Polycyclic Aromatic Hydrocarbons in Red Snapper, *Lutjanus campechanus*, and Sediment Samples After the Deepwater Horizon Oil Spill

by

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Abstract

The Deepwater Horizon oil spill occurred on 20 April 2010, which led to approximately 4.9 million barrels of oil entering the northern Gulf of Mexico. Red snapper, *Lutjanus campechanus*, are an important species in the Gulf of Mexico, and were potentially exposed to polycyclic aromatic hydrocarbons (PAHs) from this oil spill. To assess this potential PAH exposure, red snapper tissue samples were collected from 2010 to 2014, and analyzed for several PAHs. All red snapper tissue samples showed mean total PAH concentrations < 10 ppb. Significant differences were observed in total (± SD) PAH by year, with muscle tissue in 2011 having the highest concentration (5.4 ± 2.5 ppb) among all samples types and years. Sediment samples were also analyzed for PAHs and these showed no evidence of contamination above background levels. Several condition indices were used to determine if any physiological changes occurred in red snapper condition, gonadal tissue, or liver tissue following the oil spill. Significant differences were observed in gonadosomatic index and Fulton's K (condition factor); however these were not attributed to the oil spill and more likely normal yearly variations due to temperature and nutrition status. Lesions were observed in 10 of 3,934 (0.25%) red snapper collected during this study. These levels of lesions were at similar levels to non-oil exposed fishes. Based on low levels of PAH in red snapper and sediment samples, and low rate of external lesions it is unlikely that adult red snapper in the

northern Gulf of Mexico on the Alabama-Mississippi continental shelf were affected by the Deepwater Horizon oil spill.

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INTRODUCTION

On 20 April 2010 in the northern Gulf of Mexico, the Deepwater Horizon (DWH) exploratory well exploded, caught fire, and eventually sank in 1500 m of water. This well is located in Mississippi Canyon Block 252, approximately 77 km southeast of Louisiana (Atlas and Hazen, 2011). The well leaked for 84 days before the flow was stopped. Approximately 4.9 million barrels of oil entered the environment, making it the largest spill in the Gulf of Mexico, and the second largest in the world (Atlas and Hazen, 2011). After cleanup efforts, natural dispersion, and degradation, approximately 26% of the oil remains in the environment (The Federal Interagency Solutions Group: Oil Budget Calculator Science and Engineering Team, 2010).

In the Gulf of Mexico (GOM), there are several ways in which petroleum can enter the aquatic environment. Human input includes platform leaks, tanker spills, industrial runoff, and river runoff, all of which contribute polycyclic aromatic hydrocarbons (PAHs) to the GOM. The GOM also has natural seeps, which contribute about 140,000 tons of petroleum per year, resulting in oil exposure as a natural component of the environment (National Research Council, 2003). This natural seepage has allowed microorganisms such as bacteria, fungi, and archaea to adapt and utilize petroleum hydrocarbons as sources of energy, leading to hydrocarbon degradation (Atlas and Hazen, 2011). Fortunately, these bacteria have assisted in cleanup efforts, thus decreasing the amount of oil available to negatively affect other organisms. However, even in areas that can be considered pristine and oil free, such as in Low Island and Dallmann Bay, Antarctica, evidence of PAH contamination exists (McDonald et al., 1992). From these locations in Antarctica, McDonald et al. (1992) reported 13-145 ppb

(dry weight) total PAH (tPAH) concentrations in pooled fish tissues. Similarly, Johnson et al. (1985) reported 18 ppb tPAH concentrations in sediments from an undeveloped area in Alaska.

Petroleum from the DWH spill was "light Louisiana crude", and composed of 3.9% PAHs, by weight, with a total of 2.1 x 10^{10}g being released during the spill (Reddy et al., 2012). The remainder of the petroleum was composed of various sulfur, oxygen, and nitrogen-containing organic compounds (Speers and Whitehead, 1969). Polycyclic aromatic hydrocarbons are toxic to marine life, and previous studies have focused on these effects (Boehm and Page, 2007). Lighter and medium molecular weight hydrocarbons tend to remain at the surface and volatilize or degrade over time. In contrast, heavier molecular weight hydrocarbons sink into the sediment (Wolfe et al., 1994; Ho et al., 1999; Reed et al., 1999) where they can remain in anoxic environments for years, retaining their toxic properties (Sammarco et al., 2013).

Polycyclic aromatic hydrocarbons are composed of carbon and hydrogen atoms arranged in two or more benzene rings (Sims and Overcash, 1983; Eisler, 1987). These ring structures can vary greatly in size, number of carbon and hydrogen atoms, and presence or absence of substituted groups (Eisler, 1987). Polycyclic aromatic hydrocarbons are solid, but partially soluble in water, and have the ability to quickly absorb into organic and inorganic particulates (Tuvikene, 1995). The highest concentrations of PAHs are typically found in sediments, with aquatic biota carrying medium concentrations, and lowest concentrations in the water column (Tuvikene, 1995). Some of these compounds are toxic, mutagenic and/or carcinogenic to aquatic organisms

(Rose et al., 2012). The PAHs of primary concern are those with molecular weights from 128.16 g (naphthalene) to 300.36 g (coronene; Eisler, 1987).

From an environmental standpoint, one of the largest concerns related to oil spills is how the components of oil will affect aquatic organisms. Oil can affect marine life in a variety of ways, the most apparent being the detrimental effects of contact with viscous crude oil from surface slicks (Allan et al., 2012). Another more chronic risk is the persistence of PAHs in the environment, because they can easily diffuse across cell membranes to affect organisms (Neff, 1985).

One commonly studied group of organisms after oil spills are fishes, particularly economically important species. After the Exxon Valdez spill, studies examined oil effects on survival, growth, feeding, and abundance of herring and salmon (Brown et al., 1996; Carls et al., 1996a; Carls et al., 1996b; Wertheimer and Celewycz, 1996). Fishes can also be used to assess potential bioaccumulation of PAHs in chronically polluted waters (Rose et al., 2012) and to monitor pollution levels in coastal areas (Escartin and Porte, 1999).

Fish can be exposed to PAHs in several ways; these include contact with contaminated water or sediments and contaminated food resources. Once exposed, PAHs can easily diffuse across cell membranes due to their lipophilic nature. Upon entering a cell, PAHs bind to receptor proteins, causing cytochrome P4501A induction (CYP1A). Cytochrome P4501A induction catalyzes phase I enzymes, which introduce a polar group to xenobiotic (pollutant) molecules. Next, phase II enzymes produce water-soluble conjugates from the phase I metabolites, these conjugates can be easily excreted by fish

(Tuvikene, 1995). This process allows fish to expel hydrocarbons two to three days after exposure (Pointet and Milliet, 2000).

Numerous exogenous and endogenous factors can influence the rates of uptake, metabolism, and excretion of PAHs in aquatic organisms (McElroy et al., 1989). These factors include water temperature, sex, life stage, salinity, species, and length and concentration of exposure (Neff, 1985). Differences in metabolic rates among different species are most influenced by percent lipid content in tissues (Varanasi et al., 1989). Decreased water temperature increases bioaccumulation of PAHs (Varanasi et al., 1981) and increased salinity increases fish sensitivity to PAHs (Levitan and Taylor, 1979). It is also generally accepted that egg and larval life stages are most vulnerable to potential oil spill effects and rates of metabolism are shown to vary by life stage (Rosenthal and Alderdice, 1976). Solbakken et al. (1984) showed that cod eggs could not eliminate benzo(a)pyrene, whereas larvae actively expelled it. This is likely due to an increase in the activity of xenobiotic metabolizing enzymes in larval fish compared to the egg stage (Solbakken et al., 1984). Another route of exposure for eggs is via the transfer of PAHs from parental fish to developing gametes (Varanasi et al., 1989). This is important to consider for oil spills occurring during spawning season, such as the DWH, which occurred during the red snapper, *Lutjanus campechanus*, spawning season. The juvenile stages from such spawning may be affected from parental transfer and from exposure to water-borne PAHs. Gender can also influence PAH metabolism, with males showing higher mixed-function oxygenase (MFO) activity than females; additionally testosterone induces CYP1A activity and estrogen inhibits activity (Stegeman and Chevion, 1980; Vodicnik and Lech, 1983). These two factors lead to greater bioaccumulation of PAHs in

female than male fish, particularly during spawning season when estrogen and testosterone release are high.

Polycyclic aromatic hydrocarbons are lethal to aquatic organisms at concentrations of 200-10,000 ppb, however sublethal effects can be seen at concentrations as low as 5 ppb in fishes (Neff, 1985). Sublethal effects include immune suppression (Payne and Fancey, 1989), decreased reproductive potential (Adams et al., 1989), reduced juvenile growth (Heintz et al., 2000), and liver lesions (Myers et al., 1992). The toxicity of PAHs is not from the parent PAHs, but from their metabolites (Tuvikene, 1995). These metabolites often interfere with cell membrane functions and associated enzyme systems (Neff, 1985). Residues of PAHs in fish tissues can also pose health problems for human consumption (Varanasi et al., 1989).

An important aspect of oil spill research is determination of the oil source. This is particularly true in the Gulf of Mexico where there are a variety of sources of potential PAH contamination. However, identifying the oil source is complicated due to the oil weathering over time, which alters the proportion of components in the original oil. Several studies have suggested the use of PAH ratios to identify if the contaminants come from petrogenic (derived from petroleum) or pyrogenic (derived from combustion) sources. Ke et al. (2002) used ratios of phenanthrene to anthracene (PHEN:ANTH) and fluoranthene to pyrene (FLUO:PYRE) to determine the origin of PAHs shown in sediment samples and mangrove leaves following an oil spill. Baumard et al. (1998) suggested that PHEN:ANTH > 8 and FLUO:PYRE < 1 indicated petrogenic sources, and PHEN:ANTH < 8 and FLUO:PYRE > 1 indicated pyrogenic sources. Fitzgerald and Gohlke (2014) used ANTH/ANTH+PHEN and FLUO/FLUO+PYRE ratios from fish

tissue samples to compare the proportion of petrogenic to pyrogenic hydrocarbon sources following the Deepwater Horizon oil spill. They showed that the ratios detected in four samples suggested primarily pyrogenic contamination and six samples suggested petrogenic contamination

Condition indices have also been used to monitor PAH pollution effects. In contrast to most PAH analysis techniques, condition indices are rapid and have lower cost, which makes them beneficial as initial screening biomarkers or useful when funding is limited. Commonly used indices include gonadosomatic index (GSI), hepatosomatic index (HSI), and condition factor (K). The hepatosomatic index is a ratio of liver weight to body weight, and is often used because the liver is responsible for biotransformation and elimination of xenobiotics (Pointet and Milliet, 2000). Montenegro and González (2012) showed higher HSI in *Labrisomus philippii* (chalapo clinid) from a site with high anthropogenic activity, than from a site with low anthropogenic activity. Condition factor, a measurement of body weight to length, showed significantly higher values at control sites than sites influenced by heavy anthropogenic discharge in fish from the Paraiba do Sul watershed (Linde-Arias et al., 2008). The GSI index is a ratio of gonad weight to body weight, and has also been affected by chronic exposure to pollutants that caused gonadic deterioration and reduced GSI values (Linderoth et al., 2006; Marchand et al., 2008; Louiz et al., 2009).

In the northern Gulf of Mexico, red snapper are one of the most commercially and recreationally important fishes, valued at $60 million per year (Gallaway et al., 2009). Due to their economic importance, the stock has been fished extensively in the past and has been considered overfished (SEDAR 31, 2013). There has been extensive study of

this species to help management rebuild the stock. These studies have included estimations of life history parameters, diets, mortality, habitats, and movements and productivity (Gallaway et al., 2009). Oil spills have been shown to alter some of these traits in other fish species. For example, Heintz et al. (2000) found that pink salmon showed delayed effects on growth and marine survival after exposure to oil from the Exxon Valdez oil spill as embryos. Weights in oil exposed versus unexposed fish differed by 5% at the end of the experiment, and exposed salmon showed a 15% decrease in survival rate compared to unexposed salmon after two years (Heintz et al., 2000).

Previous studies have also examined oil-related contamination in fishes in the northern Gulf of Mexico. Nulton and Johnson (1981) studied PAH concentrations in large oil platform-associated fishes (including red snapper) and showed PAH concentrations between 10-220 ppb (wet weight), with most fish showing < 70 ppb in tissue samples. Studies have also examined the effects of oil platforms and the occurrence of lesions on fish. For example, Grizzle (1986) showed gross lesions in 30 of 523 collected fish, but did not detect any oil platform effect. Only limited research has been conducted examining lesions following the DWH oil spill. Murawski et al. (2014) reported 0.1 to > 6% skin lesions from long-line collections of several marine fish species, with about 3.5% of red snapper showing lesions. Arias et al. (2013) concluded that one of the isolated bacteria from the lesions, *Photobacterium damselae,* were a part of the normal microbiota for red snapper, and therefore should not be considered a pathogen at this time.

Another concern often associated with oil spills is the potential for a year class failure due to eggs/juveniles being particularly vulnerable to the effects of oil. Red

snapper spawn from April through September (Bradley and Bryan, 1975; Futch and Burger, 1976; Render, 1995; Collins et al., 1996; Jackson et al., 2007) and larvae remain pelagic until they settle to benthic habitat after approximately 28 days (Szedlmayer and Conti, 1999; Rooker et al., 2004). Red snapper life history is representative of many other commercially important fish species in the Gulf of Mexico. To date there are few reported studies that have examined year class failure or any other detrimental effects of the DWH incident on red snapper. However, Szedlmayer and Mudrak (2014) have addressed potential red snapper year class failure, and failed to find a reduction in postsettlement age-0 red snapper in the fall 2010. Additionally, the authors showed that age-1 red snapper of the 2010-year class were abundant on reefs sampled in 2011. This was consistent with a finding of no effects detected from the oil spill on juvenile fish species within seagrass habitats (Fodrie and Heck, 2011).

To determine if lesions, year class failure, or any other potential changes in red snapper populations can be attributed to the DWH spill, it is necessary to access the body burden of PAHs in fish tissues. Thus far, in the northern Gulf of Mexico, there have been studies published on a variety of coastal fishes (Fodrie and Heck, 2011) and marsh species (Whitehead et al., 2012; Moody et al., 2013), but fewer on offshores species such as red snapper (Murawski et al., 2014; Szedlmayer and Mudrak, 2014). In the present study, gas chromatography/mass spectrometry were used to measure PAHs in red snapper tissue and sediment samples from fish collected on the Mississippi-Alabama continental shelf immediate north (103 km) of the DWH spill site. In addition, condition indices were used to examine if there have been any physiological changes in red snapper following the DWH spill.

METHODS

Study area

Red snapper were collected from randomly selected reefs 20-65 km south of Mobile Bay, Alabama within three artificial reef permit areas, located 103 to 173 km from the DWH wellhead (Figure 1). Reefs were located in water depths from 18 to 41 m. Artificial reef types sampled included small to medium reefs such as steel cages, concrete pyramids, army tanks, and miscellaneous structures; and larger reefs such as ships, barges, ship dry-docks and bridge structures (Figure 2).

Capture procedure

Red snapper were caught with hook-and-line and baited fish traps. Fish capture procedures followed Syc and Szedlmayer (2012). During hook-and-line fishing, two individuals fished for 30 minutes using double 6/0 J hooks baited with gulf menhaden (*Brevoortia patronus*), 27.2 kg test monofilament line, and 45.3 kg test monofilament leader. Fishing was stopped when there were gear malfunctions, and resumed when two fishers were ready. After completion of hook-and-line sampling, additional fish were collected with a baited trap (1.2 × 1.5 × 0.6 m; Collins, 1990). The trap was baited with gulf menhaden and set for four 15 minutes intervals at each sampled reef. All collected red snapper were immediately packed on ice and returned to the laboratory for further analyses (Jaxion-Harm and Szedlmayer, 2015).

Laboratory processing

Red snapper size (SL, FL, and TL mm) and weight (0.1 g) were measured in the laboratory within 48 h of capture. Twenty randomly selected fish had tissue samples removed (liver, gall bladder, and muscle) for PAH analysis. All tissues were weighed, wrapped in aluminum foil and placed in individual plastic bags labeled with the fish number, tissue type, and date collected. All samples were then stored at -20° C until extraction. For GSI analyses, all fish caught from May 2012 – December 2014 were identified as male or female and gonads weighed. Fish were visually inspected for any external skin lesions or abnormalities, and if present photographed.

Sediment Sample Collection

Divers (SCUBA) collected sediment samples at each sample site (Figure 2) using VWR TraceClean Quality-Assured 250 ml amber glass jars. At the surface they were packed on ice and returned to the lab and stored frozen at -20° C until extraction.

Extraction and PAH Quantification

We extracted and analyzed the PAHs in fish tissues and sediment samples using a modified NOAA protocol (Sloan et al., 2004). One modification was the use of diatomaceous earth as a drying agent after homogenization of fish tissues. A second modification was the addition of sodium sulfate directly to the extracts to remove water. We used a control blank (all chemicals but no tissue), a spiked sample (spiked with known quantities of 20 different PAHs in benzene), and a duplicate sample, in each pressure extraction procedure (21-22 samples). Lastly, we did not use size exclusion

chromatography-high performance liquid chromatography to fractionate chlorinated and aromatic hydrocarbons. After the extract was filtered through silica/alumina columns, the solvent was evaporated with a RapidVap Vertex dry evaporator and then analyzed by gas chromatography/mass spectrometry (GC/MS). Polycyclic aromatic hydrocarbons were quantified in parts-per-billion (ppb). For all tissue types and sediment, only samples with weights > 3.0 g were analyzed. Samples were corrected for any analytes > 1 ppb detected in the control blank. The extraction and GC/MS procedures were considered acceptable if the control blank contained < 5 types of analytes exceeding 2X limit of detection (LOD). The LOD for the GC/MS used in these analyses was ≤ 1.0 ppb.

All collected sediment samples were analyzed except for duplicates from the same site, and all muscle tissue samples were analyzed through 27 September 2012. All 2010 pre-spill liver samples (collected prior 01 June 2010) were analyzed, while a random selection of 20-21 samples were analyzed from June and July 2010. In 2011, all liver samples were analyzed (n=32). In 2012, 2013, and 2014, 20-21 liver samples per month were randomly selected for analyses from May, June, and July. Gall bladder samples were pooled by collection date and site due to small individual weights. All collection dates and sites with pooled gall bladder weight > 3.0 g were analyzed (Table 1).

Data Analysis

We calculated all statistics using SAS 9.2. (Statistical Analysis System, Cary, NC). Total PAH (tPAH) included naphthalene, acenaphthylene, acenapthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene including those values < LOD. Total

tissue and sediment PAH were compared by Welch's analysis of variance (Welch's ANOVA; Lix et al., 1996). If significant differences were detected a Games-Howell test was applied to show specific differences (Toothaker, 1992). A linear regression was applied to examine the relationship between sediment tPAH and distance from the DWH spill site (Zar, 2010).

In addition, two ratios (phenanthrene:anthracene and fluoranthene:pyrene) of individual analytes were used to assess potential exposure sources (Baumard et al., 1998). These ratios were calculated for tissue and sediment by year.

Three different biological indices were used to assess potential oil spill effects on reproductive organs, liver size, and fish growth. The gonadosomatic index (GSI) was calculated for each fish (GSI = [gonad weight g/ fish weight g] * 100; Nikolsky, 1963). A Kruskal-Wallis test was used to compare GSI from 2012, 2013, and 2014, during peak spawning season (June-August; Zar, 2010). All immature fish and any fish with a size < 200 mm TL were removed from GSI estimates. A hepatosomatic index (HSI) was used to evaluate any changes in liver size (HSI = [liver weight g/ fish weight g] * 100; Slooff et al., 1983). Analysis of variance was used to compare HSI by year for pre-spill 2010, and post-spill 2010, 2011, 2012, 2013, and 2014 (Zar, 2010). Lastly, condition factor was compared (Fulton's K = [fish weight g/size mm TL^3] *100; Nikolsky, 1963). Analysis of variance was used to compare K by year for mature fish outside of spawning season (October – December) from post-spill 2010, 2011, 2012, 2013, and 2014. If significant differences were detected ($p < 0.05$), a Tukey-Kramer test was applied after ANOVA, a Wilcoxon's rank sum tests with a Bonferroni correction after a Kruskal-

Wallis test, or a Games-Howell test after Welch's ANOVA (Toothacker, 1992; Zar, 2010).

RESULTS

Polycyclic Aromatic Hydrocarbons

Muscle tissue

Polycyclic aromatic hydrocarbons were extracted and analyzed for red snapper muscle samples (total n = 697) from pre-spill 2010 (n = 99), and post-spill: 2010 (n = 121), 2011 (n = 32), and 2012 (n = 445). Significant differences were observed in mean (±SD) tPAH by year, with pre-spill 2010 = 0.02 ± 0.06 ppb, and post-spill 2010 = 3.89 ± 4.41 ppb, 2011 = 5.42 ± 2.54 ppb, and 2012 = 2.28 ± 3.73 ppb (Welch's ANOVA, $F_{3,116.8}$ =132.2, P < 0.0001; Figure 3). A Games-Howell test indicated that all years were significantly different from each other except post-spill 2010 and 2011.

Liver

Red snapper liver samples (total n = 349) were analyzed for PAHs from pre-spill 2010 (n = 75), and post-spill: 2010 (n = 52), 2011 (n = 32), 2012 (n = 65), 2013 (n = 63), and 2014 (n = 62). Significant differences were detected in mean (±SD) tPAH by year. Pre-spill 2010 tPAH (1.5 ± 2.1 ppb) was significantly less than post-spill 2010 tPAH (4.6 ± 6.9 ppb). In later years, 2011 tPAH (0.1 ± 0.1 ppb), 2012 tPAH (0.6 ± 2.8 ppb), 2013 tPAH (0.1 ± 0.1 ppb), and 2014 tPAH (0.1 ± 0.2 ppb) were all significantly lower than pre spill 2010 tPAH (Welch's ANOVA: $F_{5,150.4}$ = 11.7, P < 0.0001; Figure 4). A Games-Howell test indicated that post-spill 2010 liver tPAH was significantly greater than all other years.

Gall Bladder

Red snapper gall bladder samples (pooled into 72 samples for extraction) were analyzed for PAHs from pre-spill and post-spill samples by year. No significant differences were detected among years for gall bladder samples (Kruskal-Wallis, H_5 = 6.1, P = 0.30). Mean (± SD) gall bladder tPAH for pre-spill 2010 = 0.2 ± 0.2 ppb, and for post-spill years: 2010 = 0.1 ± 0.2 ppb, 2011 = 0.0 ± 0.0 ppb, 2012 = 0.2 ± 0.3 ppb, 2013 = 0.1 ± 0.1 ppb, and 2014 = 0.1 ± 0.1 ppb (Figure 5).

Sediment

Sediment samples were analyzed for PAH from 103 red snapper sample sites (n = 28 in 2011, n = 31 in 2012, n = 20 in 2013, and n = 24 in 2014). Mean (± SD) tPAH for 2011 = 0.03 ± 0.14 ppb was significantly lower than 2012 = 0.69 ± 0.96 ppb, and significantly higher than 2014 = 0.01 ± 0.01 ppb, but no difference was detected for 2013 = 0.41 ± 0.97 ppb (Welch's ANOVA: $F_{3,40.9}$ = 6.5, P = 0.0011; Figure 6). No significant distance from spill effects were detected for tPAH (linear regression: n = 103, R^2 = 0.009, P = 0.33; Figure 7).

Ratio comparisons

Phenanthrene:anthracene (PHEN:ANTH) and fluoranthene:pyrene (FLUO:PYRE) were compared over years in both red snapper tissue and sediment samples. Mean (±SD) tissue PHEN:ANTH ratios for pre-spill 2010 = 1.76 ± 1.00 (n = 6), post-spill 2010 = 2.60 ± 2.20 (n = 23), 2011 = 1.35 ± 1.38 (n = 9), 2012 = 2.52 ± 2.24 (n = 43; Figure 8). Both PHEN and ANTH were only detected in sediments for

2012 (PHEN:ANTH ratio = 2.87 ± 2.36, *n* = 4; Figure 8). Pre-spill 2010 FLUO:PYRE ratios = 1.20 (*n* = 1), and post-spill 2010 = 1.03 ± 0.86 (*n* = 65), 2011 = 1.12 ± 1.51 (*n* = 16), 2012 = 2.20 ± 5.08 (*n* = 58), and 2013 = 1.00 ± 0.01 (*n* = 2; Figure 9). For sediment, 2012 FLUO:PYRE = 0.88 ± 0.33 (*n* = 15) and 2013 = 0.27 (*n* = 1; Figure 9).

Biological Indices

Gonadosomatic Index

Gonadosomatic index was calculated for male and female red snapper during the peak spawning season (June-August) of each year. Mean (± SD) female GSI during peak spawning in 2012 = 1.2 ± 0.9% (*n* = 102), 2013 = 1.4 ± 1.2% (*n* = 35), and 2014 = 0.6 ± 1.1% (*n* = 132). Female GSI in 2012 and 2013 were significantly higher than 2014, but no significant differences were detected between 2012 and 2013(Kruskal-Wallis, $H_2 = 61.8$, $P < 0.0001$; Figure 11). Male GSI ± SD in 2012 = 1.5 ± 1.2% (*n* = 128), and was significantly higher than 2014 = 1.1 ± 1.4% (*n* = 107), but not significantly different from 2013 = 1.4 ± 1.5% (*n* = 30; Kruskal-Wallis, $H_2 = 20.9$, $P<0.0001$; Figure 10).

Hepatosomatic Index

Red snapper HSI (*n* = 1,663) were compared from pre-spill 2010 to post-spill 2010 to 2014 (Table 2). No significant differences were detected in red snapper HSI among years (ANOVA, $F_{5, 1657} = 1.0$, $P = 0.44$; Figure 11).

Fulton's K

Fulton's K was calculated for mature red snapper (n = 1047) outside of spawning season (October – December) from post-spill 2010 to 2014 (Table 2). There were significant differences in K among years, with K being the highest for 2014 (ANOVA $F_{4, 1042} = 60.6$, $P < 0.0001$; Figure 12).

Lesions in Red Snapper

External lesions in red snapper were rare. Among all red snapper collected (n = 3934) from 2010 through 2014, only 10 (0.25 %) showed external lesions or abnormalities.

DISCUSSION

Polycyclic Aromatic Hydrocarbon Analyses

Oil entered our sampling area on 1 June 2010 and remained for approximately 40 days, based on NOAA's nearshore surface oil forecasts (NOAA OR&R, 2010). This indicates the potential for hydrocarbon contamination to both sediment and biota. However, the present study only detected very low levels (means < 6 ppb tPAH) across all tissue types and sediment samples. This study failed to show any evidence of PAH contamination above background levels after 2012 in any tissue type or in sediment samples. Despite finding significant differences in tPAH concentrations among years, all PAH levels in this study were well below the levels of concern (i.e., 32,700 ppb naphthalene) set by the EPA and FDA (FDA, 2010), and equal or lower than PAH levels reported from pristine environments (~2.6 – 26 ppb wet wt; McDonald et al., 1992)

Assessing the effects of acute pollution events is complicated for a variety of reasons. First, in the Gulf of Mexico, there are historical natural and anthropogenic sources as well as contemporary natural oil sources that include natural seeps, tanker and oil rig spills, and run off from the Mississippi River and Mobile Bay. Therefore it is important to identify the origin of oil contamination before making evaluations on the effects of the DWH oil spill. Ratios of different PAHs have been used to identify the source of oil contamination in water, sediment, or tissue (Galarneau, 2008). These methods have been applied in several studies following the DWH oil spill (Mitra et al., 2012; Fitzgerald and Gohlke, 2014) and with oil spills in other areas (Ke et al., 2002). There are several possible ratios that could be used (Tobiszewski and Namieśnik, 2012). In the present study, ratios of PHEN:ANTH and FLUO:PYRE were used to assess

whether the PAH's detected in samples were petrogenic or pyrogenic origin. According to Baumard et al. (1998), PHEN:ANTH > 8 and FLUO:PYRE < 1 indicate petrogenic sources. In this study, for all tissue and sediment samples, PHEN:ANTH ratios were < 8. All tissue samples had FLUO:PYRE > 1, but in sediment samples FLUO:PYRE < 1. There were only 81 tissue samples and 4 sediment samples across all years with detectable levels of PHEN and ANTH, and 142 tissue samples and 16 sediment samples with detectable levels of FLUO and PYRE. The ratios in the present study suggested that PAH's were a mixture of both petrogenic and pyrogenic sources, and were consistent with another study that reported PAH ratios for reef fish including red snapper, red grouper (*Epinephelus morio*), yellowedge grouper (*Hyporthodus flavolimbatus*), scamp (*Mycteroperca phenax*), gag (*Mycteroperca microlepis*), snowy grouper (*Epinephelus niveatus*), and tilefish (*Lopholatilus sp*.) after the DWH oil spill (Fitzgerald and Gohlke 2014). Most likely, fish sampled in the present study showed contamination from various sources, based on the PAH ratios. These sources may or may not be related to the DWH oil spill.

Second, different areas can show different exposure levels. Thus, oil spill effects cannot be assumed similar across the areas that were exposed. For example, Tronczyński et al. (2004) reported that if oil slicks wash up on shorelines they can act as a sink for hydrocarbons, leading to chronic contamination problems. As another example, it might be expected that the deep sea environment directly surrounding the wellhead would show more PAH contamination compared to shelf habitat. Diercks et al. (2010) measured the distribution of deep subsurface hydrocarbons following the DWH oil spill. They reported that a subsurface oil plume extended as far as 13 km southwest of the wellhead site. The

present study sites might be considered in between these two extremes (beach and deep sea), with a mean distance 138 km from the DWH site. Due to the distance of the present sample sites from the DWH site, it was not surprising that PAHs were not detected in sediment samples in the present study (~ 0.3 ppb across all years). The sites in the present study were primarily exposed to surface oil, which was heavily degraded due to dissolution and evaporation (Brown et al., 2010; Gong et al., 2010), and sediments were probably not exposed to significant PAH. In contrast sediment contamination was reported primarily in samples collected within 2.7 km of the wellhead (Operational Science Advisory Team, 2010).

Third, different species, life stages, and tissues can show different responses to PAH contamination. Often invertebrates, such as molluscs and crustaceans, are used to assess the presence or absence of oil in an environment because they lack efficient metabolism of hydrocarbons (Law and Biscaya, 1994), while fish can metabolize hydrocarbons efficiently and may be less suitable for PAH evaluations. However, red snapper are an important fish to monitor due to their economic importance. If red snapper have been negatively affected by the oil spill, managers must account for this additional stressor in setting restrictive regulations. Additionally, red snapper were actively spawning during the peak oil spill months, and resultant eggs and larvae may be more susceptible to an oil spill event (Teal and Howarth, 1984).

In this study muscle samples had the highest mean tPAH, in contrast to other studies that reported the highest PAH contamination in liver samples, due to the liver's role in metabolism of PAHs and its high lipid content (Hamelink and Spacie, 1977; Pritchard, 1993). One possible explanation for this could be that higher PAH in skin

tissues contributed to muscle tissue levels (Neff, 1985), as muscle tissues were stored with attached skin tissue until the extraction process. This may have resulted in the higher tPAH concentrations in muscle tissue than in liver tissue seen in the present study.

Liver tissue showed contamination in our pre-spill (May 2010) samples. It is unlikely that oil from the DWH had reached our sites in May, because these samples were collected on 5 May 2010 and 14 May 2010, and the surface oil was still approximately 64 km south of our sites at this time (NOAA OR&R, 2010). Also unlikely is that these fish moved into our sampling array from a more contaminated location, as red snapper home ranges (radius~ 30 m) are small relative to the distance between the DWH spill and fish collections (Topping and Szedlmayer, 2011; Piraino and Szedlmayer, 2014), therefore, the PAH levels observed in our pre-spill liver samples were most likely from a source unrelated to the DWH spill.

Despite the detected trends in muscle and liver tissue PAH, actual concentrations were very low in both tissue types. There are several potential explanations for the low PAH levels observed: 1) It could be an artifact of adult fish being less susceptible to hydrocarbon contamination (Payne et al., 2003), 2) not catching the fish during the two to three day window prior to metabolism of PAH's (Pointet and Milliet, 2000), or 3) the effects of the oil spill were less encompassing than originally suspected (Fodrie and Heck, 2011; Moody et al., 2013; Szedlmayer and Mudrak, 2014).

Lastly, studies use different levels of PAH's to assess contamination effects on physiology. Some studies that showed negative effects of PAH contamination used PAH levels at much higher levels (parts-per-million) compared to the levels (parts-per-billion) shown in the present study. For example, Anderson et al. (1974) and Moles (1980)

measured acute toxicity effects (mortality of 50% of the test population) in sheepshead minnow (*Cypriondon variegatus*) and juvenile coho salmon (*Oncorhynchus kisutch*). Sheepshead minnow had 50% mortality within 24 hours with treatment of 2.4 ppm naphthalene (Anderson et al., 1974) and juvenile coho salmon had 50% mortality within 96 hours of treatment with 3.2 ppm naphthalene (Moles, 1980).

Spotted seatrout (*Cynoscion nebulosus*) exposed to ~ 0.077 ppm and ~0.725 ppm total petroleum hydrocarbon concentrations, showed no significant changes in growth rates for larval fish, but significantly reduced growth rates in juvenile fish after 3 days of depuration. After four weeks of depuration there were no significant differences in growth rates (Brewton et al., 2013).

However other studies have used low PAH levels similar to levels measured in the present study. For example, embryos of yellowfin tuna (*Thunnus albacares*), southern bluefin tuna (*Thunnus maccoyii*), and yellowtail amberjack (*Seriola lalandi*) were exposed to artificially weathered source oil and surface-skimmed oil with tPAH concentrations ranging from ~3-14 ppb (Incardona et al., 2014). These exposures resulted in gross morphological defects such as accumulation of pericardial edema, lack of actinotrichia, reduction in the outgrowth of the finfolds or finfold blisters, reduced eye growth, dorsal curvature of the body axis, and cardiac function defects (Incardona et al., 2014). In another study, pacific herring (*Clupea pallasi*) eggs were exposed to varying concentrations of PAHs from weathered Alaska North Slope crude oil for 16 days. The authors showed significantly elevated egg mortality after exposure to 7.6 ppb tPAH (Carls et al., 1999).

When comparing among studies, it is important to keep in mind that different studies assess different suites of PAHs. For example, some studies only examined bile metabolites (Aas and Klungsøyr, 1998; Aas et al., 2000), others considered parent PAHs (Fitzgerald and Gohlke, 2014), or a combination of parent and alkylated PAHs (Tronczyński et al., 2004). What is apparent from these studies is that the effects of oil exposure can vary over a large range of PAH concentrations in water, sediment, or tissue. However, for the most part, effects have been detected in larval and juvenile fish with little evidence for significant effects on adults.

Lesions

An apparent increase in the occurrence of external lesions has been attributed to the DWH oil spill (Murawski et al., 2014). They reported a high rate of lesions in red snapper that exceeded 6%, but sample size was low with only 50 red snapper examined. The present study was based on a large red snapper sample size (n = 3934) and showed little evidence of increased lesions from the DWH oil spill (lesion occurrence = 0.25 %). Another difference may be that Murawski's et al. (2014) samples were from the deeper De Sota Canyon, while present study samples were from the relatively shallower continental shelf. In addition the 'high' rate of lesions may in fact still be within the range of naturally occurring rates. For example, Grizzle (1986) collected fish in the northwestern Gulf of Mexico on two drilling platforms and near the Flower Garden Banks, and showed no significant differences in lesion prevalence between the experimental (drilling platforms) and control (Flower Garden Banks) sites and reported a lesion rate of 5.7% in fish collected.

Also, despite experiencing 'high' oil exposure on the inner-continental shelf, the percentage of fish with lesions in this area was very low (0.25 %) in the present study. If the occurrence of lesions was correlated with increased PAH exposure following the oil spill, then it would be expected that lesion frequency would be similar in regions exposed to similar concentrations of oil. To further illustrate this, areas outside of the oil spill plume, even as far southeast as Tampa, FL had lesion frequencies from 4.1-6.0% and > 6% (Murawski et al., 2014). Lastly, other sources of lesions include physical causes, environmental stressors, immunological causes, or nutritional deficiencies (Law, 2001); therefore, it is unlikely that the few fish with lesions in the present study were caused by the DWH oil spill.

Biological Indices

One of the objectives of this study was to examine whether there have been any physiological effects on red snapper from the oil spill. The gonadosomatic index is typically used to assess spawning occurrence in fish species (Collins et al., 1996; Brown-Peterson et al., 2009; White and Palmer, 2004), but it has also been useful as a quick and inexpensive method to examine pollution effects (Vandermeulen and Mossman, 1996; Louiz et al., 2009; Montenegro and González, 2012). Most studies that used GSI to evaluate pollution effects on fish health examined chronically polluted environments, rather than short-term accidental oil spills (Louiz et al., 2009; Montenegro and González, 2012). Pollution-related GSI studies typically find decreased GSIs from affected sites, compared to controls (Linderoth et al., 2006; Marchand et al., 2008).

Male red snapper GSI during peak spawning showed significant differences between 2012 and 2014. Female red snapper during peak spawning showed a significant decrease in GSI between 2012 and 2014, and 2013 and 2014, but these differences could be attributed to a multitude of factors. Red snapper are asynchronous spawners, with spawning events influenced by photoperiod and water temperature (Collins et al., 1996; Phelps et al., 2009; Woods et al., 2003). Variations in either of these parameters could explain the differences seen in GSI among years.

Another influence on GSI could be pollution from other sources besides the DWH oil spill. Rivers contribute approximately 28% of the petroleum hydrocarbon input in the sea worldwide (Pollino and Holdway, 2002), and the present study sites are heavily influenced by input from rivers that empty into Mobile Bay and Mississippi Sound (Rabalais, 1992). Because of these other influencing factors, it is difficult to attribute the statistically significant differences observed in the present study to the DWH oil spill.

Most likely, the significant differences shown in the present study are normal annual variation in GSI. This is supported by historical data on red snapper GSI from the northeastern Gulf of Mexico. One study sampled red snapper in northwestern Florida from 1991-1993 and reported that females GSI values varied from 0.78% to 1.74% and males varied from 0.41% to 0.63% during peak spawning (Collins et al., 1996). The present study GSI estimates were similar to these previous estimates for female red snapper, and higher than previous values reported for male red snapper. Additionally, the GSI values in the present study are not consistent with the timeline of the oil spill. Had GSI been influenced by the oil contamination, there should have been a steady increase in

GSI, with the highest values occurring in 2014 which had the greatest time period since the DWH oil spill, but in fact showed the lowest GSI compared to previous years.

In the present study there were no significant differences in HSI in comparisons of pre-spill and post-spill red snapper. In contrast, previous studies have reported an increase in HSI from polluted areas compared to control areas (Montenegro and González, 2012; Pointet and Milliet, 2000). Little variation in HSI by year indicated that red snapper liver sizes have remained stable from May 2010 to December 2014, and were not affected by the DWH oil spill.

The present study detected significant differences in Fulton's K among years. This index can be affected by pollution (Amara et al. 2009), but also by water temperature and several other variables (Nikolsky, 1963). However, in the present study there was not a decreasing trend, as would be expected if there were a chronic pollution effect. The pattern in K showed a significant decrease from post-spill 2010 to 2011, there were no significant differences from 2011 through 2013, then in 2014, K significantly increased. The significant differences seen in this study are unlikely due to the DWH oil spill for a number of reasons. First, evidence of decreased K doesn't occur until 2011, at which point no residual surface slick was present at the sites in this study, and no sediment contamination was detected. Juvenile sea bass (*Dicentrarchus labrax*) kept in field cages at stations with varying pollutant concentrations showed decreased K within 38 days of deployment (Kerambrun et al., 2012), which indicated that if red snapper had experienced reduced K in response to the oil spill it would have been detected in the 2010 samples. Second, other studies with similar spill characteristics as it the present study (i.e., primarily influenced by drifting oil slicks), did not detect decreased fish condition or

K values in samples from more heavily contaminated sites (Claireaux et al., 2004; Lancaster et al., 1998). Third, previous studies showed that variations in fish condition were more influenced by heavy metal contaminants than PAH pollution (Bervoets et al., 2009; Fang et al., 2009). Most likely, the significant differences observed in the present K estimates were normal yearly variations. This inter-annual normal variation was supported by the low variability among all years at $\leq 0.13\%$.

CONCLUSIONS

The present study concluded that the DWH oil spill had little detrimental effect on adult red snapper during and following the DWH oil spill. This conclusion was based on the low levels of PAHs detected in red snapper tissues and the lack of biologically significant changes in fish condition indices. Additionally, it is unlikely that there will be any chronic contamination problems in the present study area due to low levels of detected PAHs in sediment samples in the four years following the spill. However, monitoring of PAH's in red snapper and sediments should be continued as the potential for long term effects caused by lingering oil still exists. This was shown in the Exxon Valdez oil spill, where the long-term detection continued for several years, and in some cases more than a decade, following the spill. For example, one long-term monitoring study showed PAH's in whole mussel tissue and sediments biannually, in areas that were impacted by the Exxon Valdez spill, but also locations that undergo potential chronic contamination from other sources. The authors also showed that PAHs showed decreasing trends over the course of the study (1993 – 2006), and that any remnants of Exxon Valdez oil had stabilized or reached levels which no longer appear in mussel tissue (Payne et al., 2008). Another study showed elevated cytochrome P4501A expression in masked greenling (*Hexagrammos octogrammus*) and crescent gunnel (*Pholis laeta*) collected in 1999 (ten years after the spill) from sites within the original spill trajectory (Jewett et al., 2002). So, while it's possible there will be no evidence of chronic oil spill effects, the only way to verify this is to continue the monitoring effort.

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Table 1. Summary of the selection methods for PAH analysis of red snapper, *Lutjanus campechanus*, for each sample type.

Type	Method
Muscle	All samples analyzed from May 2010-September 2012.
Liver	All samples analyzed from May 2010. 20 samples randomly selected from both June and July 2010. All samples from 2011. 20 samples randomly selected from each month, May, June, and July from 2012-2014.
Gall Bladder	All samples were pooled by collection date and site, except those with insufficient sample.
Sediment	All sites except duplicates and those with insufficient sample.

Table 2. Red snapper, *Lutjanus campechanus*, mean (± SD) and samples size for HSI and K by year.

Year	HSI (%)	K (%)
Pre-spill 2010	0.74 ± 0.25 (*n* = 100)	
Post-spill 2010	0.69 ± 0.19 (*n* = 240)	1.49 ± 0.10 (*n* = 245)
2011	0.77 ± 0.34 (*n* = 36)	1.41 ± 0.08 (*n* = 106)
2012	0.70 ± 0.41 (*n* = 582)	1.40 ± 0.11 (*n* = 208)
2013	0.68 ± 0.22 (*n* = 326)	1.40 ± 0.14 (*n* = 114)
2014	0.71 ± 0.23 (*n* = 379)	1.52 ± 0.12 (*n* = 374)

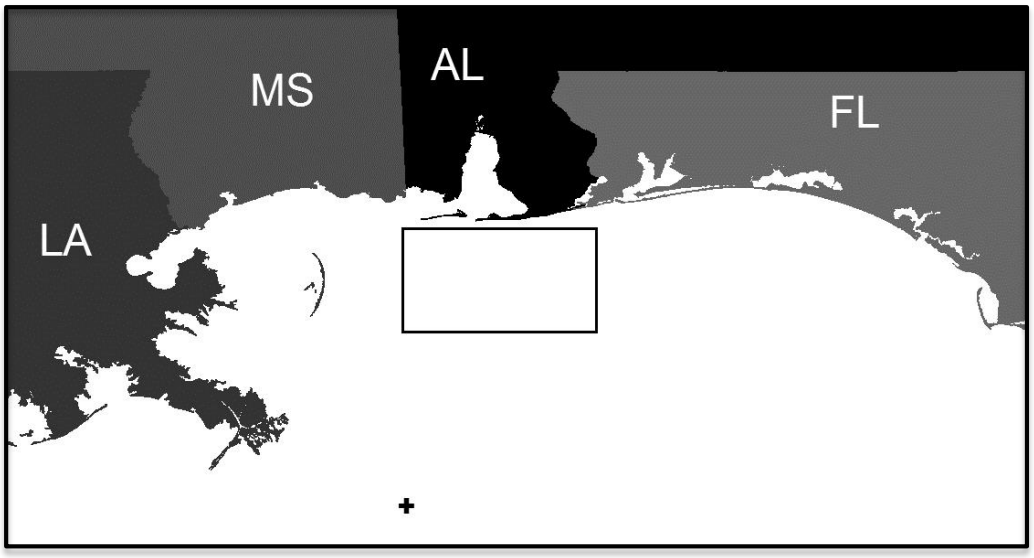

Figure 1. Study sites (black box) and DWH location (black cross).

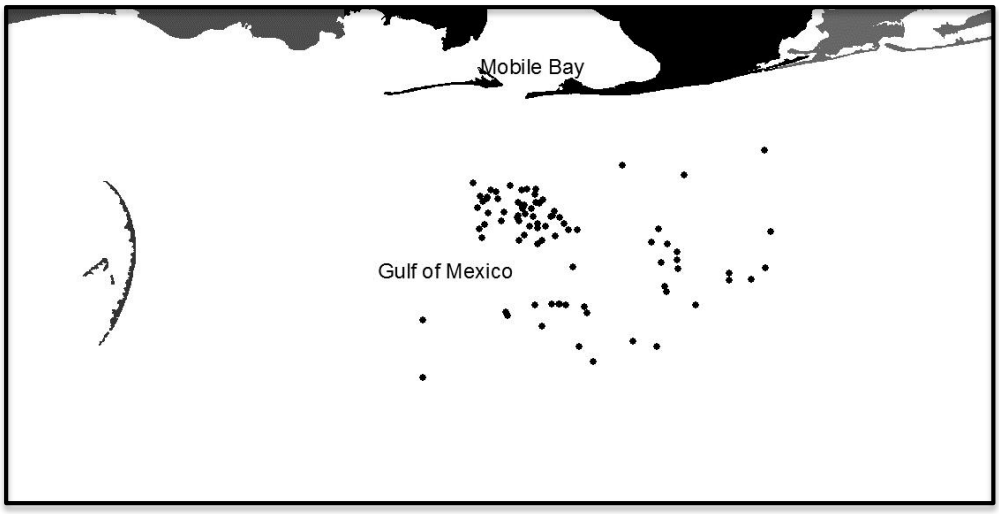

Figure 2. Sample locations for red snapper, *Lutjanus campechanus*, tissue and sediment PAH analyses following the DWH oil spill.

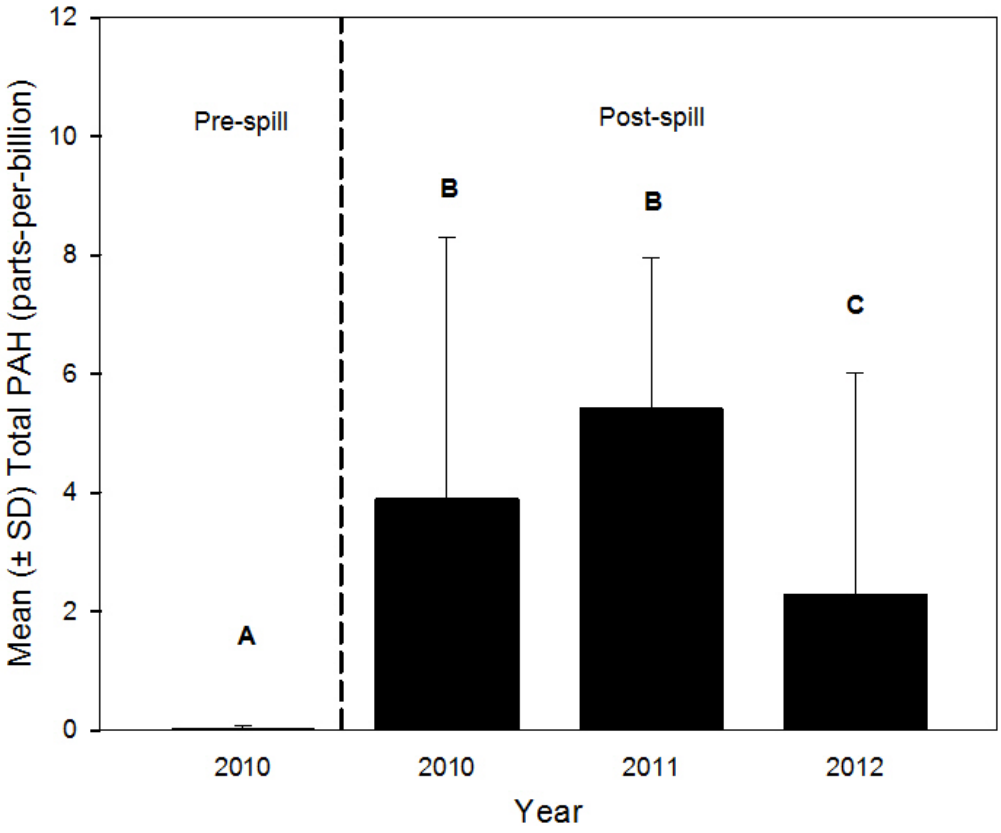

Figure 3. Red snapper, *Lutjanus campechanus*, mean (± SD) muscle tissue PAH concentrations by year. Different letters indicate significant differences.

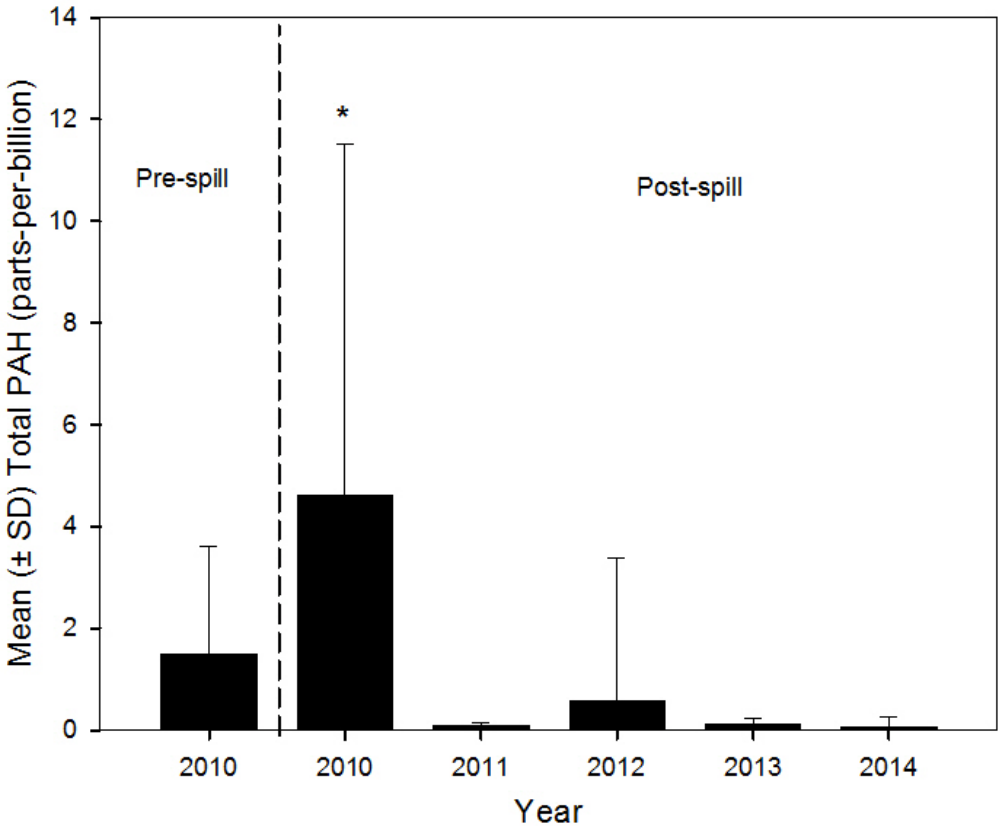

Figure 4. Red snapper, *Lutjanus campechanus*, mean (± SD) liver tissue PAH concentrations by year. Asterisk indicates significant difference.

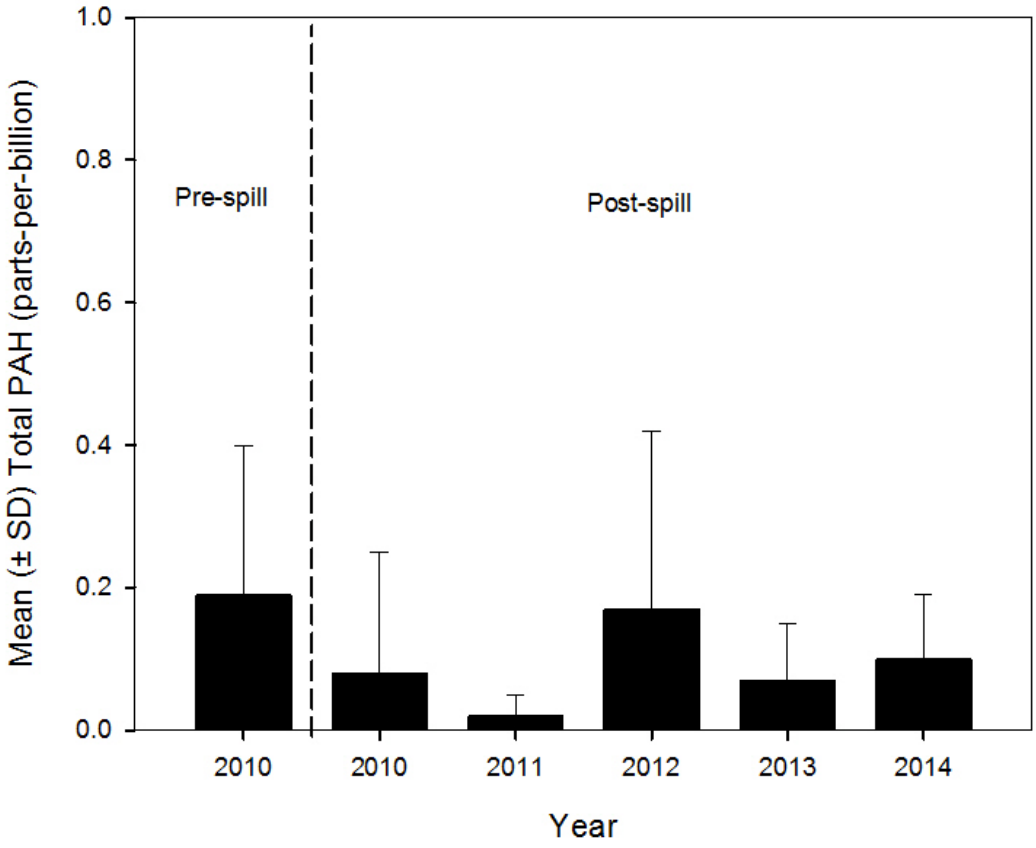

Figure 5. Red snapper, *Lutjanus campechanus*, mean (± SD) gall bladder tissue PAH concentrations by year.

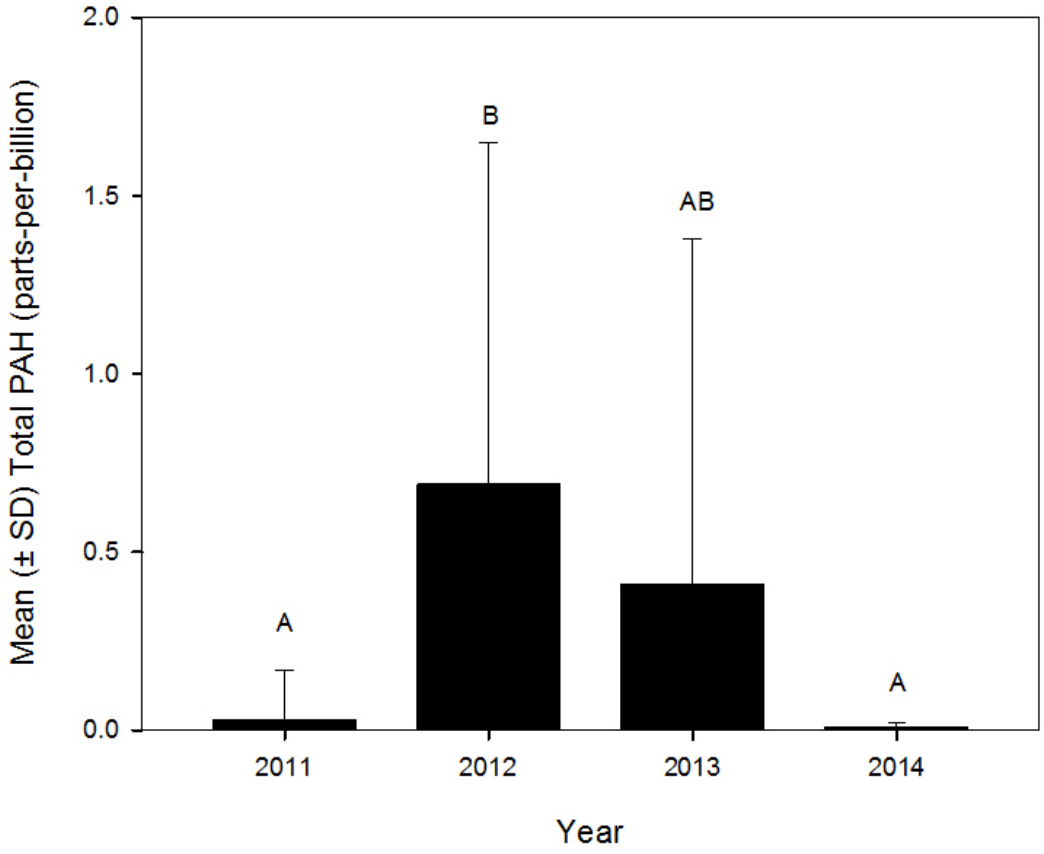

Figure 6. Mean (± SD) sediment PAH concentrations by year. Different letters indicate significant differences.

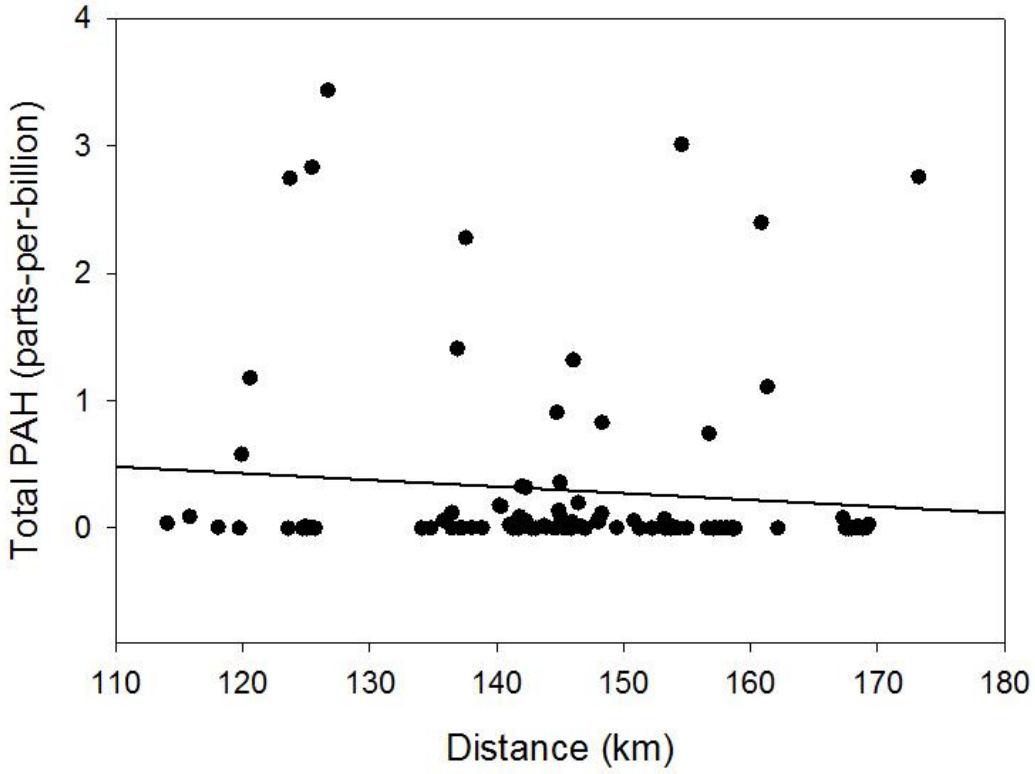

Figure 7. Sediment PAH concentrations by site distance from DWH wellhead location. (n = 103, R^2 = 0.009, P = 0.33).

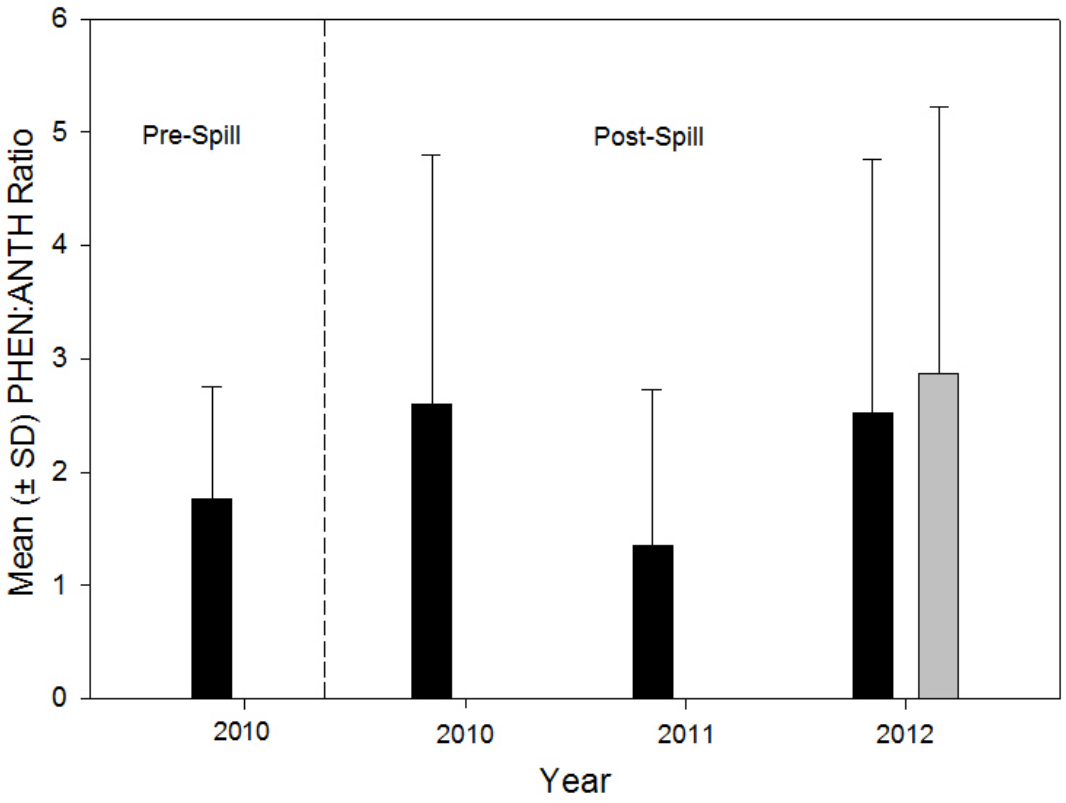

Figure 8. Red snapper, *Lutjanus campechanus*, mean (± SD) PHEN:ANTH ratios for tissue (black bars) and sediment (gray bars) by year. Values > 8 ppb indicate petrogenic sources.

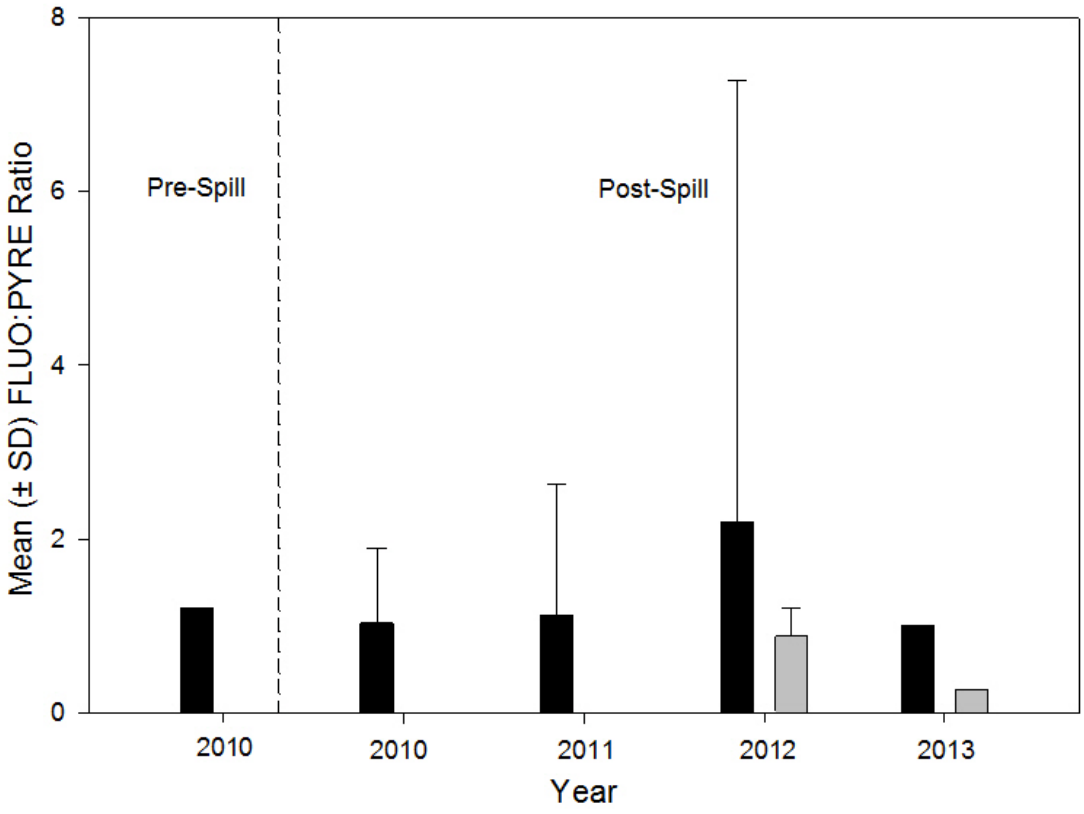

Figure 9. Red snapper, *Lutjanus campechanus*, mean (± SD) FLUO:PYRE ratios for tissue (black bars) and sediment (gray bars) by year. Values < 1 ppb indicate a petrogenic source.

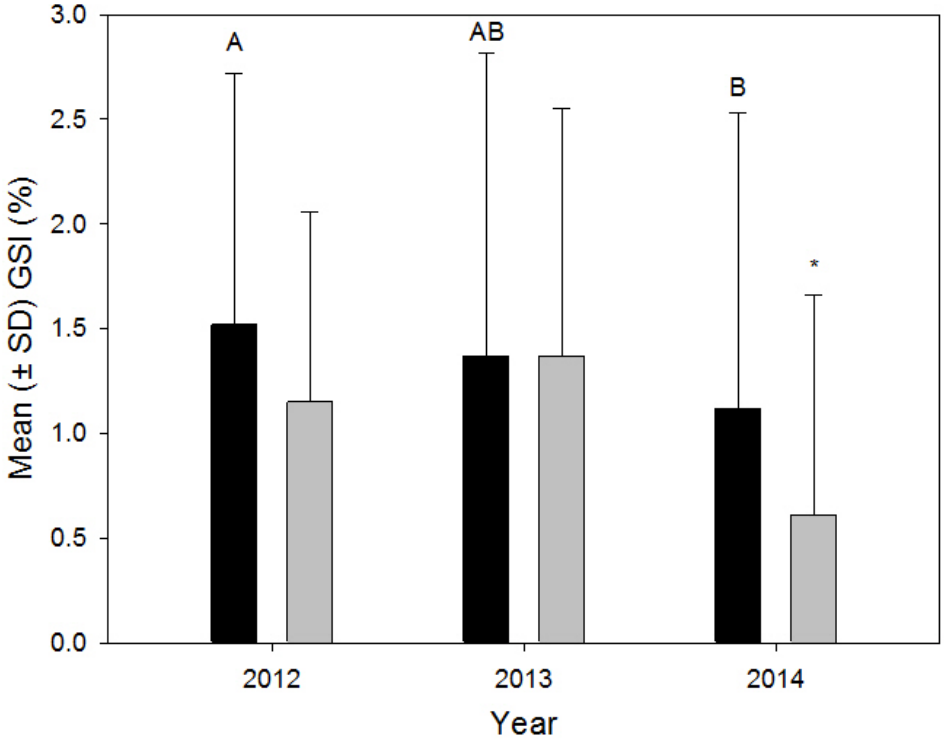

Figure 10. Red snapper, *Lutjanus campechanus*, (black bars) and female (gray bars) mean (± SD) GSI by year during peak spawning (June-August). For female fish, significant differences are indicated by an asterisk. For male fish, different letters indicate significant differences.

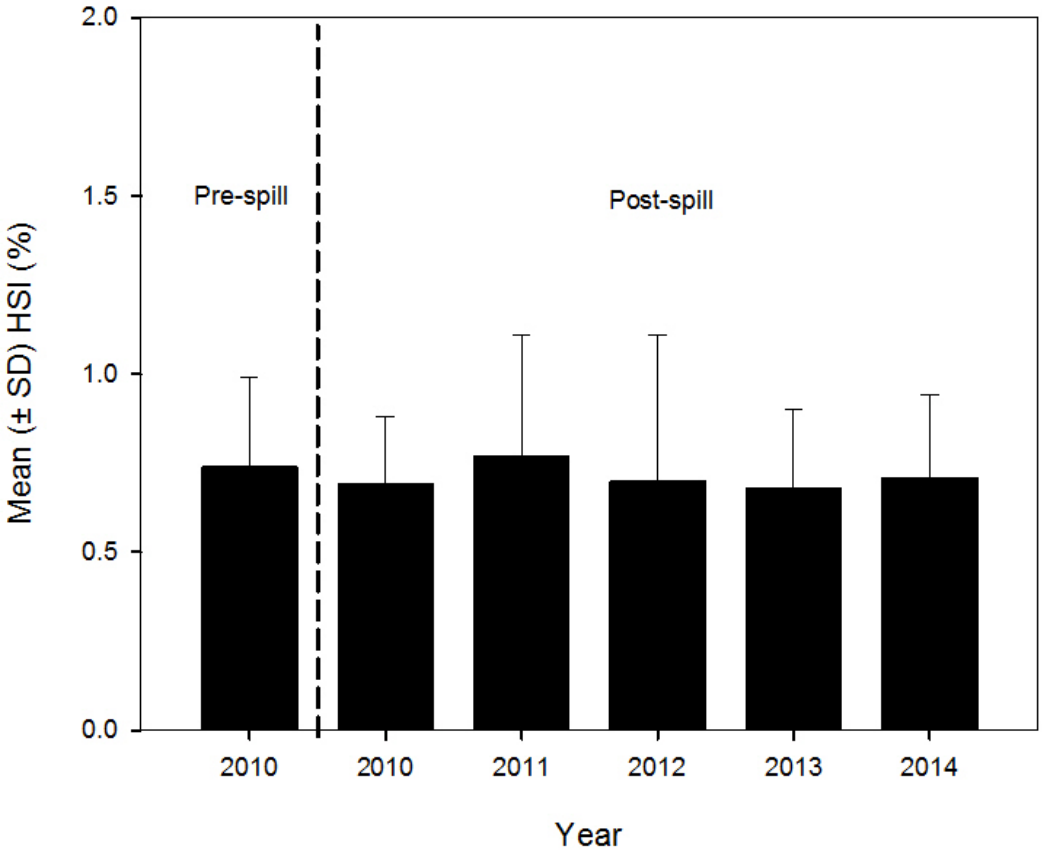

Figure 11. Red snapper, *Lutjanus campechanus*, mean (± SD) HSI by year.

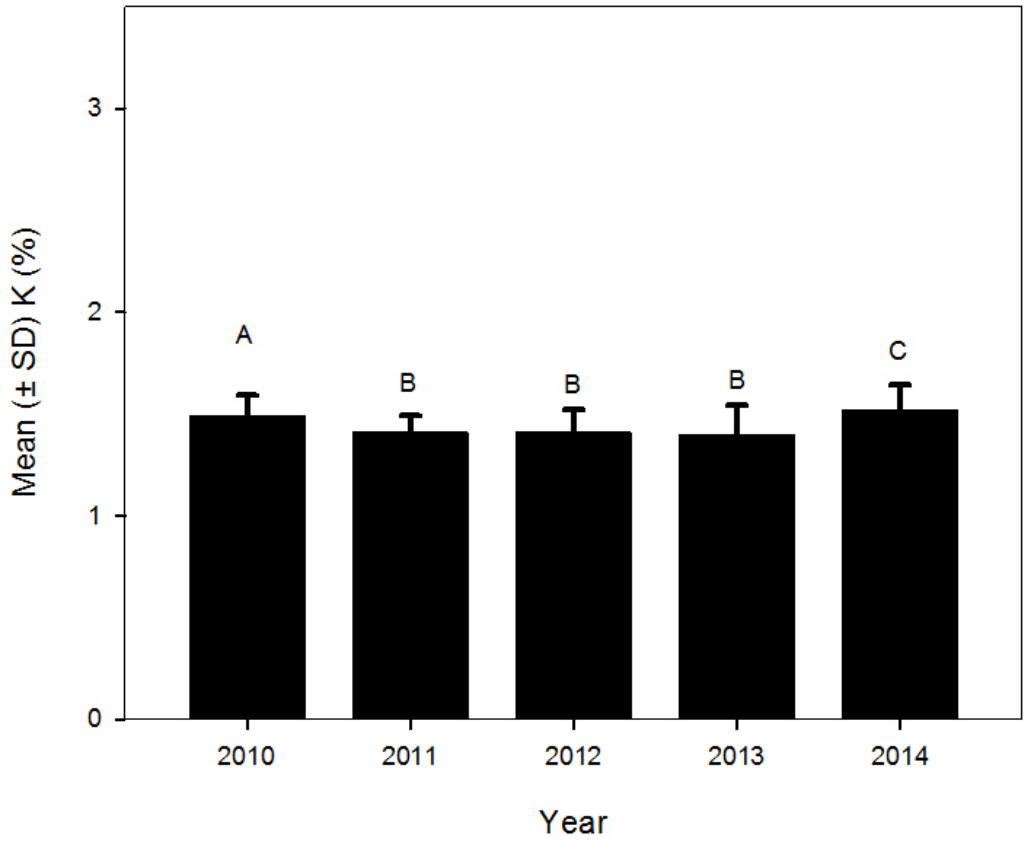

Figure 12. Red snapper, *Lutjanus campechanus*, mean (± SD) K by year. Different letters indicate significant differences.