Texas A&M University at Galveston
1001 Texas Clipper Road
Galveston, TX 77554

FIRST INTERIM PERFORMANCE REPORT

AUGUST 31ST, 2021

Project Title: The Fate and Toxicity of Microplastics and Persistent Pollutants in the Shellfish and Fish of Matagorda Bay

Submitted To:

Matagorda Bay Mitigation Trust

Performing Laboratory:

Texas A&M University on behalf of Texas A&M University at Galveston

Authors:

Ms. Emily Meese (Ph.D. student)

Mr. Asif Mortuza (Ph.D. student)

Mr. Marcus Wharton (Ph.D. student)

Dr. David Hala, Ph.D.

Dr. Karl Kaiser, Ph.D.

Dr. David Wells, Ph.D.

Dr. Lene H. Petersen, Ph.D.

Dr. Antonietta Quigg, Ph.D.

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The Fate and Toxicity of Microplastics and Persistent Pollutants in the Shellfish and Fish of Matagorda Bay

Personnel

Principal Investigator(s):

Drs. David Hala, Karl Kaiser, David Wells, Lene H. Petersen, Antonietta Quigg

Consulting MBMT Project Coordinator:

Mr. Steven J. Raabe

Location(s):

Texas A&M University at Galveston

Project Duration:

01 June 2021 – 31 August 2024

Objectives:

Objective 1: Quantify the extent of microplastics pollution in the surface waters and biota of Matagorda Bay.

Objective 2: Measure levels of persistent pollutants in surface waters, adsorbed to microplastics, and bioaccumulated in the biota of Matagorda Bay.

Objective 3: Study the toxicity of microplastics and adsorbed pollutants using embryo-larval life stages of sheepshead minnow.

Objective 4: Public educational outreach to local high school students on the science of ecosystem health monitoring.

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1. INTRODUCTION

1.1 Background

The pollution of the Matagorda Bay system by microplastics particles released from the Formosa Plastics Corporation (as recorded from 2016-2018) has caused concern for the widespread exposure of resident biota (shellfish and fish) (Conkle, 2018; Wilson, 2018). Microplastics (i.e. particles <5 mm in diameter) can also act as important carriers of pollutants in the marine environment. The ingestion of such tainted plastic particles by aquatic organisms can lead to the increased exposure and body-burdens (or bioaccumulation) of persistent organic pollutants (Hirai et al., 2011; Hüffer and Hofmann, 2016), and contribute to the toxicity of the ingested particles (Vázquez and Rahman, 2021).

This project is studying the extent of microplastics and persistent pollutant exposure of resident biota (shellfish and fish) sampled from Matagorda Bay, and also assessing any likely toxicity effects due to exposure. The *new knowledge* gained from the successful completion of this project will contribute to an understanding of the long-term fate and toxicity of microplastics (and adsorbed pollutants) in the Matagorda Bay system.

In this first quarterly interim report (June 1st – August 31st, 2021) we present the Methods and Results of our research to date. The Methods and Results sections is organized as per the key objectives of this project. It is our expectation that organized in this manner, the interim report will provide a clearer roadmap of achievements (to date) and expectations (in the near future).

2. METHODS AND RESULTS

As of the period encompassing the first interim report (June 1st – August 31st, 2021), the key achievements associated with each stated objective are detailed below.

Objective 1: Quantify the extent of microplastics pollution in the surface waters and biota of Matagorda Bay.

- The collection of fish and water samples from Matagorda Bay has commenced.

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- The GCMS-pyrolysis system, which will allow the quantification of microplastics particles in the surface waters and biota of Matagorda Bay, has been procured and is being optimized for use (**Fig. 1**).

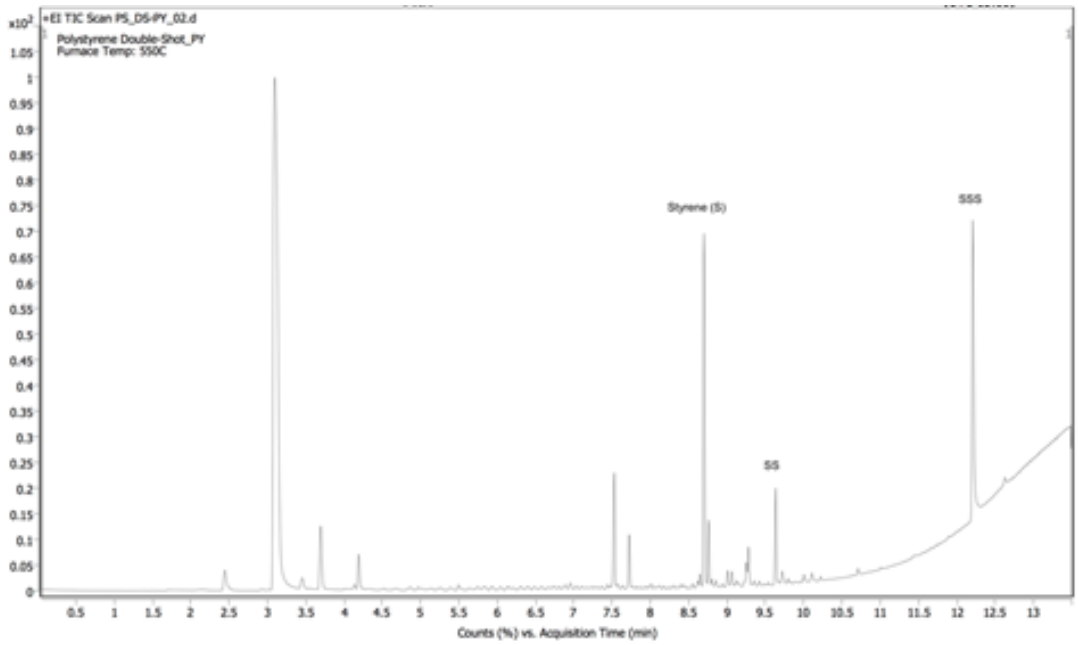

Fig. 1. Pyrogram of polystyrene. The relative distribution of peaks with associated mass spectra serve as a chemical fingerprint, and quantification can be achieved by calibrating a selected decombustion product.

2.1. Biota sampling from Matagorda Bay

A variety of catch methods have been used to sample fish from various sites across Matagorda Bay. These include the use of bag seines, gill nets, and entanglement nets. The fish have been sampled monthly from May – July 2021, and the species of fish collected thus far include: spotted seatrout (*Cynoscion nebulosus*, n=6), hardhead catfish (*Ariopsis felis*, n=46), gafftopsail catfish (*Bagre marinus*, n=6), black drum (*Pogonias cromis*, n=6), red drum (Scianops ocellatus, n=4), spot (Leiostomus xanthrus, n=1), Atlantic croaker (*Micropogonias undulates*, n=10), Gulf whiting (*Menticirrhus americanus*, n=1), Gulf menhaden (Brevoortia patronus, n= 42), gizzard shad (*Dorosoma cepedianum*, n=9), striped mullet (*Mugil cephalus*, n=27), bluefish (*Pomatomus saltatrix*, n=3), ladyfish (*Elops saurus*, n=2), and pinfish (*Lagodon rhomboids*, n=1).

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In total, 165 fish have been sampled from Matagorda Bay over May, June, and July 2021 (please see **Fig. 2** for a map of the sampling sites), and with additional sampling planned over the remainder of 2021 (and subsequently into 2022). For each of the fish sampled, the weight, standard/pre-caudal length, fork length, and stretch total length was recorded. And the tissues comprising muscle, liver, and entire gut contents (comprising the entire length of intestine and stomach), were excised and stored at -20^{o}C until needed for analysis.

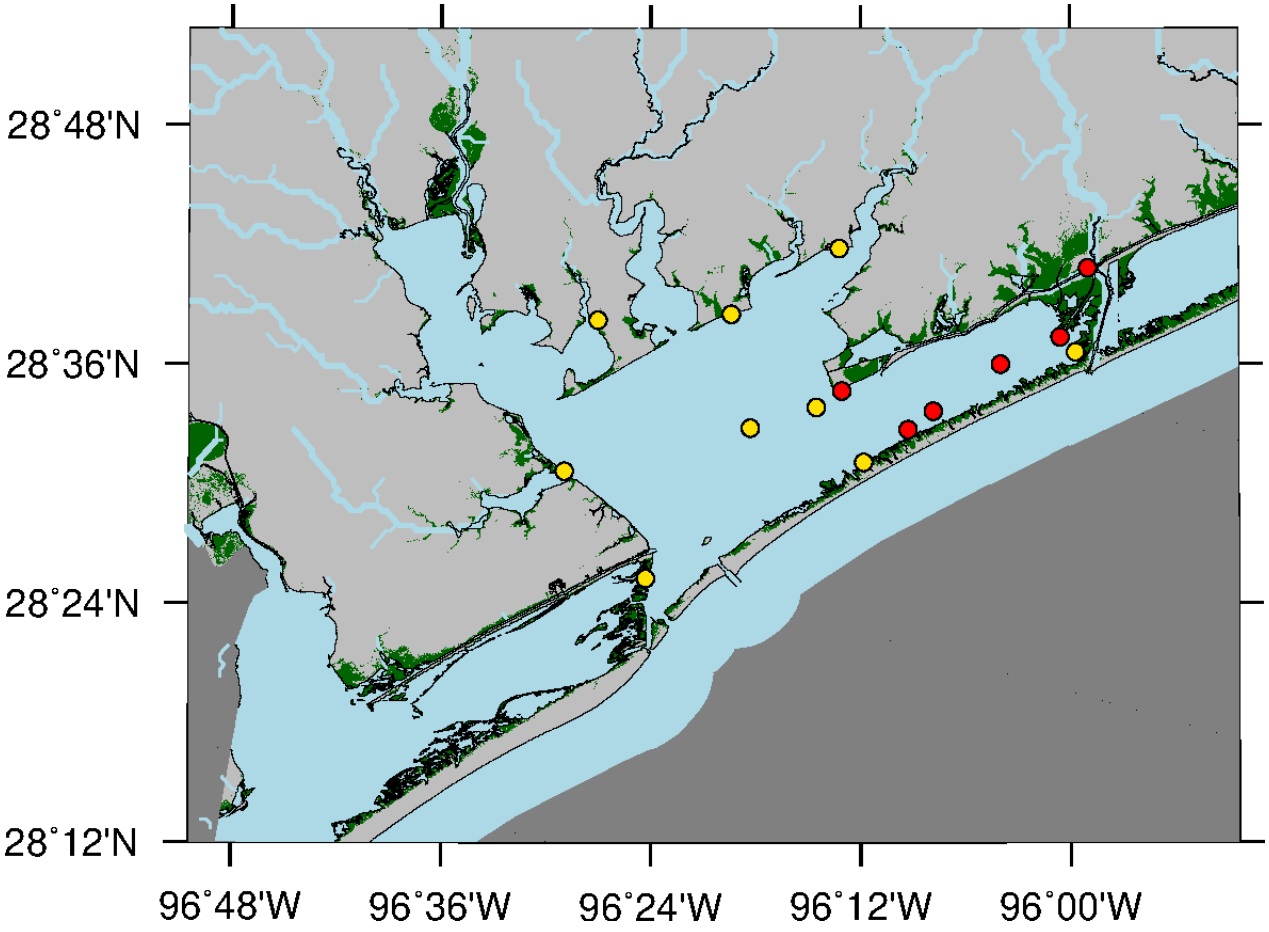

Fig. 2. Map of Matagorda Bay showing the various sites from which various fish species have already been (May – July 2021) (shown as yellow circles); and sites from which water samples were recently collected (August 2021) (shown as red circles).

In order to assess the well-being of the fish sampled during the period from May – July 2021, the condition factor of fish was calculated using the formula (Williams, 2000) (**Fig. 3**):

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$$K = W * 10^5 / L^3$$

Where K is the condition factor, W is the fish weight in grams, L is the fish fork length in millimeters, and 10^5 is a factor to bring the value of K near unity. Overlayed onto the Figure we demarcate the various K value thresholds as determined for fish health in salmonids (Barnham and Baxter, 1998).

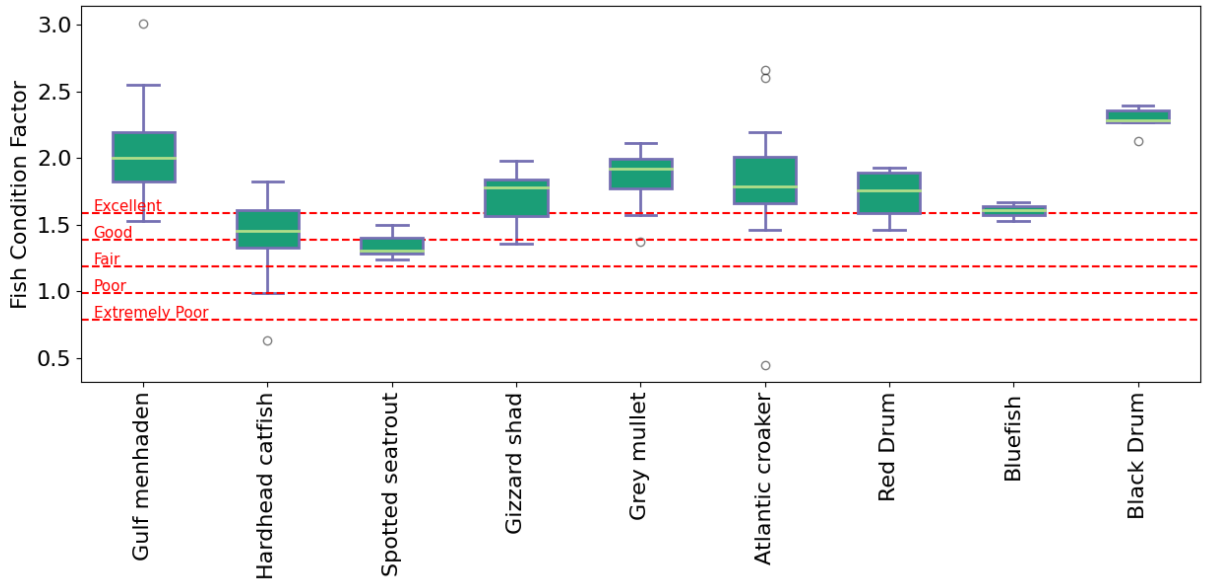

Fig. 3. Box-and-whisker plots of the condition factors for select fish species sampled in Matagorda Bay from May – July 2021. Overlayed onto the Figure are condition factor thresholds typically used to indicate fish health for salmon and trout (from Barnham and Baxter, 1998). The thresholds are only shown to qualitatively indicate the condition of fish from Matagorda Bay.

2.2. Water sampling from Matagorda Bay

Buoyant microplastic particles were collected from the surface waters of Matagorda Bay by towing a neuston net (200 µm) during planned quarterly water sampling (per year for first two years of project) aboard a chartered fishing vessel (RedFishMatagorda.com). Please see **Fig. 2** for a map of the sampling sites. Tow samples were transferred into glass jars and stored at 4°C until analysis.

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From each collected sample, microplastics will be separated with a 500 µm stainless steel sieve into particles >500 µm for identification by attenuated total reflectance fourier-transform infrared spectroscopy (ATR-FTIR), and particles <500 µm for analysis by gas chromatography mass spectrometry-pyrolysis (GCMS-pyrolysis). The combined application of ATR-FTIR and GCMS-pyrolysis will increase the analytical sensitivity for the detection of a wide size range of microplastics particles.

A GCMS-pyrolyzer was recently (August 18th, 2021) purchased from Quantum Analytics (Oak Ridge North, TX) by using contributing funds from the MBMT project. This instrument is currently being set-up and augmented to an existing GCMS system (Agilent 7010B GC/MS Triple Quad coupled with Agilent 8890 GC system).

Objective 2: Measure levels of persistent pollutants in surface waters, adsorbed to microplastics, and bioaccumulated in the biota of Matagorda Bay.

- The optimization of the use of an Accelerated Solvent Extraction (ASE) system for the analysis of select persistent organic pollutants, namely polycyclic aromatic hydrocarbons (PAHs) and polychlorinated biphenyls (PCBs), has commenced.

2.3. Accelerated solvent extraction (ASE) of PAHs and PCBs from biota samples

The organic pollutants will be extracted from muscle, liver and digestive tract samples (~1 gram for each) excised from the fish sampled from Matagorda Bay. The extraction will be done using an Accelerated Solvent Extraction (ASE) system (Dionex 350). Each tissue sample will be packed into a 34 mL stainless steel vessel, with remainder volume packed with Ottawa sand. The solvent mixture that will be used for extractions is 1:1 $^{v}/_{v}$ hexane:dichloromethane (DCM). The extraction conditions are: pressure 1500 psi, temperature 100ºC, heating time 5 mins, preheat 5 mins, static phase 4 mins, flush rate 60%, purge time 300s, 2 cycles.

The resulting extracts will be collected in a 50 mL amber glass bottle, spiked with the internal standards of Benzo[a]pyrene-d_{12} at 25 ng/mL and PCB65-d5 at 25 ng/mL (parts per billion or ppb), and dried under a gentle stream of nitrogen (N_2). The lipid content will be determined gravimetrically. The resulting residue will be reconstituted with DCM and transferred to a smaller

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(2 mL) amber glass. The recovered volume will be dried under N_2 with residue reconstituted with 0.2 mL acetonitrile (ACN), and transferred to a 0.2 mL small-volume insert. All samples will be subsequently frozen at -20^{o}C to allow the precipitation of lipids or other organic debris. Subsequently, a 0.05 mL aliquot of solvent supernatant will be removed, transferred to a new 2 mL amber vial and dried under N_2. The residue will be finally reconstituted into 0.05 mL DCM and analyzed via GCMS.

2.4. Gas Chromatography and Mass Spectrometry (GCMS) analysis of PAHs and PCBs in biota samples

The body-burdens of 16 PAHs and 29 PCB congeners (all EPA priority pollutants) will be quantified using gas chromatography mass spectrometry (GCMS). The 16 PAHs include: naphthalene, acenaphthene, acenaphthylene, fluorene, anthracene, phenanthrene, fluoranthene, chrysene, pyrene, benzo[a]anthracene, benzo[b]fluoranthene, benzo[k]fluoranthene, benzo[a]pyrene, dibenz[a,h]anthracene, benzo[g,h,i]perylene, and indeno[1,2,3-cd]pyrene. The 29 PCB congeners include PCBs 1, 18, 28, 33, 52, 95, 101, 81, 77, 149, 123, 118, 114, 153, 105, 138, 126, 187, 183, 128, 167, 177, 171, 156, 157, 180, 169, 170, and 189. Of the 29 PCB congeners, 12 are dioxin-like: PCBs 77, 81, 105, 114, 118, 123, 126, 156, 157, 167, 169, and 189. All PCBs are identified according to the IUPAC numbering system. The GCMS analysis methods proposed for use in this project have already been developed and published by the P.I. (Hala) (Bacosa et al., 2020; Cullen et al., 2019; Hernout et al., 2020; Lawson et al., 2021; Steichen et al., 2020). GCMS analysis will be conducted on a Agilent 7010B GC/MS Triple Quad coupled with Agilent 8890 GC system. Samples will be injected in splitless mode (2 μL) onto a DB-5MS (J&W Scientific) capillary column (30 m x 0.25 mm i.d.; 0.25 μm film thickness). Helium will be used as the carrier gas at a flow rate of 1.0 mL/min. Temperatures at the front inlet and the MS interface are set at 250 and 280°C, respectively. Following injection of the sample, the GC oven program will begin at 40°C, held for 1 min, then ramped up to 180°C at 20°C/min, and finally ramped up to 300°C at 5°C/min, and then held for 10 min. The MS will be operated in electron impact (EI) mode at an electron energy of 70 eV while the MS source temperature will be maintained at 230°C. Selected ion monitoring (SIM) mode will be used for identification and quantification of all 45 analytes. The quantification of each PAH and PCB will be performed

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against linear 13-point calibration curves using serially diluted standards that are prepared in DCM (10,000 to 2.5 ng/mL). Sample quality assurance and quality control measures will be conducted by running a sample blank and a mixed standard addition sample (into a representative tissue matrix being analyzed). An acceptable percent accuracy for the standard addition sample for select PAHs and PCBs will be within the range of 75 – 125% (and coefficient of variation ≤25%). The limit of detection (LOD) will be set to a signal-to-noise ratio of ≥5x for the lowest detectable calibration point. Blanks will also be checked for signs of external contamination above the LOD.

Objective 3: Study the toxicity of microplastics and adsorbed pollutants using embryo-larval life stages of sheepshead minnow.

- This objective will be engaged with starting in January 2022 and onwards.
- An Animal Use Protocol (AUP) to perform *in vivo* experimentation with early life-stages of embryo-larval sheepshead minnows (*Cyprinodon variegatus*) has been approved by the Texas A&M University's Institutional Animal Care and Use Committee (IACUC) (please see **Appendix I**).

Objective 4: Public educational outreach to local high school students on the science of ecosystem health monitoring.

- This objective will be engaged with in summer 2022.
- At present, an educational module that involves hands-on learning by students, and includes the assessment of various pollution sources into a Gulf of Mexico estuary (and the complexity associated with their mitigation), has been approved by the Director of Outreach for Texas A&M University at Galveston's Sea Camp Program, Ms. Daisy Dailey (please see **Appendix II**).

3. KEY PROGRESS AND FURTHER WORK

Key progress encompassing the project duration from 1st June – 31st August (2021) are summarized below:

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1) Two graduate students, Mrs. Marcus Wharton and Asif Mortuza, have been recruited to work on the project. Mr. Wharton's research will focus on microplastics quantification, whereas Mr. Mortuza will quantify PAHs/PCBs and perform toxicology trials.
2) A GCMS-pyrolysis system has been procured for the analysis of microplastics particles in surface waters and biota from Matagorda Bay.
3) 165 fish have been sampled from Matagorda Bay over May, June, and July 2021 (by Ms. Emily Meese). Each fish has been dissected to collect muscle, liver, and the entire gut (comprising the entire length of intestine and stomach), for microplastics and pollutant (PAHs/PCBs) analysis
4) 12 water samples have been collected from Matagorda Bay in August 2021. Currently these samples are being processed to remove organic debris, making the samples amenable to GCMS-pyrolysis analysis for the detection of microplastics. Water samples will also be analyzed for PAHs and PCBs.
5) An Animal Use Protocol (AUP) to perform *in vivo* experimentation with early life-stages of embryo-larval sheepshead minnows (*Cyprinodon variegatus*) has been approved by the Texas A&M University's Institutional Animal Care and Use Committee (IACUC).
6) An educational module has been developed to engage high school students visiting the annual summer Sea Camp at TAMUG, with a hands-on lab practical on the science of environmental monitoring.

<u>Further planned work</u> for completion over the duration of the second interim report are as follows:

1) Continue to collect biota and water samples from Matagorda Bay. Additional biota sampling is planned for September 2021, with additional water sampling also planned for September and December 2021.
2) Complete the development of a quantitative analytical method for microplastics quantification in biota and water samples using the GCMS-pyrolyzer.
3) Commence PAH and PCB analysis in biota tissues.

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4. REMAINDER BUDGET AND EXPENDITURES

Please see **Appendix III** for a summary of expenditures incurred over the duration of the first interim report from 1st June – 31st August (2021).

Reviewed by:

8/30/2021

_____ Date: _____

Dr. David Hala, TAMUG, P.I.

Approved by:

_____ Date: August 30,2021

Mr. Steven J. Raabe, Trustee

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Galveston, TX 77554

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APPENDIX I

(AUP Approval)

DIVISION OF RESEARCH

April 14, 2021

MEMORANDUM

TO: Dr. David Hala
TAMU - TAMUG - Marine Biology

FROM: Dr. Mark Westhusin, Chair
Institutional Animal Care and Use Committee
College Station/Galveston: OLAW Assurance D16-00511 / USDA Registration 74-R-0012

SUBJECT: **Approval of Animal Use Protocol IACUC 2021-0036**
Title: The toxicity of microplastics and persistent pollutants on embryo-larval sheepshead minnows (Cyprinodon variegatus)
Reference Number: 122654
Funding Source: Matagorda Bay Mitigation Trust
AUP Approval Date: 04/14/2021
AUP Expiration Date: 04/13/2024
Species: fish

The above referenced animal use protocol (AUP) has been approved by the IACUC for a period of 3 years. **The Principal investigator (PI) is responsible for all activities conducted on this protocol. As such, the PI must ensure that:**

- The AUP will be conducted in accordance with all applicable state and federal regulations, System policies or regulations, and University rules or procedures as described in TAMU Rule 15.99.07.M1.; as well as all guidance and procedures promulgated by the IACUC.
- All procedures involving animals will be carried out humanely and as described in the approved AUP.
- IACUC approval will be secured before initiating any change in the study design or procedures listed in this AUP. Protocol participants understand that all amendments must be approved by the IACUC prior to implementation.
- All individuals working autonomously on this AUP are qualified to conduct procedures involving animals, are competent in the techniques cited in the AUP which they will perform, and will maintain appropriate and complete animal records. All untrained participants will be sufficiently supervised until competency is achieved.
- The IACUC is notified regarding any unanticipated or adverse events impacting the health or safety of animals that are not clearly described within the approved AUP.
- The IACUC is notified of potential noncompliance including accidental or intentional failure to comply with state and federal regulations, System policies or regulations, University rules or procedures, IACUC guidance, or the requirements to conduct research, teaching or testing using animals; including adherence to the approved animal use protocol.
- Work performed without IACUC approval is not published with certification of IACUC approval.

A copy of this approval will be sent to the housing facility. You must consult with the housing facility manager or designee prior to ordering animals to ensure that space is available.

Best of success in your research endeavors.

Cc: Housing facility management
Comparative Medicine Program

750 Agronomy Road, Suite 2701
1186 TAMU
College Station, TX 77843-1186
Tel. 979.458.1467 Fax. 979.862.3176
http://rcb.tamu.edu**/animals**

APPENDIX II

(Educational Module)

Chapter 1. Applying Environmental Science - Investigation of Chemical Contaminants

1. Objectives

After completing this experiment you should be able to:

- Distinguish the many varieties of environmental contaminants.
- Identify the variety of sources for pollution inputs into the environment.

2. Introduction

In the United States, many cities are dominated by a principal industry, such as a company that supports the community through an extensive tax base and serves as the primary source of employment. These include coalmines in West Virginia, the technology industry in southern California and the steel mills of Pittsburgh and Birmingham. The Gulf Coast of Texas is also an excellent example, with scores of large, diverse chemical and petroleum-refining companies contributing huge sums of money to local economies. Often, a single large company will exist within a community's boundaries, pumping untold dollars into the economy. But with this tax base comes the potential for ethical problems.

This lab presents a hypothetical town of Indianola, whose community's welfare is intricately tied to the success or failure of the main local industry. In our scenario, a chemical manufacturing company is being implicated in polluting the city's river. If the plant is forced to close, the town will in all likelihood wither and die. If the plant thrives, the town will continue to thrive, but at what cost? In situations with conflicting self-interests, ethics come into play. What if the chemical plant is indeed the source of the pollution and yet is unwilling to rectify the matter? The decision could then become one of economic welfare versus the physical health of the community itself, resulting in a risk-versus-risk or bite-the-hand-that-feeds-you situation.

Our imaginary town, Indianola, is a Texas coastal town, located on the banks of an important estuarine water body known as Karankawa Creek (**Fig. 1**). The creek has been the center of commerce and commercialization for over a hundred years. Additionally, the source of the creek, Lake Karankawa, has been Indianola's principal source of freshwater for as long as people can remember. Karankawa Creek is a classic estuarine system, serving as a breeding ground for myriad saltwater species, and for many years the creek has been a viable fishery supporting a large shrimping fleet. The creek has also served as a source of power and transportation, with luxury home building increasing along its scenic banks.

In the early 1900s, the Texas Pulp and Paper Company (TPPC) was founded on Karankawa Creek, strategically located to utilize the creek as a convenient source of water for manufacturing

processes and especially as the primary means of transportation (**Fig. 1**). With the founding of TPPC, Indianola's economy was booming, and the small town became a thriving community of 15,000 people. Acquired by new owners in the 1940s, TPPC moved away from the paper business and became a specialty chemical company, focusing on the manufacture of a no-knock gasoline additive, tetraethyl lead (TEL). Not wanting to change a name that had become synonymous with the community, management decided that TPPC would become the official name of their company. Business boomed and Indianola thrived, with its population increasing to over 40,000 citizens.

Unfortunately for TPPC, in the late 1970s the U.S. government mandated that lead be taken out of gasoline. As demand for TEL plummeted, TPPC and Indianola faced hard times. In order to save the factory, TPPC bought a consortium of small chemical companies, obtaining the patents to a diverse variety of chemicals. One of these processes involved production of polysilicon crystals as a by-product, but at that time the crystals were discarded as they had no known practical use. Shortly thereafter, a chemist working at TPPC, who just happened to be a graduate of the Texas A&M University at Galveston, applied her knowledge of polysilicon crystals to a new application, computer chips. Within one year, TPPC stock soared on the NYSE, rising from a low of 1⅓ to its current price of 115½. Indianola was again thriving, and the population increased from 30,000 to 80,000 in only two years.

In order to accommodate this huge increase in population, the area east of town, but south of TPPC, became highly populated. This location was considered very desirable, with high priced homes being built along the banks of beautiful Karankawa Creek. With the advent of the Karankawa Creek development and the extension of development with Phase II, something happened to the environment of Indianola (**Fig. 1**). Changes came slowly at first, but problems soon began to rise dramatically. Karankawa Creek, the pride of the community, began to stink. The odor was horrible. The Texas State Department of Health found high levels of bacteria (*coccus*) and organic compounds in water samples taken at the Balinese Landing south of the city. In addition, wild animal populations were observed to have problems with locomotive functioning (disruption of their central nervous system) and the residents of the town suffered a higher rate of cancer than the national average.

Because Karankawa Creek serves such a vital role for industry and residential uses in Indianola and surrounding areas, an environmental consulting firm, Texas Industrial and Chemical – Karankawa Services (your Group), has been hired to find out the source of the pollution problem and to ultimately suggest a remedy. To aid you in your quest, your team of experts will be provided with the necessary information to successfully complete your task. On the next page is a map of Indianola, with pertinent information labeled.

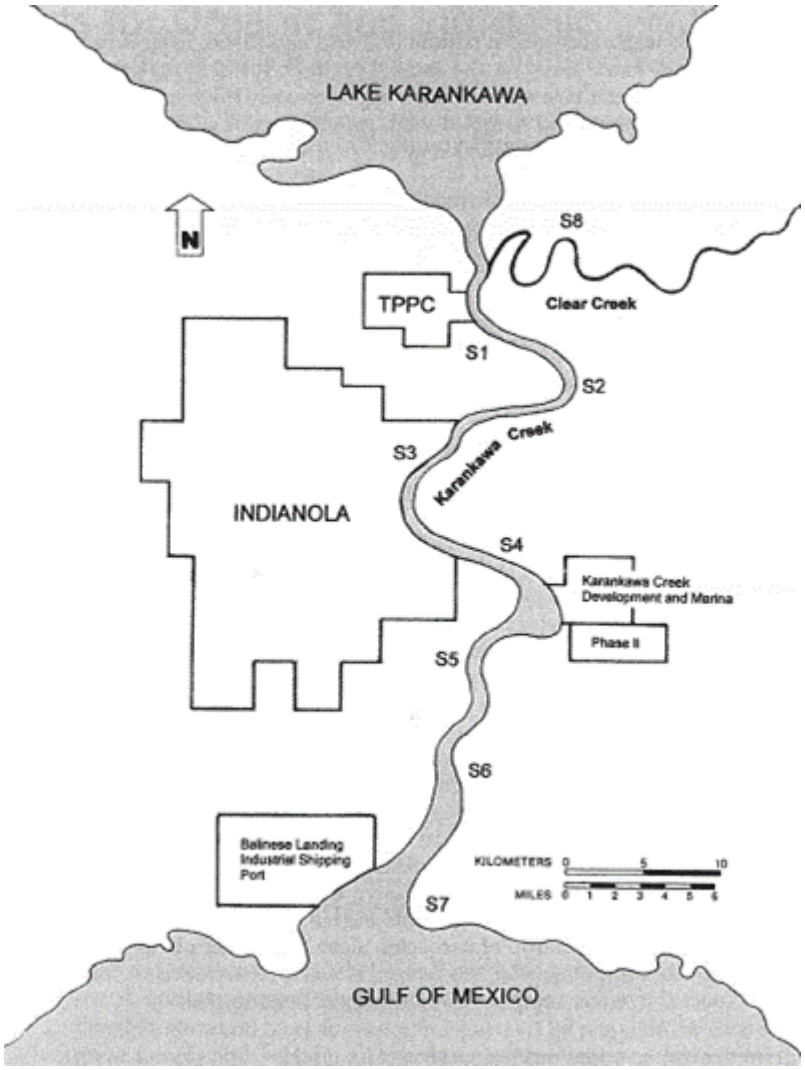


Fig. 1. Site map of Indianola and surrounding area. North is to the top of the map. Karankawa Creek flows from the north to the southwest at a fairly constant rate. The creek is fed by Lake Karankawa.

Water samples W1 – W7 were taken from running water in Karankawa Creek while W8 was taken from the largely undisturbed Clear Creek, north of the TPPC plant. S1 – S8 are sediment pore water samples that have corresponding numbers (see map) to the water samples. These were therefore taken at the same location as the water samples.

3. Summary of Lab Experiment

In this experiment, you will be presented with a simulated scenario involving contaminant pollution in a growing, industry-dominated community. Though only a simulation, this exercise replicates numerous aspects of real-life situations currently being played out in towns throughout the world. Your Group is part of a local consulting firm and your job description is to find the source of pollution problem and suggest a remedy. As a consulting firm you are not hired to place guilt, merely state what causes the pollution problem. Your task is to look for four common

contaminants: lead, mercury, polychlorinated biphenyls (PCBs) and dioxins. Based on the history of TPPC it would be expected to find some or all of these around Indianola. Therefore, you will need to answer the following two research questions:

a) Is Karankawa Creek polluted with lead, mercury, PCBs and dioxins?

b) Which area of Karankawa Creek is most polluted?

Samples of water and sediment pore water (W1–W8 and S1–S8, respectively) were collected at the same time, on the same day. Each water sample was taken at a depth of one meter below the surface, approximately three meters from the bank of the stream. Sediment samples were collected using a grab sampler, directly below the corresponding water sample.

(a) <u>Background of Tested Materials</u>

Dioxin – A compound formed as a waste product of various industrial processes, especially from processes involving chlorine. In the class of chemicals known as dioxins, dioxin is a prevalent waste product from pulp and paper mills, and it is often found in conjunction with the defoliant 2,4,5-Trichlorophenoxyacetic acid (2,4,5-T), otherwise known as Agent Orange. This chemical was used in large quantities in Vietnam in the late 1960s. Agent Orange was a suspected cancer agent in soldiers exposed to this chemical mixture during the Vietnam conflict. Dioxin is only marginally soluble in water; therefore, it may be expected that the majority of this chemical will be found in the sediment at the bottom of the creek.

Polychlorinated biphenyls (PCBs) – These compounds were used as insulation in plastic coated wire and for insulation purposes in transformers and capacitors. PCBs have also been used in computer and electronic applications. These compounds are not very soluble in water and may be expected to be present in the sediments of streams. PCBs are renowned for their environmental persistence.

Lead – Lead compounds were used predominately in exterior and, occasionally, interior oil based paints. Paints containing lead were preferred for use in arts and crafts work because they produced excellent color with superior color retention over years of wear. Lead compounds are extremely soluble in water, and thus would be expected to be present in the water of Karankawa Creek.

Mercury – Mercury compounds have many of the same properties as lead. Additionally, mercury compounds are used in marine bottom paints. Mercury in paint prevents the build-up of barnacles, algae and water plants on the bottom of boats, piers and pilings. For this reason, if contamination occurs, mercury would be expected in water samples near industry or boating areas.

***Note* This is a simulated exercise and you are not be actually working with these harmful chemicals in this lab exercise.**

4. Materials

- Sample bottles labeled S1–S8 and W1–W8.
- Sample bottles for pH measurements; water and sediment pore water samples.
- Single use plastic disposable transfer pipettes.
- Multi-well plates.
- pH indicator solution.
- Reagents specific for dioxin, PCBs, mercury and lead analysis.

5. Experimental Procedure

(a) Investigations of Chemical Contaminants

- Tests will be conducted to analyze for presence of organic compounds (TCDD and polychlorinated biphenyls) and inorganic contaminants (lead and mercury).

- Results will be reported in a semi-quantitative manner. You will report your results by using a positive **(+)** mark to indicate that a substance is present. If a substance is present in high concentrations, notate it by recording its presence as (++**)**. Negative **(-)** results should be reported for any chemical test that shows no positive result.

- The purpose of this activity is to provide data on the presence or absence of the chemicals in question. As an environmental analyst, your job is to report results to the best of your ability.

(b) Tests for Organic and Inorganic Compounds in Samples

- The following experimental protocol must be followed to accurately test for the various contaminants of concern in this study. The testing reagents give the following color indications (changes) if the listed compound is present. Please note that samples have been pre-treated for each reaction. Therefore, you must use the sample labeled for each compound for the experiment to work.

Safety Note: The reagents and some of the samples have been pre-treated with chemicals that may cause irritation to the eyes and skin. Therefore, you should wear gloves while performing this experiment.

- In summary, your experimental procedure will look as follows:

1) Organic Compounds (Organic compound + reagent → colored complex)

a) **PCB** + reagent → **pink complex** (light pink at low concentrations and dark pink at high concentrations)

b) **Dioxin** + reagent → **white precipitate** (density of precipitate is proportional to concentration)

c) Absence of **PCB** or **Dioxin** → **no color (clear)**

2) Inorganic Heavy Metals (Inorganic compound + reagent → colored complex)

a) **Lead** + reagent → **Green complex** (light green color is indicative of a low concentration whereas dark green is indicative of a high concentration)

b) Absence of **Lead** → **Blue complex**

c) **Mercury** + reagent → **Medium to Dark blue complex** (darker the blue, the higher the concentration)

d) Absence of **Mercury** → **Red complex**

(c) Experimental Protocol 1 – Analysis of Sediment Pore-Water Samples

- Each sediment pore water sample is labeled as S1 to S8 and corresponds to the numbered locations on your site map (**Fig. 1**).

- Obtain a multi-well plate. Use one well for each sediment pore-water sample and perform all tests on the same plate.

- Transfer one (1) drop of the **sample** specially prepared for each chemical to the well labeled for that chemical test.

- Add one (1) drop of each **reagent** to the sample in the well. Be careful not to mix reagents and samples.

- Observe your results based on the information listed above (*(b) Tests for Organic and Inorganic Compounds in Samples*), and record your results in **Table 1**.

Table 1. Presence (low +, high++) or absence (-) of organic and inorganic contaminants in sediment samples (S1–S8).

	S1	S2	S3	S4	S5	S6	S7	S8
PCBs								
Dioxin								
Mercury								
Lead								

(d) <u>Experimental Protocol 2 – Analysis of Water Samples</u>

- Each water sample is labeled as W1 to W8 and corresponds to the numbered locations on your site map (same sampling sites as sediment pore-water samples).
- Obtain a multi-well plate. Use one well for each water sample and perform all tests on the same plate.
- <u>Transfer one (1) drop of the **sample** specially prepared for each chemical to the well labeled for that chemical test</u>.
- <u>Add one (1) drop of each **reagent** to the sample in the well. Be careful not to mix reagents and samples</u>.
- Observe your results based on the information listed above (*(b) Tests for Organic and Inorganic Compounds in Samples*), and record your results in **Table 2**.

Table 2. Presence (low +, high ++) or absence (-) of organic and inorganic contaminants in water samples (W1–W8).

	W1	W2	W3	W4	W5	W6	W7	W8
PCBs								
Dioxin								
Mercury								
Lead								

- ***Please Note*** All tests conducted in this study are simulations. The tests are simplifications of a complex system of analyses with which chemists actually detect various compounds. In reality, the actual analysis for specific chemical contaminants involves tedious extraction of the analyte and sophisticated instrumentation (e.g., GC-MS or LC-MS/MS) to detect substances often present in only trace concentrations.

6. Data Analysis and Interpretation

- Your samples may look as the results shown in **Fig. 2**.

- In order to conclude your observations and interpretation of the data obtained, please complete the questions detailed in **Concept Check#6**.

- As a class, we may discuss your answers to discuss the results and their implications.

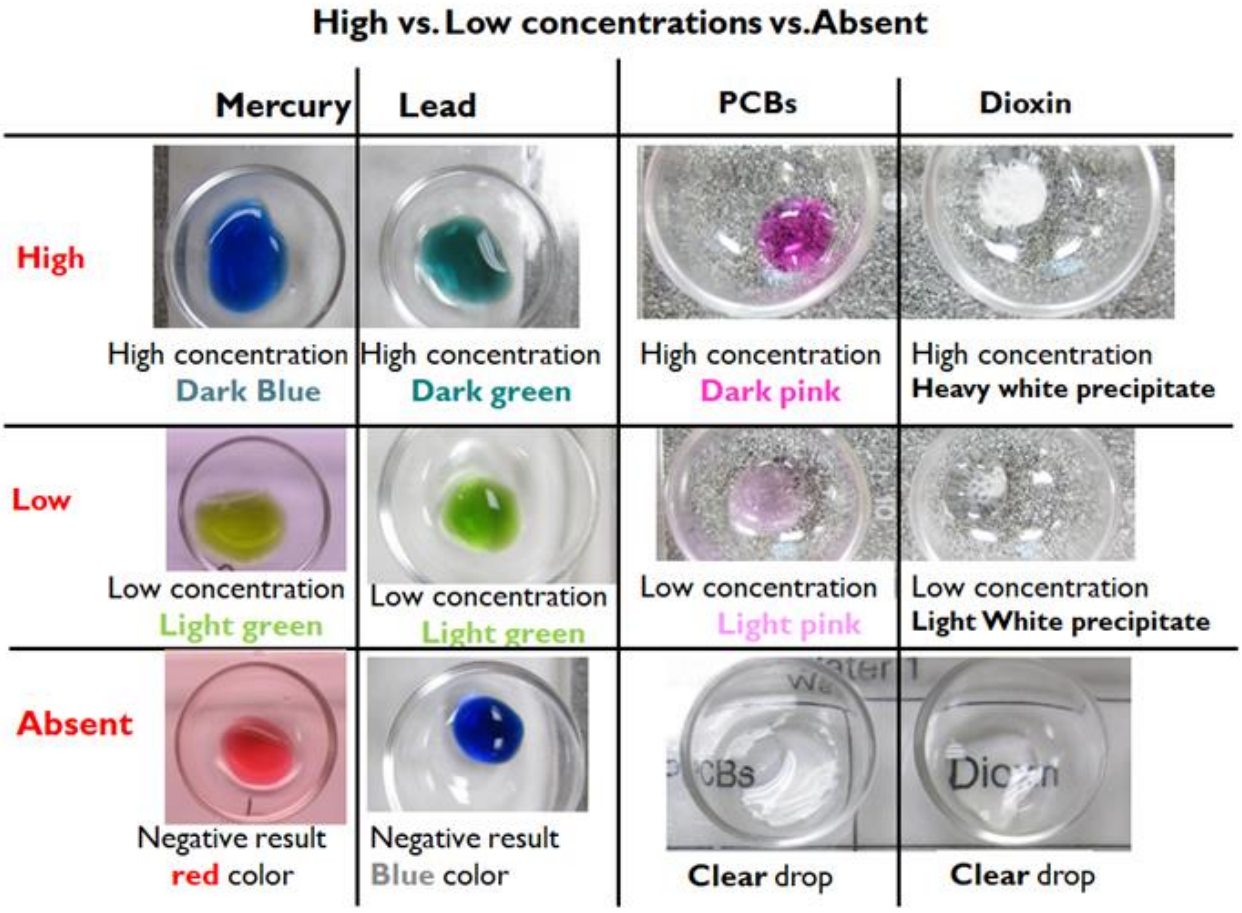


Fig. 2. Color rubric showing the expected results from the pollutant analysis of water and sediment pore-waters from various sites along Karankawa Creek.

Concept Check#1

Student Name (and Group#): **Date:**

1. **Which area of Karankawa Creek is the most polluted? (few words answer) (20 points)**

2. **What evidence do you have to support the conclusion stated above? (a few sentences) (20 points)**

3. **Is the Texas Pulp and Paper Company responsible for the pollution found in Karankawa Creek? What evidence do you have to support your answer? (a few sentences) (20 points)**

4. **What suggestions do you have for remedying the pollution problems? (a few sentences) (20 points)**

5. **Given the health crises at Indianola, what is your assessment of the relationship between wildlife and human health? (a few sentences) (20 points)**

Chapter 2. Unknown Contaminant Analysis using Mass Spectrometry: Liquid Chromatography and Tandem Mass Spectrometry

1. Objectives

After completing this exercise you should be able to:

- Describe the key working principles of liquid chromatography and tandem mass spectrometry (LC-MS/MS).
- Apply the key methods used for unknown compound detection, such as multiple reaction monitoring (MRM).
- Calculate unknown analyte concentration using an internal standard.

2. Introduction

Environmental pollution is a major concern for wildlife and human health and well-being. Such concern is warranted as various monitoring studies show ecosystems stressed from rapid population growth and increased demand for energy prospecting and consumption. Taken together, the confluence of such factors can contribute to environmental pollution and habitat loss. As a result, pollution monitoring is a key strategy towards mitigating adverse environmental impacts. A key tool used in such monitoring efforts includes liquid chromatography and tandem mass spectrometry or LC-MS/MS.

Mass spectrometers convert an analyte molecule of interest to a charged (or ionized) state. Significant developments in a novel form of ionization, the so called **electrospray ionization** (or **ESI**), has revolutionized the ability to ionize various molecules in a sample. The detection of such ions (and any associated molecular fragments) is done on the basis of their mass to charge (or $^{m}/_{z}$) ratios. LC-MS/MS works well with moderately polar compounds, and is highly suited for the analysis of metabolites, xenobiotics and peptides. Key components of the LC-MS/MS system are described below (adapted from Pitt 2009):

Analyte separation using Liquid Chromatography: chromatographic separation of a complex mixture of compounds in solution (which can include the analyte of interest) occurs by the differential partitioning of compounds between a stationary phase and a mobile phase (comprising the solvent) (**Fig. 1**). High Performance/Pressure Liquid Chromatography (HPLC) is ideal for the separation and analysis of relatively polar organic molecules, such as proteins, polymers, ionic compounds and pharmaceuticals. The most common type of HPLC used is reversed-phase HPLC, which allows analyte separation between a polar (usually water-containing) mobile phase and a non-polar stationary phase. The stationary phase comprises hydrophobic and silanophilic compounds (such as octadecyl carbon chain (C18) – bonded silica) packed into a metal cylinder

(or column). When samples are introduced onto the column, constituent compounds distribute between the stationary phase (which is packed into the column) and the mobile phase (solvent) passing through the column (**Fig. 2**). A sample compound (or analyte) is only carried forward when it is in the mobile phase. A sample component that spends most of its time in the stationary phase will have a low migration velocity and later elution time off the column. Whereas, a compound whose distribution favors the mobile phase, will be swept through the system more rapidly and have an early elution time off the column (**Fig. 1**).

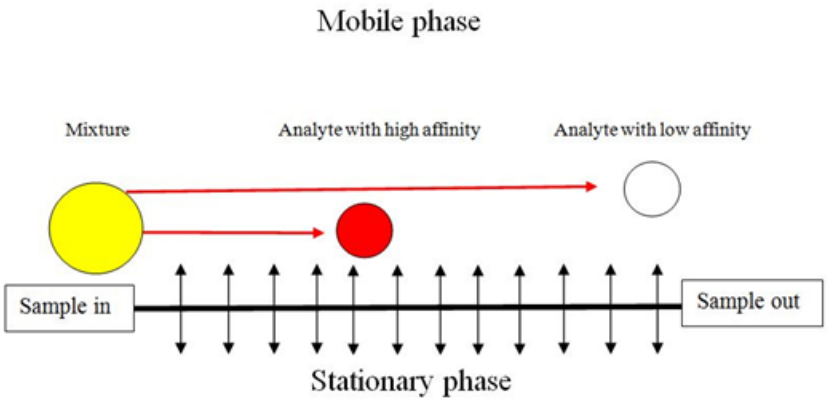


Fig. 1. Schematic showing how the differential portioning of various analyte molecules in a complex mixture, between the mobile phase of solvent and stationary phase of a chromatographic column, can lead to analyte separation. (Image from: http://archive.cnx.org/contents/1f082563-3aba-49cc-9841-5761af01187c@2/dynamic-headspace-gas-chromatography-analysis)

The result of chromatographic separation is that a mass spectrometer can detect various analytes as a function of time. Therefore each peak detected in a chromatogram will have a characteristic retention time and associated mass selectivity and accuracy (as determined by the mass spectrometer).

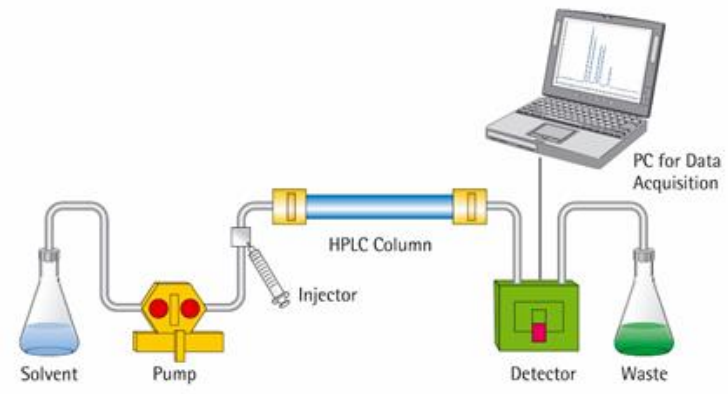


Fig. 2. Overview of an HPLC system showing the mobile phase (solvent) reservoir, column containing the stationary phase and subsequent detectors and recording instruments used to analyze specific analytes of interest.
(Image from: https://www.linkedin.com/pulse/what-do-you-know-hplc-crystal-wang)

Analyte ionization using Electrospray Ionization (ESI): Once analytes of interest are 'separated' in a column, they are introduced into the ionization source of a mass spectrometer through a metal capillary (the nebulizer needle) to form a fine spray of charged droplets (**Fig. 3**). These droplets are rapidly evaporated by applying heat and a 'curtain' of nitrogen gas. As the mobile phase used can be acidic (due to addition of a small proportion of formic acid or ammonium formate), residual electric charge in the mobile phase can be transferred to the analytes. This 'soft

ionization' allows the ionization of analytes that generally gain a single mass unit ($M+H^+$). The ionized analytes are then transferred into the high vacuum of the mass spectrometer via a series of small apertures and focusing voltages, which allow propulsion of ions into the mass spectrometer (**Fig. 3**).

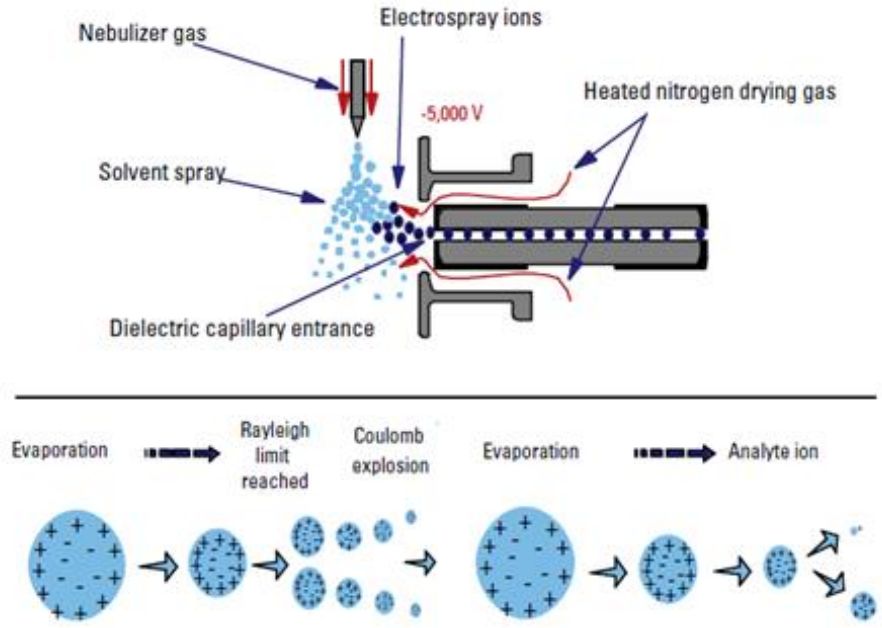


Fig. 3. Schematic overview of electrospray ionization and subsequent desolvation of acidic mobile phase into positively charged analytes that enter the mass spectrometer via application of various voltages to the mass spectrometer entrance.

Analyte detection using Quadrupole Mass Analyzers: The main selection of desired analyte mass to charge ($^m/_z$) ratios is achieved using quadrupole mass analyzers (**Fig. 4**).

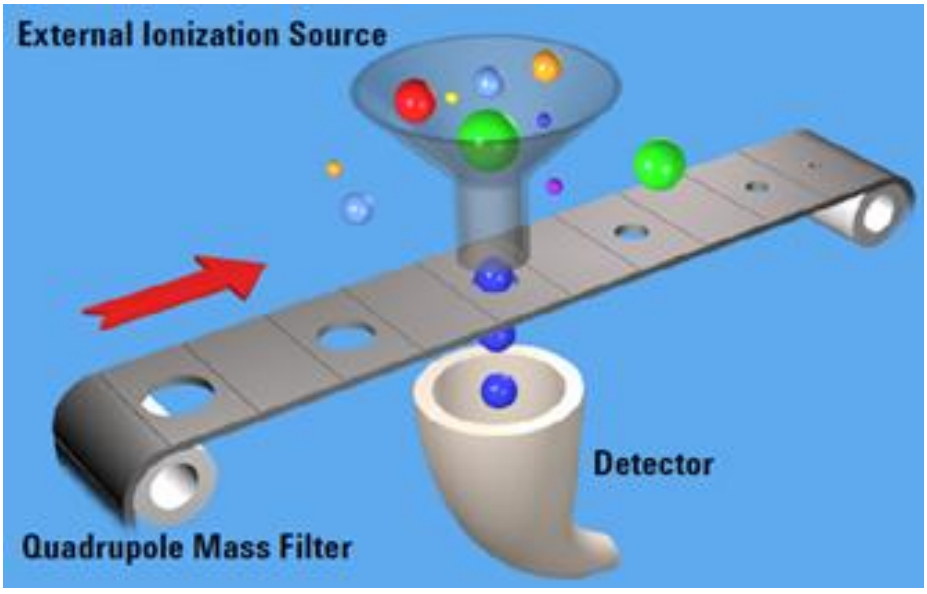


Fig. 4. A conceptual model of how a quadrupole mass analyzer can help select specific $^m/_z$ charged analyte ions. The balls of different colors and sizes represent different analyte ions that have different $^m/_z$ values. The quadrupole mass analyzer is represented as a moving belt that filters various ions as they pass through to the detector.

The quadrupole mass analyzer comprises a set of four parallel metal rods to which constant and varying radio frequency (RF) voltages are applied. These voltages allow selective transmission of specific $^{m}/_{z}$ ions along the axis of the rods. By varying voltages it is possible to scan across a range of $^{m}/_{z}$ values, resulting in a mass spectrum (**Fig. 5**).

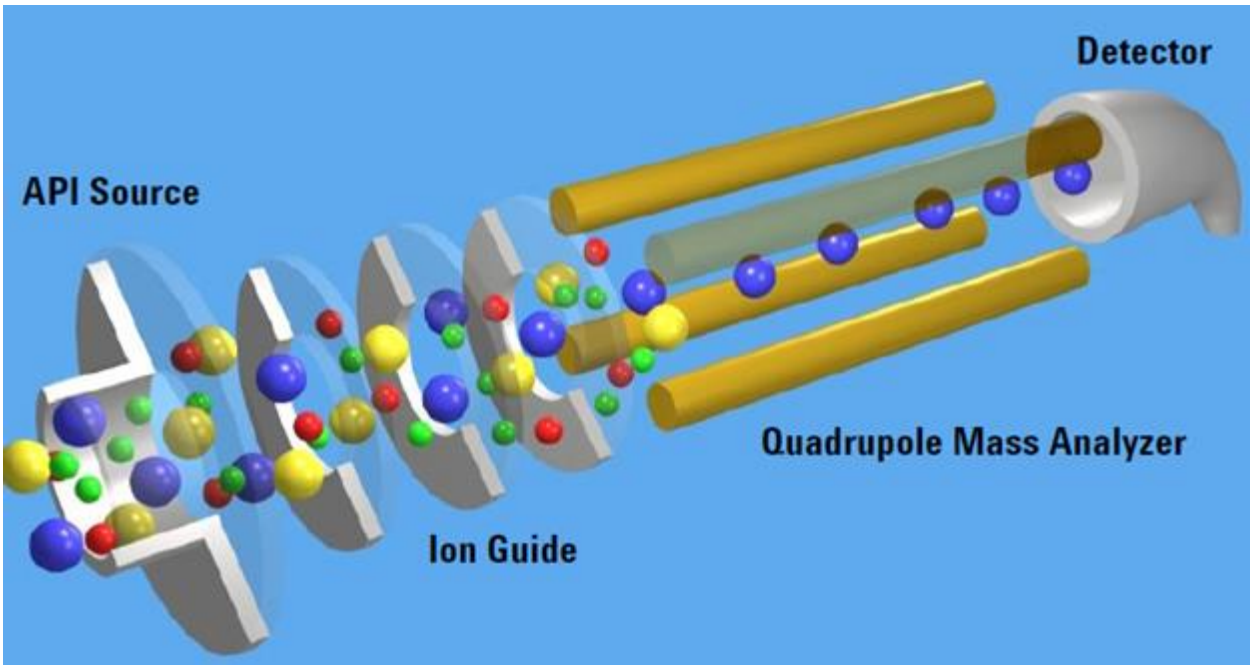


Fig. 5. Schematic showing the overall path of analyte ions across a quadrupole mass analyzer. Various ion optics are used to guide and accelerate ions into to the quadrupole mass analyzer, which in turn only allows ions with a particular $^{m}/_{z}$ (represented by blue balls) to pass through to the detector.

If we scan for masses across a wide range, we are able to generate a **total ion chromatogram** (**TIC**), which can span from a low mass to a high mass range (such as 50 to 500 $^{m}/_{z}$). In addition to scanning, the quadrupoles can also set to monitor specific $^{m}/_{z}$ values, using a method called **selective ion monitoring** (**SIM**). SIM is highly effective at precisely monitoring selected ionized analytes. However, a final (and preferred) method for analyte detection using quadrupole mass analyzers involves fragmenting analyte ions by a process called **collision induced dissociation** (**CID**) (**Fig. 6**). Collision of analyte ions with inert gas, such as nitrogen or argon, enables ion fragmentation to produce characteristic 'daughter' ions.

A useful mass spectrometric configuration is to place the collision cell (or second quadrupole) between the first and third quadrupoles. This combination allows for triple quadrupole mass spectrometry or tandem mass spectrometry in which two or more stages of mass analysis can be used independently (**Fig. 6**). The first and third quadrupoles can be simultaneously controlled to allow different parent (precursor) to daughter (product) $^{m}/_{z}$ masses to be created and monitored. This process of precursor>product ion monitoring is called **multiple reaction monitoring** (**MRM**). As most precursor ions fragment to yield characteristic product ions, the most abundant of these can be used as a 'fingerprint' to confirm the identity of the parent compound with great certainty. Therefore, the advantage of triple quadrupole mass spectrometry is the increased

specificity that it allows for compound identification and detection, enabling widespread applications in the biomedical and environmental sciences.

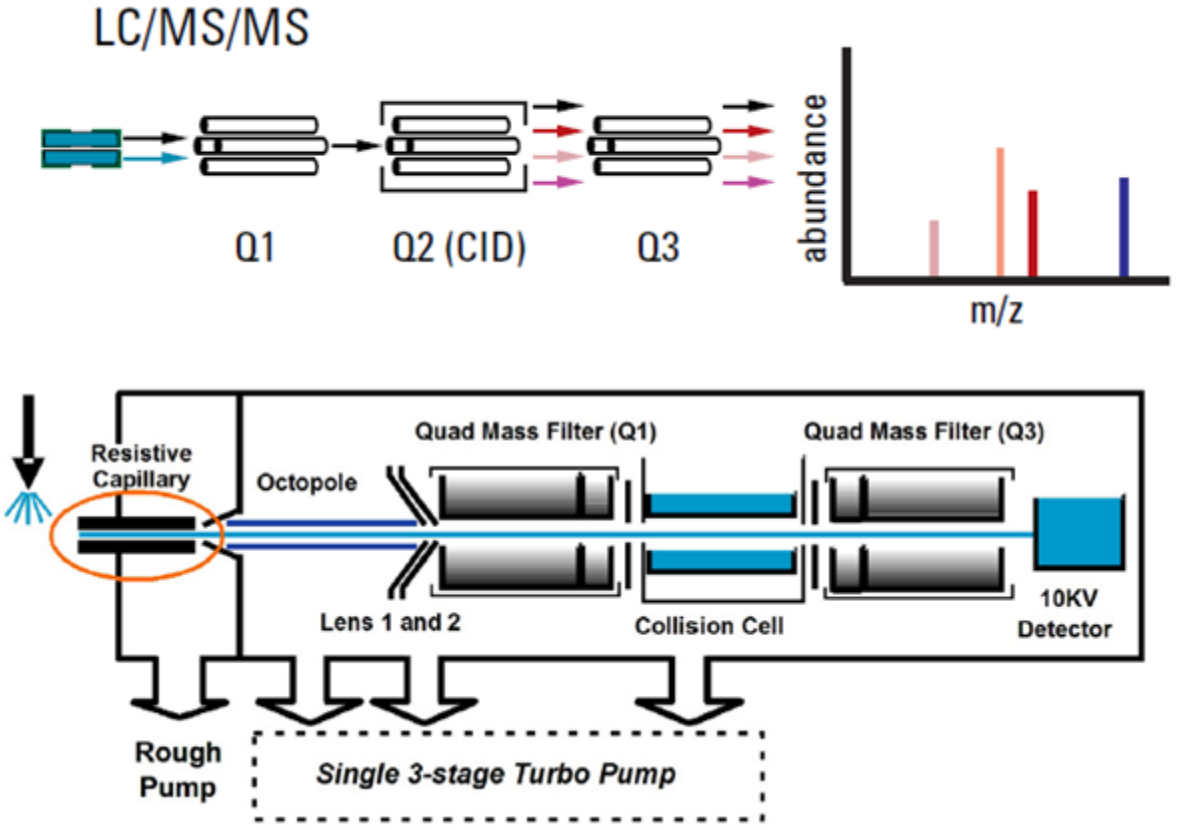


Fig. 6. Overall schematic of the triple quadrupole mass spectrometer used in this lab. These images show sequential assembly of the three quadrupoles that allow for precursor ion fragmentation to product ions.

Finally, while mass spectrometry is highly efficient at identifying analytes, their quantification requires the use of stable isotope versions of the analyte of interest or some other representative compound. The stable isotope is called an **internal standard** and tend to have identical chemical properties as the analyte(s) of interest. We use an internal standard as it is not accurate to quantify analyte concentrations based off of absolute mass spectrometer responses only. This is due to the fact that variations in sample extraction efficiency and sample ionization can cause unreliable measurements. The use of an internal standard corrects for such variation as it enables quantifications based on relative ratios of known internal standard concentration and accompanying area under curve, to the area under curve of the unknown analyte whose concentration we are trying to determine.

Therefore, a technique called **stable isotope dilution** can be used to accurately quantify unknown analytes if the concentration of internal standard (or the stable isotope) is known. The method relies on quantifying a response ratio (using area under chromatographic curve) of the internal standard:unknown analyte in order to quantify an equivalent concentration of analyte (**Fig. 7**).

While this method is overall quite acceptable, more accurate results can be obtained by plotting analyte:internal standard response ratio vs. analyte concentration (Pitt 2009).

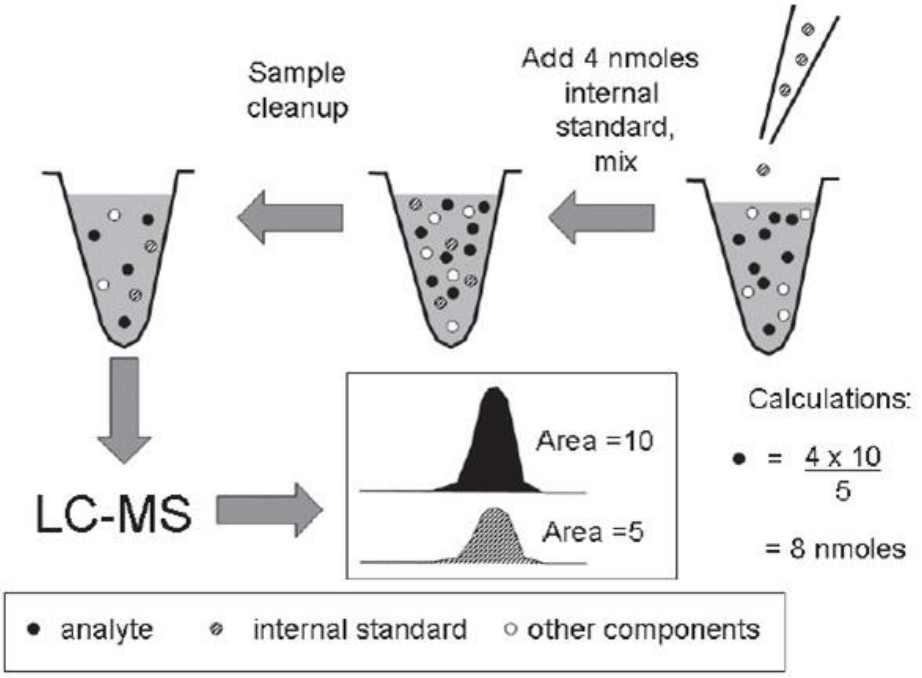


Fig. 7. Schematic showing the principle of stable isotope dilution whereby a known amount of internal standard is added to a sample containing an unknown analyte. The response ratios of area under chromatographic curves for the internal standard relative to the unknown analyte (1:2 in this case) can be used to calculate the concentration of the analyte (Image from Pitt, 2009).

3. Summary of Lab Experiment

In this lab each group will use mass spectrometry to identify the particular analyte extracted using SPE in the previous week. In doing so, each team member will gain knowledge of how to run various mass spectrometric analyses to identify an unknown compound and also use the stable isotope dilution method to quantify the unknown analyte. The analysis work flow is as follows:

- Inject your unknown sample onto the LC-MS/MS and use the mass spectrometer in MRM (multiple reaction monitoring) mode to acquire a total ion chromatography (TIC). Confirm and identify the specific analyte in your sample.

- Quantify the analyte in your sample using the stable isotope dilution method.

4. Materials

- Processed sample in solvent (already prepared by TA).
- Mobile Phase (already prepared for you):
  - Solvent A = MilliQ Water + 5 mM Ammonium Formate
  - Solvent B = Methanol + 5 mM Ammonium Formate

- Agilent Poroshell EC-C18 column (3x50 mm, 2.7 µm) (provided).
- Agilent 6420 LC-MS/MS (OCSB Bldg.#3029, Room# 308).
- **Please see Appendix 1.6, 1.7, 1.8, 1.9 and 2.0 for safety information.**

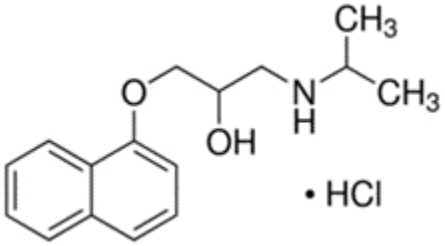
5. Experimental Procedure

(a) Analytes Assigned to the Lab

- Each group has to identify and quantify the concentration of a single unknown analyte that is present in their sample.

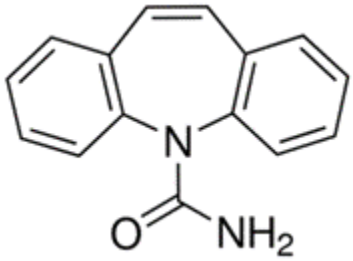
- Overall, one of the following analytes was assigned to each group (your task is to find which one is in your sample).

1) Propranolol ($C_{16}H_{21}NO_2$, Molecular Mass: 259.34 gram/mol)



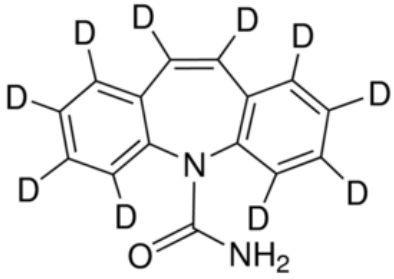
Cardiac β-blocker used for anti-anxiety and high blood pressure treatment. (Image from: Sigma-Aldrich)

2) Carbamazepine ($C_{15}H_{12}N_2O$, Molecular Mass: 236.27 gram/mol)



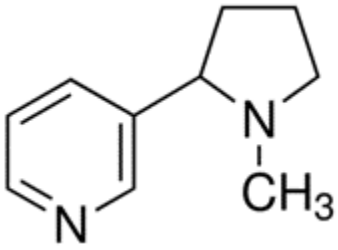
Anti-epileptic/anti-convulsant medication. (Image from: Sigma-Aldrich)

3) Carbamazepine-d10 ($C_{15}D_{10}H_2N_2O$, Molecular Mass: 246.33 gram/mol)



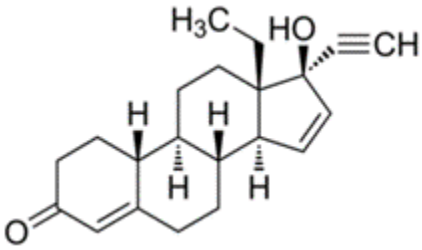
Internal standard to carbamazepine. This analyte will be used as a representative internal standard for all other analytes during this practical (Image from: Sigma-Aldrich)

4) Nicotine ($C_{10}H_{14}N_2$, Molecular Mass: 162.23 gram/mol)



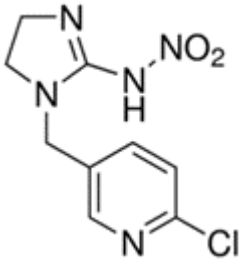
Alkaloid parasympathetic neuron stimulant. (Image from: Sigma-Aldrich)

5) Gestodene ($C_{21}H_{26}O_2$, Molecular Mass: 310.43 gram/mol)



Synthetic progestin used in combination with estrogens in hormonal contraceptives. (Image from Sigma-Aldrich)

6) Imidacloprid ($C_9H_{10}ClN_5O_2$, Molecular Mass: 255.66 gram/mol)



Insecticide or insect neurotoxin. (Image from Sigma-Aldrich)

- As you do not know which <u>one</u> of these compounds is in your 'unknown' sample, your Group will first have to detect the analyte mass spectra that is present in your sample (described below).

(b) <u>Multiple Reaction Monitoring (MRM) of Analyte Precursor>Product Ions</u>

- Inject your unknown sample onto the LC-MS/MS and monitor for specific precursor>product ion transitions for each compound. As described before, MRM allows greater accuracy for analyte detection as each compound exhibits a unique fragmentation pattern, enabling the 'fingerprinting' of each compound.

- Previous analysis has identified product ions for each analyte (**Table 1**). These precursor>product ion mass fragments will be used to confirm the identity of the analyte in your sample.

Table 1. Precursor>product mass fragments for each analyte in unknown samples. The identification of a specific fragmentation spectra in your unknown sample will confirm the identity and presence of a specific analyte.

Compound	MRM Precursor>product $^{m}/_{z}$
Propranolol	260.2>74.3, 116.1, 183
Carbamazepine	237.1>165.0, 179.0, 194.0
Carbamazepine-d10	247.2>204.1
Nicotine	163.1>130.1, 84.2
Gestodene	311.2>81.2, 91.2, 109.1
Imidacloprid	256.1>84.2, 175.0, 209.0

- An example MRM spectra for propranolol showing characteristic precursor>product ion fragments can be seen in **Fig. 8**.

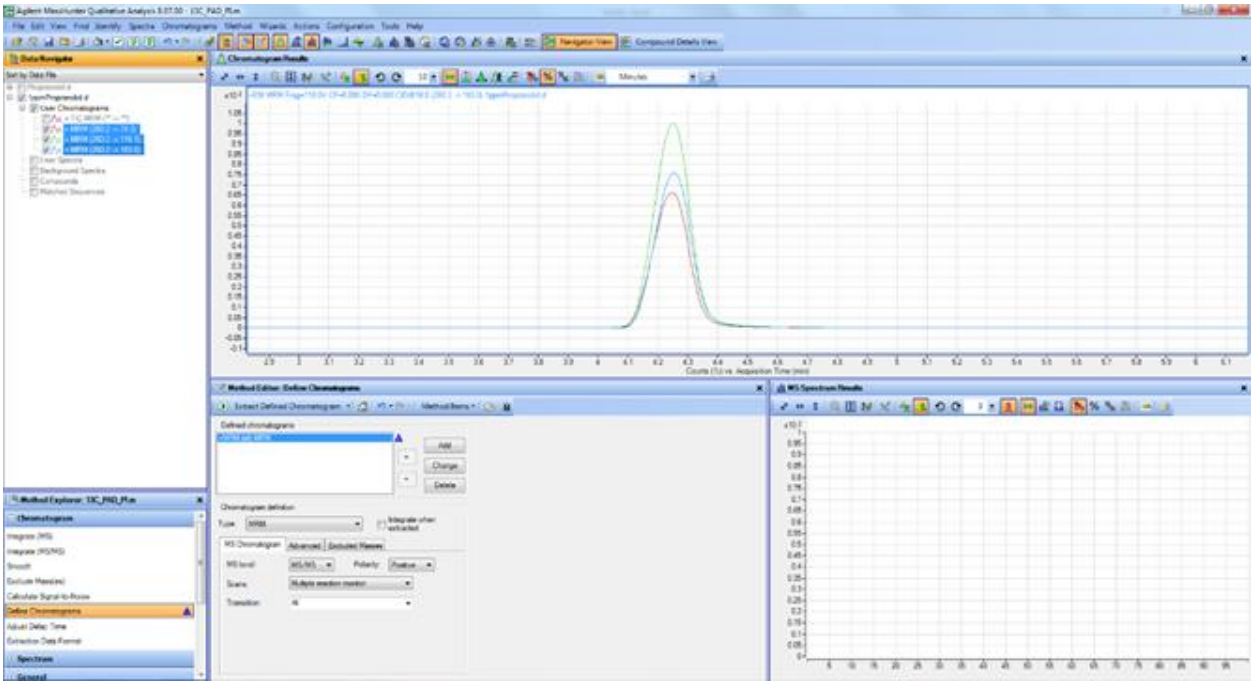


Fig. 8. Qualitative analysis screen shot showing propranolol's MRM ions.

(c) Data Analysis and Interpretation

- The TA will help with analysis of your respective samples.

- Integrate the area under the curve for the MRM ions yielding the highest response for the now identified analyte and internal standard (carbamazepine-d10, which should be in all samples).

- Use the stable isotope dilution calculation as shown below to calculate the concentration of the analyte in your sample:

$$\frac{[I.S.concentration]}{I.S.area\ under\ curve} = \frac{[Analyte\ concentration]}{Analyte\ area\ under\ curve}$$

$$[Analyte\ concentration] = \frac{[I.S.concentraion] * Analyte\ area\ under\ curve}{I.S.area\ under\ curve}$$

- please calculate and enter the identity of the analyte and its concentration below:

ANALYTE AND CONCENTRATION: _____ .

Concept Check#2

Student Name (and Group#): **Date:**

1. **What are the three key steps involved with performing a Solid-Phase Extraction? (few words answer) (20 points)**

2. **How does an analyte molecule gain a positive charge (and proton, increasing its mass by 1 atomic mass unit) during electrospray ionization? (a few sentences) (20 points)**

3. **How does MRM allow the 'fingerprinting' of an analyte molecule? (a few sentences) (20 points)**

4. **What was the 'Collision Energy' (or CE) setting used to identify the MRM spectra of the analyte assigned to your group? (one to two words) (20 points)**

5. **Why do we need to use an internal standard for analyte quantification? (a few sentences) (20 points)**

Chapter 3. Computational Ecology Lab: The Sheep-Wolf Model

1. Objectives

After completing this lab you should be able to:

- Explain how predator-prey interactions result in ecosystem stability.
- Carry out simulations to identify which variables are essential for ecosystem stability.
- Interpret changes in predator-prey population trends in order to describe long-term ecosystem behavior.

2. Introduction

The natural world is inherently complex. This complexity is manifest from the interdependent interactions of organisms that inhabit any given environment. Taken together this view leads us to the definition of an **ecosystem**, which encompasses biological systems (or organisms) and their environment.

In order to study ecosystem functions, we have to understand how energy and matter 'flow' amongst the organisms comprising an ecosystem. This flow is represented as a **food chain** in which, producers (plants) absorb sunlight and use its energy (along with carbon dioxide and water) to synthesize organic materials or food for primary consumers, such as herbivores (sheep) (**Fig. 1**). In turn, secondary consumers such as carnivores (wolves) feed on herbivores, acquiring the energy assimilated by herbivores. For each organism in a food chain, the acquired energy is used for growth, renewal and reproduction. Finally, decomposers (bacteria, fungi) liberate the energy assimilated by producers, herbivores and carnivores upon their death (**Fig. 1**). Basically a food chain (or more complex food web) represents the feeding interactions in an ecosystem.

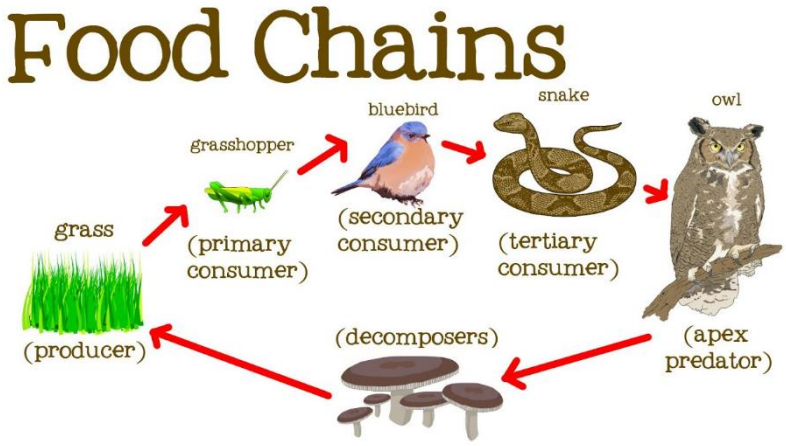


Fig. 1. Schematic summarizing an idealized food chain. The arrows indicate feeding interactions (i.e. flow of energy and matter) amongst organisms in the ecosystem. (Image from: https://www.youtube.com/watch?v=hLq2datPo5M).

3. Summary of Lab Experiment

In this lab, you will use a computer game-based program called NetLogo. This program provides us with an animated world (or ecosystem) in which we can explore how a food chain functions. We will also understand how the food chain can be impacted if certain biological components stop functioning properly.

You will perform the following simulations:

(a) To evaluate a simple relationship between primary producer (grass) and primary consumer (sheep).
(b) To evaluate a more complicated relationship between primary producer (grass), primary consumer (sheep) and secondary consumers (wolves).

4. Materials

- Laptop computers with NetLogo installed (version 6.0.3).
- Grass-Sheep-Wolf NetLogo program installed on the desktop.
- Pen and notepad to record any observations (optional).

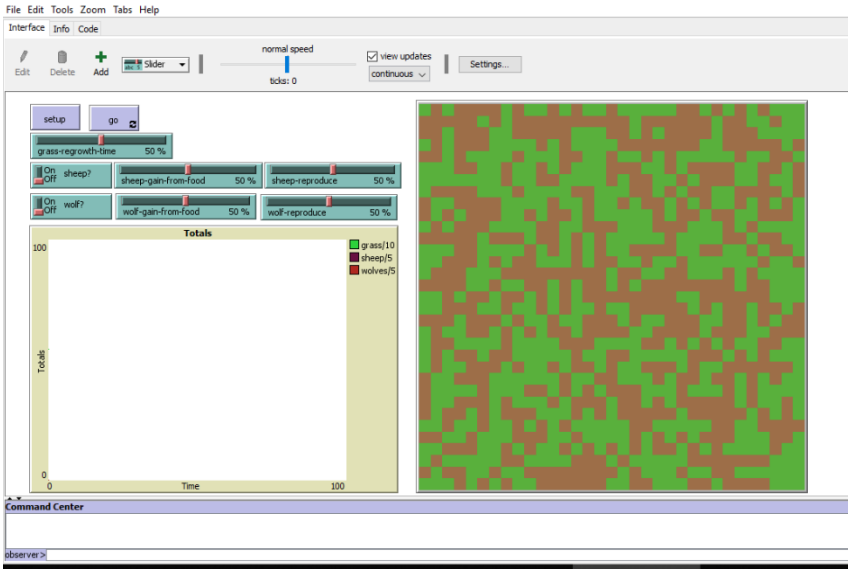
5. Experimental Procedure

(a) Launch the Grass-Sheep-Wolf NetLogo program

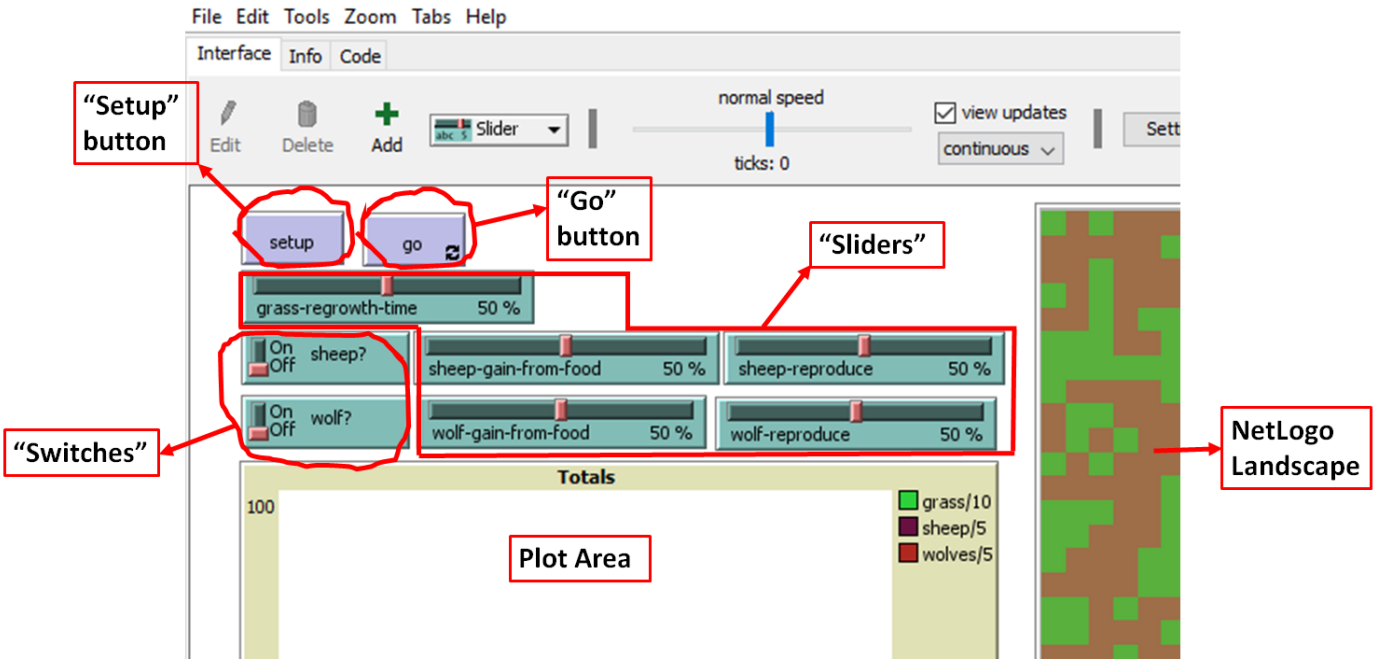
- Please work in teams.
- Double-click and launch (or open) the Grass-Sheep-Wolf program that is on your desktop.

(b) Some orientation of the NetLogo game environment

- When you launch the game you should see something that looks as follows:



- Key features of this game environment are as follows:



- The key controllers are:
  - Setup button = initializes the game environment to its initial start settings.
  - Go button = advances the model through time increments (or ticks). To stop the game you will need to press the "go" button again.
  - Switches = allow us to turn-on or turn-off the presence of 'agents' or model variables that interact in the game environment.
  - Sliders = enables the user to change the magnitude of certain model variables. These variables control the behaviors of agents in the game environment.
  - Plot Area = displays model outputs by tracking changes in model variables during the run-time of a game.
  - NetLogo Landscape = model simulations (of interacting agents and their environment) occur in this view once the "go" button is activated.

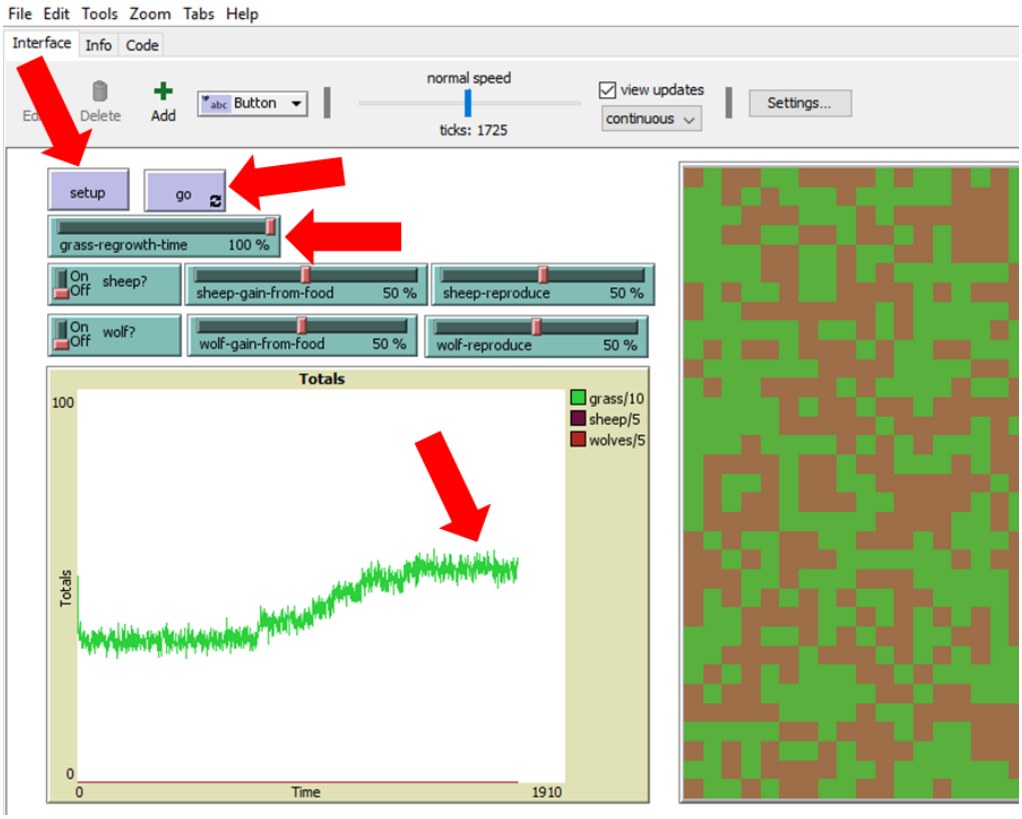
(c) <u>Running game simulations</u>

- Now that we have some orientation of the game environment, let's start playing with the Gass-Sheep-Wolves program.
- The best way to approach this is to incrementally simulate various aspects of the game and then to run all variables in one cohesive 'ecosystem' simulation.

(1) Simulate primary producers (grass)

- The organisms in a food chain can be classified according to their position or trophic (or 'feeding') level. Primary producers comprise the base of the grazer food chain and belong to the first trophic level.
- The productivity of primary producers is dependent on various abiotic factors, such as amount of sunlight received, water availability and nutrient supply of the soil.
- In this game we will represent the culmination of these factors by the model slider titled: *grass-regrowth-time*

<u>Exercise 1:</u> With Sheep and Wolf variables switched to 'off', assess the effects of changing *grass-regrowth-time* on the amount of grass growth in the model. Please record your observations below:

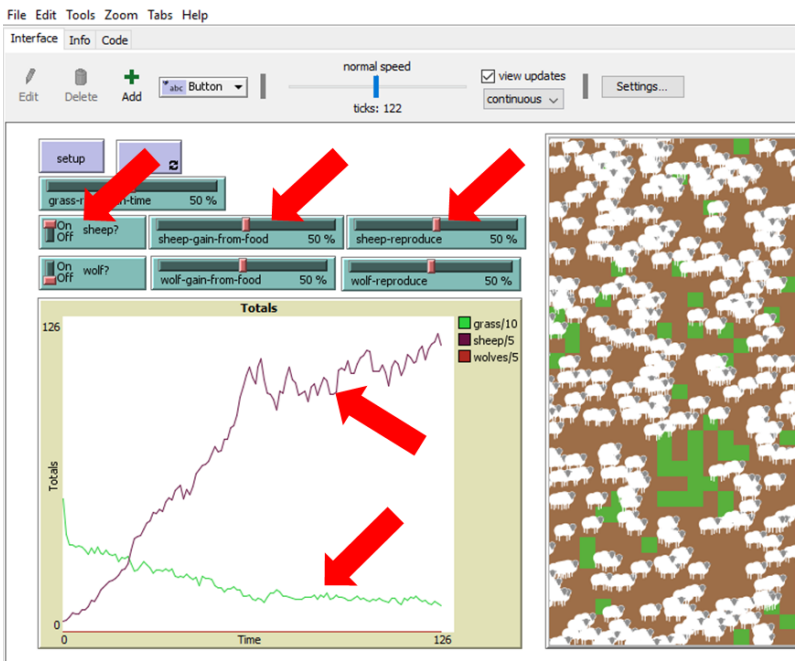


(**Hints** Click on 'setup' and then 'go', and watch the grass plot over time in the 'Totals' plot area. As the game is running, change the *grass-regrowth-time* slider up or down.)

(2) Simulate primary producers (grass) – primary consumers (sheep)

- Grazers (such as sheep) are part of the second trophic level. Plants absorb sunlight and convert it to organic materials or biomass. The biomass of the first trophic level is the raw material for the second trophic level.
- In order to simulate this first link in our food chain, we will simulate sheep grazing on grass.

<u>Exercise 2:</u> With the Sheep variable switched to 'on', assess the effects of changing *grass-regrowth-time, sheep-gain-from-food* (i.e. how much energy sheep get from eating grass) and *sheep-reproduce* (i.e. how often the sheep produce offspring). Record your observations on changes in grass growth and the numbers of sheep in the model. (To try: changes in which variable can cause the extinction of sheep?)

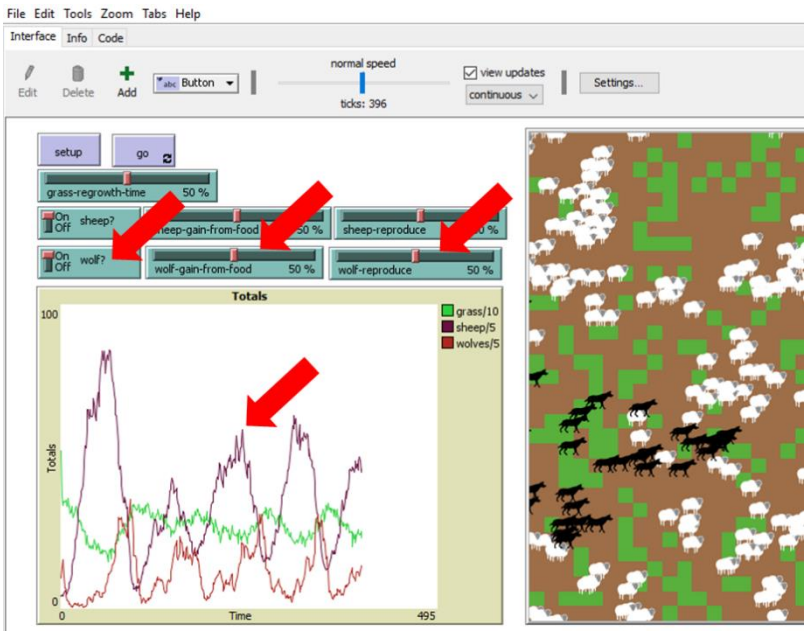


(**Hints** It may be best to start the initial simulation by having all slider settings at '50'. Turn the sheep? button to 'on', and then click on 'setup' and then 'go' to start the simulation. As the simulation runs, keep watch of the grass and sheep plots over time in the 'Totals' plot area. As the game is running, change the *grass-regrowth-time*, *sheep-gain-from-food* and *sheep-reproduce* sliders up or down.)

(3) Simulate the entire food chain, primary producers (grass) – primary consumers (sheep) – secondary consumers (wolves)

- We finally complete our food chain with carnivores. Carnivores (or secondary consumers) feed on grazers and comprise the third trophic level.
- Real-world trophic interactions are much more complex, and more like food 'webs' (than chains). However, our simple example of a food chain allows us to study trophic interactions, and appreciate how the organisms comprising an ecosystem form an interdependent community of life.
- We will now include the final link in our food chain, the secondary consumers (wolves), and simulate the entire grass-sheep-wolves ecosystem.

<u>Exercise 3:</u> With the Wolves variable switched to 'on', assess the effects of changing various sliders on the stability of the ecosystem. Record your observations on changes in grass growth, numbers of sheep and wolves in the model.



(**Hints** Start the initial simulation by having all slider settings at '50'. Turn the sheep? and wolves? buttons to 'on', and then click on 'setup' and then 'go' to start the simulation. As the simulation runs, keep watch of the grass, sheep and wolves plots over time in the 'Totals' plot area. As the game is running, change the various sliders up or down to see how your ecosystem behaves.)

Chapter 4. TAMUG Waste Water Treatment Plant (WWTP) Tour

1. Objectives

After completing this field trip you should be able:

- Demonstrate a basic understanding of the various treatment processes of a WWTP.
- Explain the important role of WWTPs in mitigating environmental pollution.

2. Introduction

Waste water treatment plants (WWTPs) constitute a 'barrier' to the output of anthropogenic pollution into the environment. A vast network of sewers across cities and towns collect wastewater from homes, businesses and industries, and deliver it to WWTPs for treatment prior to discharge into the environment. As a result, WWTPs constitute a key system mitigating the export of various domestic and industrial pollutants into the environment. Overall, most WWTPs constitute two basic treatment processes, *primary* and *secondary*, which are described below (from EPA 833-F-98-002).

Primary Treatment: Raw sewage entering the WWTP flows through a screen to remove large floating objects (sticks, rags etc.) that might clog pipes or pumps in the works. Sewage can be also be passed through a grit chamber that allows sand or small stones to settle. Subsequently, sewage flows into a *sedimentation tank* in which the speed of flow is greatly reduced, allowing minute particles (suspended solids) to settle out (**Fig. 1**). The settled particles are rich in organics and form a biosolid sludge. This sludge can be used to irrigate bacterial biomasses in the WWTP (in the form of returned or activated sludge) or thickened (i.e. allowed to dry out) and either used as fertilizer or disposed in a land fill or incinerated.

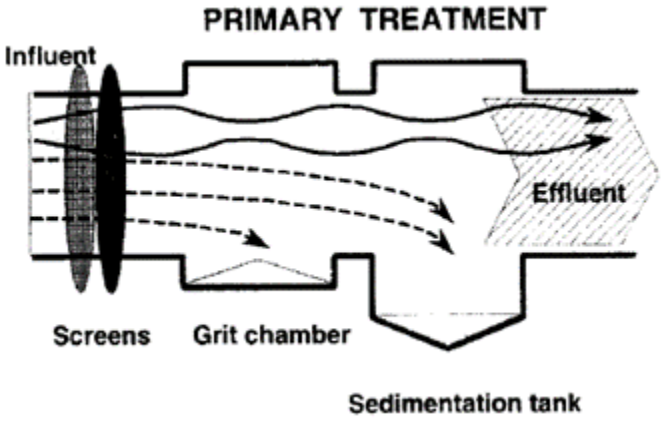


Fig. 1. Schematic showing the sedimentation processes utilized during primary treatment in a WWTP (Image from: EPA 833-F-98-002).

Secondary Treatment: This typically constitutes the second stage of treatment and encourages biological (bacteria/protist) degradation of ~85% organic matter in sewage. The key techniques used include *trickling filters* and *activated sludge process*. Trickling filters are simply a bed of stones or conglomerates of plastic substrates (3-6 feet deep) through which sewage passes. The stone or plastic matrix increases the surface area for bacterial colonization, promoting breakdown of organic matter. More recently, there has been a preference for the use of activated sludge processes. Activated sludge encourages bacterial proliferation by purging air through a proportion of sludge and returned waters (such as from primary sedimentation) (**Fig. 2**). The effluent from activated sludge flows to an additional sedimentation tank to allow particulates to settle. The resultant effluent can be treated with chlorine to kill pathogenic bacteria and reduce odor. Additional mechanisms for disinfection can include UV sterilization or ozonation prior to release of final effluent to the environment.

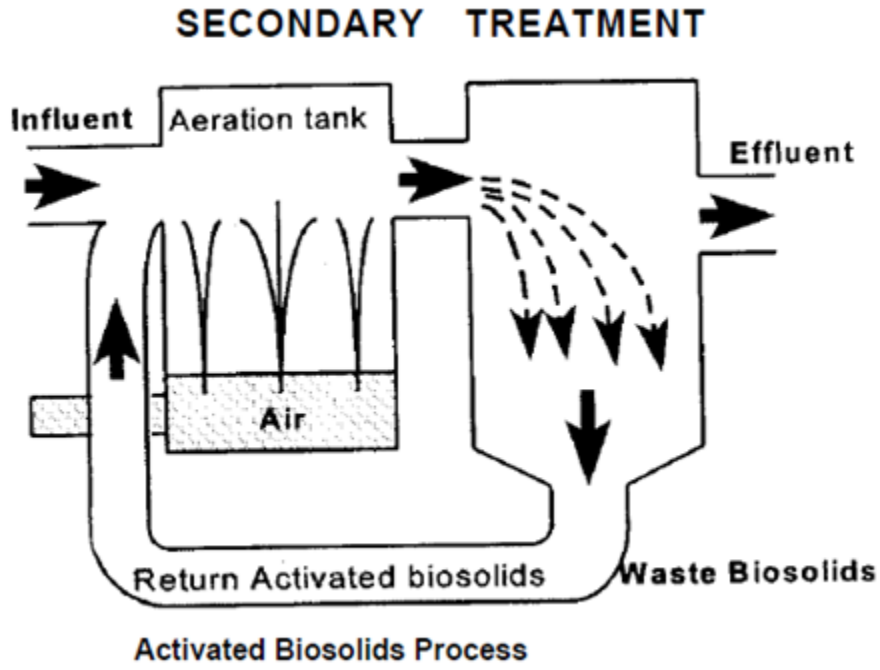


Fig. 2. Schematic of an activated sludge process showing mixing of settled (or screened) influent with returned activated sludge (or RAS) from primary settlement, and aerated to create activated sludge, rich in microorganism communities that can enhance breakdown of organic materials (Image from: EPA 833-F-98-002).

1. Summary of Field Trip

The TAMUG WWTP has architecture similar to the description above, i.e. it has a primary and secondary treatment processes (including UV sterilization of final effluent). The overall structure of the works is shown in **Fig. 3**. The goal of this field trip is to demonstrate these processes to you and to show how the domestic wastes generated by our small campus of ~2500 students/staff and faculty is treated prior to discharge into Galveston Bay. It is encouraged that as you tour, to take notes on the various processes and their functions.

2. Materials

- Please ensure that on the day of the field trip that you wear:
    - Appropriate clothing to protect exposed skin from cuts, scrapes or insect bites.
    - Closed-toed shoes/trainers.

3. Field Trip Procedure

- A schematic summary of the WWTP is shown in **Fig. 3**. Please use this schematic as a guide when on-site and annotate any notes as appropriate.

- The lab TA and instructor will help to coordinate the tours and provide commentary on each treatment process. Overall, each Group will tour seven sites, some of which include WWTP treatment processes.

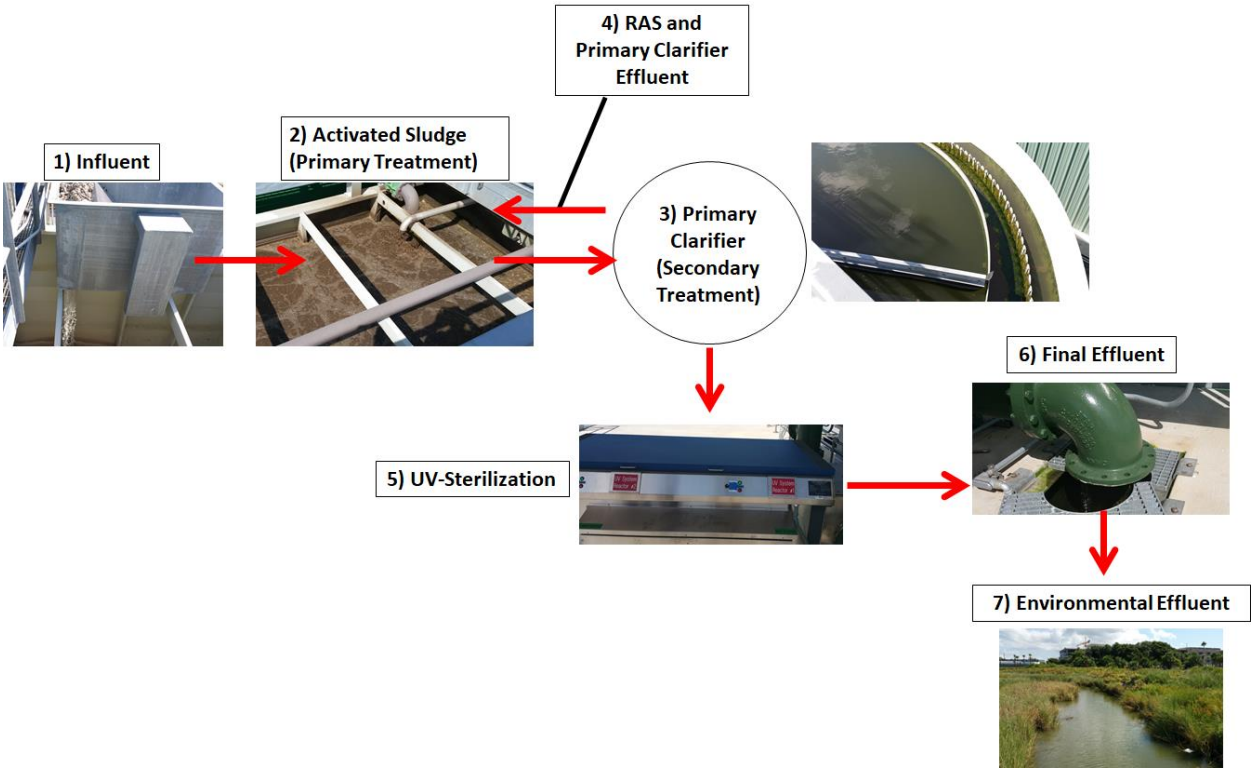


Fig. 3. Schematic of TAMUG WWTP showing the seven sites that will be visited during the field trip. Most sites correspond with WWTP treatment processes that include primary and secondary treatment processes.

- Please make notes as needed below:

1) Influent:

2) Activated Sludge:

3) **Primary Clarifier:**

4) **Return Activated Sludge (RAS) and Primary Clarifier Effluent:**

5) **UV Sterilization:**

6) **Final Effluent:**

7) **Environmental Effluent:**

- Please see below for map of TAMUG campus and location of the WWTP (opposite to Bldg#3104, dashed circle).

Concept Check#3

Student Name (and Group#): **Date:**

1. **What is the key purpose of a waste water treatment plant (WWTP)? (few words answer) (20 points)**

2. **Which treatment process is responsible for the greatest removal of organic material (including likely pollutants)? (few words) (20 points)**

3. **How many treatment processes comprise the TAMUG WWTP? (few words) (20 points)**

4. **What is the key purpose of the Primary Clarifier (Secondary Treatment) in the TAMUG WWTP? (a few sentences) (20 points)**

5. **What is the environmental fate of the Final Effluent released from the TAMUG WWTP? (a few sentences) (20 points)**

APPENDIX III

(Expenditures)

SRS Budget Worksheet

SRS Proposal #:	**2103841**
Project Dates:	**06/01/2021 - 8/31/2024**
Sponsor:	**Matagorda Bay Mitigation Trust**

Cumulative Budget Request

Category						
A. Sr Personnel					**Year 1**	**First Interim Report**
Name	**Project Role**					**Expenditures (6/1/21 - 8/31/21)**
David Hala	Principal Investigator	Person Months			1.00	
(MARB)		Salary	$		8,221	
		Fringe	$		1,521	
		Insurance	$		771	
		Total Fringe	$		2,292	
Antonietta Quigg	Co-PI	Person Months			0.12	
(MARB)		Salary	$		2,006	
		Fringe	$		371	
		Insurance	$		93	
		Total Fringe	$		464	
David Wells	Co-PI	Person Months			0.50	
(MARB)		Salary	$		6,362	
		Fringe	$		1,177	
		Insurance	$		386	
		Total Fringe	$		1,563	
Karl Kaiser	Co-PI	Person Months			0.50	
(MARS)		Salary	$		6,369	
		Fringe	$		1,178	
		Insurance	$		386	
		Total Fringe	$		1,564	
Lene Petersen	Co-PI	Person Months			0.50	
(MARB)		Salary	$		3,422	
		Fringe	$		633	
		Insurance	$		386	
		Total Fringe	$		1,019	
Subtotal Salaries Senior Personnel				$	26,380	
Subtotal Benefits Senior Personnel				$	6,902	
Subtotal Senior Personnel				$	33,282	
B. Other Personnel						
Name	**Project Role**				**Year 1**	
Marcus Wharton	Graduate Student	Person Months			6.00	
(Kaiser)		# of Persons			1	
		Salary	$		23,400	
		Fringe	$		2,574	
		Insurance	$		3,348	
		Total Fringe	$		5,922	
Asif Mortuza	Graduate Student	Person Months			6.00	
(Hala)		# of Persons			1	
		Salary	$		23,400	
		Fringe	$		2,574	
		Insurance	$		3,348	
		Total Fringe	$		5,922	
To Be Named	Graduate Student	Person Months			-	
(Wells)		# of Persons			1	
		Salary	$		-	

	Fringe	$	-
	Insurance	$	-
	Total Fringe	$	-
	Total Graduate Student Salary	$	46,800
	Total Graduate Student Fringe	$	11,844
Subtotal Salaries Other Personnel		$	46,800
Subtotal Benefits Other Personnel		$	11,844
Subtotal Other Personnel		$	58,644
Total Salaries		**$**	**73,180**
Total Benefits		**$**	**18,746**
	Total Personnel Costs	**$**	**91,926**

DIRECT COSTS

Travel: Domestic

Trip Information		**# Trips/Yr**	0	
		# Persons/Trip	0	
City & Purpose:		**# Days Per Diem/Trip**	0	
		# Days Lodging/Trip	0	
	Item			
TBD: Conference travel to disseminate project results	Per diem		$ -	
	Lodging		$ -	
	Airfare		$ -	
	Rental Car		$ -	
	Mileage		$ -	
	Total Trip		*$* -	
	Total Domestic Travel		**$** -	

Materials & Supplies

	Research consumables	$	10,600	**$2,919.20**
	Supplies for Educational Outreach	$	500	
	Total Supplies	**$**	**11,100**	

Publication Costs/Documentation/Dissemenation

	$	1,000	
Total Publications	**$**	**1,000**	

Other Costs

	Ship Time	$	5,000	**$700.00**
	Total Other Costs	**$**	**5,000**	

Capital Equipment: $5,000 or more

	Pyrolyzer	$	15,000	**$14,898.00**
	Total Capital Equipment	**$**	**15,000**	

Graduate Student Tuition & Fees

Name	**Project Role**			
Marcus Wharton	Graduate Student	$	11,496	**$437.12**
Asif Mortuza	Graduate Student	$	11,496	
To Be Named	Graduate Student	$	-	
	Total Tuition & Fees	**$**	**22,992**	

Modified Total Direct Costs (MTDC)	**$**	**109,026**
Total Direct Costs (TDC)	**$**	**147,018**

INDIRECT COSTS	**Rate**	**15.0%**	
	Base	**Direct Salaries**	
	$	10,977	
TOTAL REQUEST FROM SPONSOR (TRS)	**$**	**157,995**	**$18,954.32**

UNRECOVERED IDC		
Notes:		
IDC on Modified Total Direct Costs (MTDC)		**51.5%**
	$	56,148

Sponsored Allowed IDC*:	**Direct Salaries**		**15.0%**
		$	10,977
	TOTAL UNRECOVERED IDC	**$**	**45,171**

For this section, please use MTDC, TDC or TRS, for the calculation of Unrecovered Indirect based on sponsor guidelines.