine model organism, was exposed to environmentally relevant levels of EE2 and the estrogenic insecticide bifenthrin for 14 days at 22 and 28 degrees Celsius prior to spawning in tandem to their F1 generation embryos being exposed to the same exposure for 21 days. Results indicate that F1 generations exposed to higher water temperatures had less viable offspring and an increased prevalence of developmental deformities; even greater effects were exhibited in the juveniles of the F2 generation despite them not receiving any e-EDC exposure (DeCourten et al. 2017). Later research indicates that these transgenerational effects of e-EDC exposure act by downregulating the expression of key developmental genes in both F1 and F2 larvae (DeCourten et al. 2018). These findings have profound implications for the future conditions of the SFE. As climate change progresses, the surface waters of the estuary system are anticipated to rise, and as the results of previous research indicate, this could have implications of the prevalence of sexual dysfunction in fish species within the estuary across multiple generations. Furthermore, ecosystem-level consequences may arise from e-EDC exposure and elevated water temperatures. It has been demonstrated that exposure to E1 and elevated water temperatures resulted in a notable reduction in predator avoidance behaviors in fathead minnows (Korn 2018). Alterations in behavior such as these decrease survivorship in fish species while also contributing to the transfer of estrogenic compounds to higher trophic levels within the estuary food web. Comparable results that show the reduction in predation activity by *R.rutilus* on phytoplankton in a Canadian lake dosed with 5.0-6.0 ng/L of EE2 resulted in the increased abundance of both phytoplankton and copepods (Kidd et al. 2014). Examples such as these demonstrate the indirect effects of e-EDC exposure across both generations as well as trophic levels and give context to the disruptive potential these compounds possess.

Given the estimated output concentrations of e-EDCs from WWTP discharge points in the SFE in the context of rising water temperatures and the indirect influence of exposure on broader ecosystem structure, it is apparent that current risk assessment models have not appropriately accounted for the adverse impact of e-EDC exposure on the overall health of the SFE. The primary source of E1, E2, E3, and EE2 in the estuary system is via human excretion. As a consequence of the San Francisco Bay area being a popular locations for job opportunities, investment, and recreation, it is not unreasonable to assume the regional population will continue to increase in the near future. As a direct result of this, the average daily cumulative excretion of natural and synthetic estrogens to WWTPs and ultimately the SFE will also continue to increase. In tandem to increasing water temperatures from climate change, the increased daily loading of estrogenic compounds to the SFE may produce conditions even more conducive to sexual dysfunction and decreasing ecosystem health than are currently realized.

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REFERENCES

Atkinson, S. K., V. L. Maralatt, L. E. Kimpe, D. R. S. Lean, V. L. Trudeau, and J. M. Blais. 2012. The occurrence of steroidal estrogens in south-eastern Ontario wastewater treatment plants. Science of the Total Environment 430: 119-125.

Bittner, G. D., C. Z. Yang, and M. A. Stoner. 2014. Estrogenic chemicals often leach from BPA-free plastic products that are replacements for BPA-containing polycarbonate products. Environmental Health 13 https://doi.org/ 10.1186/1476-069X-13-41.

Briciu, R. D., A. Kot-Wasik, and J. Namiesnik. 2009. Analytical challenges and recent advances in the determination of estrogens in water environments. Journal of Chromatographic Science 47: 127-139.

Caldwell, D. J., F. Mastrocco, P. D. Anderson, R. Länge, and J. P. Sumpter. 2012. Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estadiol, estriol, 17α-ethinylestradiol. Environmental Toxicology and Chemistry 31: 1396-1406.

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

DeCourten, B. M., and S. M. Brander. 2017. Combined effects of increased temperature and endocrine disrupting pollutants on sex determination, survival, and development across generations. Scientific Reports 7 https:// doi.org/10.1038/s41598-017-09631-1.

DeCourten, B. M., R. E. Connon, and S. M. Brander. 2018. Direct and indirect parental exposure to endocrine disruptors and elevated temperature influences gene expression across generations in a euryhaline model fish. PeerJ http://doi.org/10.7717/peerj.6156.

Dotan, P., A. Tal, and S. Arnon. 2017. A simple model for estimating the concentrations of natural estrogens in raw wastewater. Science of the Total Environment 575: 588-594.

Drewes, J. E., J. Hemming, S.J. Ladenburger, J. Schauer, and W. Sonzogni. 2005. An assessment of endocrine disrupting activity changes during wastewater treatment through the use of bioassays and chemical measurements. Water Environment Research 77: 12-23.

Ellis, J. B. 2006. Pharmaceutical and personal care products (PPCPs) in urban receiving waters. Environmental Pollution 144 https://doi.org/10.1016/j.envpol.2005.12.018.

EMEA, 2005. Note for Guidance on Environmental Risk Assessment of Medicinal Products for Human Use, CMPC/SWP/4447/draft. The European Agency for the Evaluation of Medicinal Products (EMEA). London, England.

FDA-CDER. 1998. Guidance for Industry-Environmental Assessment of Human Drugs and Biologics Applications, Revision 1. FDA Center for Drug Evaluation and Research. Rockville, Maryland, United States of America.

Fent, K., A. A. Weston, and D. Caminada. 2005. Ecotoxicology of human pharmaceuticals. Aquatic Toxicology 76 http://doi:10.1016/j.aquatox.2005.09.009.

Fuhrman, W. F., A. Tai, and S. Arnon. 2015. Why endocrine disrupting chemicals (EDCs) challenge traditional risk assessment and how to respond. Journal of Hazardous Materials 286: 589-611.

Fleming, M., G. Achari, Q. K. Hassan, and C. A. Ng. 2016. Modeling the loading and fate of estrogens in wastewater treatment plants. Cogent Environmental Science 2 http://dx.doi.org/10.1080/23311843.2016.1222690.

Grabicova, K., R. Grabic, M. Blaha, V. Kumar, D. Cerveny, G. Fedorova, and T. Randak. 2015. Presence of pharmaceuticals in benthic fauna living in a small stream affected by effluent from a municipal sewage treatment plant. Water Research 72 http://dx.doi.org/10.1016/j.watres.2014.09.018.

Head, J. A. 2014. Patterns of DNA methylation in animals: an ecotoxicological perspective. Integrative and Comparative Biology 54: 77–86.

Hecker, M. and H. Hollert. 2011. Endocrine disruptor screening: regulatory perspectives and needs. Environmental Sciences Europe 23 https://doi.org/ 10.1186/2190-4715-23-15.

Hibberd, A., K. Maskaoui, Z. Zhang, and J. L. Zhou. 2009. An improved method for the simultaneous analysis of phenolic and steroidal estrogens in water and sediment. Talanta 77: 1315–1321.

Hinck, J. E., V. S. Blazer, C. J. Schmitt, D. M. Papoulias, and D. E. Tillitt. 2009. Widespread occurrence of intersex in black basses (micropterus spp.) from U. S. rivers. Aquatic Toxicology 95: 60-70.

IPCS. 2002. Global Assessment of the State-of-the-Science of Endocrine Disruptors. World Health Organization's International Programme on Chemical Safety. Geneva, Switzerland.

Jarošová, B., L, Bláha, J. P. Giesy, and K. Hilscherová. 2013. What level of estogenic activity determined by *in vitro* assays in municipal waste waters can be considered as safe? Environment International 64: 98-109.

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

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

Johnson, A.C., A. Belfroid, and A. Di Corcia. 2000. Estimating steroid estrogen inputs into activated sludge treatment works and observations on their removal from the effluent. Science of the Total Environment 256: 163–173.

Johnson, A. C., and R. J. Williams. 2004. A model to estimate influent and effluent concentrations of estradiol, estrone, and ethinylestradiol at sewage treatment works. Environmental Science and Technology 38: 3649-3658.

Johnson, A. C., and Y. Chen. 2017. Does exposure to domestic wastewater effluent (including steroid estrogens) harm fish populations in the UK? Science of the Total Environment 589: 89–96.

Kidd, K. A., M. J. Paterson, M. D. Rennie, C. L. Podemski, D. L. Findlay, P. J. Blanchfield, and K. Liber. 2014. Direct and indirect responses of a freshwater food web to a potent synthetic oestrogen. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 369 https://doi.org/10.1098/rstb.2013.0578.

Kolpin, D. W., E. T. Furlong, M. T. Meyer, E. M. Thurman, S. D. Zaugg, L. B. Barber, and H. T. Buxton. 2002. Pharmaceuticals, hormones, and other organic wastewater contaminants in

U.S. streams, 1999–2000: a national reconnaissance. Environmental Science and Technology 36: 1202-1211.

Korn, V. 2018. Effects of Estrone and Temperature on the Predator-Prey Relationship Between Bluegill Sunfish and Fathead Minnows. Thesis, St. Cloud State University, St. Cloud, Minnesota, USA.

Lambert, M. R., G. S. J. Giller, L. B. Barber, K. C. Fitzgerald, and D. K Skelly. 2015. Suburbaization, estrogen contamination, and sex ratio in wild amphibian populations. Proceedings of the National Academy of Sciences U. S. A. 112: 11881-11886.

Lavado, R., J. E. Loyo-Rosales, E. Floyd, E. P. Kolodziej, S. A. Snyder, D. L. Sedlak, and D. Schlenk. 2009. Site-specific profiles of estrogenic activity in agricultural areas of California's inland waters. Environmental Science and Technology 43: 9110-9116.

Liao, P-H., S-H. Chu, T-Y. Tu, S-H. Wang, A. Y-C. Lin, and P-J. Chen. 2014. Persistent endocrine disruption effects in medaka fish with early life-stage exposure to a triazole-containing aromatase inhibitor (letrozole). Journal of Hazardous Materials 277: 141-149.

Liu Z. H., G. N. Lu, H. Yin, and Z. Dang. 2015. Do we underestimate the concentration of estriol in raw municipal wastewater? Environmental Science and Pollution Research 22: 4753-4758.

Loyo-Rosales, J. E., C. P. Rice, and A. Torrents. 2010. Fate and distribution of the octyl-and nonlyphenol ethoxylates and some carboxylated transformation products in the Back River, Maryland. Journal of Environmental Monitoring 12: 614– 621.

Ma, X., Zhang, C., Deng, J., Song, Y., Li, Q., Guo, Y., and Li, C. 2015. Simultaneous degradation of estrone, 17β-ethinyl estradiol in an aqueous UV/H_2O_2 system. International Journal of Environmental Research and Public Health 12: 12012-12029.

Magi, E., C. Scapolla, M. D. Carro, and C. Liscio. 2010. Determination of endocrine-disrupting compounds in drinking waters by fast liquid chromatography-tandem mass spectrometry. Journal of Mass Spectrometry 45: 1003–1011.

Marcus, M. 2009. Women's reproductive health and the environment epidemiological evidence and research needs. Reproductive Health and Endocrine Disrupting Chemicals Conference, Israeli Environment and Health Fund, 23 May. Tel Aviv, Israel.

Martínez, N. A., S. V. Pereira, F. A. Berolino, R. J. Schneider, G. A. Messina, and J. Raba. 2011. Electrochemical detection of a powerful estrogenic endocrine disruptor: ethinylestradil in water samples through bioseparation procedure. Analytica Chimica Acta 723: 27-32.

Miller, J. 2019. San Francisco Bay area and delta protection. Center for Biological Diversity. https://www.biologicaldiversity.org/campaigns/san_francisco_bay_area_and_delta_protection/index.html.

Mohagheghian, A., R. Nabizadeh, A. Mesdghinia, N. Rastkari, A. H. Mahvi, M. Alimohammadi, M. Yunesian, R. Ahmadkhaniha, and S. Nazmara. 2014. Distribution of estrogenic steroids in municipal wastewater treatment plants in Tehran, Iran. Environmental Health Science & Engineering 12: http://doi.10.1186/2052-336X-12-97.

Noguera-Oviedo, K., D. S. Aga. 2016. Lesson learned from more than two decades of research on emerging contaminants in the environment. Journal of Hazardous Materials 316: 242-251.

Oulton, R. L., T. Kohn, and D. M. Cwiertny. 2010. Pharmaceuticals and personal care products in effluent matrices: A survey of transformation and removal during wastewater treatment and implications for wastewater management. Journal of Environmental Monitoring 12: 1956-1978.

Park, B. J., and K. Kidd. 2005. Effects of the synthetic estrogen ethinylestradiol on early life stages of mink frogs and green frogs in the wild and in situ. Environmental Toxicology and Chemistry 24: 2027–2036.

Pastorova, I. B., H. Vissers, G. Kearney, and J. A. Van Leedeam. 2001. Screening and identification of unknown contaminants in water with liquid chromatography and quadrupole-orthogonal acceleration-time-of-flight tandem mass spectrometry. Journal of Chromatography 929: 63-74.

Pereira, R. O. C. Postigo, M. L. de Alda, L. A. Daniel, and D. Barceló. 2011. Removal of estrogens through water disinfection processes and formation of by-products. Chemosphere 82: 789-799.

Purdom, C. E. 1993. Genetics and Fish Breeding. Chapman and Hall, London, England.

Routledge, E. J., J. Parker, J. Odum, J. Ashby, and J. P. Sumpter. 1998. Some alkyl hydroxyl benzoate preservatives (parabens) are estrogenic. Toxicology and Applied Pharmacology 153: 12-19.

San Francisco Bay Conservation & Development Commission (SFBCDC). 2015. San Francisco Bay and Estuary. http://www.bcdc.ca.gov/bay_estuary.html.

Schaider, L. A., K. M. Rodgers, and R. A. Rudel. 2017. Review of organic wastewater compound concentrations and removal in onsite wastewater treatment systems. Environmental Science and Technology 51: 7305-7317

Schlenk, D., R. Lavado, J. E. Loyo-Rosales, W. Jones, L. Maryoung, N. Riar, I. Werners, and D. Sedlak. 2012. Reconstitution studies of pesticides and surfactants exploring the cause of estrogenic activity observed in surface waters of the San Francisco Bay Delta. Environmental Science and Technology 46: 9106-9111.

Shore, L. S., M. Shemesh. 2003. Naturally produced steroid hormones and their release into the environment. Pure Applied Chemistry 75: 1859-1871.

Silva, C. P., M. Otero, and V. Esteves. 2012. Processes for the elimination of estrogenic steroid hormones from water: a review. Environmental Pollution 165: 38-58.

Snyder, S. A and M. J. Benotti. 2010. Endocrine disruptors and pharmaceuticals: implications for water sustainability. Water Science and Technology 61: 145-154.

Spearow, J. L., R. S. Kota, and D. J. Ostrach. 2011. Environmental contaminant effects on juvenile striped bass in the San Francisco Estuary, California, USA. Environmental Toxicology Chemistry 30: 393-402.

Stackelberg, P. E., E. T. Furlong, M. T. Meyer, S. D. Zaugg, A. K. Henderson, D. B. Reissman. 2004. Persistence of pharmaceutical compounds and other organic wastewater contaminants in a conventional drinking-water-treatment plant. Science of the Total Environment 329: 99-113.

Stumpf, M., T. A. Ternes, R. D. Wilken, S. V. Rodrigues, W. Baumann. 1999. Polar drug residues in sewage and natural waters in the state of Rio de Janeiro, Brazil. The Science of the Total Environment 225http://doi:10.1016/S0048-9697(98)00339-8.

Sumpter, J. P. and S. Jobling. 1993. Male sexual development in "a sea of Oestrogen." Aquatic Toxicology 27: 361-372.

Swan, S. H. 2009. Human Exposure to Endocrine Disrupting Chemicals Evidence of Impacts on Public Health. Reproductive Health and Endocrine Disrupting Chemicals Conference, Israeli Environment and Health Fund, 23 May. Tel Aviv, Israel.

Tambosi, J. L., L. Y. Yamanaka, H. J. José, R. F. P. M. Moreira, and H. F. Schröder. 2010. Recent research data on the removal of pharmaceuticals from sewage treatment plants (STP). Química Nova 33http://dx.doi.org/10.1590/S0100-40422010000200032.

Ternes, T. A. 1998. Occurrence of drugs in German sewage treatment plants and rivers. Water Research 32: 3245-3260.

Ting, Y. F., S. M. Praveena, A. Z. Aris, S. N. S. Ismail, and I. Rasdi. 2017. Mathematical modeling for estrogenic activity prediction of estradiol and 17α-ethynylestradiol mixtures in wastewater treatment plants effluent. Ecotoxicology 26: 1327-1335.

Umali, H. M., S. A. Pagsuyoin, and W. J. Parker. 2012. Estimation of influent concentrations of estrogens and select prescription drugs in wastewater treatment plants. Proceedings of the 2012 Institute of Electrical and Electronics Engineers (IEEE) Systems and Information Design Symposium, Charlottesville, Virginia, USA.

USEPA [United States Environmental Protection Agency]. 2000. Supplementary guidance for conducting health risk assessment of chemical mixtures. Risk Assessment Forum. USEPA, Washington, DC.

Vandenberg, L. N., T. Colborn, T. B. Hayes, J. J. Heindel, D. R. Jacobs, D-H. Lee, T. Shioda, A. M. Soto, F. S. vom Saal, W. V. Welshons, R. T. Zoeller, and J. P. Myers. 2012. Hormones and endocrine disrupting chemicals: low dose effects and nonmonotonic dose responses. Endocrine Reviews 33: 378–455.

Vandenberg, L. N., T. Colborn, T. B. Hayes, J. J. Heindel, D. R. Jacobs, D-H. Lee, T. Shioda, A. M. Soto, F. S. vom Saal, W. V. Welshons, R. T. Zoeller, and J. P. Myers. 2013.

Regulatory decisions on endocrine disrupting chemicals should be based on the principles of endocrinology. Reproductive Toxicology 38: 1-15.

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

Vega-Morales, T., Z. Sosa-Ferrera, and J. J. Santana-Rodriquez. 2013. Evaluation of the presence of endocrine disrupting compounds in dissolved and solid wastewater treatment plant sample of Gran Canaria Island (Spain). BioMed Research International 2013: http://dx.doi.org/10.1155/2013/790570.

WHO [World Health Organization]. 2005. Carcinogenicity of combined hormonal contraceptives and combined menopausal treatment. Department of Reproductive Health and Research. World Health Organization, Geneva, Switzerland

Windsor, F. M., S. J. Ormerod, and C. R. Tyler. 2018. Endocrine disruption in awuatic systems: up-scaling research to address ecological consequences. Biological Reviews 93: 626-641.

Yang, C. Z., S. I. Yaniger, V. C. Jordan, D. J. Klein, and G. D. Bittner. 2011. Most Plastic Products Release Estrogenic Chemicals: A Potential Health Problem That Can Be Solved. Environmental Health Perspectives 7: 989-996.

Yost, R. A., and C. G. Enke. 1978. Selected ion fragmentation with a tandem quadrupole mass spectrometer. Journal of the American Chemical Society 100: 2274-2275.