Age frequency, growth, mortality, and PAH levels of roughtongue bass (*Pronotogrammus martinicensis*) following the Deepwater Horizon oil spill~~.~~

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Abstract

Age, growth, mortality, and polycyclic aromatic hydrocarbons (PAHs) in roughtongue bass (*Pronotogrammus martinicensis*) were examined in the northern Gulf of Mexico following the 2010 Deepwater Horizon oil spill. Fish ($N = 1090$) were collected from September 2014 to July 2015 from the Alabama Alps (54 km from the spill site) and Roughtongue Reef (111 km from the spill site). Sites were dominated by the 2010 year-class. Growth rates were significantly lower for fish from Alabama Alps compared to Roughtongue Reef ($p < 0.001$) and likely linked to proximity of the Mississippi River discharge. Mean total PAH ± SD was 50 ± 52.6 and ranged from 0~~–~~ to 220 ppb. These PAH levels were below a 300-ppb minimum effect level and not significantly different between sites. The dominant 2010 year-class, low PAH levels, and similar growth rates to pre-spill measures indicated that the Deepwater Horizon oil spill had little effect on roughtongue bass.

Keywords: Pinnacle reefs; von Bertalanffy growth; ~~p~~Polycyclic aromatic hydrocarbons; ~~m~~Mesophotic habitats

~~1.~~1 Introduction

In April 2010, the Deepwater Horizon (DWH) Macondo well, located 73 km off the southeast coast of Louisiana, exploded and sank. After the explosion, the well leaked oil for three months, resulting in an estimated 779,000 m^3 (4.7–5.5 million barrels) of oil entering the Gulf of Mexico (Thibodeaux et al., 2011). The spill has subsequently been labeled as the worst recorded in U.S. history.

There have been several studies documenting the negative effects of large quantities of oil entering marine systems. These studies have detected effects from oil in the aquatic environment at the biochemical, organismal, population, and community levels (Capuzzo, 1987). A few well-known examples include the 1989 EXXON Valdez spill and the 1979 Ixtoc-1 spill, which resulted in both immediate wildlife mortalities and longer-lasting effects on the surrounding ecosystems (Incardona et al., 2015; Jernelov, 2010; Jewett et al., 2003; Piatt et al., 1990; Soto et al., 2014; Thorne and Thomas, 2008).

Aside from suffocation and direct oiling, polycyclic aromatic hydrocarbons (PAHs) are often to blame for long term deleterious effects of oil spills (Albers, 2003; Diercks et al., 2010). Approximately 4~~-~~% of the DWH oil that was released into the marine ecosystem consisted of PAHs, resulting in 2.1~~x~~ $\times 10^{10}$ g of PAHs entering the Gulf of Mexico. There are 16 identified PAHs established by the US Environmental Protection Agency (US EPA, 1987) as being particularly toxic to aquatic organisms, and include acenaphthylene, acenaphthene, anthracene, benzo[*a*] anthracene, benzo[*a*]pyrene, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*ghi*]perylene, chrysene, dibenzo[*a*,*h*]anthracene, fluoranthene, fluorene, indeno[1,2,3-*cd*]pyrene, naphthalene, phenanthrene, and pyrene (Latimer and Zheng, 2003; US EPA, 1987). These PAHs were detected at concentrations of 189 ppb in depths of 1320 m, within 13 km of the spill site, three weeks after the explosion (Diercks et al., 2010; Reddy et al., 2012). Petrogenic PAHs can degrade in marine environments, but within anaerobic subtidal sediments of aquatic environments they can persist for many years after their initial release into the ecosystem and create the possibility for continuous exposure for benthic species (Albers, 2003; Diercks et al., 2010).

Since the spill there have been several studies to identify the potential residual effects of the DWH oil and it~~’~~'s PAH effects on fish species, both from initial exposure and chronic exposure (Beyer et al., 2016; Fodrie and Heck, 2011; Mendelssohn et al., 2012; Szedlmayer and Mudrak, 2014; Whitehead et al., 2012). Sub-lethal DWH oil concentrations were reported to cause changes in genome expression and tissue morphology impairment in gulf killifish (*Fundulus grandis*; ~~(~~Whitehead et al., 2012). Hydrocarbons in PAHs have also been linked to the alteration of mixed-function oxygenase enzymes (Akcha et al., 2003; Sims and Overcash, 1983) and increased mutagenic and carcinogenic events (Albers, 2003; Tuvikene, 1995). PAH concentrations in water at 1~~-~~–15 ppb has also been documented as having damaging effects on larvae formation, causing heart dysfunction, and malformation in several fish species, e.g., larval dolphinfish (*Coryphaena hippurus*; ~~(~~Carls et al., 2008; Edmunds et al., 2015; Incardona et al., 2015, 2014). Based on the chemical make-up of the DWH oil, immunotoxicity effects could include increased parasitism and reduced disease resistance, which could affect population reproduction (Barron, 2012). However, there has yet to be any detected year-class failures or recruitment failures associated with the DWH spill (Beyer et al., 2016; Fodrie and Heck, 2011; Szedlmayer and Mudrak, 2014).

One of the specific environments potentially affected by the DWH spill was a natural rock reef system on the continental slope off the Alabama-Mississippi coastline (the “Pinnacles”). This habitat has been described as a “mesophotic” reef system and is located approximately 54 to 111 km northeast of the DWH spill site (McBride et al., 2009; Thurman et al., 2004; Weaver et al., 2002). The reef system consists of a series of rock outcrops at depths ranging from 68 to 100 m (Thurman et al., 2004). This system has a diverse array of at least 53 demersal fish species, including commercially important Serranidae, Carangidae, and Lutjanidae (Thurman et al., 2004; Weaver et al., 2002). These deep slope habitats are the closest mesophotic reef structures to the DWH oil spill site and as such may have been exposed to PAH contamination in 2010 (Silva et al., 2016). Two reefs within the Pinnacles, the Alabama Alps and Roughtongue reef were the focus of the present study.

The roughtongue bass (*Pronotogrammus martinicensis*) is found throughout the Gulf of Mexico, South Atlantic, Caribbean to Brazil, at depths ranging from 45 to 250 m, and is one of the most abundant fish species residing on the Pinnacles mesophotic reefs (Anderson and

Heemstra, 1980; Dennis and Bright, 1988). Studied extensively prior to the DWH oil spill, this brightly colored, small (<200 mm) reef fish species in the family Serranidae, spawns primarily from February to July, a time which coincides with the DWH spill (Continental Shelf Associates Inc. Texas A&M University, 2001; McBride et al., 2009; Thurman et al., 2004; Weaver et al., 2002). Also, nearly all age-1 of this species are females because they are protogynous hermaphrodites, meaning they mature first as females and then switch to males around age-2 (Coleman, 1981; McBride et al., 2009; Thurman et al., 2004). Lastly, roughtongue bass are secondary consumers in the mesophotic reef system and also prey for larger reef predators, such as almaco jack (*Seriola rivoliana*) and sand tilefish (*Malacanthus plumieri*) making them an important trophic link for energy transfer to the deeper reef systems (McBride et al., 2009; Thurman et al., 2004; Weaver et al., 2002). The abundance, year-class vulnerability, and trophic position of the roughtongue bass have important ecological, economic, and management implications, which make it a species well suited for assessing potential effects of the DWH spill (George et al., 2007).

The present study measured the effects of the DWH oil spill through comparisons of pre-spill to post-spill year-class abundances, growth rates, and mortality rates. The use of year-class abundance as a measure of pollution effects is a well-documented assessment technique. For example, a previous study has indicated significant reductions in fish recruitment and population abundances in highly polluted streams, while less polluted streams had higher fish recruitment and higher adult abundance (Siligato and Böhmer, 2001). In addition to direct mortalities, another well documented indicator of pollution is reduced growth rate. For example, significantly reduced growth rates of juvenile sole (*Solea solea*) were caused by decreased environmental quality (Amara et al., 2007). Thus, comparison of pre to post DWH spill measures of these selected life history parameters in this deep water Serranidae is a suitable method of evaluating oil spill effects on fishes from these mesophotic reef habitats.

The present study also measured PAH content in roughtongue bass. Different levels of the various PAHs from fish tissues can be used to identify sources of pollution as well as magnitude of exposure (Yunker et al., 2002). Also, ratios of specific PAHs can help distinguish between pyrogenic and petrogenic sources (Yunker et al., 2002). There are multiple sources of PAHs within the marine environment, including watershed from terrestrial habitats, boat exhaust, and diffusion from the atmosphere (Latimer and Zheng, 2003). Therefore, quantifying the different PAHs is critical for estimating the extent of exposure and determining if roughtongue bass were exposed to DWH oil. In addition, PAH quantification within fish tissue can be compared with life history parameters (e.g., year-class strength, mortality, and growth rates) for a more comprehensive evaluation of potential DWH oil spill effects. Thus, the present study examined life history parameters, and measured PAH levels in roughtongue bass to evaluate the potential effects of the DWH oil spill on this ecologically important reef fish species from mesophotic reef habitats.

~~2.~~2 Materials and ~~M~~methods

~~2.1.~~2.1 Study area

Study sites were located within the Pinnacles reef area on the continental slope approximately 70 km south of the Mississippi-Alabama coastline. Particular locations are defined as the Alabama Alps (AA: N 29.252, W 88.337) and Roughtongue Reef (RR: N 29.442, W 87.579). These sites have shown high abundances of roughtongue bass prior to the DWH spill (Continental Shelf Associates Inc. Texas A&M University, 2001; McBride et al., 2009; Weaver et al., 2002) and are within close proximity to the spill site (AA is 54 km and RR is 111 km from the DWH site; Fig. 1). Each site has been previously characterized for habitat and fish diversity (Thurman et al., 2004). Roughtongue Reef has a ~~diameter~~maximum length of approximately 400 m, with a depth of 68 to 78 m and Alabama Alps has a ~~diameter~~maximum length of approximately 1000 m, with a depth of 72 to 88 m (Thurman et al., 2004) (Fig. 2).

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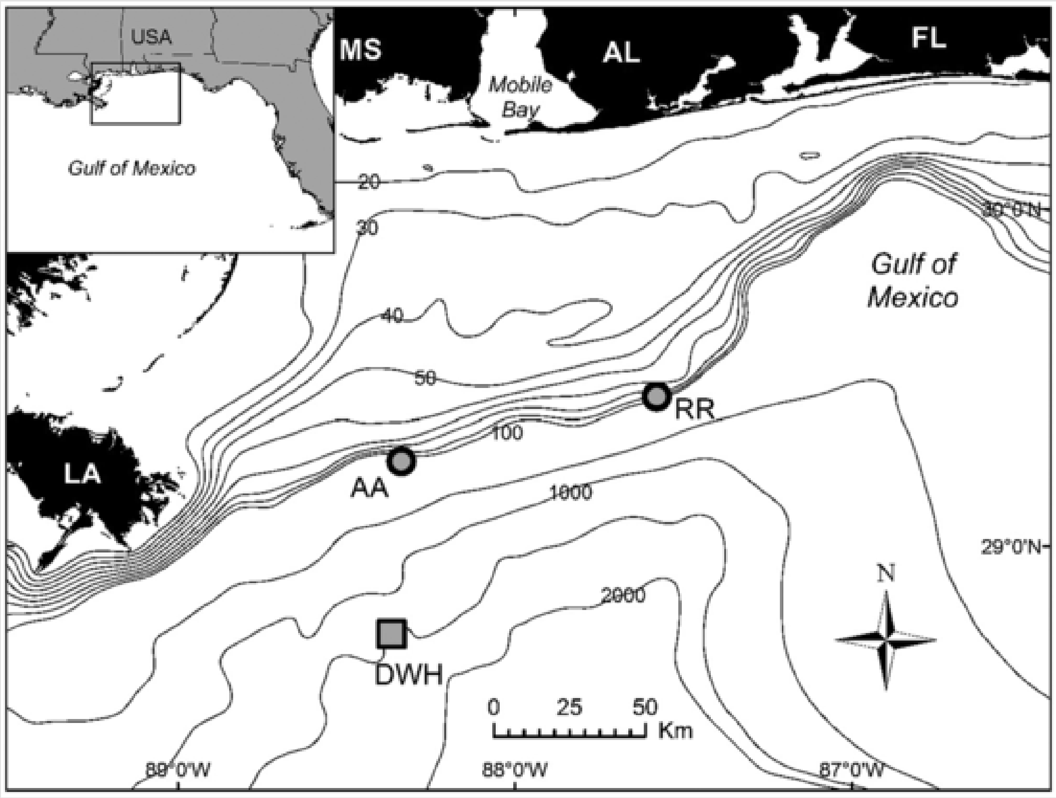

Sample sites for roughtongue bass (*Pronotogrammus martinicensis*) in the northern Gulf of Mexico. Two sites from the Pinnacles reefs included the Alabama Alps = AA (N 29.252, W 88.337) and Roughtongue Reef = RR (N 29.442, W 87.579). The gray square is the DWH spill site (N 28.738, W 88.366).

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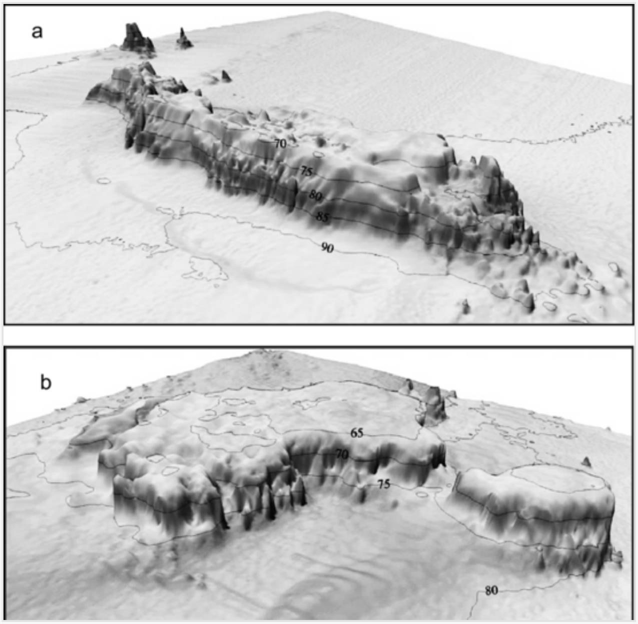

Three dimensional images of (a) Alabama Alps (N 29.252, W 88.337) and (b) Roughtongue Reef (N 29.442, W 87.579). Isobaths depths = m, and the horizontal distance across the image is approximately 700 ~~meters~~m, and the vertical exaggeration is 5~~x~~× (Multibeam maps, USGS).

~~2.2.~~2.2 Fish collections

Roughtongue bass were sampled from reef tops, reef slopes, and reef base at both AA and RR. Four seasons were sampled over one year (fall = September–October 2014, winter = December 2014, spring = March 2015, summer = June–July 2015). After arrival at a reef site, the research vessel anchored on the reef and drifted back and forth over the reef as fish were collected. The exact positions (latitude and longitude) for each fish collected were recorded. The research vessel was repositioned haphazardly several times (>20) over each reef site for 2~~—~~–5 day periods during each seasonal survey to sample the entire reef area.

Roughtongue bass were collected with hook-and-line and sabiki rigs (number 4~~—~~–6 hook size) with six hooks for each fishing gear. Weights were varied from 227 to 454 g depending on current and sea state. The fishing gear included Daiwa deep-drop rods and Tanacom-Bull 750 electric reels. Reels were filled with 22.7 kg braided line. Four deep-drop gears were fished simultaneously at each site.

When fishing started the time and location were recorded. Once the lines reached the seafloor, the fishing depth was recorded from the electric reels. The fishing continued until a fish was caught or a 5-min limit was reached at which time the line was retrieved and checked (for fish or lost hooks) and then fishing continued. If there were less than 5 hooks when the fishing line was checked, the sabiki rig was replaced with a new 6-hook rig. After capture, fish were removed with a de-hooker and placed in ice chilled seawater. All captured fish were measured and identified on the research vessel. Fish were placed into plastic bags, labeled with waterproof paper, and frozen on the research vessel for transport back to the laboratory.

~~2.3.~~2.3 Age estimation and annual growth rates

In the laboratory roughtongue bass were weighed to the nearest 0.01 g on an Ohaus Scout-Pro 200 g scale and otoliths were extracted by dorsal dissection. Both sagittal otoliths were removed, rinsed with de-ionized water, and stored in sealed vials. Vials contained an internal label with capture date and fish ID number, and an external label with the fish ID number.

Otoliths were stored and dried for a minimum of two months before age estimates. After the drying period, otoliths were viewed with a Leica-MZ6 stereomicroscope at 20~~x~~× magnification, and illuminated with fiber optic transmitted light (Wetzlar, Germany). Digital images of the whole otolith were captured at 2046~~ x ~~ × 1536 pixel resolution (5165~~ x ~~ × 3878 µm) with a Lumenera Infinity-I digital camera and Infinity Capture program version 6.1.0 (Ottawa, Ontario; Fig. 3).

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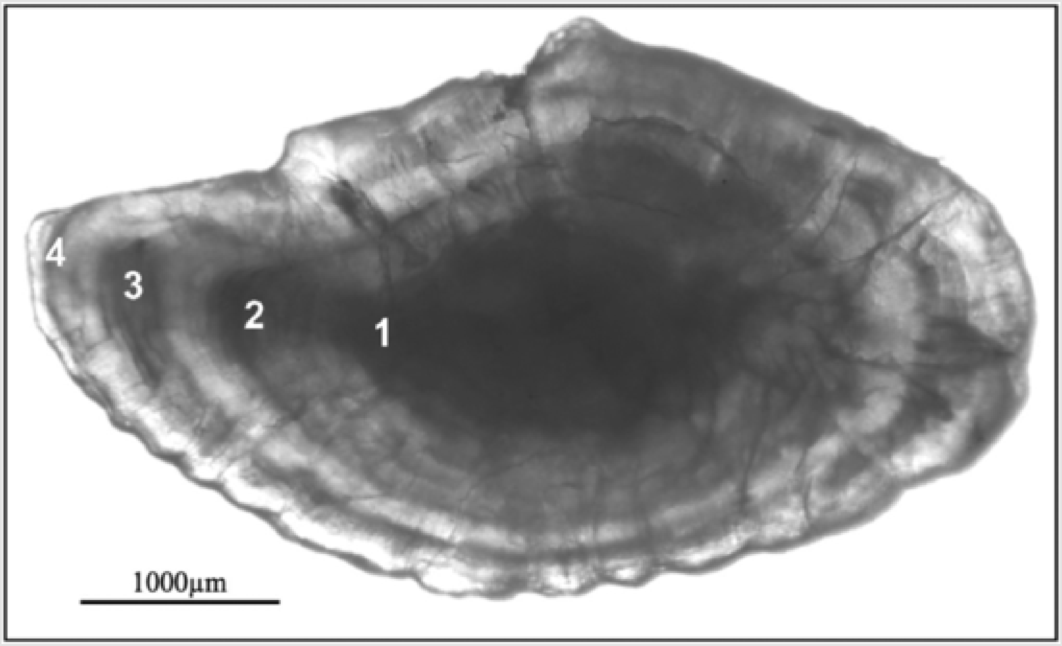

Whole sagittal otolith from an age-4 (94 mm SL) roughtongue bass (*Pronotogrammus martinicensis*). Opaque ~~band~~bandsare marked with numbers that correspond to age. This fish was captured in March 2015 with a newly formed opaque edge (age-4) and was from the 2011 year-class.

After otolith images were captured, readers were assigned a random set of otoliths to count increments. The following counting protocol for whole otoliths was developed after unsuccessful attempts to count sectioned otoliths. In the present study (N = 17) sectioned otoliths from fish ranging from 72 to 85 mm SL showed diffuse or missing opaque bands that were much clearer and distinct in whole otoliths. Initial examination of whole otoliths from small sized fish (<~~–~~90 mm SL) indicated that the first increment was formed within 1767–2271 µm diameter across the core, but as fish age increased this first band would become obscure due to increased otolith diameter. Subsequently, the maximum diameter of the first visible increment was measured, if this distance was >~~-~~2398 µm one increment count was added for an assumed obscured first increment at 1767–2271 µm diameter in larger otoliths. Also, the smallest fish (54–90 mm SL) sometimes showed an apparent false increment that was visible at 1010–1262 µm. This increment was assumed a false increment based on previous age and growth studies, i.e., the observed small size was difficult to accept as an age-2 fish (McBride et al., 2009; Thurman et al., 2004). Annuli were assumed to be deposited in winter (McBride et al., 2009; Thurman et al., 2004). Marginal increment analysis was previously used to verify that increments were formed annually (Thurman et al., 2004).

When an increment count was completed, it was used along with date of capture and determination of translucent or opaque edge band to estimate year-class. For example, under transmitted light, a fish captured in the fall of 2014 with an increment count = 4 was from the 2010 year-class. A fish captured in the spring 2015 with an increment count = 4 and a translucent edge band was also from the 2010 year-class, while a fish captured in spring 2015 with an increment count = 4 and an opaque edge band was from the 2011 year-class. Lastly, all fish captured in summer 2015 with an increment count = 4 were from the 2011 year-class. All otoliths were independently counted by two readers. Counts and year-class were then compared between readers and disagreements were reexamined by both readers. If consensus was not reached the otolith count was not used in further analyses.

~~2.4.~~2.4 Length-age relations

A length at age von Bertalanffy growth model was used to estimate growth rate for all roughtongue bass, and separately for fish from AA and RR. Parameters were estimated with the non-linear regression procedure in Statistical Analysis Software 9.4 (SAS Institute Inc., Cary, North Carolina, USA) for the equation:

Previous Version

Updated Version

where L_t = the mean standard length (mm) at age t, L_∞ = the asymptotic standard length predicted by the equation, K = the growth coefficient, and t_0 = the age at which standard length was equal to zero. Von Bertalanffy growth curves were compared between sites and between pre and post DWH oil spill with a “sums-of-squares reduction test” (Schabenberger and Pierce, 2002). The sums-of-squares reduction test compares residuals between full and reduced models to test for significant differences between nonlinear growth curves. Mean age and length (SL) were compared between sites with a *t*-test, if variances differed between sites, a Satterthwaite correction was applied. Differences were considered significant if $p \leq 0.05$ (Zar, 2010).

~~2.5.~~2.5 Mortality and survival

Linear regression of the natural log of abundance on age was used to estimate total mortality (Z) for roughtongue bass for AA, RR, and pooled over both sites. Annual survival was estimated as S = e~~-~~$^{-Z}$ (Beverton and Holt, 1957; Ricker, 1975). Year-class rather than age was used in this mortality estimation. The 2011 and 2012 year-classes were only partially collected by the fishing gear, while fish from the 2013 year-class were not collected. Mortality estimations were therefore, based on 2010 and earlier year-classes. Mortality Z (regression slope) estimates were compared between sites with analysis of covariance (ANCOVA). Mortality estimates were significantly different between sites if there was a significant interaction effect between site and age. Roughtongue bass are not caught by either sport or commercial fishers, therefore total mortality was equal to natural mortality ($Z = M$).

~~2.6.~~2.6 PAH

Roughtongue bass ($N = 171$) were randomly selected for PAH measurement from the earliest sampled season (Fall 2014) to minimize the time between collections and the DWH spill. Similar sample sizes were measured from AA ($N = 86$) and RR ($N = 85$). A modified NOAA method was used for PAH extraction (Roberts and Szedlmayer, 2020; Sloan et al., 2004). In the present study the entire fish was used for the PAH extraction, because individual tissue weights were too small for the present extraction method.

Gas chromatograph mass spectrometer (GC/MS) analysis was used to detect and identify potential PAHs after the extraction procedure of the fish samples. The limit of detection for the GC/MS (Waters GCT Premier) was 5 ppb PAH. Control blanks were used to detect and correct for analytes not associated with the sample. Control blanks were considered acceptable when PAHs measured were <~~-~~2~~x~~× the detection limit of the GC/MS (Roberts and Szedlmayer, 2020). An analyte was considered significant in a sample if the peak presented was at least 3~~x~~× the limit of detection. Peak PAH areas were quantified with Waters MassLynx version 4.1 SCN 569.

The present study tested for eight different PAHs: naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, and pyrene. Two control samples in each analysis were spiked with 10 µl of PAH solution that was at a concentration of 10 ppm, as standards for the any PAH detected in the samples and for conversion of peak areas into ppb. Total PAH and mean PAH were then calculated for each fish. Total PAH was the pooled quantities of the eight PAHs measured. If a particular PAH signal was below the limit of detection it was not included in the Total PAH estimate. Fish PAHs were compared between sites with a *t*-test. Analysis of variance was used to compare PAHs among year-class and among fish length (SL mm) (Zar, 2010).

Marine environmental PAHs are not limited to petrogenic sources (oil) and may also originate from pyrogenic sources (combustion), therefore, the present study also analyzed PAHs in fish tissues for source identification. Source of PAHs can be identified from ratios of phenanthrene to anthracene (PHEN: ANTH) and fluoranthene to pyrene (FLUO:PYRE) within fish samples. If PHEN:ANTH was >10 and FLUO: PYRE < 1 then the PAHs detected were considered petrogenic (Budzinski et al., 1997; Ke et al., 2002; Yunker et al., 2002).

~~3.~~3 Results

~~3.1.~~3.1 Life history (size, age, growth, mortality)

A total of 1090 roughtongue bass were collected and measured from the Pinnacles reef area from September 2014 to July 2015. Mean size ± SD was 92.7 ± 10.4 mm SL and ranged from 54 to 135 mm. Mean sizes of roughtongue bass from AA (mean SL = 79.3 ± 9.4 mm, N = 216) were significantly smaller compared to RR (mean SL = 96.0 ± 7.7 mm, N = 874; $t_{289} = 24.1$, $p < 0.001$; Fig. 4).

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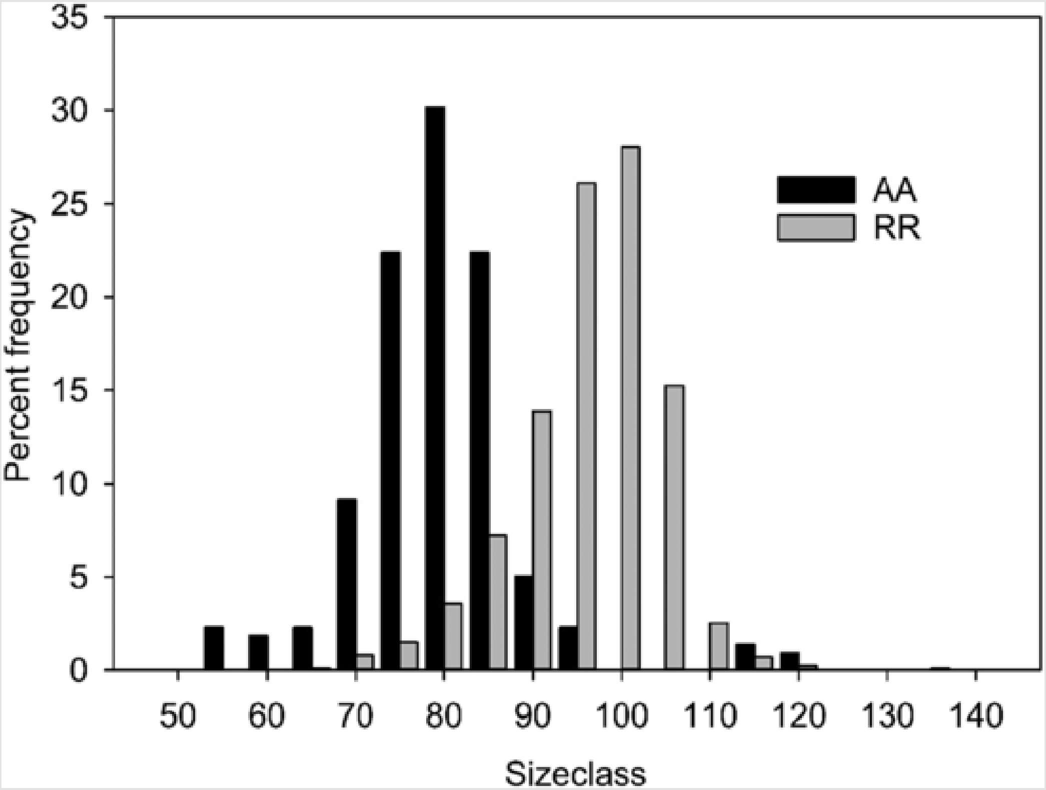

Comparison of size frequency distributions for roughtongue bass (*Pronotogrammus martinicensis*) from Roughtongue Reef (RR, *N* = 874) and Alabama Alps (AA, *N* = 216) in the northern Gulf of Mexico.

Initial otolith increment counts showed an 82% agreement between readers (*N* = 889 out of *N* = 1090 that were successfully extracted). However, upon reexamination of these increment count disagreements (*N* = 201) by both readers a consensus was reached, and all otoliths were used in final analysis (*N* = 1090). The first increment was visible in 56% of all otoliths, but the percentage was higher for younger fish and decreased with age (age ≤ 3, 87%; age-4, 64%; age-5, 44%; age-6, 36%; age ≥ 7, 8%). The most abundant age was age-4 from the 2010 year-class and few juvenile fish were collected, e.g., no age-1 and only six age-2 (Fig. 5). Roughtongue bass from AA (mean = 4.1 ± 0.76 years, *N* = 216) were significantly younger ~~that~~than those from RR (mean = 4.5 ± 0.83 years, *N* = 874; $t_{1088} = 7.7$, $p < 0.001$), but most fish were still from the 2010 year-class for both sites (Fig. 5).

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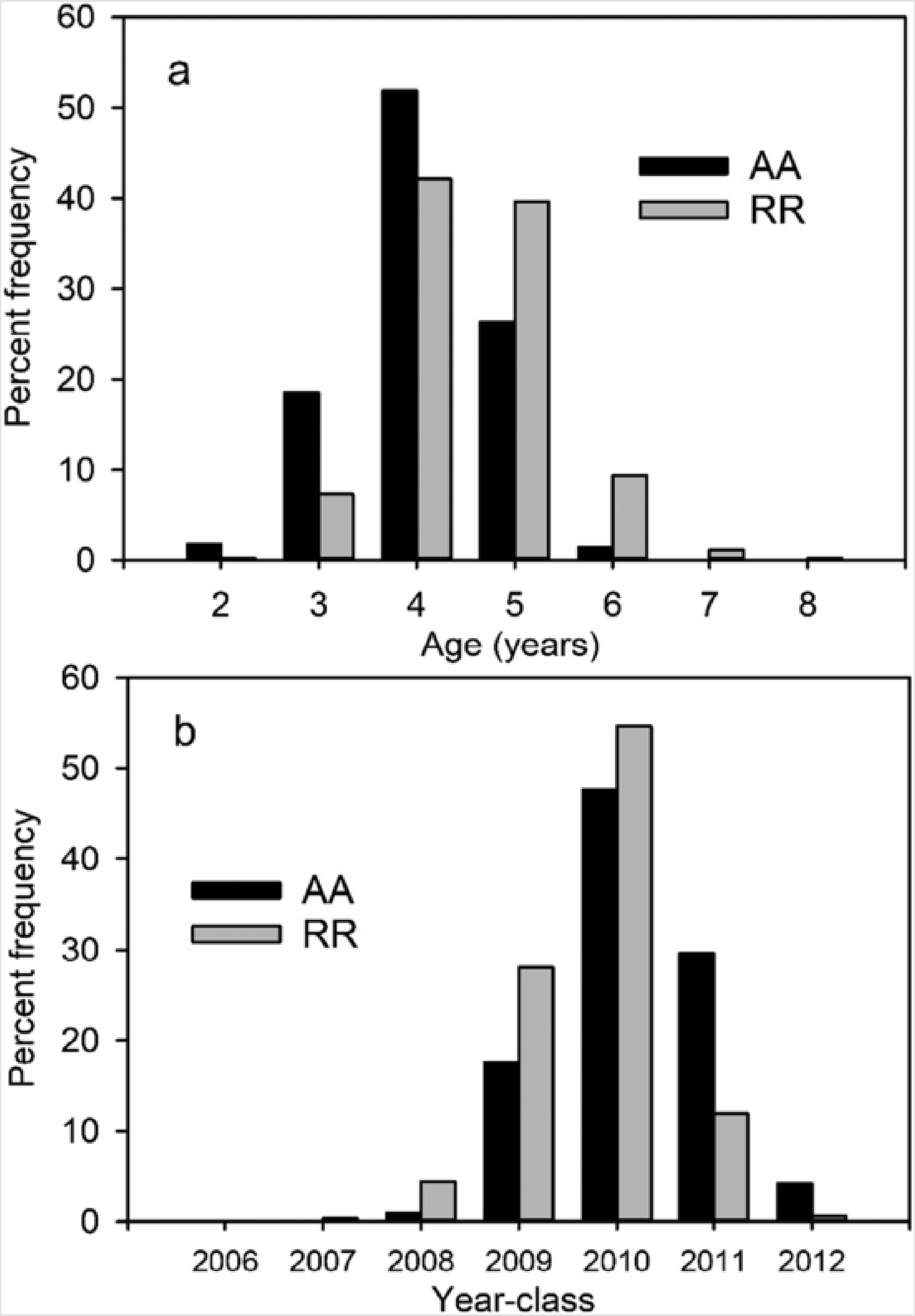

Comparison of age and year-class frequencies for roughtongue bass (*Pronotogrammus martinicensis*) from Roughtongue Reef (RR, *N* = 874) and Alabama Alps (AA, *N* = 216) in the northern Gulf of Mexico. Age frequency (a), and year-class frequency (b).

Von Bertalanffy (VB) growth rates for all roughtongue bass were similar to pre-spill collections (McBride et al., 2009) (Fig. 6). Separated by site, von Bertalanffy (VB) growth rates were significantly slower for roughtongue bass from AA compared to RR ($F_{9,6} = 52.6$, $p < 0.001$; Fig. 7).

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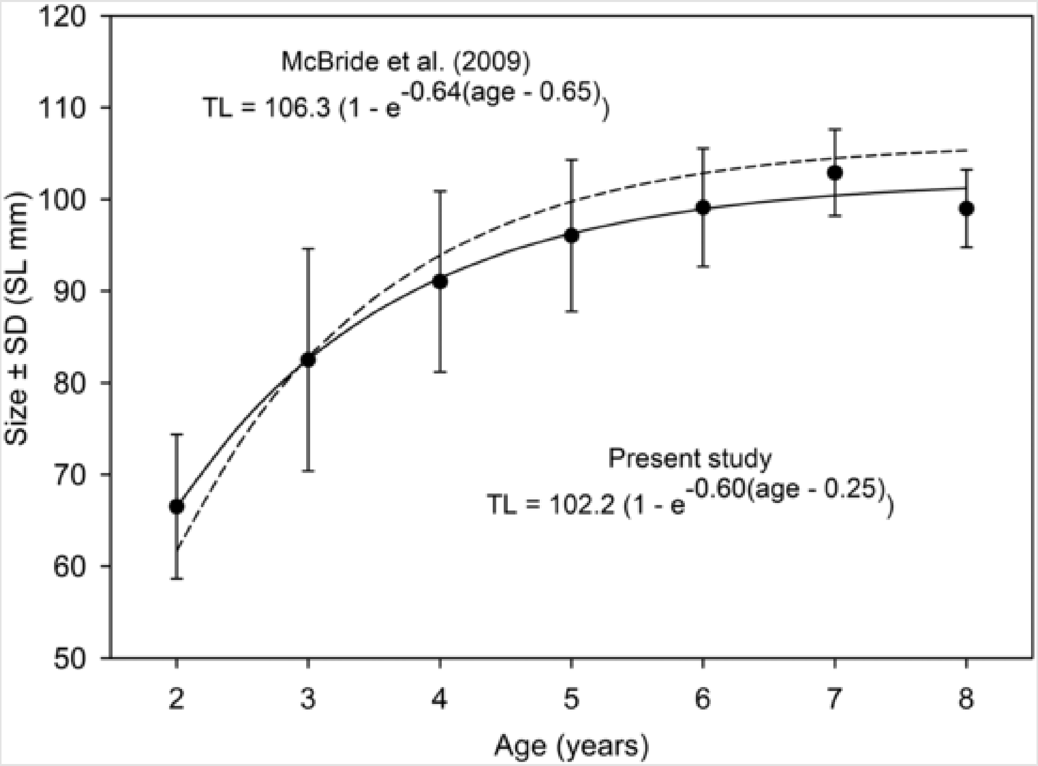

Comparison of von Bertalanffy growth curves for roughtongue bass (*Pronotogrammus martinicensis*) from the present study (solid line; pseudo-R^2 = 0.99) to fish collected before the DWH oil spill (dashed line; McBride et al., 2009). Dots and error bars = mean SL ± SD for each age from the present study.

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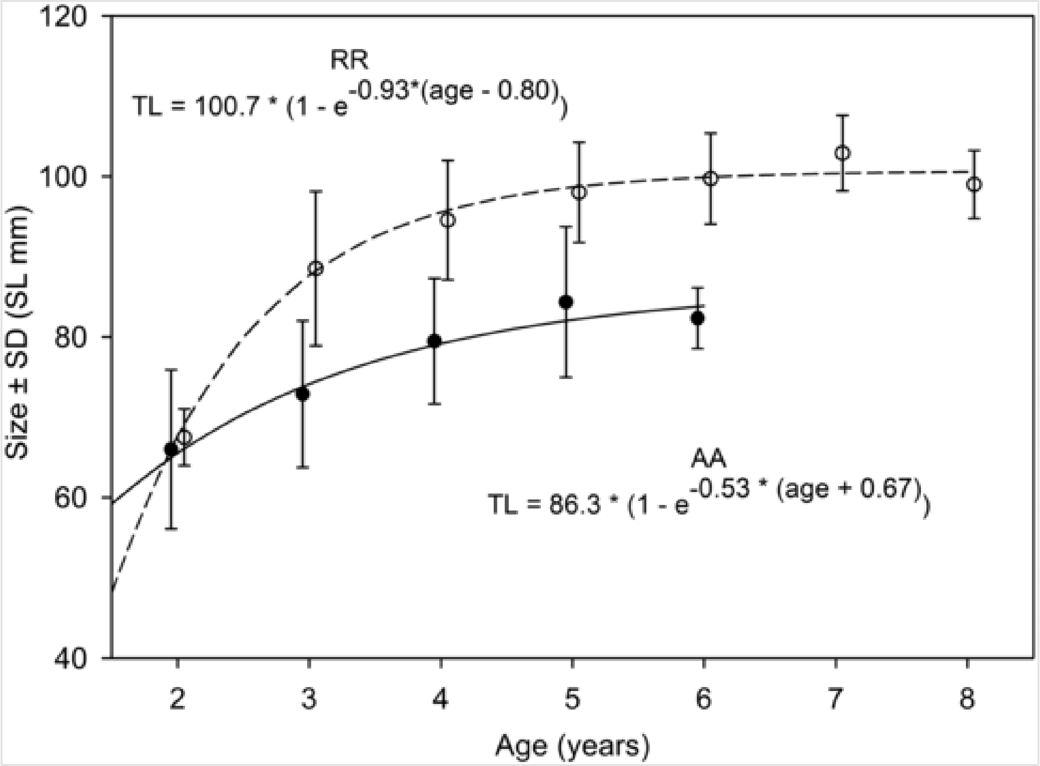

Comparison of von Bertalanffy growth curves for roughtongue bass (*Pronotogrammus martinicensis*) from Alabama Alps (AA = solid line; pseudo-R^2 = 0.96) and Roughtongue Reef (RR = dashed line; pseudo-R^2 = 0.99) in the northern Gulf of Mexico. Black dots and error bars = mean SL ± SD for AA, and open circles and error bars = mean SL ± SD for RR. Growth curves were significantly different ($F_{9,6} = 52.6$, $p < 0.001$).

Annual mortality for roughtongue bass greater than age-3 was 1.73 ($R^2 = 0.97$). Mortality rates were not significantly different between the sites AA ($Z = 1.98$, $R^2 = 0.93$) and RR ($Z = 1.67$, $R^2 = 0.97$; $F_{1,4} = 0.4$, $p = 0.56$; Fig. 8).

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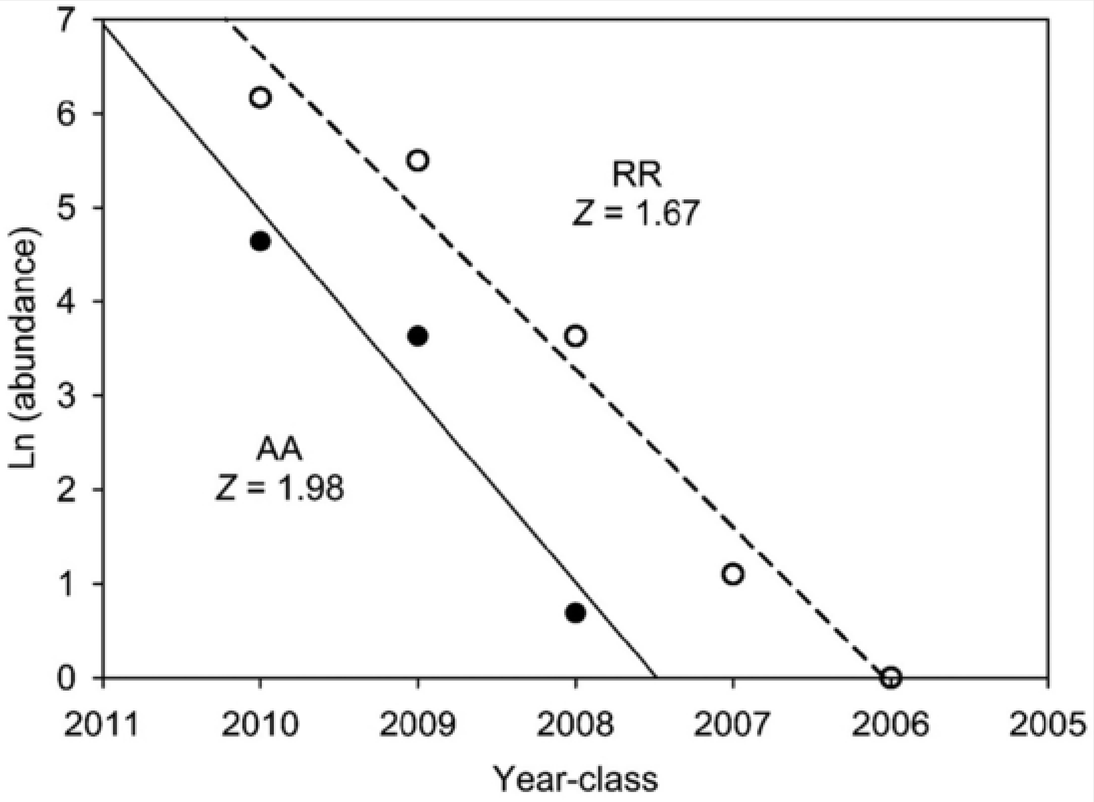

Mortality (Z) estimates for roughtongue bass (*Pronotogrammus martinicensis*) from Alabama Alps (AA = dots and solid line) and Roughtongue Reef (RR = open circles and dashed line) in the northern Gulf of Mexico.

~~3.2.~~3.2 PAH

We analyzed 171 roughtongue bass for PAHs, and 38 produced acceptable results (AA $N = 19$, RR $N = 19$). Invalid extraction procedures were indicated by the lack of PAH detections for spiked controls. Total PAH levels detected in valid extractions for individual roughtongue bass ($N = 38$) ranged from 0 to 220 ppb. Among the valid extractions 79% ($N = 30$) contained detectable levels of PAH and 21% ($N = 8$) had no detectable PAHs. The mean total PAH ± SD was 50 ± 52.6 ppb. Mean total PAH ± SD for fish from AA (42 ± 35.6 ppb) was not significantly different compared to fish from RR (59 ± 65.3 ppb; $F_{1,36} = 1.02$, $p = 0.32$).

There was a significant inverse correlation between phenanthrene and SL ($N = 38$, $r = -0.47$, $p = 0.003$). There were no significant effects of PAH types (naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene) on year-class (Fig. 9). Fluoranthene and pyrene were not detected.

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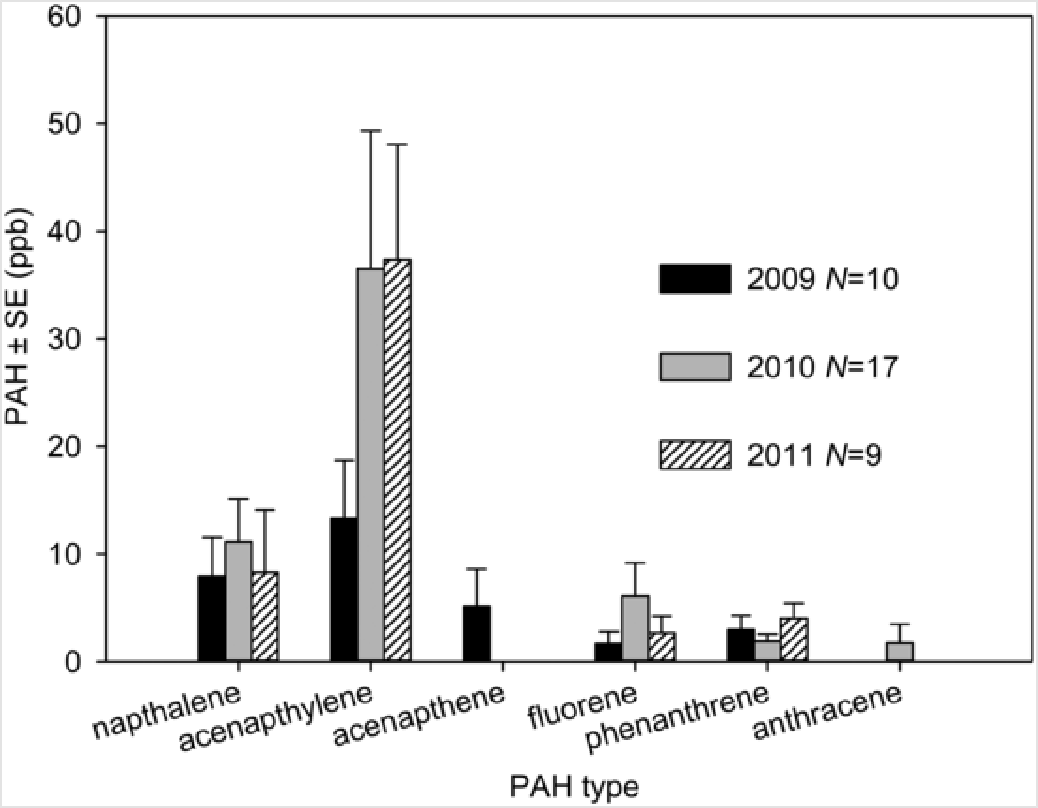

Comparisons of the total mean ± SE of different PAH types detected among roughtongue bass (*Pronotogrammus martinicensis*) year-class. No significant differences were detected for any PAH type among year-class (ANOVA, $p > 0.05$).

There were no significant correlations between total PAH and SL (mm) for both sites combined ($N = 38$, $r = 0.14$, $p = 0.40$), or separated by site (RR, $N = 19$, r = ~~-~~ −0.04, $p = 0.86$; AA, $N = 19$, $r = 0.18$, $p = 0.45$; Fig. 10). There were also no significant differences in total PAH by year-class ($F_{3,33} = 1.40$, $p = 0.26$; Fig. 10).

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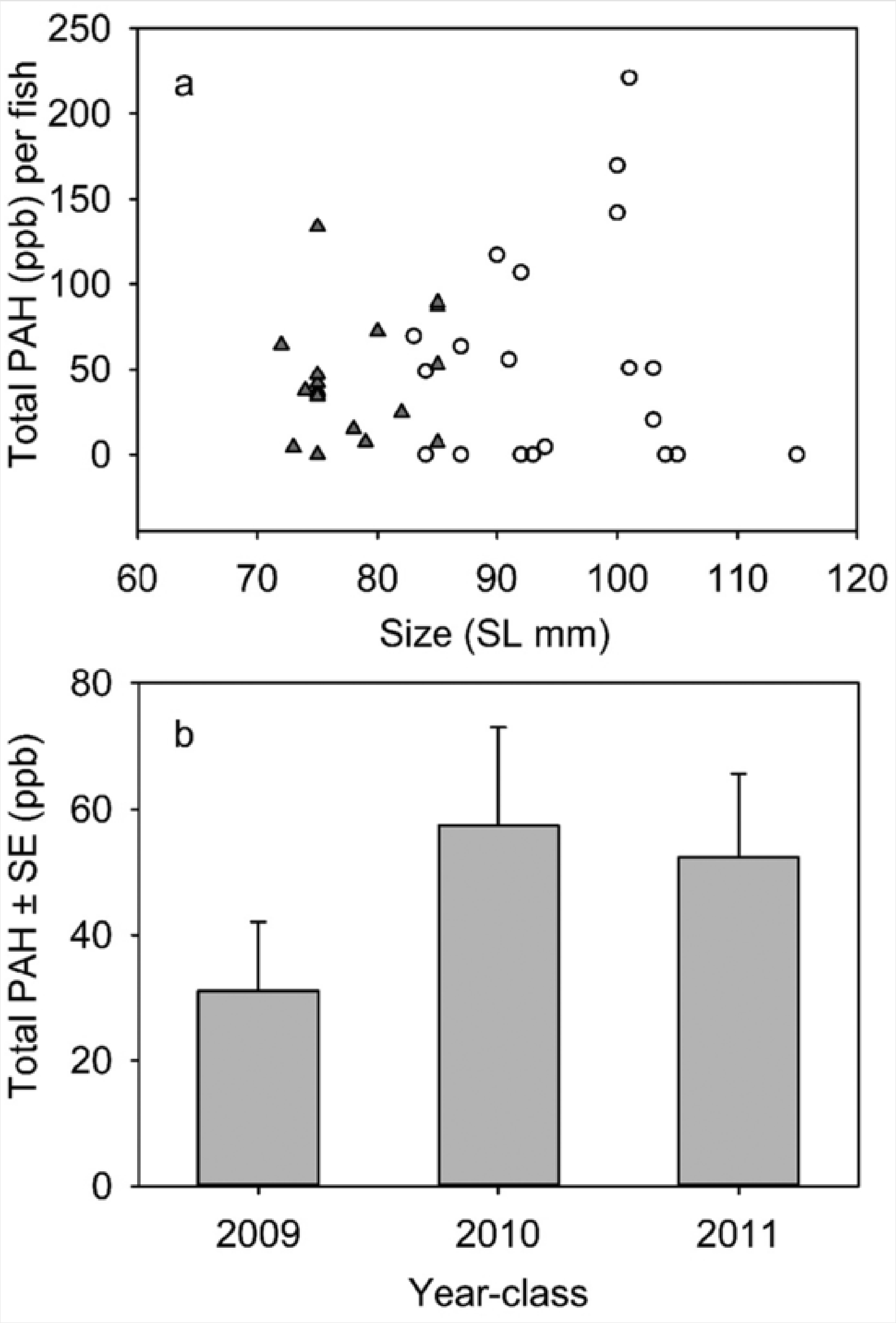

Comparison of total PAH level to fish size, Roughtongue Reef = triangles, Alabama Alps = circles (a) and mean total ± SE of PAH per year-class (b) for roughtongue bass (*Pronotogrammus martinicensis*) from the Pinnacle reefs in the northern Gulf of Mexico.

~~4.~~4 Discussion

~~4.1.~~4.1 Size and age

The size frequency distributions of roughtongue bass in the present study were similar to previous studies from the same study sites (McBride et al., 2009; Thurman et al., 2004). Size ranges were similar, but truncated (55–135 mm SL) compared to previous studies (31–143 mm SL, Thurman et al., 2004; 15–143 mm SL, McBride et al., 2009). The size ranges in these earlier studies were greater most likely because they used multiple sampling methods (hook-and-line and trawls) compared to only hook-and-line in the present study.

Age frequencies in the present study were also similar to pre-spill studies in the same locations. The modal age-4 in the present study was the same as in Thurman et al. (2004), but one year older than age-3 in McBride et al. (2009). In contrast, maximum age differed among studies: maximum age was 8 years in the present study, 9 years in Thurman et al. (2004) and 15 years in McBride et al. (2009). These differences in maximum ages between pre-spill and post-spill were again likely due to sampling and otolith analysis methods. The present study and Thurman et al. (2004) both used whole otoliths for increment counting, while McBride et al. (2009) used sectioned otoliths. Sectioned otoliths that are clear will provide a more accurate estimate of maximum age (McBride et al., 2009), but most sectioned otoliths in the present study showed difficulties with viewing increments. Whole otolith increments were clear and age comparisons among different readers were consistent (82% agreement on independent counts). The first increment was visible in 56% and obscured in 44% of all otoliths, and there was an increasing percentage of obscured first increments with age. It might be argued that sectioned otoliths are more appropriate for older aged fish. However, the present study accounted for these obscured first increments by measuring the diameter of the first increment and adding one when this measure exceeded 2398 µm (i.e., the first increment at 1767–2271 µm was obscured).

Roughtongue bass year-classes were dominated by the 2010 year-class (age-4; Fig. 5). This 2010 year-class dominance was the exact opposite of what would be predicted if there was a significant effect of the DWH oil spill in 2010. The lack of year-class failure or any indication of reduction in 2010 implies that the DWH spill had little effect on roughtongue bass early life-stage survival.

There are several possible explanations why these young of year were unaffected. It is possible that roughtongue bass from the Pinnacles reefs had minimal exposure to DWH oil, i.e., the oil did not reach the sampling sites. This explanation is consistent with a study performed by Silva et al. (2016) that showed sediment samples from 2010 and 2011, from the same sites as the present study had only slight increases in total PAH and no significant differences. In fact, the sediment samples analyzed by Silva et al. (2016) showed that PAH sediment detections in some samples actually had lower total PAH values in 2010 and 2011 than what were present in 2000. This indicated that little to no oil exposure from the DWH spill occurred at the Pinnacle reef sample sites in the present study.

Another more complicated explanation is that the roughtongue bass recruited from distant unoiled areas. Abundant populations of roughtongue bass have been identified in surrounding regions at much greater distances from the DWH spill site compared to the Pinnacle reefs, and most likely these distant areas were not exposed to oil in 2010 (Nuttall et al., 2014). Also, small numbers of roughtongue bass larvae have been collected in ichthyoplankton surveys of the loop current, which was largely uncontaminated by DWH oil spill (Liu et al., 2013; Richards et al., 1993). Distant populations and planktonic larvae both open the possibility that roughtongue bass on the Pinnacle reefs may have recruited from areas distant from the DWH oil spill. However, there are many unanswered questions that need to be addressed for this remote recruitment to function and at the time of the present study there was little information on the early life history or movement patterns of roughtongue bass.

Natural biological resilience due to conditioning could also help to explain the abundance of the 2010 year-class, as resident fish may have already been resistant to PAH exposure. Petroleum has been present in the Gulf of Mexico from other sources prior to the DWH event. There are natural petroleum seeps in the Gulf of Mexico as well as land runoff sources and atmospheric deposition (Latimer and Zheng, 2003; National Research Council, 2003). Thus, it should be recognized that these fish and their environments have most likely been previously exposed to petroleum and its derivatives for some time, and may have the genetic capability to metabolize petroleum sources and PAHs more readily without experiencing negative effects (National Research Council, 2003). For example, mummichog (*Fundulus heteroclitus*) from chronically polluted sites showed heritable resilience to PAH toxicity and were also resistant to multiple insecticides (Clark et al., 2012; Meyer and Di Giulio, 2002). Similarly, another study on the DWH spill concluded that preadaptation to oil exposure was the reason for not detecting differences in fish species composition, abundance, or size from oiled versus non-oiled sites (Able et al., 2015). This resilience would likely need to be present in roughtongue bass larval stages to explain the 2010 year-class dominance, if in fact they were exposed to excess oil. At the time of the present study it was difficult to validate an oil resistant hypothesis especially for roughtongue bass as they were difficult to retrieve from deep colder waters, keep alive, and hold captive for oil exposure studies.

~~4.2.~~4.2 Site differences in growth rates

Growth rates in the present study were also similar to previous studies (McBride et al., 2009; Thurman et al., 2004). Variance around the VB growth relation in the present study (pseudo-$R^2 = 0.99$), was less than a previous growth relation with $R^2 = 0.49$ (McBride et al., 2009). This reduced variance here may be that mean lengths at age were used in the calculation of the VB relation, which is a typical approach, but details of regression were not reported in McBride et al. (2009). However, this earlier VB growth curve reported by McBride et al. (2009) was still within one SD of the present study curve (Fig. 6).

Roughtongue bass growth rates were significantly different between AA and RR (Fig. 7). Similarly, Thurman et al. (2004) also detected slower growing roughtongue bass from AA. In the present study, collection methods were the same at both sites, ruling out gear selectivity as a possible cause. There are several possible factors that may explain growth rate site differences between AA and RR. Growth rate differences in fish can result from changes in prey type or other environmental habitat characteristics (Weatherly and Gill, 1987). In the present study there are several environmental factors that potentially differ between sites. The Mississippi River outflow is likely a major factor due to its much closer proximity to AA compared to RR. The Mississippi River is one of the top ten largest rivers in the world and carries sediment in quantities of 150~~x~~ × 10^9 kg~~-~~–$^{\mathrm{yr}}$ that are then deposited into the Gulf of Mexico (Dagg and Breed, 2003; Meade, 1996). The closer proximity of AA (68 km) to the Mississippi River outflow likely caused reduced water quality for roughtongue bass that inhabit AA compared to RR that is further away (142 km). The Mississippi River plume has been shown to extend out 60 km to the northeast of the Pass-a-Loutre, which would put AA near the boundaries of the sediment outflow from the Mississippi River (Walker, 1996). In support of this contention, ROV video surveys during September~~-~~–October 2014 showed that AA were much more turbid, with a substantial sediment layer (that appeared about 5 cm in depth) compared to RR (Szedlmayer, unpublished data). Similarly, previous studies have reported silt deposition up to 0.5 m at the same AA study sites as the present study (Continental Shelf Associates Inc. Texas A&M University, 2001). Roughtongue bass are visual feeders, and reduced visibility alone can significantly decrease feeding success in fish (Kestemont and Baras, 2008). Also, increased sediment suspension and eutrophication have been specifically documented to harm corals by reducing light penetration, smothering, and increasing competition for space for filter feeders (Gabrica and Bell, 1993). For example, a reduction of coral encrusting communities was shown with closer proximity to riverine inputs in Rio Bueno, north Jamaica (Mallela, 2007) and decreased coral taxa has been linked to increasing proximity to the Mississippi River at the same sites as the present study (Continental Shelf Associates Inc. Texas A&M University, 2001). These types of structures provide shelter to roughtongue bass and their reduction would increase predation rates as well as reduce foraging time, feeding success, and subsequently cause reduced growth rates.

In addition to reduced water clarity caused by the Mississippi River outflow, there is also nitrogen loading and phosphorus influx due to fertilizers and freshwater volumes of 350 km^3 per year (Dagg and Breed, 2003; Meade, 1996). The Mississippi River is responsible for 1.8~~x~~ $\times 10^9$ kg of nitrogen entering the Gulf of Mexico each year (Dagg and Breed, 2003; Howarth et al., 1996). This nitrogen loading results in eutrophication of waters and ultimately causes hypoxia events and dead zones in the Gulf of Mexico (Rabalais et al., 2001, 1996). Reduced oxygen has been shown to cause decreases in growth (Kestemont and Baras, 2008). Steady increase in fertilizers and nutrient inputs in the last 50 years has coincided with increasing dead zones in the Gulf of Mexico (Galloway et al., 2004; Goolsby et al., 2001; Rabalais et al., 2001, 1996). In addition, freshwater influx from the Mississippi River has been shown to effectively reduce salinity over the entire Gulf of Mexico (Dagg and Breed, 2003; Grimes and Finucane, 1991). Salinity variation away from the optimal ranges has been shown to affect fish growth rates (Kestemont and Baras, 2008). Again, closer proximity of AA to the Mississippi River outflow suggest that this site may experience lower oxygen levels and lower salinity levels at more frequent intervals than RR, which ultimately could have reduced growth rates at AA.

A final environmental factor to consider is presence of chemical contaminants. Exposure to contaminants can cause an organism to expend energy otherwise used for growth in attempting to eliminate or avoid certain toxins (Kestemont and Baras, 2008; Meador et al., 2006). Additionally, some toxins may inhibit the uptake of certain nutrients in food sources that can further reduce growth rates (Kestemont and Baras, 2008). Again, proximity to the Mississippi River outflow, which contains runoff from 3.2~~x~~ $\times 10^9$ $\text{km}^2$of the United States is likely an overwhelming source of chemical contaminants in the Gulf of Mexico (Howarth et al., 1996; Meade, 1996). Studies in estuaries have shown that there is an extensive list of contaminants in riverine discharge that are capable of causing deleterious effects to marine organisms (Kennish, 1991). When tested for contaminants the Mississippi River outflow showed evidence of substantial concentrations of wastewater sewage, agriculture, and industrial point source contaminants (Meade, 1996). Within these groupings are specific contaminants such as alkyl benzene sulfonate and ammonia, which have been shown to cause reduced swimming capability and reduced growth in juvenile rainbow trout (*Oncorhynchus mykiss*) (Hofer et al., 1995). Again, due to closer proximity to the Mississippi River outflow, reef fish residing at AA may receive greater exposure to such contaminants compared to RR.

~~4.3.~~4.3 Mortality

Roughtongue bass mortality (Z) was 1.7 in the present study and greater than a previous estimate of 0.5 (McBride et al., 2009), but similar to an earlier estimate of 1.3 (Thurman et al., 2004). It is likely that mortality and survival differences observed in the present study compared to these previous studies were at least in part due to differences in otolith increment counting methods. The present study used whole otoliths for aging and spent a considerable amount of time and effort in establishing a method and validating counting precision. As part of that effort it was determined that roughtongue bass otoliths would often show a false increment (2 opaque bands in the first year). If these false increments were counted in previous estimates it would result in reduced mortality rates. In previous studies both sectioned otoliths (McBride et al., 2009) and whole otoliths (Thurman et al., 2004) were used but neither study reported a false first increment. Thus, differences in mortality rates between the present and previous studies were likely due to otolith counting methods rather than pre-spill versus post-spill mortality rates. Due to the difficulty in counting sectioned roughtongue bass otoliths observed in the present study, we do not recommend sectioning otoliths for future age estimation of roughtongue bass.

~~4.4.~~4.4 The DWH spill and PAH

An important question to consider is the effect of the DWH oil spill. The sample site AA was closer (54 km) to the spill site than RR (111 km), increasing the possibility of oil exposure. There is some evidence that levels of PAH in sediments were higher at AA compared to RR, but significant differences were not detected (Silva et al., 2016). The present study detected PAH levels in roughtongue bass with a maximum of 220 ppb in one fish, but no significant difference between AA and RR. Unfortunately, PAH baseline levels and thresholds for toxicity in roughtongue bass were not available. However, PAH levels in the present study were below the minimum level of 300 ppb that had any effect on organisms in the marine environment (US EPA, 1987). This EPA minimum is 70 ppb greater than the highest recorded total PAH content for a single roughtongue bass, and 250 ppb above the mean total PAH content in all roughtongue bass in the present study. However, more recent studies have indicated that PAH concentrations in water at 1~~-~~–15 ppb can cause substantial growth and cardiac developmental issues in fish larvae (Carls et al., 2008; Incardona et al., 2015, 2014). Despite these studies, there were no indications that PAH levels in the present study affected roughtongue bass growth rates or caused year-class failures, which would be expected if PAHs were affecting reproductive or developmental ability in this fish species.

Although PAH levels within roughtongue bass in the present study were all very low and unlikely to affect ~~developmental~~development, it is worth considering the position of roughtongue bass within the trophic chain. Roughtongue bass as mentioned earlier are prey species for many larger predatory fish found near the Pinnacles (Weaver et al., 2002). As a result, even if they carry low levels of PAH, roughtongue bass may be a source for PAH accumulation and biomagnification in larger fish species (van der Oost et al., 2003).

However, an important caveat in the examination of PAH effects are the biological limitations in measuring oil exposure in fish. PAHs are rapidly metabolized by most fish and are often out of the system before a fish can be examined (Altenburger et al., 2003; van der Oost et al., 2003). Therefore, PAH detection or lack of detection may not adequately represent actual fish exposure to oil. Additionally, when PAH levels begin to fall below ppm and are at ppb levels their detection is difficult and often inconsistent. For example, a change in GC/MS analysts or machine variation can result in variations > 200 ppb. The present study encountered difficulties in detection of PAHs and after extracting and analyzing 171 roughtongue bass samples, only 38 were considered usable based on adequate detection of PAHs in spiked control samples. The difficulty in detecting low levels of PAH affected the present attempts to analyze PAH ratio values for source identification. In this attempt only one fish had detectable levels of both phenanthrene and anthracene, and although the ratio indicated a non-petrogenic source, results were inconclusive due to the small sample size. Similar difficulties and variations were also apparent in the study performed by Silva et al. (2016) that showed several inconsistencies in PAH detections. Thus, the PAH levels detected here as well as other studies may be compromised due to this inherent variation when measuring at ppb levels and results should be interpreted with caution. An alternative method is the analysis of PAH biliary metabolites as biomarkers of exposure and should be considered in future oil exposure assessment in roughtongue bass (van der Oost et al., 2003).

The present study provided important ecological information on a little studied reef fish species, roughtongue bass, from mesophotic reef habitats after the DWH oil spill. This species showed a dominant 2010 year-class that appears to have recruited the same year as the DWH spill. Also, post-spill growth rates were similar compared to pre-spill studies. There were significant growth rate differences detected between sites, but these were more likely linked to Mississippi River discharge rather than the DWH oil spill. Different mortality rates between pre-spill studies and the present study were most likely linked to otolith aging methods rather than actual differences in mortality rates. Detected PAH levels in roughtongue bass tissue were well below toxicity levels, but these low levels should be interpreted with caution due to the difficulty of measuring contaminants at the ppb level. Based on the dominant 2010 year-class, similar pre-spill to post spill growth rates, and the low levels of PAH, the present study indicates that there was little effect of the DWH oil spill on roughtongue bass from these mesophotic reef habitats.

Authors' contributions

Both authors contributed equally to all aspects of this study.

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Compliance with ethical standards

All applicable international, national, and institutional guidelines for the care and use of animals were followed. All work was carried out under Auburn University Animal Care and Use Committee approval under Protocol 2013-2251.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Queries
0
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