EVALUATING THE EFFECT OF SYNTHETIC ESTROGEN (*17α-ETHINYLESTRADIOL*) CONTAMINATION UPON FOUNTAIN DARTER (*ETHEOSTOMA FONTICOLA*) POPULATION

An Undergraduate Research Scholars Thesis

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ABSTRACT

Evaluating the effect of Synthetic Estrogen (*17α-Ethinylestradiol*) Contamination upon Fountain Darter (*Etheostoma Fonticola*) Population

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Found in the headwaters of the Comal and San Marcos River, the Fountain Darter (*Etheostoma fonticola*) is on average a three-centimeter length fish that feeds upon small invertebrates. Considered endangered by the United States and the International Union for Conservation of Nature (IUCN), the darter has been controversial due to its location in the Edwards Aquifer in south-central Texas. This aquifer is recognized worldwide for its aquatic species of flora and fauna, many of which are endangered or threatened like the Fountain Darter. The Edwards aquifer is also the sole water source supporting the industrial, agricultural, municipal, and recreational needs of nearly 2 million people. The endangered darter are generally poor competitors and are the first species affected by habitat disruption, making them a focal point for controversies involving the Endangered Species Act, State of Texas Groundwater Law, and Private Property Rights. An age and sex-structured population model for the Fountain Darter will be created using pharmaceutical data and initial darter population dynamics. The model will also extend to include population dynamics under scenarios of increased contamination that could occur as a result of an environmental spill or increased urban construction.

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KEY WORDS

EE_2	17α-Ethinylestradiol
E_2	Estradiol
EPA	Environmental Protection Agency
FDA	Food and Drug Administration

CHAPTER I

INTRODUCTION

The Edwards Aquifer is famous for its exotic flora and fauna as well as the many endangered animal and plant species that live within it. Located in central Texas centered around San Antonio and covering 1,250 square miles, the Edwards is a vital and sole source supporting the industrial, municipal, agricultural and recreational needs of 2 million people (Earl and Wood, 2002). Managing the demand of an ever-increasing population and a limited surface water supply is of great concern to the federal and state agencies charged with protecting this aquatic system. The aquifer supports the headwaters and rivers containing many endangered and threatened species including: The Comal salamander (E*urycea neotenes*), the Peck's Cave Amphipod (*Stygobromus pecki*), the Texas Blind salamander (*Eurycea rathbuni*), the Barton Springs salamander (*Eurycea sosorum*), the Comal Springs dryopid beetle (*Stygoparnus comalensis*), the Comal Springs riffle beetle (*Heterelmis comalensis*) and the Fountain Darter (*Etheostoma fonticola*) (USFWS 2015). While detailed population demographic parameters have not been conducted there have been noted decreases in Fountain Darter population that coincided with a decrease in primary food sources, namely filamentous algae (Schenck and Whiteside 1976). The indication that the darter's population fluctuates significantly in poor habitats suggests the need for more research into how the Fountain Darter will respond to other changes in habitat.

As humans and society continues to progress through the technological and computer age, advancements in medicine and treating the sick and wounded have increased exponentially. However, with the dissemination and availability of pharmaceuticals, the concentrations and variety of pharmaceuticals that are found in surface water sources have also increased, bringing about possible unknown consequences to the natural flora and fauna that call these sources home. Recent studies have shown that only portions of active components in prescribed drugs are actually metabolized (Ternes 1998). The non-metabolized components will enter the natural water environment as waste discharge. This leads to trace amounts of pharmaceuticals, yet still measurable, in our streams, rivers, and lakes as well as the groundwater. Since pharmaceuticals are designed to have activity in relatively low concentrations, trace amounts could have serious ramifications on aquatic species and humans. The Environmental Protection Agency (EPA) requires pharmaceutical companies to perform environment assessments for new drugs entering the market (U.S. FDA 2009), with predicted effluent concentrations based on both high-end sales and worst case discharges. However, pharmaceuticals are not regulated, meaning that even after the wastewater is treated the same amounts of pharmaceuticals will be present and will enter into the natural water environment. In the early 2000s, the United States Geological Survey studied this nation’s streams and found many pharmaceuticals in the water, including antibiotics, antidepressants and oral contraceptives (Rodriguez-Mozaz and Weinburg 2010). One of the most common pharmaceuticals is 17α-Ethinylestradiol (EE_2) which is found in human, livestock and even aquaculture environments (Aris 2014). Initial studies have linked EE_2 to altering sex determination, delaying sexual maturity, and decreasing secondary sexual characteristics (Aris 2014).

For the federal agencies in charge of regulating the many waterways of Texas, managing the quality of the water is not only important for the many people who rely on the rivers to survive, but also for the many species of flora and fauna that call the waterways their home. The Fountain Darter is unique in that it has been identified for having a low potential for full recovery and delisting (USFWS 1995). With a limited distribution, specific habitat requirements and difficulty rebounding after harmful events necessitates the need to properly study the Fountain Darter and any factor that could affect its population or survivability. With such low population numbers to begin with, an estimated 45,900 in 1993 (Linam 1993), knowing whether EE_2 will have an effect upon Fountain Darter populations is crucial to ensure that the Fountain Darter population is kept stable and sustainable. This study will focus on estimating the effect of the introduction of pharmaceuticals on the Fountain Darter population.

CHAPTER II

METHODS

Research Area

The area in question centers on the Edwards Aquifer, a vibrant and unique aquifer with a 1250-square mile recharge zone. More specifically, the Fountain Darter is found almost exclusively in San Marcos River Watershed, a 522-square mile area found in the Edwards Aquifer in south-central Texas. Sourced by the San Marcos Springs, the river itself runs 75 miles Southeast through Luling, Texas before flowing into the larger Guadalupe River (Figure 1). With an elevation of 575 feet at the source, the river drops 300 feet to 275 feet at its mouth into the Guadalupe (San Marcos Source 2015, San Marcos Discharge 2015).

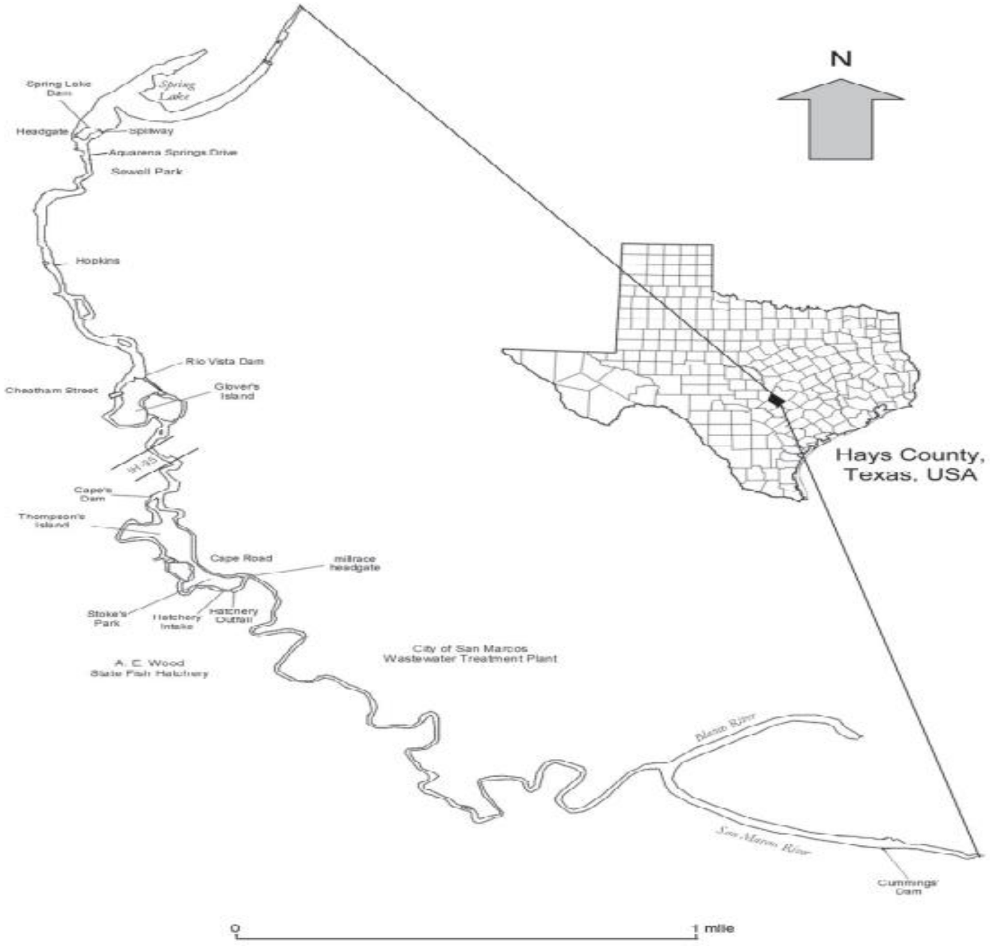


Figure 1: Location of study area in Texas (Mora et al. 2013)

The Comal River itself begins and ends within the city limits of New Braunfels, Texas. Sourced at the Comal Springs in Landa Park, the river flows only 2.5 miles before flowing into the Guadalupe River (Comal Source 2016, Comal Discharge 2016). The river only drops 45 feet in its entire length, going from 645 feet at the source to 600 feet at the mouth (Comal Source 2016, Comal Discharge 2016). The Comal river resides in a highly urban area, completely within the New Braunfels city limits; a city that boasted the second highest growth rate of all cities with over 50,000 residents in 2015 (Quesada 2016). The San Marcos River runs through both urban and rural areas, beginning in the industrialized San Marcos city, running through Texas State’s campus, through rural south Texas, and finally flowing into the Guadalupe River. San Marcos is no stranger to rapid growth as well, with its 2012 census showing an 8% growth (Solomon 2014).

Both rivers maintain a moderate climate, maintaining an average year-round temperature of about 72 degrees Fahrenheit (GBRA 2013). For the San Marcos River, the median pH is 7.67, median dissolved oxygen 9.35 mg/l, and the median chloride and sulfate concentrations were 19.2 and 25.3 mg/l respectively (GBRA 2013).

Fountain Darter

The Fountain Darter (*Etheostoma fonticola)* is a small aquatic freshwater fish that is generally 1-inch long (Whiteside et. al 2013). The Fountain Darter generally has 6-7 dorsal fins and less than 77 scales in a lateral line (Whiteside et. al 2013). It is only found in the headwaters of the Comal and San Marcos rivers, mostly spending its time in the filamentous green algae beds found at the bottom of the rivers (Whiteside et. al 2013). Fountain Darters mainly feed on small aquatic invertebrates that also live in the filamentous green algae. Generally living 1-2 years, the Fountain Darter females can lay eggs all year round, but generally have peaks of egg production in the early spring and fall (Whiteside et. a l 2013).

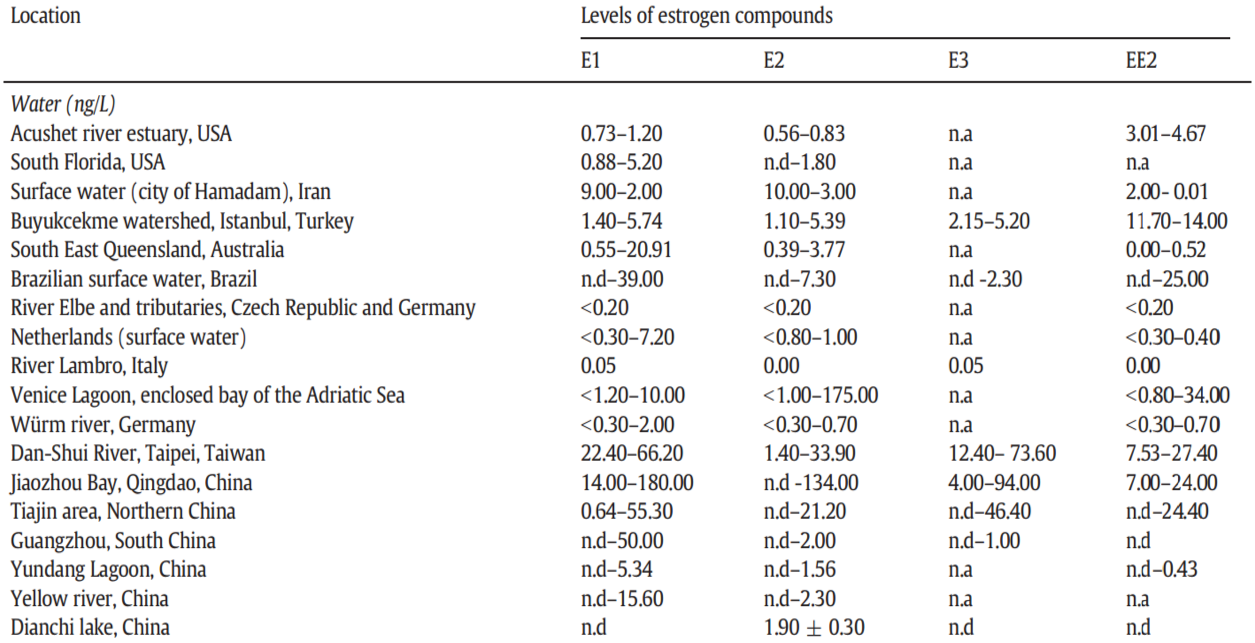
Consistent water temperature is required for successful and consistent reproduction and survival in early life stages (Bonner et al., 1998). Consistent water discharge is also needed order to maintain population as well. The Fountain Darter population remains unaffected by low spring flow up until 2.8 m^3/s and reduces sharply at 1.68 m^3/s (Mora et. al 2012). The lower water discharge can have an effect on the filamentous green algae and reduce habitable area for the surviving darters as well (Saunders et al., 2001).

17α-Ethinylestradiol

17α-Ethinylestradiol (EE_2) is a synthetic estrogen derived from the natural counterpart, estradiol (E_2). From the properties of synthetic estrogens, it should be noted that compared to its natural counterparts, EE_2 is more resistant to biodegradation and has a higher bonding affinity to the estrogen receptor, up to one to two times higher in humans and up to five times higher in certain fish species (Aris et al. 2014). For these reasons, EE_2 is of the most commonly used chemicals in contraceptive pills (Delclos et al. 2009). Also, EE_2 showed the highest estrogenic potency compared to other estrogens; *in vitro* tests in fish showed EE_2 was 11-30 times more potent than E_2 (de Mes et al. 2005).

EE_2 can enter the environment from several different human, industrial, and livestock wastes, including from wastewater treatment plants, septic systems, industrial sources, or agricultural runoff when manure is used as a fertilizer (Aris et al. 2014). While the primary source of EE_2 is human urine, recent studies have suggested that livestock waste was at the same magnitude or even higher than concentrations from human sources (Aris et al. 2014, S. Liu et al. 2012). Even when human waste are processed through an activated sludge wastewater process, a widely used wastewater treatment design, activated sludge is ineffective in reducing EE_2 concentrations (Forrez et al. 2009). While EE_2 concentration reduces by half 25 kilometers downstream from a wastewater treatment plant, possibly due to EE_2's low solubility, it can still be detected up to 100 kilometers downstream (Barel-Cohen et al. 2006). Table 1 shows detected EE_2 concentrations at several sites around the world. Although most concentrations shown are single recordings and do not show the change in EE_2 concentration over time, it does show that high EE_2 concentrations are found in surface water systems around the globe.

Table 1: detected natural and synthetic estrogen concentrations in surface waterways around the globe (Aris et al. 2014)



Location	Levels of estrogen compounds			
	E1	E2	E3	EE2
Water (ng/L)				
Acushet river estuary, USA	0.73–1.20	0.56–0.83	n.a	3.01–4.67
South Florida, USA	0.88–5.20	n.d–1.80	n.a	n.a
Surface water (city of Hamadam), Iran	9.00–2.00	10.00–3.00	n.a	2.00- 0.01
Buyukcekme watershed, Istanbul, Turkey	1.40–5.74	1.10–5.39	2.15–5.20	11.70–14.00
South East Queensland, Australia	0.55–20.91	0.39–3.77	n.a	0.00–0.52
Brazilian surface water, Brazil	n.d–39.00	n.d–7.30	n.d -2.30	n.d–25.00
River Elbe and tributaries, Czech Republic and Germany	<0.20	<0.20	n.a	<0.20
Netherlands (surface water)	<0.30–7.20	<0.80–1.00	n.a	<0.30–0.40
River Lambro, Italy	0.05	0.00	0.05	0.00
Venice Lagoon, enclosed bay of the Adriatic Sea	<1.20–10.00	<1.00–175.00	n.a	<0.80–34.00
Würm river, Germany	<0.30–2.00	<0.30–0.70	n.a	<0.30–0.70
Dan-Shui River, Taipei, Taiwan	22.40–66.20	1.40–33.90	12.40– 73.60	7.53–27.40
Jiaozhou Bay, Qingdao, China	14.00–180.00	n.d -134.00	4.00–94.00	7.00–24.00
Tiajin area, Northern China	0.64–55.30	n.d–21.20	n.d–46.40	n.d–24.40
Guangzhou, South China	n.d–50.00	n.d–2.00	n.d–1.00	n.d
Yundang Lagoon, China	n.d–5.34	n.d–1.56	n.a	n.d–0.43
Yellow river, China	n.d–15.60	n.d–2.30	n.a	n.a
Dianchi lake, China	n.d	1.90 ± 0.30	n.d	n.d

Model Formulation

The model represents the effect of synthetic estrogen (*17α-Ethinylestradiol*) on the Fountain Darter in the egg, larval, juvenile and adult stages of its life. An age-structured population model was created to model a control life cycle and multiple cycles when synthetic estrogen (EE_2) is introduced. The duration of each stage is defined by: egg (6 days), larval (60 days), juvenile (120 days) and adult (550 days) (Mora et al. 2013). Due to the lack of available data on Fountain Darter and the effect of aquatic contamination on their lifecycle, a similar surrogate species, the Fathead Minnow (*Pimephales promelas*) and its lifecycle response to several EE_2 concentrations was used. Using the four-stage structure, the corresponding population matrix **A**, is shown below.

$$\mathbf{A} = \begin{matrix} P_1 & 0 & 0 & F_4 \\ G_1 & P_2 & 0 & 0 \\ 0 & G_2 & P_3 & 0 \\ 0 & 0 & G_3 & P_4 \end{matrix}$$

P_i is the probability of surviving and staying in stage i, G_i is the probability of surviving and growing from stage i to stage i+1, and F_i is the recruitment of stage i.

To represent a normal population with no exposure to EE_2, the normal matrix $\mathbf{A_n}$ is shown below.

$$A_n = \begin{matrix} 0.872 & 0 & 0 & 0.340 \\ 0.116 & 0.987 & 0 & 0 \\ 0 & 0.0105 & 0.994 & 0 \\ 0 & 0 & 0.00514 & 0.999 \end{matrix}$$

The probability that an egg survives and becomes a larval ($G_1 = .116$) was estimated from the overall survival percentage converted into survival per day percentage. P_1 is then estimated as $P_1 = 1 - G_1 - M_1$ where M_1 is the mortality rate of the egg stage per day. The subsequent G_i and P_i probabilities were found in the same method. P_4 is considered .999, or 99.9% probability of survival in the adult stage, however we assume death after the 550 days. To find the recruitment of the adult, the average eggs per female per day is multiplied by the proportion of females producing eggs per day. Since the Fountain Darter produces eggs throughout the whole year, a proportion of producing females per month is found (Mora et al. 2013). These two numbers multiplied together gives the recruitment for the specified EE_2 exposure. The probability shown in the F_4 location uses the average proportion of producing females for the whole year (.0179; Mora et al. 2013).

To represent the effect due to the EE_2 concentrations, specifically 0.2 ng/l, 1.0 ng/l, and 4.0 ng/l were used (Länge et al. 2001). Larger concentrations of EE_2 of 16 ng/l and 64ng/l were initially studied, however, in those concentrations the Fathead Minnow was unable to survive through adulthood and all study fish in these high concentrations were killed before the tests were finished (Länge et al. 2001). For this reason, those concentrations were ignored. Representing the 0.2 ng/l concentration, a second population matrix $\mathbf{A}_{.2}$ is shown below.

$$A_{.2} = \begin{matrix} 0.869 & 0 & 0 & 0.234 \\ 0.111 & 0.988 & 0 & 0 \\ 0 & 0.0108 & 0.994 & 0 \\ 0 & 0 & 0.00531 & 1.0 \end{matrix}$$

The probability that an egg survives and becomes a larval ($G_1 = .111$) was estimated from the overall survival percentage converted into survival per day percentage. P_1 is then estimated as $P_1 = 1 – G_1 - M_1$ where M_1 is the mortality rate of the egg stage per day. The subsequent G_i and P_i probabilities were found in the same method. P_4 is considered 1, or 100% probability of survival in the adult stage, however we assume death after the 550 days. To find the Fountain Darter egg production (13.09), a proportion was applied that was found from the decrease of egg production in the Fathead Minnow due to EE_2 (Länge et al. 2001). Since the Fountain Darter produces eggs throughout the whole year, a proportion of producing females per month is found (Mora et al. 2013). These two numbers multiplied together gives the recruitment for the specified EE_2

exposure. The probability shown in the F_4 location uses the average proportion of producing females for the whole year (.0179; Mora et al. 2013).

The 1.0 ng/l concentration population matrix, $\mathbf{A_1}$, is shown below.

$$A_1 = \begin{matrix} 0.872 & 0 & 0 & 0.206 \\ 0.115 & 0.986 & 0 & 0 \\ 0 & 0.0098 & 0.993 & 0 \\ 0 & 0 & 0.00491 & 0.999 \end{matrix}$$

The P_i and G_i probabilities are found using the same formulas as the 0.2 ng/l concentration above. For the recruitment F_4, the modified Fountain Darter egg production was found to be 11.49 when exposed to 1.0 ng/l (Länge et al. 2001).

For the 4.0 ng/l concentration, the population matrix $\mathbf{A_4}$ is shown below.

$$A_4 = \begin{matrix} 0.870 & 0 & 0 & 0 \\ 0.1134 & 0.9876 & 0 & 0 \\ 0 & 0.0108 & 0.9926 & 0 \\ 0 & 0 & 0.00527 & 0.999 \end{matrix}$$

The P_i and G_i probabilities were found using the same method as the 0.2 ng/l concentration except for the P_4 variable. From the Fathead Minnow research, adult survivability was not calculated. However, since P_i and G_i probabilities did not change with increasing EE_2 concentration the 1.0 ng/l P_4 variable was chosen. The fertility rate F_4 was parameterized as zero due to EE_2 effect on sex determination. After 172 days post hatching, females were found with appropriate sexual characteristic however, no males were found (Länge et al. 2001). This effect reduced the effective reproduction to zero.

In order determine the 4.0 ng/l scenario with a return to control conditions, a special control matrix must be created. Due to the 4.0 ng/l concentration, even after minnows were depurated in non-contaminated water, only 50% of the fish were functionally reproductive (Länge et al. 2001). Therefore, the control matrix $\mathbf{A_{n4}}$ was created to represent this fact.

$$A_{n4} = \begin{matrix} 0.872 & 0 & 0 & .0857 \\ 0.116 & 0.987 & 0 & 0 \\ 0 & 0.0105 & 0.994 & 0 \\ 0 & 0 & 0.00514 & 0.999 \end{matrix}$$

All P_i and G_i probabilities are taken from control conditions, while the F_4 variable is taken as half of the egg production of fish within the 4.0 ng/l concentration.

To evaluate the model, a time-step of one day ($\Delta t=1$ day) was selected. This small time-step was chosen so the short duration of the egg cycle could be modeled. An initial population vector, **n**, of 11 larvae, 28 juveniles, and 61 adults was chosen ($\mathbf{n}(0) = [0, 11, 28, 61]$) (Bio-West 2006).

In order to discover the effect of EE_2 on the Fountain Darter population, a two-year simulation (730 days) will be used for the control, 0.2 ng/l, 1.0 ng/l, and 4.0 ng/l concentrations. Separately, at the one year mark, the 0.2 ng/l, 1.0 ng/l, and 4.0 ng/l will be returned to control concentrations to determine whether the Fountain Darter population could rebound after exposure to EE_2.

$\mathbf{n}(730)=\mathbf{A}_{\mathbf{n}}^{730}\mathbf{n}(0)$

$\mathbf{n}(730)=\mathbf{A}_{\mathbf{.2}}^{730}\mathbf{n}(0)$

$\mathbf{n}(730)=\mathbf{A}_{\mathbf{1}}^{730}\mathbf{n}(0)$

$\mathbf{n}(730)=\mathbf{A}_{\mathbf{4}}^{730}\mathbf{n}(0)$

$\mathbf{n}(730)=\mathbf{A}_{\mathbf{.2}}^{365}\mathbf{A}_{\mathbf{n}}^{365}\mathbf{n}(0)$

$\mathbf{n}(730)= \mathbf{A}_{\mathbf{1}}^{365}\mathbf{A}_{\mathbf{n}}^{365}\mathbf{n}(0)$

$\mathbf{n}(730)= \mathbf{A}_{\mathbf{4}}^{365}\mathbf{A}_{\mathbf{n4}}^{365}\mathbf{n}(0)$

CHAPTER III

RESULTS

Assumptions

With every model, certain assumptions must be made about system components of the model. The main assumption involved the use of the surrogate species (the Fathead Minnow) in order to parameterize the matrix. The Fathead Minnow is a temperate freshwater fish found in most of North America waterways and is one of the most prevalent fish species in the eastern section of North America (Sommer 2011). The Fathead Minnow is often used as test species due to its ability to survive in poor conditions, like high temperature, low oxygen levels, or high turbidities (Texas Parks & Wildlife). This competition is much higher than the Fountain Darter, which requires a very specific habitat in order to survive. This difference could lead to an overestimation of the Fountain Darter's ability to survive in a habitat with EE_2 concentration. In order to normalize the data to Fountain Darter population dynamics, the Fathead Minnow data was appropriated to Fountain Darter stage durations and the fertility rate was normalized to Fountain Darter due to the difference in egg production between each species. Even with these differences, the Fathead Minnow was chosen as the surrogate species due to its similar size, diet, and ideal habitat.

The model also assumed constant concentrations of EE_2 in the water supply. The existence of synthetic estrogen in the watersupply through wastewater (Länge et al. 2001) could result in variable concentrations depending on location to the wastewater outlet or up-network condition changes. If the human population contributing to the wastewater increases its consumptions of synthetic estrogens over time, an increasing concentration gradient within the rivers would likely be seen. From tests in the world's waterways, EE_2 concentrations were varied over time, but more tests need to be completed in order to understand the change over time (Aris 2014).

Results

After calculation, the long-term population growth rate for each of the five scenarios is depicted in Figure 2. In the 4.0 ng/l scenario, the population growth rate drops below 1, signifying a drop in population.

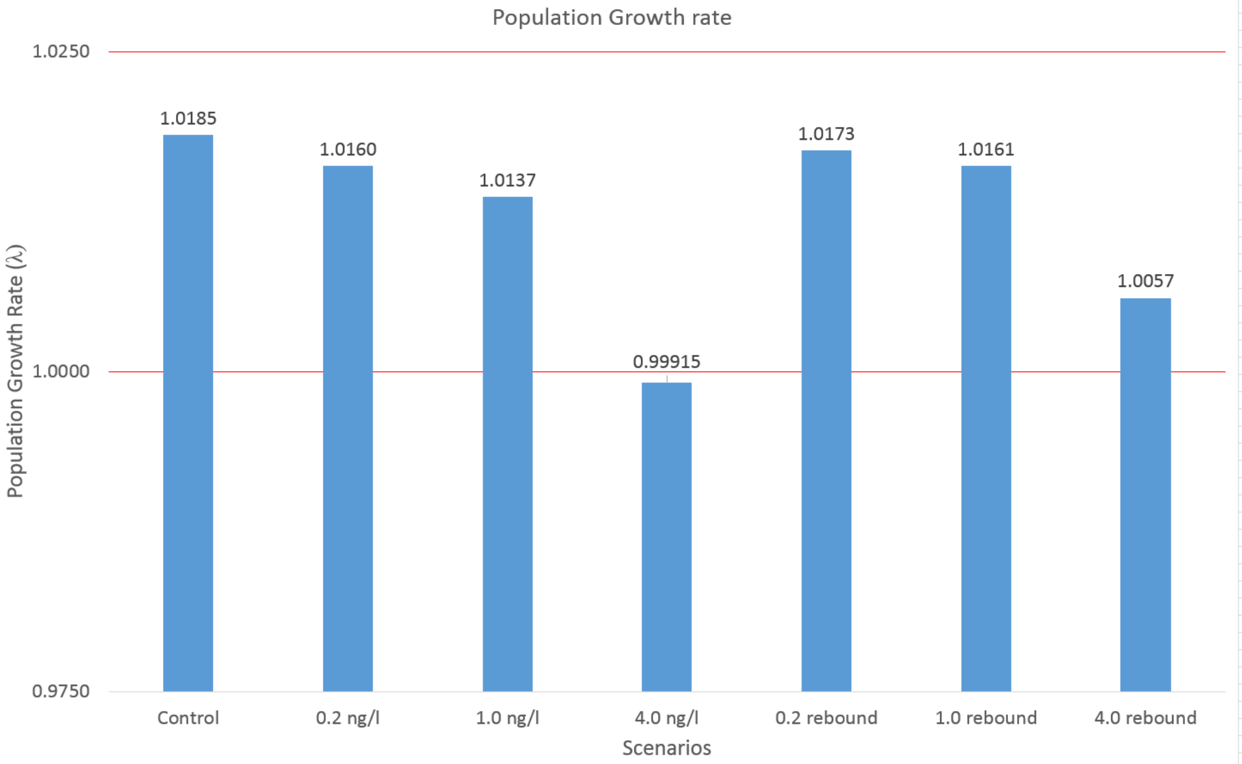


Figure 2: Population growth rate for each scenario

For the 0.2 and 1.0 ng/l rebound scenarios with one year possessing a higher EE_2 concentration and the second year possessing no EE_2 concentrations, the population growth rate is within .1% for the 0.2 ng/l scenario and .25% for the 1.0 ng/l scenario. The 4.0 ng/l rebound scenario difference is more significant with a difference of 1.25%. Even still, the population growth rate stays above one.

In order to understand the accuracy of our population matrix, the elasticity was calculated for every parameter. Shown in Figure 3, the elasticity of the control matrix was found. We see that the population growth rate is more affected by the later-stage parameters and most affected by the survival probability of the adult stage at 2.12×10^{-4}.

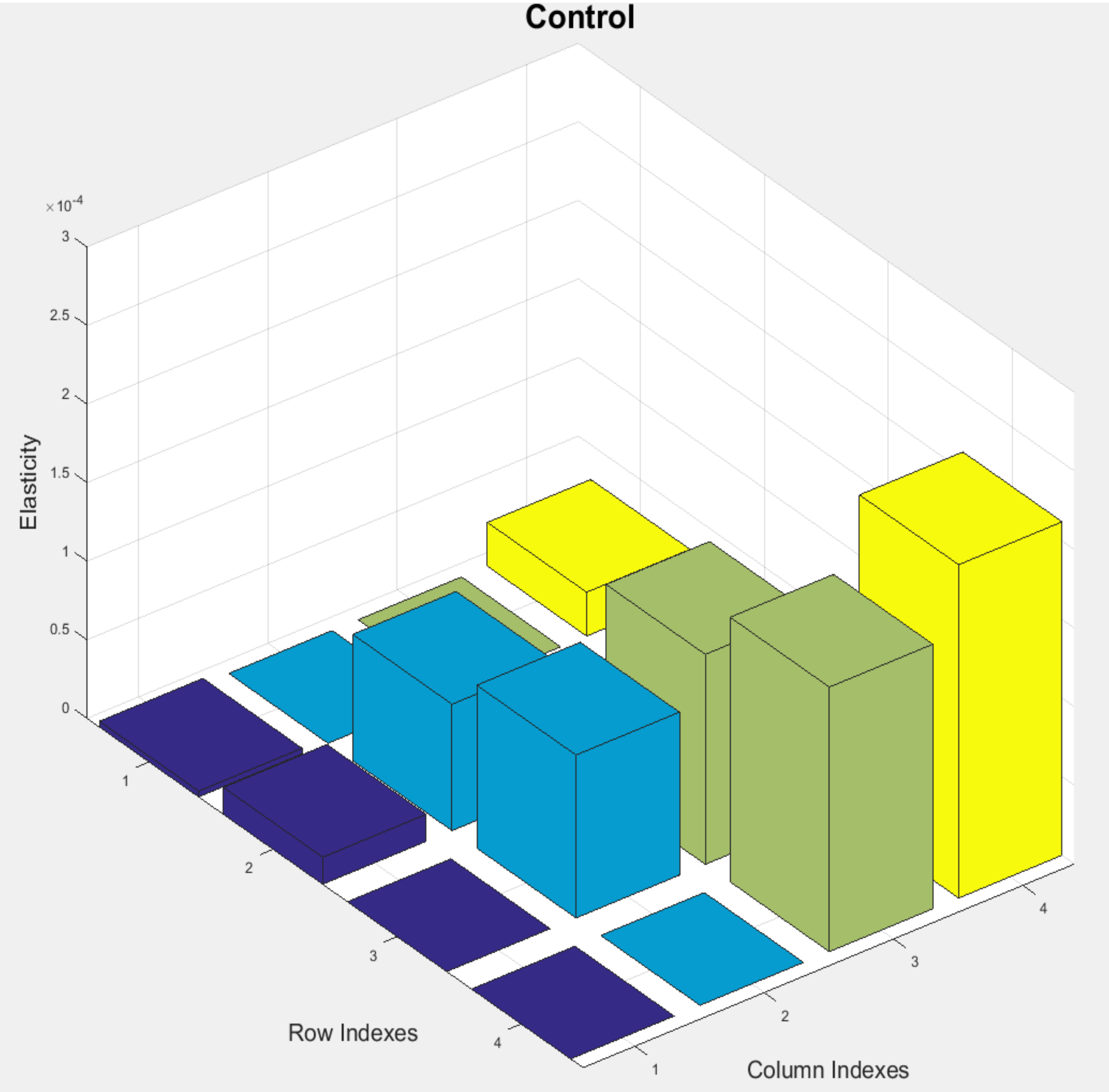


Figure 3: Elasticity of Control Population matrix

The elasticities of the several scenarios were calculated as well. In Figure 4, we see that the 0.2 and 1.0 ng/l scenario's elasticities match the format of the control elasticity matrix. The values of elasticity for the P_4 parameter were slightly higher, with the highest elasticity for the set occurring in the 1.0 ng/l rebound scenario (Figure 3d) with a value of 3.07×10^{-4}. This value is a 37% change from control elasticity on the P_4 parameter, but only an 8.32×10^{-5} overall difference.

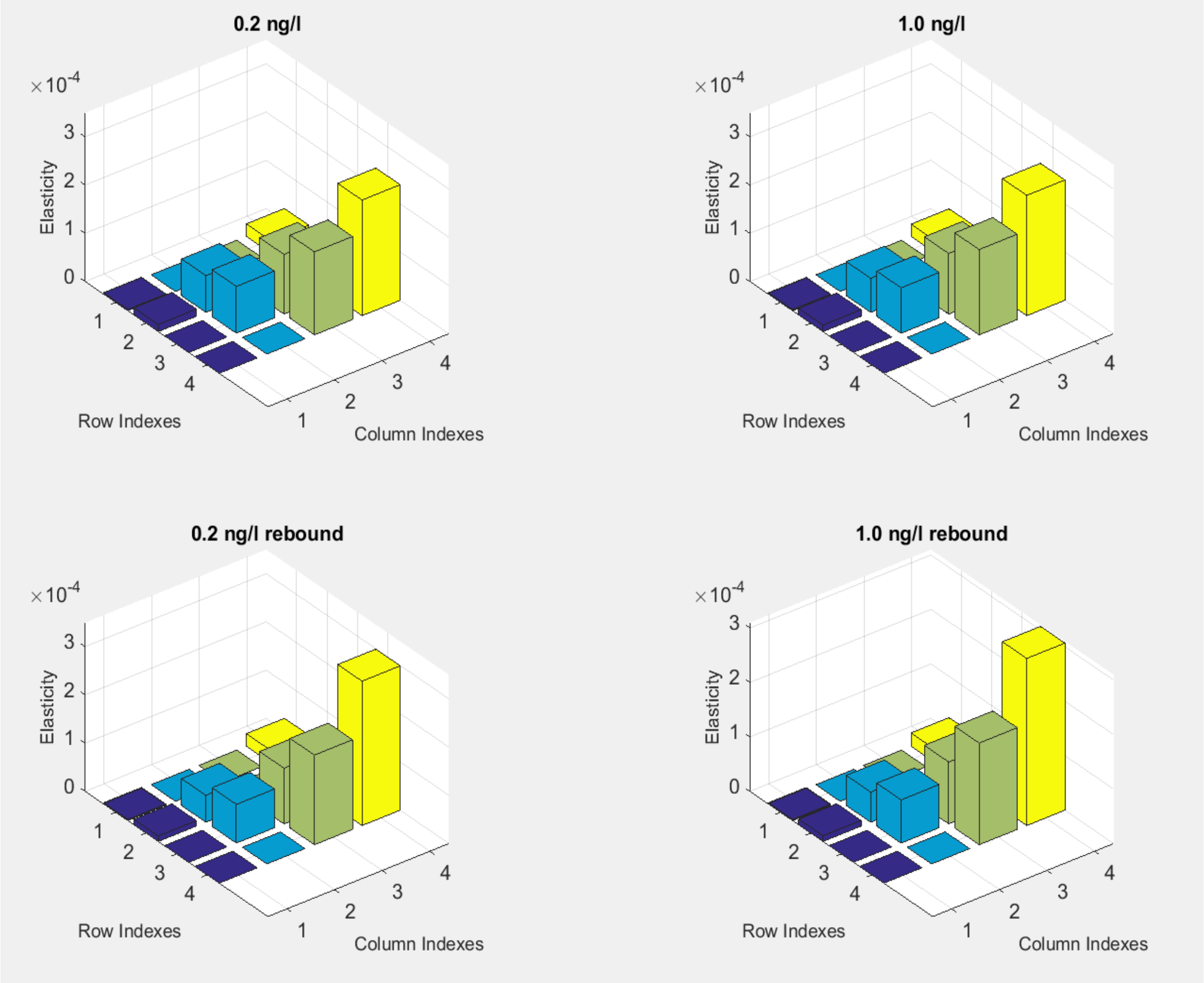


Figure 4: Elasticities of 0.2 and 1.0 ng/l EE_2 concentration scenarios. **a)** 0.2 ng/l concentration; **b)** 1.0 ng/l concentration; **c)** 0.2 ng/l concentration with rebound; **d)** 1.0 ng/l concentration with rebound

Finally, in Figure 5 the elasticities of the 4.0 ng/l and 4.0 ng/l are shown. These elasticities vary from the other scenarios with the population growth rate being only affected by the adult survivability parameter. The magnitude increased as well, with the highest occurring in the 4.0 ng/l scenario at 0.0014. This is a significant increase of over 520%, even if it is a 0.00116 overall change.

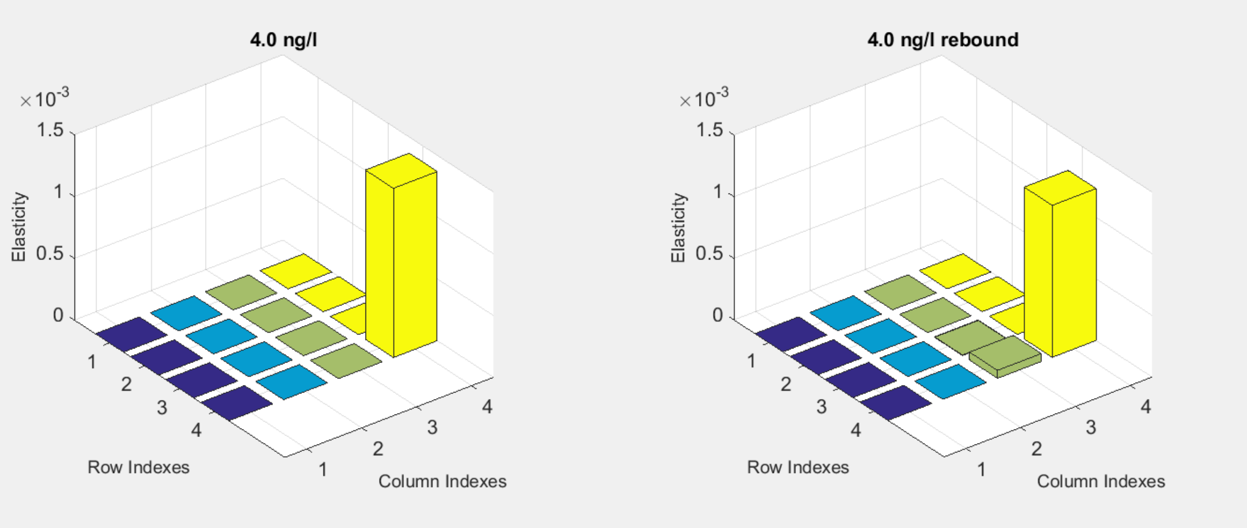


Figure 5: Elasticities of 4.0 ng/l EE_2 concentration scenarios. **a)** 4.0 ng/l concentration; **b)** 4.0 ng/l concentration with rebound

CHAPTER IV

DISCUSSION

Survivability in Adult Stage

In each scenario, it is seen that the survivability of the adult stage is unusually high, never reducing below 0.999. This is due to several factors. First, through normalizing the data to fit Fountain Darter life stages, the long adult stage of the Fountain Darter (550 days) trends the survivability to one. Secondly, this could be an over-estimation of the Fountain Darter survivability due to the Fathead Minnow's ability to survive wide ranging environmental conditions and habitats (Texas Parks & Wildlife). While it is not known if a study has been completed testing the Fathead Minnows response to EE_2 concentrations with other similar aquatic species, it is possible that the Fathead Minnow can survive in higher concentrations of EE_2 than other species, especially against the Fountain Darter which requires a very specific habitat to survive. Finally, this could be due to the highly controlled environment in which the study was performed. In the study, each test tank was supplied with a tightly controlled flow rate including magnetic stirrers to ensure mixing of the EE_2 concentration (Länge et al. 2001). The Fountain Darter population drops once the spring flow drops below 2.8 m^3/s, and drastically reduces once flow drops below 1.68 m^3/s (Mora et al. 2013). If an increase in EE_2 concentration were to coincide with a reduction in spring flow, the Fountain Darter's population could be severely affected.

Physical Mutation of Fish Anatomy

Over the multiple test groups exposed to EE_2 concentrations, varying levels of mutation or retarded growth was seen. Stunted growth was seen in the larval life stages in concentrations as low as 1.0 ng/l and more obviously in the 4.0 ng/l and above concentrations (Länge et al. 2001). This reduced growth could also indicate long-term effects as well, like gonadal development in sexually mature Fountain Darters. As for the adult life stages, reduced growth was once again seen in the 1.0 ng/l concentrations, but it is unknown whether this is due to the initial effect on the larval life stage or if EE_2 can affect growth throughout the entire life cycle. At the 4.0 ng/l concentrations, gonad development was affected as well. In the 4.0 ng/l concentrations, no male secondary sexual characteristics were found at any age; when test fish were dissected to study their anatomy further, no ovarian tissue was found (Länge et al. 2001). Slight male gonad degeneration was even seen as low as 1.0 ng/l, which is supported by the reduced fertility rates seen in the 1.0 ng/l matrix (Länge et al. 2001).

In Länge et al. (2001) Fathead Minnows were studied at 16 ng/l and 64 ng/l concentrations. Due to the minnow's inability to survive long-term in those concentrations, those tests were terminated and no modeling could be performed. However, during the minnow's exposure to these high concentrations severe deformities were found, among which included anal protrusion, distended abdomens, curvature of the spine, and hemorrhaging. These abnormalities extend far beyond the effects of genital anatomy, signifying that higher concentrations are able to reduce survival probabilities in all life stages and not just the fertility rates.

Reduction in Fish Fertility after Exposure

As explained above, fish exposed to 4.0 ng/l EE_2 concentrations saw no male gonadal development, with the sex ratio 100% female once the adult stage was reached. After re-exposure to control conditions and control male fish, the females previously exposed to 4.0 ng/l EE_2 concentrations had a 50% reduction in fertility rate compared to their control counterparts (Länge et al. 2001). However, this data is difficult to interpret because visual identification of fish sex is impossible since no male characteristics are seen in the fish exposed to EE_2. While all traditional males developed ovarian tissue during its exposure to 4.0 ng/l of EE_2, at this time it is unknown if these fish have any reproductive ability at all. Further study of depurated fish at possible lower concentrations are necessary to see if permanent reproductive effects on the female and male populations are seen.

CONCLUSION

This model is a static life stage structured population model on the Fountain Darter (*Etheostoma fonticola*) attempting to model future darter populations under the effect of synthetic estrogen (*17α-Ethinylestradiol*). In order to parameterize the several matrices, available data on EE_2's effect on the Fathead Minnow (*Pimephales Promelas*) was used and normalized to the Fountain Darter's lifecycles and egg production to represent the reaction that an individual Fountain Darter population would have when exposed to EE_2.The Fathead Minnow was chosen due to its similar life stages (egg, larvae, juvenile, adult), similar anatomy and size, and similar habitat and food (Sommer 2011).

After parameterization, seven different scenarios were evaluated based on varying levels of EE_2 concentrations. The population growth rates of these scenarios were calculated to determine in which scenarios the Fountain Darter population would decrease. Through calculations, in the 4.0 ng/l scenario, a decaying population growth rate was found. In order to verify the matrices themselves, the elasticity of each matrix was found. The elasticities of each matrix was found to be relatively low, signifying changes to matrix parameters would not drastically change the population growth rates.

With available lifecycle and egg production data, this model could be used to represent the effect of EE_2 on other small freshwater fish due to the use of a surrogate species. The model could also be related to available past Fountain Darter population data (Schenck and Whiteside, 1976; Linam et al. 1993) to predict total Fountain Darter populations currently and in the future under EE_2 concentrations. A study into the effect of EE_2 on the next generation of fish within the contaminated habitat could be conducted with available data (Länge et al. 2001) in order to fully understand successive years and populations with sustained EE_2 concentration. All of these separate model modifications could describe the Fountain Darter within their delicate habitat.

LID practices and designs should be incorporated in San Marcos and effluent wastewater parameters should be stringent in order to reduce the amount of contaminant reaching the San Marcos River. The increase in contamination reaching the San Marcos and Comal Rivers is not only detrimental to the Fountain Darter, but all unique aquatic flora and fauna that live within these waterways. With the new knowledge from this model, decline of these unique species can be reduced as well as accurately passing regulations limiting the amount of EE_2 and other synthetic and natural estrogens from entering Edwards Aquifer’s surface waterways.

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