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PAH Concentrations Found within Gulf Menhaden (Brevoortia patronus) Populations Located off of the South/Southeast Coast of Louisiana

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PAH CONCENTRATIONS FOUND WITHIN GULF MENHADEN (BREVOORTIA PATRONUS) POPULATIONS LOCATED OFF OF THE SOUTH/SOUTHEAST COAST OF LOUISIANA

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ABSTRACT

In April 2010, large quantities of crude oil were released into the Gulf of Mexico (GoM) raising questions about the possible contamination of marine organisms with constituents of concern known as Polycyclic Aromatic Hydrocarbons (PAHs). In order to determine the impact of the Deepwater Horizon (DWH) crude oil spill, Gulf menhaden (*Brevoortia patronus*) were harvested from two coastal regions of Louisiana. Tissue analysis to determine total PAH concentration was conducted using an adapted matrix solid phase dispersion (MSPD) method; a Soxhlet extraction process was used to determine fish oil to mass ratios for "small" (fork length < 16 cm) and "large" (fork length > 16 cm) menhaden. Menhaden oil and meal, harvested prior to the DWH spill, was used to create menhaden facsimiles for baseline total PAH concentrations. Gulf menhaden were harvested off of the coast of Louisiana from July 2011 through October 2011. Sampling occurred at sites around Vermilion Bay (VB) as well as Grand Isle (GI) and the menhaden were analyzed by region as well as size to determine if the concentrations of PAHs varied based on these factors. PAH concentrations were quantified along with total Benzo[a]pyrene mutagenic (B[a]P-MEQ) and Benzo[a]pyrene toxic equivalencies (B[a]P-TEQ) and all analysis was completed using Gas Chromatography/Mass Spectroscopy (GC/MS). Results were reported in ng of total PAH/g of dry weight tissue and the detection limits of the GC/MS method were between 0.4 ng/g dry weight and 4.4 ng/g dry weight. In conclusion, the two Louisiana coastal regions were not statistically different and therefore cannot be used to identify the impact of the DWH spill in a one-year sampling event. However, PAH concentrations were statistically different based on month with a significant interaction based on size. Mean concentrations for "small" and "large" menhaden were not statistically different; the B[a]p-TEQs were highly significant suggesting the larger menhaden were exposed to more

carcinogenic PAHs throughout their life due to variations in feeding patterns. Continuing the study for a second year will provide further elucidation on species life cycle exposure to PAHs and possible impacts to fisheries.

INTRODUCTION

The release of large quantities of crude oil into the Gulf of Mexico (GoM) during 2010 has raised concerns dealing with possible contamination of marine organisms because of this continued oiling event. The major constituents of concern within this crude oil encompass the fraction that remains left behind due to its inability to volatilize. These compounds, known as Polycyclic Aromatic Hydrocarbons (PAHs) can be characterized by multiple conjoined ring structures with naphthalene and its alkylated forms being the smallest (molecular mass of 128.17 g/mol) (Haritash & Kaushik, 2009). The higher the molar mass of the PAHs results in less volatilization, which in turn allows those compounds to remain in nature far longer than other lighter constituents of oil (Feng *et al*., 2009). This leads to the possibility of bioaccumulation within the adipose fraction of marine organisms and possible biomagnification within the trophic structure of the GoM.

PAHs are considered compounds of concern according to the United States Environmental Protection Agency (USEPA) because of their ability to accumulate within adipose tissue (USEPA, 2008). There are several PAHs that are considered mutagenic as well as carcinogenic, making their possible presence in a commercial fishery a major concern for GoM coastal fisheries (Nisbet & LaGoy, 1992; Durant *et al*., 1996; USEPA, 2008). In an attempt to quantify the PAH concentrations that are found within the commercial fishery of the GoM, an initial phase assessment of this fishery must be completed. This study is an attempt to understand the concentrations of PAHs within a commercially viable fish that is harvested in great multitudes from the GoM.

The Gulf menhaden (*Brevoortia patronus*) was identified as the second largest commercial harvest from 2005–2010 and selected as the principle organism to study (Van

Voorhees & Lother, 2011). This decision was further validated by the fact that menhaden are collected due to the amount of fats and oils that can be extracted from them and refined for consumer use, which is important because of the lipophilic nature of PAHs (Vaughan *et al*., 1998; Franklin, 2007). This fish is also significant because of its position within the food web of the Gulf of Mexico. The obligate filter feeding nature of this organism has two very important ramifications: 1) this fish will be in contact with surface and subsurface oil not just with dermal contact but through direct ingestion as well. 2) The feeding method and the sheer amount of Gulf menhaden in the GoM suggest that menhaden are the main link between producers and secondary consumers (Vaughan *et al*., 1998; Franklin, 2007; Van Voorhees & Lother, 2011).

This research is crucial in understanding trophic level transfer of PAHs within marine ecosystems such as that of the GoM. The importance of this research will be significant to the Atlantic Menhaden (*Brevoortia tyrannus*) fisheries along the eastern coast of the United States of America due to the fishery's proximity to many industrialized areas such as New Jersey. Many individuals use the GoM as a recreational fishing area, meaning that this research will indirectly shape their fishing habits. If the principal prey for the sport fish caught in the GoM has the ability to accumulate PAHs, then there is the opportunity for these PAHs to accumulate within the sport fish themselves.

To summarize, The GoM has experienced the largest oil spill in the history of the United States. It is important to determine the impact of this spill on the GoM fishery, hence the use of the gulf menhaden (the largest commercial catch in the GoM) as the principal organism of interest. Total PAH (constituent of crude oil that can remain in the environment) concentrations for each sample will be identified through the use of an adapted sonication assisted MSPD extraction method. This will allow for not only the quantification of total PAH concentration but

the generation of Benzo[a]pyrene mutagenic and toxic equivalencies for the test organisms using a GC/MS analytical method designed to identify 43 aromatic (PAHs) compounds found in crude oil. Using these parameters it will be possible to determine if the DWH oil spill is affecting the trophic structure of the GoM.

LITERATURE REVIEW

Gulf Menhaden (*Brevoortia patronus*)

Gulf Menhaden (*Brevoortia patronus*) is a smaller menhaden compared to the other menhaden species found along the east coast of the United States, with fork lengths of no more than 22 cm. It is an obligate filter feeding fish that consumes anything collected within its gill rakers as a result of schooling through the water (Vaughan *et al*., 1998). Fishing grounds tend to be as far east as Florida and stretch west to Mexico. From roughly April to October (as late as November) each year these fish form large schools and are collected for industrial refining of the oil that they so readily produce (Vaughan *et al*., 1998; Franklin, 2007). Menhaden oil is refined and used in a variety of commercial products ranging from makeup to over-the-counter supplements and is another reason that this particular fish was selected (Franklin, 2007). Again, PAHs are lipophilic and will accumulate within the adipose tissue of an organism (Larsen *et al.*, 2002). The menhaden itself is full of fats and oils that can readily dissolve PAHs, which could not only accumulate within the fish, but could possibly magnify through trophic transfer as it is consumed. Menhaden are a standard forage food for various fish, birds, and marine mammals and represents the primary connection between produces and secondary consumers within the GoM (Vaughan *et al*., 1998; Franklin, 2007). Another reason this fish was selected is the fact that Gulf menhaden undergo no major longitudinal migrations. These fish are in coastal waters seasonally and spend their first year within estuarine waters (Vaughan *et al*., 1998). This means that Gulf menhaden will continue to reside in areas that were affected by the Deepwater Horizon (DWH) oil spill for the duration of their life, moving between deep GoM waters (roughly 80 km off shore) and GoM coastal waters (Vaughan *et al*., 1998). Gulf menhaden spawning usually takes place between October and March with peak spawning taking place between December

and January (Raynie & Shaw, 1994) suggesting that April–October is the optimal time for harvesting.

GoM Sublittoral Current Systems

Currents within the GoM had a great impact on the distribution of oil released from the DWH spill. During the time of the oil spill the movement of the Gulf was influenced greatly by the Loop Current (LC) as well as the Eddy Franklin (EF), which detached in June of 2010 (Hamilton *et al.* 2011) (Figure 1). The LC moves between the latitudes of 24–28°N on varying timescales (0.5–18.5 months) after entering the Yucatan Channel. During its maximum penetration the LC turns anticyclonically and exits through the Florida straight. The LC is comprised of salty (36.7–36.8) warm water (25–26°C) (Vukovich 2007; Hamilton *et al.* 2011). The LC has a baroclinic flow structure, with the majority of the flow being above 800 m with the habitual non-chaotic northward branching of the LC contributing to the upper level mesoscale variability among marine species of the Eastern Gulf. The LC enters the Gulf at 23–27 Sverdrups and at its northernmost position usually sheds an eddy (Hamilton *et al.* 2011). LC eddies also contribute to the upper layer mesoscale distribution as well as transfer mass, heat, momentum, and salt from the eastern to the western Gulf basins (Vukovich 2007; Hamilton *et al.* 2011). Another phenomenon that creates movement within the GoM is known as frontal or cold cyclonic waves, which are located along the edge of the LC as well as the fringes of LC eddies. This phenomenon is not well understood; however, it has been suggested that these cold cyclonic waves help the shedding of LC eddies (Walker *et al.* 2003). This implies that anterior eddies fluctuate systematically around the LC, becoming largest at the northern edge, intermediate on the eastern side, and smallest on the southern edge (Vukovich 2007; Walker *et al.* 2009). During May and June of 2010 measurable particulates freely suspended in the water showed that EF was

displaying a closed anticyclonic flow with intense southwestward currents between the Campeche bank and the west Florida slope (Hamilton *et al.* 2011). (Figure 1)

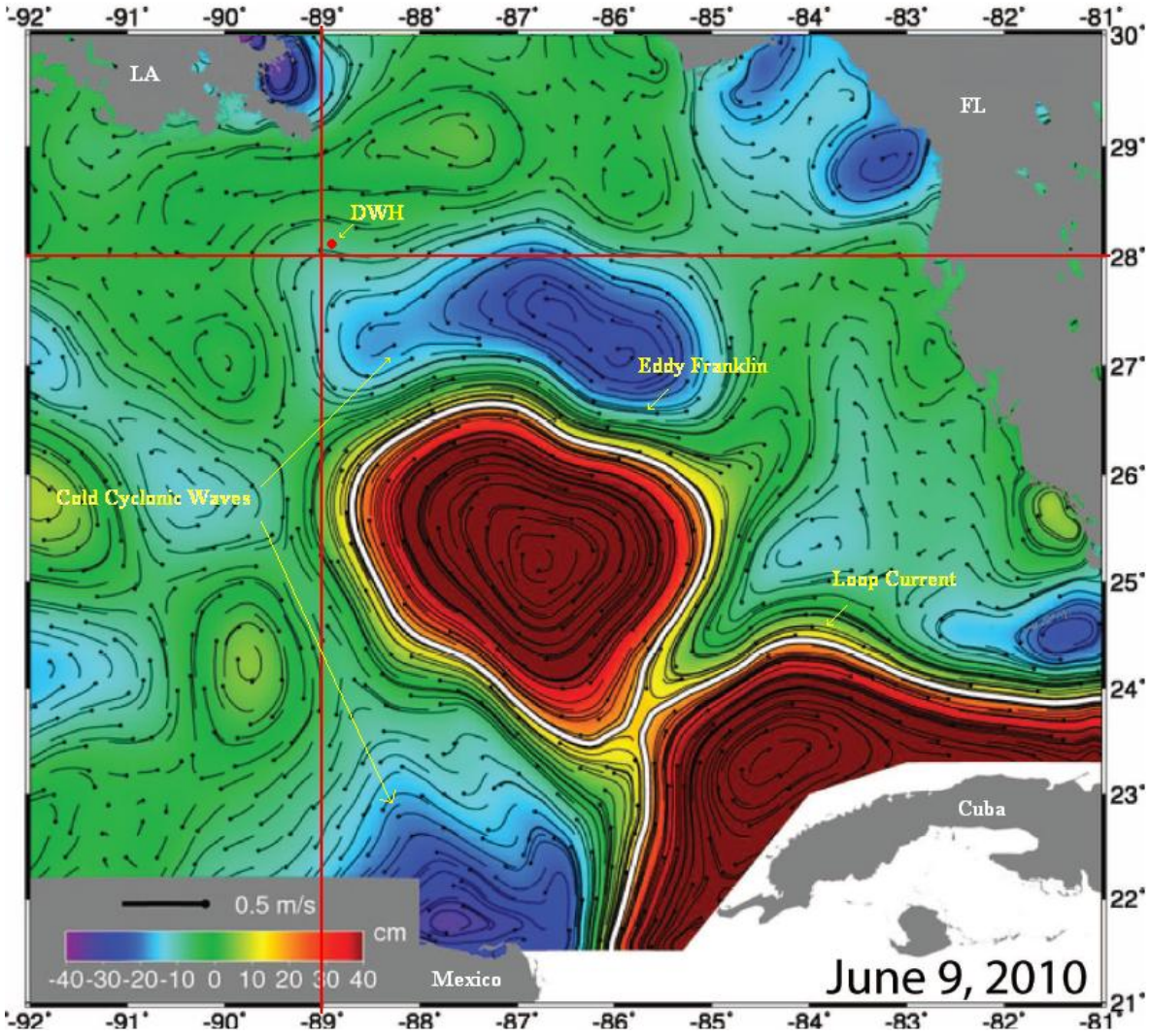


Figure 1: Lagrangian particle path computed from geostrophic flow fields derived from sea surface height (SSH) maps for the selected LC intrusion event associated with the separation of Eddy Franklin in 2010. This shows Eddy Franklin at the time of the first observed detachment from the LC. This event exhibited a deep intrusion into the northern Gulf, a large LC eddy detachment, and a significant retreat of the LC northern boundary after eddy detachment to well south of 25°N. Labeled within the map is the location of the Deep Water Horizon oil platform (DWH), the Loop Current, Eddy Franklin, and the Cold Cyclonic Wave systems surrounding both EF and the LC. (Adapted from Hamilton et al. 2011)

The detailed Lagrangian particle path computed from geostrophic flow fields derived from sea surface height (SSH) maps for the selected LC intrusion event associated with the separation of Eddy Franklin in 2010. Being that the major contributor to the movement of Gulf water during this timeframe is clearly the LC as well as EF, it is important that the smaller currents surrounding the DWH platform should not be overlooked when interpreting the possible fate and distribution of the oil.

Physical and Chemical Properties of PAHs

PAHs are found naturally within crude oil as well as coal resulting from conversion over time of natural compounds (such as steroids) to various aromatic hydrocarbons (Roy, 1995). Remember that PAHs are lipophilic and will not easily solubilize in water. PAHs tend to adsorb onto organic materials found within the substrate they are located. For example within soils they generally do not penetrate beyond the organic fraction, which limits leaching into groundwater (Larsen *et al*., 2002). Although PAHs of a lesser mass are semi-volatile, most of the PAHs found in our environment are heavier and preferentially combine with particulate material. This combination is the standard route of atmospheric deposition of PAHs (Edwards, 1983; Neilson *et al*., 1996). Two/three ring PAHs are almost entirely found in vapor form, with four-ring PAHs being intermediately positioned between the two/three ring and five or greater ring PAHs. Five or greater ringed PAHs are found predominantly adsorbed to other materials (Neilson *et al*., 1996). From here, these particulates can settle from the atmosphere and land in fresh and/or marine water. Again, it is important to remember that these PAHs are adsorbed onto organic materials as well as particulates (> 2.5 μm in diameter) and will either be consumed by some organism or eventually settle to the sediment layer (Larsen *et al*., 2002). The majority of aquatic organisms that come into contact with PAHs have the ability to bio-transform and eliminate them

from their bodies,. However, certain filter-feeding organisms tend to bio-accumulate these PAHs because of the nature of their feeding methods as well as their inability to bio-transform them (these organisms include bivalves such as oysters) (Baumard *et* al., 1998; Larsen *et al*., 2002). In an attempt to determine whether these PAHs are moving through the trophic levels within the Gulf of Mexico a much larger commercial organism that is an obligate filter feeder found all over the GoM was selected (Gulf menhaden).

Abiotic and Biotic Degradation of PAHs

One major issue with the structure of PAHs comes from their inherent stability. This is compounded by the fact that they are not readily broken down through the process of hydrolysis (Howard *et al*., 1991). These compounds, however, can go through photodissociation as well as oxidation within the environment. How rapidly this takes place is largely controlled by where the particular PAH is residing within the environment. Exposed, PAHs can have half-lives of hours to days depending on mass and structure, but within particular substrates such as soil these can change into months and years (Parks *et al.*, 1990). It is important to note that even though these compounds do abiotically degrade, their oxidized derivatives can be just as dangerous in the environment. An example would be the Nitro-PAHs, which are associated with lung cancer (Larsen *et al*., 2002). Biologically, PAHs are metabolized by the cytochrome p-450 super family of enzymes. The bio-transformation efficiency is directly related to the cytochrome p-450 dependant mixed function oxidase activity harbored by a specific organism (James, 1989). It has been reported that in invertebrates the initial transformation of the compounds takes longer than it does with vertebrates. It has also been shown that the invertebrate's ability to eliminate the resulting metabolites is much slower as well (IPCS, 1998). It should be noted that the alkylated

PAHs do not necessarily behave as their non-alkylated counterparts during the processes of abiotic and biotic degradation.

Routes of Exposure

Routes of exposure to PAHs must be understood in order to quantify the impact of the DWH oil spill on the PAH concentrations found within the GoM. These compounds are generally a part of air pollution and can be released because of incomplete combustion of several different fossil fuels such as coal, oil, gasoline, as well as burned garbage (Larsen *et al*., 2002). They are found readily along areas of high motor vehicle use because of the nature of the exhaust produced during the combustion process (Butler *et al*., 1984). PAHs are also produced by many industrial processes such as incineration. They are also found in wood preservatives that are composed of tar and/or creosote. The disposal of many things by incineration (such as tires, treated wood, and garbage in general) results in even more PAH production (IPCS, 1998). PAHs can also enter our atmosphere from natural events such as volcanic eruptions and forest fires (Hites *et al*., 1980). PAHs are found within the air, soil, and water through the processes of deposition and transference. The PAHs in the air are deposited either onto soil or water due to their tendency to adsorb to organics as well as particulates. The PAHs in the soil are transferred to the water (usually through some form of weathering), and finally the surface water is contaminated by both atmospheric deposition and soil transfer of PAHs, regardless of their origin (Larsen *et al*., 2002). These are the many ways that PAHs can contaminate the environment, and it is necessary to understand this when attempting to identify the impact of PAH concentration within a particular species. There will be a background level of PAHs within the organism selected for study, and this background level needs to be identified through the use of a control in order to assign meaning to the values identified within this study.

Chromatographic Approach to PAH Analysis

The Matrix Solid Phase Dispersion (MSPD) extraction method is characterized by the total disruption of the sample through the use of an appropriate bonded phase or other solid support material such as octadecylsilyl (ODS)-derivatized silica (C-18 Silica), which is ground with the sample. Once this step has taken place the material is packed into a container suitable for a series of elutions with the desired solvent. This creates a new phase consisting of the sample and bonded phase material and allows for distinctive sample fractionation (Barker, 2007; García-López *et al.*, 2008). For this experiment a lipophilic bonding phase of C-18 silica was used; however, the use of C-8 silica would have been a possible alternative for binding lipids (Barker, 2007). Within the procedure used for MSPD extraction we applied a negative pressure at the receiving end of the process. This form of MSPD extraction is known as pressurized-liquid extraction (PLE) or accelerated solvent extraction (ASE) (Barker, 2007). Generally, the eluate collected from this process is sufficiently "clean" enough to run on analytical instruments; however, additional cleanup measures can be conducted, such as co-column cleanup, where other support materials are added to the bottom of the container (Barker, 2007). In the case of the eluate collected from menhaden the only secondary cleanup method employed was a standard settling period of approximately 24 hrs after the extraction process. This allowed any material large enough to pass through the glass microfiber filter time to settle out. This method along with a dilution with hexane is sufficient enough to create a sample that will not cause damage to the GC/MS columns.

MATERIALS AND METHODS

Solvents, Reagents, and Chemicals

Only pesticide /reagent grade solvents were used in all standard preparations, sample analysis, and dish washing procedures. The dichloromethane (DCM) and hexane were obtained through the university supply store and were originally purchased from Mallinckrodt Chemicals (St. Louis, MO). The RediSep C-18 silica (40–60 µm) was obtained though the university supply store and was originally purchased from Teledyne Isco, Inc (Lincoln, NE). The sodium sulfate is certified A.C.S. (anhydrous) (10–60 mesh) and was also purchased through the university supply store; it was obtained originally from Fisher Scientific (Waltham, MA).

Calibration Standards

A commercially prepared oil analysis standard, available through Absolute Standards (Hamden, CN), was used to prepare the five-point calibration standards. Calibration standard solutions were stored in amber-colored vials with PTFE-lined caps. The calibration standards were checked frequently for signs of degradation or evaporation and were replaced if the quality control check sample indicated a problem.

A five-point calibration curve was performed quarterly. A continuing calibration standard (one point of the initial five-point calibration standard) was analyzed in each batch of samples or each 12-hour period during which analyses were performed. The acceptance criterion for the continuing calibration standard was ± 20% of the average relative response factor calculated from the initial five-point curve. If the acceptance criterion was not met, all analyses were stopped until the instrument was performing satisfactorily. Any instrument maintenance or troubleshooting might have required that a new five-point calibration curve be performed.

Internal Standard Solutions

The internal standards were naphthalene-d_8, acenaphthene-d_{10}, chrysene-d_{10}, and perylene-d_{12}. The internal standards were bought from AccuStandard Inc (New Haven, CT) and stored individually until they were mixed to make the internal standard solution.

Reference Oil Standard

The usual laboratory reference oil was Alaska North Slope Crude Oil (ANSCO); however, the reference oil standard used for the analyses in this study was Macondo 252 (MC 252), which was collected directly from the riser of the DWH oil rig. Reference oil standards were prepared by extracting 1 gram of pure oil in 40 mL of solvent (or equivalent ratio of 1g: 40 mL, e.g. 0.50 g: 20 mL). The laboratory reference oil was analyzed in each sample batch as an additional QA/QC sample (a laboratory control sample).

Surrogate Standards

The surrogate standards were 5α-androstane (alkanes) and phenanthrene-d_{10} (aromatics). The surrogate standards were purchased from AccuStandard Inc and stored individually until they were mixed to make the needed concentration of surrogate standard. The extraction efficiency for each sample was evaluated based on the percent recovery of the surrogate standard with an acceptable percent recovery range of 70–120%.

Sample Collection

Menhaden were sampled at locations around Grand Isle (GI) and Vermilion Bay (VB), Louisiana. The samples were taken through the use of a five-panel gill net approximately 700 ft long with distinct plastic mesh panels. The menhaden were caught within these panels for use in tissue extraction as well as for fixing in a 10% buffered formalin solution. The fish that were fixed in formalin were later sent to a laboratory located at the New Brunswick campus of Rutgers

for histology analysis. No sampling events took place without the help of the Louisiana Department of Wildlife and Fisheries (LDWF); therefore all sampling protocols that were followed were designated by the LDWF agents. The menhaden were separated by length, bagged in plastic freezer bags, and placed on ice until they could be frozen in a –4 °C deepfreeze.

Protocol for Preparing the Menhaden for Tissue Extraction

Six samples of menhaden with fork lengths of 16 cm or less ("small" menhaden) were removed from the deep freeze for each location. They were chopped into small indiscriminant pieces (the amount of which was dependent upon the initial size of the menhaden) so that the entire fish could be held in the beaker. These pieces were then placed into a 150–200 ml beaker (400–500 ml beaker for the "large" menhaden). All beakers had been washed and tared prior to contact with the menhaden. The pieces of menhaden were then compressed into the beaker with a clean glass pestle unless all pieces fit so that no portion of the menhaden was protruding from the top of the beaker. The samples were then placed in a –86 °C freezer and allowed to freeze solid. During the freezing process a freeze dryer was prepared to receive the menhaden samples once they were removed from the –86 °C freezer. The samples were then lyophilized for 24–36 hours. Once finished, these samples were then removed to a dessicator for at least 24 hours. This process was repeated with six menhaden having fork lengths greater than 16 cm ("large" menhaden) from each location. These menhaden were allowed to freeze dry for 36–48. Once removed, these samples were allowed to finish drying in a dessicator for at least 24 hours. (See Appendix A for Detailed Protocol)

Protocol for Extracting PAHs from Tissue

The menhaden were removed from the dessicator and their final dry mass was recorded. The menhaden pieces were then placed in a grinding apparatus and ground until all major pieces

were comminuted. A 5–10 gram subsample was then removed and a 1:1 ratio of C-18 silica was added to the sample. This mixture was then ground. resulting in a material that was of a powdery consistency (roughly 200 mesh). Then approximately 3–5 g of sodium sulfate (enough to cover the top of the sample) was added and mixed in with a spatula. At this point the sample was then spiked with 1 ml of the surrogate spiking solution (Appendix A). This material was then sonicated for 20 minutes with dichloromethane (DCM) and then transferred to a 350-ml glass Büchner funnel fitted with a Whatman glass microfiber filter (934-AH 90 mm diameter) topped with a 10-g layer of sodium sulfate. The funnel was then attached to a side-arm flask affixed to a vacuum manifold. The beaker used to lyophilize the sample was then rinsed three times with DCM into the homogenized sample to ensure complete transfer of all materials. The resulting slurry was then inundated with DCM until the elution became clear. This was done under a vacuum of 5–10 inches of water. The resulting elution was then placed into a flat-bottom Florence flask and rotary evaporated until all excess DCM was removed. The residual material was then reconstituted in hexane and transferred to a 100-ml glass graduated cylinder. An appropriate amount of hexane was then used to dilute the resulting material to a whole number volume in ml (this amount is not set, enough hexane was used to dilute the sample to sufficient clarity deemed by the GC/MS operator, with smaller samples ranging from 20 to 35 ml of hexane and larger samples ranging from 40 to 80 ml of hexane). The final volume was then recorded and the solution homogenized in the graduated cylinder with a Pasteur pipette via aspiration and stirring. Then 20 ml were removed from the solution and collected in a volatile organic analysis (VOA) bottle. From here any excess particulates were allowed to settle overnight (or longer depending on the solution) and 1 ml of this liquid was placed (using a gas tight syringe) into an amber GC bottle. An aliquot of 10 µl of a prepared internal standard

(Appendix A) was then added to the contents of the GC bottle before it was capped and placed in refrigeration. Once this had been preformed the sample could then be analyzed on the GC/MS. (See Appendix A for Detailed Protocol)

Protocol for Extracting the Menhaden Oil

A sample of menhaden were homogenized and then mixed with roughly 5–10 g of sodium sulfate to bind up any moisture possibly present within the lyophilized fish. The sample was packed into a cellulose extraction thimble, spiked with 1 ml of surrogate spike solution (Appendix A), and placed into a Soxhlet extraction column. An aliquot of roughly 100 ml of DCM was placed into a tared, flat-bottom Florence flask resting on a hot plate. The column was connected to the flat-bottom Florence flask and the DCM was heated to a boil. The resulting process ran for 16–18 hours (overnight) and extracted the lipid and oil fractions that were within the menhaden. The flask was then removed to a rotary evaporator and all excess DCM was evaporated. The resulting material was weighed within the flask and the initial mass of the flask was subtracted from the total. This provided the total amount of oil extracted from the menhaden in grams. This number was compared to the total mass of the wet menhaden, and an oil content percentage was determined. This was done for each sampling location and for both small and large menhaden. A mean oil percentage per fish along with a mean mass of oil per gram of menhaden was determined for each size category. (See Appendix A for Detailed Protocol)

Protocol for GC/MS Analysis

A calibration standard (see Appendix A) as well as a source oil standard (Macando 252) was analyzed along with every sample. The analytical method I used identified 71 key constituents of crude oil with 43 components identified as aromatic. There were two surrogates used to identify recovery for both the alkane and aromatic portions of the sample. The surrogate

used to validate the recovery of the alkanes was 5α-androstane. The surrogate used to validate the recovery of the aromatics/PAHs was phenanthrene d_{10}. The portion of the extraction that is significant to this study was the aromatic concentration found within each menhaden sampling group. The samples were individually integrated and compared to the known peaks of the 71 key constituents used to identify crude oil. From these integrations, retention times, and response times the concentration in ng/g of dry wt tissue was computed. (See Appendix A for Detailed Protocol)

Protocol for Data Analysis

Menhaden PAH/raw oil ratios were analyzed using an analysis of covariance with a type I error rate (α) of 0.05 to determine significance between region as well as size. A student's t-test with an α of 0.05 was preformed to assess the significance of the difference of the means between October 2010 and October 2011 GI small menhaden. All months were individually compared using one-way analysis of variance with an α of 0.05. All multiple variable comparisons were made using two-way analysis of variance along with nested analysis of variance both using an α of 0.05. All statistical analyses were performed using the MATLAB r2009a software package and Microsoft Excel. (See Appendix A for Detailed Figures and Tables)

Quality Assurance/Quality Control Measures

All menhaden were captured in accordance with the LDWF standard operating procedure for collecting gill net samples. All water quality measurements were compared to those taken by the LDWF when I was on the boat. Otherwise the measurements came directly from the LDWF sample reports.

All materials used within the extraction method were washed as per the washing procedure found in Appendix A of this document. All extracting devices were rinsed and primed as per the Pasteur pipette/gas tight syringe procedure found in the C-18 Silica extraction process steps 8A and 8B located in Appendix A. All experimental and analytical procedures used surrogates and standards as well as method blanks to validate the methods preformed during the whole of this endeavor (see Surrogates and Standards section in Appendix A).

EXPERIMENT AND RESULTS

Site Locations and Conditions

Site selection of the two locations in the Louisiana coastal zone was determined by several factors. The location had to be 1) feasible for sampling throughout the timeframe of this study; 2) readily accessible; 3) control versus experimental locations for impacted and non-impacted coast affected by the DWH spill. Advice from LDWF also contributed to the site selection decision-making process in that the fishery scientists had stored datasets for specific locations of these Louisiana coastal zones. At the recommendation of the division of fisheries for LDWF, Vermilion Bay (VB) Louisiana was selected as the "minimally impacted" sampling location for this study. The Grand Isle (GI) Barataria Bay location was and remains an impacted location from the DWH spill. Water quality measurements on samples collected at all locations during the course of this investigation are shown in Table 1. Overall pH at all sampled sites throughout this study did not vary significantly (pH range: 7.5–8.5) and were well within the historic range of values for these locations. As such, they were not included in Table 1. As shown in Figure 2, salinity varied by location and month over the course of this study ($p < 0.10$). Similarly, dissolved oxygen (DO) levels also varied by location and month (Figure 3, $p < 0.02$). All latitude and longitude measurements were reported in degree, minutes, and seconds (including decimals). These regions are two very different costal habitats and are influenced by several different factors including: fresh water river systems, annual rainfall, annual flooding, seasonal current movement, varying oceanic influence, differing anthropogenic influences, etc.

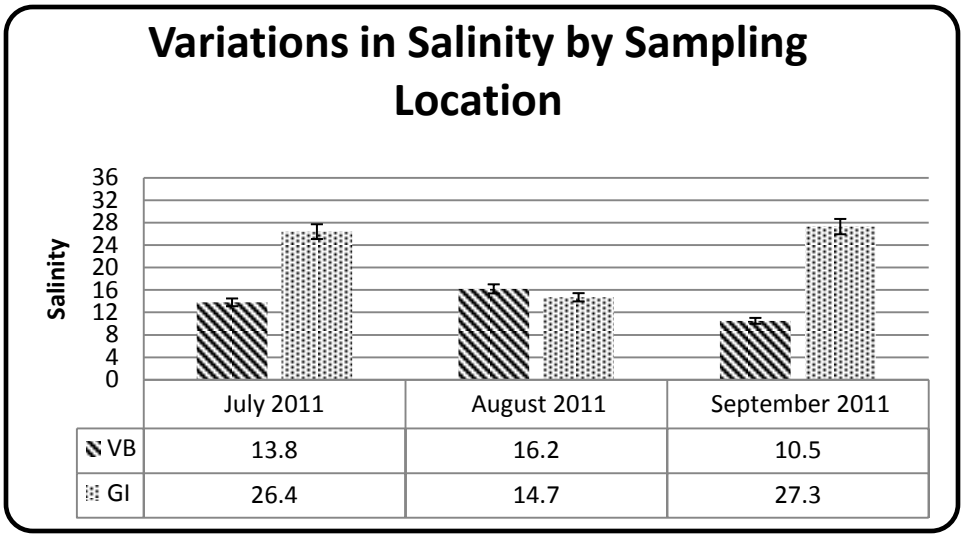


Figure 2: Salinity levels for each sampling event. (Note: September VB levels are a mean of six events).

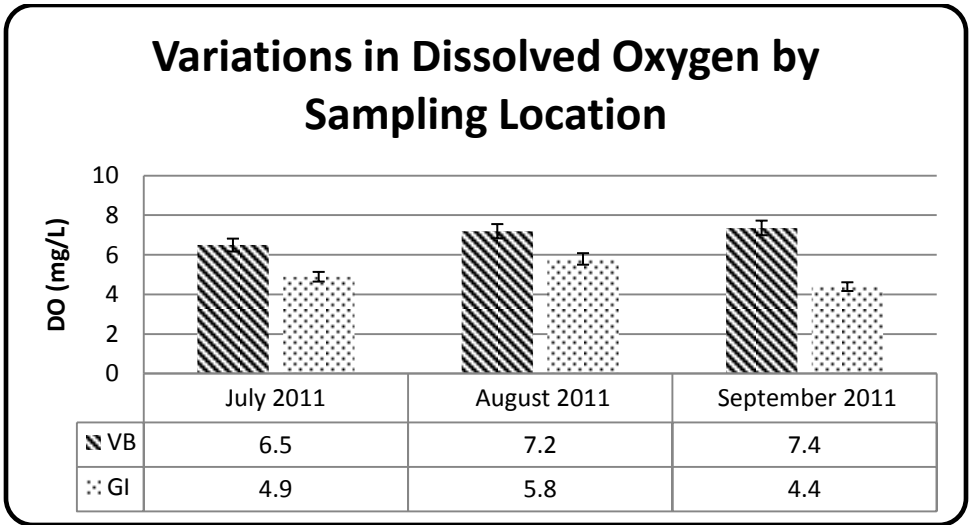


Figure 3: Dissolved oxygen levels in mg/L for each sampling event (Note: September VB levels are a mean of six events).

These two locations are tidal-connected bays of the GoM and are therefore considered arms of the sea. Conversely, they are also coastal habitats dominated by seasonal detrital deposition from upland riverine locations. Finally, these locations are not pristine and have a legacy of oil exploration, production, and distribution. An adjusted total PAH analysis showed moderate to

low concentrations during the course of this study. LDWF agents reported an oil spill of undetermined origin in upper Vermilion Bay during the month of August 2011, an area with minimal impact from the DWH spill, an indication that this region, even though it was minimally impacted by the DWH, has oiling events that can contribute to PAH concentrations.

Table 1: 2011 Gulf Menhaden Sample Locations with associated Water Quality Measurements

Date	Location	Lat Long	Time (24)	Water Temp (°C)	Dissolved Oxygen (mg/L)	Salinity
7/6/2011	VB	29°33'23.17"N 92°1'18.79"W	1150	33.1	6.5	13.8
7/28/2011	GI (Grand Terre)	29°15'58.27"N 89°56'34.31"W	1330	29.9	4.9	26.4
8/23/2011	VB	29°33'23.17"N 92°1'18.79"W	1015	32.5	7.2	16.2
8/25/2011	GI	+29° 17' 11.04" -89° 56' 20.72"	1000	32.1	5.8	14.7
9/15/2011	VB	+29° 35' 50.43" -91° 46' 52.58"	1030	29.2	10.9	4.7
9/21/2011	VB (Mound Pt)	+29° 28' 20.93" -91° 49' 57.77"	1320	28.3	6.3	12.0
9/21/2011	VB (South Pt)	+29° 29' 37.73" -91° 46' 7.55"	1229	27.8	6.7	10.1
9/27/2011	VB (Shark Island)	+29° 47' 26.60" -91° 50' 59.30"	1437	28.2	6.2	5.7
9/27/2011	VB (Tete Butte)	+29° 34' 54.00" -92° 5' 36.00"	1149	28.4	6.8	16.0
9/27/2011	VB (Pavy Reef)	+29° 33' 30.64" -92° 1' 1.63"	1230	28.6	7.3	14.5
9/13/2011	GI (Elmer's Island)	+29° 10' 35.74" -90° 3' 41.34"	1400	29.9	4.4	27.3

Gulf Menhaden Mass and Lengths

Mass and length datasets were collected by catch and quantified for size and location. As normally practiced by fisheries scientists, fish samples were measured to their fork lengths and further separated by size into two major groups. "Small" menhaden had fork length less than 16 cm; "Large" menhaden had fork links greater than 16 cm. Differentiation by size was more of a function of type of net used in sample collection. Samples collected in this study were subsets of

larger fish and invertebrate collections under the supervision of LDWF scientists. The gill net mesh size allowed for differentiation of menhaden length was 16 cm.

A total of 330 menhaden with fork lengths ranging from 10.5 cm to 22.7 cm were collected from the Grand Isle location. The mass range for harvested menhaden spanned a fresh mass of 21.8 g to 218.1 g for GI. Similarly, a total of 307 fish were collected and measured from the Vermilion Bay sampling location. The menhaden ranged from 6.5 cm (caught in a trawl) to 23.5 cm in fork length with a fresh mass ranging from 4.3 g to 227.3 g.

In quantifying the age of menhaden, a mass to length ratio was use to calculate possible variations in age for each sampling location. Equation 1 was used to provide an estimate of Gulf menhaden age. Fishery scientists and commercial fishermen report that menhaden can possibly live up to 3.5–4.0 years, with juveniles routinely collected by net or trawl between the 3–6 month timeframe (0.25–0.50 years). That equation is:

Equation 1: Equation used to estimate gulf menhaden age. Based on the maximum fork length of a menhaden being directly proportional to its maximum age in nature.

$$\frac{M:L}{2.5} = EAy$$

Where: *M:L* = Mass to Length Ratio
EAy = Estimated Age in Years
2.5 = Age Adjustment Constant

Use of Equation 1 indicates that the estimated age in years for menhaden collected at the Grand Isle impacted site was 0.8–3.9 years. Menhaden at the unaffected site in Vermilion Bay had an estimated age range of 0.3–3.9 years. Figure 4 documents the exponential relationship between increasing fork length and increases in total fresh mass.

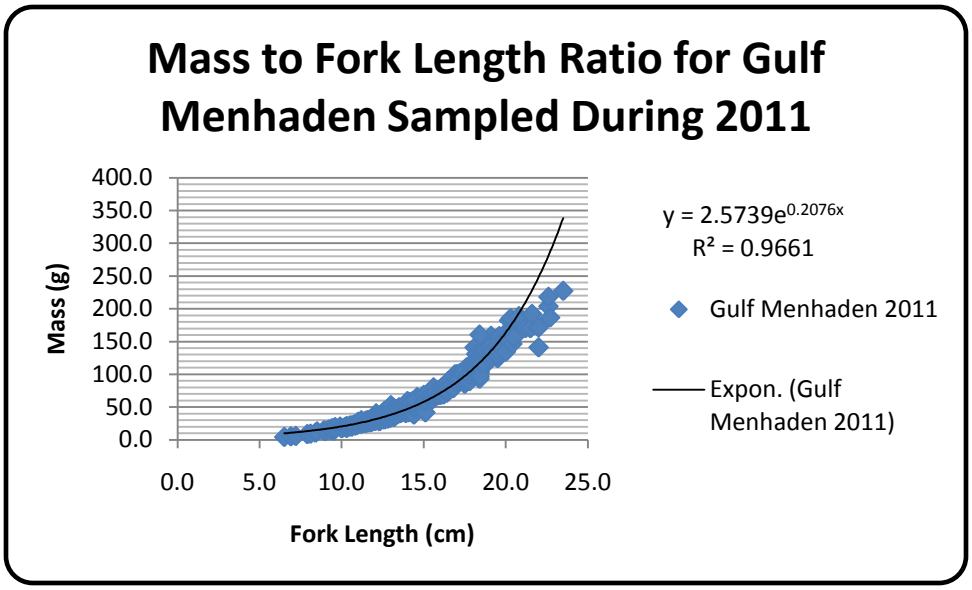


Figure 4: Menhaden mass plotted against fork length. Data represent all menhaden that were collected in this study (n = 637).

Gulf Menhaden Oil Concentrations

As mentioned in the literature review, menhaden are harvested primarily for the commercial value of fish oil as well as for the proteinaceous meal produced from refining. From an environmental risk perspective, the lipophilic nature of PAHs suggests that increases due to exposure can be determined by a careful analysis of the lipophilic fraction collected from menhaden. A careful quantification of fish oil content is first necessary to complete a total mean PAH content material balance for this oily fish. Quantifying oil yields for menhaden based on size was necessary to determine proper oil to fishmeal ratios. This ratio is important in establishing baseline concentrations of total PAHs for analysis of both commercial and field-collected menhaden after the DWH accident.

Figure 5 presents data on raw oil, namely the unrefined lipophilic fraction extract, for fish samples collected at both field locations. The supposition that larger menhaden contained higher quantities of extractable fish oil was confirmed ($P_{July} < 6.05x10^{-7}$ $P_{Aug} < 1.04x10^{-4}$ $P_{Sept} <$

$1.54x10^{-4}$). Additionally, the amount of oil found within the "large" fish stock was fairly consistent. The "small" menhaden showed seasonal increases in oil concentration.

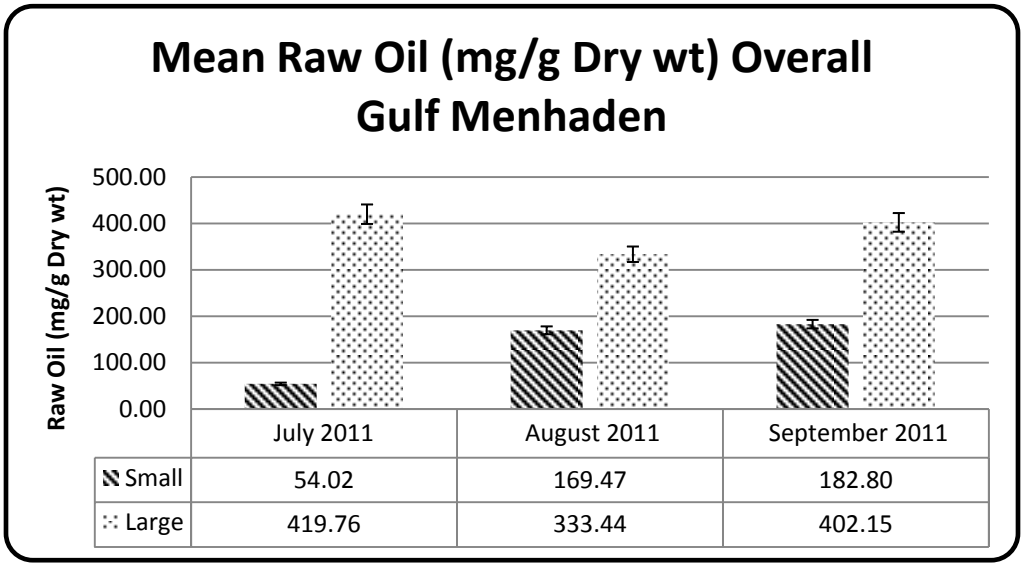


Figure 5: Raw oil collected from harvested menhaden based on fork length. "Raw" oil indicates unrefined fish oil collected directly from the menhaden.

As shown in Figures 6 and 7, raw oil amounts were consistent at both the Grand Isle and Vermilion Bay locations. Again, raw oil concentrations increased for "small" menhaden during the months of July, August, and September, 2011 but remained similar ($P_{July} > 0.41$ $P_{Aug} > 0.24$ $P_{Sept} > 0.38$). As small fish grazed in these coastal waters, they grew in length and mass and subsequently raw oil content. Mean raw oil yields for "large" menhaden dropped slightly in August 2011 compared to previous months this change was statistically significant ($P_{July} > 0.80$ $P_{Aug} < 0.03$ $P_{Sept} > 0.09$). Tables 2a, 2b, and 2c provide specifics on extracted raw oil by size and location.

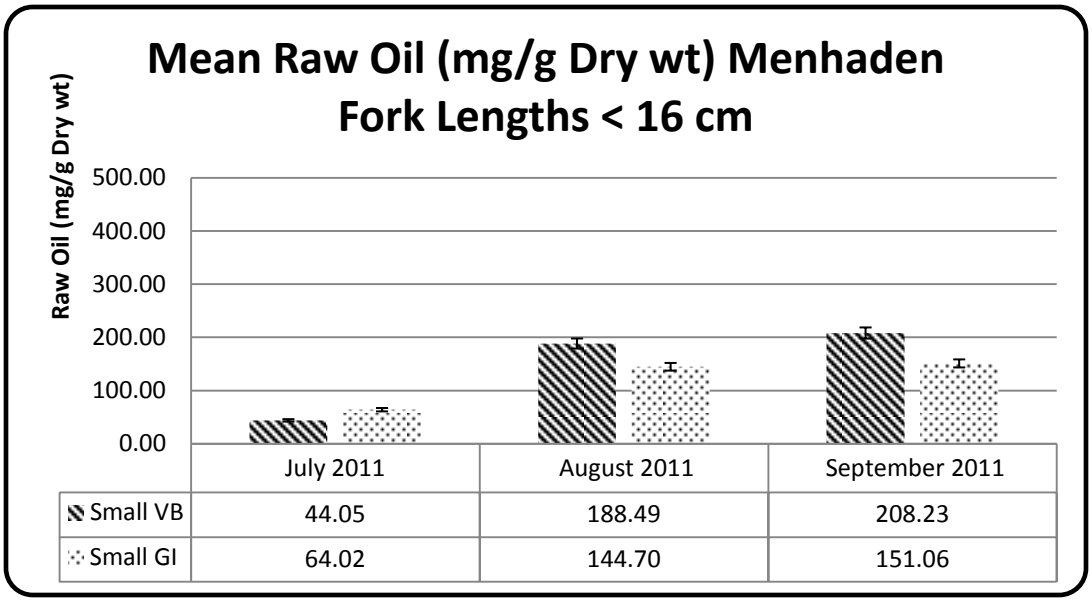


Figure 6: Raw oil harvested from menhaden sampled with fork lengths < 16 cm based on location. "Raw" oil indicates unrefined fish oil collected directly from the menhaden

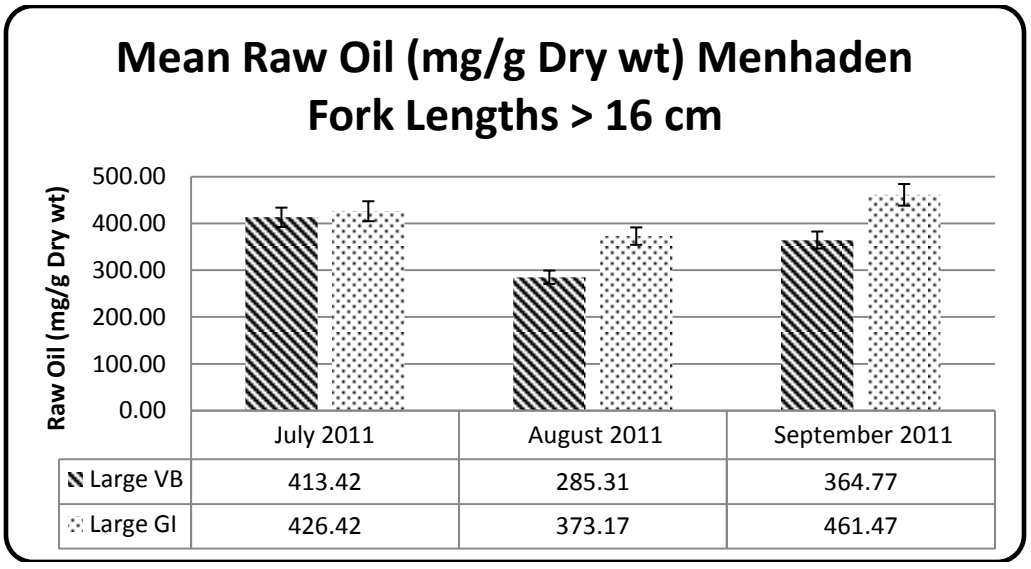


Figure 7: Raw oil harvested from menhaden sampled with fork lengths > 16 cm based on location. "Raw" oil indicates unrefined fish oil collected directly from the menhaden.

Table 2a: Raw Oil (extracted mean) from harvested menhaden separated by size and location: July 2011

Size	Mean Fresh Mass (g)	Mean Dry Mass (g)	Mean Fork Length (cm)	Mean Raw Oil (g/whole fish)	Mean Raw Oil (mg/g fresh fish)	Mean Fresh %	Mean Raw Oil (mg/g dry fish)	Mean Dry %
Large VB	126.30	48.70	18.93	20.13	159.41	12.62%	413.42	32.73%
Large GI	116.27	46.43	18.17	19.80	170.30	14.65%	426.42	36.68%
Large Total	121.28	47.57	18.55	19.97	164.63	13.57%	419.76	34.61%
Small VB	43.43	15.13	13.67	0.67	15.35	3.53%	44.05	10.14%
Small GI	43.70	15.10	13.27	0.97	22.12	5.06%	64.02	14.65%
Small Total	43.57	15.12	13.47	0.82	18.75	4.30%	54.02	12.40%

Table 2b: Raw Oil (extracted mean) from harvested menhaden separated by size and location: August 2011

Size	Mean Fresh Mass (g)	Mean Dry Mass (g)	Mean Fork Length (cm)	Mean Raw Oil (g/whole fish)	Mean Raw Oil (mg/g fresh fish)	Mean Fresh %	Mean Raw Oil (mg/g dry fish)	Mean Dry %
Large VB	99.30	46.97	17.80	13.40	134.94	13.6%	285.31	28.73%
Large GI	127.00	56.90	18.80	21.23	167.19	13.2%	373.17	29.38%
Large Total	113.15	51.93	18.30	17.32	153.04	13.5%	333.44	29.47%
Small VB	61.43	16.80	15.30	3.17	51.55	8.4%	188.49	30.68%
Small GI	48.67	12.90	14.03	1.87	38.36	7.9%	144.70	29.73%
Small Total	55.05	14.85	14.67	2.52	45.72	8.3%	169.47	30.79%

Table 2c: Raw Oil (extracted mean) from harvested menhaden separated by size and location: September 2011

Size	Mean Fresh Mass (g)	Mean Dry Mass (g)	Mean Fork Length (cm)	Mean Raw Oil (g/whole fish)	Mean Raw Oil (mg/g fresh fish)	Mean Wet %	Mean Raw Oil (mg/g dry fish)	Mean Dry %
Large VB	159.73	59.03	19.97	21.53	134.8	8.44%	364.77	22.84%
Large GI	102.43	37.20	17.63	17.17	167.6	16.36%	461.47	45.05%
Large Total	131.08	48.12	18.80	19.35	147.6	11.26%	402.15	30.68%
Small VB	51.47	13.77	14.13	2.87	55.7	10.82%	208.23	40.46%
Small GI	42.93	11.03	13.53	1.67	38.8	9.04%	151.06	35.18%
Small Total	47.20	12.40	13.83	2.27	48.0	10.17%	182.80	38.73%

Phenanthrene d_{10} Surrogate Recoveries

Table 3: Average surrogate recovery, corrected total PAH concentration, adjusted PAH concentration, and standard deviations on the menhaden sampled for this study.

Treatment	Mean Dry mass (g)	Mean Fork Length (cm)	Mean Recovery	Mean Corrected* PAHs (ng/g)	Mean C3-Phenanthrenes Adjusted** PAHs (ng/g)	Standard Deviation of Recovery	Sample (n)
Spiked Before Freeze Drying	40.16	18.25	87% (±8%)	8415	4830	0.08	7
Spiked After Freeze Drying	36.94	15.83	89% (±3%)	6485	1790	0.03	29
Average/Total of Whole Study	37.56	16.30	88% (±5%)	6860	2381	0.05	36
Controls	N/A	N/A	87% (±1%)	3501	46.8	0.01	6

* Corrected for surrogate recovery
** Adjusted to remove C3-phenanthrenes from the overall PAH total

The spiking surrogate solution containing phenanthrene d_{10} was administered at two different times of the study in order to show method validity. The samples spiked prior to lyophilizing had a mean recovery of 87% of the phenanthrene d_{10}. The samples spiked after the 10-g subsample was taken had a mean recovery of 89%. The standard deviation of the samples spiked prior to the lyophilizing process was 8%, and those spiked after the lyophilizing process had a standard deviation of 4%. This shows that there is not a significant loss in phenanthrene d_{10} recovery in the lyophilizing process. As can be clearly seen by the recoveries throughout this study, the adaptation of a sonication-assisted MSPD extraction can yield recoveries in excess of 90%. The lowest recorded recovery among the samples spiked after the lyophilizing process was 80%, with the lowest recorded recovery among the samples spiked before the lyophilizing process being 75%. The highest recorded recovery among the samples spiked after the lyophilizing process was 96%, with the highest recorded recovery among samples spiked before the lyophilizing process being 93%. The number of samples before the lyophilizing process was 7, and the number of samples after the freeze drying process was 29. The overall study summary

statistics are a recovery mean of 88%, a standard deviation of 5% , and a range from 75% to 96% with a total sample size of 36 (Table 3). The samples were then broken down via size in an attempt to identify size-specific issues with PAH recoveries. The only month with a significant difference in recoveries versus size and location was July 2011. In Figure 8 we can see that the difference in recoveries was 10% between "large" menhaden caught around Vermilion Bay, LA and "large" menhaden

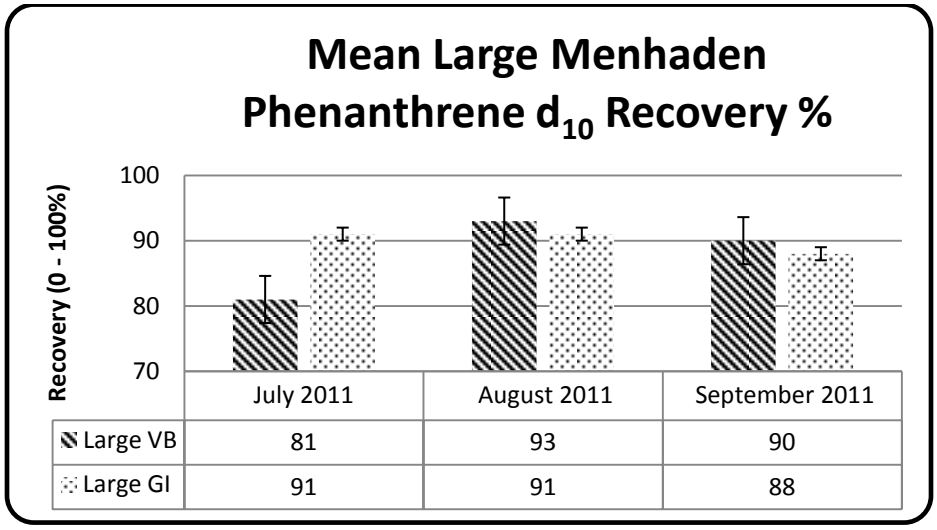


Figure 8: Mean "large" menhaden phenanthrene d_{10} recoveries based on site and month: Summer 2011

caught around Grand Isle, LA, however this does not represent a significant difference in recovery ($P_{July} > 0.14$ $P_{Aug} > 0.28$ $P_{Sept} > 0.29$). For the rest of the study it can be seen from Figure 8 and Figure 9 that the other recovery numbers remain very consistent (not varying more than 2%, Figure 9 $P_{July} > 0.99$ $P_{Aug} > 0.52$ $P_{Sept} > 0.61$).

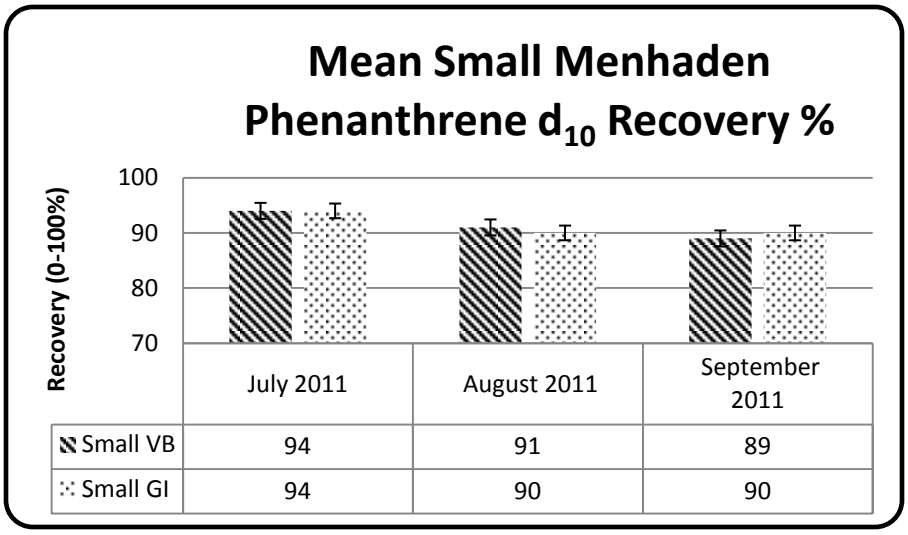


Figure 9: Mean "small" menhaden phenanthrene d_{10} recoveries based on site and month: Summer 2011

July is again the only month that recoveries between "large" and "small" menhaden differed however this was not a significant difference can be seen in Figure 10 (overall *p*-value > 0.09). The fact that this disparity was only noted in July, both between location (Figures 8 and 9) as well as size (Figure 10), indicates that the difference in recoveries stems from human error. The recoveries suffered in this month due to the inefficiency of the operator (the first month constitutes the first attempts at this method), therefore this was more of an experimental error.

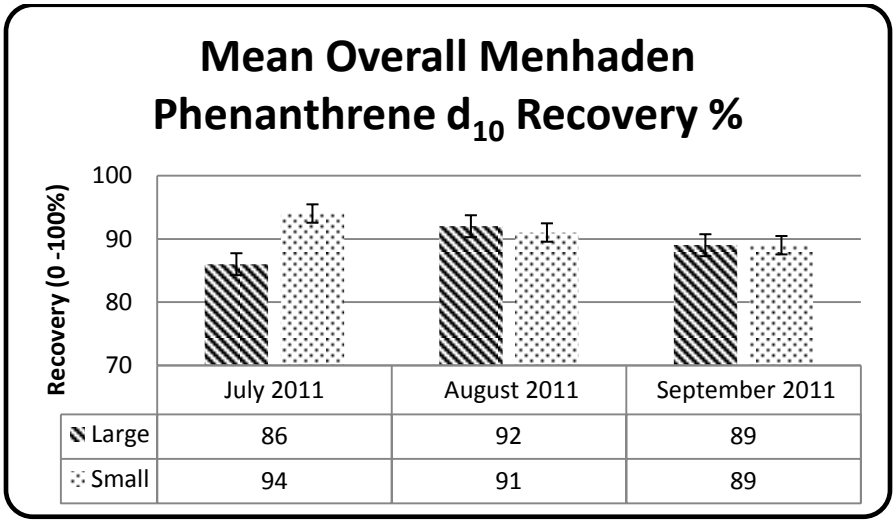
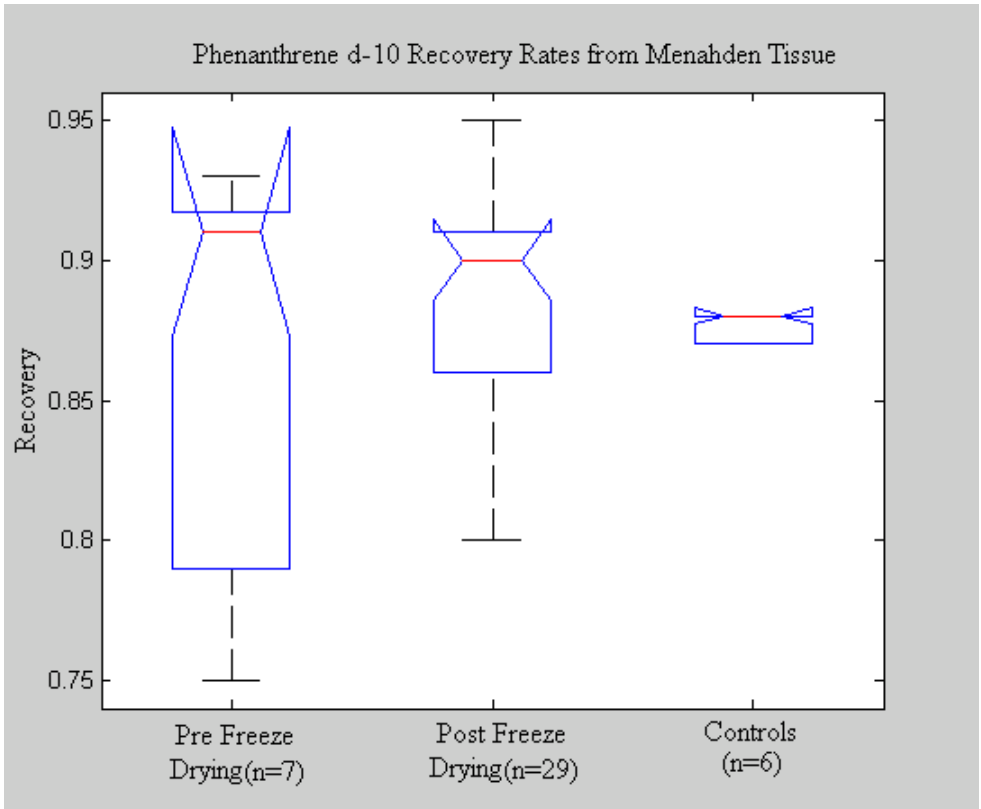


Figure 10: Overall mean phenanthrene d_{10} recoveries based on size and month: Summer 2011

It should be noted that these inefficiencies still resulted in an 81% recovery when comparing phenanthrene d_{10} by site and an 86% recovery when comparing phenanthrene d_{10} by size, which falls between the acceptable method range of 70–120%. Also, the *p*-values associated with these results do not indicate any significant difference in recovery. Statistical analysis of the recoveries using a one-way analysis of variance with an α of 0.05 showed that when comparing all sample means (Pre, Post, and Control) there was no significant difference. The *p*-value for the analysis was 0.57 identified in Figure 11.



ANOVA Table

Source	SS	df	MS	F	Prob>F
Groups	0.00212	2	0.00106	0.57	0.5686
Error	0.07224	39	0.00185		
Total	0.07436	41			

Figure 11: Analysis of variance of phenanthrene d_{10} recoveries.

Whole Fish Total PAH Concentration

With the ability to quantify raw oil, total PAH concentrations were determined for the lipid fraction. Again, these concentrations can be differentiated by fork length and location. As shown in Figure 12 ($P_{July} > 0.54$ $P_{Aug} > 0.17$ $P_{Sept} > 0.16$), large menhaden showed appreciable concentrations of total PAH concentration for July and September, 2011. A notable reduction in total PAH concentration was seen at both field locations for August, 2011 ($p < 4.5 \times 10^{-3}$).

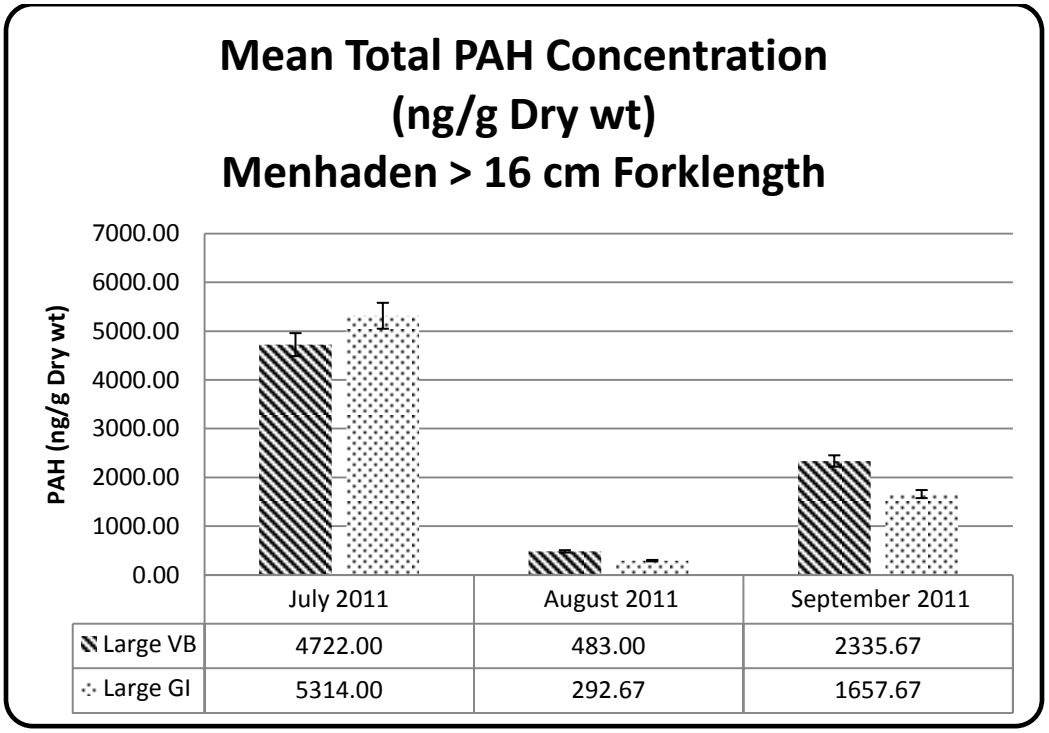


Figure 12: Mean Total PAH concentrations (ng/g dry wt) from large menhaden by location.

Figure 13 ($P_{july} > 0.11$ $P_{Aug} > 0.94$ $P_{Sept} > 0.48$) presents data for small menhaden by location. The data indicate that for the smaller fish, concentrations of total PAHs are highest in August. Total PAHs for all fish (see Figure 14) suggest that there is a relationship with seasonal feeding activity. Datasets are difficult to interpret based on a one-year sampling period. However, current

movement and seasonal feeding as well as a possible delay in aromatic accumulation should be more carefully tracked in future studies.

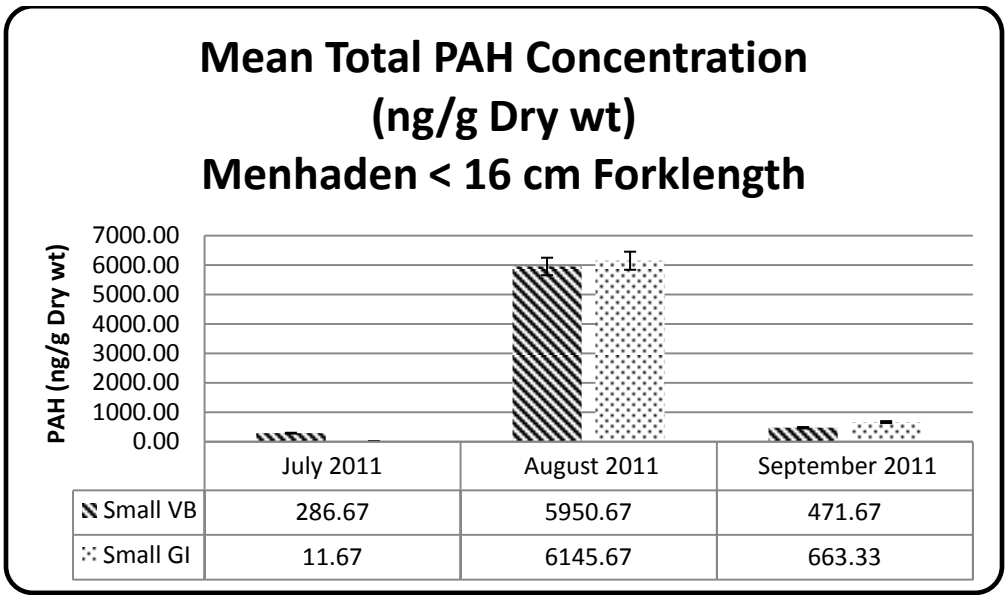


Figure 13: Mean Total PAH concentrations (ng/g dry wt) from small menhaden by location.

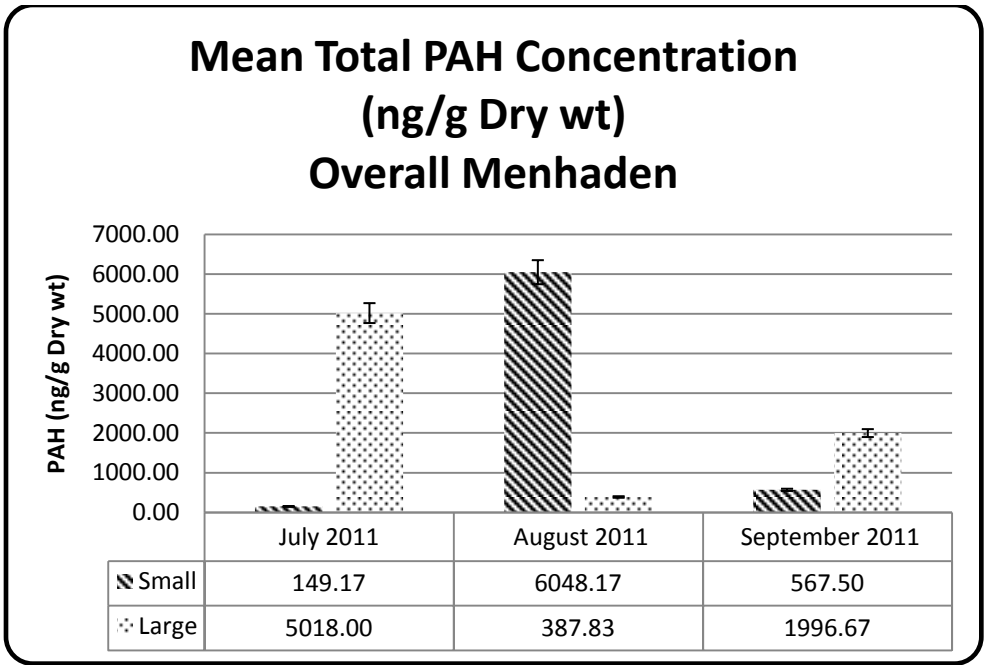


Figure 14: Mean Total PAH concentrations (ng/g dry wt) from all sampled menhaden by size.

Statistically, the site ($p > 0.98$) and size ($p > 0.94$) results were not significant at an α of 0.05 over the entirety of the study. The overall means for the "large" menhaden and "small" menhaden were 2468 ng/g (± 3082) and 2255 ng/g (± 2077) dry mass, respectively. However, when an analysis of variance was done on each individual month, PAH concentration (ng/g) was always statistically different (see appendix A for detailed ANOVA data).

As mentioned earlier, understanding PAH content in fish tissue and oil prior to the DWH spill may provide further insight into variations in content over seasons. A control menhaden facsimile tissue was formulated using meal and oil collected during June 2009 from a commercial source. Determining the appropriate oil/meal ratio for both "small" menhaden as well as "large" menhaden allowed for the creation of these facsimile controls. Datasets were generated using the same Soxhlet extraction method for raw oil. Table 4 presents data on the homogenized meal/commercial oil extracts.

Table 4: Menhaden facsimile samples based on calculated oil yields from menhaden samples.

ID	Control For	Phenanthrene d-10 Surrogate Recovery	Corrected Total PAHs (ng/g)	Adjusted Total PAHs (ng/g) for C3-Phenanthrenes	% C3-Phenanthrens
C1	Small*	0.88	2806	47	98.33%
C2	Small*	0.88	2940	47	98.40%
C3	Small*	0.88	3162	46	98.55%
C4	Large**	0.87	4030	47	98.83%
C5	Large**	0.88	4140	47	98.86%
C6	Large**	0.87	3929	47	98.80%

*Small ≈ 0.13 g menhaden oil/g dry tissue **Large ≈ 0.39 g menhaden oil/g dry tissue

Table 5a: Total PAH mean concentration by site and size: July 2011

Size	Avg Fresh Mass (g)	Avg Dry Mass (g)	Avg Fork Length (cm)	Avg Total PAH (ng/mg)	Avg Surrogate Recovery %	Avg Corrected* Total PAH (ng/g)	Avg Adjusted** Total PAH (ng/g)
Large VB	133.20	44.47	19.37	6708.67	80.67%	8398.67	4722.00
Large GI	112.95	37.35	18.30	8359.50	91.00%	9154.00	5314.00
Large Total	123.08	40.91	18.83	7534.08	85.83%	8776.33	5018.00
Small VB	79.03	25.27	12.78	3835.67	94.33%	4074.67	286.67
Small GI	77.67	21.63	12.73	2174.33	94.33%	2313.67	11.67
Small Total	78.35	23.45	12.76	3005.00	94.33%	3194.17	149.17

Corrected*= increased based on surrogate recovery. Adjusted**= C3-phenanthrenes removed

Table 5b: Total PAH mean concentration by site and size: August 2011

Size	Avg Fresh Mass (g)	Avg Dry Mass (g)	Avg Fork Length (cm)	Avg Total PAH (ng/mg)	Avg Surrogate Recovery %	Avg Corrected* Total PAH (ng/g)	Avg Adjusted** Total PAH (ng/g)
Large VB	119.00	47.07	18.47	2592.00	92.67%	2784.00	483.00
Large GI	115.53	46.60	18.37	2352.33	91.00%	2595.67	292.67
Large Total	117.27	46.83	18.42	2472.17	91.83%	2689.83	387.83
Small VB	109.60	31.00	14.65	7777.33	91.00%	8581.33	5950.67
Small GI	67.60	17.87	12.72	7314.00	90.00%	8122.00	6145.67
Small Total	88.60	24.43	13.69	7545.67	90.50%	8351.67	6048.17

Corrected*= increased based on surrogate recovery. Adjusted**= C3-phenanthrenes removed

Table 5c: Total PAH mean concentration by site and size: September 2011

Size	Avg Fresh Mass (g)	Avg Dry Mass (g)	Avg Fork Length (cm)	Avg Total PAH (ng/mg)	Avg Surrogate Recovery %	Avg Corrected Total PAH (ng/g)	Avg Adjusted Total PAH (ng/g)
Large VB	114.47	40.33	17.77	14044.67	90.33%	15563.00	2335.67
Large GI	121.30	45.50	18.60	9285.33	87.67%	10520.33	1657.67
Large Total	117.88	42.92	18.18	11665.00	89.00%	13041.67	1996.67
Small VB	91.30	48.13	13.48	4279.00	89.00%	4795.67	471.67
Small GI	87.57	44.77	13.58	3063.67	89.67%	3423.67	663.33
Small Total	89.43	46.45	13.53	3671.33	89.33%	4109.67	567.50

Corrected*= increased based on surrogate recovery. Adjusted**= C3-phenanthrenes removed

Table 5a, 5b, and 5c show total PAH mean concentrations by site and fish size over the three-month harvest in 2011. Datasets indicated that commercial oil and meal had notable total PAH concentrations. These whole fish concentrations fell within the range of both "small" and "large" fish. Importantly, the data suggest that characterization of whole fish Total PAH concentrations alone does not provide a complete picture of possible exposure and uptake of weathered oil in these locations.

Raw Oil/ PAH Correlations to Size and Location

The culmination of this study was to determine the PAH concentrations found within Gulf Menhaden off of the south/southeastern coast of Louisiana. Two sites were sampled for four months (October was not included due to an incomplete sampling), and the resulting data set was generated. All raw data points for every section within the Experiment and Results section can be found within Appendix B: Data. The results from the raw oil extractions coupled with the results from the extraction of the PAHs allow for the identification of oil-to-PAH ratios. Plotting the two parameters against each other results in a scatter plot that shows the Raw oil/PAH breakdown by size in Figure 15 ($P_{slopes} > 0.93$ $P_{elevations} > 0.10$) and by sample location in Figure 16 ($P_{slopes} > 0.61$ $P_{elevations} > 0.91$). Remember that the test for slopes is the first identifying test to determine if the best fit lines are the same, conversely the test for elevations should only matter if the test for slopes is inconclusive. This helps determine if the best fit lines are parallel or if they are in fact the same lines. Differentiating all plotted points by size and site should give an indication as to the distribution of the raw oil as compared to the total PAH concentrations measured within the gulf menhaden sampled throughout this study.

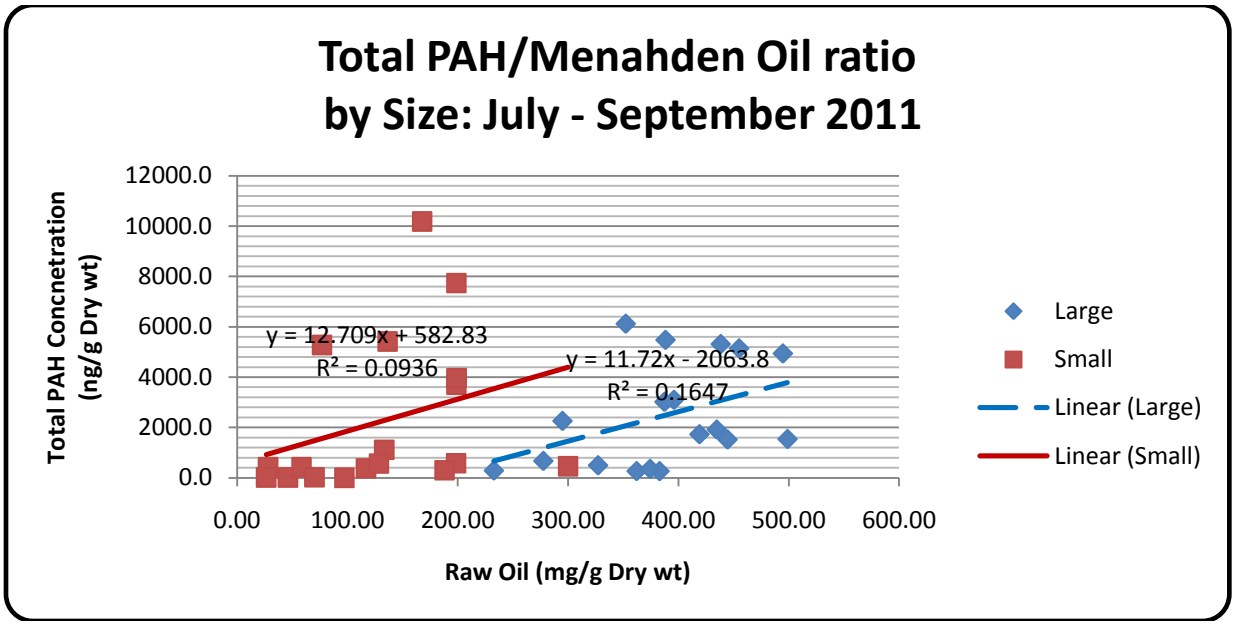


Figure 15: Variations in total PAH concentration for raw fish oil yield by size.

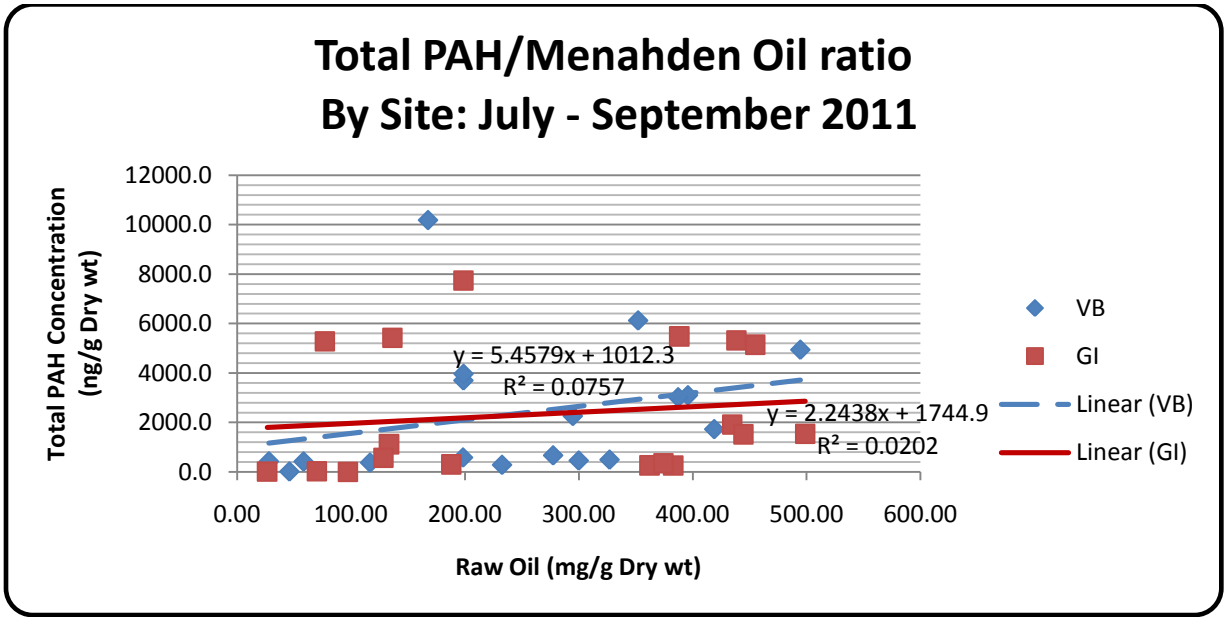


Figure 16: Variation in total PAH concentration for raw fish oil yield by site.

An analysis of covariance revealed no difference between size and location. If there was an effect on the coast of Louisiana, it was universal and could not be quantified with population sampling throughout one year. Sampling regions were chosen with the assumption that VB was a

possible control site based on Shoreline Cleanup Assessment Technique (SCAT) maps as reported by the EPA. The SCAT maps indicated the shorelines around VB were “clean” . However, this assumption proved to be inaccurate according to the collected data. Lack/presence of oiled shoreline was not a good indicator of pollution levels in the water column.

Table 6: Menhaden oil and corrected/adjusted PAH concentrations based on recovery of Phenanthrene d_{10}: Summer 2011

Month/Size	Site	Oil/Fish (mg/g dry fish)	Corrected/Adjusted** Total PAH (ng/g dry fish)
Jul Large	VB	352.17	6125.00
Jul Large	VB	395.91	3099.00
Jul Large	VB	494.60	4942.00
Jul Large	GI	388.21	5481.00
Jul Large	GI	454.92	5144.00
Jul Large	GI	438.26	5317.00
Jul Small	GI	97.22	0.00
Jul Small	GI	26.32	14.00
Jul Small	GI	70.06	21.00
Jul Small	VB	27.97	425.00
Jul Small	VB	45.98	15.00
Jul Small	VB	58.39	420.00
Aug Small	VB	198.76	3700.00
Aug Small	VB	198.86	3966.00
Aug Small	VB	167.66	10186.00
Aug Small	GI	198.68	7739.00
Aug Small	GI	136.36	5419.00
Aug Small	GI	76.92	5279.00
Aug Large	VB	277.51	669.00
Aug Large	VB	327.09	495.00
Aug Large	VB	232.67	285.00

Continuation of Table 6: Menhaden oil and corrected/adjusted PAH concentrations based on recovery of Phenanthrene d_{10}: Summer 2011

Aug Large	GI	362.04	264.00
Aug Large	GI	382.81	262.00
Aug Large	GI	374.27	352.00
Sept Small	VB	116.79	374.00
Sept Small	VB	300.00	459.00
Sept Small	VB	198.41	582.00
Sept Small	GI	133.33	1118.00
Sept Small	GI	128.44	570.00
Sept Small	GI	188.03	302.00
Sept Large	VB	418.99	1729.00
Sept Large	VB	387.28	3018.00
Sept Large	VB	294.85	2260.00
Sept Large	GI	434.67	1915.00
Sept Large	GI	498.80	1539.00
Sept Large	GI	444.44	1519.00

**Adjusted refers to the removal of C3-phenanthrenes from Total PAH based on the control menhaden facsimiles created with menhaden tissue and oil collected before the DWH spill.

Assessment of Menhaden Total PAH Concentrations Based on October 2010 and October 2011

During the Month of October 2010, menhaden samples were taken and kept frozen until the designed sonication-assisted MSPD method could be applied. The concentrations of PAHs from Oct 2010 as compared to Oct 2011 can be seen in Figure 17. This was the only sample that was taken by our lab during the 2010 gulf menhaden season. Unfortunately the affected areas were closely guarded and monitored, making proper sampling during this time frame quite difficult. It should be noted that the data generated from one month during the 2010 season should not be used to characterize the entire menhaden catch of 2010. This is simply an attempt to gain a better understanding of the possible effects of the DWH spill and to put future data into an appropriate perspective.

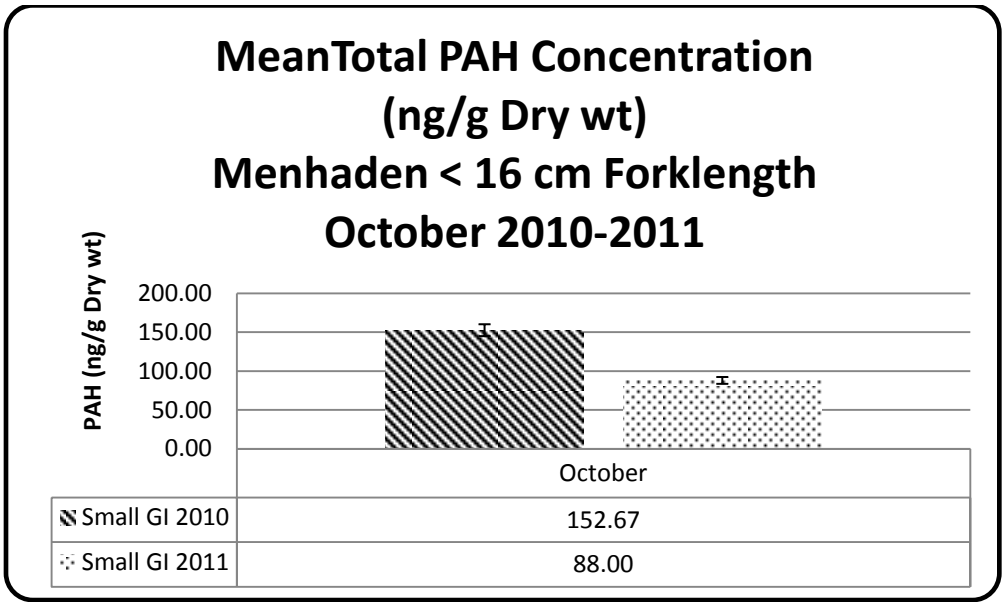


Figure 17: Mean total PAH concentrations collected from menhaden with fork lengths < 16cm based on location and time. Note: C3-phenanthrenes have been removed based on controls.

Analysis of variance was performed on and the results indicated that at an α of 0.05 the means of the total PAH concentrations between October 2010 and October 2011had a *p*-value of 0.59 which indicates that there is not a significant difference between October 2010 and October 2011 total PAH concentrations. It therefore cannot be concluded with any certainty that these separate sampling times are different.

Benzo[a]pyrene Toxic Equivalencies and Mutagenic Equivalencies

It is important to understand the Benzo[a]pyrene toxic equivalencies (B[a]P-TEQs) as well as Benzo[a]pyrene mutagenic equivalencies (B[a]p-MEQs) of the PAHs isolated from each menhaden. In the current study no allowance has been made for alkylated PAH other than within the values for the sum of all PAH determined. Parent compound data are the only data to have been used in the calculation of B[a]P-TEQ and B[a]p-MEQ values. In order to calculate B[a]P-

TEQs, the proper toxic equivalency factor (TEF) must be used to augment the concentration. These TEFs (proposed by Nisbet and LaGoy, 1992) are listed on Table 8. From this point the PAH concentrations were multiplied by their respective TEF to give the individual concentration per menhaden. These numbers were summed to give a "total" B(a)P-TEQ in ng/g dry mass. PAHs not found on the Nisbet and LaGoy list as well as compounds that were found in Nisbet and LaGoy but not isolated during this study were not factored into the total B[a]P-TEQ for each sample. The B[a]p-MEQs were determined using the minimum mutagenic concentrations (MMCs) found in Durant et al. (1996).

Table 7: List of Toxic Equivalency Factors and Mutagenic Equivalency Factors used to quantify the total Benzo[a]pyrene Toxic Equivalencies as well as the Benzo[a]pyrene Mutagenic Equivalencies.

Compound	TEF*	MEF**
Dibenz[a,h]Anthracene	5	0.29
Benzo[a]Pyrene	1	1
Indeno[1,2,3 - cd]Pyrene	0.1	0.31
Pyrene	0.001	0
Benzo[b]Fluoranthene	0.1	0.25
Benzo[k]Fluoranthene	0.1	0.11
Benzo[g,h,i]Perylene	0.01	0.19
Fluoranthene	0.001	0
Benzo[a]Anthracene	0.1	0.082
Chrysene	0.01	0.017
Anthracine	0.01	[2]na
[1]Acenaphthene	0.001	0
[1]Acenaphthylene	0.001	0.00056
Fluorene	0.001	[2]na
[1]2-Methylnaphthalene	0.001	[2]na
Naphthalene	0.001	[2]na
Phenanthrene	0.001	[2]na

*TEF = Toxic Equivalency Factor (see Nisbet and LaGoya, 1992)
**MEF = Mutagenic Equivalency Factor (Minimum Mutagenic Concentration [B(a)P]/MMC [Selected PAH] where [ng/ml] see Durant et al., 1996)
1 = Compound was not an analyte of interest and was therefore not quantified for this study.
2= Compound was not analyzed in Durant et al., 1996

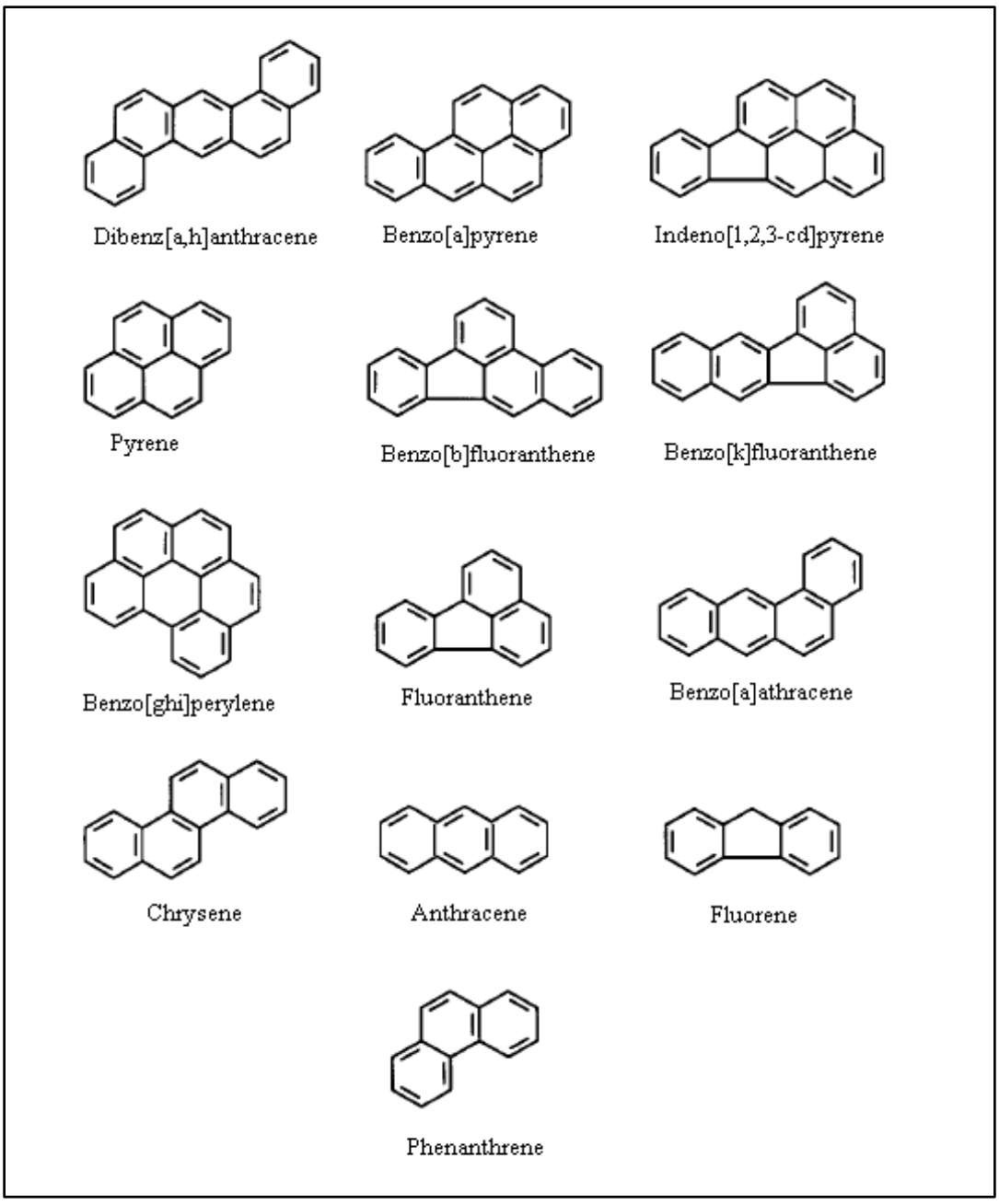


Figure 18: PAHs with identified B[a]P TEFs

Dividing the MMC of B[a]P by the MMC of the desired PAH, a mutagenic equivalency factor (MEF) can be generated and applied to the concentration of a particular PAH. Using Durant et al. (1996) MEFs were identified for the majority of the same compounds with TEFs and can be seen on Table 7.

From these data it was possible to determine an overall mean for both the B[a]P-TEQs and B[a]P-MEQs as well as monthly means broken down by size as can be seen in Table 7. Figure 19 shows the significant difference in the B[a]P-TEQs between "large" and "small" menhaden over the summer of 2011. This difference demonstrates that the "large" menhaden are more carcinogenic than the "small" menhaden ($P_{July} < 0.05$ $P_{Aug} < 9.0\text{x}10^{-4}$ $P_{Sept} > 0.41$).

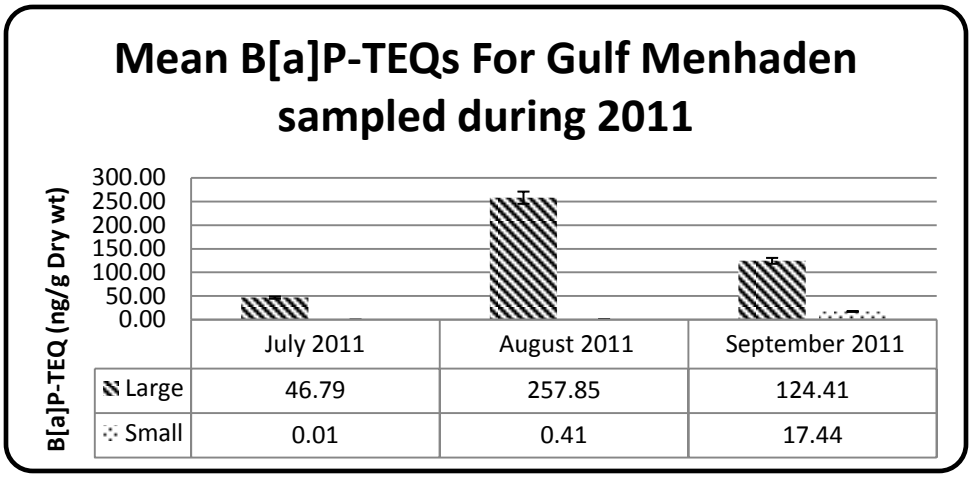


Figure 19: Monthly B[a]P-TEQs for both "large" and "small" menhaden.

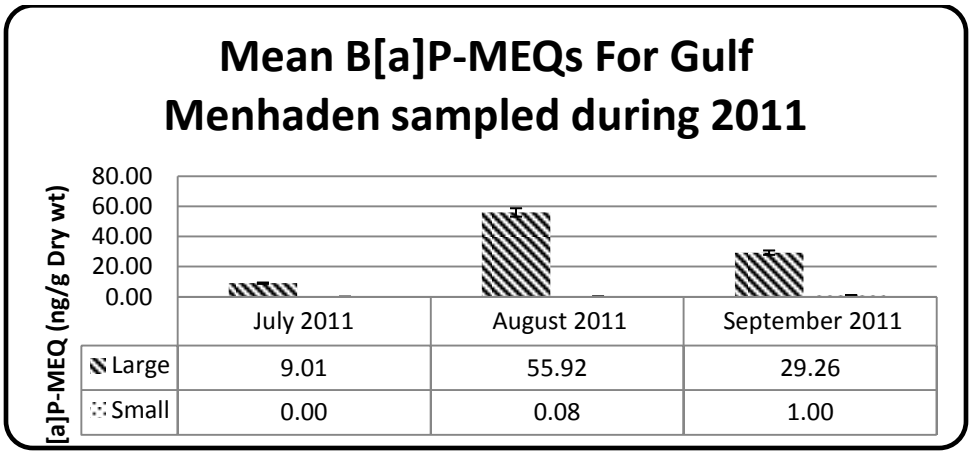


Figure 20: Monthly B[a]P-MEQs for both "large" and "small" menhaden.

Table 8: Toxic and Mutagenic equivalencies for Gulf Menhaden sampled during the summer of 2011.

	Size	Mean B[a]P-TEQ*	Mean B[a]P-MEQ**
July 2011	All	23.40	4.51
	Lg	46.79	9.01
	Sm	0.01	0.00
Aug 2011	All	129.13	28.00
	Lg	257.85	55.92
	Sm	0.41	0.08
Sept 2011	All	70.92	15.13
	Lg	124.41	29.26
	Sm	17.44	1.00
Overall	All	74.48	15.88
	Lg	143.02	31.40
	Sm	5.95	0.36

* = Summation of applicable compounds listed on Table 7.
** = Summation of compounds with applicable MEF values from Table 7.

The B[a]P-MEQs follow a similar pattern, showing that the "large" menhaden have a more mutagenic quality to them than the "small" menhaden in Figure 20 ($P_{July} < 4.3x10^{-3}$ $P_{Aug} < 0.05$ $P_{Sept} > 0.31$). Seasonally, the B[a]P-MEQs were not significantly different ($p > 0.52$) but the B[a]P-TEQs were ($p < 7.2 \times 10^{-3}$), suggesting that the "large" menhaden are significantly more carcinogenic than the "small" menhaden.

Examination of the B[a]P-TEQs and B[a]P-MEQs for the October 2010 menhaden sampled from GI and comparison of them to the 2011 values does show a significant difference between years ($p > 0.37$ and $p > 0.31$ respectively). These data have to be taken lightly because there was only one sampling event from this region during 2010. The differences can be seen in

Figures 21 and 22, and show that even though the overall PAH concentrations were similar for these two months (see Assessment of Menhaden Total PAH Concentrations based on October. 2010 and October 2011) the B[a]P-TEQs were very different

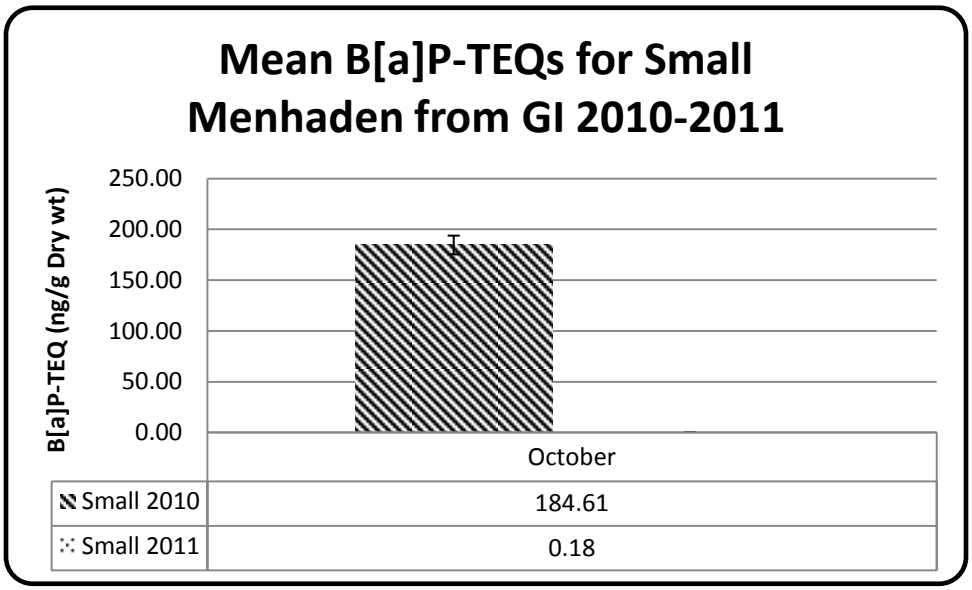


Figure 21: Mean B[a]p-TEQs for "small" menhaden sampled at GI during October 2010 and 2011

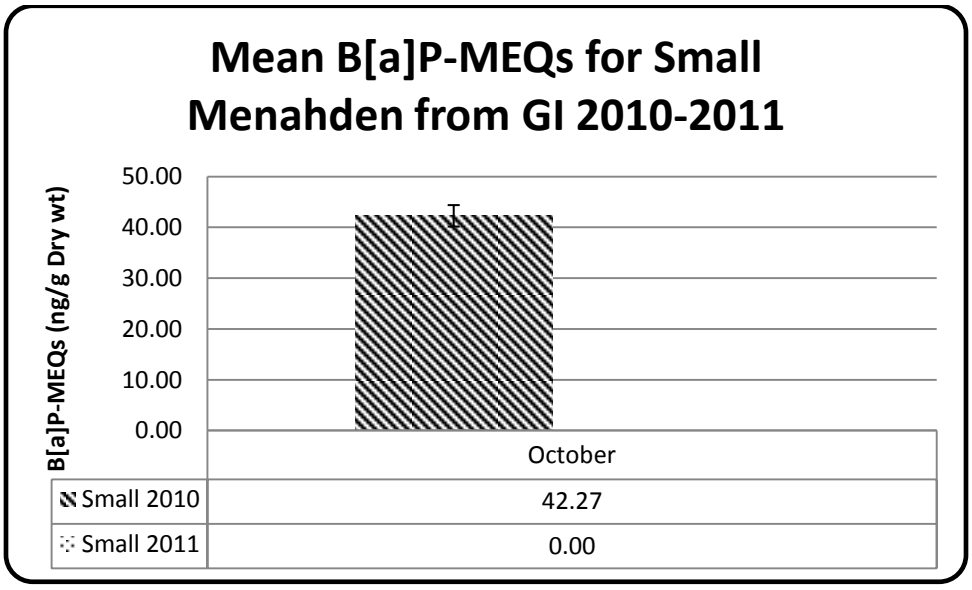


Figure 22: Mean B[a]P-MEQs for "small" menhaden sampled at GI during October 2010 and 2011

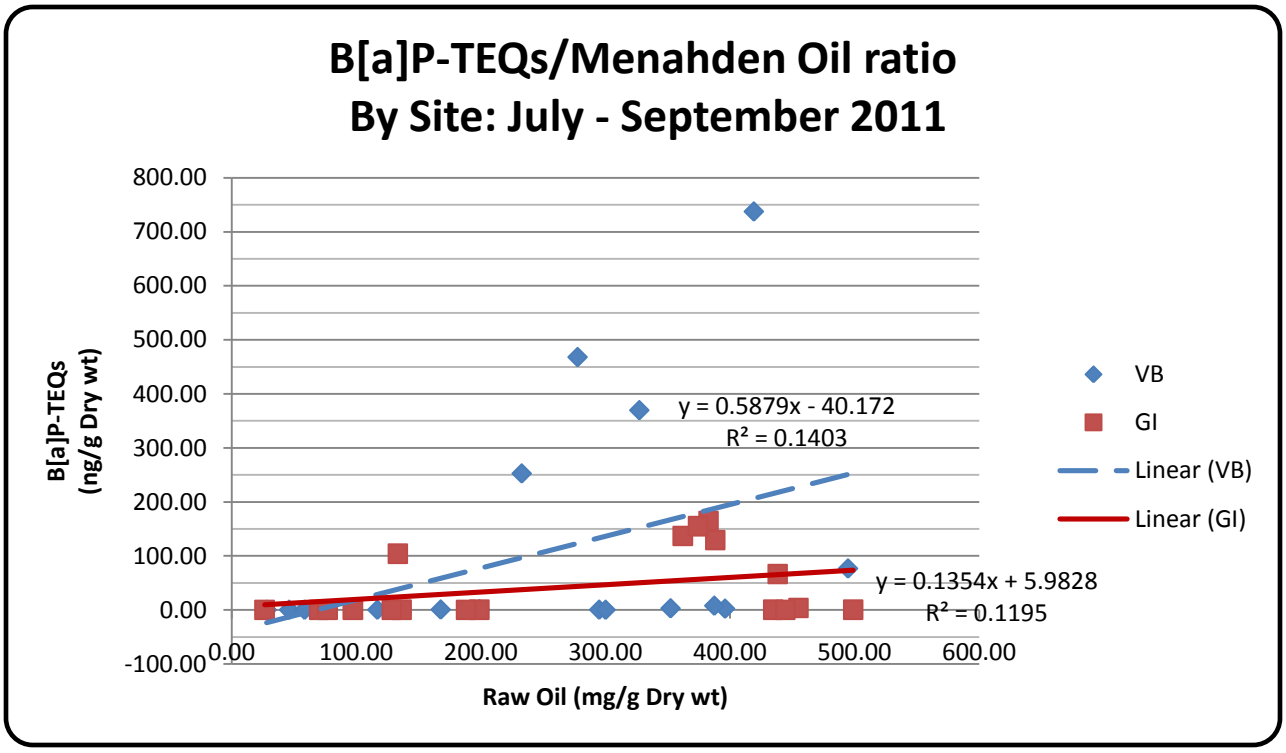


Figure 23: Variations in total B[a]P-TEQ concentration for raw fish oil yield by site.

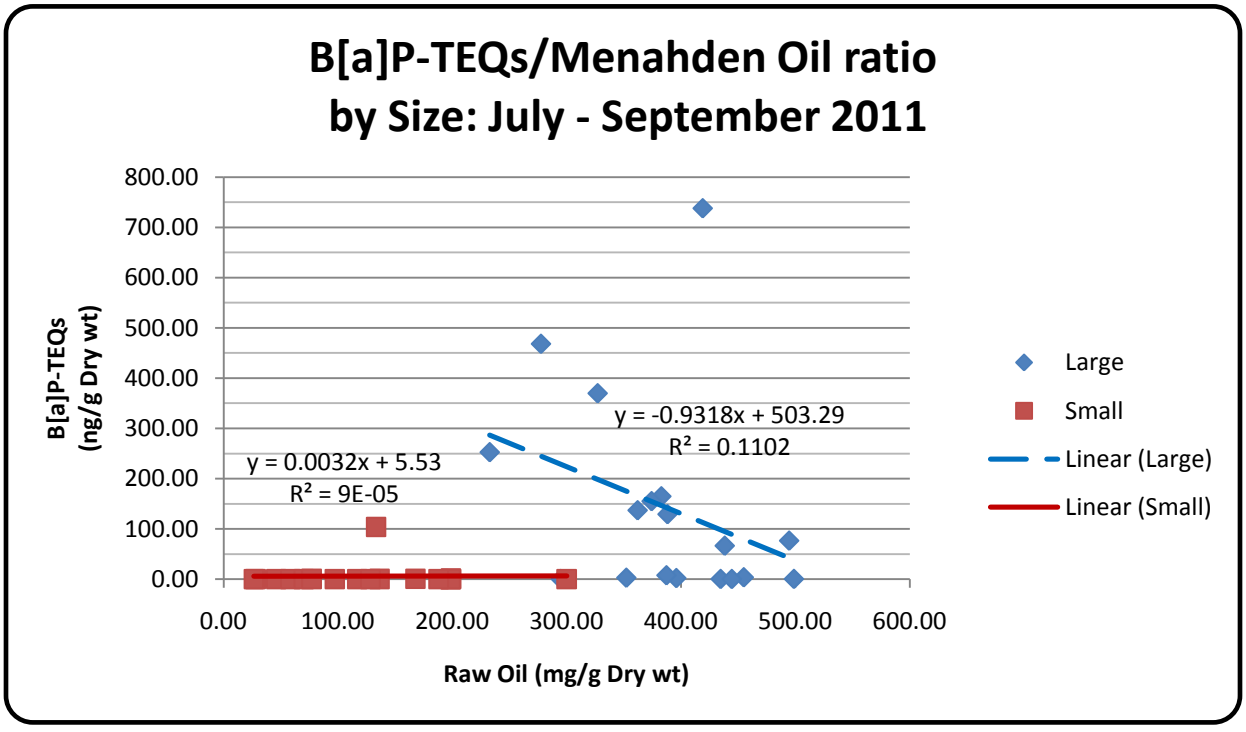


Figure 24: Variations in total B[a]P-TEQ concentration for raw fish oil yield by size.

Figure 21 shows that menhaden caught the previous year were exposed to more carcinogenic PAHs than those caught in 2011. The same can be said about the concentration of mutagenic PAHs with respect to year collected. Again it should be noted that these differences are not significant enough to make an assessment about the status of the Gulf of Mexico during 2010. More sampling events could have given a better indication; however, the data needed to properly assess the impact of the DWH spill were not generated during 2010. Figure 23 shows that there is no discernable difference in B[A]P-TEQs (because there was no significant difference between B[a]P-MEQs over the course of the study, $p > 0.52$, an analysis of covariance was not applied to that data) between sampling locations ($P_{slopes} > 0.21$ $P_{elevations} > 0.17$). Figure 24 ($P_{slopes} > 0.16$ $P_{elevations} < 0.01$), however, shows that there is a significant difference in the elevations for each data point. This means that the "large" menhaden have significantly different concentration of carcinogenic PAHs as well as raw menhaden oil.

DISCUSSION

Regional Variations Due to Potential Oiling

Based on the analysis of covariance performed on the current dataset, it can be concluded that the two sampling regions were not statistically different with respect to PAH concentration versus raw menhaden oil. Therefore it cannot be determined that the existing PAH concentrations are a result of the 2010 DWH oil spill.

Based on analysis of B[a]P-TEQs and MEQs it can be concluded that the overall "large" menhaden population has significantly higher carcinogenic (B[a]P-TEQs) PAH concentrations than "small" menhaden. This suggests that these fish were exposed to an event that the smaller menhaden were not. It is important to remember that the 2011 "large" menhaden were juvenile/"small" menhaden from 2010. The data was not conclusive for the October 2010 and 2011 comparison, however, elevated levels of carcinogenic/mutagenic PAHs were measured in the 2010 menhaden. The PAH concentrations found in the 2011 "large" menhaden may be a legacy of the oil spill and continued analysis of menhaden throughout successive years will provide data to make conclusions on the legacy of the DWH oil spill.

In order to accurately determine if there was a significant change in PAH concentrations within menhaden populations off the coast of Louisiana, the study should be conducted for an additional 2–3 years based on the whole life cycle of a gulf menhaden. Further sampling events at both VB and GI would reveal whether or not PAH concentrations as well as B[a]P-TEQs diminish and/or increase over time. Yearly changes in PAH concentration B[a]P-TEQs could be correlated subsequent to the DWH oil spill, and it may be possible to assign contributing sources to these changes. Additional enumeration of data would allow researchers to answer the question: Did the DWH oil spill effect PAH concentrations within Gulf Menhaden?

Temporal Factors Contributing to PAH concentrations in Menhaden

Several factors contributed to the overall PAH concentrations found within Gulf Menhaden. It is important to identify the initial concentrations in order to better quantify the data collected over time. This study attempted to determine if PAH concentrations differed spatially from GI to VB as well as with size based on fork length. An analysis of covariance indicated that neither location nor size demonstrated a statistically significant difference in total PAH concentration. October data from 2010 were compared to October data from 2011 for small menhaden in order to determine if there was a possible difference. A one way analysis of variance was performed on the total PAH concentration means to determine if there was a significant difference. A trend was identified showing that these two temporal events demonstrated means that were not statistically different in total PAH concentrations. However, these data represented only one month sampled from 2010 and 2011 and cannot be used to adequately quantify the differences between years. The need for more temporal data has been clearly identified based on the results found in this study.

Conclusions

The concentration of PAH's as well as raw menhaden oil were measured within GoM Menhaden. The menhaden were extracted via a modified MSPD extraction process using C-18 silica followed by Gas Chromatography analysis using a method that identifies 71 key constituents within crude oil, 43 components identified as aromatic (contains the PAHs), and a simple Soxhlet extraction was used to extract the raw menhaden oil. The filter-feeding nature of Gulf Menhaden presented a vector for possible PAH accumulation within menhaden and possibly trophic level transfer to organisms that feed on these menhaden. The PAH levels in both "small" (fork length < 16 cm) and "large" (fork length > 16 cm) menhaden was compared during

the months from July to October of 2011. Based on one- and two-way ANOVA analysis using an α of 0.05, it was concluded that the location of the sampling events did not show statistical difference in total PAHs. The individual months, however, did show statistical differences from each other. Total PAH concentrations were never statistically similar between months for both "large" and "small" menhaden, this suggests differences in feeding patterns as well as movement patterns. The nested ANOVA did not show significance between sizes; however, upon further analysis between the interaction of these variables it was determined that the size of the menhaden (depending on month) had either a synergistic or antagonistic affect on the total PAHs measured within the samples. Because of the strong influence temporal sampling had on the total PAH concentrations for a season; the individual months were analyzed using a one-way

Table 9: Statistical analysis of PAH content in menhaden by site, size and month (summary of PAH 2 way and nested ANOVA results. See appendix A for detailed ANOVA data).

ANOVA2 Total PAH (ng/g) **Large** menhaden by site and month							
Fsite	0.0800	<	FstatSt	4.7472	Accept	Means are not statistically different*	pvalue 0.7821
Fmonth	69.4855	>	FstatM	3.8853	Reject	Means are statistically different*	pvalue 0.0001
Finteraction	1.2903	<	FstatI	3.8853	Accept	Interaction is not statistically significant*	pvalue 0.3108
						* significance of 0.05	
ANOVA2 Total PAH (ng/g) **Small** Menhaden by site and month							
Fsite	0.0142	<	FstatSt	4.7472	Accept	Means are not statistically different*	pvalue 0.9071
Fmonth	24.9729	>	FstatM	3.8853	Reject	Means are statistically different*	pvalue 0.0001
Finteraction	0.0362	<	FstatI	3.8853	Accept	Interaction is not statistically significant*	pvalue 0.9646
						* significance of 0.05	
Nested ANOVA **Total** PAH Concentrations (ng/g) in Menhaden 2011							
Fsite	0.1618	<	FstatSt	2.5082	Accept	Means are not statistically different*	pvalue 0.9845
Fmonth	196.9703	>	FstatM	4.5337	Reject	Means are statistically different*	pvalue 0.0001
Fsize	0.0083	<	FstatSi	7.7086	Accept	Means are not statistically different*	pvalue 0.9381
						* significance of 0.05	
ANOVA2 **Total** PAH (ng/g) by month and size 2011							
Fsize	0.1601	<	FstatSz	4.1709	Accept	Means are not statistically different*	pvalue 0.6919
Fmonth	9.1172	>	FstatM	3.3158	Reject	Means are statistically different*	pvalue 0.0008
Finteraction	66.5907	>	FstatI	3.3158	Reject	Interaction is statistically significant*	pvalue 0.0001
						* significance of 0.05	

analysis of variance to determine if on a month-to-month basis size was a determining factor in total PAH concentrations. It was found that for all months sampled to completion, the "large"

menhaden and “small” menhaden were always significantly different at an α of 0.05 as seen in Figure 25.

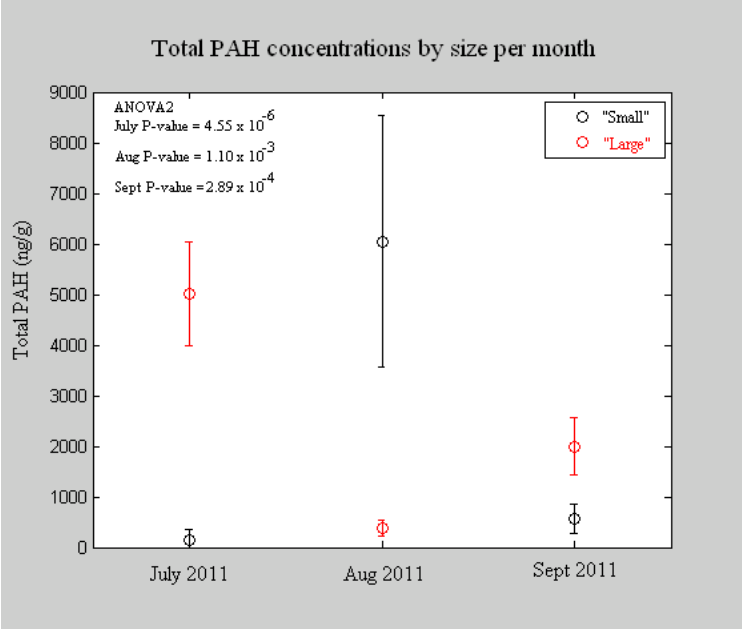


Figure 25: Total PAH concentrations found in gulf menhaden by size per month. Note: Data indicates statistically significant difference in size over time

As can be seen on Table 9, the significance of the PAH concentrations was not present over the whole study. The mean “small” total PAH concentration of approximately 2255 ng/g dry tissue was not statistically different from the mean “large” total PAH concentration of 2468 ng/g dry body tissue. An examination of the B[a]P-TEQs and B[a]P-MEQs, however, shows a significant statistical difference, with “large” menhaden averaging 143 ng/g and 31.4 ng/g respectively while “small’ menhaden had a mean of 5.95 ng/g and 0.36 ng/g respectively. Based on this study, it can be concluded that “large” menhaden contain higher concentrations of carcinogenic PAHs than “small” menhaden, even though the overall total PAH concentrations are not statistically different. This possible legacy from 2010 menhaden should be used to identify lingering effects on the GoM fishery. Further analysis will show whether the

carcinogenic PAHs found in adult/"large" menhaden came from a specific event or if they are present in all adult menhaden.

Future Research

The need for more temporal data has been demonstrated clearly with the results of this initial study. Based on the results the regions sampled were either affected equally of not at all by the DWH spill. Only subsequent years of sampling and PAH concentration quantification can show an increase (more oil spills would contribute to this), show a decrease, (either the DWH spill did affect the PAH concentration or oil spill frequency is decreasing), or show no change at all (the DWH had little to no effect on PAH concentrations). Menhaden generally live for three years, so it would be pertinent to conduct this study for at least two more years in order to have whole-life datasets on these menhaden (2011–2013). Similar studies should be conducted in all of the major industrialized waters of the United States. It may be possible to identify specific chemical signatures and correlate them to an urban area, allowing for a better understanding in terms of the fate of particular industrialized, commercial, and residential processes.

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APPENDIX A: EXPERIMENTAL PROTOCOLS

MSPD C-18 Silica Extraction Process

Purpose

The purpose of this method is to extract Polycyclic Aromatic Hydrocarbons (PAHs) from menhaden tissue.

****
Where applicable all materials including chemicals should be solvent rinsed and then dried before use (Not the C-18 silica). All Plastic components should be only solvent rinsed with Methanol.
****

Materials

150-200 ml Beakers
400-600 ml Beakers
100 ml Glass Graduated Cylinder
Spatula
Sonicator
Rotary Evaporator
Büchner flask (250 ml)
Büchner funnel with a sintered glass disc (350 ml)
Filter paper
Blender/Coffee Mill (Sunbeam Heritage Series Kitchen Assistant 2774 or similar item)
500 ml Beaker
Glass Funnel
1 ml Gas Tight Syringe
25 µl Gas Tight Syringe
GC Bottles and Caps
Capping Device

Chemicals

Dichloromethane (DCM)
Hexanes
Methanol
C-18 Silica
Sodium Sulfate

Washing

All materials should be washed using the following method.

Soak in hot soapy water over night.
Wash with hot soapy water again.
Rinse with hot water 3 times (if the item holds liquids fill to the top 3 times)
Rinse with DI water 3 times (if the item holds liquids fill to the top 3 times)
Rinse with methanol to remove water.
Rinse with DCM and allow the item to flash in a hood.
Bake in a vented oven until completely dry.
Cover any open areas with aluminum foil until use.

The glass wool and the sodium sulfate need to be solvent rinsed. Do this by placing an amount of the wool or sodium sulfate in a beaker (250 ml is fine, but this is up to you) then rinse with DCM. Allow this to flash in the hood overnight and then remove to a vented oven and bake until dry. Cover with aluminum foil and store until needed.

Pasture Pipettes and the Graduated Syringes should be rinsed as per step 9A and 9B below.

Procedure

1. Take frozen menhaden and cut them into pieces. Arrange them into a labeled beaker and using a glass pestle gently compress the menhaden pieces into the beaker. Make a note as to how many organisms were used for the sample. Cover with foil and puncture two to three holes into the top.

2. Cool sample to -60°C or lower, then place in a freeze dryer for 36-48 hours. Remove and store in a dessicator until ready for step 3.

3. Homogenize the freeze dried material until all of the tissue has been evenly distributed. Remove a 10 g subsample of the tissue back into the grinder and add a 1:1 ratio dry weight C-18 Silica. Homogenize further until the material is powdery and well broken down. Add an amount of sodium sulfate to cover the top of the contents in the beaker. Do not blend the sodium sulfate, simply mix with a spatula until evenly dispersed. (Between samples rinse the blender/coffee mill with water, then apply methanol to a cloth or paper towel and wipe the blades and container and then allow it to dry under the hood.

A. Excess Tissue:

Place the excess tissue on a large piece of foil and fold into a square. Place this enclosed foil into a piece of butcher paper (standard printer paper is also acceptable) and fold into a square. Label and store in a 4°C freezer for possible later use.

4. Fill the beaker with DCM until the tissue is covered, then using the solvent rinsed spatula mix thoroughly. Sonicate for 20 minutes. After sonication, use the spatula to again mix thoroughly.

5. Using a Büchner flask (attached to a vacuum) with a Büchner funnel (with a sintered glass disc) filter the tissue extract into the flask. Once the flask is full remove the contents to a labeled flat bottom flask.

6. Evaporate the extract in a rotary evaporator until there is no DCM left. Transfer the extract to a graduated cylinder using hexanes and rinse the flat bottom flask with hexanes into the graduated cylinder for final volume measurement three times (This allows for the hexanes exchange). It may be necessary to reconstitute the material with Hexanes first before the contents are transferred.

A. If the material within the flat bottom flask is minimal then the contents should be transferred to a 15 ml graduated concentrator tube for final volume measurement (7A).

7. Using hexanes dilute the recovered extract to a whole number volume. Mix thoroughly with a solvent rinsed pasture pipette. Make a final volume measurement and record in ml. Transfer 20 ml of the resulting material using a pasture pipette to a volatile organic analysis (VOA) bottle for storage. Allow to settle for 24 hours.

A. Using the graduated concentrator tube:

Attach a Snyder column to the concentrator tube and heat in a water bath until you are left with 1 ml of extract. If the material seems to be rather dark in coloration you will need to dilute with pure hexanes (this will always be the case with menhaden).

i. <u>Dilute the sample:</u>

Use the graduated concentrator tube to measure the volume of added hexanes. Fill the tube to 10 ml using pure hexanes and then transfer to the previously used VOA bottle associated with this sample. Remember to rinse this VOA bottle with hexanes 3 times before making the transfer. Fill the graduated concentrator tube back to the 10 ml mark again and then transfer this amount to the VOA bottle. There is now a final volume of 20 ml which can then be sampled to run on the GC/MS. This final volume will be required to interpret the data.

step 8 is only followed if the sample is relatively clear, otherwise follow Step 9 for dilution and simply remove 1 ml as needed to be analyzed on the GC/MS

8. It is important to concentrate to 1 ml or less (this is for samples that will not be diluted). If you use the 1 ml syringe to remove all the liquid and there is more than 1 ml in the syringe place the liquid back in the concentrator tube and continue to evaporate until there is 1 ml or less. If there is exactly 1 ml, then transfer to a GC bottle and then add the internal standard (this will usually be done with the graduated 25µl syringe). If the volume is less than 1 ml, pull an amount of clean hexanes into the syringe that will give a final volume of 1 ml.

9. If you are pulling from a diluted sample simply remove 1 ml from the 20 ml dilution and place in the GC bottle. Once this is completed, cap the GC bottle and store at 4°C until step 10.

A. Cleaning the syringe and adding "clean" hexanes:

It is important to have two VOA bottles marked clean hexanes and waste. Fill the VOA bottle labeled clean hexanes with clean hexanes. Any time that you need to add hexanes to a sample as mentioned above use this volume of hexanes. Remember to rinse the needle of the syringe with hexanes before placing it into the "clean" hexanes bottle. If you simply want to clean the syringe, rinse the needle and then draw in a full amount of hexanes. Expel this into the VOA bottle marked waste. Repeat this process 3 times. This can be done when working with DCM as well. Simply follow the steps, but instead of hexanes use DCM.

B. Preparing the syringe:

Once the syringe has been cleaned as stated above (9A) it is important to remove the possible dilution factor of residual Hexanes or DCM left in the syringe. This can be done by simply drawing in a small amount of the liquid to be transferred with the syringe and then drawing that liquid back and forth into the syringe several times. Discard the amount of liquid drawn into the syringe in the usual manner. Repeat as needed.

10. When ready run the samples on a GC/MS.

Raw Oil Recovery Using Soxhlet Extraction Procedure

Purpose

The purpose of this method is to extract Raw (un refined) Menhaden Oil from selected individuals.

****

Where applicable all materials including chemicals should be solvent rinsed and then dried before use. All plastic components should be only solvent rinsed with Methanol.

****

Materials

150-200 ml Beakers
400-600 ml Beakers
100/250 ml Glass Graduated Cylinder
Spatula
Soxhlet Extraction Tubes
Soxhlet Condenser Tubes
Rotary Evaporator
Blender/Coffee Mill (Sunbeam Heritage Series Kitchen Assistant 2774 or similar item)
Glass Funnel

1 ml Gas Tight Syringe
Cellulose Extraction Thimbles
Boiling Stones
250 ml Florence Flasks (flat bottom)
Hot Plate

Chemicals

Dichloromethane (DCM)
Hexanes
Methanol
Sodium Sulfate

Washing

All materials should be washed using the following method.

Soak in hot soapy water over night.
Wash with hot soapy water again.
Rinse with hot water 3 times (if the item holds liquids fill to the top 3 times)
Rinse with DI water 3 times (if the item holds liquids fill to the top 3 times)
Rinse with methanol to remove water.
Rinse with DCM and allow the item to flash in a hood.
Bake in a vented oven until completely dry.
Cover any open areas with aluminum foil until use.

The glass wool and the sodium sulfate need to be solvent rinsed. Do this by placing an amount of the wool or sodium sulfate in a beaker (250 ml is fine, but this is up to you) then rinse with DCM. Allow this to flash in the hood overnight and then remove to a vented oven and bake until dry. Cover with aluminum foil and store until needed.

Pasture Pipettes and the Graduated Syringes should be rinsed as per step 9A and 9B in the MSPD C-18 Silica extraction Protocol.

Procedure

1. Take frozen menhaden and cut them into pieces. Arrange them into a labeled beaker and using a glass pestle gently compress the menhaden pieces into the beaker. Make a note as to how many organisms were used for the sample. Cover with foil and puncture two to three holes into the top.

2. Cool sample to -60°C or lower, then place in a freeze dryer for 36-48 hours. Remove and store in a dessicator until ready for step 3.

A. Preparing the Florence flasks

i. During this time gather the amount of Florence flasks that will be needed. Add 1-2 boiling stones and label them according to sample. Then record the flasks mass.

ii. Once the flasks are massed, add 100 ml of DCM to the flasks and cover with foil until step 5.

3. Homogenize the freeze dried material with sodium sulfate until all of the tissue has been evenly distributed. Return the material to the beaker that it was freeze dried with and gather the proper amount of cellulose extraction thimbles.

4. Pack the comminuted material into the cellulose extraction thimbles (generally the smaller menhaden will fit within one cellulose extraction thimble where as the larger menhaden will need at least two). Spike the tissue with the surrogate spiking solution (for the larger menhaden that require two or more thimbles you will only need to spike one of the thimbles) and place into the soxhlet tube. *It has been observed that placing a bit of glass wool at the bottom of the soxhlet extraction tube prevents the majority of the particulates that will float out from entering the sample.

5. Fill the soxhlet extraction tube with just enough solvent to reach the small bubble in the evacuation arm and then place the extraction tube into the Florence flask. Place this combined apparatus with the condenser tube hooked to a coolant. Make sure that the flat bottom of the flask is sitting flush with the hot plate. Turn on the hot plate to medium heat and allow for the solvent to boil. Then reduce the heat slightly below medium and allow for 16-18 hour extraction (usually over night).

A. Preparing to remove the Tubes.

Turn off the hot plates and allow the apparatus to cool for 45 minutes. Rinse the condenser tube with DCM into the soxhlet tube and evacuate the solvent into the Florence flask.

6. Remove the soxhlet extraction tubes from the condenser tubes and evacuate all additional solvent to the Florence flask. Discard the extraction thimble and glass wool. Use a rotary evaporator to drive off all of the DCM and then allow the “Raw Menhaden Oil” to air dry over night.

7. Once the “Raw” oil is dry, mass the Florence flasks again and record the results. The resulting difference between the final and the initial mass will be grams of raw oil per menhaden. Simply divide the amount of oil by the mass of the menhaden to get grams of oil/ gram of fish.

8. Transfer the extract to a graduated cylinder using hexanes and rinse the flat bottom flask with hexanes into the graduated cylinder for final volume measurement three times (This allows for the hexanes exchange). It may be necessary to reconstitute the material with hexanes first before the contents are transferred.

9. Using hexanes dilute the recovered extract to a whole number volume. Mix thoroughly with a solvent rinsed pasture pipette. Make a final volume measurement and record in ml. Transfer 20 ml of the resulting material using a pasture pipette to a volatile organic analysis (VOA) bottle for storage. Allow to settle for 24 hours before any material is used.

10 This material is simply stored until further use is required. Remember that this elution has not been cleaned up and will need to undergo further preparation if is to be analyzed on laboratory equipment.

Surrogates and Standards

There will be three sets of standards used during the process of tissue extraction and analysis. The primary standard is the **surrogate spike solution** added to the tissue at the beginning of the extraction process. This is simply a deuterated PAH solution of known concentration added to the initial processes of both protocols. These standards can be obtained from Supelco pre mixed. They can then be further diluted to fit within the analytical range of the GC being used. The secondary standard will be the **GC/MS internal standard solution**. This again is a mixture of deuterated PAHs at varying molecular weights used to maintain the validity of the instrument. The last standard will be the **calibration curve standards** passed through the MS to verify that the MS is in a fully functional state.

Surrogate Spike Solution:

1. 1.0 ml of 5-alpha Androstane at 10 mg/ml (dissolved in DCM) is added to 500 ml DCM in a 500 ml volumetric flask.

2. Mass 0.0100 g (10 mg) of Phenanthrene - d10 (neat) and add to the 500 ml DCM.

3. Allow time for the Phenanthrene - d10 to dissolve.

Final Volume = 500 ml
Final Concentration = 20 mg/ml

Store in aliquots determined by need using amber glass.

This surrogate is added to each sample at 1 ml. per sample extracted.

GC/MS Internal Standard Solution:

1. Add 1 ml of the following to a 5 ml amber vial
   Napthalene - d8 at 4.0 mg/ml in DCM
   Acenapthene - d10 at 4.0 mg/ml in DCM
   Chrysene - d12 at 4.0 mg/ml in DCM
   Perylene - d12 at 4.0 mg/ml in DCM

Final Volume = 4.0 ml
Final Concentration = 1000 mg/ml

This internal standard is added at 10µl to each GC bottle.

Calibration Curve Standard:

- Surrogate Spike for Calibration Standards
Add 3.0 ml of DCM to an 8 ml amber vial.
Add 1.0 ml of 5-alpha Androstane at 1000 µg/ml in DCM to the 8 ml amber vial.
Add 1.0 ml of Phenanthrene - d10 at 1000 µg/ml in DCM to the 8 ml amber vial.

Final Volume = 5.0 ml
Final Concentration = 200 µg/ml

- Oil Analysis Standard (44 oil constituents) 100µg/ml in Hexanes/DCM (9:1)
Order from http://www.absolutestandards.com/ Absolute Standards part # 90311

0.5 ppm = 10.0µl Oil Analysis Standard in 1.985 ml DCM
5.0µl Surrogate Spike for Calibration Standard Final Volume = 2.0 ml

1.0 ppm = 20.0µl Oil Analysis Standard in 1.97 ml DCM
10.0µl Surrogate Spike for Calibration Standard Final Volume = 2.0 ml

5.0 ppm = 100µl Oil Analysis Standard in 1.85 ml DCM
50µl Surrogate Spike for Calibration Standard Final Volume = 2.0 ml

10.0 ppm = 200µl Oil Analysis Standard in 1.70 ml DCM
100µl Surrogate Spike for Calibration Standard Final Volume = 2.0 ml

25.0 ppm = 500µl Oil Analysis Standard in 1.25 ml DCM
250µl Surrogate Spike for Calibration Standard Final Volume = 2.0 ml

These are placed in a GC bottle that has been adapted to hold 0.2 ml.

The final ppm will depend on the range you set the GC/MS for the sample. Any of these will be fine as long as you make a note as to which one used for that particular sample run.

GC/MS Protocol

The instrumental analysis and data processing aspects focus directly on the generation of data using a list of target compounds (listed in Table 1) applicable to petroleum oil identification and includes petrogenic and pyrogenic sources of polycyclic aromatic hydrocarbons (PAHs) as well as, straight chain alkanes in the range of nC_{10} - nC_{35}.

GC Operation

All GC/MS analyses used an Agilent 5890 GC system configured with a 5% diphenyl/95% dimethyl polysiloxane high resolution capillary column (30 meter, 0.25 mm ID, 0.25 micron film) directly interfaced to an Agilent 5972 mass selective detector system. An Agilent 6890 series Auto Injector is used for sample introduction into the GC/MS system. The GC flow rates are optimized to provide a required degree of separation, particularly n-C_{17} and pristane should be near baseline resolved, and n-C_{18} and phytane should be baseline resolved. The injection temperature is set at 250°C and only high-temperature, low thermal-bleed septa are used in the GC inlet. The GC is operated in the temperature program mode with an initial column temperature of 60°C for 3 minutes then increased to 280°C at a rate of 5°C/minute and held for 3 minutes. The oven is then heated from 280°C to 300°C at a rate of 1.5°C/min and held at 300°C for two minutes. Total run time is 65.333 minutes per sample. The interface to the MS is maintained at 280°C. Ultra High Purity (UHP) Helium is the carry gas for the GC/MS system.

MS Operation

The MS is operated in the Selective Ion Monitoring (SIM) to maximize the detection of several trace target constituents unique to crude oil. The instrument is operated such that the selected ions for each acquisition window are scanned at a rate greater than 1.5 scans/sec with a dwell time of 60 milli-seconds. At the start of each analysis period or every twelve hours, the MS is tuned to PFTBA, an internal instrument standard. Laboratory reference standards such as a reference oil and a continuing calibration standard are also analyzed prior to the analysis of the unknown sample extracts. This standard operating procedure ensures quality assurance/quality control of the instrument conditions prior to sample analysis.

Data Analysis and Report Generation

Quantitative Analysis

Spectral data is processed by Chemstation™ Software using a customized data analysis method developed by LSU-RCAT. The customized data processing method creates a custom report that contains the raw integration data analysis method which is then exported to a spreadsheet for quantitative analysis. Integration results for each data file are carefully reviewed and reintegrated as required. In addition to the raw integration data, a macro printout is also generated and contains the extracted ion chromatography data, or oil fingerprints, to be qualitatively compared to the source oil.

Analyte concentrations are calculated in the spreadsheet and are based on the internal standard method that uses mean relative response factors calculated from the 5-point calibration curve. The calibration curve standards contain the parent (non-alkylated) hydrocarbons and alkylated

homologues are quantified using the response factor of the parent, and are therefore, only semiquantitative. This is the standard procedure since alkylated standards are not available.

Calculations

CONCENTRATION OF ANALYTES IN A SAMPLE:

$$\text{Conc (ng/mg or ng/mL)} = (A_x * I_s * V_t * DF * 1000) / (A_{is} * RRF * V_i * M \text{ or } V)$$

A_x	=	area of analyte
I_x	=	concentration of internal standard injected (ng)
V_t	=	final volume of the total extract (mL)
DF	=	dilution factor
A_{is}	=	area of internal standard
RRF	=	mean relative response factor
V_i	=	volume injected (µL)
M or V	=	mass if solid (mg) or volume if liquid (mL)

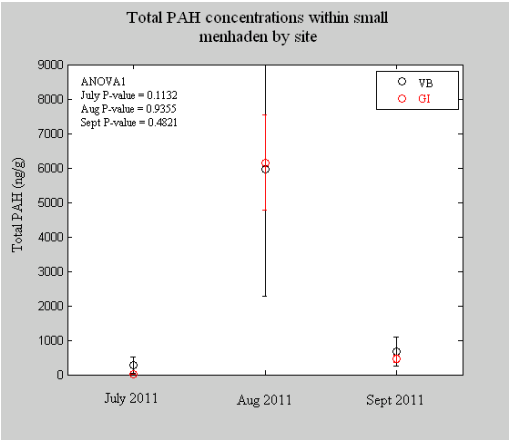
RELATIVE RESPONSE FACTOR:

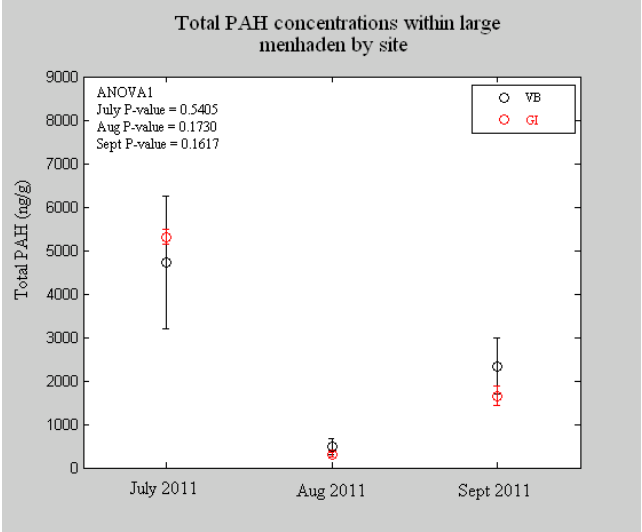
$$RRF = (A_x * C_{is}) / (A_{is} * C_x)$$

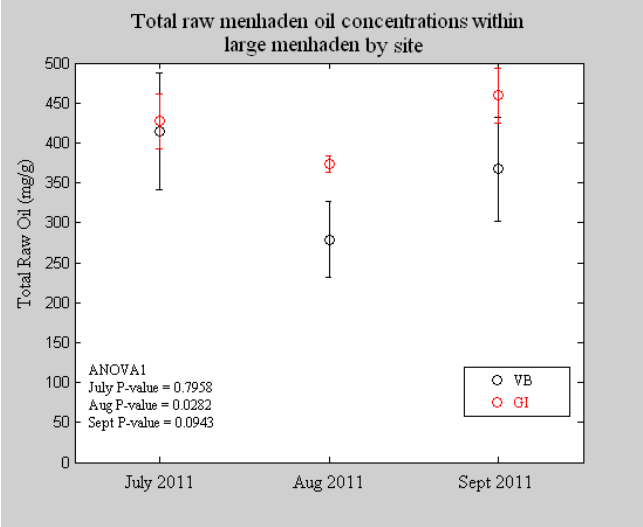
A_x	=	area of analyte in calibration standard
C_{is}	=	concentration of the internal standard
A_{is}	=	area of the internal standard
C_x	=	concentration of calibration standard

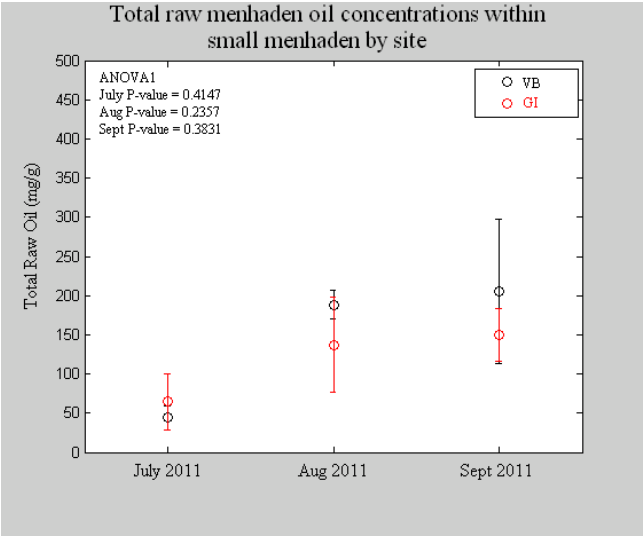
Results for all analytical methods are reported as a function of volume, wet weight, or dry weight values depending on the circumstances and sample. The final results of the quantitative analysis are reported at three significant figures.

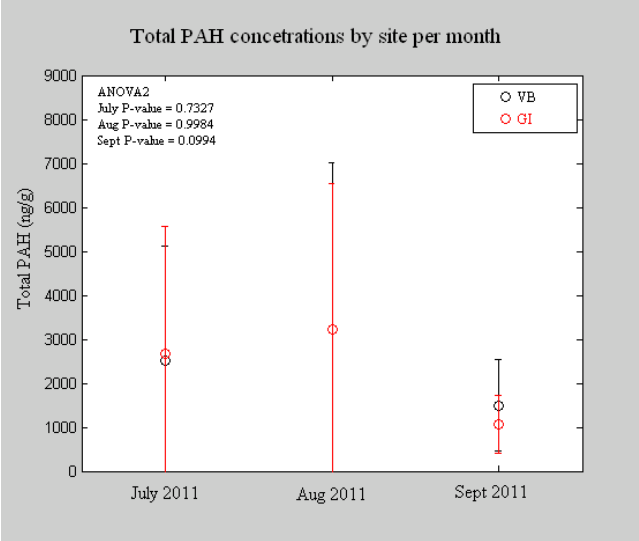
ANOVA Graphs and Tables

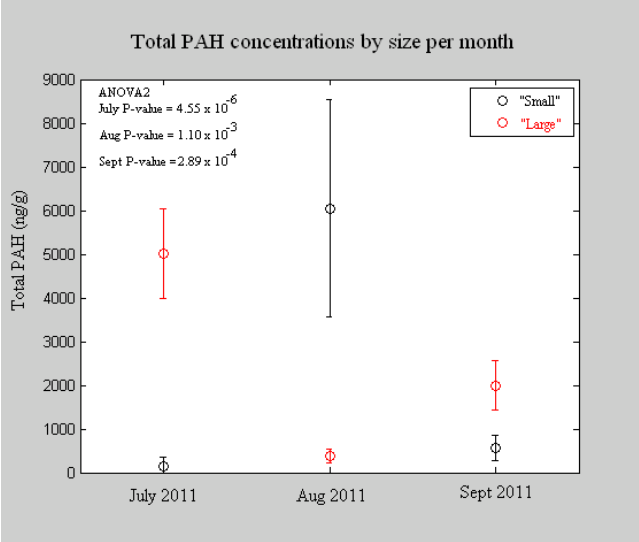


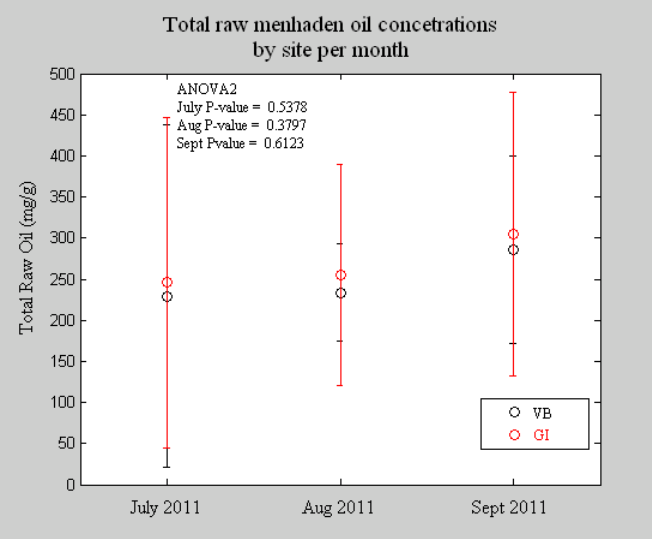

Total PAH concentrations within large menhaden by site
ANOVA1
July P-value = 0.5405
Aug P-value = 0.1730
Sept P-value = 0.1617
VB
GI
Total PAH (ng/g)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
July 2011
Aug 2011
Sept 2011

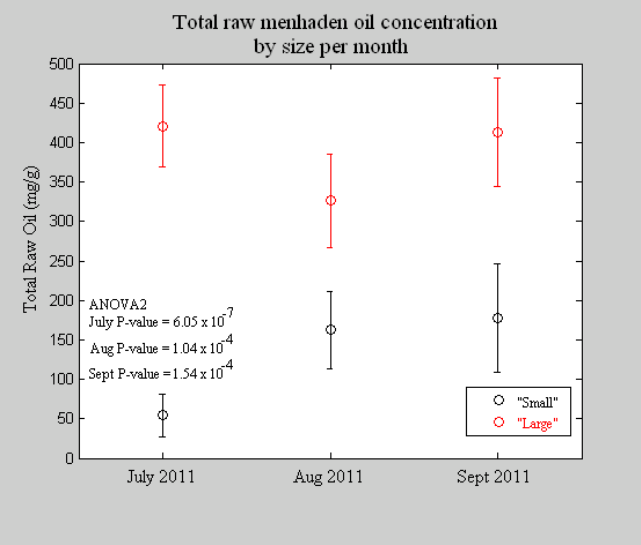

Total raw menhaden oil concentrations within large menhaden by site
ANOVA1
July P-value = 0.7958
Aug P-value = 0.0282
Sept P-value = 0.0943
VB
GI
Total Raw Oil (mg/g)
500
450
400
350
300
250
200
150
100
50
0
July 2011
Aug 2011
Sept 2011

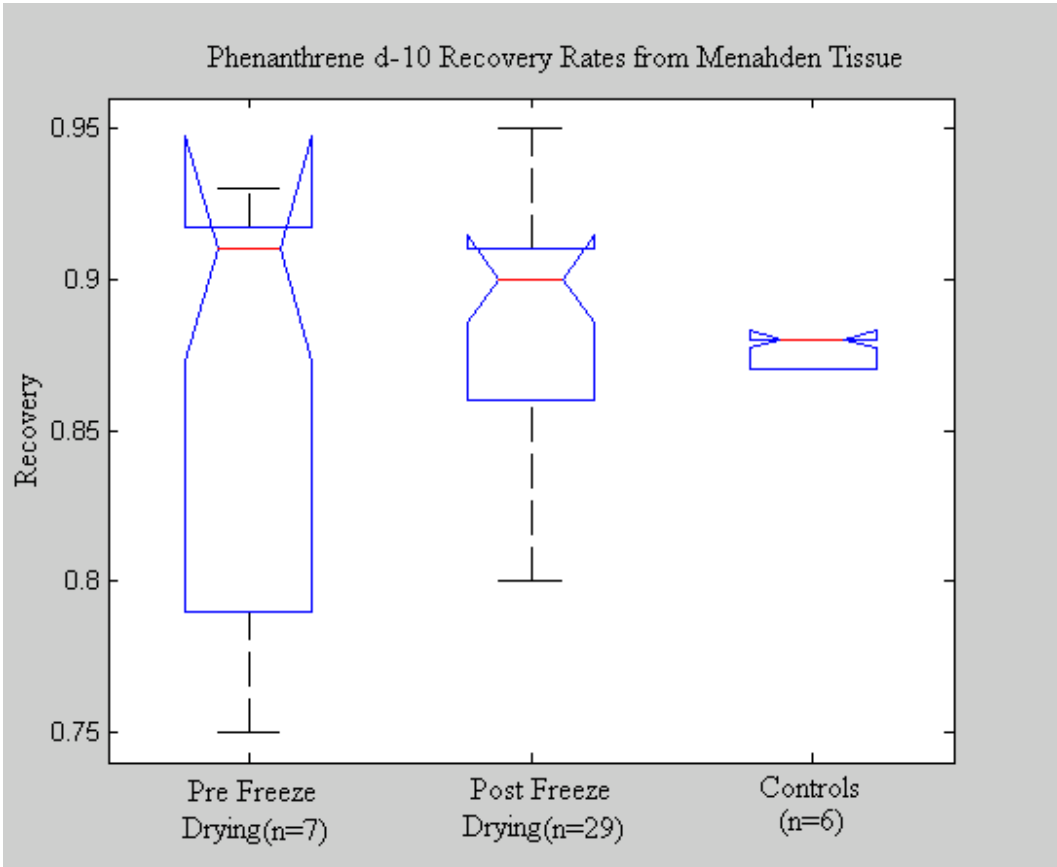

Total raw menhaden oil concentrations within small menhaden by site
ANOVA1
July P-value = 0.4147
Aug P-value = 0.2357
Sept P-value = 0.3831
VB
GI
Total Raw Oil (mg/g)
500
450
400
350
300
250
200
150
100
50
0
July 2011
Aug 2011
Sept 2011


Total PAH concetrations by site per month
ANOVA2
July P-value = 0.7327
Aug P-value = 0.9984
Sept P-value = 0.0994
VB
GI
Total PAH (ng/g)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
July 2011
Aug 2011
Sept 2011


Total PAH concentrations by size per month
ANOVA2
July P-value = 4.55 x 10^-6
Aug P-value = 1.10 x 10^-3
Sept P-value = 2.89 x 10^-4
"Small"
"Large"
Total PAH (ng/g)
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
July 2011
Aug 2011
Sept 2011


Total raw menhaden oil concetrations
by site per month
ANOVA2
July P-value = 0.5378
Aug P-value = 0.3797
Sept Pvalue = 0.6123
VB
GI
Total Raw Oil (mg/g)
500
450
400
350
300
250
200
150
100
50
0
July 2011
Aug 2011
Sept 2011





ANOVA Table

Source	SS	df	MS	F	Prob>F
Groups	0.00212	2	0.00106	0.57	0.5686
Error	0.07224	39	0.00185		
Total	0.07436	41			

ANOVA2 Total PAH (ng/g) Large menhaden by site and month

	vb		gi
July 2011	6125		5481
	3099		5144
	4942		5317
group mean	4722	group mean	5314
Aug 2011	669		264
	495		262
	285		352
group mean	483	group mean	292.67
Sept 2011	1729		1915
	3018		1539
	2260		1519
group mean	2335.67	group mean	1657.67

Overall	total mean	2467.5
Site	VB mean	2513.5556
Site	GI mean	2421.4444
Month	Jul mean	5018
Month	Aug mean	387.83333
Month	Sept mean	1996.6667

		DF
SStotal	73305890.5	17
SSerror	5725842	12
SSmonth	66310486.33	2
SSsite	38180.05556	1
SSinteraction	1231382.111	2

Fsite	0.0800	<	FstatSt	4.7472	Accept	Means are not statistically different*	pvalue 0.7821
Fmonth	69.4855	>	FstatM	3.8853	Reject	Means are statistically different*	pvalue 0.0001
Finteraction	1.2903	<	FstatI	3.8853	Accept	Interaction is not statistically significant*	pvalue 0.3108

* significance of 0.05

ANOVA2 Total PAH (ng/g) Small Menhaden by site and month

	VB		GI
July 2011	425		0
	15		14
	420		21
group mean	286.666667	group mean	11.66667
Aug 2011	3700		7739
	3966		5419
	10186		5279
group mean	5950.66667	group mean	6145.667
Sept 2011	1118		374
	570		459
	302		582
group mean	663.333333	group mean	471.6667

Overall	total mean	2254.9444
Site	VB mean	2300.2222
Site	GI mean	2209.6667
Month	Jul mean	149.16667
Month	Aug mean	6048.1667
Month	Sept mean	567.5

		DF
SStotal	161486530.9	17
SSerror	31239130	12
SSmonth	130021821.8	2
SSsite	36901.38889	1
SSinteraction	188677.7778	2

Fsite	0.0142	<	FstatSt	4.7472	Accept	Means are not statistically different*	pvalue 0.9071
Fmonth	24.9729	>	FstatM	3.8853	Reject	Means are statistically different*	pvalue 0.0001
Finteraction	0.0362	<	FstatI	3.8853	Accept	Interaction is not statistically significant*	pvalue 0.9646

* significance of 0.05

ANOVA2 Total PAH (ng/g) by month and size

	sm		lg
July 2011	425		6125
	15		3099
	420		4942
	0		5481
	14		5144
	21		5317
group mean	149.166667	group mean	5018
Aug 2011	3700		669
	3966		495
	10186		285
	7739		264
	5419		262
	5279		352
group mean	6048.16667	group mean	387.8333
Sept 2011	1118		1729
	570		3018
	302		2260
	374		1915
	459		1539
	582		1519
group mean	567.5	group mean	1996.667

Overall	total mean	2361.2222
Size	sm mean	2254.9444
Size	lg mean	2467.5
Month	Jul mean	2583.5833
Month	Aug mean	3218
Month	Sept mean	1282.0833

		DF
SStotal	235199040.2	35
SSerror	38460113.33	30
SSmonth	23376640.39	2
SSsize	205222.3889	1
SSinteraction	170739103.7	2

Fsize	0.1601	<	FstatSz	4.1709	Accept	Means are not statistically different*	pvalue	0.6919
Fmonth	9.1172	>	FstatM	3.3158	Reject	Means are statistically different*	pvalue	0.0008
Finteraction	66.5907	>	FstatI	3.3158	Reject	Interaction between size and month is significant*	pvalue	0.0001

* significance of 0.05

Nested ANOVA Total PAH Concentrations (ng/g) in Menhaden 2011

		DF
SStotal	235199040	35
SSerror	36964972	24
SSsite	1495141.3	6
SSmonth	196332308	4
SSsize	406618.78	1

Fsite	0.1618	<	FstatSt	2.5082	Accept	Means are not statistically different*	pvalue	0.9845
Fmonth	196.9703	>	FstatM	4.5337	Reject	Means are statistically different*	pvalue	0.0001
Fsize	0.0083	<	FstatSi	7.7086	Accept	Means are not statistically different*	pvalue	0.9381

* significance of 0.05

2361.222222											
Total											
2254.944444						2467.5					
Small						Large					
149.1666667		6048.166667		567.5		5018		387.8333333		1996.666667	
July		Aug		Sept		July		Aug		Sept	
286.6667	11.666667	5950.667	6145.667	663.3333	471.6667	4722	5314	483	292.6667	2335.667	1657.667
VB	GI	VB	GI	VB	GI	VB	GI	VB	GI	VB	GI
425	0	3700	7739	1118	374	6125	5481	669	264	1729	1915
15	14	3966	5419	570	459	3099	5144	495	262	3018	1539
420	21	10186	5279	302	582	4942	5317	285	352	2260	1519

APPENDIX B: DATA

Mass and Length

Grand Isle			Vermilion Bay	
Length (cm)	Mass (g)		Length (cm)	Mass (g)
10.5	21.8		6.5	4.3
10.7	22.3		6.9	5.2
10.8	23.4		7.2	5.8
10.9	22.0		7.9	8.7
11.0	24.5		8.1	9.2
11.2	25.0		8.4	10.8
11.2	27.9		8.5	12.7
11.2	28.4		8.9	14.1
11.3	26.9		9.0	13.0
11.4	25.4		9.0	14.0
11.4	26.7		9.2	15.6
11.4	28.1		9.3	15.2
11.5	25.6		9.4	15.3
11.5	28.7		9.4	17.5
11.5	29.5		9.5	13.8
11.6	27.1		9.5	16.2
11.6	29.3		9.5	16.3
11.7	26.3		9.5	16.4
11.7	28.1		9.5	16.5
11.7	28.3		9.5	17.1
11.7	28.8		9.6	16.9
11.7	29.9		9.6	19.3
11.8	27.2		9.9	20.0
11.8	28.1		10.0	17.6
11.8	28.9		10.0	17.6
11.9	28.9		10.2	19.4
11.9	29.5		10.3	17.9
11.9	30.2		10.3	20.5
11.9	30.7		10.3	20.7
11.9	31.5		10.4	19.6
11.9	31.8		10.6	19.7
12.0	28.5		10.6	21.8
12.0	30.7		10.8	21.6
12.0	30.8		10.8	22.1
12.0	31.2		10.8	23.5

12.0	31.4		10.9	23.4	
12.0	31.5		11.0	23.6	
12.0	32.1		11.0	24.0	
12.0	32.4		11.0	26.4	
12.0	35.3		11.0	27.4	
12.1	29.0		11.2	24.1	
12.1	30.1		11.2	29.4	
12.1	30.5		11.2	29.5	
12.1	30.6		11.4	26.6	
12.1	30.6		11.5	28.3	
12.1	32.6		11.6	26.7	
12.1	33.0		11.6	29.9	
12.1	34.3		11.7	29.2	
12.1	35.0		11.8	27.2	
12.1	36.3		11.8	27.9	
12.1	40.3		11.8	30.6	
12.2	30.9		11.8	30.7	
12.2	31.4		11.9	29.5	
12.2	31.8		11.9	30.6	
12.2	33.0		12.0	30.4	
12.2	34.1		12.0	30.5	
12.2	34.4		12.0	31.3	
12.2	35.9		12.0	32.0	
12.3	28.1		12.0	32.2	
12.3	30.1		12.0	33.6	
12.3	31.5		12.1	30.7	
12.3	32.7		12.1	31.6	
12.3	33.2		12.2	33.5	
12.3	33.4		12.2	36.7	
12.3	34.3		12.3	34.8	
12.4	31.3		12.4	34.6	
12.4	32.6		12.5	34.4	
12.4	32.8		12.5	37.0	
12.4	33.4		12.5	37.3	
12.4	34.3		12.5	40.6	
12.4	34.8		12.6	36.0	
12.4	35.5		12.6	37.2	
12.4	36.1		12.6	38.4	
12.4	36.8		12.6	40.0	
12.4	38.4		12.6	40.9	
12.5	31.9		12.7	34.2	

12.5	33.0		12.7	34.9	
12.5	33.5		12.7	38.9	
12.5	34.1		12.7	41.4	
12.5	34.7		12.8	35.7	
12.5	36.5		12.8	36.9	
12.5	37.8		12.8	39.1	
12.5	37.9		12.8	39.3	
12.5	37.9		12.8	40.8	
12.6	30.7		12.8	40.9	
12.6	32.0		12.9	34.8	
12.6	32.3		12.9	37.9	
12.6	32.4		12.9	39.9	
12.6	33.9		12.9	41.7	
12.6	34.4		12.9	43.9	
12.6	34.8		13.0	34.1	
12.6	35.8		13.0	38.7	
12.6	35.9		13.0	39.2	
12.6	36.7		13.0	39.6	
12.6	38.2		13.0	39.6	
12.6	38.4		13.0	39.7	
12.6	43.9		13.0	40.2	
12.7	34.0		13.0	41.0	
12.7	35.6		13.0	42.8	
12.7	35.7		13.0	47.3	
12.7	36.5		13.0	52.7	
12.7	37.7		13.1	38.6	
12.7	38.9		13.1	39.2	
12.8	32.0		13.1	40.0	
12.8	33.6		13.1	40.3	
12.8	37.1		13.1	40.4	
12.8	37.4		13.1	41.7	
12.8	37.5		13.1	42.0	
12.8	38.8		13.1	42.5	
12.8	40.0		13.1	45.5	
12.8	40.8		13.1	45.9	
12.9	33.7		13.2	34.7	
12.9	33.8		13.2	39.7	
12.9	34.0		13.2	40.3	
12.9	34.1		13.2	41.5	
12.9	35.8		13.2	41.6	
12.9	35.8		13.2	42.6	

12.9	36.9		13.2	44.0	
12.9	37.2		13.2	44.9	
12.9	37.8		13.2	47.2	
12.9	38.5		13.3	39.3	
12.9	38.9		13.3	41.6	
12.9	42.7		13.4	39.2	
13.0	35.2		13.4	39.7	
13.0	35.2		13.4	42.1	
13.0	35.3		13.4	42.9	
13.0	36.4		13.4	44.1	
13.0	36.4		13.4	44.7	
13.0	37.2		13.4	45.0	
13.0	37.3		13.4	45.2	
13.0	38.1		13.4	45.3	
13.0	38.3		13.4	47.2	
13.0	39.3		13.5	40.1	
13.0	41.3		13.5	42.6	
13.0	42.6		13.5	43.0	
13.1	36.9		13.5	43.4	
13.1	37.3		13.5	44.5	
13.1	42.1		13.5	44.8	
13.1	42.9		13.5	45.5	
13.1	45.4		13.5	45.9	
13.2	39.1		13.5	46.4	
13.2	39.8		13.5	46.9	
13.2	42.0		13.5	48.8	
13.2	42.6		13.5	49.4	
13.2	43.1		13.6	41.3	
13.2	47.1		13.6	44.1	
13.2	47.8		13.6	44.2	
13.3	41.8		13.6	44.6	
13.3	44.3		13.6	44.8	
13.4	41.3		13.6	45.5	
13.4	41.5		13.6	46.0	
13.4	42.4		13.6	47.1	
13.4	43.5		13.7	42.3	
13.4	44.5		13.7	46.0	
13.4	46.6		13.7	46.7	
13.4	47.1		13.7	46.7	
13.5	41.2		13.7	48.2	
13.5	41.4		13.7	49.2	

13.5	41.8		13.8	44.0	
13.5	42.0		13.8	44.7	
13.5	42.2		13.8	45.9	
13.5	44.8		13.8	46.5	
13.6	40.9		13.8	46.8	
13.6	45.1		13.9	40.7	
13.6	46.5		13.9	42.5	
13.6	47.7		13.9	42.9	
13.7	47.1		13.9	44.0	
13.7	47.2		13.9	45.3	
13.8	46.4		13.9	47.2	
13.8	46.8		13.9	49.6	
13.8	48.1		13.9	50.8	
13.9	46.6		13.9	54.3	
13.9	47.6		14.0	41.6	
13.9	48.4		14.0	42.7	
13.9	52.4		14.0	44.5	
14.0	49.2		14.0	44.6	
14.0	49.9		14.0	47.5	
14.0	50.1		14.0	49.8	
14.0	51.4		14.0	49.8	
14.0	51.8		14.0	49.9	
14.0	54.4		14.0	50.9	
14.1	50.8		14.0	58.9	
14.1	51.0		14.1	42.1	
14.1	52.0		14.1	46.7	
14.1	57.1		14.1	47.0	
14.1	57.2		14.1	47.3	
14.1	57.8		14.1	47.5	
14.2	45.6		14.1	47.9	
14.2	55.0		14.1	49.0	
14.2	55.5		14.1	51.8	
14.3	52.6		14.1	51.9	
14.3	58.2		14.1	53.2	
14.3	58.5		14.2	49.8	
14.4	51.4		14.2	49.9	
14.4	52.0		14.2	50.7	
14.4	52.9		14.2	50.8	
14.4	58.3		14.2	56.1	
14.5	53.0		14.2	56.5	
14.5	53.3		14.3	47.9	

14.5	55.6		14.4	38.4	
14.6	65.2		14.4	49.5	
14.7	52.7		14.4	49.8	
14.8	54.4		14.4	53.1	
14.8	55.0		14.5	49.6	
14.9	48.4		14.5	52.2	
14.9	65.4		14.8	54.1	
14.9	62.7		14.6	55.8	
15.0	62.1		14.5	56.5	
15.0	62.7		14.8	56.7	
15.1	51.1		14.5	59.0	
15.1	61.5		14.6	61.0	
15.2	59.4		14.8	61.3	
15.4	65.6		14.5	61.4	
15.4	65.7		15.0	56.1	
15.5	63.4		15.0	56.6	
15.5	66.5		15.0	59.2	
15.5	70.1		15.0	59.8	
15.5	70.9		15.0	61.6	
15.5	71.5		15.0	68.3	
15.6	80.0		15.1	41.7	
15.7	67.4		15.1	58.9	
15.9	69.6		15.1	59.3	
15.9	76.0		15.1	60.8	
16.0	76.1		15.3	65.2	
16.3	74.9		15.4	62.7	
16.4	79.7		15.5	62.5	
16.4	84.9		15.5	64.1	
16.5	83.5		15.5	66.1	
16.8	86.8		15.6	63.4	
16.8	95.8		15.6	67.3	
16.9	85.0		15.7	71.5	
16.9	87.1		15.7	71.8	
16.9	93.8		15.8	67.2	
16.9	99.8		16.0	68.4	
17.0	90.5		16.0	68.5	
17.0	93.7		16.1	72.5	
17.0	96.0		16.1	79.8	
17.0	100.0		16.2	68.9	
17.1	100.0		16.3	75.6	
17.2	101.3		16.3	76.2	

17.2	101.4		16.3	77.2	
17.3	94.8		16.4	71.7	
17.3	101.8		16.4	75.1	
17.4	101.6		16.4	75.3	
17.4	102.8		16.4	80.4	
17.4	103.7		16.5	74.3	
17.4	94.1		16.5	88.4	
17.4	95.7		16.5	88.8	
17.4	102.3		16.5	89.7	
17.4	104.7		16.6	80.8	
17.5	100.3		16.6	81.5	
17.5	101.6		16.8	78.6	
17.5	103.3		16.9	90.7	
17.5	103.4		17.0	88.3	
17.5	104.1		17.1	92.0	
17.6	96.3		17.1	94.6	
17.6	100.3		17.2	90.8	
17.6	101.5		17.2	93.1	
17.6	106.1		17.3	101.2	
17.6	108.4		17.4	101.0	
17.6	110.2		17.5	85.3	
17.7	89.7		17.5	107.3	
17.8	104.0		17.6	89.6	
17.8	110.2		17.6	96.8	
17.9	113.6		17.7	92.8	
18.0	100.2		17.8	89.5	
18.0	101.5		17.8	97.3	
18.0	102.8		17.8	98.4	
18.0	104.4		17.9	104.3	
18.0	109.9		17.9	111.6	
18.0	113.4		18.0	107.4	
18.1	104.3		18.0	108.7	
18.1	109.5		18.1	111.3	
18.1	111.5		18.2	107.5	
18.1	112.6		18.2	116.5	
18.1	113.1		18.3	118.4	
18.1	116.2		18.4	93.1	
18.1	119.0		18.4	96.8	
18.1	119.7		18.4	160.5	
18.1	121.3		18.5	118.5	
18.1	141.0		18.6	117.5	

18.2	103.9	
18.2	110.3	
18.2	112.2	
18.2	131.1	
18.3	103.9	
18.3	109.8	
18.3	116.1	
18.3	120.7	
18.4	101.9	
18.4	104.3	
18.4	111.7	
18.4	122.0	
18.4	122.3	
18.5	110.5	
18.5	128.7	
18.6	121.7	
18.6	125.3	
18.6	133.7	
18.7	121.4	
18.7	127.4	
18.7	136.2	
18.7	137.4	
18.8	119.5	
18.8	126.4	
18.9	118.5	
18.9	127.7	
19.0	128.5	
19.0	130.8	
19.1	125.0	
19.1	158.6	
19.4	127.0	
19.4	132.0	
19.4	132.5	
19.4	137.2	
19.4	153.4	
19.5	142.2	
19.5	153.3	
19.6	133.7	

19.0	123.3		
19.0	141.2		
19.1	127.5		
19.2	136.0		
19.5	124.5		
19.5	129.1		
19.5	137.1		
19.5	155.6		
19.6	133.8		
19.6	158.1		
19.6	148.4		
19.7	140.8		
19.7	144.3		
19.8	138.8		
19.9	154.2		
20.0	134.3		
20.1	153.8		
20.4	146.5		
20.4	160.4		
20.4	160.5		
20.5	156.6		
20.8	188.6		
21.2	170.4		
21.5	170.2		
21.6	192.0		
22.0	171.5		
23.5	227.3		
Total Avg Length (cm)	Total Avg Mass	Total STD Length	Total STD Mass
14.15	58.08	2.933836079	38.23803596

19.9	156.4		
20.0	144.6		
20.0	145.8		
20.0	163.3		
20.2	182.1		
20.3	185.4		
20.6	165.4		
22.0	141.1		
22.0	173.3		
22.6	203.7		
22.6	218.1		
22.7	186.6		
Total Avg Length	Total Avg Mass	Total STD Length	Total STD Mass
14.74	65.73	2.752821063	40.28440204

Raw Menhaden Oil

Date	ID	Oil/Fish (mg/g dry fish)	Dry % Oil
7/6/2011	VB13	352.17	27.69%
7/6/2011	VB14	395.91	29.22%
7/6/2011	VB15	494.60	42.56%
7/28/2011	GI16	388.21	31.72%
7/28/2011	GI17	454.92	38.23%
7/28/2011	GI18	438.26	40.81%
7/28/2011	GI37	97.22	23.43%
7/28/2011	GI38	26.32	5.87%
7/28/2011	GI39	70.06	15.64%
7/6/2011	VB40	27.97	6.91%
7/6/2011	VB41	45.98	9.31%
7/6/2011	VB42	58.39	14.45%
8/23/2011	VB49	198.76	33.86%
8/23/2011	VB50	198.86	31.07%
8/23/2011	VB51	167.66	27.22%
8/24/2011	GI52	198.68	38.21%
8/24/2011	GI53	136.36	26.74%
8/24/2011	GI54	76.92	17.89%
8/23/2011	VB55	277.51	30.07%
8/23/2011	VB56	327.09	27.74%
8/23/2011	VB57	232.67	26.53%
8/24/2011	GI58	362.04	31.45%
8/24/2011	GI59	382.81	36.77%
8/24/2011	GI60	374.27	23.13%
9/21/2011	VB67	116.79	21.08%
9/21/2011	VB68	300.00	60.85%
9/21/2011	VB69	198.41	39.92%
9/13/2011	GI70	133.33	32.68%
9/13/2011	GI71	128.44	29.06%
9/13/2011	GI72	188.03	42.93%
9/21/2011	VB79	418.99	26.07%
9/21/2011	VB80	387.28	27.18%
9/27/2011	VB81	294.85	16.75%
9/13/2011	GI82	434.67	40.47%
9/13/2011	GI83	498.80	45.55%
9/13/2011	GI84	444.44	49.16%
10/5/2011	VB97	200.00	46.19%
10/5/2011	VB98	209.30	43.79%

10/5/2011	VB99	31.01	7.44%
10/11/2011	GI100	71.75	12.48%
10/11/2011	GI101	76.92	14.09%
10/11/2011	GI102	75.00	13.49%
10/11/2011	GI103	359.13	36.35%
10/11/2011	GI104	390.53	39.17%
10/11/2011	GI105	438.98	26.73%

Total PAH Concentrations

Date	ID	Phenanthrene d-10 Surrogate Recovery	Corrected Total PAH(ng/g)	Adjusted Total PAH for C3 Phenanthrenes
7/6/2011	VB19	0.75	11438.00	6125.00
7/6/2011	VB20	0.75	6233.00	3099.00
7/6/2011	VB21	0.92	7525.00	4942.00
7/28/2011	GI22	0.91	8988.00	5481.00
7/28/2011	GI23	0.91	9320.00	5144.00
7/28/2011	GI 24	0.91	9154.0	5317.00
8/23/2011	VB25	0.93	6245.00	3700.00
8/23/2011	VB26	0.90	6458.00	3966.00
8/23/2011	VB27	0.90	13041.00	10186.00
8/24/2011	GI28	0.89	10132.00	7739.00
8/24/2011	GI29	0.92	7227.00	5419.00
8/24/2011	GI30	0.89	7007.00	5279.00
8/23/2011	VB31	0.91	2517.00	669.00
8/23/2011	VB32	0.92	2422.00	495.00
8/23/2011	VB33	0.95	3413.00	285.00
8/24/2011	GI34	0.91	3229.00	264.00
8/24/2011	GI35	0.92	1892.00	262.00
8/24/2011	GI36	0.90	2666.00	352.00
9/13/2011	GI43	0.89	2860.00	1118.00
9/13/2011	GI44	0.89	4429.00	570.00
9/13/2011	GI45	0.91	2982.00	302.00
9/21/2011	VB46	0.88	3672.00	374.00
9/21/2011	VB47	0.88	4838.00	459.00
9/21/2011	VB48	0.91	5877.00	582.00
9/21/2011	VB61	0.91	13128.00	1729.00
9/21/2011	VB62	0.90	20523.00	3018.00
9/21/2011	VB63	0.90	13038.00	2260.00
9/13/2011	GI64	0.86	8438.00	1915.00
9/13/2011	GI65	0.85	10216.00	1539.00
9/13/2011	GI66	0.92	12907.00	1519.00
10/5/2011	VB73	0.83	6728.00	769.00
10/5/2011	VB74	0.83	5685.00	260.00
10/5/2011	VB75	0.84	6326.00	329.00
10/11/2011	GI76	0.86	2306.00	94.00
10/11/2011	GI77	0.80	1890.00	98.00

10/11/2011	GI78	0.85	2210.00	72.00
10/25/2010	GI85	0.86	1570.00	368.00
10/25/2010	GI86	0.92	1541.00	90.00
10/25/2010	GI87	0.89	1218.00	0.00
7/6/2011	VB88	0.92	5283.00	425.00
7/6/2011	VB89	0.96	1901.00	15.00
7/6/2011	VB90	0.95	5040.00	420.00
7/28/2011	GI91	0.95	2952.00	0.00
7/28/2011	GI92	0.95	1916.00	14.00
7/28/2011	GI93	0.93	2073.00	21.00
10/11/2011	GI94	0.91	2299.00	357.00
10/11/2011	GI95	0.88	3684.00	522.00
10/11/2011	GI96	0.90	5109.00	944.00
12/13/2011	MTHD BLK	0.99	0.00	0.00
1/13/2012	MB 2	0.85	0.00	0.00
1/13/2012	MB 3	0.86	0.00	0.00

Menhaden Oil and PAHs

Month/Size	Date	ID	Corrected PAH (ng/g)	Oil/Fish (mg/g dry fish)
Jul Large	7/6/2011	VB	6125.0	352.17
Jul Large	7/6/2011	VB	3099.0	395.91
Jul Large	7/6/2011	VB	4942.0	494.60
Jul Large	7/28/2011	GI	5481.0	388.21
Jul Large	7/28/2011	GI	5144.0	454.92
Jul Large	7/28/2011	GI	5317.0	438.26
Jul Small	7/6/2011	VB	425.0	27.97
Jul Small	7/6/2011	VB	15.0	45.98
Jul Small	7/6/2011	VB	420.0	58.39
Jul Small	7/28/2011	GI	0.0	97.22
Jul Small	7/28/2011	GI	14.0	26.32
Jul Small	7/28/2011	GI	21.0	70.06
Aug Large	8/23/2011	VB	669.0	277.51
Aug Large	8/23/2011	VB	495.0	327.09
Aug Large	8/23/2011	VB	285.0	232.67
Aug Large	8/24/2011	GI	264.0	362.04
Aug Large	8/24/2011	GI	262.0	382.81
Aug Large	8/24/2011	GI	352.0	374.27
Aug Small	8/23/2011	VB	3700.0	198.76
Aug Small	8/23/2011	VB	3966.0	198.86
Aug Small	8/23/2011	VB	10186.0	167.66
Aug Small	8/24/2011	GI	7739.0	198.68
Aug Small	8/24/2011	GI	5419.0	136.36
Aug Small	8/24/2011	GI	5279.0	76.92
Sept Large	9/21/2011	VB	1729.0	418.99
Sept Large	9/21/2011	VB	3018.0	387.28
Sept Large	9/21/2011	VB	2260.0	294.85
Sept Large	9/13/2011	GI	1915.0	434.67
Sept Large	9/13/2011	GI	1539.0	498.80

Sept Large	9/13/2011	GI	1519.0	444.44
Sept Small	9/21/2011	VB	374.0	116.79
Sept Small	9/21/2011	VB	459.0	300.00
Sept Small	9/21/2011	VB	582.0	198.41
Sept Small	9/13/2011	GI	1118.0	133.33
Sept Small	9/13/2011	GI	570.0	128.44
Sept Small	9/13/2011	GI	302.0	188.03
Oct Small	10/5/2011	VB	769.0	200.00
Oct Small	10/5/2011	VB	260.0	209.30
Oct Small	10/5/2011	VB	329.0	31.01
Oct Small	10/11/2011	GI	94.0	71.75
Oct Small	10/11/2011	GI	98.0	76.92
Oct Small	10/11/2011	GI	72.0	75.00

Benzo[a]pyrene Mutagenic and Toxic Equivalencies

		B(a)P-TEQ (ng/g dry tissue)	BAP-MEQ (ng/g dry tissue)
July Large	VB	2.78	3.86
		2.11	3.30
		76.52	17.30
	GI	129.21	14.89
		3.66	4.85
		66.44	9.87
Aug Small	VB	0.34	0.00
		0.56	0.49
		0.51	0.00
	GI	0.48	0.00
		0.30	0.00
		0.29	0.00
Aug Large	VB	468.08	155.68
		369.69	103.64
		252.53	44.18
	GI	136.96	13.49
		164.74	9.54
		155.07	8.98
Sept Small	GI	103.98	6.03
		0.17	0.00
		0.16	0.00
	VB	0.11	0.00
		0.04	0.00
		0.16	0.00
Sept Large	VB	737.74	161.95
		7.60	13.61
		0.20	0.00
	GI	0.29	0.00
		0.40	0.00
		0.24	0.00
Oct Small	VB	0.24	0.00
		0.26	0.00
		0.33	0.00
	GI	0.09	0.00
		0.10	0.00
		0.07	0.00
Oct 2010	GI	547.47	114.96

Small			
		6.37	11.85
		0.00	0.00
July Small	VB	0.00	0.00
		0.01	0.00
		0.00	0.00
	GI	0.00	0.00
		0.01	0.00
		0.02	0.00
Oct Large	GI	429.87	99.13
		0.04	0.00
		0.09	0.00
Method	Blank	0.00	0.00
Controls	Small	0.05	0.00
		0.05	0.00
		0.05	0.00
	Large	0.05	0.00
		0.05	0.00
		0.05	0.00

GC/MS Raw Data

See next page for the beginning of the data.

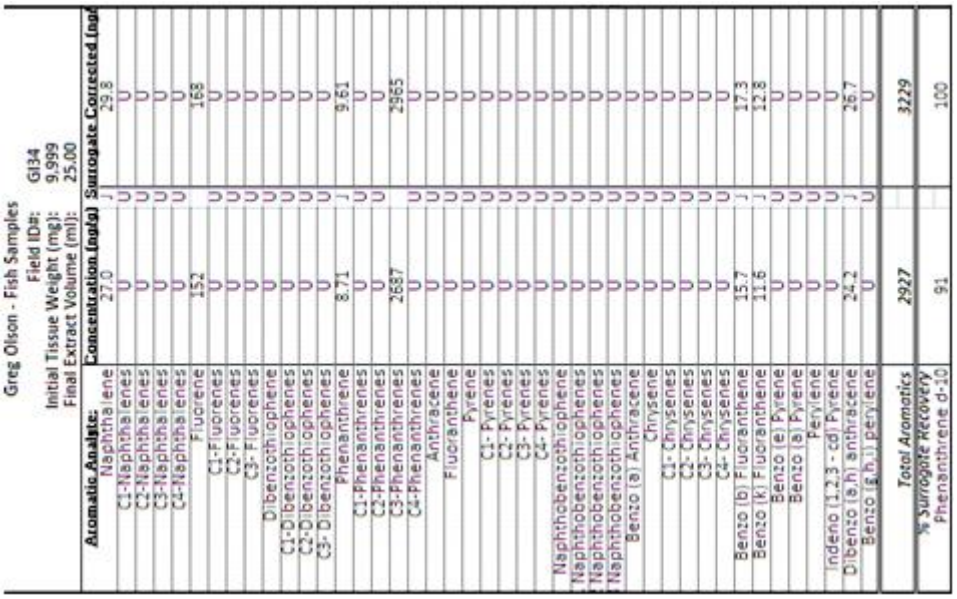
Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	VB19			Field ID#:	VB20			Field ID#:	VB21		
Initial Tissue Weight (mg):	34,200			Initial Tissue Weight (mg):	50,700			Initial Tissue Weight (mg):	48,500		
Final Extract Volume (ml):	50.00			Final Extract Volume (ml):	55.00			Final Extract Volume (ml):	50.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	65.9		87.9	Naphthalene	U	U	U	Naphthalene	41.6		45.3
C1-Naphthalenes	33.7		45.0	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	302		404	Fluorene	278		373	Fluorene	154		168
C1-Fluorenes	U	U	U	C1-Fluorenes	112		150	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	138		185	C2-Fluorenes	237		258
C3-Fluorenes	221		295	C3-Fluorenes	U	U	U	C3-Fluorenes	380		413
Dibenzothiophene	12.4	J	16.6	Dibenzothiophene	5.77	J	7.75	Dibenzothiophene	13.8		15.1
C1-Dibenzothiophenes	182		243	C1-Dibenzothiophenes	75.4		101	C1-Dibenzothiophenes	194		211
C2-Dibenzothiophenes	1139		1521	C2-Dibenzothiophenes	547		734	C2-Dibenzothiophenes	857		934
C3-Dibenzothiophenes	539		720	C3-Dibenzothiophenes	170		228	C3-Dibenzothiophenes	497		541
Phenanthrene	48.3		64.5	Phenanthrene	21.9		29.4	Phenanthrene	43.7		47.6
C1-Phenanthrenes	556		742	C1-Phenanthrenes	318		427	C1-Phenanthrenes	626		681
C2-Phenanthrenes	1395		1861	C2-Phenanthrenes	580		778	C2-Phenanthrenes	1372		1493
C3-Phenanthrenes	3980		5313	C3-Phenanthrenes	2336		3134	C3-Phenanthrenes	2373		2583
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	9.27	J	12.37	Anthracene	U	U	U	Anthracene	7.43	J	8.09
Fluoranthene	16.4		21.8	Fluoranthene	8.33	J	11.2	Fluoranthene	11.7		12.7
Pyrene	44.7		59.6	Pyrene	21.6		29.0	Pyrene	46.9		51.0
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	15.5		20.8	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	8.77	J	11.7	Benzo (b) Fluoranthene	7.77	J	10.4	Benzo (b) Fluoranthene	14.2		15.5
Benzo (k) Fluoranthene	6.35	J	8.48	Benzo (k) Fluoranthene	4.68	J	6.28	Benzo (k) Fluoranthene	10.6	J	11.5
Benzo (e) Pyrene	8.36	J	11.2	Benzo (e) Pyrene	6.12	J	8.21	Benzo (e) Pyrene	12.3		13.4
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	7.71	J	8.40
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	11.9		13.0
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*8569*		*11438*	*Total Aromatics*	*4646*		*6233*	*Total Aromatics*	*6911*		*7525*
% Surrogate Recovery				*% Surrogate Recovery*				*% Surrogate Recovery*			
Phenanthrene d-10	75		100	Phenanthrene d-10	75		100	Phenanthrene d-10	92		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	GI22			Field ID#:	GI23			Field ID#:	VB25		
Initial Tissue Weight (mg):	37,000			Initial Tissue Weight (mg):	37,700			Initial Tissue Weight (mg):	35,600		
Final Extract Volume (ml):	80.00			Final Extract Volume (ml):	80.00			Final Extract Volume (ml):	45.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	307		336	Fluorene	252		276	Fluorene	151		162
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	275		301	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	379		415	C3-Fluorenes	348		373
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	10.1	J	10.9
C1-Dibenzothiophenes	222		243	C1-Dibenzothiophenes	187		205	C1-Dibenzothiophenes	125		133
C2-Dibenzothiophenes	1138		1246	C2-Dibenzothiophenes	1076		1178	C2-Dibenzothiophenes	752		805
C3-Dibenzothiophenes	738		809	C3-Dibenzothiophenes	541		593	C3-Dibenzothiophenes	479		513
Phenanthrene	57.2		62.6	Phenanthrene	45.6		49.9	Phenanthrene	41.6		44.6
C1-Phenanthrenes	722		791	C1-Phenanthrenes	585		641	C1-Phenanthrenes	511		547
C2-Phenanthrenes	1680		1840	C2-Phenanthrenes	1263		1383	C2-Phenanthrenes	989		1059
C3-Phenanthrenes	3202		3507	C3-Phenanthrenes	3814		4176	C3-Phenanthrenes	2376		2545
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	11.3		12.3	Anthracene	8.63	J	9.25
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	58.0		63.5	Pyrene	48.9		53.5	Pyrene	39.5		42.3
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	11.3		12.3	Chrysene	11.6		12.7	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	20.7		22.6	Benzo (b) Fluoranthene	12.7		13.9	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	14.8		16.2	Benzo (k) Fluoranthene	9.58	J	10.5	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	12.6		13.7	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	22.8		24.9	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*8207*		*8988*	*Total Aromatics*	*8512*		*9320*	*Total Aromatics*	*5830*		*6245*
% Surrogate Recovery				*% Surrogate Recovery*				*% Surrogate Recovery*			
Phenanthrene d-10	91		100	Phenanthrene d-10	91		100	Phenanthrene d-10	93		100

	Greg Olson - Fish Samples Field ID#: VB26 Initial Tissue Weight (mg): 10,001 Final Extract Volume (ml): 30.00			Greg Olson - Fish Samples Field ID#: VB27 Initial Tissue Weight (mg): 9998.94 Final Extract Volume (ml): 25.00			Greg Olson - Fish Samples Field ID#: GI28 Initial Tissue Weight (mg): 9999.12 Final Extract Volume (ml): 25.00		
rected (ng/g)	**Aromatic Analyte:**	**Concentration (ng/g)**	**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**	**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**	**Surrogate Corrected (ng**
	Naphthalene	U U	U	Naphthalene	U U	U	Naphthalene	U U	U
	C1-Naphthalenes	U U	U	C1-Naphthalenes	U U	U	C1-Naphthalenes	U U	U
	C2-Naphthalenes	U U	U	C2-Naphthalenes	U U	U	C2-Naphthalenes	U U	U
	C3-Naphthalenes	U U	U	C3-Naphthalenes	U U	U	C3-Naphthalenes	U U	U
	C4-Naphthalenes	U U	U	C4-Naphthalenes	U U	U	C4-Naphthalenes	U U	U
2	Fluorene	125	139	Fluorene	126	141	Fluorene	161	182
	C1-Fluorenes	U U	U	C1-Fluorenes	U U	U	C1-Fluorenes	U U	U
	C2-Fluorenes	U U	U	C2-Fluorenes	U U	U	C2-Fluorenes	U U	U
3	C3-Fluorenes	U U	U	C3-Fluorenes	2279	2536	C3-Fluorenes	1657	1867
9	Dibenzothiophene	13.3	14.8	Dibenzothiophene	21.1 J	23.4	Dibenzothiophene	21.0 J	23.7
3	C1-Dibenzothiophenes	173	193	C1-Dibenzothiophenes	338	376	C1-Dibenzothiophenes	288	324
5	C2-Dibenzothiophenes	872	974	C2-Dibenzothiophenes	1470	1636	C2-Dibenzothiophenes	1085	1223
3	C3-Dibenzothiophenes	484	540	C3-Dibenzothiophenes	948	1055	C3-Dibenzothiophenes	714	804
6	Phenanthrene	58.6	65.4	Phenanthrene	102	114	Phenanthrene	91.5	103.1
7	C1-Phenanthrenes	578	645	C1-Phenanthrenes	1186	1320	C1-Phenanthrenes	899	1013
8	C2-Phenanthrenes	1160	1295	C2-Phenanthrenes	2449	2726	C2-Phenanthrenes	1782	2008
5	C3-Phenanthrenes	2233	2492	C3-Phenanthrenes	2565	2855	C3-Phenanthrenes	2124	2393
	C4-Phenanthrenes	U U	U	C4-Phenanthrenes	U U	U	C4-Phenanthrenes	U U	U
5	Anthracene	U U	U	Anthracene	U U	U	Anthracene	U U	U
	Fluoranthene	U U	U	Fluoranthene	38.4	42.7	Fluoranthene	37.3	42.0
3	Pyrene	62.2	69.5	Pyrene	195	217	Pyrene	132	149
	C1- Pyrenes	U U	U	C1- Pyrenes	U U	U	C1- Pyrenes	U U	U
	C2- Pyrenes	U U	U	C2- Pyrenes	U U	U	C2- Pyrenes	U U	U
	C3- Pyrenes	U U	U	C3- Pyrenes	U U	U	C3- Pyrenes	U U	U
	C4- Pyrenes	U U	U	C4- Pyrenes	U U	U	C4- Pyrenes	U U	U
	Naphthobenzothiophene	U U	U	Naphthobenzothiophene	U U	U	Naphthobenzothiophene	U U	U
	C-1 Naphthobenzothiophenes	U U	U	C-1 Naphthobenzothiophenes	U U	U	C-1 Naphthobenzothiophenes	U U	U
	C-2 Naphthobenzothiophenes	U U	U	C-2 Naphthobenzothiophenes	U U	U	C-2 Naphthobenzothiophenes	U U	U
	C-3 Naphthobenzothiophenes	U U	U	C-3 Naphthobenzothiophenes	U U	U	C-3 Naphthobenzothiophenes	U U	U
	Benzo (a) Anthracene	U U	U	Benzo (a) Anthracene	U U	U	Benzo (a) Anthracene	U U	U
	Chrysene	25.9	28.9	Chrysene	U U	U	Chrysene	U U	U
	C1- Chrysenes	U U	U	C1- Chrysenes	U U	U	C1- Chrysenes	U U	U
	C2- Chrysenes	U U	U	C2- Chrysenes	U U	U	C2- Chrysenes	U U	U
	C3- Chrysenes	U U	U	C3- Chrysenes	U U	U	C3- Chrysenes	U U	U
	C4- Chrysenes	U U	U	C4- Chrysenes	U U	U	C4- Chrysenes	U U	U
	Benzo (b) Fluoranthene	U U	U	Benzo (b) Fluoranthene	U U	U	Benzo (b) Fluoranthene	U U	U
	Benzo (k) Fluoranthene	U U	U	Benzo (k) Fluoranthene	U U	U	Benzo (k) Fluoranthene	U U	U
	Benzo (e) Pyrene	U U	U	Benzo (e) Pyrene	U U	U	Benzo (e) Pyrene	U U	U
	Benzo (a) Pyrene	U U	U	Benzo (a) Pyrene	U U	U	Benzo (a) Pyrene	U U	U
	Perylene	U U	U	Perylene	U U	U	Perylene	U U	U
	Indeno (1,2,3 - cd) Pyrene	U U	U	Indeno (1,2,3 - cd) Pyrene	U U	U	Indeno (1,2,3 - cd) Pyrene	U U	U
	Dibenzo (a,h) anthracene	U U	U	Dibenzo (a,h) anthracene	U U	U	Dibenzo (a,h) anthracene	U U	U
	Benzo (g,h,i) perylene	U U	U	Benzo (g,h,i) perylene	U U	U	Benzo (g,h,i) perylene	U U	U
5	***Total Aromatics***	***5785***	***6458***	***Total Aromatics***	***11717***	***13041***	***Total Aromatics***	***8992***	***10132***
0	***% Surrogate Recovery*** Phenanthrene d-10	90	100	***% Surrogate Recovery*** Phenanthrene d-10	90	100	***% Surrogate Recovery*** Phenanthrene d-10	89	100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	GI29			Field ID#:	GI30			Field ID#:	VB31		
Initial Tissue Weight (mg):	10000			Initial Tissue Weight (mg):	10001			Initial Tissue Weight (mg):	10,000		
Final Extract Volume (ml):	30.00			Final Extract Volume (ml):	35.00			Final Extract Volume (ml):	30.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	6.998	J	7.655
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	73		79	Fluorene	96.3		108	Fluorene	124		136
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	1206		1305	C3-Fluorenes	1234		1379	C3-Fluorenes	U	U	U
Dibenzothiophene	13.2	J	14.3	Dibenzothiophene	11.9	J	13.3	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	200		216	C1-Dibenzothiophenes	184		206	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	821		888	C2-Dibenzothiophenes	775		866	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	471		510	C3-Dibenzothiophenes	442		494	C3-Dibenzothiophenes	U	U	U
Phenanthrene	69.9		75.6	Phenanthrene	60.0		67.0	Phenanthrene	U	U	U
C1-Phenanthrenes	650		703	C1-Phenanthrenes	606		677	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	1370		1482	C2-Phenanthrenes	1212		1355	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	1672		1808	C3-Phenanthrenes	1546		1728	C3-Phenanthrenes	1689		1848
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	21.1	J	22.9	Fluoranthene	19.5	J	21.8	Fluoranthene	U	U	U
Pyrene	112		121	Pyrene	81.2		90.8	Pyrene	U	U	U
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	66.2		72.4
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	51.6		56.4
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	26.3	J	28.8
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	67.0		73.3
Perylene	U	U	U	Perylene	U	U	U	Perylene	62.3		68.1
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	58.6		64.1
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	68.5		74.9
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	79.4		86.8
Total Aromatics	***6681***		***7227***	***Total Aromatics***	***6269***		***7007***	***Total Aromatics***	***2300***		***2517***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	92		100	Phenanthrene d-10	89		100	Phenanthrene d-10	91		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	VB32			Field ID#:	VB33		
Initial Tissue Weight (mg):	9,998			Initial Tissue Weight (mg):	9,999		
Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	30.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	5.89	J	6.21
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	105		113	Fluorene	147		155
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U	C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U	C3- Dibenzothiophenes	U	U	U
Phenanthrene	U	U	U	Phenanthrene	U	U	U
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	1780		1927	C3-Phenanthrenes	2969		3128
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U
C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U
C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	47.7		51.7	Benzo (b) Fluoranthene	23.3	J	24.6
Benzo (k) Fluoranthene	36.1	J	39.1	Benzo (k) Fluoranthene	19.8	J	20.8
Benzo (e) Pyrene	20.2	J	21.8	Benzo (e) Pyrene	9.50	J	10.0
Benzo (a) Pyrene	34.6	J	37.5	Benzo (a) Pyrene	21.5	J	22.7
Perylene	40.2		43.5	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	53.3		57.7	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	58.5		63.3	Dibenzo (a,h) anthracene	42.7		45.0
Benzo (g,h,i) perylene	61.8		66.9	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*2237*		*2422*	*Total Aromatics*	*3239*		*3413*
% Surrogate Recovery				*% Surrogate Recovery*			
Phenanthrene d-10	92		100	Phenanthrene d-10	95		100



Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	GI35			Field ID#:	GI36			Field ID#:	GI43		
Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,002		
Final Extract Volume (ml):	30.00			Final Extract Volume (ml):	30.00			Final Extract Volume (ml):	20.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	20.2	J	22.5	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	50.14		55.89	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	198		215	Fluorene	206		229	Fluorene	10.4	J	11.6
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	23.8	J	26.6
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	316		354
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	93.1		104
Phenanthrene	12.6	J	13.7	Phenanthrene	12.5	J	13.9	Phenanthrene	8.59	J	9.60
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	66.5		74.3
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	265		296
C3-Phenanthrenes	1497		1630	C3-Phenanthrenes	2076		2314	C3-Phenanthrenes	1559		1742
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U	Pyrene	10.0	J	11.2
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	188		210
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	30.2	J	32.9	Dibenzo (a,h) anthracene	27.8	J	31.0	Dibenzo (a,h) anthracene	18.6	J	20.8
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***1738***		***1892***	***Total Aromatics***	***2392***		***2666***	***Total Aromatics***	***2559***		***2860***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	92		100	Phenanthrene d-10	90		100	Phenanthrene d-10	89		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	GI44			Field ID#:	GI45			Field ID#:	VB46		
Initial Tissue Weight (mg):	9,999			Initial Tissue Weight (mg):	10001.67			Initial Tissue Weight (mg):	10001.52		
Final Extract Volume (ml):	20.00			Final Extract Volume (ml):	25.00			Final Extract Volume (ml):	20.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (**
Naphthalene	5.380	J	6.067	Naphthalene	U	U	U	Naphthalene	5.60	J	6.35
C1-Naphthalenes	62.7		70.7	C1-Naphthalenes	U	U	U	C1-Naphthalenes	50.1		56.9
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	10.9	J	12.3	Fluorene	9	J	10	Fluorene	7	J	8
C1-Fluorenes	63.1		71.1	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U	C3- Fluorenes	U	U	U	C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U	C3- Dibenzothiophenes	U	U	U	C3- Dibenzothiophenes	U	U	U
Phenanthrene	11.8	J	13.3	Phenanthrene	13.8	J	15.2	Phenanthrene	16.1	J	18.3
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	339		382	C2-Phenanthrenes	224		247	C2-Phenanthrenes	242		275
C3-Phenanthrenes	3423		3859	C3-Phenanthrenes	2430		2680	C3-Phenanthrenes	2905		3298
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	11.9	J	13.5	Anthracene	11.0	J	12.1	Anthracene	7.47	J	8.48
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	16.6	J	18.3	Pyrene	U	U	U
C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***3928***		***4429***	***Total Aromatics***	***2704***		***2982***	***Total Aromatics***	***3234***		***3672***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	89		100	Phenanthrene d-10	91		100	Phenanthrene d-10	88		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	VB47			Field ID#:	VB48			Field ID#:	VB61		
Initial Tissue Weight (mg):	9999			Initial Tissue Weight (mg):	9999			Initial Tissue Weight (mg):	10,000		
Final Extract Volume (ml):	25.00			Final Extract Volume (ml):	20.00			Final Extract Volume (ml):	50.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (r**
Naphthalene	8.61	J	9.75	Naphthalene	6.63	J	7.31	Naphthalene	U	U	U
C1-Naphthalenes	85.8		97.2	C1-Naphthalenes	127		140	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	U	U	U	Fluorene	7	J	8	Fluorene	123		135
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U	C3- Fluorenes	U	U	U	C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U	C3- Dibenzothiophenes	U	U	U	C3- Dibenzothiophenes	U	U	U
Phenanthrene	12.8	J	14.5	Phenanthrene	29.0		32.0	Phenanthrene	56.1		61.5
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	288		326	C2-Phenanthrenes	310		341	C2-Phenanthrenes	993		1087
C3-Phenanthrenes	3867		4379	C3-Phenanthrenes	4802		5295	C3-Phenanthrenes	10405		11399
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	8.32	J	9.17	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	6.42	J	7.08	Fluoranthene	U	U	U
Pyrene	11.2	J	12.6	Pyrene	21.9		24.2	Pyrene	U	U	U
C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	12.2	J	13.4	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	74.7		81.9
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	60.1		65.9
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	89.4		98.0
Perylene	U	U	U	Perylene	U	U	U	Perylene	68.2		74.8
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	114		125
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***4273***		***4838***	***Total Aromatics***	***5330***		***5877***	***Total Aromatics***	***11983***		***13128***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	88		100	Phenanthrene d-10	91		100	Phenanthrene d-10	91		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	VB62			Field ID#:	VB63			Field ID#:	GI64		
Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,000		
Final Extract Volume (ml):	50.00			Final Extract Volume (ml):	50.00			Final Extract Volume (ml):	40.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	265		296	Fluorene	101		111	Fluorene	190		221
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	385		430	C3-Fluorenes	U	U	U	C3-Fluorenes	209		243
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U
Phenanthrene	66.3		74.0	Phenanthrene	46.4	J	51.4	Phenanthrene	37.1	J	43.2
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	1898		2120	C2-Phenanthrenes	1863		2062	C2-Phenanthrenes	1186		1378
C3-Phenanthrenes	15670		17505	C3-Phenanthrenes	9738		10778	C3-Phenanthrenes	5611		6523
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	22.8	J	25.5	Pyrene	31.8	J	35.2	Pyrene	25.7	J	29.8
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	36.3	J	40.6	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	28.2	J	31.5	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*18371*		*20523*	*Total Aromatics*	*11780*		*13038*	*Total Aromatics*	*7258*		*8438*
% Surrogate Recovery				*% Surrogate Recovery*				*% Surrogate Recovery*			
Phenanthrene d-10	90		100	Phenanthrene d-10	90		100	Phenanthrene d-10	86		100

Greg Olson - Fish Samples			
Field ID#:	GI65		
Initial Tissue Weight (mg):	10.000		
Final Extract Volume (ml):	35.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U
C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U
Fluorene	295		345
C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U
Phenanthrene	31.9	J	37.4
C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	977		1144
C3-Phenanthrenes	7410		8677
C4-Phenanthrenes	U	U	U
Anthracene	U	U	U
Fluoranthene	U	U	U
Pyrene	11.4	J	13.4
C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U
C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U
Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*8725*		*10216*
% Surrogate Recovery			
Phenanthrene d-10	85		100

Greg Olson - Fish Samples			
Field ID#:	GI66		
Initial Tissue Weight (mg):	10.000		
Final Extract Volume (ml):	30.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U
C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U
Fluorene	118		128
C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U
Phenanthrene	98.5		107
C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	1181		1284
C3-Phenanthrenes	10476		11388
C4-Phenanthrenes	U	U	U
Anthracene	U	U	U
Fluoranthene	U	U	U
Pyrene	U	U	U
C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U
C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U
Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*11873*		*12907*
% Surrogate Recovery			
Phenanthrene d-10	92		100

Greg Olson - Fish Samples			
Field ID#:	VB73		
Initial Tissue Weight (mg):	10.000		
Final Extract Volume (ml):	30.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U
C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U
Fluorene	164		198
C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U
Phenanthrene	34.1	J	41.2
C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	439		530
C3-Phenanthrenes	4940		5959
C4-Phenanthrenes	U	U	U
Anthracene	U	U	U
Fluoranthene	U	U	U
Pyrene	U	U	U
C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U
C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U
Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*5577*		*6728*
% Surrogate Recovery			
Phenanthrene d-10	83		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	VB74			Field ID#:	VB75			Field ID#:	GI76		
Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,000		
Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	40.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	215		261	Fluorene	223		265	Fluorene	80.8		93.5
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U
Phenanthrene	U	U	U	Phenanthrene	53.6		63.7	Phenanthrene	U	U	U
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	4481		5425	C3-Phenanthrenes	5047		5997	C3-Phenanthrenes	1911		2212
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U	Pyrene	U	U	U
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***4696***		***5685***	***Total Aromatics***	***5324***		***6326***	***Total Aromatics***	***1992***		***2306***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	83		100	Phenanthrene d-10	84		100	Phenanthrene d-10	86		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	GI77			Field ID#:	GI78			Field ID#:	GI85		
Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	5,000		
Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	30.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	78.7		97.8	Fluorene	60.6		71.7	Fluorene	U	U	U
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U
Phenanthrene	U	U	U	Phenanthrene	U	U	U	Phenanthrene	26.9	J	31.4
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	1442		1792	C3-Phenanthrenes	1809		2138	C3-Phenanthrenes	1031		1202
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U	Pyrene	U	U	U
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	61.7		72.0
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	61.5		71.7
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	31.54	J	36.8
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	52.9	J	61.7
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	80.8		94.3
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***1521***		***1890***	***Total Aromatics***	***1870***		***2210***	***Total Aromatics***	***1346***		***1570***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	80		100	Phenanthrene d-10	85		100	Phenanthrene d-10	86		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	GI86			Field ID#:	GI87			Field ID#:	VB88		
Initial Tissue Weight (mg):	5,000			Initial Tissue Weight (mg):	5,000			Initial Tissue Weight (mg):	10,000		
Final Extract Volume (ml):	30.00			Final Extract Volume (ml):	30.00			Final Extract Volume (ml):	40.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	U	U	U	Fluorene	U	U	U	Fluorene	U	U	U
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U
Phenanthrene	24.2	J	26.4	Phenanthrene	U	U	U	Phenanthrene	U	U	U
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	392		425
C3-Phenanthrenes	1327		1451	C3-Phenanthrenes	1085		1218	C3-Phenanthrenes	4473		4858
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U	Pyrene	U	U	U
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	31.8	J	34.8	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	26.2	J	28.7	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***1410***		***1541***	***Total Aromatics***	***1085***		***1218***	***Total Aromatics***	***4865***		***5283***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	92		100	Phenanthrene d-10	89		100	Phenanthrene d-10	92		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	VB89			Field ID#:	VB90			Field ID#:	GI91		
Initial Tissue Weight (mg):	10.000			Initial Tissue Weight (mg):	10.000			Initial Tissue Weight (mg):	10.000		
Final Extract Volume (ml):	35.00			Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	40.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	U	U	U	Fluorene	U	U	U	Fluorene	U	U	U
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U
Phenanthrene	14.0	J	14.5	Phenanthrene	U	U	U	Phenanthrene	U	U	U
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	401		420	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	1820		1886.4	C3-Phenanthrenes	4407		4620	C3-Phenanthrenes	2803		2952
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U	Pyrene	U	U	U
C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*1834*		*1901*	*Total Aromatics*	*4808*		*5040*	*Total Aromatics*	*2803*		*2952*
% Surrogate Recovery				*% Surrogate Recovery*				*% Surrogate Recovery*			
Phenanthrene d-10	96		100	Phenanthrene d-10	95		100	Phenanthrene d-10	95		100

Greg Olson - Fish Samples			
	Field ID#:	GI92	
	Initial Tissue Weight (mg):	10,000	
	Final Extract Volume (ml):	35.00	
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U
C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U
Fluorene	U	U	U
C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U
Phenanthrene	13.9	J	14.6
C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	1812		1902
C4-Phenanthrenes	U	U	U
Anthracene	U	U	U
Fluoranthene	U	U	U
Pyrene	U	U	U
C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U
C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U
Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*1826*		*1916*
% Surrogate Recovery			
Phenanthrene d-10	95		100

Greg Olson - Fish Samples			
	Field ID#:	GI93	
	Initial Tissue Weight (mg):	10,000	
	Final Extract Volume (ml):	35.00	
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U
C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U
Fluorene	U	U	U
C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U
Phenanthrene	18.9	J	21.0
C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	1839		2052
C4-Phenanthrenes	U	U	U
Anthracene	U	U	U
Fluoranthene	U	U	U
Pyrene	U	U	U
C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U
C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U
Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*1858*		*2073*
% Surrogate Recovery			
Phenanthrene d-10	90		100

Greg Olson - Fish Samples			
	Field ID#:	GI94	
	Initial Tissue Weight (mg):	10,000	
	Final Extract Volume (ml):	40.00	
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U
C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U
Fluorene	32.9	J	36.1
C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	44.9		49.3
C3- Dibenzothiophenes	U	U	U
Phenanthrene	30.5	J	33.5
C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	1754		1924
C4-Phenanthrenes	U	U	U
Anthracene	U	U	U
Fluoranthene	U	U	U
Pyrene	U	U	U
C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U
C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	37.8	J	41.5
Benzo (k) Fluoranthene	42.0		46.1
Benzo (e) Pyrene	30.9	J	33.9
Benzo (a) Pyrene	57.3		62.9
Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	65.3		71.6
Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*2055*		*2255*
% Surrogate Recovery			
Phenanthrene d-10	91		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	GI95			Field ID#:	GI96			Field ID#:	Method Blk		
Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	10,000			Initial Tissue Weight (mg):	3,800		
Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	40.00			Final Extract Volume (ml):	30.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	U	U	U	Fluorene	25.9	J	28.7	Fluorene	U	U	U
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	26.7	J	30.3	C3-Dibenzothiophenes	237		262	C3-Dibenzothiophenes	U	U	U
Phenanthrene	35.0	J	39.8	Phenanthrene	43.2		47.8	Phenanthrene	U	U	U
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	398		452	C2-Phenanthrenes	539		597	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	2782		3162	C3-Phenanthrenes	3764		4165	C3-Phenanthrenes	U	U	U
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	7.92	J	8.77	Pyrene	U	U	U
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*3241*		*3684*	*Total Aromatics*	*4616*		*5109*	*Total Aromatics*	*0.000*		*0.000*
% Surrogate Recovery				*% Surrogate Recovery*				*% Surrogate Recovery*			
Phenanthrene d-10	88		100	Phenanthrene d-10	90		100	Phenanthrene d-10	99		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	C1			Field ID#:	C2			Field ID#:	C3		
Initial Tissue Weight (mg):	2,870			Initial Tissue Weight (mg):	2,849			Initial Tissue Weight (mg):	2,858		
Final Extract Volume (ml):	20.00			Final Extract Volume (ml):	35.00			Final Extract Volume (ml):	25.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	U	U	U	Fluorene	U	U	U	Fluorene	U	U	U
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U
Phenanthrene	41.5	J	47.3	Phenanthrene	41.6	J	47.4	Phenanthrene	40.5	J	45.8
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	2419		2759	C3-Phenanthrenes	2539		2893	C3-Phenanthrenes	2751		3116
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U	Pyrene	U	U	U
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U	C1-Chrysenes	U	U	U
C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U	C2-Chrysenes	U	U	U
C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U	C3-Chrysenes	U	U	U
C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U	C4-Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***2461***		***2806***	***Total Aromatics***	***2580***		***2940***	***Total Aromatics***	***2792***		***3162***
% Surrogate Recovery				***% Surrogate Recovery***				***% Surrogate Recovery***			
Phenanthrene d-10	88		100	Phenanthrene d-10	88		100	Phenanthrene d-10	88		100

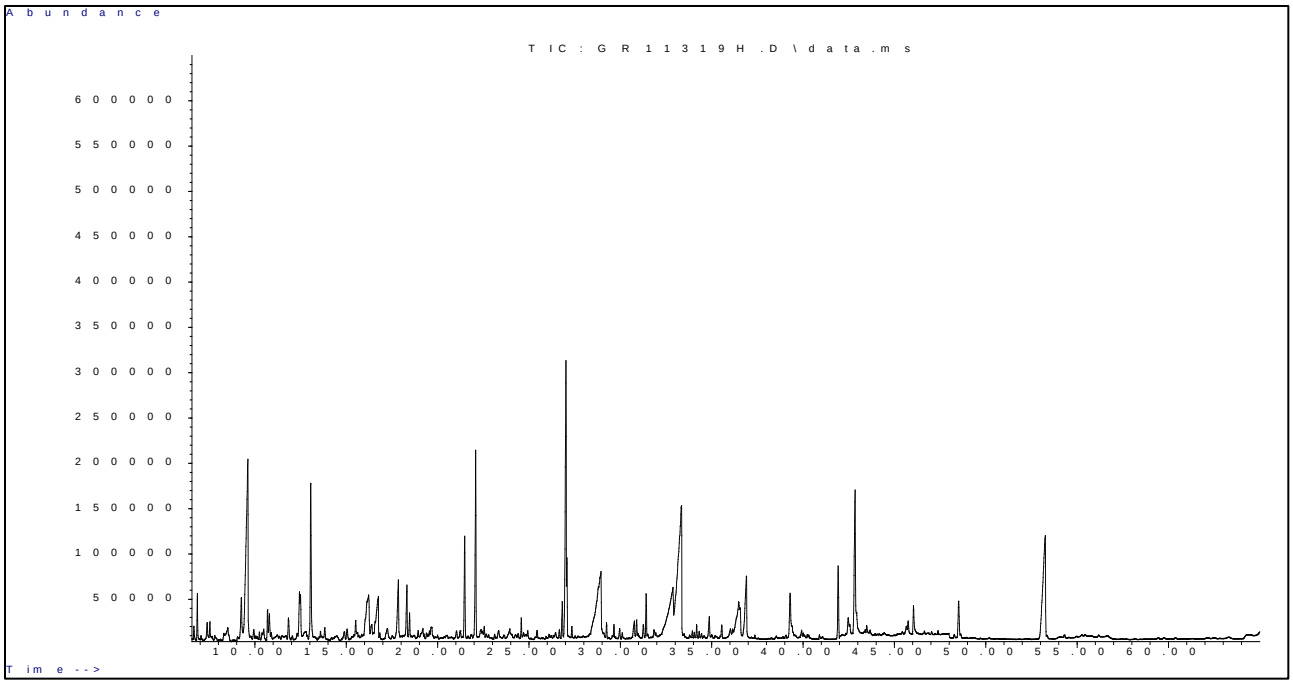
Greg Olson - Fish Samples				Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	C4			Field ID#:	C5			Field ID#:	C6		
Initial Tissue Weight (mg):	3,470			Initial Tissue Weight (mg):	3,460			Initial Tissue Weight (mg):	3,434		
Final Extract Volume (ml):	25.00			Final Extract Volume (ml):	25.00			Final Extract Volume (ml):	25.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	U	U	U	Fluorene	U	U	U	Fluorene	U	U	U
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U	C3-Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U	C3-Dibenzothiophenes	U	U	U
Phenanthrene	40.8	J	47.0	Phenanthrene	41.8	J	47.3	Phenanthrene	40.2	J	46.4
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	3461		3983	C3-Phenanthrenes	3614		4093	C3-Phenanthrenes	3367		3882
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U	Pyrene	U	U	U
C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U	C1-Pyrenes	U	U	U
C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U	C2-Pyrenes	U	U	U
C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U	C3-Pyrenes	U	U	U
C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U	C4-Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U	Chrysene	U	U	U
C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	*3502*		*4030*	*Total Aromatics*	*3655*		*4140*	*Total Aromatics*	*3407*		*3929*
% Surrogate Recovery				*% Surrogate Recovery*				*% Surrogate Recovery*			
Phenanthrene d-10	87		100	Phenanthrene d-10	88		100	Phenanthrene d-10	87		100

Greg Olson - Fish Samples				Greg Olson - Fish Samples			
Field ID#:	MB2			Field ID#:	MB3		
Initial Tissue Weight (mg):	5,000			Initial Tissue Weight (mg):	5,000		
Final Extract Volume (ml):	45.00			Final Extract Volume (ml):	35.00		
Aromatic Analyte:	**Concentration (ng/g)**		**Surrogate Corrected (ng/g**	**Aromatic Analyte:**	**Concentration (ng/g)**		**Surrogate Corrected (ng/g)**
Naphthalene	U	U	U	Naphthalene	U	U	U
C1-Naphthalenes	U	U	U	C1-Naphthalenes	U	U	U
C2-Naphthalenes	U	U	U	C2-Naphthalenes	U	U	U
C3-Naphthalenes	U	U	U	C3-Naphthalenes	U	U	U
C4-Naphthalenes	U	U	U	C4-Naphthalenes	U	U	U
Fluorene	U	U	U	Fluorene	U	U	U
C1-Fluorenes	U	U	U	C1-Fluorenes	U	U	U
C2-Fluorenes	U	U	U	C2-Fluorenes	U	U	U
C3- Fluorenes	U	U	U	C3- Fluorenes	U	U	U
Dibenzothiophene	U	U	U	Dibenzothiophene	U	U	U
C1-Dibenzothiophenes	U	U	U	C1-Dibenzothiophenes	U	U	U
C2-Dibenzothiophenes	U	U	U	C2-Dibenzothiophenes	U	U	U
C3- Dibenzothiophenes	U	U	U	C3- Dibenzothiophenes	U	U	U
Phenanthrene	U	U	U	Phenanthrene	U	U	U
C1-Phenanthrenes	U	U	U	C1-Phenanthrenes	U	U	U
C2-Phenanthrenes	U	U	U	C2-Phenanthrenes	U	U	U
C3-Phenanthrenes	U	U	U	C3-Phenanthrenes	U	U	U
C4-Phenanthrenes	U	U	U	C4-Phenanthrenes	U	U	U
Anthracene	U	U	U	Anthracene	U	U	U
Fluoranthene	U	U	U	Fluoranthene	U	U	U
Pyrene	U	U	U	Pyrene	U	U	U
C1- Pyrenes	U	U	U	C1- Pyrenes	U	U	U
C2- Pyrenes	U	U	U	C2- Pyrenes	U	U	U
C3- Pyrenes	U	U	U	C3- Pyrenes	U	U	U
C4- Pyrenes	U	U	U	C4- Pyrenes	U	U	U
Naphthobenzothiophene	U	U	U	Naphthobenzothiophene	U	U	U
C-1 Naphthobenzothiophenes	U	U	U	C-1 Naphthobenzothiophenes	U	U	U
C-2 Naphthobenzothiophenes	U	U	U	C-2 Naphthobenzothiophenes	U	U	U
C-3 Naphthobenzothiophenes	U	U	U	C-3 Naphthobenzothiophenes	U	U	U
Benzo (a) Anthracene	U	U	U	Benzo (a) Anthracene	U	U	U
Chrysene	U	U	U	Chrysene	U	U	U
C1- Chrysenes	U	U	U	C1- Chrysenes	U	U	U
C2- Chrysenes	U	U	U	C2- Chrysenes	U	U	U
C3- Chrysenes	U	U	U	C3- Chrysenes	U	U	U
C4- Chrysenes	U	U	U	C4- Chrysenes	U	U	U
Benzo (b) Fluoranthene	U	U	U	Benzo (b) Fluoranthene	U	U	U
Benzo (k) Fluoranthene	U	U	U	Benzo (k) Fluoranthene	U	U	U
Benzo (e) Pyrene	U	U	U	Benzo (e) Pyrene	U	U	U
Benzo (a) Pyrene	U	U	U	Benzo (a) Pyrene	U	U	U
Perylene	U	U	U	Perylene	U	U	U
Indeno (1,2,3 - cd) Pyrene	U	U	U	Indeno (1,2,3 - cd) Pyrene	U	U	U
Dibenzo (a,h) anthracene	U	U	U	Dibenzo (a,h) anthracene	U	U	U
Benzo (g,h,i) perylene	U	U	U	Benzo (g,h,i) perylene	U	U	U
Total Aromatics	***0.000***		***0.000***	***Total Aromatics***	***0.000***		***0.000***
% Surrogate Recovery				***% Surrogate Recovery***			
Phenanthrene d-10	85		100	Phenanthrene d-10	86		100

APPENDIX C: CHROMATOGRAMS

Grand Isle

GI22



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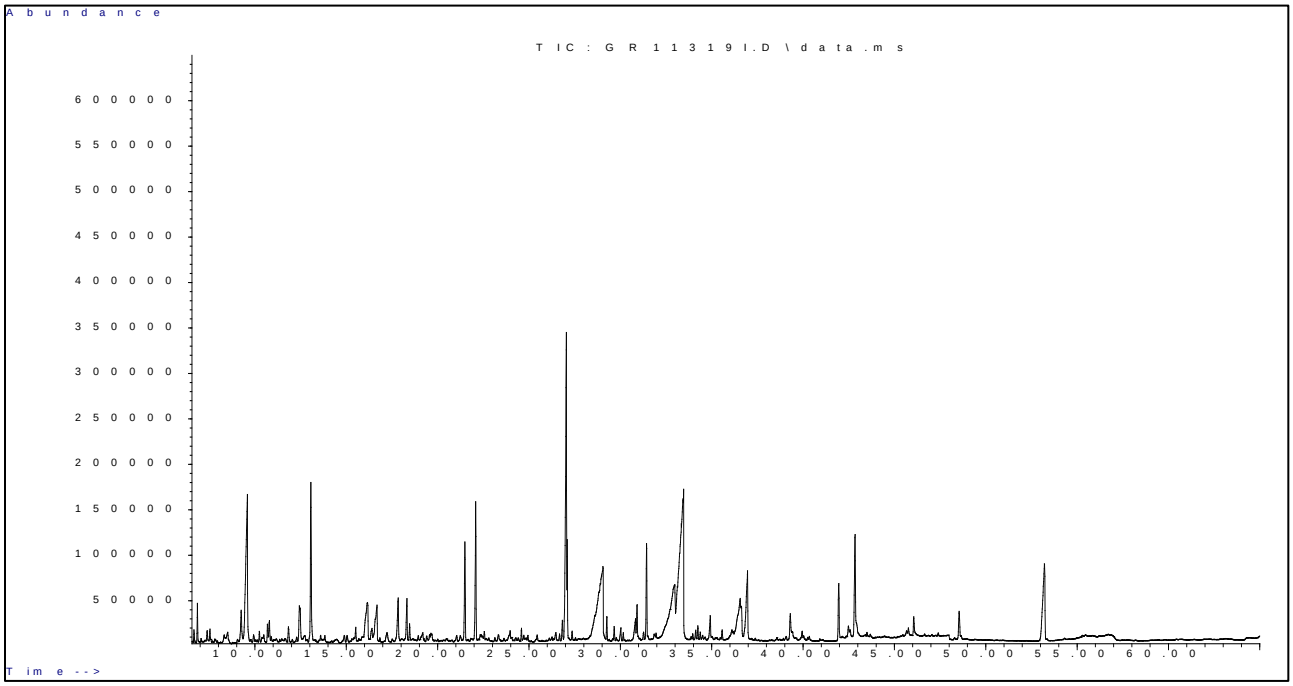

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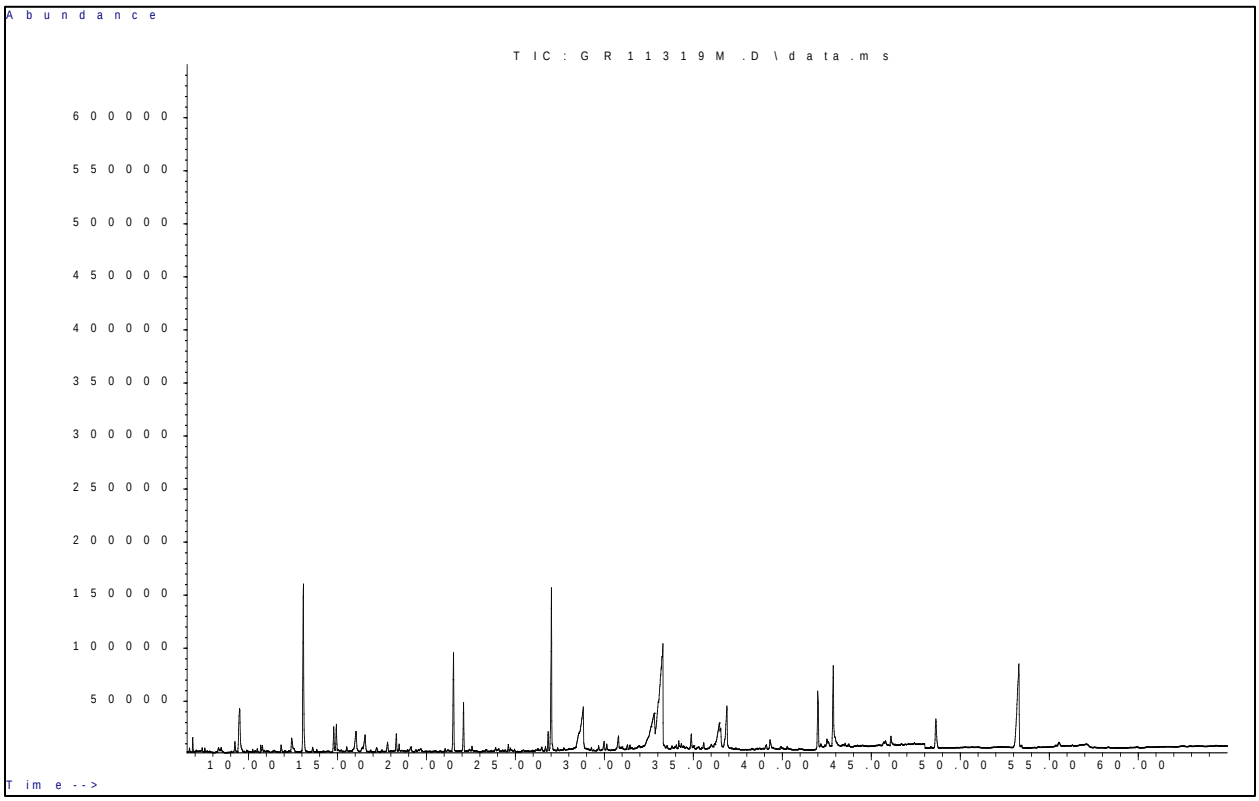
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Time -->

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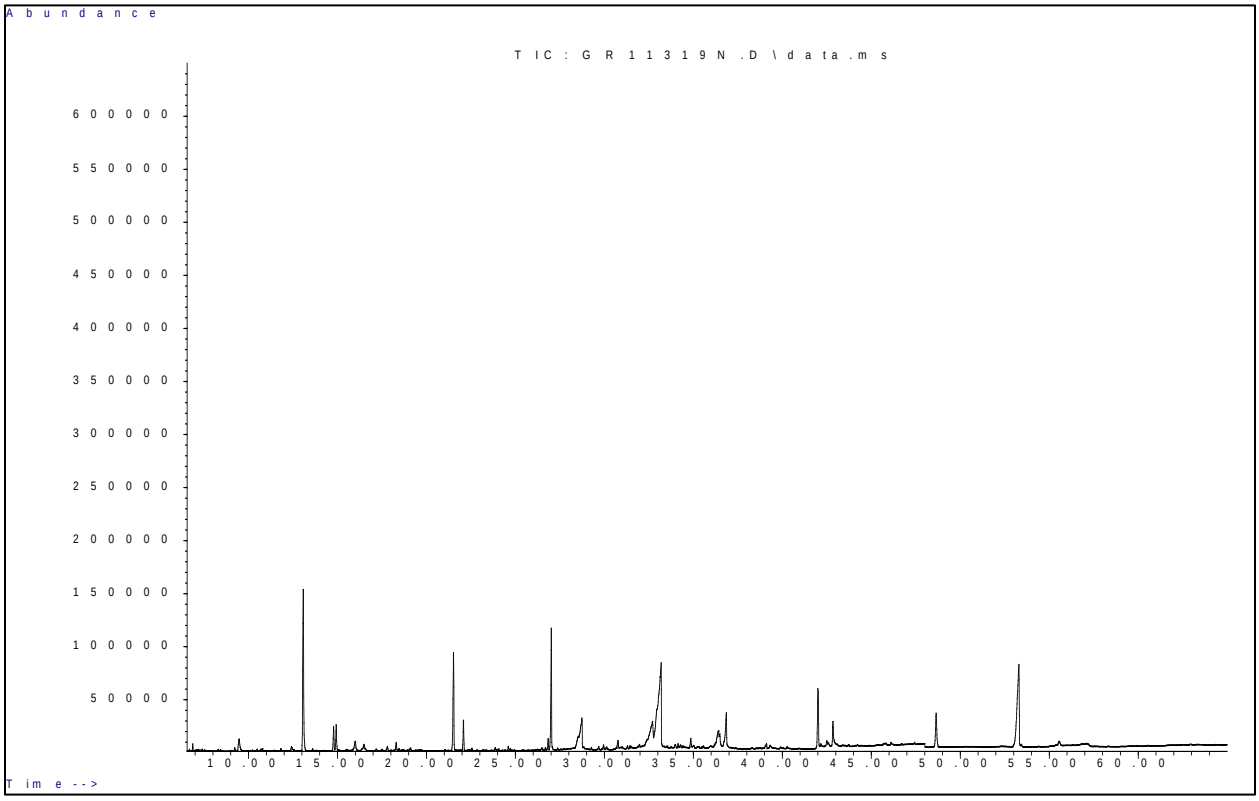
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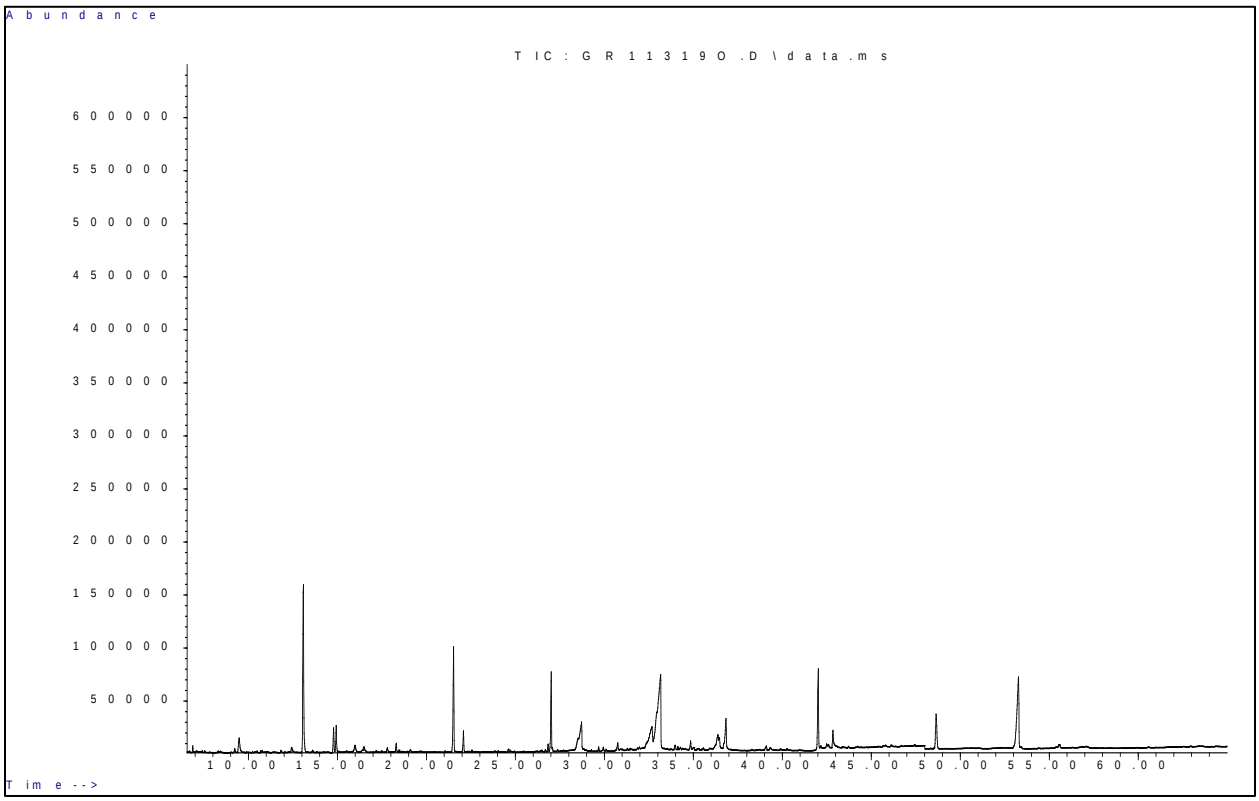
GI30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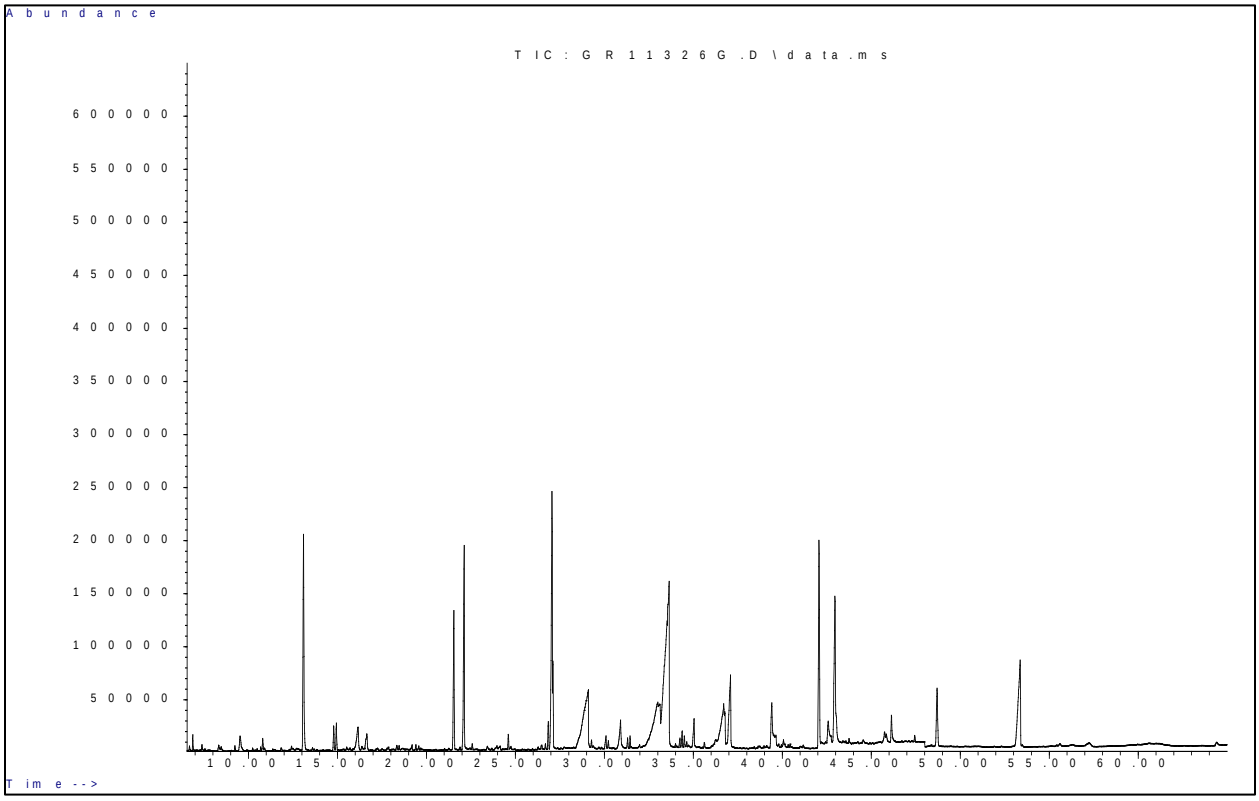
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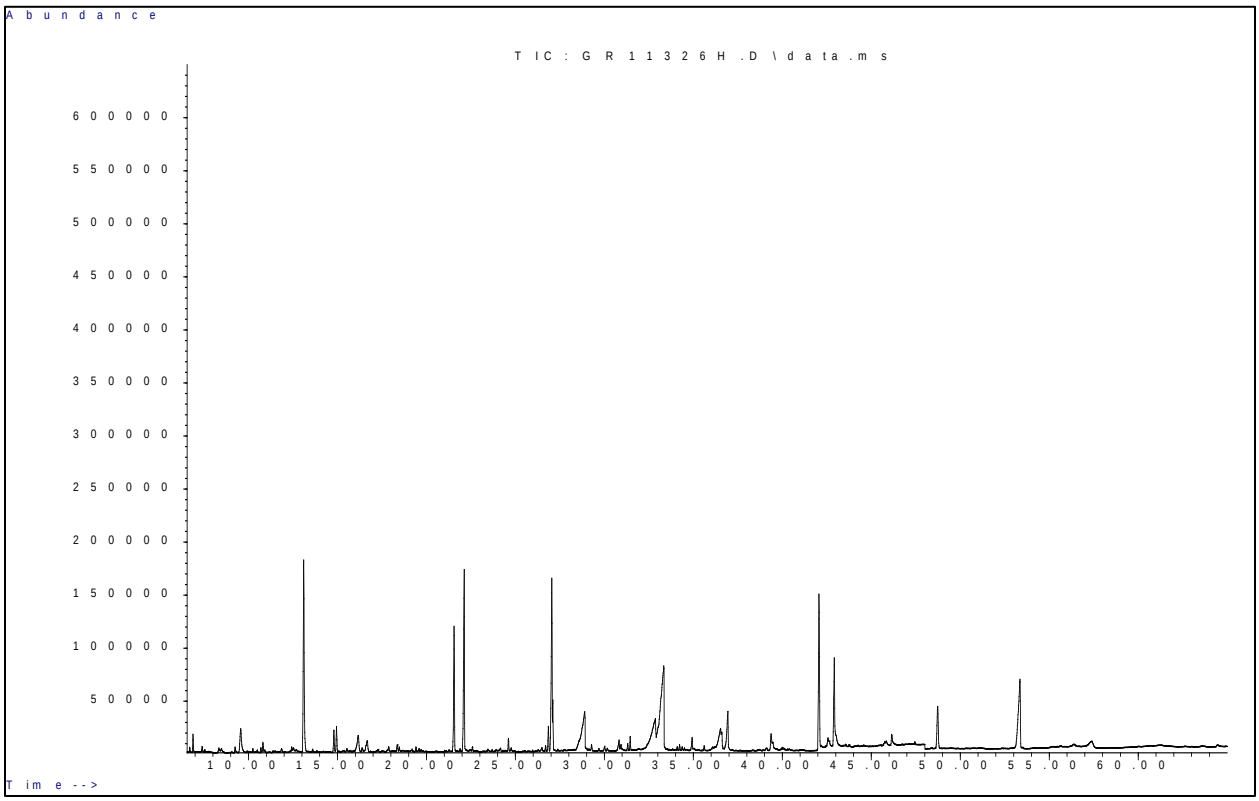
Time -->

GI35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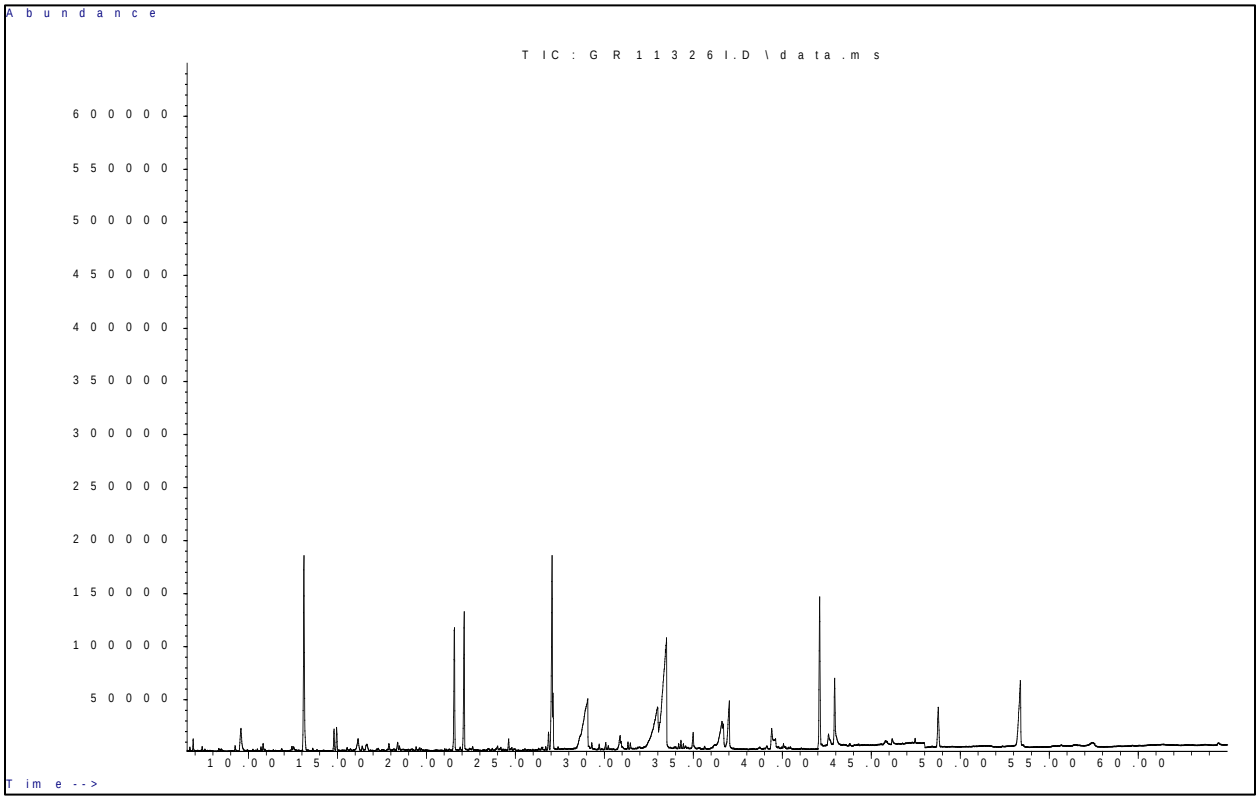
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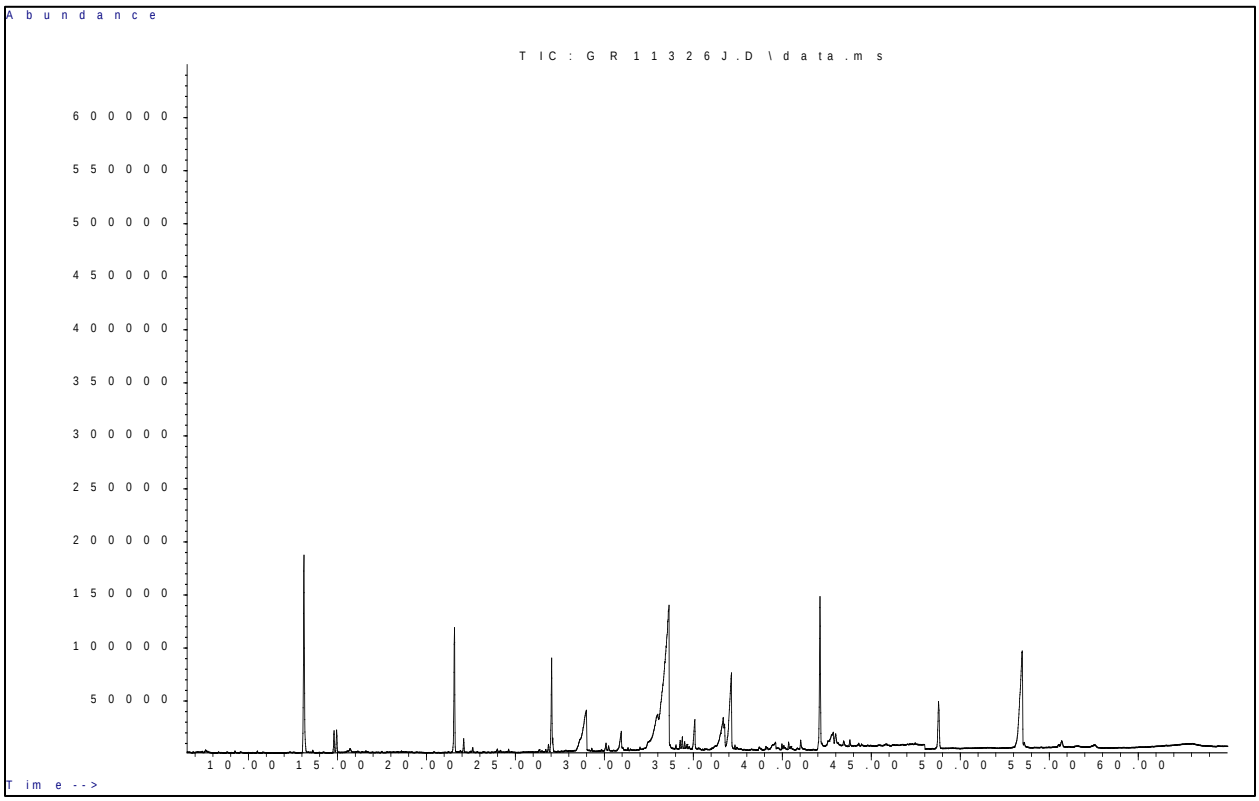
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Time -->

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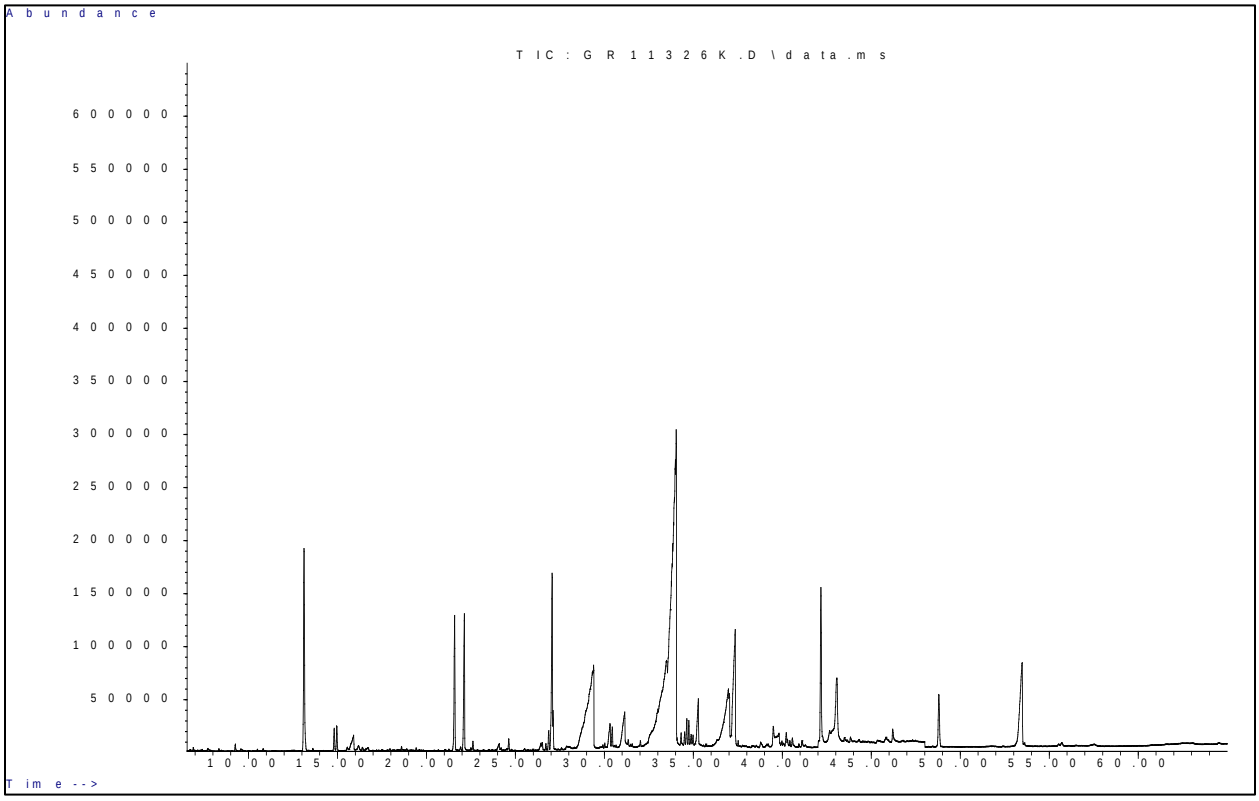
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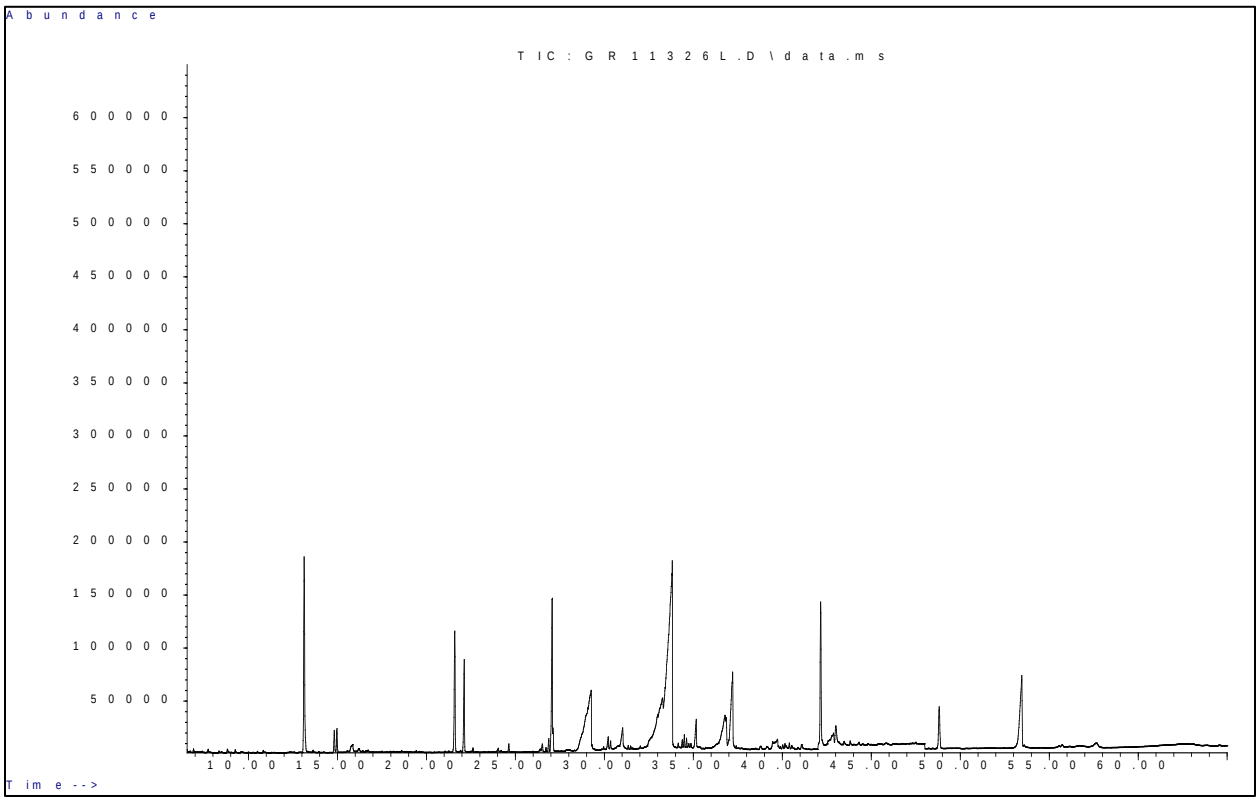
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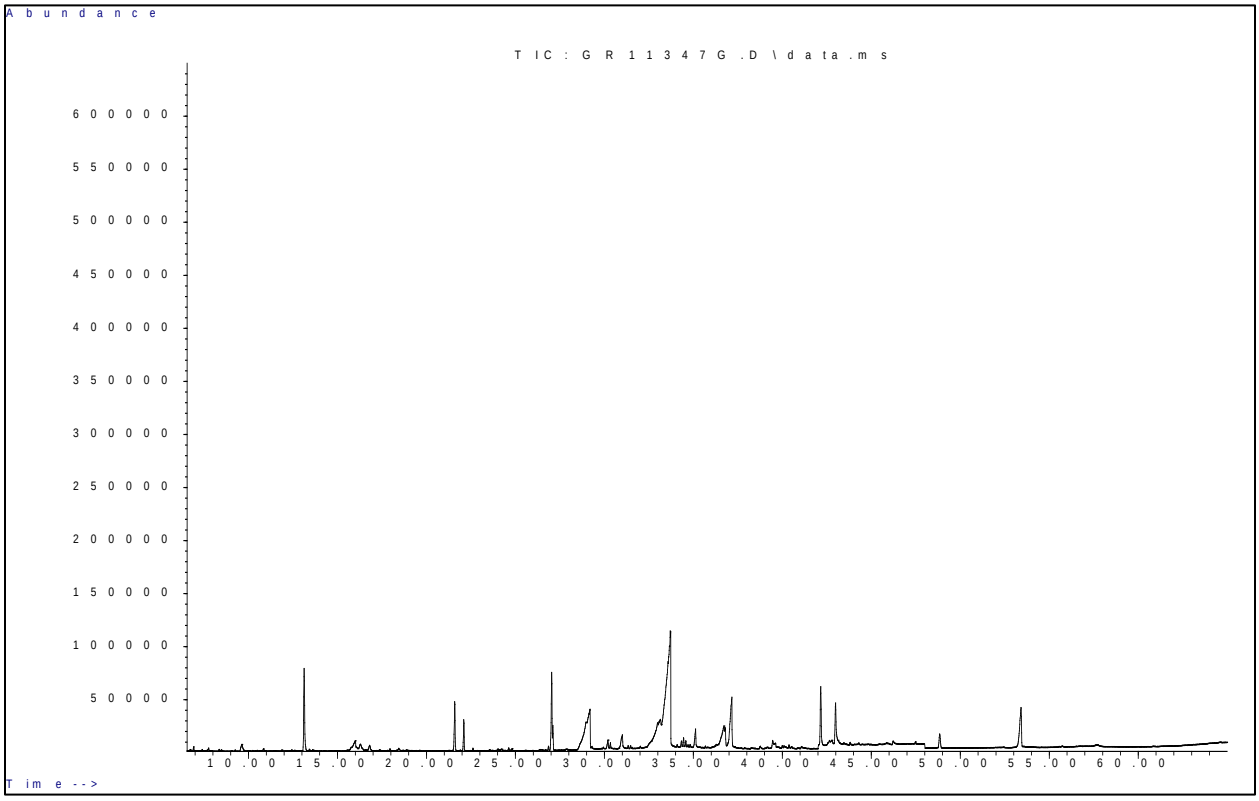
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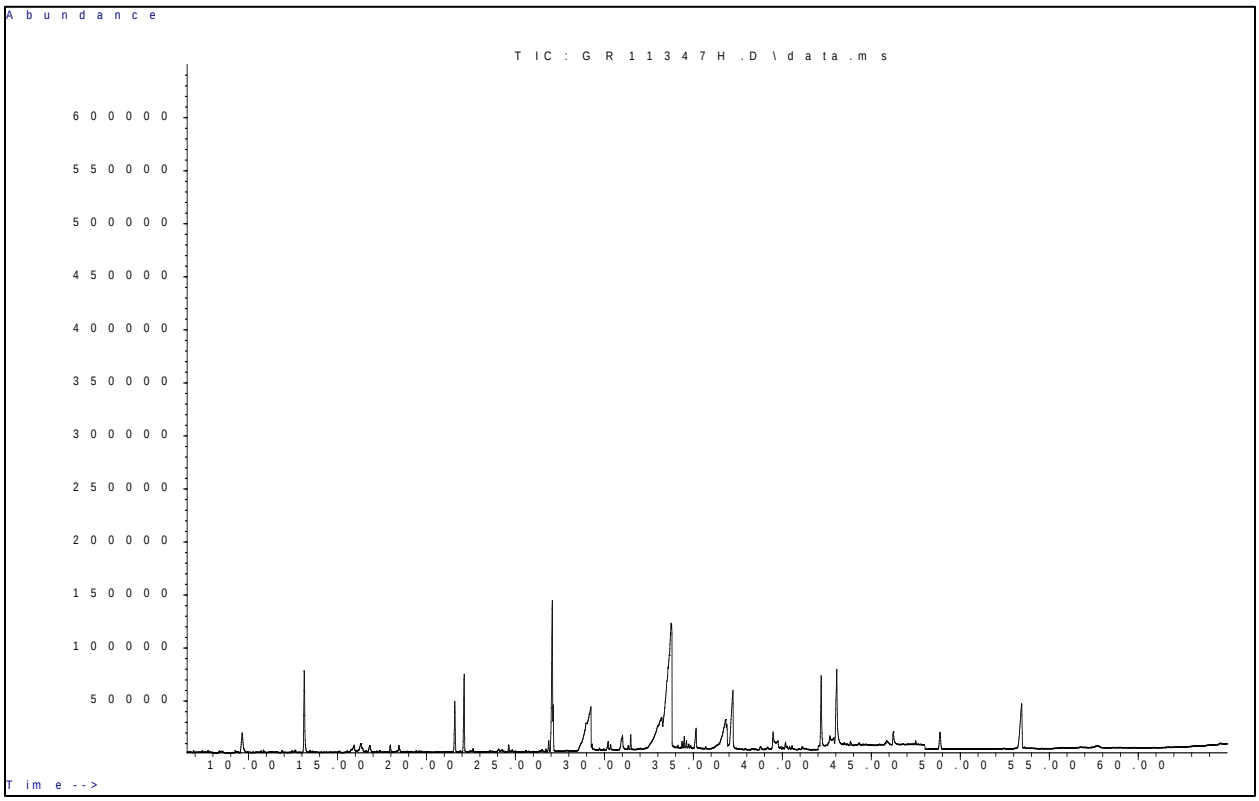
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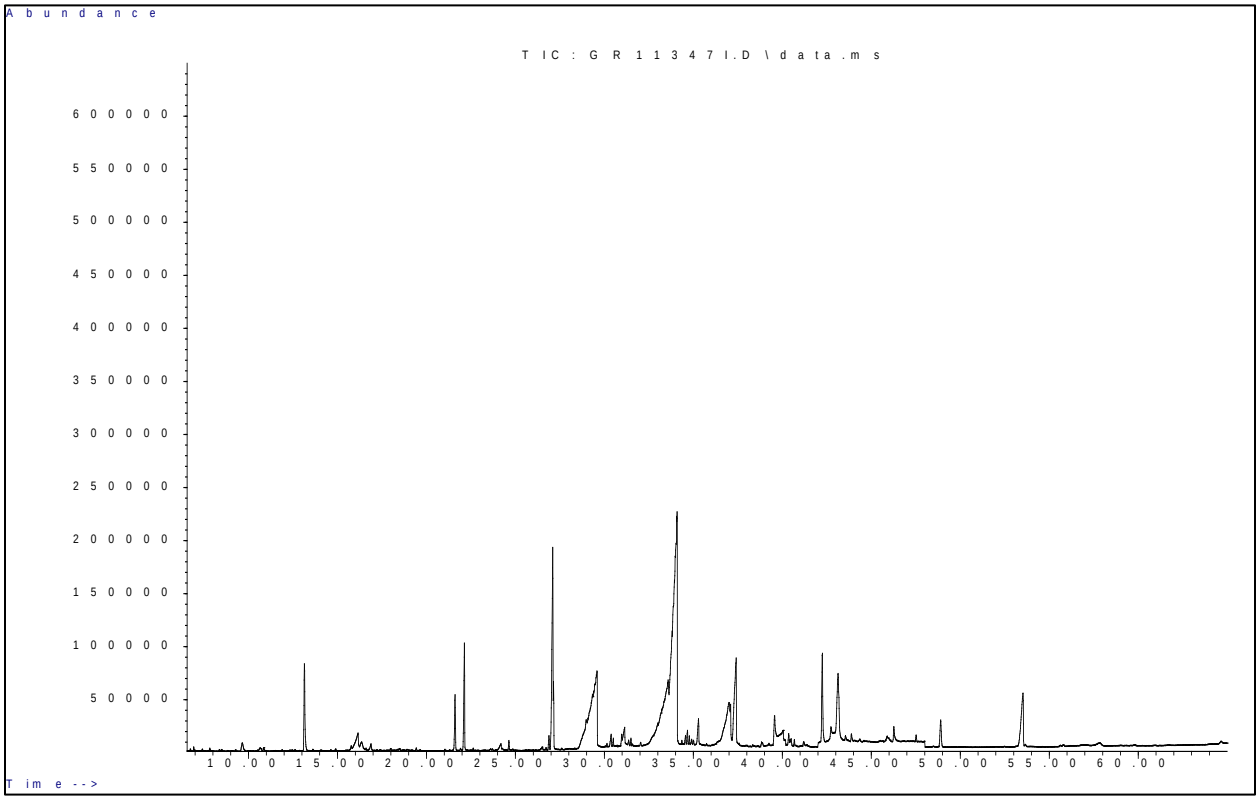
GI66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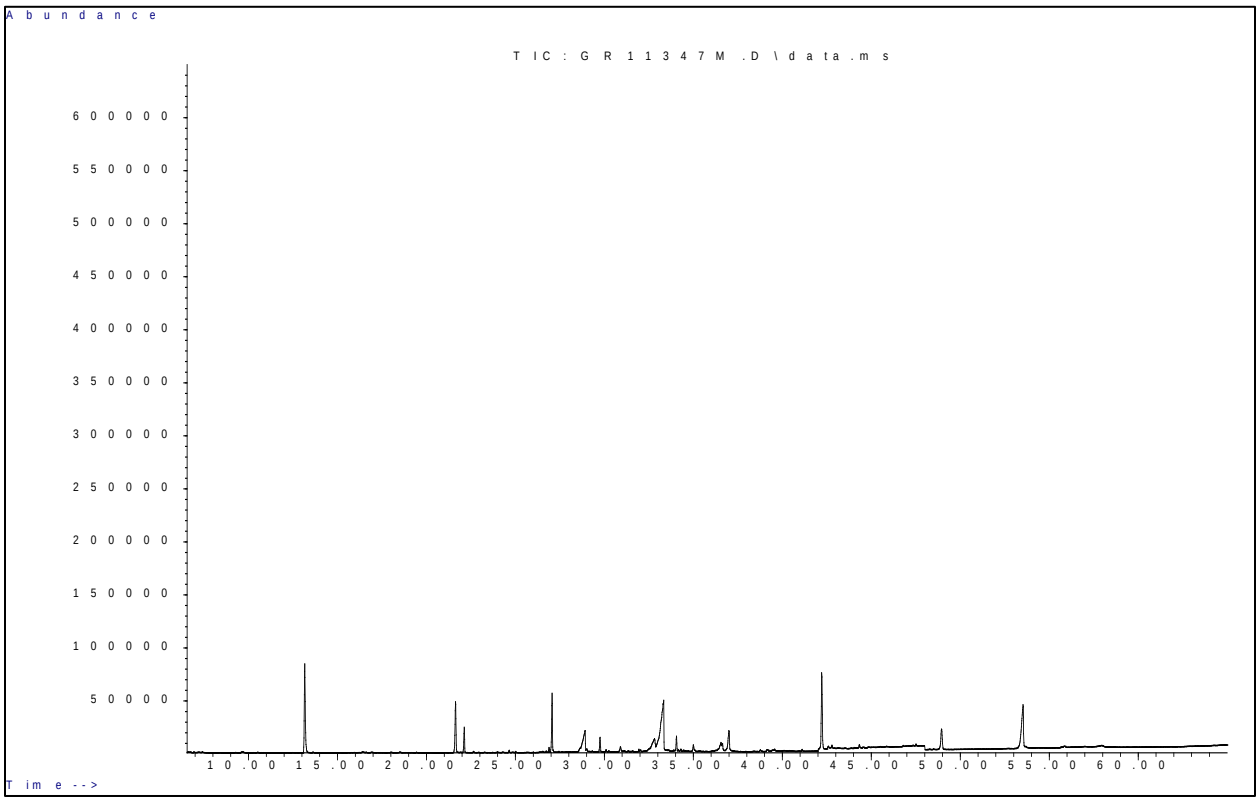
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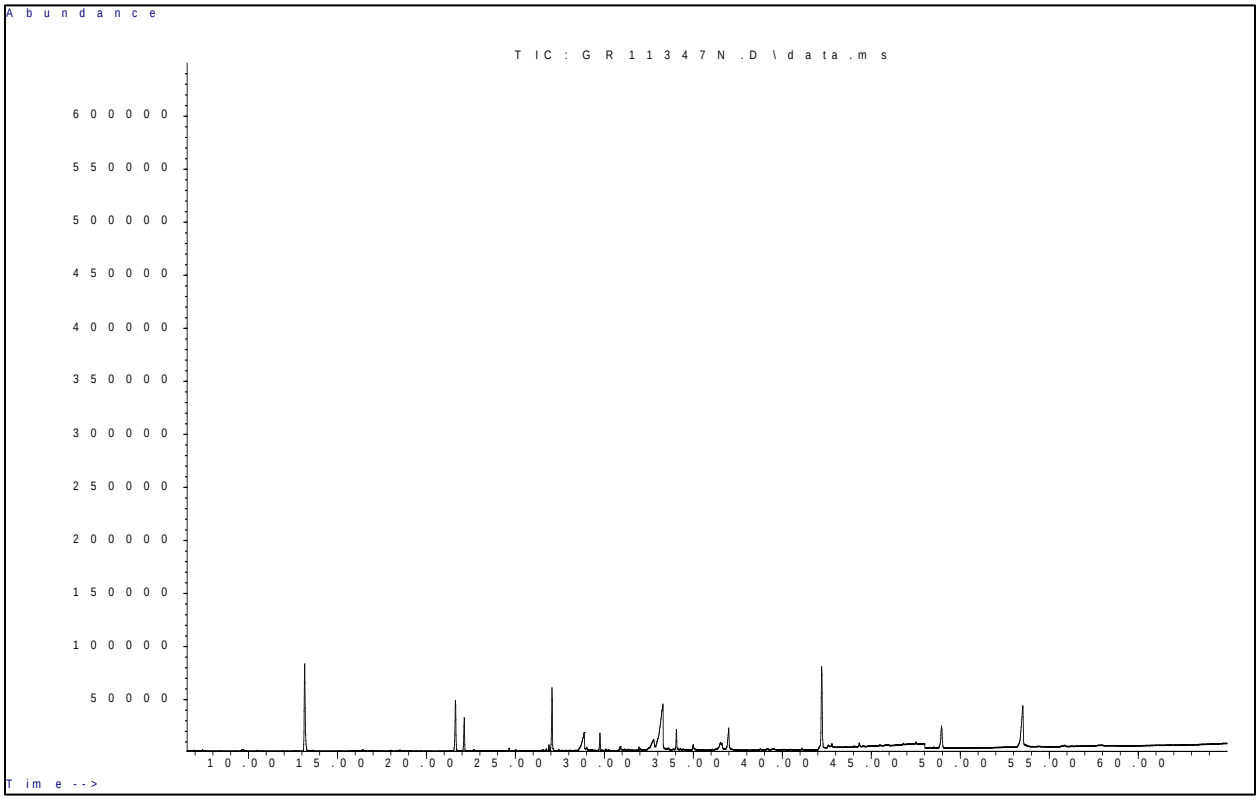
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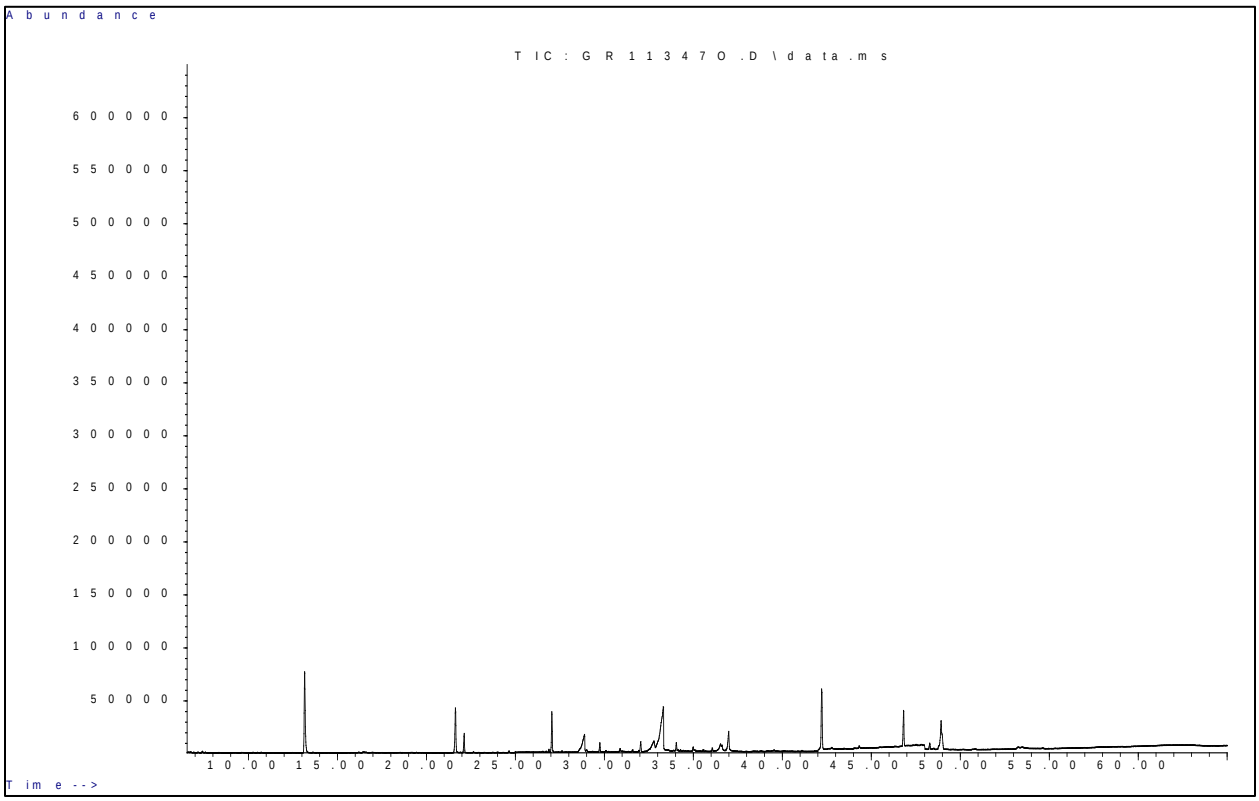
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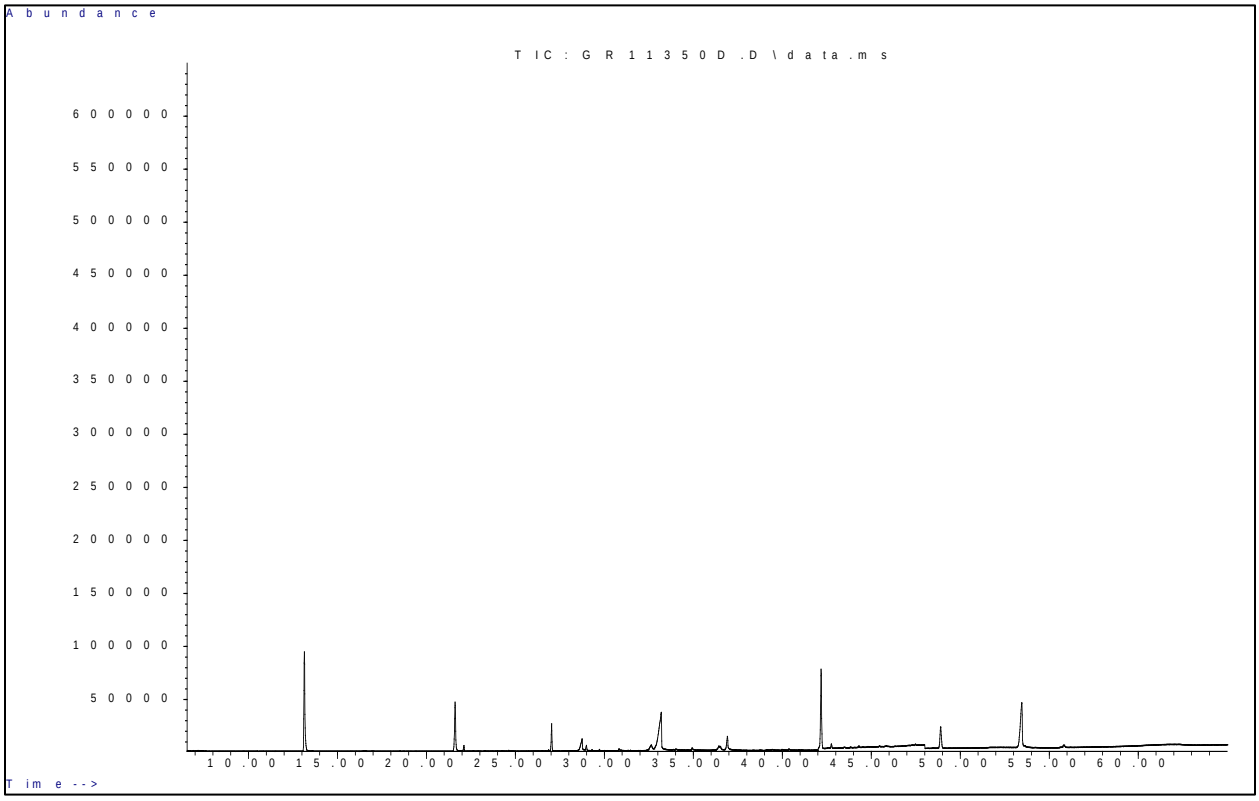
GI85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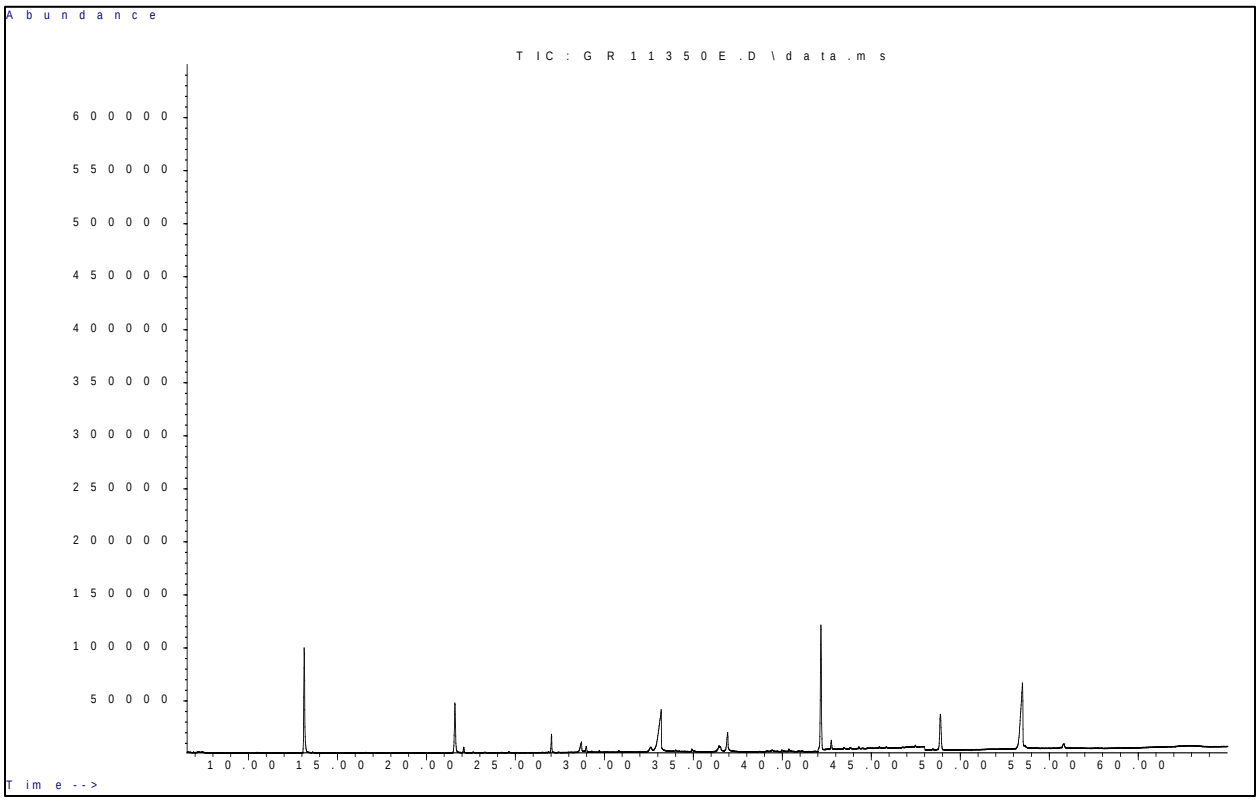
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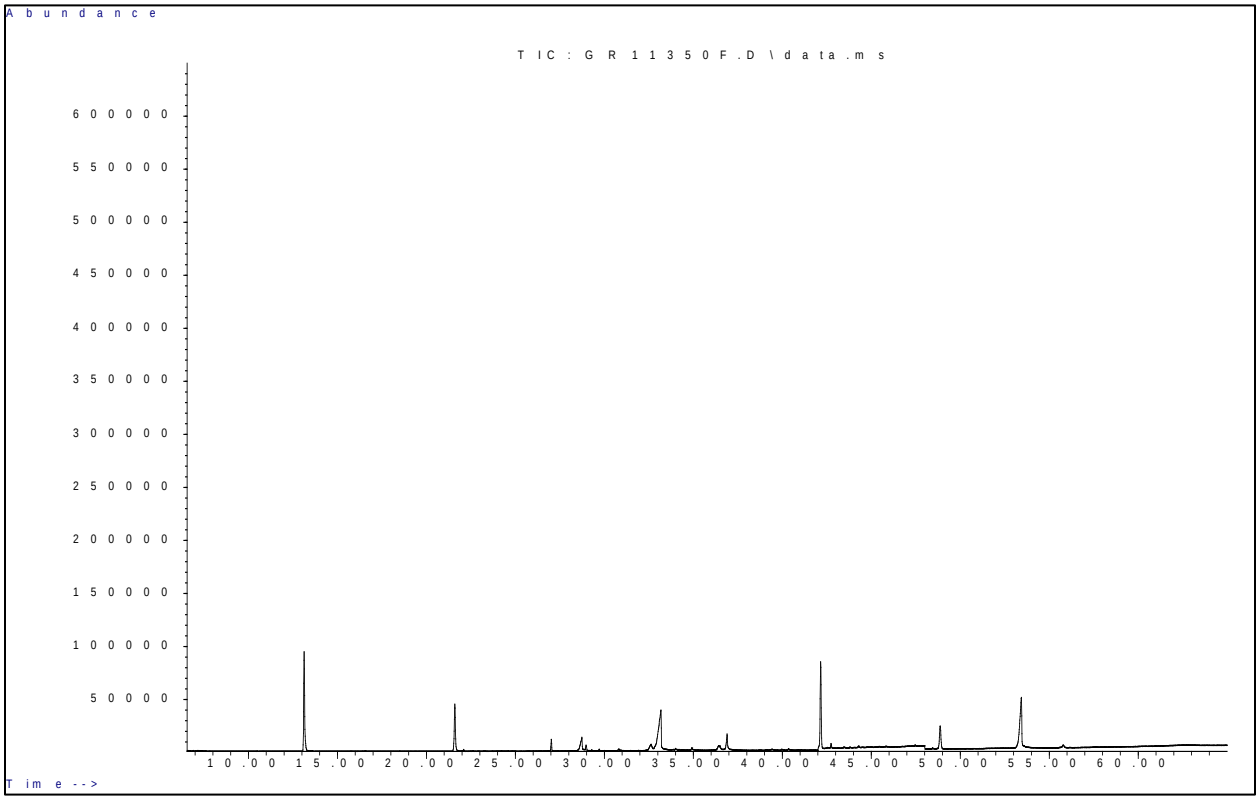
T i m e -->

GI87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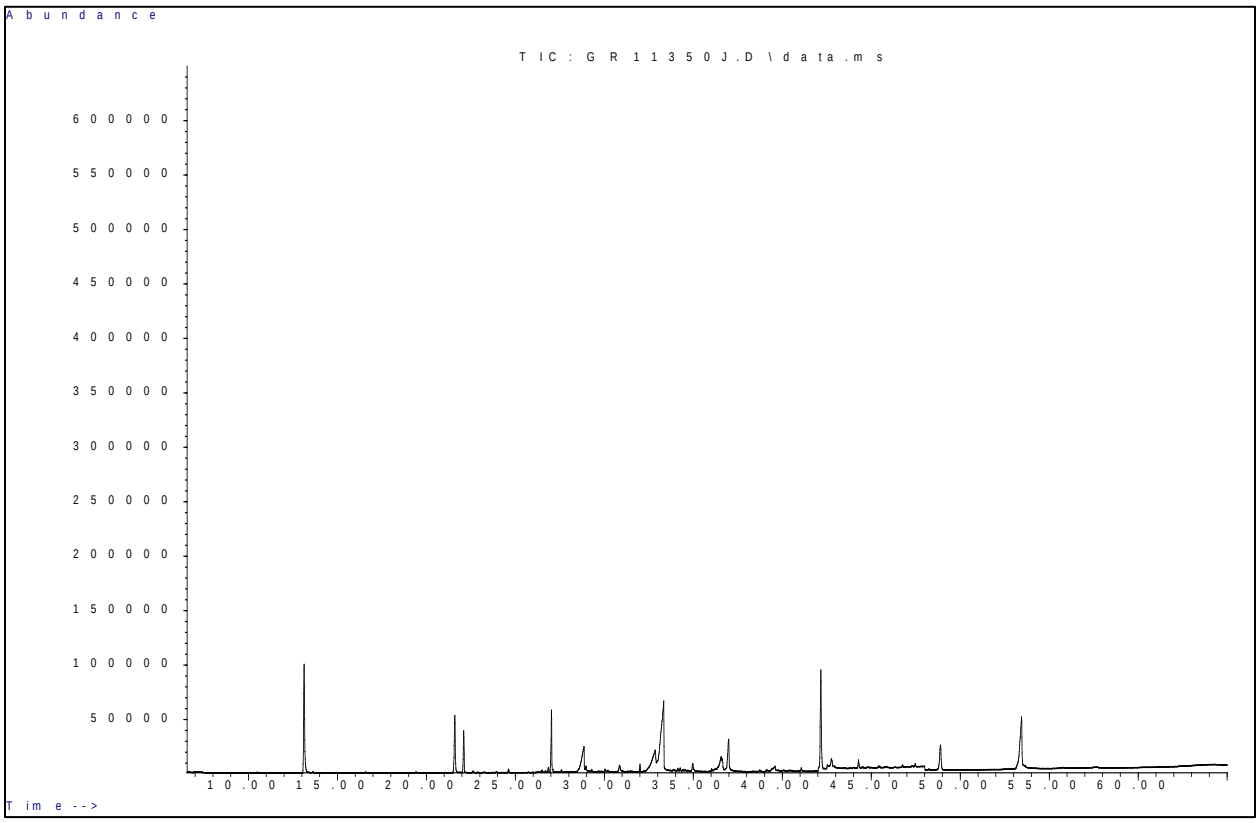
4 0 0 0 0 0
3 5 0 0 0 0
3 0 0 0 0 0
2 5 0 0 0 0
2 0 0 0 0 0
1 5 0 0 0 0
1 0 0 0 0 0
5 0 0 0 0
1 0 . 0 0 1 5 . 0 0 2 0 . 0 0 2 5 . 0 0 3 0 . 0 0 3 5 . 0 0 4 0 . 0 0 4 5 . 0 0 5 0 . 0 0 5 5 . 0 0 6 0 . 0 0
T i m e -->

GI91


A b u n d a n c e
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4 0 0 0 0 0
3 5 0 0 0 0
3 0 0 0 0 0
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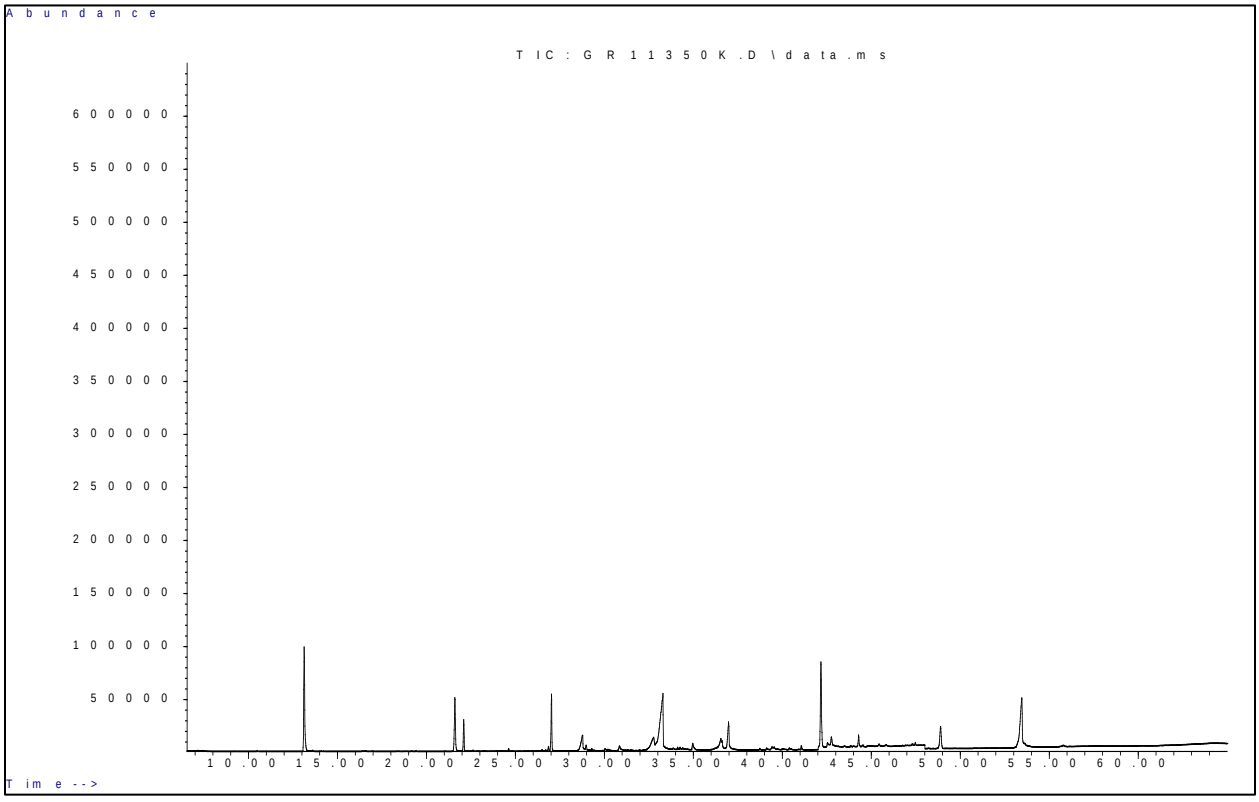
5 0 0 0 0
1 0 . 0 0 1 5 . 0 0 2 0 . 0 0 2 5 . 0 0 3 0 . 0 0 3 5 . 0 0 4 0 . 0 0 4 5 . 0 0 5 0 . 0 0 5 5 . 0 0 6 0 . 0 0
T i m e -->

GI92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4 0 0 0 0 0
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2 5 0 0 0 0
2 0 0 0 0 0
1 5 0 0 0 0
1 0 0 0 0 0
5 0 0 0 0
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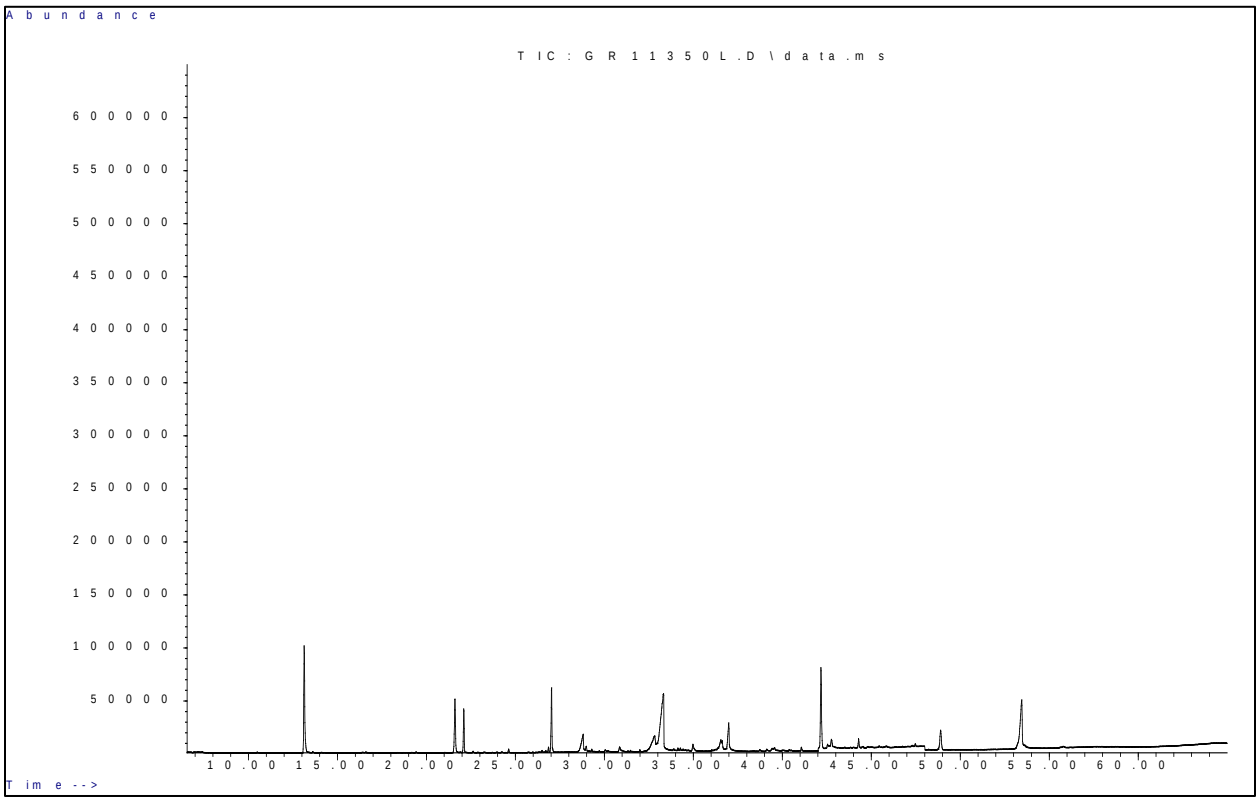
T i m e -->

GI93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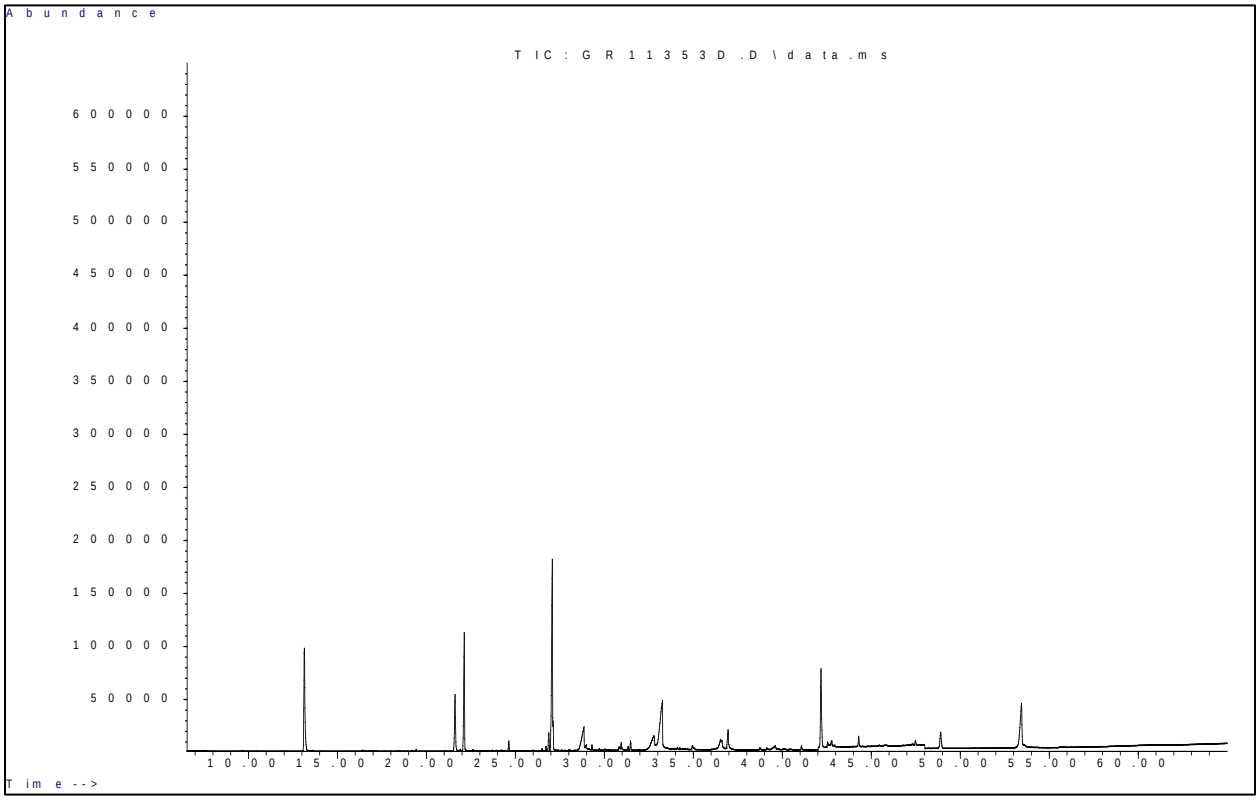
Time -->

GI94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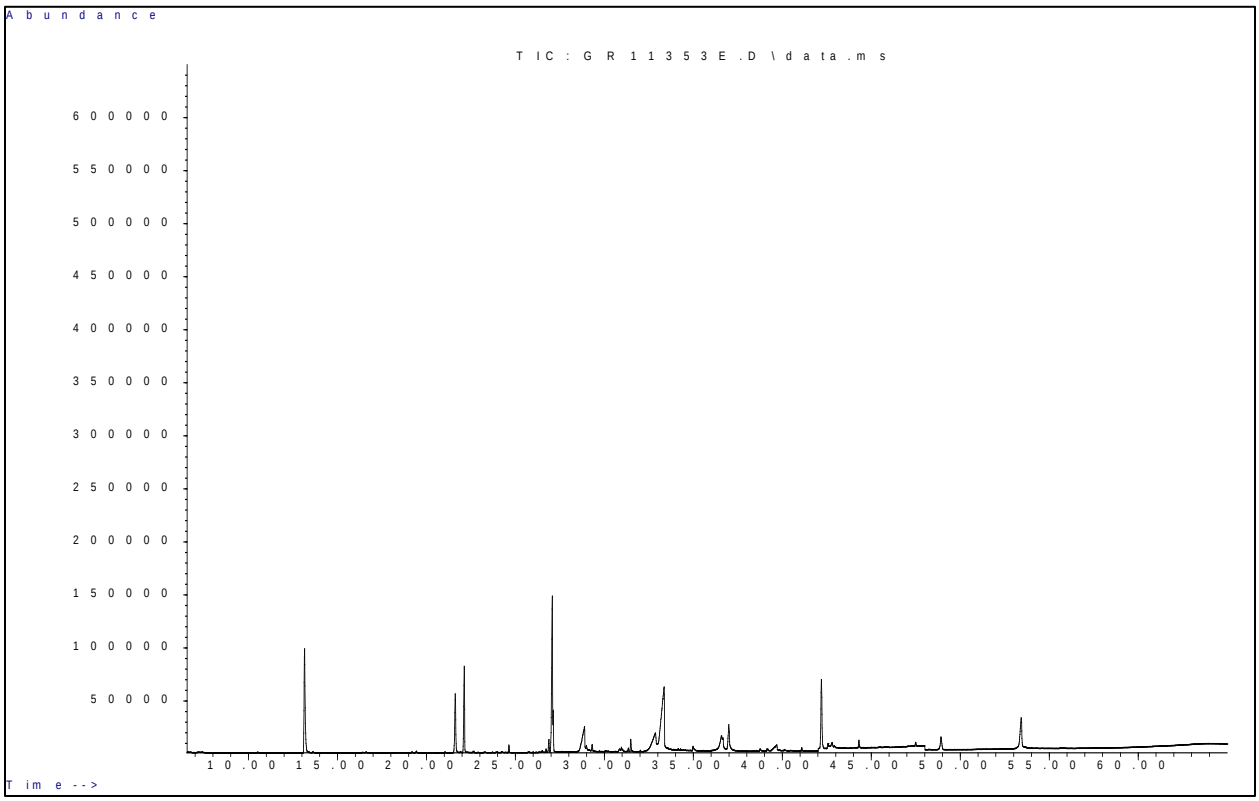
Time -->

GI95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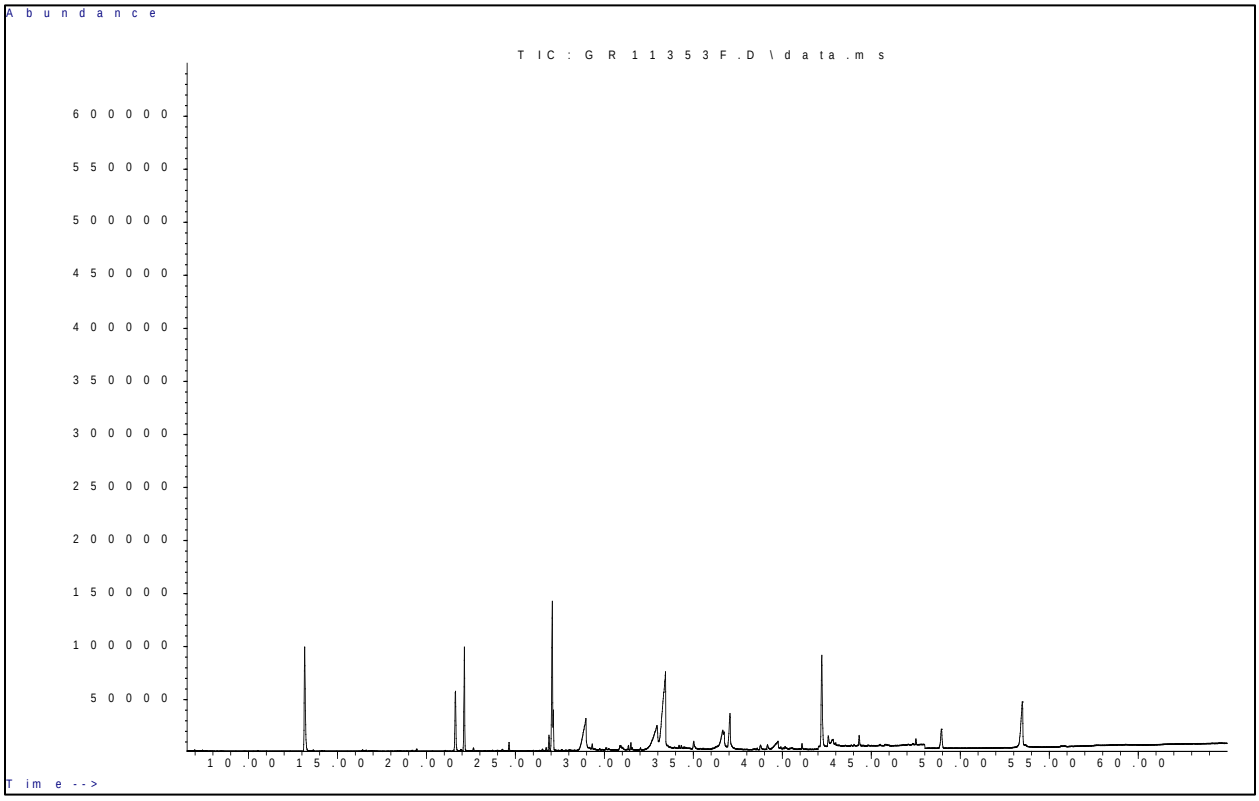
Time -->

GI96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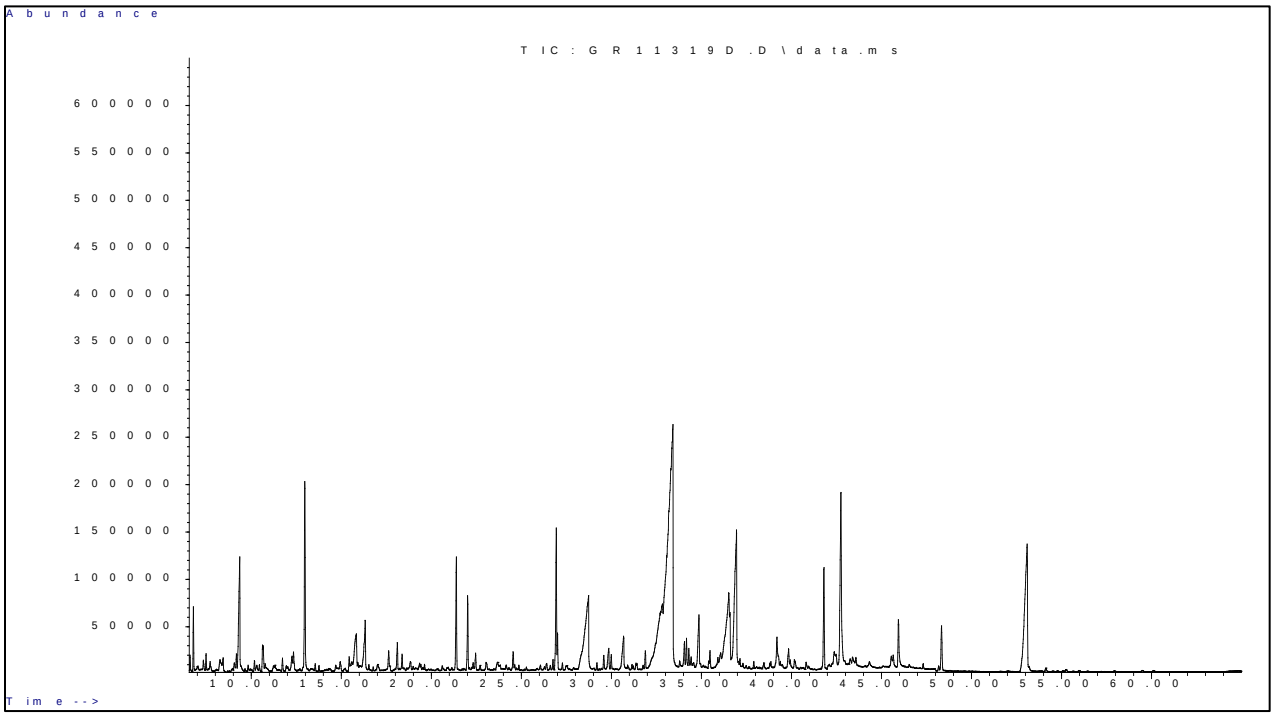
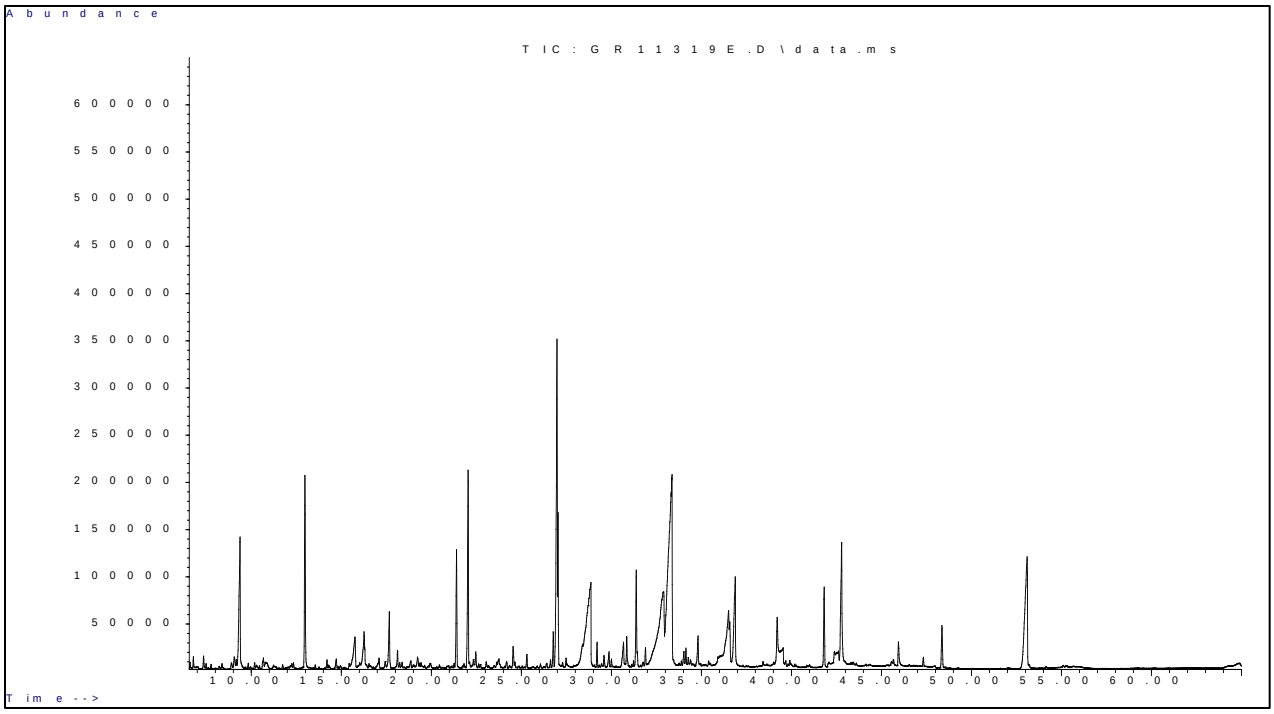
Time -->

Vermilion Bay

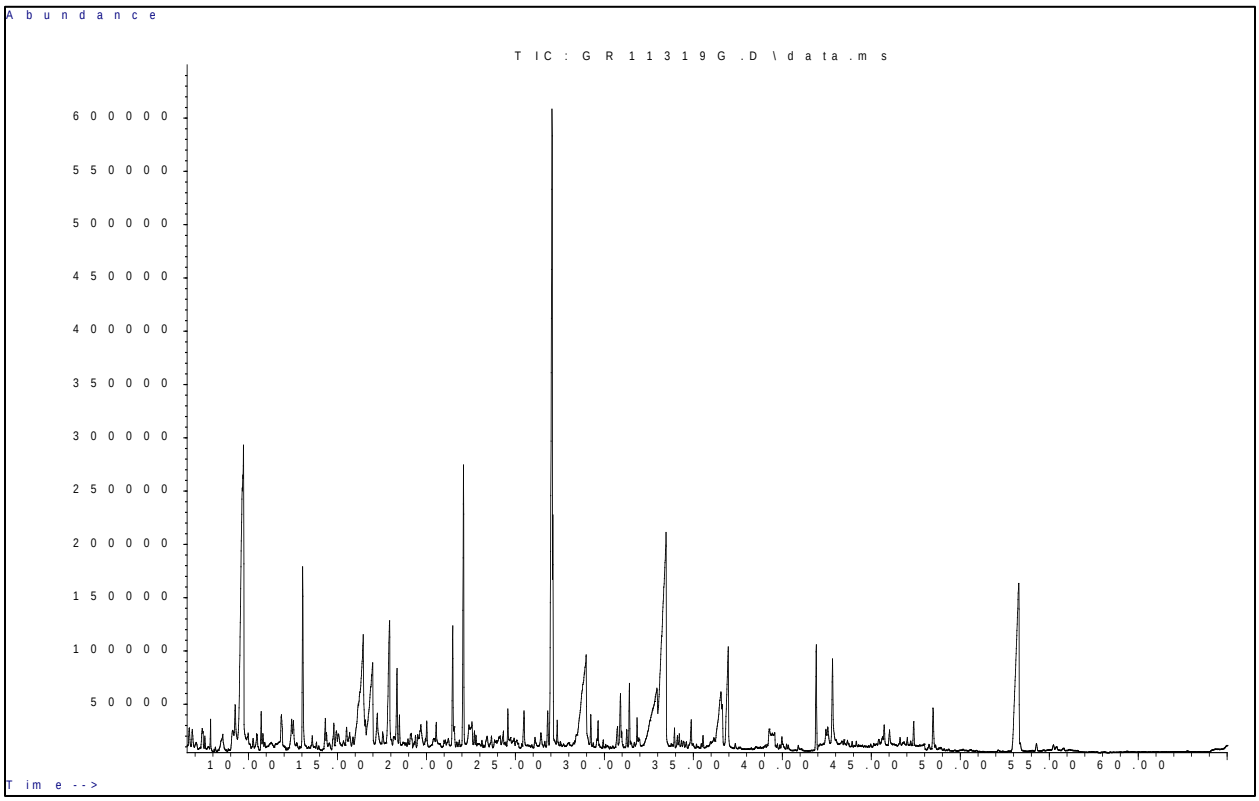
VB19



VB20



VB21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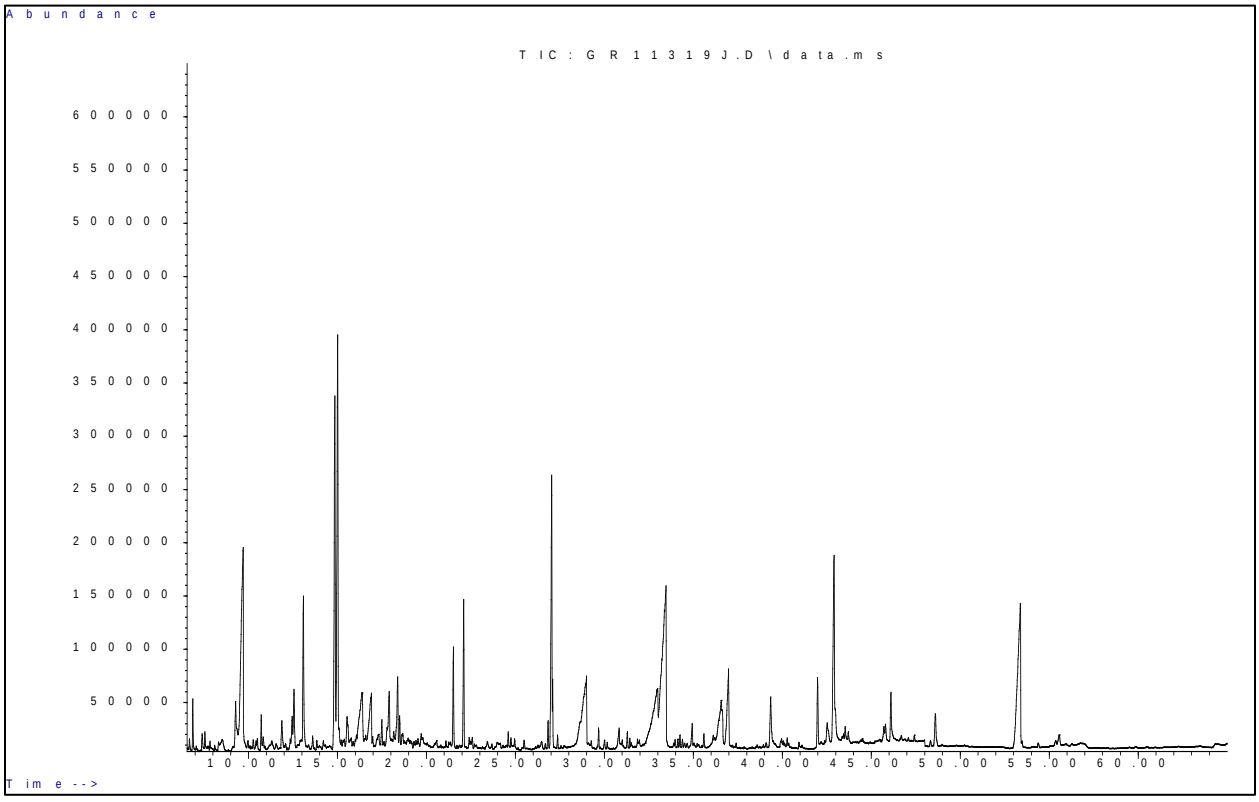
Time -->

VB25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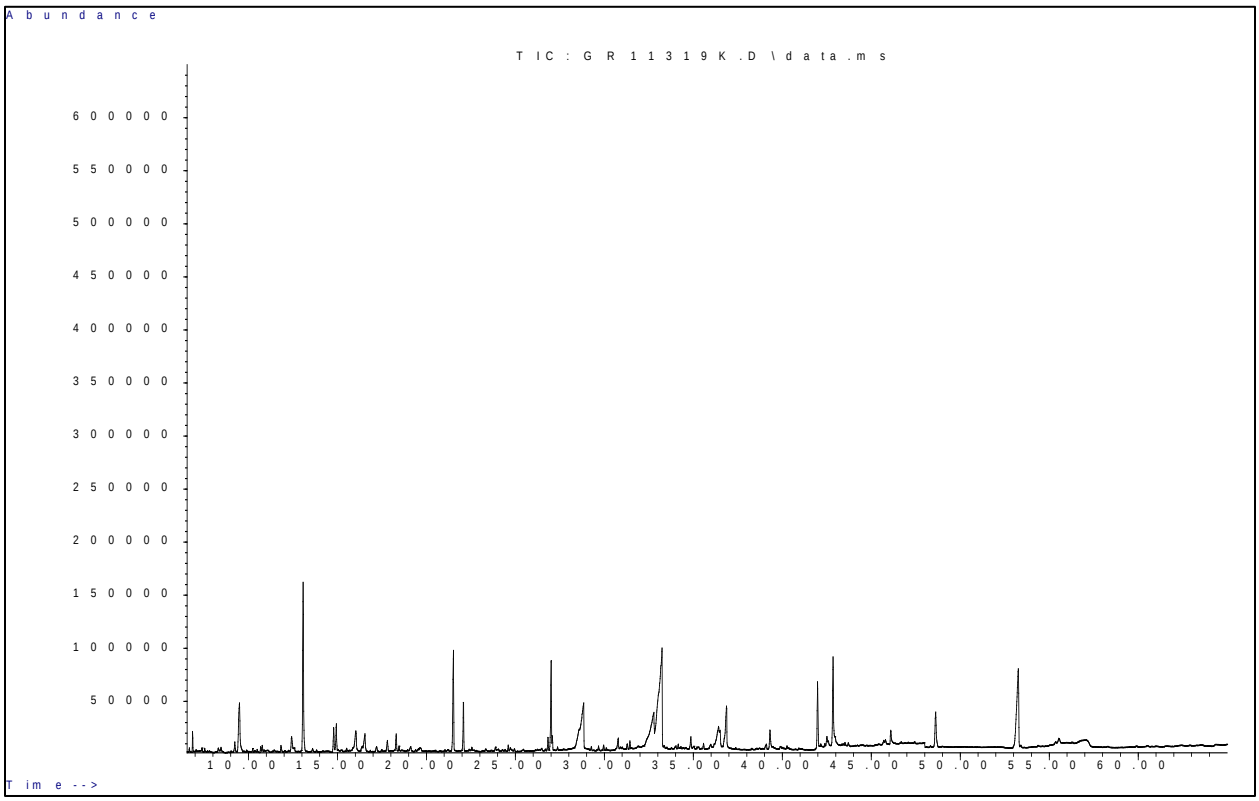
Time -->

VB26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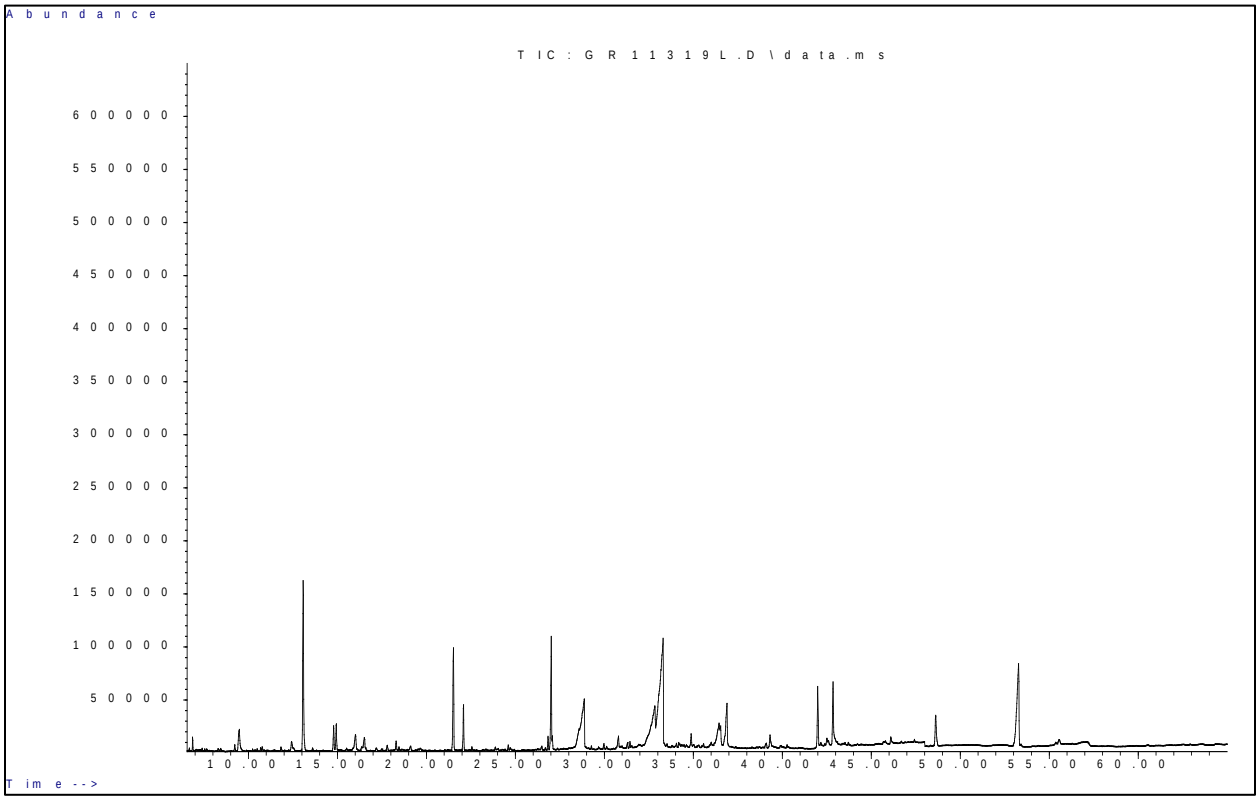
Time -->

VB27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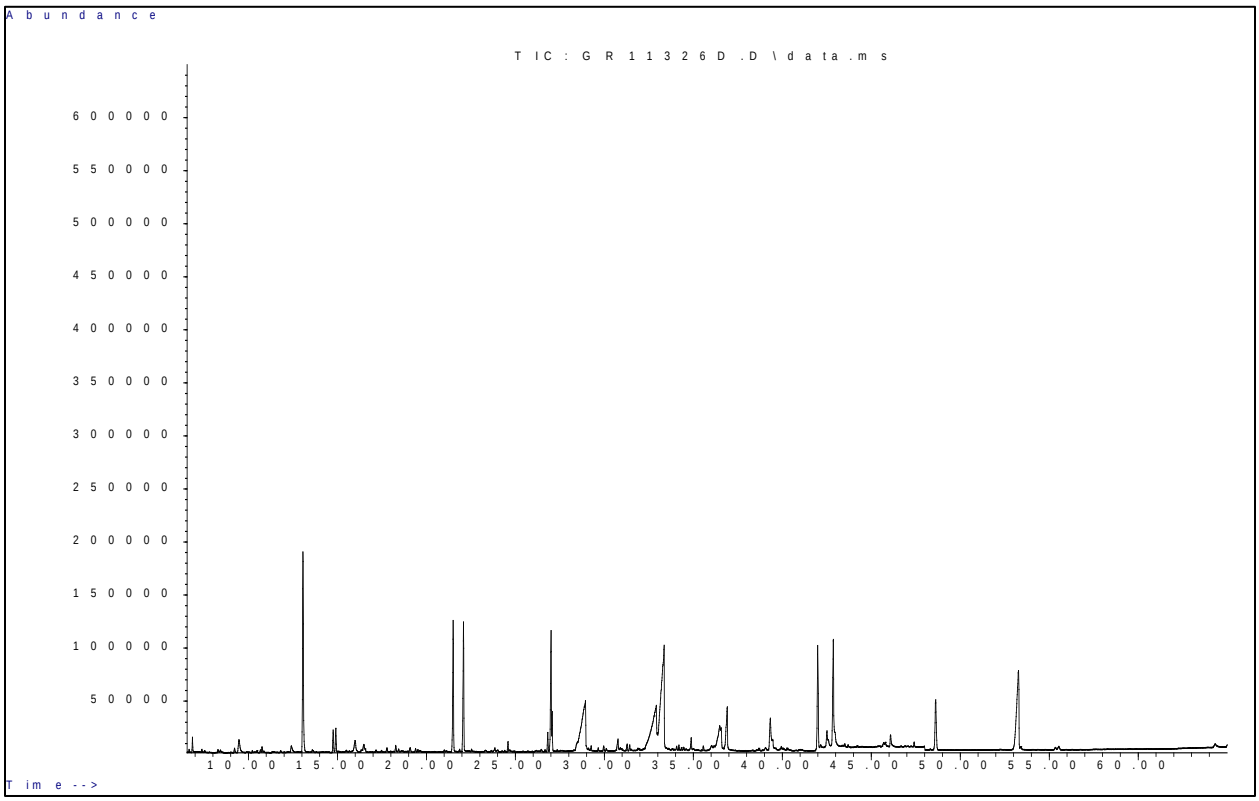
Time -->

VB31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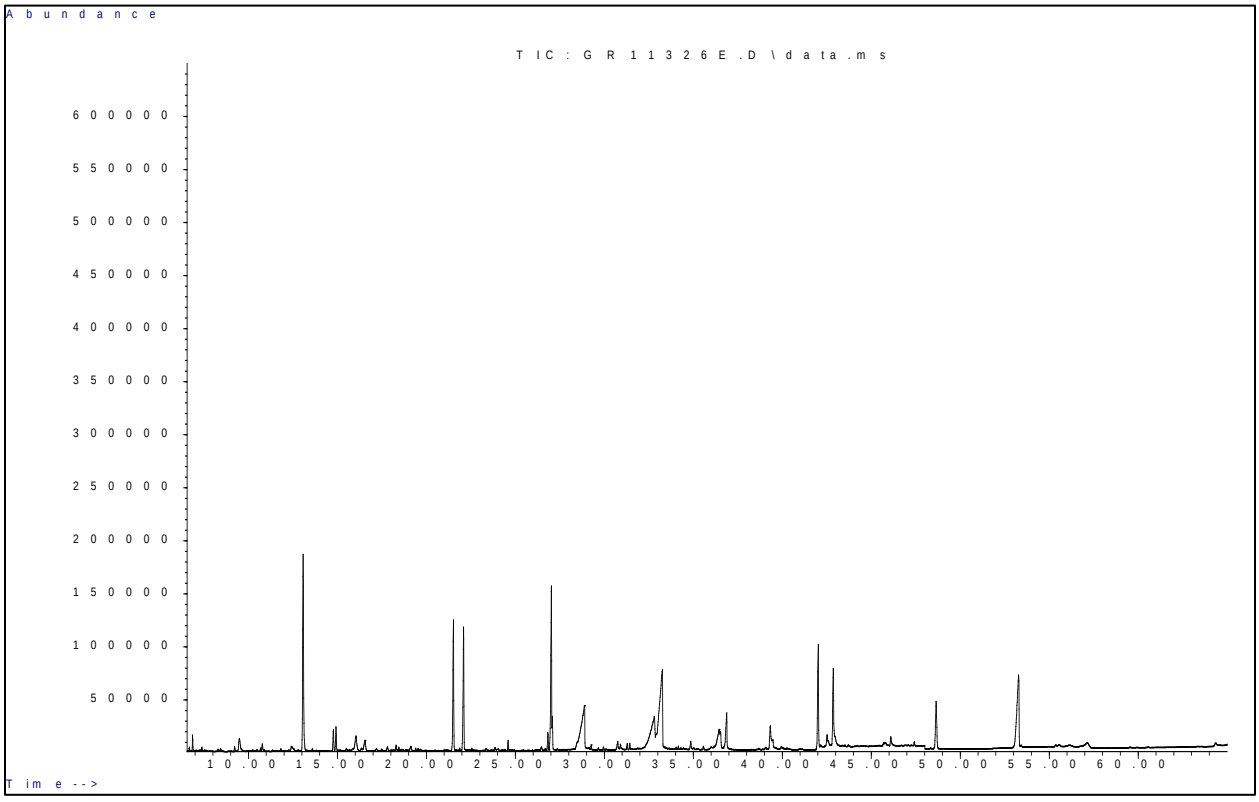
Time -->

VB32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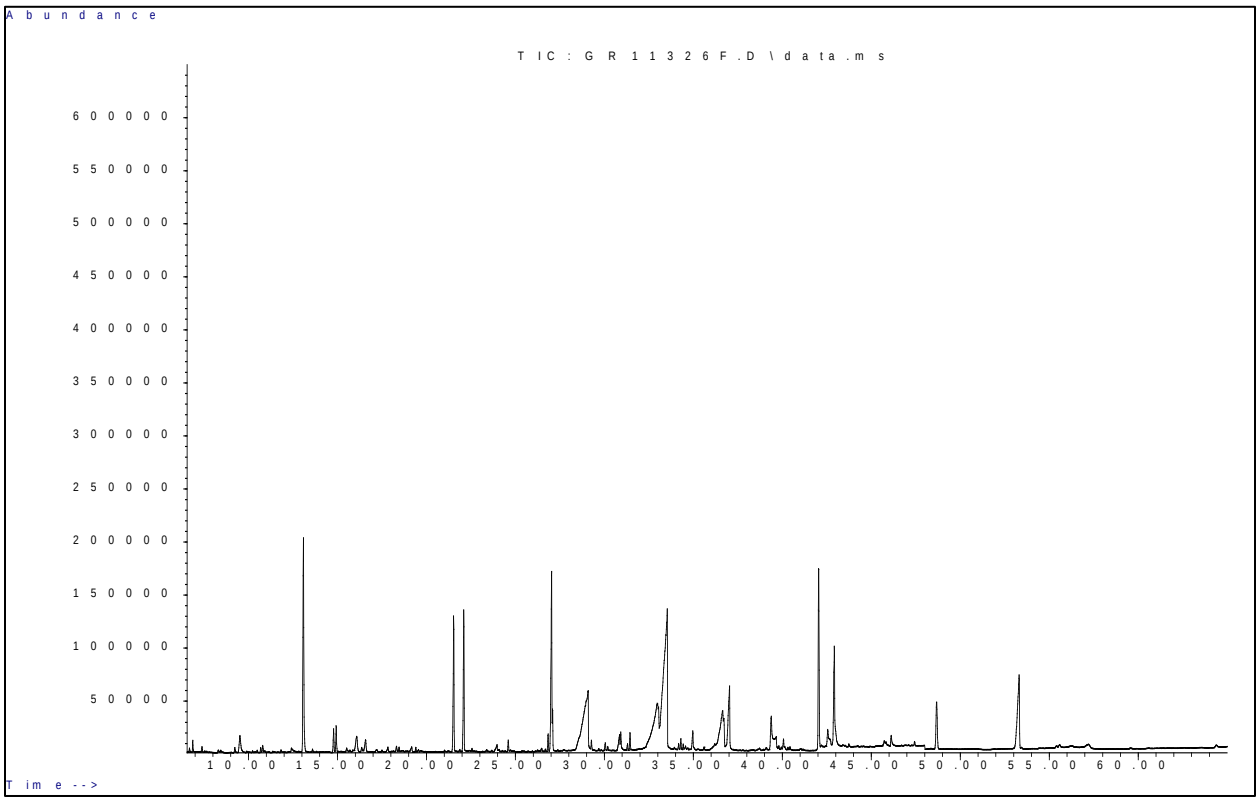
Time -->

VB33


A b u n d a n c e
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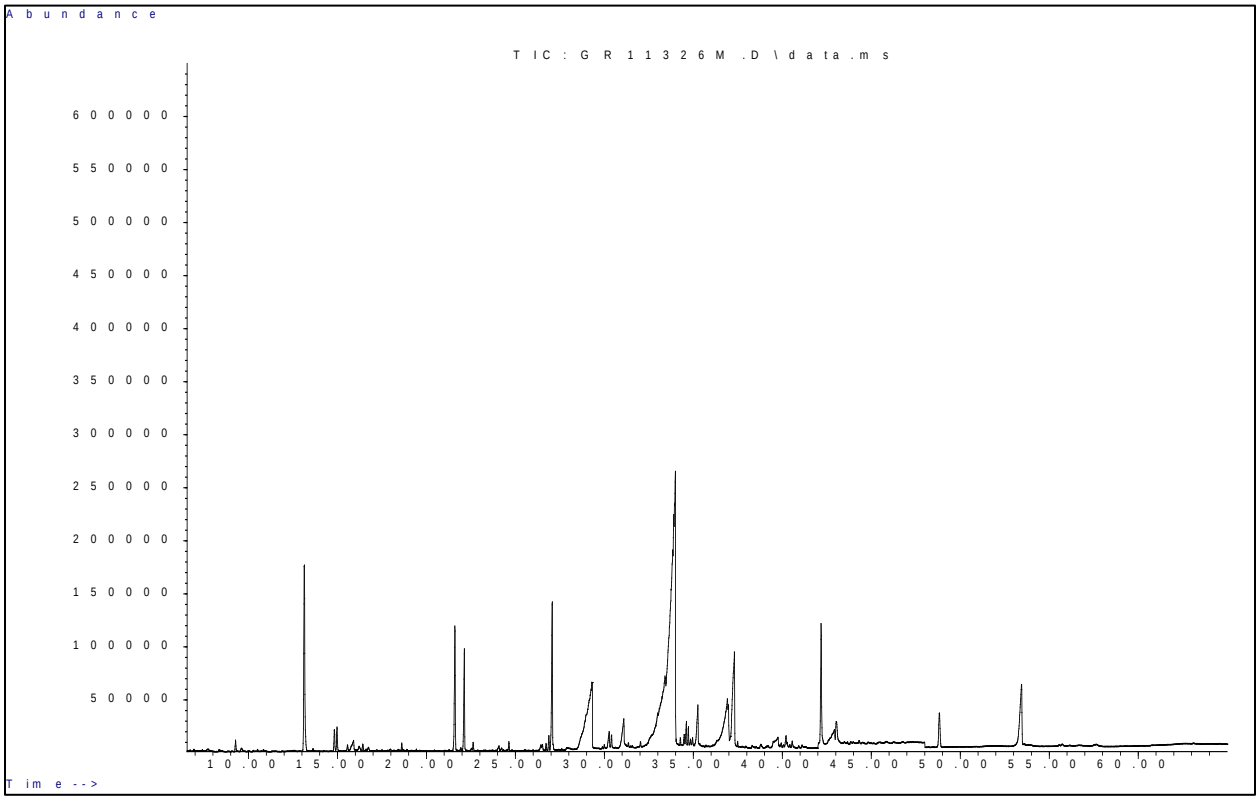
5 0 0 0 0
1 0 . 0 0 1 5 . 0 0 2 0 . 0 0 2 5 . 0 0 3 0 . 0 0 3 5 . 0 0 4 0 . 0 0 4 5 . 0 0 5 0 . 0 0 5 5 . 0 0 6 0 . 0 0
T i m e -->

VB46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2 5 0 0 0 0
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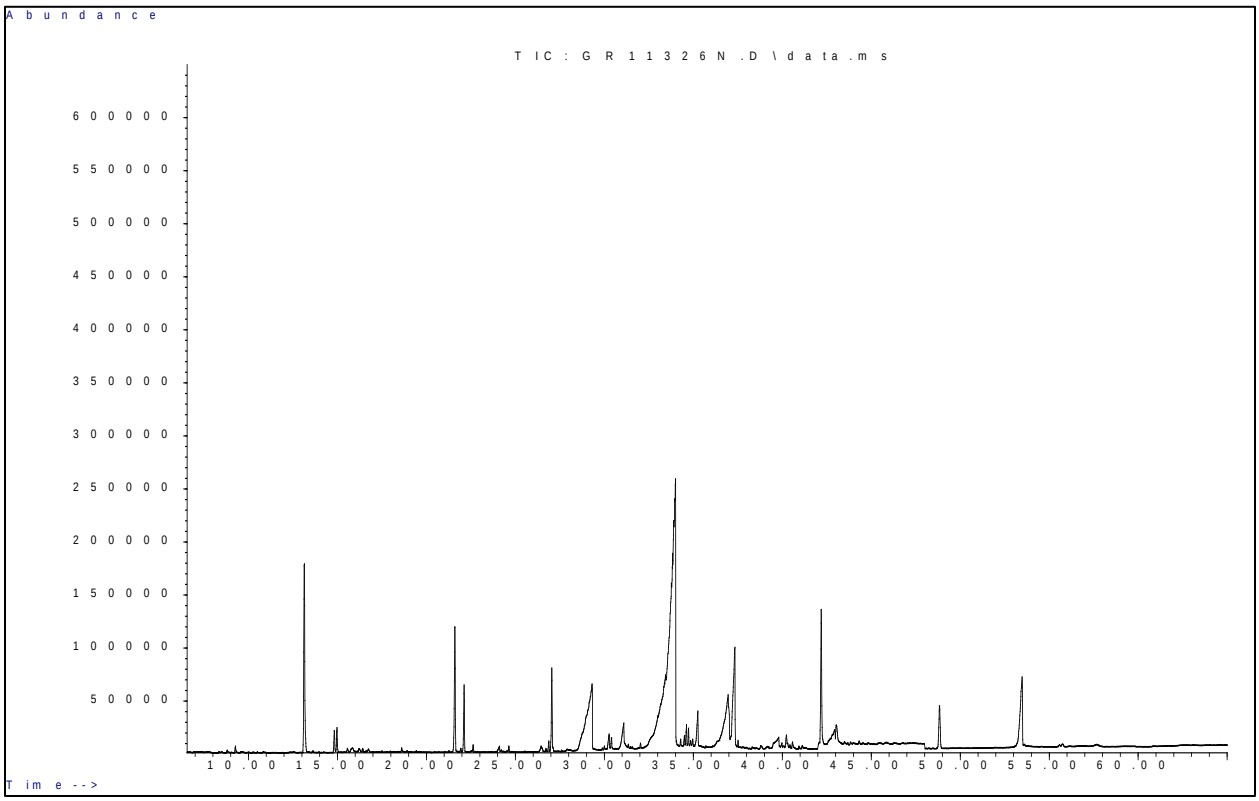
5 0 0 0 0
1 0 . 0 0 1 5 . 0 0 2 0 . 0 0 2 5 . 0 0 3 0 . 0 0 3 5 . 0 0 4 0 . 0 0 4 5 . 0 0 5 0 . 0 0 5 5 . 0 0 6 0 . 0 0
T i m e -->

VB47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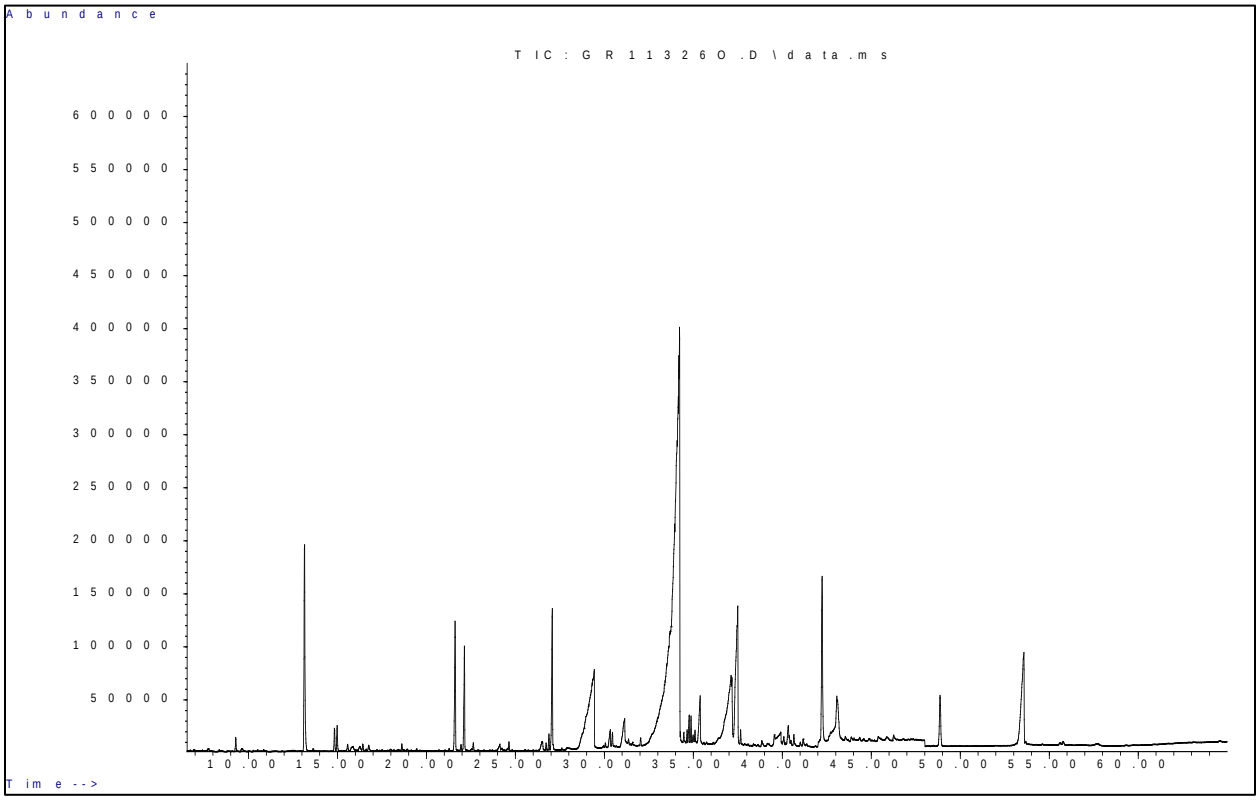
Time -->

VB48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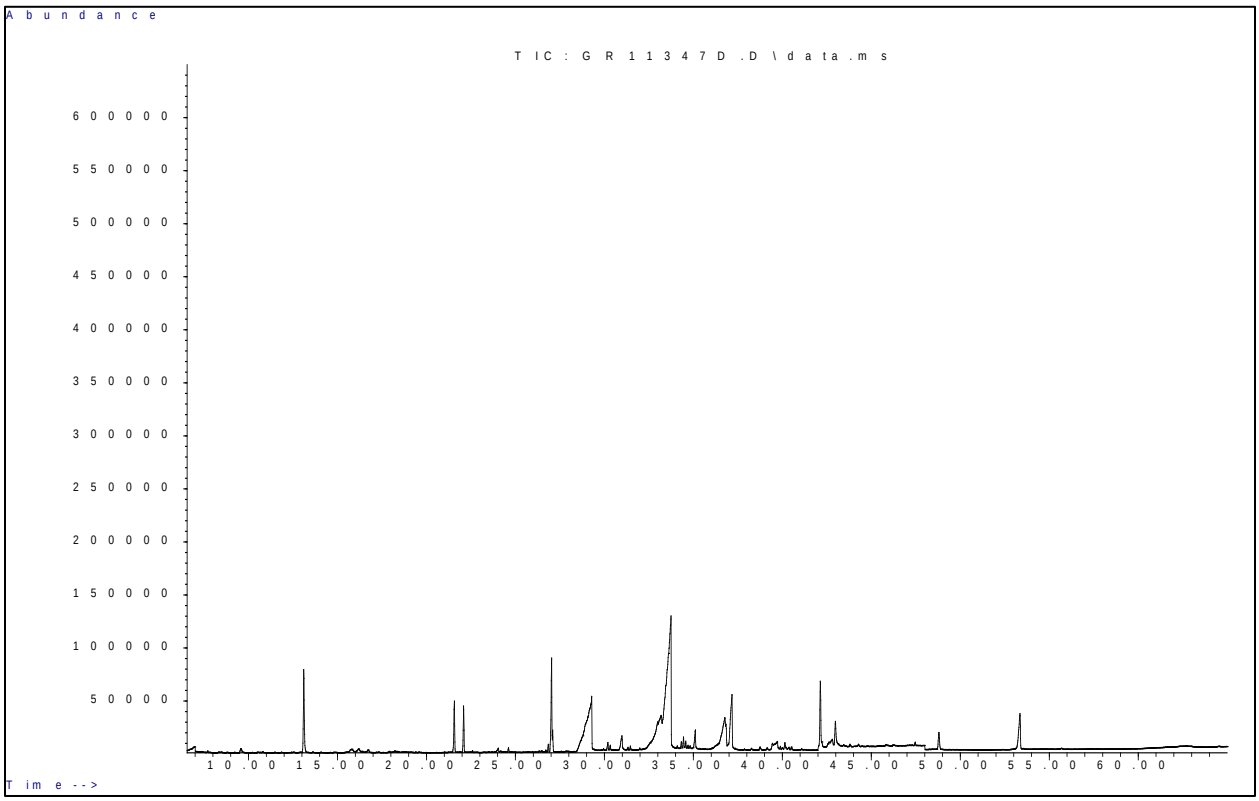
Time -->

VB61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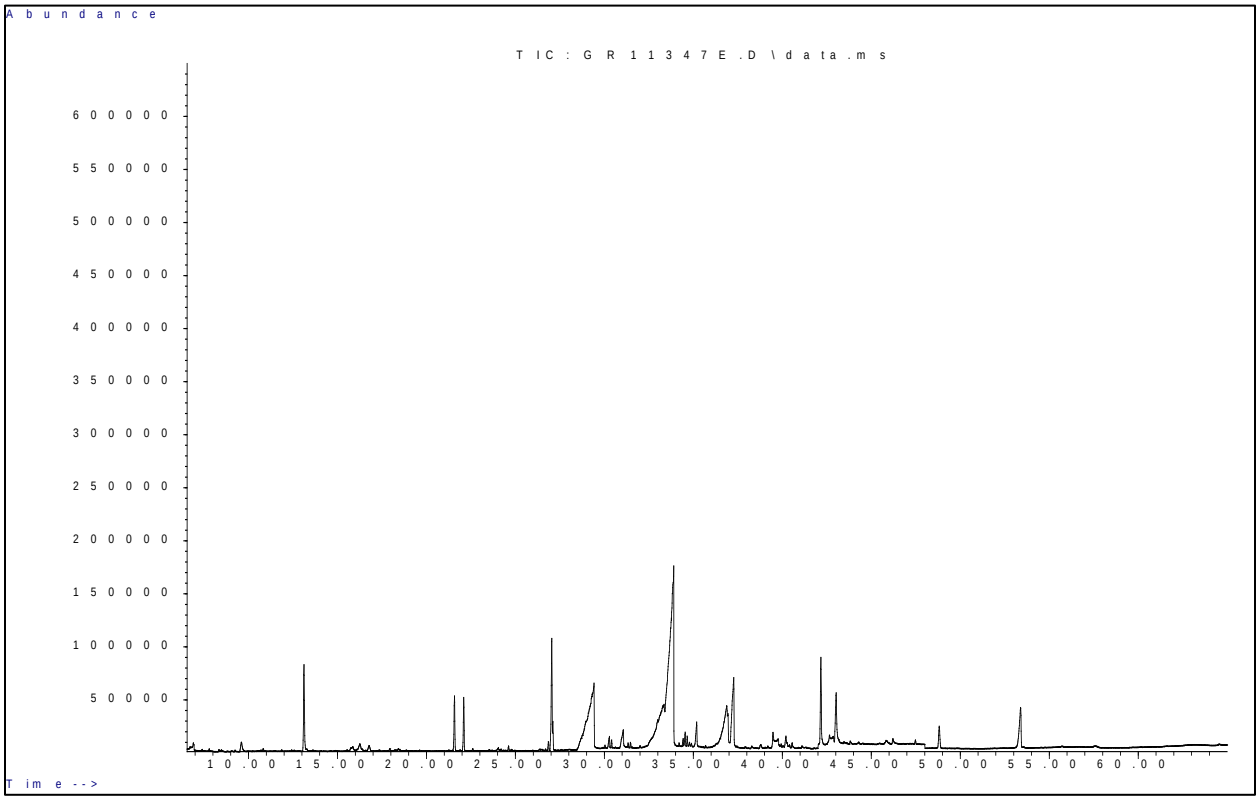
Time -->

VB62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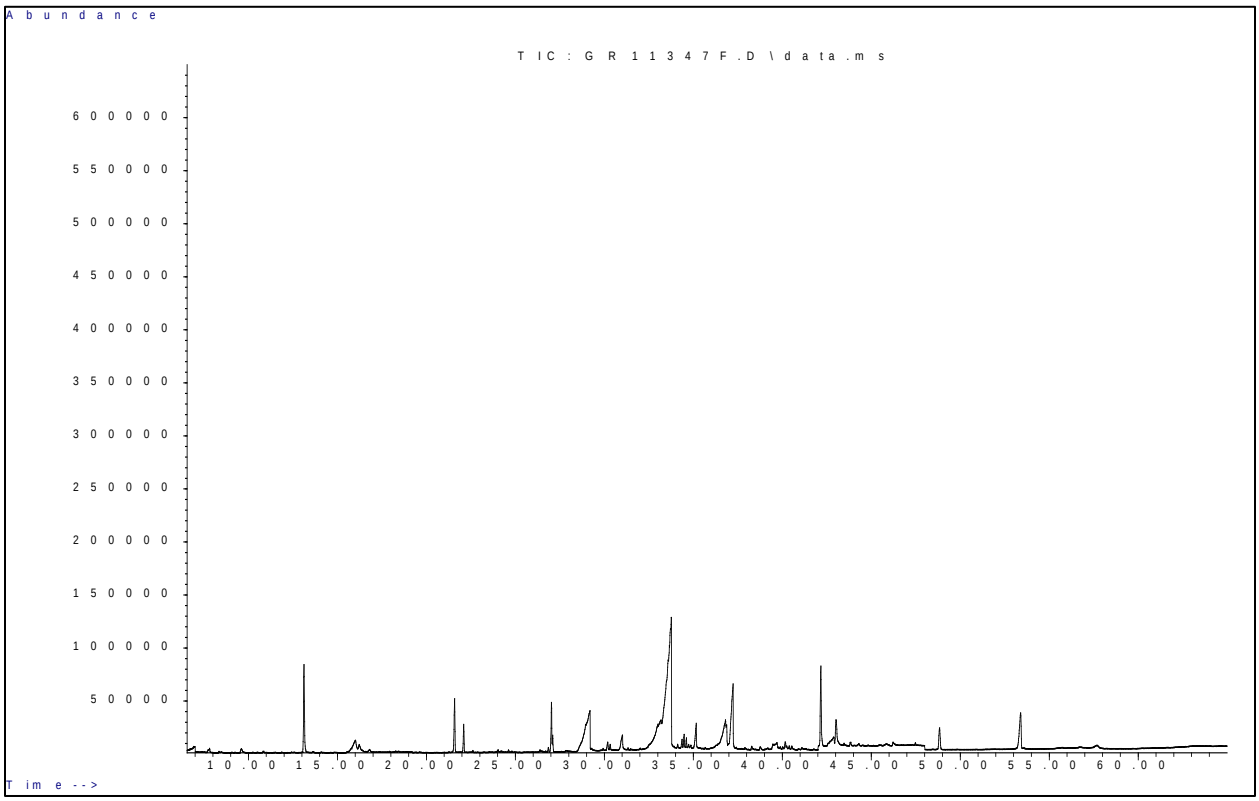
Time -->

VB63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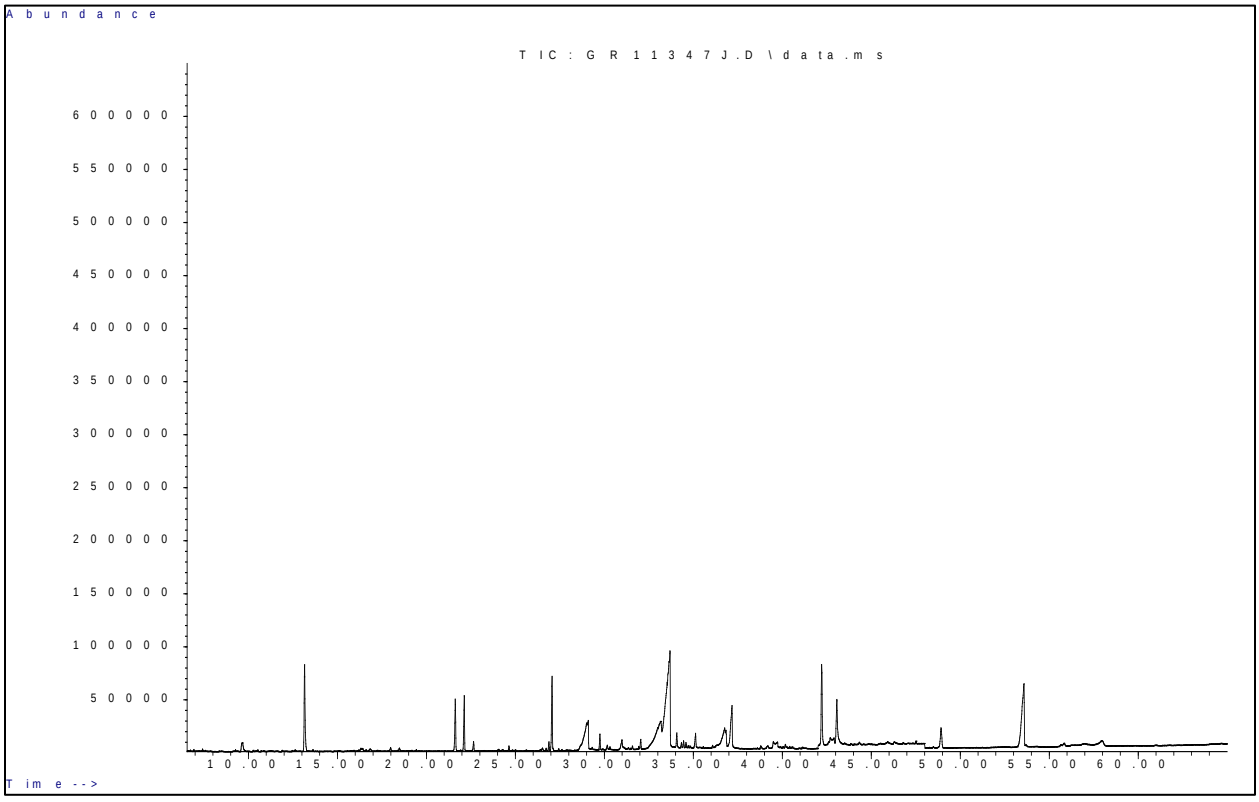
Time -->

VB73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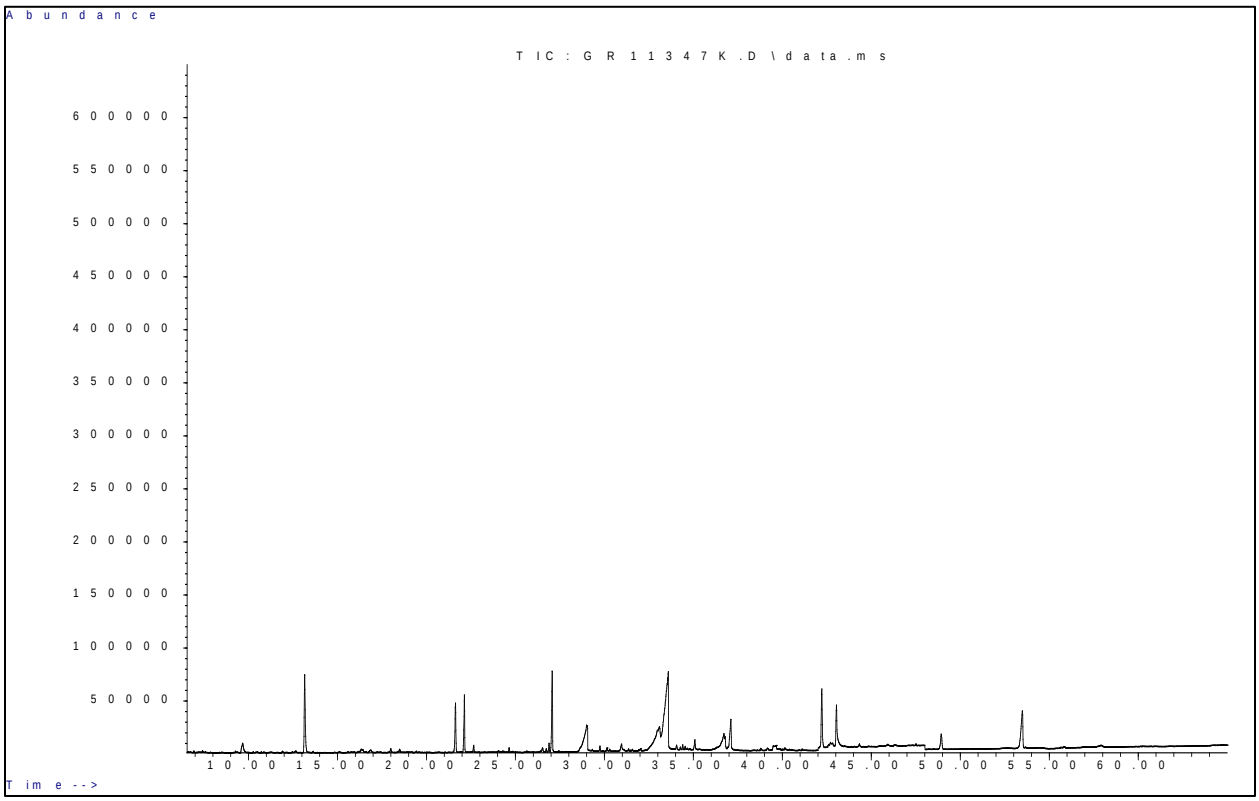
Time -->

VB74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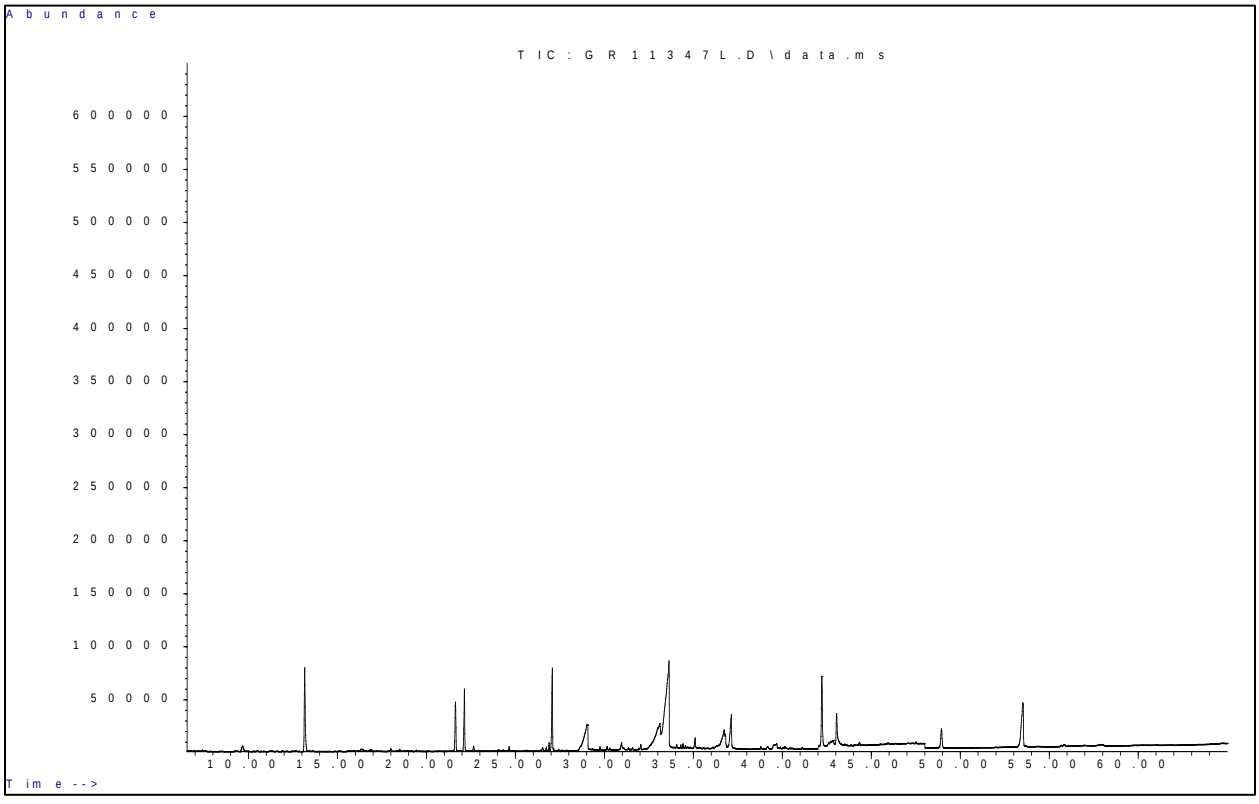
Time -->

VB75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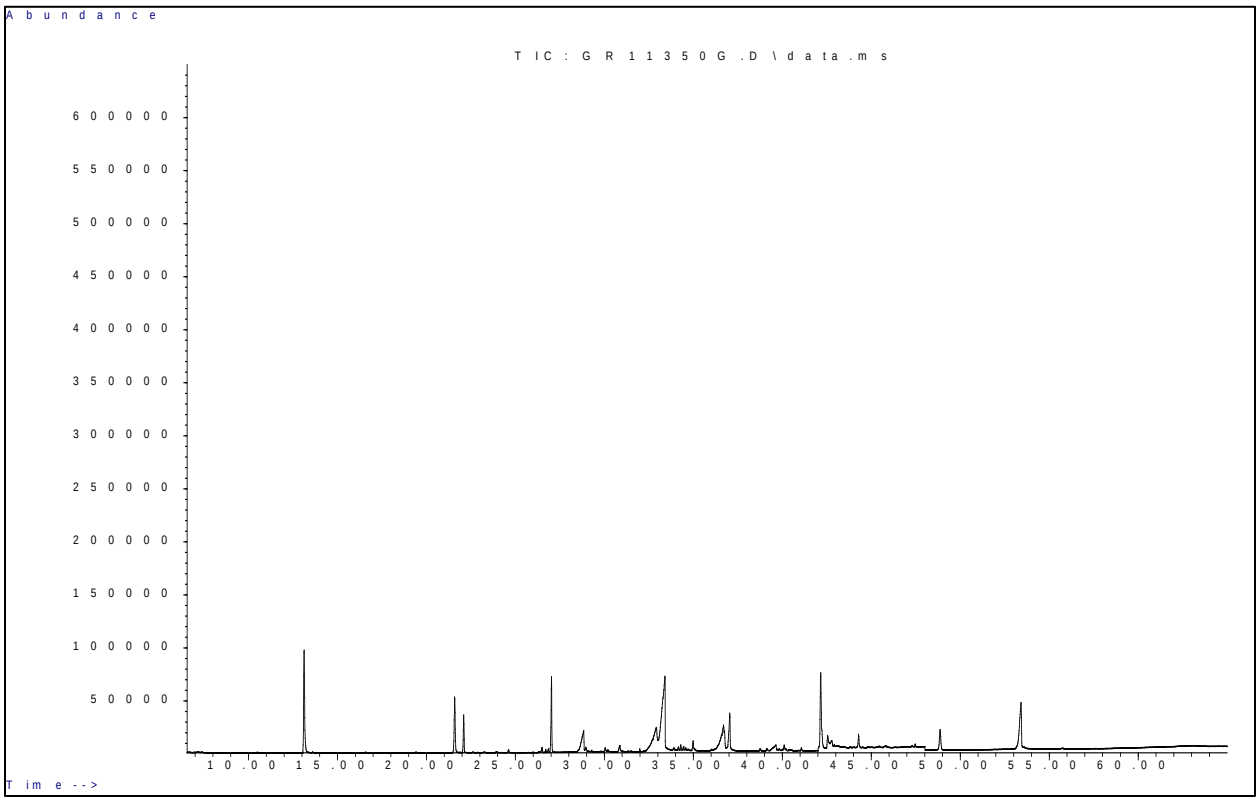
Time -->

VB88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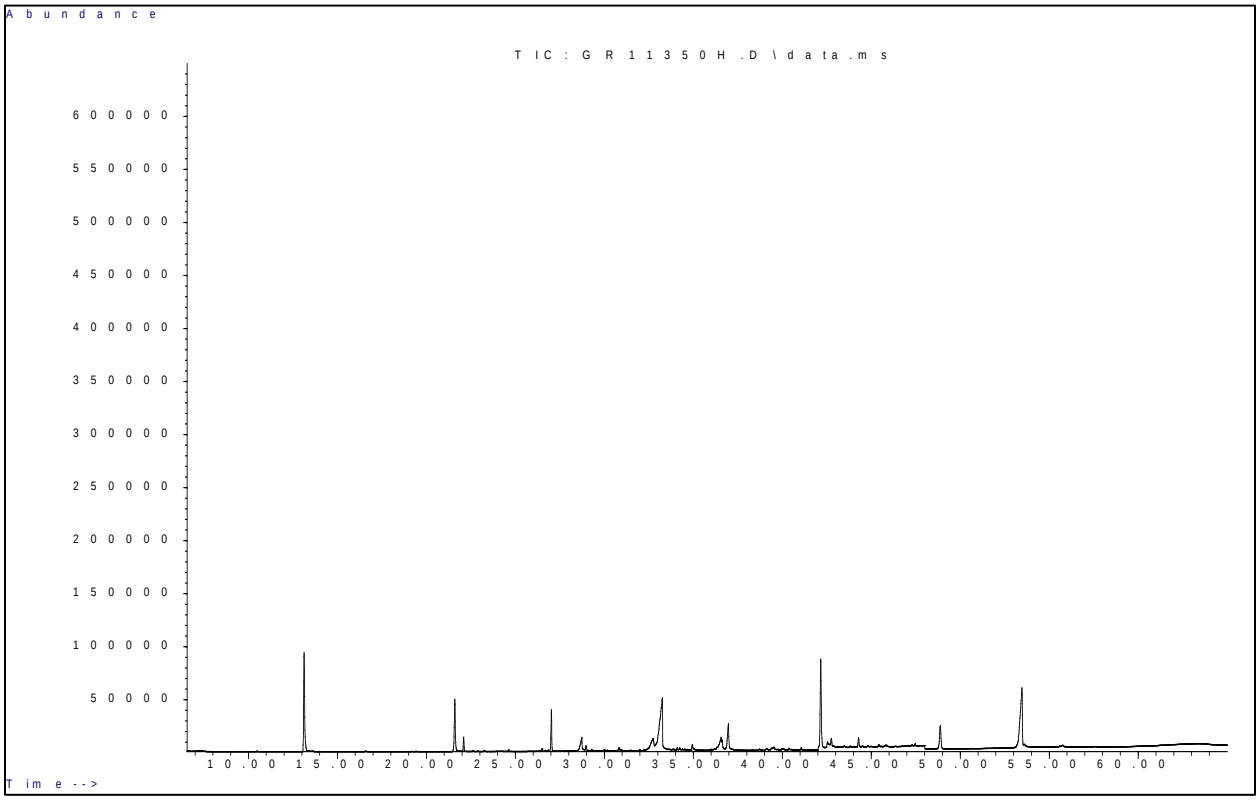
Time -->

VB89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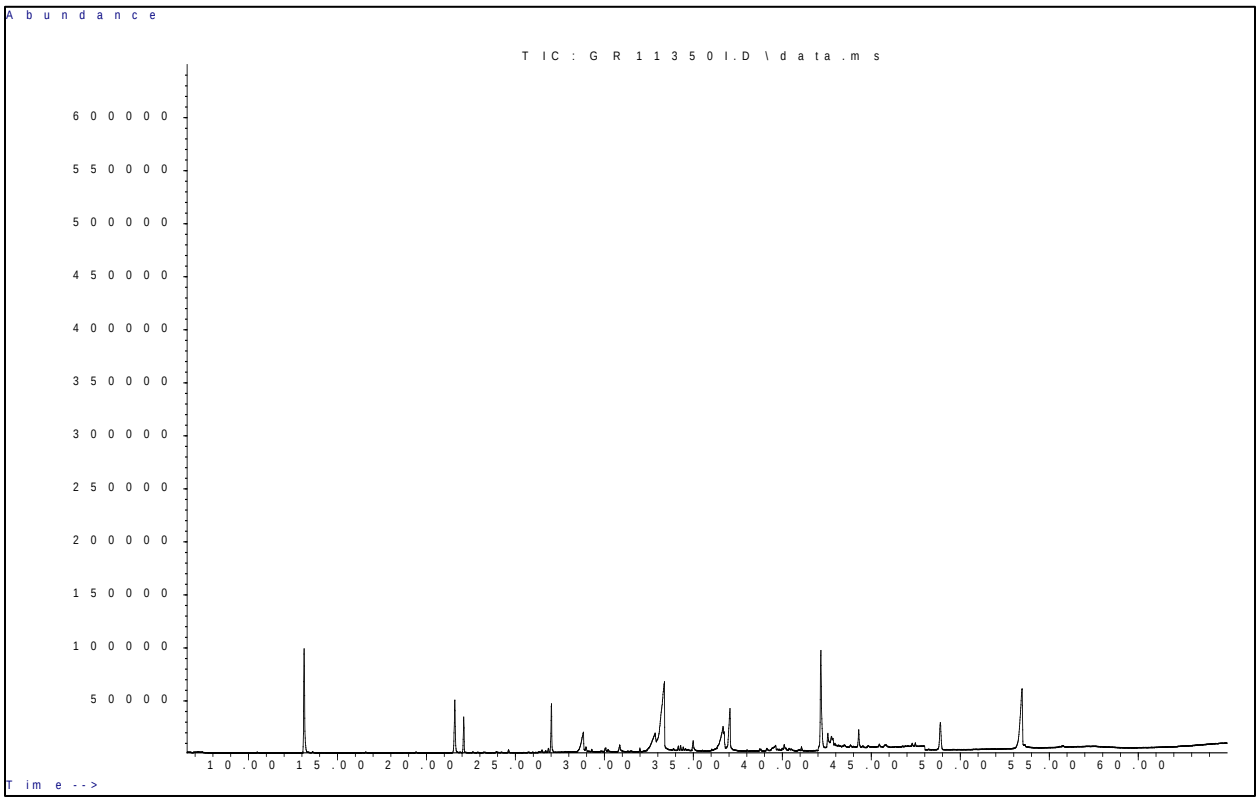
Time -->

VB90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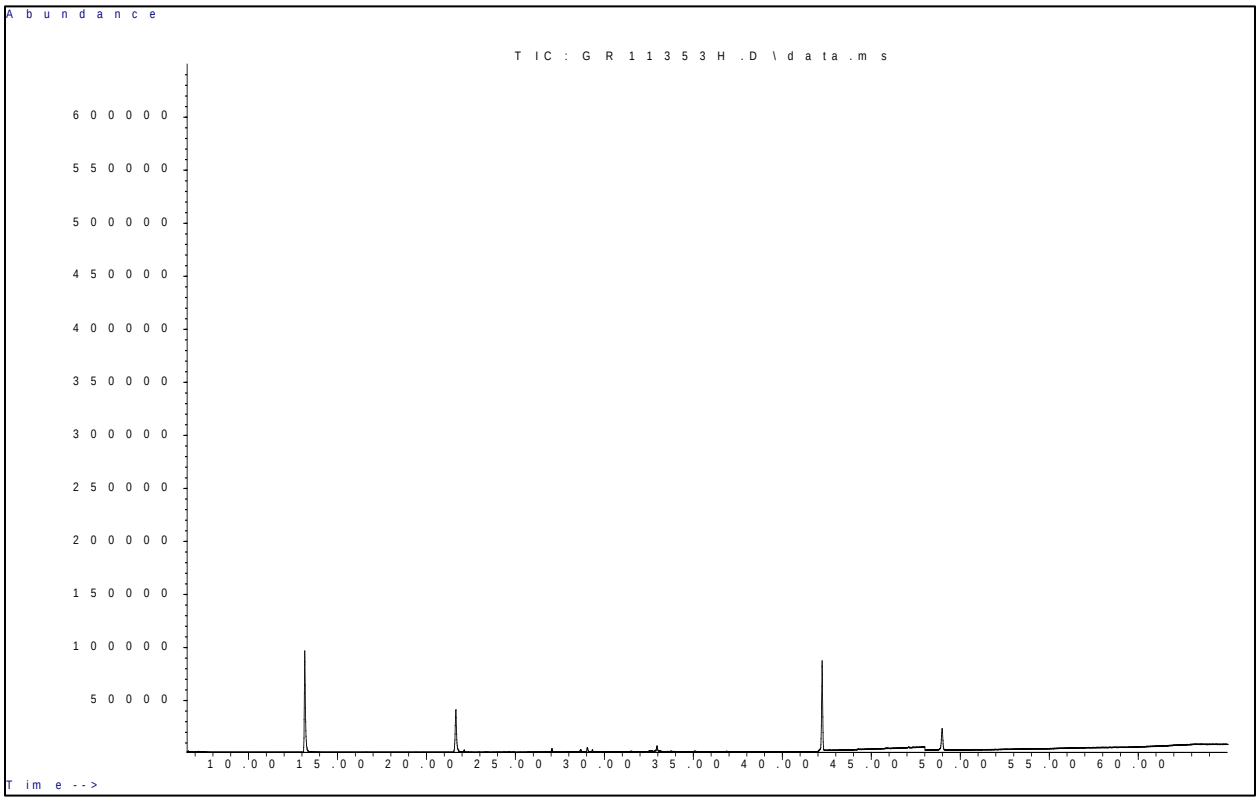
Time -->

Method Blank and Controls

Method Blank



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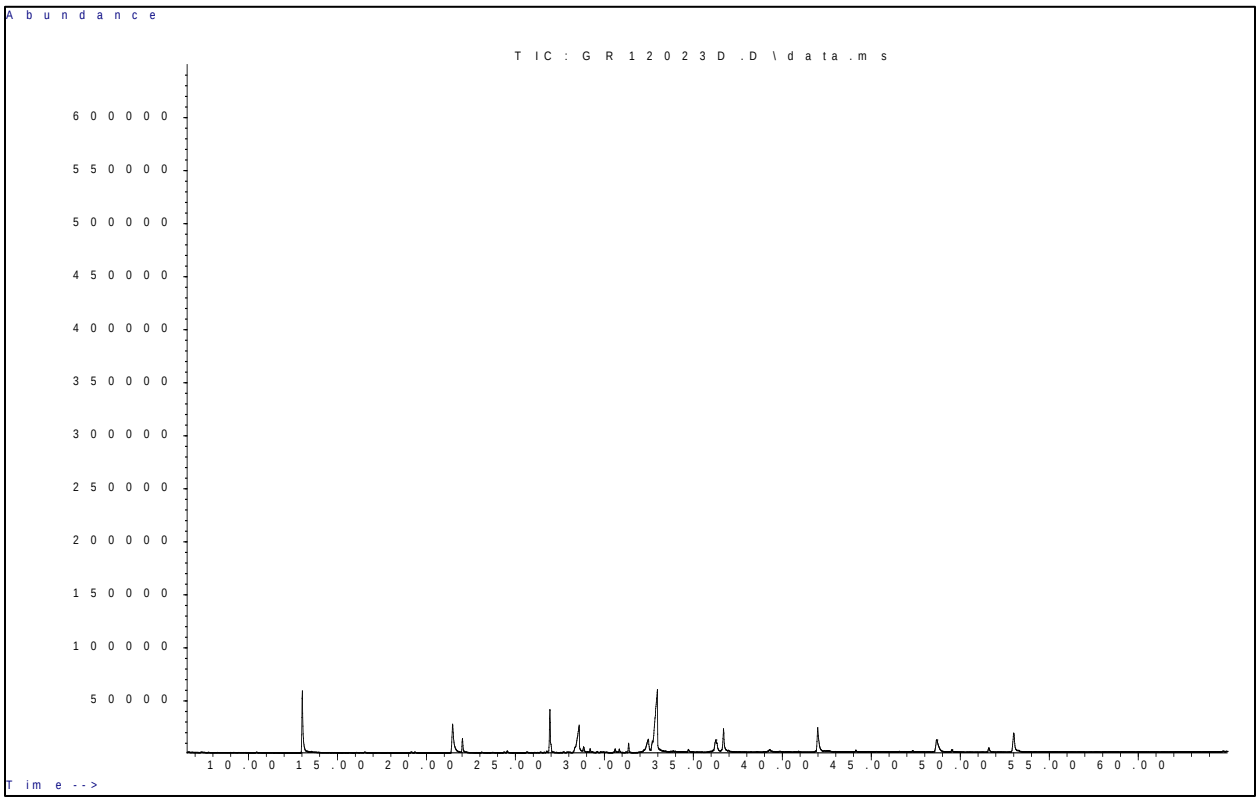
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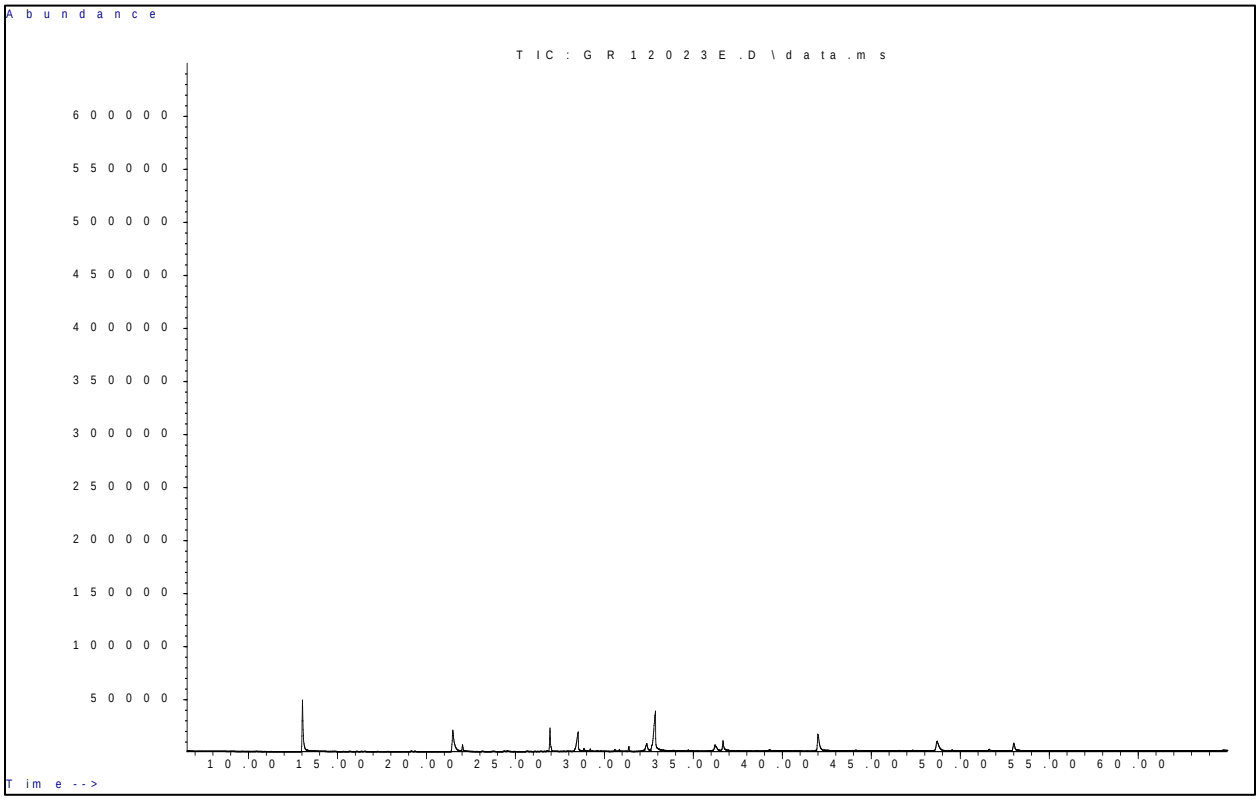
Time -->

C2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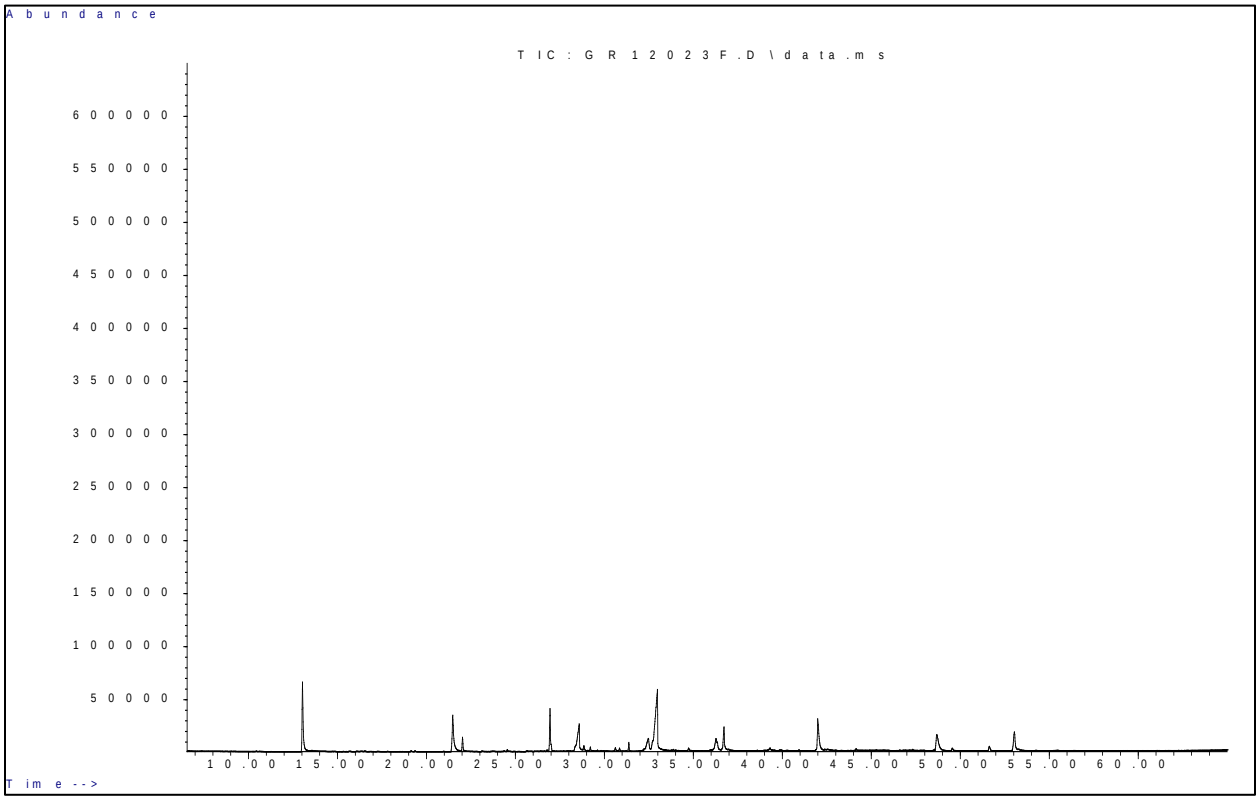
Time -->

C3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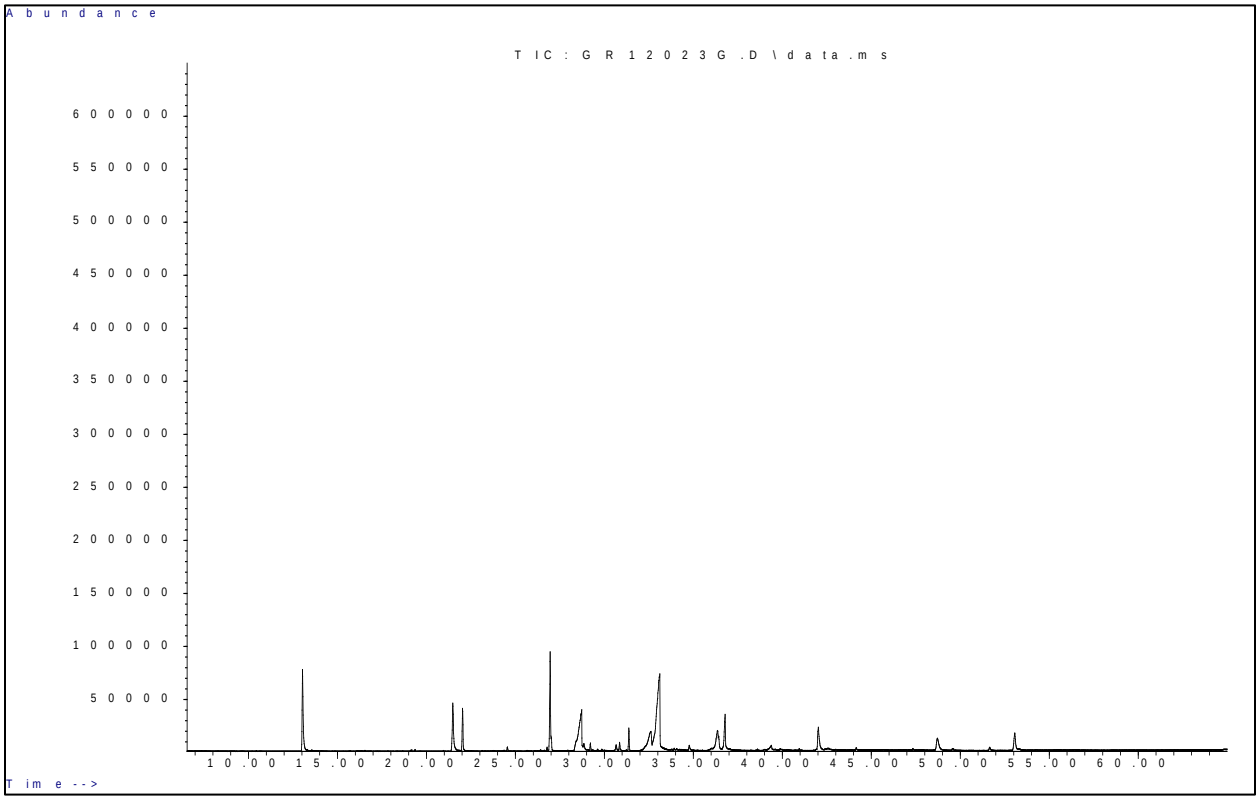
Time-->

C4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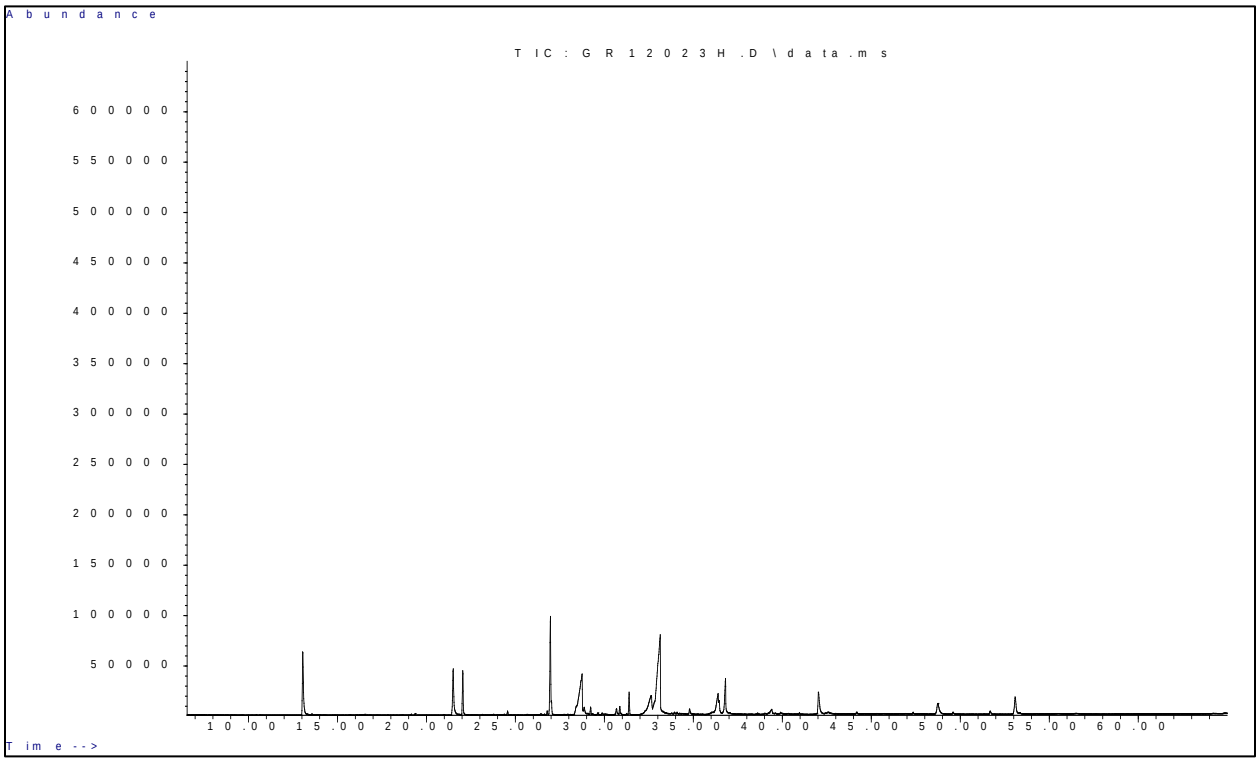
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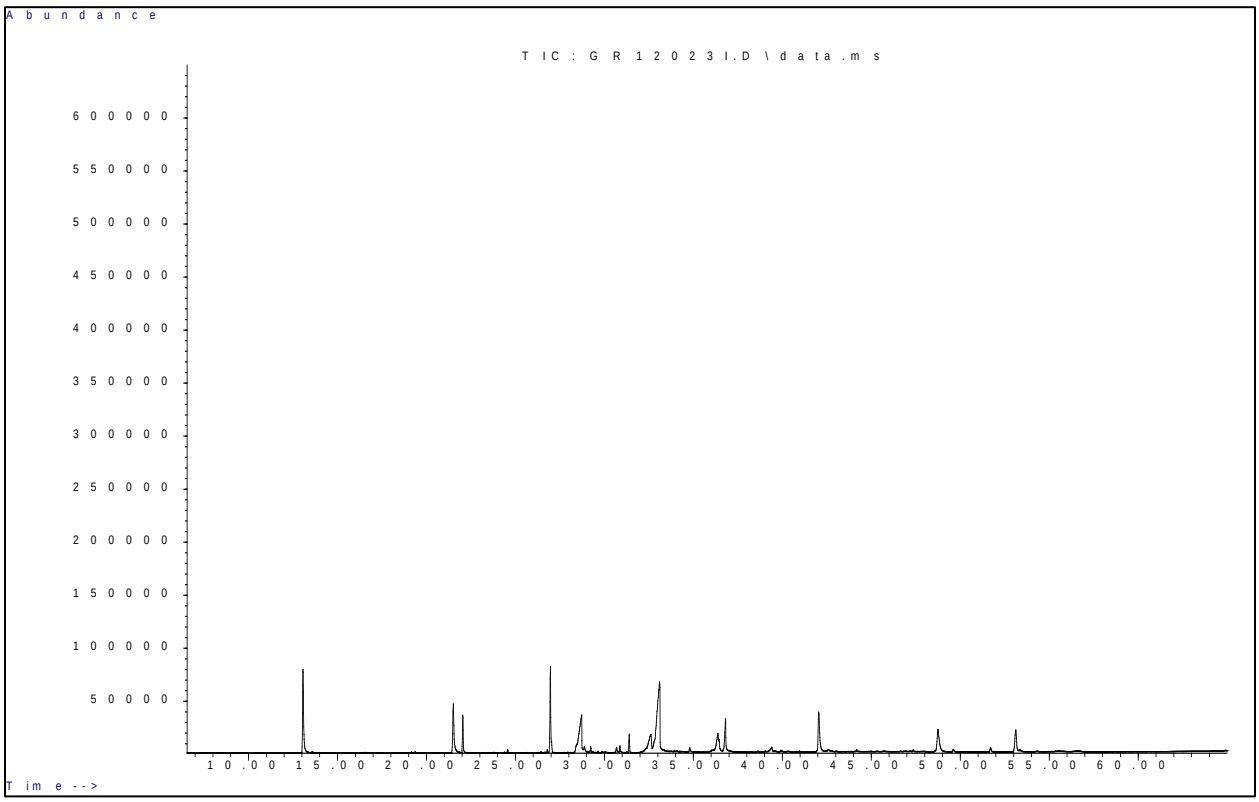
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APPENDIX D: IACUC ACUP FORMS FOR VERTEBRATE USE

ACUP Protocol

PROTOCOL NUMBER: _____ APPROVAL DATE: _____

LSU PROTOCOL FOR ANIMAL CARE AND USE

SECTION 1: Principal Investigator

Name: Dr. Ralph J. Portier	Department: Environmental Sciences
Office Phone: 225-578-4287 Home Phone: 225-921-1518	E-mail Address: rportie@lsu.edu

SECTION 2:

A. Project Title (Enter the name of your project/course number below.)

A comparative and correlative study of PAH accumulation within Gulf and Atlantic menhaden populations versus Gulf coastal and Atlantic coastal oyster populations.

B. Anticipated Project Start Date

Summer 2011

SECTION 3:

A. Animal Species

Species (common name): *Brevoortia patronus* and *Brevoortia tyrannus* (Gulf and Atlantic Menhaden)	Strain:

Number of animals needed: Year 1: **876** Year 2:_____ Year 3:_____ TOTAL: 876	Maximum number needed at one time: 51	Number of animals to be placed in each group: 17

Yes:	No: X	Are you using wild, invasive, or non-native species for which permits are necessary? (ATTACH COPY OF PERMIT) Note: a copy of the permit(s) must be received before animal work begins.

B. Source of Animals

	Order through DLAM
X	Other (list source): Natural capture off the Louisiana, Florida, and New Jersey coasts as well as tanks located at LUMCON in Cocodrie, LA. Other possible locations of tanks: Key West, Florida and in New Jersey
	Transfer from Approved Protocol (list protocol number):

C. Location of Animal Housing

	DLAM Vivarium
	Life Sciences Vivarium
	SVM Barns (list site):
	SVM Fish Building
	Research Herd
	LAES (list site):
X	Other (list site): LUMCON (Louisiana Universities Marine Consortium) Marine Center Cocodrie, LA
	Field Study (Do not complete D and E)

Animal housing and veterinary care have been coordinated with DLAM office or LSU Agricultural Center Unit.

Yes: _____
No: ___**X**___

Name of Animal Housing Representative Contacted (typed):

Signature (required): _____

Mr. Michael Keowen's signature is also required below if you plan to use animals from the EHSP Herd:

_____

D. Special Husbandry Requirements

Do your animals have special needs to be address by DLAM?

xX	Housing under the direct care of DLAM is not required. (e.g. SVM fish building)
	NO. Animals will be cared for according to standard operating procedures of DLAM.
	YES (complete table below)

TEMPERATURE RANGE	(F)	Humidity:	(%)	
LIGHT CYCLE	Hours light:	Hours dark:		
CAGING	Type:	Size:	ABSL2:	ABSL3:
BEDDING/LITTER	Type:	Autoclaved:	Changes/week:	

WATER	Sterile: De-ionized: Acidified: Tap: Other:
DIET	List Special Feeding Requirements:
OTHER SPECIAL NEEDS	List:

E. Animal Management

Individual (or groups of) animals are identified by:

	Tag
	Tattoo
X	Cage, Tank, or Stall Card
	Other. List type of identification:

Check all applicable below:

CARE OF SICK ANIMALS		DISPOSAL OF DEAD ANIMALS		PEST CONTROL	
X	Call Investigator	X	Call Investigator		Call Investigator
	Clinician to Treat		Necropsy		Pesticides OK
	Euthanasia		Disposal. List any special requirements:	X	No Pesticides

F. Disposition of Animals

What will be done with any animals at the conclusion of the project? Mark all that apply.

xX	Animals will be euthanized.
	DLAM/LAES has permission to REASSIGN animals to another IACUC-approved protocol.
	TRANSFER animals to the following IACUC-approved protocol(s). List Protocol Number(s):
	Catch and release (applies to field studies).
	Return to owner/supplier.
	Other (please state):
	TRANSFER animals to another institution (please state):

SECTION 4: Layman's Summary of Research/Teaching

Provide a brief (100 word maximum), non-scientific (i.e., no jargon) explanation of the purpose, materials, and methods in the block below for the benefit of reviewers and animal handlers who need to understand the research project.

Menhaden will be caught in naturally occurring waters and packed in ice. Once they are in the lab, they

will be placed in an ultra cold freezer (-80 degrees Celsius) and then freeze dried. They will be blended up and a tissue extraction will be done to identify PAH (Polycyclic Aromatic Hydrocarbons which are a constituent of oil) concentrations in the organism.

For those menhaden from LUMCON, they will be fed a clean diet to control tissue concentrations for 1 month. The above procedure will be carried out on them in order to determine the natural amount of PAHs in the body of menhaden.

SECTION 5: Investigator's Statement. Assurances for the Humane Care and Use of Vertebrate Animals.

By signing this form, we agree to abide by the Policy for the Care and Use of Animals of Louisiana State University. This project will be in accordance with the NIH "Guide for the Care and Use of Laboratory Animals" (except as explained in the accompanying protocol), and the Louisiana State University Animal Welfare Assurance on file with the U.S. Public Health Service.

We further assure the Committee that: 1) We will abide by all federal, state, and local laws and regulations governing the use of animals in teaching and research; 2) the investigators and technicians are adequately trained to perform the research techniques required in these studies; and 3) the fewest number of animals required to produce valid results are being used in this study. (Add additional rows as needed)

Principal Investigator Signature:	Principal Investigator Name (Typed): Ralph J. Portier	Title/Rank: Dr./Full Professor	Date:
Co-Investigator Signature:	Co-Investigator Name (Typed): Gregory M. Olson	Title/Rank: Mr./Graduate Student	Date:
Surgeon Signature:	Surgeon Name (Typed):	Title/Rank:	Date:

SECTION 6: Hazardous Materials

Will zoonotic or recombinant, radioactive, or hazardous chemical agents be **PRESENT IN THE ANIMAL ROOM?**

If zoonotic (infectious to humans) or recombinant organisms are to be used, this protocol request must be submitted to the IBRDS Committee for approval **PRIOR TO CONSIDERATION** by the IACUC. Final approval will not be granted until IBRDS approval is received by the IACUC. Similarly, if hazardous chemicals are to be used in the animal room, submit the proposal to the Chemical Safety Committee for prior approval. **P.I. MUST PROVIDE** health and safety measures

for animal technicians and facility maintenance personnel. In Standard Operating Procedure (SOP) form, describe any precautions, procedures, or personal protection required in handling animals or waste containing listed agents or compounds, or in working in or around the animal room (including air handling system), and **attach a copy of your SOP(s) to this protocol proposal.**

Will Zoonotic Agents be used? ___ YES __X__ NO

List agents:_____

Has request for use of agents been submitted to the Institutional Biological Recombinant DNA Safety (IBRDS) Committee? ___ YES __X__ NO

If not, please contact either Dr. Greg Hayes, Biological Safety Manager, at (225) 578-4658 / ghayes@lsu.edu in the Office of Occupational and Environmental Safety; or Dr. Gregg Pettis, Chair of the IBRDS, at (225) 578-2798 / gpettis@lsu.edu in the Department of Biological Sciences.

Also note that a Door Posting Form for the Animal Room is required when using zoonotic agents. Please submit this form to the IBRDS along with your request for use of agents. This form must be signed by either Dr. Hayes or Dr. Pettis. (Blank form is attached at end of protocol. It can also be obtained from Dr. Hayes.)

Will Recombinant DNA and/or Virus Vectors be used? ___ YES __X__ NO

List:_____

Has request for use been submitted to the IBRDS Committee? ___ YES __X__ NO

If not, please contact either Dr. Greg Hayes, Biological Safety Manager at (225) 578-4658 / ghayes@lsu.edu in the Office of Occupational and Environmental Safety; or Dr. Gregg Pettis, Chair of the IBRDS, at (225) 578-2798 / gpettis@lus.edu in the Department of Biological Sciences.

Will radioisotopes be used? ___ YES __X__ NO

List isotope(s):_____

Are you certified by the Radiation Safety Committee? __X__ YES ___ NO

Will hazardous chemicals be used? ___ YES __X__ NO

List compound(s):_____

Please note that approval from the Mr. Jerry Steward, Chemical Safety Manager, is required when using hazardous chemicals in the animal facilities. You can contact him at (225) 578-5640 / jsteward@lsu.edu regarding a list of hazardous chemicals, and approval of these chemicals.

SECTION 7: Type of Project and Narrative Statement

	TYPE B – Animals being bred, conditioned, or held for use in teaching or research but not yet used for such purposes. (e.g. a breeding colony of mice which will transfer individuals to experimental protocols.)
xX	**TYPE C -** Pain or distress will not be induced; animals will only be used for injections, collections, or procedures causing nothing more than minor discomfort; or will be humanely euthanized prior to the procedures that induce pain or distress.
	TYPE D - Pain or distress will be relieved by appropriate therapy, e.g. sedatives, analgesics, anesthetics, or euthanasia.
	TYPE E - Drug intervention for pain or distress would interfere with the protocol. **(If this block is checked, specific justification MUST be provided here.)**

Federal regulations mandate that you provide **written, narrative statements** for all projects.

1. You must state that "the proposed activities do not unnecessarily duplicate previous experiments". In this statement, include sources used to make such a determination (e.g., Databases, workshops, expertise in the field, etc.) If an electronic database was used, include database, years and words searched, and date of search.

The proposed activities do not unnecessarily duplicate previous experiments based on a lack of similar research within these expansive databases listed below. The hyperlinks below are linked to the exact search terms used, databases included, and years searched that were performed.

http://www.lib.lsu.edu/apps/onoffcampus.php?url=http://search.ebscohost.com/login.aspx?direct=true&db=a9h&db=syh&db=fzh&bquery=(XX+%22menhaden%22%5b100%5d+AND+(XX+%22polycyclic%22%5b76%5d+OR+XX+%22hydrocarbon%22%5b64%5d+OR+XX+%22aromatic%22%5b58%5d+OR+XX+%22accumulation%22%5b45%5d))&type=1&site=ehost-live&scope=site

http://www.lib.lsu.edu/apps/onoffcampus.php?url=http://search.ebscohost.com/login.aspx?direct=true&db=a9h&db=syh&db=fzh&bquery=(XX+%22menhaden%22%5b100%5d+AND+(XX+%22pah%22%5b79%5d+OR+XX+%22accumulation%22%5b45%5d))&type=1&site=ehost-live&scope=site

Database used: Academic Search Complete, Science & Technology Collection, Wildlife & Ecology Studies Worldwide

Years searched: 1976 – 2011

Words searched: PAH accumulation in menhaden, Polycyclic Aromatic Hydrocarbon accumulation in menhaden Date of search: May 18, 2011

Note: Address the following items only if you indicated project **Type D or E**.

2. You must indicate that you have considered alternatives to procedures producing more than momentary or slight pain or distress. Describe any alternatives available and why they are not appropriate.

3. Describe the methods you used to determine that alternatives to such procedures were not available (Databases, years and words searched, date of search etc.). Put your statements in the block below. Database used:_____ Years Searched:_____ Words Searched:_____ Date of Search:_____

SECTION 8: Animal Treatment Checklist

Check "Yes" or "No" to each of the following questions. Provide an explanation in Section 9 for any "yes" answers.

Q#	YYES	NNO		
11		xX	Will animals be restrained? *(Restraint refers to immobilization or other restrictions to normal movement beyond momentary holding for injections, etc.)*	Not applicable
22		xX	Will animals be fasted?	Not applicable
23		xX	Are any ANESTHETICS, ANALGESICS, or TRANQUILIZERS to be used? Include drug, dose, route and frequency, and how animals will be monitored in Section 9.	**Who will administer?** **_____**
44		xX	Are neuromuscular blocking agents to be used? Include drug, dose, route and frequency, and how animals will be monitored in Section 9.	**Who will administer?** **_____**

55		xX	Will surgical procedures be employed? Check all that apply! Are they: Survival_____ Multiple-Major Survival _____ Multiple-Minor Survival _____ *Major survival surgery= Any procedure which penetrates **and** exposes a body cavity or alters function. Terminal_____ In addition to describing surgical procedures in Sec. 9, you must indicate the time frame between multiple procedures. **Note**: *Survival mammalian surgeries must be conducted aseptically, and major surgical procedures performed on non-rodent species must be conducted in a dedicated surgical facility.*	**Who** will perform surgery? **_____** If survival: **1) Who** will be responsible for recovery of the animals? _____ **2) Who** will maintain post-operative records? _____ **3) Where** will records be maintained? _____ **4) Who** will provide post-operative analgesics? _____
66		xX	Do you anticipate any adverse effects of the experimental procedures on the animals (e.g., pain, discomfort, reduced growth, fever, anemia, etc)?	Not applicable.
77		xX	Is death an endpoint in your experimental procedure? **Note**: *Death as an endpoint refers to acute toxicity testing, assessment of virulence of pathogens, neutralization tests for toxins, and other studies in which animals are not euthanized, but die as a direct result of the experimental manipulation.*	Not applicable.
88		xX	Are there emergency treatments by the DLAM veterinary staff that would not be allowed?	Not applicable.
99	xX		Will animals be euthanized during or at the close of the study?	**Who will perform euthanasia?** **Gregory Olson and/or Dr. Ralph Portier__**
010		xX	Will animals be used for antibody production?	Not applicable.

111		xX	Will Complete Freund's Adjuvant be used? **Must be scientifically justified in Section 9.**	Not applicable.
112		xX	Will other adjuvants be used?	**If yes, please specify here:** **_____**
113		xX	Will blood be collected? **Note**: *Blood equal to 1.5% of the animal's body weight per 2 weeks represents the upper approvable limit, unless scientific justification is provided.*	**How often? _____** **Volume? _____** **Who will collect blood? _____**
114		xX	Will live animals be taken from approved housing facilities for procedures followed by their return later that day? **Note:** *Animals may not be housed outside of the Vivarium (e.g. in a laboratory) overnight.*	**If yes, please specify to which building and room/rooms the animals will be taken:** **Note:** *This room(s) must be approved for use before the animals can be brought there. Contact IACUC coordinator for list of approved rooms.*
115		xX	Will live animals be brought onto campus for demonstration, teaching, euthanasia, etc. for which no housing is required?	**If yes, please specify to which building and room/rooms the animals will be taken:** **Note:** *This room(s) must be approved for use before the animals can be brought there. Contact IACUC coordinator for list of approved rooms.*

SECTION 9: Summary of Procedures

Your response in this section should provide the reader with a complete description of how every animal to be used in this project is to be treated during every phase of the study. Your target audience is a faculty member from a scientific discipline unrelated to yours. Do not use jargon. **Please answer each statement in its own expanding box.**

1 a: What is the rationale for using animals?
Menhaden are filter feeding fish. They are the entry points for PAHs in the food chain as well as possible vectors of PAH transfer to humans. (Menhaden are used for fish meal as well as any commodity that has fish oil in it such as cosmetics and fish oil supplements)

1 b: Why should this study be done?
To asses PAH accumulation after the prolonged oiling of the Gulf of Mexico in juvenile menhaden. Menhaden are also an extremely important commercial species and this study will help understand the impact of the Gulf oil spill on the health of this species. Additionally they are a commercially harvested fish with potential consumer product impacts.

1 c: What hypothesis will be tested?
Hypothesis – the addition of oil due the spill has increased PAH accumulation in filter feeding marine organisms.

2. Explain how and/or why the particular animal species was selected?
It is a near shore filter feeding organism that can be compared to PAH accumulation in oysters (an immobile filter feeding organism located near shore as well)

3. Explain how you arrived at the number of animals to be used (e.g., power analysis in comparison studies, permitted animal limits in field studies, etc).

Menhaden will be collected from 2 sites here in Louisiana over the period of 6 sampling events. Menhaden will also be collected twice from a location in Florida and twice from a location in New Jersey. This is a total of 16 separate sampling events. For each location and event I will need a maximum of 51 menhaden. There will also be a collection of menhaden as a control from the facilities at LUMCON. This makes the total maximum number of menhaden 876 (17 * 51). We will be collecting fish that range from 3 to 8 inches. This means that the total number of menhaden required will vary greatly and the numbers presented here are estimates based on the maximum number needed for a 90% power. My numbers are over estimated because I am attempting to account for size and weight variation. The statistical analysis assumes uniform individuals. The power analysis was performed using GPower 3.1.2

[1] *-- Monday, May 23, 2011 -- 10:37:43*

t tests – Correlation: Point biserial model

Analysis:	A priori: Compute required sample size		
Input:	Tail(s)	=	One (Do not need to know if the PAH level is too small)
	Effect size |ρ|	=	0.1 (Want to measure a small effect change in PAH concentration)
	α err prob	=	0.05 (Will accept a possible 5% type 1 error rate)
	Power (1−β err prob)	=	0.90 (Will accept a power of 90%)
Output:	Noncentrality parameter δ	=	2.9301636
	Critical t	=	1.6466525
	Df	=	848
	Total sample size	=	850
	Actual power	=	0.9002490

4. Provide a complete description of the proposed use of the animals. Describe the experimental design of the study. Include a list of any physical, chemical or biological agents (name, dose, volume, route, frequency) that may be administered. If animals are being transported between facilities, describe conditions of transport. If multiple surgical procedures are planned you must include the time frame between those procedures. If food or fluid restriction and/or restraint are used you must include the duration of each. Use tables and outlines to indicate group assignments and study progression.

The menhaden will be used to determine PAH accumulation within filter feeding vertebrates. They will be captured via a cast net and then bagged into groups of 8-17 in a 10% TMS, MS 222 solution (based on size) and then placed on ice until they are placed in the freezer. Once the menhaden are cooled to a temperature of -80 degrees Celsius they will be placed in a freeze dryer for 24 hours to remove all moisture. They will then be homogenized with sodium sulfate to create more surface area (the sodium sulfate will remove any remaining water). The mixture will then go through the process of Ultrasonic Extraction EPA Method 3550C. They will then go through a modified cleanup technique that combines

EPA Method 3630C and 3611B. The remaining liquid will then be evaporated down to 1 ml and be analyzed through Gas Chromatography. Any menhaden that may be transported will be placed in tanks of water collected from the location of their collection. No menhaden will be transferred any more than 3 hours.

1) Collection site (includes collection from 1 time lab site)
    a) Bagged in a group of 8-17
        i) Placed in 10% TMS,MS 222 solution
        ii) Placed on ice
    b) Bagged in a group of 8-17
        i) Placed in 10% TMS,MS 222 solution
        ii) Placed on ice
    c) Bagged in a group of 8-17
        i) Placed in 10% TMS,MS 222 solution
        ii) Placed on ice
2) Lab site (transported from collection site on ice)
    a) Ultra Cold Freezer
        i) Temp down to -80 degrees Celsius
    b) Freeze Dryer
        i) Removes water
    c) Ultrasonic Extraction
        i) GC analysis of total PAHs within the composites

5 a: Describe expected adverse effects.
The menhaden will be caught via a cast net in the same manner recreational fishermen catch them. The menhaden will be euthanized prior to any adverse effects.

5 b: What is the likelihood of these effects (high, low, unknown)?
Every menhaden will be caught in a net and then euthanized.

6. Describe procedures designed to assure that discomfort and injury to animals will be limited to that which is unavoidable in the conduct of scientifically valuable research. For anesthesia and survival surgeries, include a description of post-procedural care and monitoring. Indicate how analgesic, anesthetic, and tranquilizing agents will be used where appropriate, to minimize discomfort and pain to the animals. Include any conditions where veterinary treatment would not be allowed. Specify which treatments would not be allowed, and include a scientific justification. It is advisable that you obtain input from LSU's Attending Veterinarian (Dr. David Baker) or from another veterinarian familiar with the species to be used**.**

Appropriate netting will be use to catch fish. Live fish will be transported in a manner that maximizes

their survival rate. The menhaden will be placed in a 10% solution of TMS, MS 222 and water before being placed on ice. This will alleviate any stress the fish would have felt as the Dissolved Oxygen levels go down and the temperature decreased.

Name	Online Investigator Training Course Attended? (Indicate Yes or No)	Date Attended	Species Wet Lab Taken? (Indicate Yes or No)*	Date Attended or Exempted	Training or Experience? (Indicate Yes or No)**
Dr. Ralph J. Portier	Yes	June 22, 2011	No		Yes
Gregory M. Olson	Yes	May 19, 2011	No		Yes
Dr. John R. Sowa	Yes	June 29, 2011	No		Yes
Dr.Carolyn Bentivegna			No		Yes

7. Describe any euthanasia method to be used. Even if euthanasia is not planned please provide a "What If" scenario in the event of unforeseen circumstances. Justify any deviation from AVMA Guidelines on Euthanasia, 2007. Text, viewable at http://avma.org/resources/euthanasia.pdf.

The menhaden will be placed in a 10% solution of TMS, MS 222 and water before being placed on ice.

SECTION 10: Investigator Training

In accordance with IACUC policy, all personnel conducting animal-based research must attend a Rules and Regulations Course and verify their training, experience and skills in the care and use of the animals and techniques they are responsible for.

List all persons involved in animal care and use for this study below. Add additional lines as needed.

***Exemption from wet lab training for specific procedures needed for the protocol may be obtained by written request to the IACUC. Training wet labs will be scheduled on an 'as needed' basis. Please contact Ms. Dawn Best-Desjardins at 578-9643 or dbest@vetmed.lsu.edu to sign up for these courses.**

****The person named has training/experience in assigned procedures for this protocol.**

Who will train individuals for participation in protocol procedures? Answer in the block below.

Dr. Ralph J. Portier

Personnel participating in the project must complete the online investigator training course once every three years. Those who have not attended the online course or the applicable Species Wet Lab, will have **six (6) months** from the approval date of the project to complete them.

The online investigator training course is offered through the AALAS Learning Library www.aalaslearninglibrary.org . Training wet labs will be scheduled on an **'as needed'** basis. Please contact Ms. Best-Desjardins at 578-9643 or dbest@vetmed.lsu.edu to sign up for these courses.

SECTION 11: Occupational Health and Safety

It is the responsibility of the principal investigator to conduct a hazard analysis and risk assessment to determine if personnel involved in the proposed study should participate in the Occupational Health and Safety Program administered through DLAM and the Student Health Center. Currently, there is no direct cost for participation in the program**. All persons listed in Section 10 must read the following and indicate level of participation with their signature. Add additional rows in the table as needed.**

The Division of Laboratory Animal Medicine operates an Occupation Heath Program (OHP). Participation is voluntary, and is open to all personnel with direct or indirect contact with animals used in teaching and research, their bodily products, or materials to which they may be exposed, as described in this protocol. Eligible persons include facility services personnel, animal caretakers, principal investigators, technical staff, graduate and other student workers, and post-doctoral and visiting scientists. All medical information is kept confidential, and is retained by the Student Health Center. You have the right to refuse any and all procedures recommended.

To determine the extent of your participation in the OHP, discuss with the principal investigator named on this protocol, and/or your health professional, any potential physical, chemical, or infectious hazards to which you may be exposed while working on the project. Whether or not you participate, questions related to health risks should be directed to Dr. Tim Honigman, Campus Physician, at the Student Health Center.

If you are at increased risk of illness or injury due to drug-related immune suppression, HIV infection, pregnancy, concurrent illness, musculoskeletal problems, etc., you are advised to discuss your risks with Dr. Honigman, your physician, or another health professional.

To participate in the OHP, contact Ms. Dawn Best-Desjardins at 578-9643 or dbest@vetmed.lsu.edu for information.

Printed Name:	Signature:	____I choose to participate
Dr. Ralph J. Portier		**X** I choose NOT to participate

Printed Name: Gregory M. Olson	Signature:	____I choose to participate __X__I choose NOT to participate
Printed Name: Dr. John R. Sowa	Signature:	____I choose to participate __X__I choose NOT to participate
Printed Name: Dr. Carolyn Bentivegna	Signature:	____I choose to participate __X__I choose NOT to participate

Exemption Letter

Institutional Animal Care and Use Committee
Division of Laboratory Animal Medicine
LSU School of Veterinary Medicine
Skip Bertman Drive
Baton Rouge, LA 70803

June 20, 2011

To whom it may concern:

My name is Dr. Ralph Portier of the Department of Environmental Sciences. I am completing the required protocol for using menhaden (an estuarine fish) in a study that our laboratory is conducting and it requires a wet lab training session for our proposed work. I am asking that we are granted an exemption from this wet lab training on the grounds that I have 34 years of experience with microcosms and small scale natural habitats including aquarium habitats designed for estuarine fish. All designs and decisions will be under my direct supervision and all other co-PIs will be trained to handle the aquarium systems if we actually need these laboratory scale microcosms. The need for us to maintain an estuarine system is virtually nonexistent due to our partnership with Dr. Edward Chesney at LUMCON and his lab's ability to house menhaden.

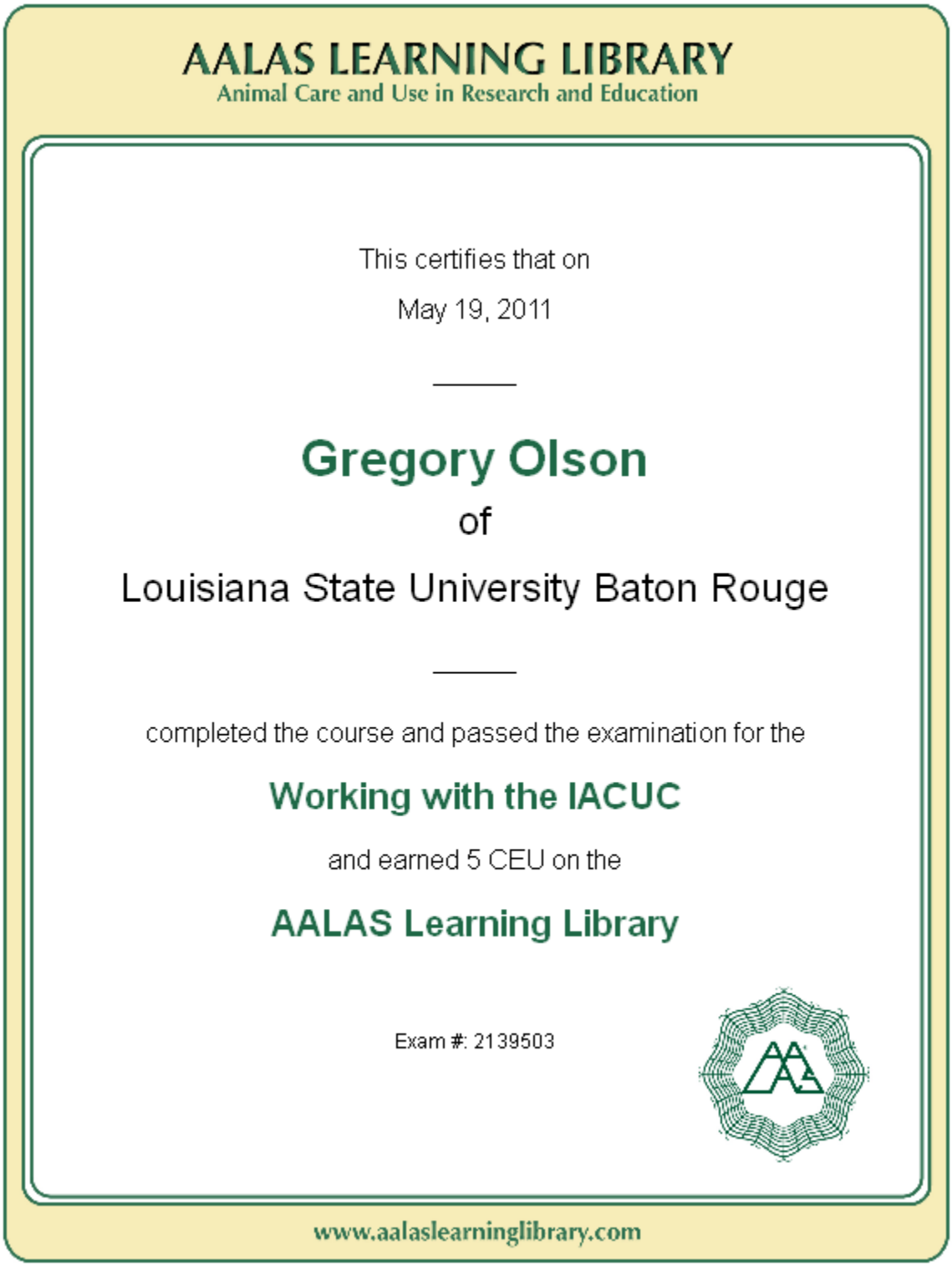
I am asking on behalf of myself and all other co-PIs for exemption on the grounds of my experience with small scale habitat systems as well as the fact that we will not be housing any live animal in our direct care.

Sincerely,

Dr. Ralph J. Portier
Professor of Environmental Sciences
School of the Coast & Environment
Louisiana State University
1165 EC&E Bldg
Baton Rouge, LA 70803

Office: (225)-578-4287
Cell: (225)-921-1518
Fax: (225)-578-4286
Email: rportie@lsu.edu

IACUC Training Certificate



AALAS LEARNING LIBRARY

Animal Care and Use in Research and Education

This certifies that on

May 19, 2011

Gregory Olson

of

Louisiana State University Baton Rouge

completed the course and passed the examination for the

Working with the IACUC

and earned 5 CEU on the

AALAS Learning Library

Exam #: 2139503

www.aalaslearninglibrary.com

VITA

Gregory Michael Olson was born to Carmen E. Olson July of 1984 in Fort Sam Houston, Texas, just outside of San Antonio. He Moved to Pickering, Louisiana, in 1987 with his mother and from here he attended Pickering Elementary and High School. He graduated in 2003 and began his undergraduate degree at McNeese State University in Lake Charles, Louisiana. He graduated in 2009 and taught in the public school system. He wanted to do more with his life so he pursued a graduate assistantship at Louisiana State University in Baton Rouge, Louisiana, and is currently studying towards a Master's in environmental science concentrating in Toxicology. He works in Dr Ralph J. Portier's a lab at LSU and plans to one day have a successful career as a collegiate educator.