A comparison of PAH exposure in Red Snapper (*Lutjanus campechanus)* around natural and artificial reefs in the northwestern Gulf of Mexico

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Abstract

Red Snapper *(Lutjanus campechanus)* are an important fishery resource in the Gulf of Mexico (GoM) and are found in abundance around artificial (e.g. oil and gas platforms) and natural habitats (e.g. coral reefs, outcroppings). Polycyclic aromatic hydrocarbons (PAHs), are a small but toxic component of crude oil released into GoM waters through both anthropogenic (e.g., oil and gas activities) and natural (e.g., hydrocarbon seeps) sources. The objectives of this study were to measure and compare PAH concentrations between tissue matrices (liver, gonad, muscle, and bile), by sex and location (e.g., artificial oil and gas infrastructure vs. natural reefs), of Red Snapper collected in the northwestern GoM. In 2016, Red Snapper (n = 88) were collected from natural and artificial reefs to evaluate potential differences in PAH exposures and body burdens. There were no significant results from the biometric analysis or their association with PAH's. Average PAH concentrations were significantly higher ($p<0.001$) in livers than in gonads and muscle tissue. No significant differences were found in PAH concentrations between sexes or between structure type for both biliary, liver, or, muscle PAH concentrations, likely due to the close proximity of the natural and anthropogenic sites sampled (within 97km radius). However, biliary PAH concentrations in fish collected outside of the densely populated oil and gas infrastructure fields had significantly lower PAH levels ($p<0.001$). Thus, the scale at which samples are collected and compared is critical to understanding the cumulative impacts of oil and gas activities and underscores the need for further research at multiple scales across the GoM.

Keywords: oil rigs, fisheries, artificial reefs, contaminants, PAHs, Red Snapper

INTRODUCTION

Red Snapper (*Lutjanus campechanus*) are a primary target species of reef fish fisheries throughout the Gulf of Mexico (GoM) and along the Atlantic coast of the United States. Red Snapper grow rapidly, initiate spawning in their second year and are relatively long-lived (40-50 years) (Wilson et al. 2001). Spawning occurs from May through early October with peak production in August (Collins et al. 1996). Juvenile Red Snapper prefer shallow, sandy bottom areas, feeding primarily on small zooplankton or squid until they experience an ontogenic shift to primarily feeding on crustaceans and other fishes while occupying hard bottom habitats as adults (Wells et al. 2008).

Adult and large juvenile Red Snapper congregate around both natural and artificial reef structures, which are thus important recreational fishing locations (Bradley et al. 1975; Streich et al. 2017b). Natural reef structures consist of coral reefs and carbonate rock outcroppings, whereas artificial reefs consist primarily of various engineered structures such as concrete slabs, reef "balls", tires, abandoned vehicles, sunken ships and other debris, which serve as aggregating locations for bottom-dwelling fishes (Rezak et al. 1990). Producing and abandoned oil infrastructures (e.g., production platforms, pipelines, pumping stations, etc.) particularly in the western GoM constitute the bulk of "artificial reefs" (Kaiser et al. 2005).

The GoM contains numerous non-operational (but potentially exuding oil) oil and gas infrastructure in the northern GoM, which function as artificial reef systems (Gallaway et al. 2009; Reynolds et al. 2018). Rigs providing large surface areas attract many animals and provide some of the only hard substrate available in various locations to support organisms (Shinn 1974). Federal legislation(s) require that abandoned rigs be removed within a year from being decommissioned (Sammarco et al. 2014; Rusco 2017). However, during the mid-1970s the

United States Department of Interior initiated the Rigs-to-Reef program (Reggio 1987) to transition decommissioned rigs into permanent artificial reefs (Kaiser et al. 2005). There are three categories of rig structures that serve as artificial reefs: (1) standing rigs that are no longer in service; (2) cutoff rigs; and (3) toppled rigs. A standing rig is one where no major structural changes have occurred. A cutoff structure is formed when the top of the oil platform is severed at a depth >26m and either taken to shore, or left on the seafloor to create a smaller system next to the larger remaining section. A toppled structure refers to a standing rig that has been toppled on its side, either in its original location or transported to a designated artificial reef zone.

With over 3,200 operational and abandoned structures found throughout the GoM (https://www.ncei.noaa.gov/maps/gulf-data-atlas/), there is a risk to marine life occupying these structures of being exposed to hydrocarbon pollution. The most toxic constituents of hydrocarbons to wildlife and humans are polycyclic aromatic hydrocarbons (PAHs) which comprise approximately 2-7% by volume in crude oil (Jourdain de Muizon et al. 1990; Maes 2004). PAHs are environmentally persistent pollutants that are both anthropogenically and naturally derived through pyrolytic processes and incomplete combustion that can be found in natural fuel deposits (e.g. crude oil) as well as the incomplete combustion or burning of organic substances (e.g. coal, oil, diesel, etc.). Hydrocarbons can be separated into three main groups based on where they have been derived: natural sources, such as plants (phytogenic) and petroleum (petrogenic), or from the anthropogenic sources (pyrogenic). While an organism can metabolize PAHs, these concentrations in fish have been linked to DNA damage, altered growth, reduced fitness, abnormalities, and epidermal lesions (Myers et al. 1994; Heintz et al. 2000; Collier et al. 2013; Murawski et al. 2014). These PAHs (petrogenic/pyrogenic) can be associated with oil rigs and have potentially negative effects due

to increased exposure to the organisms that utilize these structures (Peterson et al. 1996). An obvious concern is whether the high abundance of Red Snapper inhabiting oil and gas infrastructure result in differential exposure to hydrocarbons, potentially compromising fish health, including reproduction, disease prevalence and growth.

Understanding when and where fish congregate and if they are thus differentially exposed to harmful concentrations of PAHs is an important, but as of yet, untested assumption of the Rigs-to-Reefs campaign. The main objectives of this study were: (1) to quantify and compare PAH concentrations between sexes and matrices (i.e., liver, gonad, muscle, and bile samples) in Red Snapper collected from the northwestern GoM; and (2) to compare PAH concentrations in Red Snapper occupying natural vs. various types of artificial habitats, including standing, toppled and cut-off oil and gas rigs.

METHODS

Sample collections. — In 2016, a total of 88 Red Snapper (35 female/53 male) were collected from one natural and four artificial reefs (one toppled rig, one standing rig, and two cutoff rigs) in depths ranging from 47 to 76 m, located in the northwestern GoM. These samples were collected by researchers fromTexas A&M University- Corpus Christi Harte Research Institute as part of the center for the integrated modeling and analysis of the Gulf Ecosystem (C-IMAGE) research consortium (Table S1; Figure 1). Of the 88 Red Snapper used in this study, 11 were caught at a toppled rig, 22 at cutoff rigs, 24 at a standing rig, and 31 at a natural reef site. All samples were caught using a hook and line or vertical longline during July-November. Biometric data (i.e., length, weight, organ weight) were recorded and bile, muscle, gonad, and liver sampled from each fish. Samples were immediately frozen at -20°C and transported to the laboratory for further analysis.

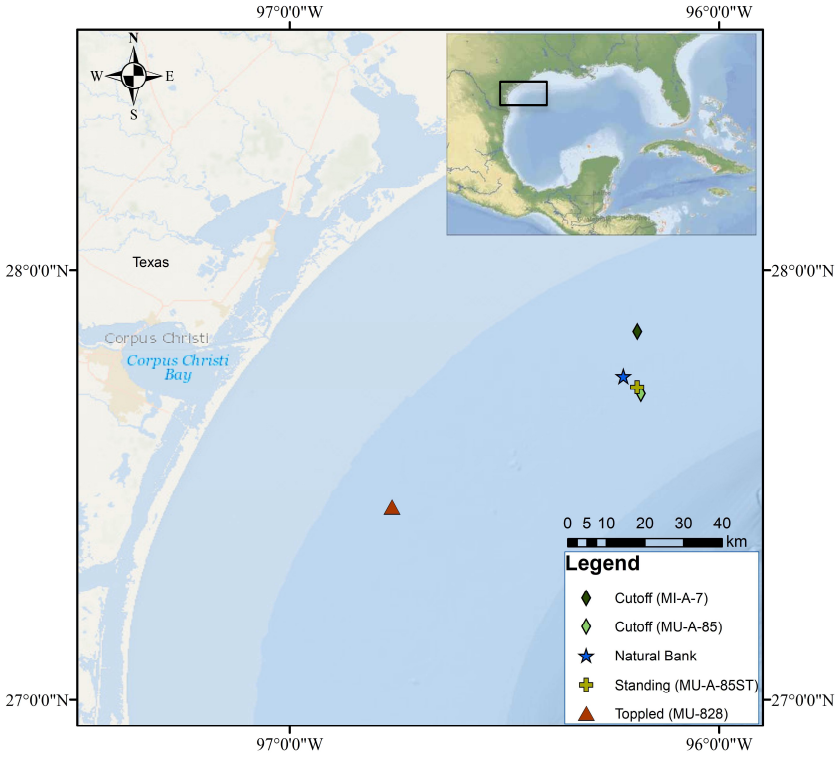

FIGURE 1: Locations of Red Snapper collected in 2016 off the coast of Texas from 1-toppled rig (triangle), 1-standing rig (cross), 2-cutoff rigs (diamonds), and 1-natural reef (star) for assessment of PAH concentrations.

Biliary PAH analyses. — Of the 88 Red Snapper collected, 78 bile samples had an adequate volume for analysis. PAH compounds, Naphthalene (NAP), phenanthrene (PHN), and benzo[*a*]pyrene (B[*a*]P) equivalents were quantified in bile samples using the semi-quantitative method of high performance liquid chromatography with fluorescence detection (HPLC-F) (Krahn et al. 1984). Briefly, 3 uL of untreated bile samples were injected into the HPLC-F system (LaChrom Elite, Hitachi High Technologies America, San Jose, CA) equipped with a C18 reverse-phase column (Synergi™ 4 µm Hydro-RP 80Å, Phenomenex, Torrance, CA) held at a constant temperature of 50 °C. The PAH equivalents were eluted with a linear gradient from 95% water/acetic acid and 5% methanol (95:5) to 100% methanol at a flow rate of 1 mL/min.

Chromatograms were recorded at wavelength pairs for two and three aromatic ring compounds (292/335 nm, e.g. naphthalene), three and four aromatic ring compounds (260/380 nm, e.g. phenanthrene), and four and five aromatic ring compounds (380/430 nm for, e.g. benzo[a]pyrene). All peaks eluting from 5 to 19 minutes were integrated for each wavelength pair and peak areas were summed. Concentrations of biliary PAH equivalents (PAHeq) were calculated using external standards of the parental PAHs, NAP (1 ng/µL), PHN (1 ng/µL) and B[a]P (250 pg/µL), and the following formula:

$$Biliary = \frac{standard\ conc\ ntration}{standard\ m\ an\ ar\ a} \times \frac{int\ grat\ d\ sampl\ ar\ a(5-19\text{min})}{d\ nsity\ of\ bil\ (0.00103\frac{g}{\mu L})} \times \frac{\mu L\ standard\ inj\ ct\ d}{\mu L\ sampl\ inj\ ct\ d}$$

Tissue extractions and PAH analysis. — Tissue samples (liver, gonad, muscle) for each of the 88 Red Snapper were extracted using the QuEChERS method (Bond Elute, Agilent Technologies, Santa Clara, CA) optimized for this species. Using this method, analytes of interest were extracted from homogenized samples using an organic solvent, followed by a cleanup method to remove excess water and other compounds.

For the QuEChERS extraction method, two grams of homogenized liver and gonad tissue and five grams of homogenized muscle tissue were individually weighed and added to a 50 mL test tube, then spiked with deuterated surrogate standard (SS) at a final concentration of 100 pg/µL. The sample and SS were vortexed (Mini vortexer MV 1, IKA Works, VWR) and allowed to marinate at room temperature. Extraction solvent (20 mL, Acetonitrile, ACN Optima, Fisher Scientific, Fair Lawn, NJ, USA) and two steel homogenizing beads were added to the sample test tube and mixed for ten minutes using a MiniG® Geno Grinder (MiniG 1600, SPEX Sample Prep, Metuchen, NJ, USA) and then centrifuged (Bio Lion, XC-L5) at 5,000 rpm for five

144 minutes. For further cleanup, 8 mL of the ACN extract were transferred to a 15 mL EMR dSPE
145 tube (Bond Elute, QuEChERS dispersive kit, Agilent Technologies, Santa Clara, CA, USA) and
146 shaken using the Mini G for five minutes at 1,000 rpm and then centrifuged (Premiere, Model
147 XC-2400) at 3,300 rpm for five minutes. The extract was decanted into a clean 50 mL test tube,
148 followed by the addition of 3.5 g of $MgSO_4$ polish (Bond Elute, QuEChERS extraction salts,
149 Agilent Technologies, Santa Clara, CA, USA) to assist in water removal. This mixture was
150 vortexed for 30 seconds and centrifuged for five minutes at 5,000 rpm. The process was repeated
151 until all water was removed from each sample. Prior to analysis, 50 µL (final concentration of
152 100 ng/µL) of the internal standard *p*-Terphenyl-d14, was added to the sample extract to monitor
153 instrument stability.
154 Two-layer sandwich injections drew 0.2 µL of analyte protectant (20 mg/mL L-
155 gulonolactone and 10 mg/mL D-sorbitol in ACN) with 2 µL of sample or standard for enhanced
156 peak signal quality (Anastassiades et al. 2003; Maštovská et al. 2005). Sample extracts were
157 simultaneously analyzed for a total of 46 PAHs ($TPAH_{46}$), including 19 parental PAHs and their
158 associated homologues, using an Agilent 7890B gas chromatograph equipped with a 7010
159 tandem mass spectrometer (GC/MS/MS) operating in electron impact (EI) and multiple reactions
160 mode (MRM). The multimode inlet and source temperatures were set to 295° C, with the transfer
161 line set to 320° C, and both quadrupoles set to 150 ° C. Chromatographic separation was
162 achieved with a Rxi-5sil MS fused silica column with an Integra-guard column (Restek
163 Bellefonte, PA, USA) utilizing ultra-high purity helium as the carrier gas at a flow rate of 1
164 mL/min. The initial GC oven temperature of 60 °C was held for three minute and then increased
165 to 120 °C at 12 °C/min, followed by an 8° C/min increase to 300 °C, and a 15° C/min increase to
166 the final temperature of 320° C with a four-minute hold, resulting in an average total run time of

35.83 minutes per sample. The mass spectrometer collision cell used ultra-high purity nitrogen as the dissociation gas with a flow rate of 1.5 mL/min and ultra-high purity helium as the quench gas with a flow rate of 2.25 mL/min. Acquisition parameters are provided in the supplementary material. All selected PAHs were identified through standards, retention time, and quantifier/qualifier ion peak ratios using the Agilent Technologies Mass Hunter Workstation Qualitative analysis software. Lipid content was also determined in all individual tissue samples (liver, gonad, muscle) to evaluate potential associations with PAH concentration. Lipids were measured following a modified Folch method (Matyash et al. 2008). Briefly, homogenized sample aliquots of 20 mg were mixed thoroughly with 1.5 mL of methanol (Optima LC/MS, Fisher Scientific, Fair Lawn, NJ, USA). Then five mL methyl-tert-butyl ether (MTBE, HPLC grade, Fisher Scientific, Fair Lawn, NJ, USA) was and added to the sample and placed on a pulsing vortex mixer (Fisherbrand, Fisher Scientific, Fair Lawn, NJ, USA) for one hour at room temperature. After one hour, 1.25 mL of MS-grade water (Optima LC/MS grade, Fisher Scientific, Fair Lawn, NJ, USA) was added and the sample was centrifuged to allow a clear separation of the organic phases. The organic phase was transferred to a pre-weighed glass vial and the remaining phase was re-extracted with two mL of MTBE: MeOH: H_2O (10:3:2.5 v/v/v) mixture. The top organic phase was transferred and combined with the previously-extracted organic phase and allowed to dry completely before re-weighing the vial containing the lipids. The following calculation was used to determine the lipid content in the tissue sampled:

$$\% \text{ Crude lipid} = \frac{\left(Vial{+}lipid\ wt(mg)\right) - \left(Empty\ vial\ wt\ (mg)\right)}{Sample\ Aliquot\ wt\ (mg)}\ x\ DF\ x\ 100$$

Quality assurance / Quality control (QA/QC) Plan. — The QA/QC plan consisted of method and solvent blanks, matrix spikes, analytical standards, and sample replicates. For biliary PAH analysis, a continuing calibration standard for each parent PAH (NAP: 2.5 ng/µL, PHN: 1.0

190 ng/μL, B[*a*]P: 250 pg/μL, Ultra Scientific, Kingstown, RI) was analyzed every six samples for
191 quantification and to ensure instrument stability in the HPLC- F system. Field samples were
192 analyzed in triplicate for biliary PAH equivalents with coefficient variations (CV) < 20%. Any
193 sample triplicates with a CV > 20% were reanalyzed until the QA/QC requirements were met.
194 Methanol solvent blanks were analyzed prior to field samples and areas were subtracted from the
195 area of field samples.

196 For tissue extractions, QA/QC procedures were adapted from the USEPA (e.g., EPA
197 Method 8270D) and NOAA (e.g., MC252 Analytical QAP, NOS ORCA 71). During the
198 QuEChERS extractions, tissue samples were spiked with the appropriate deuterated surrogate
199 and internal standards and analyzed alongside matrix matched standards (MMS), method blanks
200 and solvent blanks for each round. In addition, matrix spikes were used for each tissue (i.e., liver,
201 gonad, muscle) for species-specific optimization and to ensure method validation with recoveries
202 meeting quality control criteria (mean of 80 - 120%). Any samples that did not meet quality
203 control criteria were re-extracted and re-analyzed until criteria were met. The method detection
204 limit (MDL, 1 ng/g) was defined as the lowest matrix matched calibration standard where all
205 compounds were detectable.

206 *Data Analysis.* — For statistical purposes, any non-detects or concentrations <MDL (1.0
207 ng/g), were substituted with MDL/2 (0.5 ng/g). All statistical analyses were performed using
208 JMP 14 (SAS Institute) and all distributions were logarithmically transformed when necessary to
209 ensure normality. Any data failing the assumptions of normality were evaluated with non-
210 parametric tests and raw (non-transformed) data were used in these cases. To evaluate statistical
211 significance pairwise *post-hoc* comparisons were performed using the Steel-Dwass multiple
212 comparison method, and significance evaluated with Kruskal Wallis one-way non-parametric

analysis of variance. Non-parametric linear regressions were fit and tested with analysis of variance to assess total biliary PAH equivalents and are reported as the sum of naphthalene, phenanthrene and benzo[*a*] pyrene equivalents rounded to two significant figures as ng FAC g^{-1} bile. Tissue PAHs are reported to three significant figures as ng g^{-1} wet weight (w.w.) with an alpha value of $p < 0.05$ for statistical significant value.

To facilitate broader comparisons, biliary concentrations in fishes from this study were compared to results provided by two previous studies (Murawski et al. 2018; McDonald et al. 1996); (Figure 2). One study conducted by Murawski et al. 2018 collected fish via demersal longline sampling in a transect survey design conducted for another C-IMAGE project. Similar to my study, these C-IMAGE projects are part of a research consortium comprised of 17 different institutions from 5 countries, all studying the impacts of oil spills on the Gulf of Mexico. The McDonald et al. (1996) study is used due to reason that their research was conducted at two locations very close in proximity (< 3 miles) to those of this study.

RESULTS

Biometrics

For all 88 Red Snapper (35 female, 53 male), wet weight, liver weight, gonad weight, and lengths (total, fork, or standard) did not differ significantly by sex (Table 1). The average wet weight of Red Snapper for both sexes combined was significantly greater at the natural bank (2330 ± 79.5g) when compared to the standing rig (1790 ± 90.4g; $p < 0.001$) and a cutoff rig (1740 ± 147g; Z score= 3.08; $p = 0.018$). Average total length of Red Snapper for both sexes combined was also significantly greater at the natural bank (54.9 ± 0.70cm) when compared to

235 the standing rig (50.1 ± 0.80cm; $p < 0.001$) and a cutoff rig (49.5 ± 1.31cm; Z score = 2.97; $p =$
236 0.018).

237 Fulton's condition factor (K) was calculated to evaluate the robustness of fish as [K=
238 weight (g) / length (cm^3) x 100] to evaluate associations with PAH concentrations. In this study,
239 the mean K value was 1.34 ± 0.007 for females and 1.34 ± 0.005 for males, with an overall
240 min/max range of 1.15 to 1.52 (for both sexes), indicating relatively good health conditions (K >
241 1) for all fish. The hepatosomatic [HSI = liver weight (g) /total weight (g)] and gonadosomatic
242 [GSI = gonad weight (g) /total weight (g)] indices were calculated to determine the status of
243 energy reserve and reproductive condition. The mean HSI for females (0.63 ± 0.019) was
244 significantly higher than males (0.54 ± 0.016, $p < 0.001$) for all structure types combined. The
245 HSIs were also significantly higher for females ($p = 0.002$) at natural reefs compared to those
246 collected near artificial (standing, cutoff, and toppled rigs) habitats. In males, the mean HSI was
247 significantly higher in the natural ($p = 0.003$) and cutoff ($p < 0.001$) structure types as compared
248 to the standing structure. The mean GSI was similar between females (0.37 ± 0.067) and males
249 (0.51 ± 0.055, $p = 0.120$) for all structure types combined. In females, the GSI did not differ
250 significantly between any structure type ($p = 0.210$); however, in males the GSI was significantly
251 higher at the standing structure type compared to the cutoff ($p < 0.001$), natural ($p = 0.014$), and
252 topple ($p = 0.018$) structure types.

253 **Biliary PAH metabolite equivalents**

254 A total of 78 individual bile samples collected from 2016 Red Snapper located in the
255 northwestern region of the GoM were analyzed for the three PAH metabolite equivalents
256 (naphthalene (NAP), phenanthrene (PHN) and benzo[a]pyrene (B[*a*]P)) using HPLC-F (Table 2).
257 There were no significant differences ($p = 0.691$) observed in total biliary PAH equivalent

concentrations between males (180,000 ± 11,000 ng FAC/g bile) and females (190,000 ± 20,000 ng FAC/g bile) located at all sites, therefore male and female biliary PAH concentrations were combined. No significant differences were observed in the total biliary PAH equivalent concentrations (NAP, PHN, B[*a*]P) in Red Snapper across sites or structure type (cutoff, natural, standing, toppled; Table 2). The mean concentrations of biliary PAH equivalents in all fish were 160,000 (NAP), 26,000 (PHN) and 290 (B[*a*]P) ng FAC/g bile respectively. Biliary PAH metabolite equivalents were dominated by NAP (85.7%), followed by PHN (14.2%) and B[*a*]P (0.12%). Naphthalene was significantly higher than both PHN ($p < 0.001$) and B[*a*]P ($p < 0.001$), with PHN also being significantly higher than B[*a*]P ($p = 0.003$).

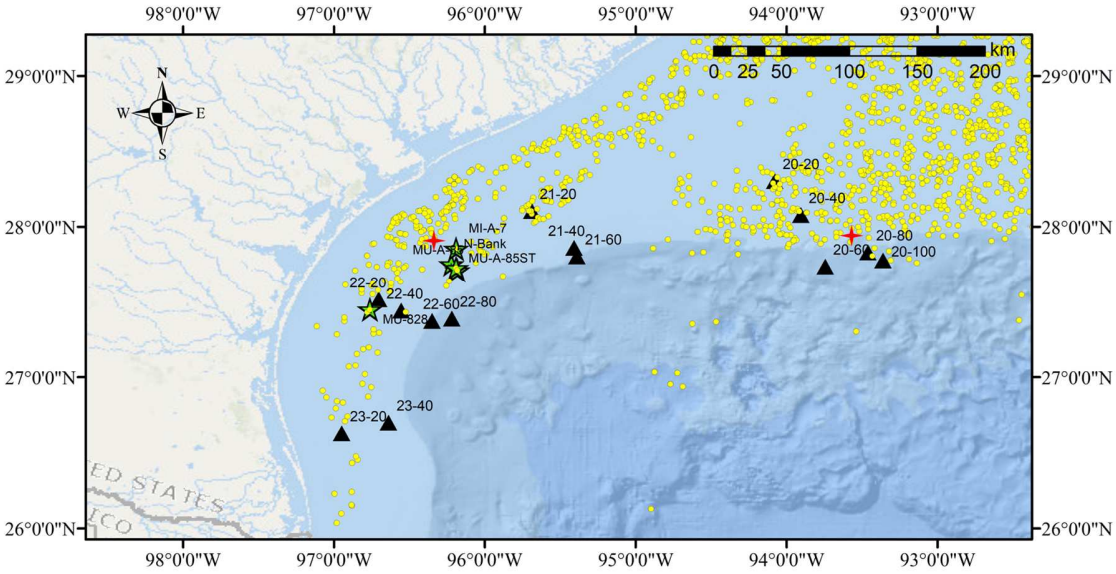

FIGURE 2: Fish sampling locations in 2016 via benthic longlining from this current project (stars), in 2016 via the C-IMAGE benthic longlining transects (triangles; (Murawski et al. 2018), in 1993 via semi-balloon otter trawling (diamonds; (McDonald et al. 1996) in the northwest Gulf of Mexico. Locations of oil/gas platforms (active/non-active) are plotted as yellow circles.

By comparing data to past records, it is possibleto address the changes over time similar to other studies that monitor PAH exposure in fishes overtime (Dearnley et al. 2020). The McDonald et al. (1996) study was conducted near the same platform locations used in this study but included various fish species other than Red Snapper. The 2016 C-IMAGE surveys also included biliary concentrations from a variety of benthic, pelagic and reef associated fish species (Pulster et al. 2020a), however only Red Snapper concentrations were used for comparison. The mean biliary concentrations collected from Red Snapper obtained in the 2016 C-IMAGE study (66,000 ± 8,000 ng FAC/g bile;(Pulster et al. 2020b) were significantly lower than both the McDonald et al. (1996) study (100,000 ± 14,000 ng FAC/g bile; $p < 0.001$) and this current study (190,000 ± 7,000 ng FAC/g bile; $p < 0.001$) (Figure 3). Mean biliary concentrations for this current study were also significantly higher than the McDonald et al. (1996) study ($p < 0.001$).

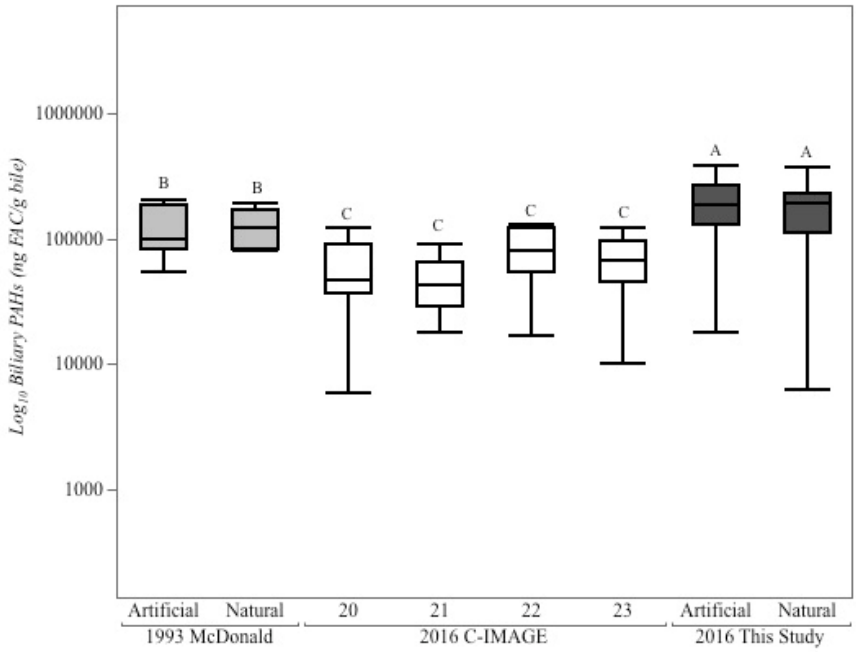

FIGURE 3: Comparison of biliary PAH equivalents (ng FAC/g bile) in samples collected in 1993 (McDonald et al. 1996), 2016 by C-IMAGE (transects 20 – 23) and 2016 samples in this study.

290 **Tissue PAH concentrations**

291 Tissue concentrations of PAHs are summarized by structure type in Table 2 and
292 individual PAH concentrations by structure and tissue type are provided in the supplementary
293 material (Tables S3 - 5). Lipid content and concentrations of 19 parent PAHs and their
294 associated alkylated-homolog compounds ($TPAH_{46}$) were measured in all tissue matrices (liver,
295 gonad, muscle) from 35 female and 53 male Red Snapper (Table 2). No significant differences
296 were found between sex for lipid content in any tissue type. Lipid content in livers (37.5% ±
297 2.70%) was similar to those in gonadal tissues (37.0% ± 4.59%) and both were significantly
298 higher than muscle (8.71% ± 0.65%, $p < 0.001$) for all structure types combined (Table 1).
299 No statistically significant relationships were observed between $TPAH_{46}$ and the lipid content in
300 liver ($r_s = -0.321$, $p = 0.018$), gonad ($r_s = 0.121$, $p = 0.268$), or muscles ($r_s = -0.174$, $p = 0.105$).
301 For all sites combined, the overall mean concentrations of PAHs in liver tissues for
302 females (505 ± 28.8 ng/g w.w.) and males (682 ± 42.3 ng/g w.w.) were both significantly higher
303 than the mean concentrations for female ($p < 0.001$) and male ($p < 0.001$) gonads and female (p
304 < 0.001) and male ($p < 0.001$) muscles. Female (142 ± 28.9 ng/g w.w.) and male (212 ± 41.5
305 ng/g w.w.) gonads were also significantly higher than the mean concentration of $TPAH_{46}$ for
306 both female muscles (18.5 ± 2.89 ng/g w.w., $p < 0.001$) and male muscles (19.3 ± 4.15 ng/g
307 w.w., $p < 0.001$). Within a particular site or structure type, there were no significant differences
308 between sexes for both liver and muscle $TPAH_{46}$ concentrations in Red Snapper (Table 2). In
309 Red Snapper collected at the cutoff structure, total PAH concentrations in male gonads were
310 significantly higher than females ($p < 0.001$), but no other differences were found across the
311 other structure types. Within a particular structure type, the $TPAH_{46}$ for muscles were
312 significantly lower than both the liver (ANOVA, $p < 0.001$) and gonadal tissues (ANOVA, $p <$

0.001) for both females and males at each structure type (Figure 4). Additonally, there were no significant differences in the muscle, liver, or gonad concentrations of PAHs between the natural and artificial habitats.

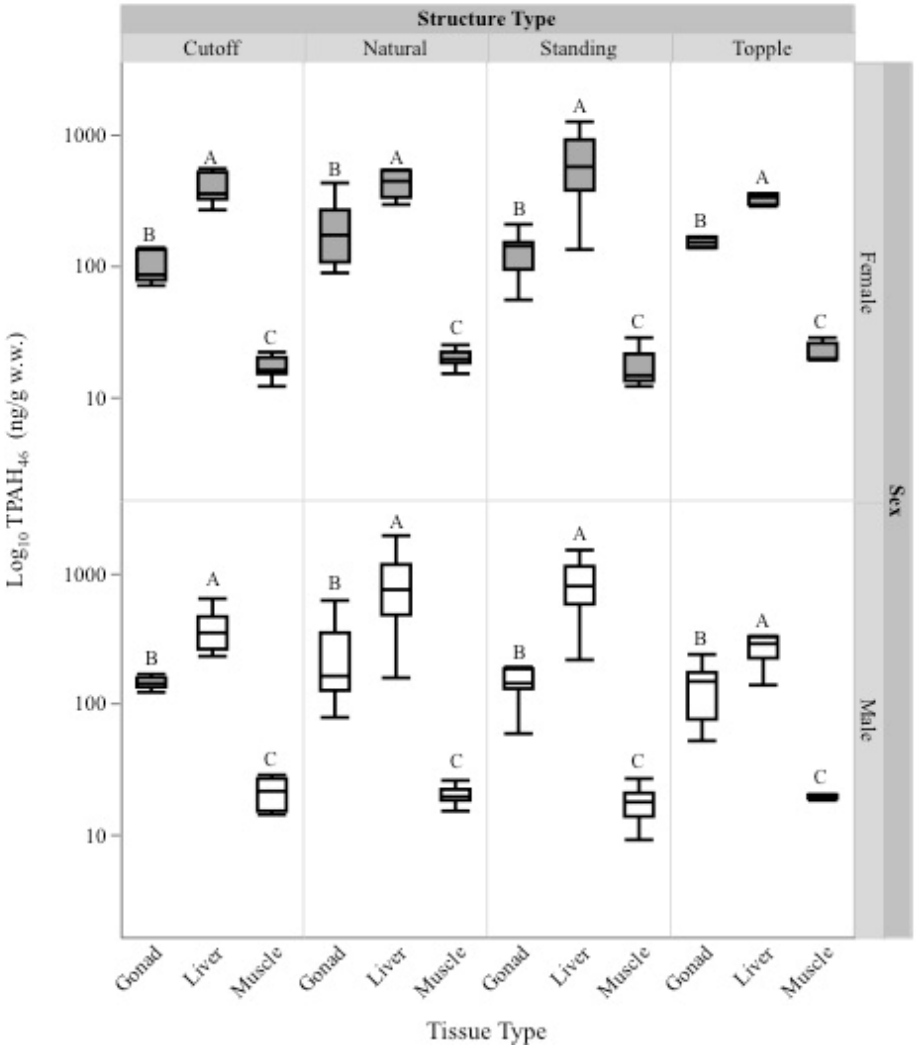

FIGURE 4: Total PAH concentrations ($Log_{10}TPAH_{46}$ ng/g w.w.) by sex and tissue matrix and structure type for Red Snapper collected in the GoM. For each sex and within a structure type, tissue matrices not connected by a letter are statistically different.

All Red Snapper samples (n = 88) were obtained and processed across six different sampling days throughout July through November 2016 (7/18, 7/29, 8/25, 8/26, 10/19, 11/15). Red Snapper collected on July 29th (n = 10) were significantly larger in total weight, gonad

weight, liver weight, and total length when compared to those caught on any other date. Although no significant results were found, there was an increase and decrease in gonadosomatic indices (GoSI) as well as mean gonadal PAH concentrations (TPAH) for both male and female Red Snapper over the study period (Figure 5).

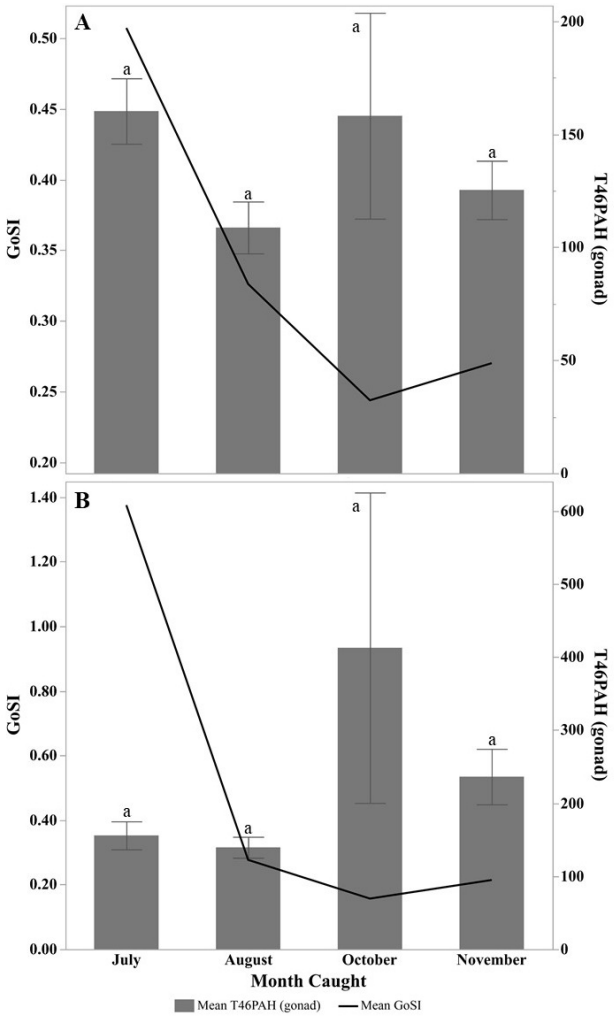

FIGURE 5: Female (A) and male (B) gonadosomatic indices (GSI) and mean gonadal PAH concentrations ($TPAH_{46}$ ng/g w.w.) by month caught. Gonadal PAH concentrations not connected by a letter are statistically different.

The two and three-ring low molecular weight (LMW) PAHs predominated the distribution in fish tissues accounting for 92 - 96% of total PAHs for all tissue types. Of the

335 PAHs, NAP and its associated homologues, was the highest contributing LMW, accounting for
336 43% in all tissue types across all structure types. The four and five-ring, high molecular weight
337 (HMW) PAHs accounted for only 4 - 8% of total PAH concentration in all tissue types.

338 **DISCUSSION**

339 The Red Snapper population in the GoM has been a crucial part of the fishery economy
340 in the GoM for many years (Warren 1898). Additional knowledge regarding their abundance and
341 health status of fish throughout the GoM can help identify whether artificial reefs are having
342 negative or positive impacts on fish survival. Building on previous work (Streich et al. 2017a;
343 Downey et al. 2018), this study adds critical information regarding PAH concentrations in Red
344 Snapper inhabiting natural and artificial reef systems in the northwestern region of the GoM.
345 This study is also the first in-depth study of red snapper specifically and supports biliary PAH
346 and temporal trends observed in other species found in the GoM including Golden tilefish
347 (Snyder et al. 2019; Pulster et al. 2021).

348 The Red Snapper samples (n = 88) were obtained and processed across six different
349 sampling days throughout July through November 2016. All Red Snapper caught in July (male
350 and female) were collected at the natural site eliminating site variability and preventing statistical
351 comparisons between habitat types. The higher GoSI and TPAH indices found in July are likely
352 due to the Red Snappers typical peak spawning month occurring in June - July; at which time
353 they typically have enlarged gonads in preparation for the release of millions of eggs (Collins et
354 al. 1996). Red Snapper collected in July had significantly higher mean (±SD) GSIs than any
355 other month, suggesting these fish were ready to spawn (Figure 5, Table 1). Additionally,
356 although not significant, between July and August there was a decrease in gonadal PAH
357 concentrations for both sexes (Figure 5). In female Red Snappers, this was followed by a 45%

358 increase in gonadal PAH concentrations by October (p = 0.91) and another decrease (21%, p =
359 0.99) by November. In male Red Snapper, there was a 197% increase in gonadal PAH
360 concentrations between August and October (p = 0.91) followed by a 43% decrease in November
361 (p = 0.99). This is an important finding in terms of PAH tissue desposition and offloading during
362 these months. These data suggest Red Snapper are offloading PAHs during spawning as
363 illustrated by the decrease in gonadal PAH concentrations between July and August and again
364 between October and November. However, other factors such as diet and feeding status may
365 influence these results.
366 The LMW compounds in general dominated the biliary and tissue concentrations of
367 PAHs in this study. This is not uncommon due to the abundance of these compounds throughout
368 the environment (Xu et al. 2011) and similar findings were also observed in other species
369 collected in the GoM (Snyder et al. 2015; Struch et al. 2019; Pulster et al. 2020a). Additionally,
370 LMW PAHs (i.e. NAP) are more commonly detected in tissues of exposure studies (Tuvikene
371 1995) as a result of uptake being largely driven by hydrophobicity. PAHs are more
372 hydrophobic as their molecular weight increases so in terms of naphthalene (least
373 hydrophobic) to benzo[g,h,i]pyrene (most hydrophobic), there is ~3.7 orders of magnitude
374 difference. This is why HMW PAHs (i.e. B[a]P) are associated with decreased uptake efficiency
375 due to their decreased bioavailability. NAP a petrogenic PAH is also much more bioavailable
376 and tends to be more imbequotous to the environment as compared to the pyrogenic B[a]P,
377 which is why we see the biliary concentrations in this study primary dominated by NAP.
378 Neither tissue nor biliary PAHs in Red Snapper differed significantly between natural and
379 artificial habitats in this study. The lack of significant differences detected between platforms
380 sampled in this study and parallel studies could be due to a number of reasons including 1) small

samples sizes; 2) annual variations;3) the relatively small geographical sampling range; 4) the density of oil platforms in this region; 5) fish movement and 6) the complex circulation patterns in this region. However, it is important to consider these findings in a wider spatial context in order to understand factors that may contribute to the lack of detectable differences between sites.

The amount of accumulation in aquatic environments depends highly upon the type of food chains, availability or persistence of a pollutant, and the physical-chemical properties of the pollutant (Spacie and Hamelink, 1985). Since bioaccumulation can only occur when the rate of uptake is higher than the rate of elimination, there would need to be an increased amount of pollutants in the surrounding environment that would burden the fishes ability to eliminate pollutants fast enough. With a fish's ability to move to different places freely, it can decrease it's level of exposure in an area. The sites in this study were all located within ~80 km of each other in the northwestern GoM embedded in the midst of a once high-density oil and gas region (Figure 2). The majority of the platforms in this region has since been decommissioned and is currently inactive (www.bsee.gov and www.boem.gov). The platforms sampled in this study were all installed during the late 1970's and early to mid-1980s and decommissioned ~20 years later, with the exception of standing platform, MU-A-85ST. This platform was installed in 1977 and is still one of the few remaining active platforms in this region.

The two cutoff platforms (MI-A-7 and MU-A-85), the standing (MU-A-85ST) and the natural bank sampled in this study are all clustered within 20 kilometers of each other whereas the toppled MU-828 platform is located approximately 80 km southwest. There are less than a dozen active platforms within 50 km northeast of the platform cluster and up to 100 kilometers

404 northeast of the MU-828 platform. There are no other active platforms within a 100 km radius of
405 any of the platforms sampled in this study, however, fish collected around these platforms still
406 had similar PAH levels as those collected near the actively producing platform (MU-A-85ST, to
407 the 80 km northeast). This suggests that their level of exposure to PAHs could also be
408 influenced by other factors including river discharge, runoff, and natural oil seeps. This
409 project takes place in a relatively small sampling area in the GoM, which could be the
410 reason for the lack of significant differences found amongst these sites with other oil and
411 PAH contaminants found influencing the surrounding area. Since there were no tags on
412 these Red Snapper, it is not known if the Red Snapper caught in this study were known to
413 reside in those specific areas or if they traveled to various distant locations. Their ability to
414 move to various locations could mean that these other oil rigs and natural seeps, outside of
415 the study areaare playing a significant role in the PAH concentrations. It is important to
416 consider the larger scale as an influential factor in this study as these other factors could be
417 influenced by oil dispersal through the many currents that flow through the Gulf of Mexico
418 basin.
419 Biliary PAHs in fish collected at the combined 2016 C-IMAGE transects 20, 21, 22, and
420 23 were significantly lower ($p < 0.001$) than all reef systems (natural and artificial) sampled in
421 both this current study and the McDonald et al. (1996) study (Figure 3). These transects were
422 located approximately 20 km (transects 21 and 22) to 200 km (transects 20 and 23) from the sites
423 sampled in the present study and locations sampled by McDonald et al. (1996). The significantly
424 lower concentrations observed in Red Snapper collected along the C-IMAGE transects could be
425 due to the complex circulation patterns in this region (Duran et al. 2018). Compared to C-
426 IMAGE transect 20, the platforms sampled in this study are located within a relatively inactive

oil region, however this area is particularly impacted by pollution based on the circulation patterns identified by Duran et al. (2018).

The density of oil infrastructure in the Northern GoM surrounding the natural and artificial reef systems should not be discounted as a potential source of PAH exposures compared to areas without any oil and gas infrastructure (e.g., the West Florida Shelf, WFS). Significant spatial gradients in biliary PAH exposures were identified when comparing locations within an area of high-density oil infrastructure to sites without any infrastructure. For example, the 2011 through 2015 mean biliary PAHs in Red Snapper collected along the WFS (32,000 ± 25,000 ng FAC/g bile) were significantly lower than in Red Snapper collected within the high density fields of oil and gas activity in the north central region (82,000 ± 65,000 ng FAC/g, $p < 0.001$) surrounding the Mississippi Delta during the same time period (Pulster et al. 2020b).

CONCLUSION

These data suggest that the scale at which the sites used in this study and previous studies were too small to detect spatial gradients. Future research should carefully consider scale and expand the site selection to include areas at least 250 km outside of the density-rich oil and gas regions of the northern GoM. This will be difficult to achieve as the oil industry expands into deeper and deeper waters and into the eastern boundary. Currently, there are no known areas in the Gulf of Mexico that have not been impacted by oil to some degree (Pulster et al. 2020a). This study is crucial to understanding the multi-scale impacts of oil and gas infrastructure in the Gulf of Mexico. In this study, PAHs were detected in all Red Snapper samples, located at both natural and artificial habitats. However, data revealed significantly higher biliary PAHs in Red Snapper collected within a high-density oil and gas region (i.e. northern Gulf of Mexico) when compared to a location without any known oil and gas activity (i.e. the West Florida Shelf). Additionally,

450 levels measured in this study were higher than those measured in fish collected over 20 years
451 previous from the same sites (McDonald et al. 1996). This indicates Red Snapper chronic
452 exposure to PAHs for at least two decades by pollution transport via circulation patterns, land-
453 based sources, natural seeps, or a combination of sources. This research not only demonstrates
454 the chronic pollution problem in the Gulf but also the scale at which samples are being collected
455 is critical to understanding the impacts of oil and gas activities and underscores the need for
456 further research on a larger scale in the Gulf of Mexico.

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464 **AUTHOR CONTRIBUTIONS**

465 TN, EP, SM, HJ- project conceptualization, TN- data collection and analysis, original draft; EP-
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467 **REFERENCES**

468 Anastassiades, M., K. Maštovská, and S. J. Lehotay. 2003. Evaluation of analyte protectants to
469 improve gas chromatographic analysis of pesticides. Journal of Chromatography A
470 1015(1):163-184.
471 Bradley, E., and C. Bryan. 1975. Life history and fishery of the Red Snapper (Lutjanus
472 campechanus) in the northwestern Gulf of Mexico 1970-1974.
473 Collier, T. K., B. F. Anulacion, M. R. Arkoosh, J. P. Dietrich, J. P. Incardona, L. L. Johnson, G.
474 M. Ylitalo, and M. S. Myers. 2013. Effects on fish of polycyclic aromatic hydrocarbons
475 (PAHs) and naphthenic acid exposures. Pages 195-255 *in* Fish physiology, volume 33.
476 Elsevier.
477 Collins, L., A. Johnson, and C. Keim. 1996. Spawning and annual fecundity of the red snapper
478 (Lutjanus campechanus) from the northeastern Gulf of Mexico. Pages 174-188 *in*

Biology, fisheries and culture of tropical groupers and snappers. ICLARM Conference Proceedings.
Dearnley, J. M., C. Killeen, R. L. Davis, V. P. Palace, and G. T. Tomy. 2020. Monitoring polycyclic aromatic compounds exposure in fish using biliary metabolites. Critical Reviews in Environmental Science and Technology:1-45.
Downey, C. H., M. K. Streich, R. A. Brewton, M. J. Ajemian, J. J. Wetz, and G. W. Stunz. 2018. Habitat‐specific reproductive potential of red snapper: a comparison of artificial and natural reefs in the western Gulf of Mexico. Transactions of the American Fisheries Society 147(6):1030-1041.
Duran, R., F. J. Beron-Vera, and M. J. Olascoaga. 2018. Extracting quasi-steady Lagrangian transport patterns from the ocean circulation: An application to the Gulf of Mexico. Scientific reports 8(1):1-10.
Gallaway, B. J., S. T. Szedlmayer, and W. J. Gazey. 2009. A Life History Review for Red Snapper in the Gulf of Mexico with an Evaluation of the Importance of Offshore Petroleum Platforms and Other Artificial Reefs. Reviews in Fisheries Science 17(1):48-67.
Glenn, H. D., J. H. Cowan Jr, and J. E. Powers. 2017. A comparison of red snapper reproductive potential in the northwestern Gulf of Mexico: natural versus artificial habitats. Marine and Coastal Fisheries 9(1):139-148.
Heintz, R. A., S. D. Rice, A. C. Wertheimer, R. F. Bradshaw, F. P. Thrower, J. E. Joyce, and J. W. Short. 2000. Delayed effects on growth and marine survival of pink salmon Oncorhynchus gorbuscha after exposure to crude oil during embryonic development. Marine Ecology Progress Series 208:205-216.
Jourdain de Muizon, M., L. d'Hendecourt, and T. Geballe. 1990. Polycyclic aromatic hydrocarbons in the near-infrared spectra of 24 IRAS sources. Astronomy and Astrophysics 227:526.
Kaiser, M. J., and A. G. Pulsipher. 2005. Rigs-to-reef programs in the Gulf of Mexico. Ocean Development and International Law 36(2):119-134.
Karnauskas, M., J. F. Walter, M. D. Campbell, A. G. Pollack, J. M. Drymon, and S. Powers. 2017. Red Snapper Distribution on Natural Habitats and Artificial Structures in the Northern Gulf of Mexico. Marine and Coastal Fisheries 9(1):50-67.
Krahn, M. M., M. S. Myers, D. Burrows, and D. C. Malins. 1984. Determination of metabolites of xenobiotics in the bile of fish from polluted waterways. Xenobiotica 14(8):633-646.
Maes, F. 2004. National Research Council, Oil in the Sea III. Inputs, Fates and Effects, Washington, The National Academies Press, 2003, 265 p. International Journal of Environment and Pollution 22:743-744.
Maštovská, K., S. J. Lehotay, and M. Anastassiades. 2005. Combination of Analyte Protectants To Overcome Matrix Effects in Routine GC Analysis of Pesticide Residues in Food Matrixes. Analytical Chemistry 77(24):8129-8137.
Matyash, V., G. Liebisch, T. V. Kurzchalia, A. Shevchenko, and D. Schwudke. 2008. Lipid extraction by methyl-tert-butyl ether for high-throughput lipidomics. Journal of lipid research 49(5):1137-1146.
McDonald, S. J., K. L. Willett, J. Thomsen, K. B. Beatty, K. Connor, T. R. Narasimhan, C. M. Erickson, and S. H. Safe. 1996. Sublethal detoxification responses to contaminant exposure associated with offshore production platforms. Canadian Journal of Fisheries and Aquatic Sciences 53(11):2606-2617.

525 Murawski, S. A., W. T. Hogarth, E. B. Peebles, and L. Barbeiri. 2014. Prevalence of External
526 Skin Lesions and Polycyclic Aromatic Hydrocarbon Concentrations in Gulf of Mexico
527 Fishes, Post-Deepwater Horizon. Transactions of the American Fisheries Society
528 143(4):1084-1097.
529 Murawski, S. A., E. B. Peebles, A. Gracia, J. W. Tunnell Jr, and M. Armenteros. 2018.
530 Comparative abundance, species composition, and demographics of continental shelf fish
531 assemblages throughout the Gulf of Mexico. Marine and Coastal Fisheries 10(3):325-
532 346.
533 Myers, M. S., C. M. Stehr, O. P. Olson, L. L. Johnson, B. B. McCain, S.-L. Chan, and U.
534 Varanasi. 1994. Relationships between toxicopathic hepatic lesions and exposure to
535 chemical contaminants in English sole (Pleuronectes vetulus), starry flounder (Platichthys
536 stellatus), and white croaker (Genyonemus lineatus) from selected marine sites on the
537 Pacific Coast, USA. Environmental Health Perspectives 102(2):200-215.
538 Peterson, C. H., M. C. Kennicutt, R. H. Green, P. Montagna, D. E. Harper, E. N. Powell, and P.
539 F. Roscigno. 1996. Ecological consequences of environmental perturbations associated
540 with offshore hydrocarbon production: A perspective on long-term exposures in the Gulf
541 of Mexico. Canadian Journal of Fisheries and Aquatic Sciences 53(11):2637-2654.
542 Pulster, E. L., S. Fogelson, B. E. Carr, J. Mrowicki, and S. A. Murawski. 2021. Hepatobiliary
543 PAHs and prevalence of pathological changes in Red Snapper. Aquatic Toxicology
544 230:105714.
545 Pulster, E. L., A. Gracia, M. Armenteros, G. Toro-Farmer, S. M. Snyder, B. E. Carr, M. R.
546 Schwaab, T. J. Nicholson, J. Mrowicki, and S. A. Murawski. 2020a. A first
547 comprehensive Baseline of Hydrocarbon pollution in Gulf of Mexico fishes. Scientific
548 reports 10(1):1-14.
549 Pulster, E. L., A. Gracia, S. M. Snyder, I. C. Romero, B. Carr, G. Toro-Farmer, and S. A.
550 Murawski. 2020b. Polycyclic aromatic hydrocarbon baselines in Gulf of Mexico fishes.
551 Pages 253-271 *in* Scenarios and Responses to Future Deep Oil Spills. Springer.
552 Reggio, V. 1987. Rigs-to-Reefs: The use of obsolete petroleum structures as artificial reefs. US
553 Department of the Interior, Minerals Management Service, Gulf of Mexico ….
554 Reynolds, E. M., J. H. Cowan, K. A. Lewis, and K. A. Simonsen. 2018. Method for estimating
555 relative abundance and species composition around oil and gas platforms in the northern
556 Gulf of Mexico, USA. Fisheries Research 201:44-55.
557 Rezak, R., S. R. Gittings, and T. J. Bright. 1990. Biotic assemblages and ecological controls on
558 reefs and banks of the northwest Gulf of Mexico. American Zoologist 30(1):23-35.
559 Rusco, F. 2017. Offshore Oil and Gas Resources: Information on Infrastructure
560 Decommissioning and Federal Financial Risk.
561 Sammarco, P. W., A. Lirette, Y. F. Tung, G. S. Boland, M. Genazzio, and J. Sinclair. 2014.
562 Coral communities on artificial reefs in the Gulf of Mexico: standing vs. toppled oil
563 platforms. Ices Journal of Marine Science 71(2):417-426.
564 Shinn, E. A. 1974. Oil structures as artificial reefs. Pages 91-96 *in* Proceedings of an
565 international conference on artificial reefs. Texas A&M University USA.
566 Snyder, S. M., E. L. Pulster, and S. A. Murawski. 2019. Associations between chronic exposure
567 to polycyclic aromatic hydrocarbons and health indices in Gulf of Mexico tilefish
568 (Lopholatilus chamaeleonticeps) post deepwater horizon. Environmental toxicology and
569 chemistry 38(12):2659-2671.

570 Snyder, S. M., E. L. Pulster, D. L. Wetzel, and S. A. Murawski. 2015. PAH exposure in Gulf of
571 Mexico demersal fishes, post-Deepwater Horizon. Environmental science & technology
572 49(14):8786-8795.
573 Streich, M. K., M. J. Ajemian, J. J. Wetz, J. D. Shively, J. B. Shipley, and G. W. Stunz. 2017a.
574 Effects of a new artificial reef complex on red snapper and the associated fish
575 community: an evaluation using a before–after control–impact approach. Marine and
576 Coastal Fisheries 9(1):404-418.
577 Streich, M. K., M. J. Ajemian, J. J. Wetz, J. A. Williams, J. B. Shipley, and G. W. Stunz. 2017b.
578 A comparison of size structure, age, and growth of Red Snapper from artificial and
579 natural habitats in the western Gulf of Mexico. Transactions of the American Fisheries
580 Society 146(4):762-777.
581 Struch, R. E., E. L. Pulster, A. D. Schreier, and S. A. Murawski. 2019. Hepatobiliary analyses
582 suggest chronic PAH exposure in hakes (Urophycis spp.) following the Deepwater
583 Horizon oil spill. Environmental toxicology and chemistry 38(12):2740-2749.
584 Tuvikene, A. 1995. Responses of fish to polycyclic aromatic hydrocarbons (PAHs). Pages 295-
585 309 *in* Annales Zoologici Fennici. JSTOR.
586 Warren, A. F. 1898. The red snapper fisheries: their past, present and future. Bulletin of the
587 United States Fish Commission 17:331-335.
588 Wells, R. J. D., J. H. Cowan, and B. Fry. 2008. Feeding ecology of red snapper Lutjanus
589 campechanus in the northern Gulf of Mexico. Marine Ecology Progress Series 361:213-
590 225.
591 Wilson, C. A., and D. L. Nieland. 2001. Age and growth of red snapper, Lutjanus campechanus,
592 from the northern Gulf of Mexico off Louisiana. Fishery Bulletin 99(4):653-665.
593 Xu, F. L., W. J. Wu, J. J. Wang, N. Qin, Y. Wang, Q. S. He, W. He, and S. Tao. 2011. Residual
594 levels and health risk of polycyclic aromatic hydrocarbons in freshwater fishes from Lake
595 Small Bai-Yang-Dian, Northern China. Ecological Modelling 222(2):275-286.
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598 TABLE 1: Mean biometrics (± standard deviation) and sample sizes (*n*) for male (M) and female (F) Red Snapper collected in 2016
599 from the northwest Gulf of Mexico used in this study.

Structure Type	Sex	*n*	Total length (cm)	Total wet weight (g)	Liver weight (g)	Gonad weight (g)	% Liver lipid content (g)	% Gonad lipid content (g)	% Muscle lipid content (g)
Cutoff 1(MI-A-7)	F	6	52.9 ± 1.62	2020 ± 160	10.3 ± 1.10	6.80 ± 1.00	24.0 ± 7.73	21.0 ± 10.4	4.18 ± 1.93
	M	7	53.6 ± 1.43	2120 ± 165	10.8 ± 1.65	2.05 ± 5.30	18.7 ± 4.83	26.6 ± 12.4	7.71 ± 1.42
Cutoff 1 mean (sexes combined)		13	53.3 ± 1.09	2080 ± 122	10.5 ± 1.06	4.24 ± 3.13	21.2 ± 2.44	23.5 ± 2.66	6.07 ± 2.44
Cutoff 2 (MU-A-85)	F	4	52.6 ± 1.91	2120 ± 200	14.1 ± 1.46	8.40 ± 1.20	39.0 ± 9.47	21.5 ± 12.7	12.2 ± 2.30
	M	5	47.0 ± 1.61	1440 ± 196	9.10 ± 1.97	4.60 ± 6.30	41.8 ± 5.71	48.8 ± 14.3	7.20 ± 1.72
Cutoff 2 mean (sexes combined)		9	49.5 ± 1.31	1740 ± 147	11.4 ± 1.27	6.36 ± 3.77	40.6 ± 6.12	36.6 ± 6.10	9.44 ± 6.10
Standing (MU-A-85ST)	F	10	51.6 ± 1.22	1930 ± 130	10.6 ± 0.83	7.20 ± 0.80	46.5 ± 5.99	37.0 ± 8.06	8.10 ± 1.29
	M	14	49.0 ± 0.92	1690 ± 117	8.10 ± 1.21	6.90 ± 3.79	37.6 ± 3.41	42.6 ± 8.54	9.28 ± 1.02
Standing mean (sexes combined)		24	50.1 ± 0.80	1790 ± 90.4	9.16 ± 0.78	7.03 ± 2.31	41.3 ± 4.33	40.3 ± 4.34	8.79 ± 4.32
Toppled (MU-828)	F	4	51.3 ± 1.93	1850 ± 200	9.60 ± 1.42	5.40 ± 1.25	61.0 ± 9.47	36.2 ± 12.7	7.00 ± 2.37
	M	7	54.4 ± 1.42	2220 ± 165	11.1 ± 1.63	8.20 ± 5.37	35.4 ± 4.81	31.8 ± 12.1	8.28 ± 1.45
Toppled mean (sexes combined)		11	53.3 ± 1.18	2090 ± 133	10.6 ± 1.15	7.19 ± 3.41	44.7 ± 6.22	33.4 ± 6.22	7.82 ± 6.23
Natural Bank (NBAK)	F	11	51.2 ± 1.21	1900 ± 120	14.1 ± 0.84	7.30 ± 0.74	36.3 ± 5.71	35.7 ± 7.69	9.82 ± 1.42
	M	20	57.0 ± 0.85	2570 ± 98	16.2 ± 0.90	22.8 ± 3.26	31.1 ± 2.85	62.4 ± 7.14	10.3 ± 0.86
Natural mean (sexes combined)		31	54.9 ± 0.70	2330 ± 79.5	15.5 ± 0.69	17.3 ± 2.03	32.9 ± 3.86	52.9 ± 3.68	10.2 ± 3.86
Mean by Sex	F	35	51.8 ± 1.25	1950 ± 690	11.9 ± 5.10	7.10 ± 4.23	40.2 ± 3.21	31.9 ± 5.11	8.31 ± 0.73
	M	53	53.2 ± 0.99	2120 ± 860	12.0 ± 8.25	12.2 ± 5.66	34.7 ± 2.31	42.0 ± 4.23	9.12 ± 0.61
Mean (all data)		88	52.5 ± 0.85	2040 ± 530	12.0 ± 7.23	9.65 ± 4.96	37.5 ± 2.70	37.0 ± 4.59	8.71 ± 0.65

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601 TABLE 2. The 2016 mean (± standard deviation) biliary PAH equivalents (PAHeq, ng FAC/g
602 bile) and tissue (ng/g w.w.) PAH concentrations for male (M) and female (F) Red Snapper by
603 structure type.
604

Structure Type (Structure ID)	Sex	*n*	Biliary PAHeq	Liver	Gonad	Muscle
Cutoff 1 (MI-A-7)	F	6	200,000 ± 46,000	412 ± 114	81.6 ± 27.1	17.2 ± 2.23
	M	7	200,000 ± 30,000	341 ± 159	137 ± 95.2	24.4 ± 1.69
Cutoff 1 mean (sexes combined)		13	200,000 ± 36,000	374 ± 105	106 ± 50.9	21.1 ± 1.38
Cutoff 2 (MU-A-85)	F	4	160,000 ± 56,000	379 ± 140	119 ± 32.3	20.0 ± 2.73
	M	5	230,000 ± 36,000	479 ± 189	146 ± 95.6	16.6 ± 2.01
Cutoff 2 mean (sexes combined)		9	20,000 ± 46,000	434 ± 126	134 ± 56.3	18.1 ± 1.65
Standing (MU-A-85ST)	F	10	180,000 ± 40,000	643 ± 88.5	129 ± 21.1	17.4 ± 1.72
	M	14	170,000 ± 22,000	895 ± 113	189 ± 56.4	17.4 ± 1.96
Standing mean (sexes combined)		24	180,000 ± 33,000	790 ± 77.6	164 ± 34.4	17.4 ± 1.01
Toppled (MU-828)	F	4	280,000 ± 65,000	328 ± 140	149 ± 33.3	21.5 ± 2.733
	M	7	140,000 ± 32,000	292 ± 160	141 ± 80.3	20.4 ± 1.69
Toppled mean (sexes combined)		11	210,000 ± 45,000	305 ± 114	144 ± 50.9	20.7 ± 1.50
Natural Bank (NBAK)	F	11	180,000 ± 38,000	542 ± 84.2	192 ± 20.2	18.8 ± 1.64
	M	20	180,000 ± 19,000	841 ± 94.6	287 ± 47.6	19.1 ± 1.00
Natural mean (sexes combined)		31	180,000 ± 23,000	735 ± 68.3	253 ± 30.3	19.0 ± 0.89
Mean by Sex	F	35	190,000 ± 20,000	505 ± 28.8	142 ± 28.9	18.5 ± 2.89
	M	53	180,000 ± 11,000	682 ± 42.3	212 ± 41.5	19.3 ± 4.15
Mean (all data combined)		88	190,000 ± 15,000	593 ± 35.6	177 ± 37.2	18.9 ± 3.15

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