Polychlorinated Biphenyls and Organochlorine Pesticides as Intrinsic Tracer Tags of Foraging Grounds of Bluefin Tuna in the Northwest Atlantic Ocean

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Abstract

Researchers have utilized chemical fingerprints in the determination of habitat utilization and movements of the aquatic animals. In the present effort, we analyzed polychlorinated biphenyl (PCB) congeners and organochlorine pesticides in the samples of juvenile bluefin tuna caught offshore of Virginia, and in larger bluefin tuna from the Gulf of Maine and near Nova Scotia. For a given specimen, or a given location, PCB concentrations were highest, followed by DDTs, and chlordanes. Average contaminant concentrations from fish captured from the three locations were not significantly different; and PCBs, DDTs, and chlordanes correlated well with each other. Trans-nonachlor/PCB 153 ratios in bluefin tuna of eastern Atlantic (i.e., Mediterranean) origin

are low compared to the corresponding ratios in fish in the western Atlantic. As the former migrate to the western Atlantic, these ratios gradually turnover due to the accumulation of biomass from forage contaminated with higher trans-nonachlor/PCB 153 ratio reflecting dissimilar use of chlordane pesticides on two sides of the Atlantic Ocean. The trans-nonachlor/PCB 153 ratio indicated that one juvenile bluefin tuna from offshore of Virginia and one large bluefin tuna from Gulf of Maine in the present study originated from foraging grounds in the Mediterranean Sea, and that they have made the trans-Atlantic migrations. The remaining individuals were determined to be either spawned in the Gulf of Mexico or the trans-nonachlor/PCB 153 ratio for the putative Mediterranean bluefin tuna was completely turned over to resemble the ratio characteristic to the western Atlantic. Based on the turnover time for trans-nonachlor/PCB 153 ratio previously determined, the residence time of juvenile bluefin tuna offshore Virginia was estimated to be at least 0.8 to 1.6 years. A discriminant function analysis (DFA) plot of total PCB normalized signatures of PCB congeners showed three separate clusters, which suggested that bluefin tuna from offshore Virginia, Gulf of Maine, and Nova Scotia could have had extended residences and foraging within the areas of capture to be able to sustain the stable signatures of PCB congeners. The DFA cluster results supported the concept of metapopulation theory of spatial ecology comprising discrete aggregates of local populations of bluefin tuna where the desired prey species are likely to be abundant. Despite their highly migratory trait and endothermic advantage of foraging in broader and colder habitats, the movements and mixing across the aggregation ranges related to feeding did not appear to be extensive. Advancement in the understanding of bluefin tuna population dynamics beyond the coarse concept of trans-Atlantic migrations to the

metapopulation hypothesis provides a novel exploratory tool in the stock assessment and resource management. As the chemical tracer tags are fortified naturally and document the foraging history, they promise to serve as the low-cost alternatives to the high-cost electronic data recording tags employed for addressing the migratory movements of bluefin tuna. Between the different potential chemical tracer tags, a distinct advantage of PCB/pesticide analysis over the otolith micro-constituent analysis is that the muscle tissue of a given individual bluefin tuna can be sampled repeatedly for PCB/pesticide analysis over different spatial and temporal scales in a non-lethal manner.

Introduction

North Atlantic bluefin tuna (*Thunnus thynnus*) is a prized species important to both the recreational and commercial fisheries. It is distributed from subtropical to subarctic regions across the North Atlantic (Mather et al., 1995; Fromentin and Powers, 2005). The species is subdivided into two units: a northwestern stock breeding in the Gulf of Mexico and an eastern stock breeding in the Mediterranean Sea (Carlsson et al., 2007; Boustany et al. 2007). Magnuson et al. (1994) estimated the population size of the latter to be at least an order of magnitude larger than the former. International Commission for the Conservation of Atlantic Tuna (ICCAT) regulates the bluefin tuna landings based on an assumption of a two stocks separated at the 45ºW Meridian and negligible intermixing (Lutcavage 1999; Rooker et al. 2007). Declines in the putative northwestern Atlantic population resulted in the reduction in the catch quota in the northwestern management unit, originally, without equivalent actions in the eastern management unit (Sissenwine et

al., 1998). The northwestern Atlantic population is showing signs of recovery, and there has been progress in the recent years in controlling the exploitation rates of the eastern Atlantic management unit (ICCAT 2014).

The results of tagging studies and the analyses of chemical markers in the otoliths and soft tissues indicate that bluefin tuna undergo extensive and complex migrations, including the trans-Atlantic movements; and up to 57% of the individuals comprising the eastern and northwestern Atlantic stocks may cross the ICCAT stock boundary line at some point in their lives (Lutcavage et al., 1999 and 2001; Block et al., 2001 and 2005, Rooker and Secor 2004; Rooker et al. 2003; Rooker et al. 2008; Dickhut et al., 2009; Graves et al., 2015). Stable isotope data indicate that the occurrence of eastern bluefin tuna in the Mid-Atlantic Bight decreases with increasing size, and that larger bluefin tuna present in Gulf of Maine and Gulf of St. Lawrence in the northwestern Atlantic are almost entirely of northwestern origin (Rooker et al., 2008). However, if the eastward dispersive behaviors of adolescents from northwestern population across the 45°W management boundary occurs at rates observed for adolescents from eastern population, the smaller and less productive northwestern population will be disproportionately affected by higher fishing rates in the eastern management zone (Rooker et al., 2008). This, in turn, can negatively impact the stability and future commercial viability of this species.

Because of differential application practices of organochlorine pesticides in Europe and North America, the origin of a given bluefin tuna can be assigned to the eastern or

northwestern origin by using the distinctive embedded ratios of certain PCB congeners and the organochlorine pesticides (Dickhut et al., 2009). Trans-nonachlor/PCB 153 ratios in bluefin tuna of eastern origin are low compared to the corresponding ratios in bluefin tuna of northwestern Atlantic origin. As the bluefin tuna of Mediterranean origin migrate to the northwestern Atlantic, these ratios gradually turnover due to their foraging in the northwestern Atlantic and the cumulative accumulation of biomass tagged with the higher trans-nonachlor/PCB 153 ratios. The origin of a given bluefin tuna can therefore be determined as Mediterranean if the trans-nonachlor/PCB 153 ratio is less than 0.164, and vice versa (Dickhut et al., 2009).

There are several hypotheses relevant to the bluefin tuna stock structure and movements. The prevailing “Overlap Model” focuses on the natal homing of the spawning stock, but with a high degree of overlap in the feeding areas on an annual and/or ontogenetic basis (Secor 2001). Based on otolith chemistry, Rooker et al. (2008) reported that the giant bluefin tuna collected from Gulf of Maine and Gulf of St. Lawrence were 94.8% and 100% of northwestern origin, and Secor et al. (2014) reported that bluefin tuna spawning in the Gulf of Mexico exhibited 100% natal homing, regardless of the sampling period. Similarly, Rooker et al. (2014) reported that bluefin tuna collected at the entrance to the Strait of Gibraltar (eastern Atlantic Ocean) and from several regions within the Mediterranean Sea (Balearic Islands, Malta, and Sardinia) were 100% eastern Atlantic fish. These observations indicate that natal homing is well developed in both populations. The alternate “Diffusion Model” is based on an undefined degree of mixing in which the trans-Atlantic migrants become expatriates, joining the alternate population (Secor 2001).

In this approach, bluefin tuna spawned in one area can spawn in the other. The "Diffusion Model" appears to be of only minimal importance based on the near 100% natal homing behaviors observed in individuals from both populations. However, based on the spawning behavior of Pacific bluefin tuna in the ocean basin and in two marginal seas, another spawning region outside the Gulf of Mexico/Florida Straits and the Mediterranean Sea cannot be ruled out (Secor 2001). Supportive of this idea, using popup satellite archival tags deployed on the adult Atlantic bluefin tuna off the coast of Nova Scotia and on the Georges Bank (northwestern Atlantic Ocean), Galuardi et al. (2010) found that during the assumed spawning period only 56% of the tagged fish occupied a known spawning area, while 44% were located in the distant oceanic regions. These observations are inconsistent with the notion of spawning site fidelity exclusively to the Gulf of Mexico. The authors also noted that the consideration of alternate spawning strategies and the recognition of a complex stock substructure may yield a more realistic view of the bluefin tuna population dynamics and could enhance the fisheries management rebuilding efforts.

The strong annual numerical index on a given side of the Atlantic Ocean, driven by the migratory behavior based recruitment differences, can result in an over-estimation of the past stock abundance, and perhaps erroneous assessments of future recovery (Secor 2001). We therefore argue that the simple northwestern Atlantic or Mediterranean characterization of bluefin tuna would be far too coarse to elucidate the stock dynamics in ways needed for the development of effective management strategies (Kritzer and Sales 2004). The primary goal of our study was to test the null hypothesis that no further fine-

scale, geographical groupings of bluefin tuna exist beyond the simple northwestern Atlantic or Mediterranean designations. We therefore examined the signatures of select polychlorinated biphenyl (PCB) congeners and organochlorine pesticides in bluefin tuna caught in the northwestern Atlantic (juveniles captured offshore of Virginia, and larger individuals from Gulf of Maine and Nova Scotia). We first computed the ratios of PCB congeners and organochlorine pesticides in individual fish and compared them to the corresponding ratios reported by Dickhut et al. (2009) for young-of-the-year (YOY) bluefin tuna from Mediterranean Sea and offshore Virginia. We then assigned feeding areas of individual bluefin tuna based on the pesticide/PCB ratio guidelines (Dickhut et al., 2009). Lastly, we performed discriminant function analysis (DFA) of the total PCB normalized signatures of PCB congeners to either support or refute the null hypothesis on the concept of metapopulation behavior of north Atlantic bluefin tuna.

Materials and Methods

Like many other non-polar organic compounds, PCBs and organochlorine pesticides are hydrophobic in nature, and they do tend to naturally bioaccumulate in the lipid-rich tissues. We therefore conceptually decided to analyze the lipid-rich liver samples of bluefin tuna, with the expectation that we will have the best success in detecting PCBs and pesticides if the bluefin tunas were indeed exposed to these chemical (for example, Stefanelli et al. 2002). However, as the study progressed, we received more muscle samples than the liver samples. We therefore adjusted our analytical protocols to include muscle as a target tissue in the subsequent analyses. Since we had planned to use the

ratios of analytes and not the absolute concentrations, it was assumed that the tissue type will not introduce significant bias in the analyses. We assumed that the ratios of lipids and analytes, and the ratios among different analyte types in the muscle and liver tissue, would be approximately similar (Dickhut 2009).

Samples of liver tissues of juvenile bluefin tuna were obtained from the fish landed by a local sport fishing fleet at Wachapreague on the Eastern Shore of Virginia (Figure 1) in 2004. Actual capture sites and physical measurements were not available for these specimens. Based on the rod and reel sport fishing in the area (Turner et al. 1993), the sampled bluefin tuna were thought to be most likely from the 4.5 - 13.6 kg juvenile fish caught from approximately 30-50 km offshore. Gender could not be readily identified as the gonads of the juvenile bluefin tunas were not fully developed.

Samples of muscle tissues of larger bluefin tuna from the Gulf of Maine and Nova Scotia were obtained in 2006 either from specimens caught by purse seine or hook-and-line. These specimens were originally caught from June to October in 2004 and from June and July in 2005. Length, weight, and gender information were not available for some specimens. However, being the commercially landed fish, all bluefin specimens from the Gulf of Maine and Nova Scotia were likely larger than 185 cm fork length which is the minimum legal size. When available, the specimens length ranged from 188 - 290 cm and weights ranged from 84 - 294 kg. The sample pool contained both male and female specimens.

Liver samples of bluefin tuna caught from offshore Virginia, and muscle samples of bluefin tuna caught from the Gulf of Maine and Nova Scotia, were analyzed for the select sets of PCB congeners and organochlorine pesticides following the procedures of Deshpande et al. (2002), with some modifications. Briefly, the twelve samples of liver tissues were freeze-dried and extracted with methylene chloride in the two extraction batches by using Organomation Soxhlet extraction apparatus (ROT-X-TRACT). Fifty seven samples of muscle tissues were separately dried with hydromatrix and extracted in three extraction batches using Dionex Accelerated Solvent Extractor (ASE-300). Method surrogate standards were added to each sample before extraction. These standards included 4,4-dibromooctafluorobiphenyl (DOB), ronnel and, PCB 198, and they were carried through the entire method. Twenty percent of the extract was used for the gravimetric determination of the lipids. Bulk interfering components in the remaining extracts were removed by using the silica-alumina-florisil glass column chromatography. Each extract was further purified on the Phenomenex styrene-divinylbenzene polymeric (Phenogel), size-exclusion, high performance liquid chromatographic (HPLC) column. HPLC internal standards were added to each sample before the HPLC cleanup step. These standards included 1,2,3-trichlorobenzene (TCB) and PCB 192. After the HPLC cleanup, the solvent was exchanged from methylene chloride to hexane. Samples were first screened for the analyte levels, and then depending upon the analyte levels, they were appropriately concentrated or diluted. Final extracts were analyzed using Agilent 5890 gas chromatograph (GC) equipped with an electron capture detector (ECD). Analytes were separated on Agilent 5% diphenyl - 95% dimethyl polysiloxane based fused silica capillary column (DB-5) by using a multi-step temperature ramping program.

GC column was heated from 50º to 155ºC at 5ºC min^{-1}, 155ºC to 210ºC at 1ºC min^{-1}, 210ºC to 315ºC at 4ºC min^{-1}, and held at this temperature for 16 min. Hydrogen was used as a carrier gas and nitrogen as a make-up gas. Analytes were quantified by using the internal standard method. Analyte concentrations and % lipid values were expressed on the wet weight basis.

Aroclor equivalent PCB concentrations were calculated by summing up the concentrations of the following PCB congeners: 18, 28, 44, 52, 66, 105, 118, 128, 138, 153, 170, 180, 187, 195, 206, 209; and multiplying this sum by a factor of 2 (NOAA 1989; ACE-EPA 1992). Total DDTs and metabolites concentration was calculated by summing up the concentrations of o,p'-DDT, p,p'-DDT, o,p'-DDD, o,p'-DDE and p,p'-DDE. Total chlordane concentration was calculated by summing up the concentrations of oxychlordane, γ-chlordane, α-chlordane and trans-nonachlor. The following coeluting pairs were not included in the quantitative or statistical analyses: α-BHC and PCB 8; dieldrin and PCB 87; endosulfan-I and PCB 101; PCB 77 and PCB 110; p, p'-DDD and cis-nonachlor; and mirex and PCB 169.

Statistical analyses were performed with Statistica (StatSoft) and SigmaStat (Systat) software programs. Information on the gender of each bluefin tuna specimen would have been helpful in the understanding of differential offloading of contaminants during spawning. However, as the gender information was not available for all specimens, data were pooled. In our previous study (Dickhut 2009), the analyses were performed by using GC-negative ion mass spectrometry which allowed the separation of p, p'-DDD and cis-

nonachlor. As this separation was not possible with GC-ECD in the present study, we used a ratio of PCB 187/PCB 153 as a surrogate for the ratio cis-nonachlor/PCB 153 in creating the scatterplots. Ratios of chemical tracers were proposed to eliminate the variability related to the changes in absolute concentrations with lipids, size, and the extent of contamination in the given area of capture (Dickhut et al. 2009). Also, since the compound ratios are used, it was assumed that the comparisons can be performed regardless of the tissue type (Dickhut et al. 2009). It is recognized that the ratio plot will not exactly mimic the ratio plot in Dickhut et al. (2009), however it was anticipated that the inclusion of trans-nonachlor as one of pesticide compounds in the ratios will provide sufficient discrimination between bluefin tuna foraging along the two sides of the Atlantic. Thus, ratios of trans-nonachlor/PCB 153 were plotted against ratios of PCB 187/PCB 153 for the juvenile bluefin tuna from offshore Virginia and the larger bluefin tuna from Gulf of Maine and Nova Scotia. In this scatterplot, we also included the ratio plots for YOY bluefin tuna from offshore Virginia and from Mediterranean Sea from our previous study (Dickhut et al., 2009). Origin of a particular bluefin tuna was determined as Mediterranean if the ratio trans-nonachlor/PCB 153 was less than 0.164 (Dickhut et al., 2009). The value of 0.164 was suggested by Dickhut et al (2009), and it was 90% of the lowest measured trans-nonachlor/PCB 153 ratio of 0.182 measured in YOY bluefin tuna collected from U.S. Mid Atlantic Bight in the Dickhut et al. (2009) study. For the discriminant function analysis (DFA), the concentrations of PCB congeners in the individual samples were normalized to the sum of the concentrations of all detected PCB congeners in an attempt to remove the effects of absolute concentrations on the first

principal component (Deshpande et al. 2016; Monosson et al. 2003; Wenning et al. 1992; Schwartz and Stalling 1991).

Quality Assurance

Measures of quality assurance included the analyses of laboratory method blanks, analyses of internal surrogate standards, replicate analyses, analyses of CARP-2 (NRC, Canada) and Lake Superior Trout SRM 1946 (NIST) reference materials, and participation in the NIST/NOAA Marine Mammal Interlaboratory Comparison Exercises. Target analytes were either not detected or their concentrations were very low in the laboratory method blanks in the both Soxhlet extraction batches; which indicated the cleanliness of the glassware and equipment. Blanks in the first and third ASE extraction batches were similarly clean; however blank for the second extraction batch contained some interfering peaks at retention times closer to PCB 153/132, PCB 183, PCB 194, and endosulfan sulfate. Average recoveries of surrogate internal standards DOB, ronnel, PCB 198, and PCB 192 ranged from 73-90% (± 10-19%) for the Soxhlet extraction batches. Internal standards were not recovered from one bluefin tuna sample, while the recoveries were 2-4 times greater than 100% for another bluefin tuna sample. TCB recovery in a majority of samples was 6-10 times greater than 100%, indicating matrix interferences in the lower retention time area of the chromatogram. For the ASE extractions, the average internal standard recoveries ranged from 57-107% (± 12-40%), with the exception of low ronnel recoveries in the second extraction batch. Each of the three ASE extraction batches also included triplicate samples. Relative standard deviation (RSD) values for

triplicate PCB determinations were below 25% for 82% of Batch-1 samples, 89% of Batch-2 samples and 96% of Batch-3 samples. RSD values for triplicate pesticide determinations were below 25% for 88% of Batch-1 samples, 63% of Batch-2 samples and 71% of Batch-3 samples. One of the triplicate samples in the first ASE extraction batch gave relatively low analyte recoveries that were inconsistent with the other two replicates in this group, and this replicate was discarded from further consideration. Average values for the other two replicates in this group were used in the statistical analyses. Recovery of PCB congeners in CARP-2 ranged from 30-129% for the Soxhlet extraction batches. For the ASE extraction batches, the recovery of PCB congeners in SRM 1946 ranged from 22-174%, while the recovery of pesticides ranged from non-recovered to 247%. About 73% of PCB determinations and 59% of pesticide determinations were within 40-140% for the combined data for CARP-2 and SRM 1946. In the NIST/NOAA Marine Mammal Interlaboratory Comparison Exercises, about 87% of PCB determinations and about 75% pesticide determinations in 2005, and about 80% of PCB determinations and about 83% pesticide determinations in 2007 were within 40-140% range. The data quality objectives were generally met with some deviations, which is not uncommon when a great number of variables are analyzed for the large groups of samples. Comparability of our data with the data of other national and international researchers provides additional support for the use of our data in understanding the significance of PCB and pesticide signatures in delineating the feeding areas of bluefin tuna.

Results

The Gulf of Maine bluefin tuna samples represented the largest number of samples analyzed (Table 2), and were they followed by bluefin tuna from Nova Scotia (Table 3) and offshore Virginia (Table 1). Considerable sample-to-sample and location-to-location variation was observed in the levels of lipids, PCBs, DDTs, and chlordanes (Figures 2-4; and Tables 1-3).

Lipids and Contaminants

Except for one relatively high value of 9.09%, lipids in the liver tissues of juvenile bluefin tuna from the offshore Virginia ranged from 0.02-1.43% (Table 1). PCBs, expressed as Aroclors, ranged from 61.3-361 ng g^{-1} wet weight; total DDTs ranged from 10.37-56.7 ng g^{-1} wet weight; and total chlordanes ranged from 2.72-19.58 ng g^{-1} wet weight. Correlations between contaminants and lipids were poor to moderate, and the regression line was mostly skewed for PCBs, DDTs, and chlordanes due to one relatively high lipid value (Figures 2A, 2B, and 2C). Correlation coefficient (r) values for the correlations between lipids and PCBs, lipids and DDTs, and lipids and chlordanes were 0.37 (Figure 2A), 0.27 (Figure 2B), and 0.67 (Figure 2C). PCBs correlated well with DDTs and chlordanes with r of 0.92 (Figure 2D) and 0.89 (Figure 2E). DDTs and chlordanes correlated with r of 0.76 (Figure 2F).

Lipids, PCBs, and chlorinated pesticides varied widely in the muscle samples of larger bluefin tuna from Gulf of Maine (Table 2). Lipid levels ranged from 0.05-43.8%, and included the values from almost negligible to medium to relatively high. PCBs, as

Aroclors, ranged from 22.5-842 ng g^{-1} wet weight; DDTs ranged from 2.95-170 ng g^{-1} wet weight; and chlordanes ranged from 1.01-42.49 ng g^{-1} wet weight. Lipids correlated moderately with the contaminants, and r values for correlations between lipids and PCBs, DDTs and chlordanes were 0.62 (Figure 2A), 0.62 (Figure 2B), and 0.66 (Figure 2C). PCBs correlated well with chlordanes and DDTs with r of 0.91 (Figure 2D) and 0.94 (Figure 2E). DDTs correlated well with chlordanes with r of 0.92 (Figure 2F).

Lipids, PCBs, and chlorinated pesticides also varied widely in the muscle samples of larger bluefin tuna from Nova Scotia (Table 3). Lipid levels ranged from 0.77-41.93%, PCBs, as Aroclors, ranged from 34.76-911 ng g^{-1} wet weight; DDTs ranged from 7.4-188 ng/g wet weight; and chlordanes ranged from 2.05-66.34 ng g^{-1} wet weight. Lipids correlated moderately with the contaminants, and r values for correlations between lipids and PCBs, DDTs, and chlordanes were 0.76 (Figure 2A), 0.66 (Figure 2B), and 0.80 (Figure 2C). PCBs correlated well with chlordanes and DDTs with r of 0.92 (Figure 2D) and 0.95 (Figure 2E). DDTs correlated well with chlordanes with r of 0.84 (Figure 2F).

For a given bluefin tuna, PCB concentrations were always highest, and followed by DDTs and chlordanes. For a given location, average PCB concentrations were always highest, and followed by DDTs and chlordanes (Figure 3). The average PCB concentration in the Gulf of Maine bluefin tuna was numerically the highest, and it was followed by bluefin tuna from the Nova Scotia and offshore Virginia. Although the average PCB concentrations were found to be different in one way ANOVA test (P=0.043), further analysis using Holm-Sidak all pairwise multiple comparison procedure

indicated that the average PCB concentrations at the three locations were not significantly different from each other. Similarly, DDTs (P=0.057) and chlordanes (P=0.067) at the three locations were not significantly different from each other in the Kruskal-Wallis one way ANOVA test. Lipid levels in the Gulf of Maine and Nova Scotia bluefin tuna were not significantly different from each other.

Ratio Plots in the Assignment of Origin of Bluefin Tuna

A scatterplot of trans-nonachlor/PCB 153 against PCB 187/PCB 153 clearly separated Mediterranean YOY bluefin tuna specimens from all northwestern Atlantic bluefin tuna specimens (Figure 4). Ratio of trans-nonachlor/PCB 153 was less than 0.164 for one large bluefin tuna from Gulf of Maine (ratio=0.14) and one juvenile fish from offshore Virginia (ratio=0.144). These two specimens were assigned as of Mediterranean Sea origin (Dickhut et al. 2009). Ratios of trans-nonachlor/PCB 153 for all other specimens were greater than 0.164. These bluefin tuna specimens could be either of Gulf of Mexico origin, or a mix of Gulf of Mexico and Mediterranean origins with a complete turnover of trans-nonachlor/PCB 153 ratios in those of putative Mediterranean origins (Dickhut et al., 2009).

PCB Metabolism and Recalcitrant PCB Congeners

PCBs undergo Phase I biotransformation reactions mediated by the hepatic cytochrome P-450 (CYP) superfamily of isozymes to yield the hydroxylated metabolites (OH-PCBs)

(Grimm et al. 2015). Mono-hydroxylated PCBs are the major metabolic products, but the di-hydroxylated, poly-hydroxylated, sulfur containing, and methyl ether PCB metabolites have also been identified. Metabolism of PCBs is generally slow compared to many other xenobiotics. OH-PCB metabolites themselves serve as the substrates for the Phase II conjugation reactions catalyzed by the sulfotransferase, UDP-glucuronosyl transferase, or glutathione S-transferase enzymes (Grimm et al. 2015). PCB metabolite conjugates are eventually excreted from the body primarily via the bile and feces (EURO WHO). CYP enzymes play an important role in determining the half-life and extent of bioaccumulation of PCBs, and in determining the potential magnitude and duration of biological harmful effects of PCBs (Clevenger et al. 1989). While PCBs bioaccumulate in the lipids and biomagnify up the food chain, all PCB congeners are not metabolized equally. The rate and extent of PCB biotransformation are dependent upon the animal species, and the number and position of chlorine atoms on the biphenyl ring structure (Schnellmann et al. 1985). The presence of adjacent unsubstituted carbon atoms on at least one of the biphenyl rings, particularly at the meta- and para- positions, facilitate the PCB metabolism, and the rate of PCB metabolism decreases with the increasing number of chlorine substitutions. Grimm et al. (2015) noted that PCB congeners with 2,3-, 2,5- and 2,6-dichlorination or 2,3,6-trichlorination patterns are most easily metabolized. Hansen (2001) referred to the PCB congeners that are metabolized much more rapidly as the “transient” PCB congeners, and he noted that these “transient” PCB congeners are not typically encountered in the biological samples unless they are sampled shortly after the exposure. Grimm et al. (2015) noted that PCB congeners with 4-, 3,4-, 3,5-, 2,4,5-, 2,3,4,6- or 2,3,5,6- chlorine substitutions are less efficiently metabolized. These

metabolism resistant PCB congeners are therefore typically observed in high proportions in the biological matrices. Fisk et al. (1998) empirically determined the biomagnification factors (BMF) for 25 organochlorine compounds. The authors reported that PCB 153 and PCB 138 exhibited the highest BMFs, followed by PCB 180 and PCB 118. The authors noted that the resistance of these PCB congeners to metabolism was partly due to their 2,4,5- substitution pattern.

As some PCB congeners are more readily and metabolized than others and readily eliminated out of the body as the metabolite conjugates (Letcher et al. 2000), the temporal congener patterns in the biota samples shift away from the PCB congener pattern of the source to which the organism was exposed. Based on the general guideline of Grimm et al. (2015) on the chlorine substitution pattern and metabolic efficiency, the following PCB congeners can be suggested to be some of the recalcitrant and prominent PCB congeners that can be detected in the biota samples across a wide range of environments: PCB 153 (2,2’,4,4’,5,5’-hexachlorobiphenyl) (4-, 2,4,5- chlorine substitutions), PCB 138 (2,2’,3,4,4’,5’-hexachlorobiphenyl) (4-, 3,4-, 3,5-, 2,4,5- chlorine substitutions) PCB 180 (2,2',3,4,4',5,5'-heptachlorobiphenyl (4-, 3,4-, 3,5-, 2,4,5- chlorine substitutions), PCB 118 (2,3',4,4',5-pentachlorobiphenyl) (4-, 3,4-, 2,4,5- chlorine substitutions), and PCB 187 (2,2',3,4',5,5',6-heptachlorobiphenyl) (4-, 3,5-, 2,4,5-, and 2,3,5,6- chlorine substitutions). These PCB congeners were indeed some of the prominent PCB congeners in bluefin tuna tissue samples in the present study, and also in bluefin tuna tissue samples in the studies of Stefanelli et al. (2002) and Carsolini et al. (2007). The overall PCB pattern in bluefin tuna from a given collection site will thus be reflective

of the relative proportions of the recalcitrant PCB congeners in the prey resources plus other PCB congeners therein that are, although subject to metabolism, can be still persistent in the tuna due to their relatively high concentrations and the generally slow rate of PCB metabolism.

Discriminant Function Analysis (DFA): Geographic Grouping of Bluefin Tuna

In the DFA of total PCB normalized concentrations of PCB congeners, the first root accounted for 71.2% of explained variance, and it was weighted heavily in the decreasing order by PCB 138, PCB 52, PCB 187, PCB 44, PCB 156, PCB 128, and PCB 201. Second DFA root was marked by the variables PCB 138, PCB 153, PCB 128, PCB 105, PCB 52, PCB 201, and PCB 49. Except for PCB 44, PCB 49, and PCB 52, all PCB congeners identified as important in the DFA analyses are resistant to metabolism as per the chlorine substitution guidelines of Grimm et al. (2015). Although PCB 44, PCB 49, and PCB 52 are subject to metabolism, as described above, their occurrence in bluefin tuna was probably detected due to their relatively high concentration in the prey resources and the generally slow rate of PCB metabolism. The most discriminative DFA power of PCB 138 was probably due to its recalcitrant nature and its occurrence in different concentrations in the different areas of collection. Although the median concentrations of PCB congeners identified in the in the first DFA root in the present study were different, the differences were not statistically significant with the exception of significant differences for PCB 44 and PCB 201 between the bluefin tuna from Gulf of Maine and offshore Virginia, and between the bluefin tuna from Nova Scotia and

Virginia. Median PCB 156 concentrations were similarly significantly different between bluefin tuna from the Gulf of Maine and Virginia. We are unable to clearly identify additional factors that would make PCB 138 the most discriminative factor in the first DFA root. Becker et al. (1998) also reported that PCB 138 was major component of the discriminant function used to separate oystercatcher breeding sites on the Wadden Sea coast.

The means of canonical variables indicated that root 1 discriminated between juvenile bluefin tuna from offshore Virginia and large bluefin tuna from the Gulf of Maine and Nova Scotia. Means of the canonical variables also indicated that DFA root 2 discriminated bluefin tuna from Nova Scotia and bluefin tuna from Gulf of Maine and offshore Virginia. DFA plot of root 1 against root 2 showed clear separation of all three subgroups of bluefin tuna within the northwestern Atlantic (Figure 5). The differences between PCB congener signatures at different locations created three different DFA groups, however as these differences were not always statistically significant, dispersions were evident within the individual groups. Predicted post-hoc DFA classification of each bluefin tuna to the respective geographical group was 100% accurate.

Discussion

Lipids and Contaminants

Lipids in the liver samples of juvenile bluefin tuna from offshore Virginia were uniformly low and, except for one specimen, all lipid values were < 1.5% (Table 1). It appears that the energy in these juvenile fish is predominantly being utilized for the somatic growth, resulting in less energy being stored in the form of lipids. Individual lipid levels in the muscle tissues of larger bluefin tuna from Gulf of Maine and Nova Scotia varied considerably. It is possible that lipid reserves in the larger specimens from the northern waters could have been depleted via a variety of physiological mechanisms. For example, the demand for lipids could arise from the need for energy for migration, metabolism, or endothermy, a mechanism by which bluefin tuna maintain their body temperature above the ambient ocean water temperature. Endothermic capacity allows bluefin tuna to become more active predators by expanding their foraging into much cooler waters. Lipids may also be mobilized for the spawning events, but this scenario seemed less likely as the northwestern Atlantic bluefin tunas are known to spawn mainly in the Gulf of Mexico. Some specimens may have indeed spawned and migrated to the northern waters, but because we lacked information on the state of gonads for some specimens, we were unable to scrutinize this possibility any further. Low lipid levels may also reflect poor nutritional status due to the shortage in the availability, abundance, and quality of the prey species. Popovic et al. (2012) reported much less fluctuation of lipid levels (11.04 ± 0.78%) in the ventral ordinary tail muscle of wild bluefin tuna obtained at a fish market from a certified trader in Croatia. We were not able to compare our lipid data with the lipid data reported by Popovic et al. (2012) for the lack of more information on those specimens.

Despite the inconsistencies in the lipid contents, the contaminant levels irrespective of sampling location were not always lowest in bluefin tuna that had the lowest lipids. The observation that contaminant levels were not uniform in the individual samples (Tables 1-3; Figures 2-4), suggests that some bluefin tuna may have depurated or redistributed the contaminants to other organs during the lipid mobilization related to spawning and/or migration. However, relatively weak correlations between lipids and contaminants suggest that the lipid mobilization may not necessarily result in quantitative depuration or redistribution. Debier et al. (2006) reported that the lipids are mobilized from blubber to match the energy requirements of the northern elephant seals (*Mirounga angustirostris*) during the post-weaning fast. The authors noted that this lipid transfer frees toxic fat-soluble pollutants like PCBs from blubber into the blood circulation, and that the increases in total PCB concentrations in the inner blubber and serum were more pronounced in the leaner animals. Johnson et al. (2013) reported that during the reproduction the lipid mobilization occurs in female teleosts to support the egg development which provides a route through which the lipophilic contaminants accumulated in other organs high in lipids like liver are transferred to gonads and eventually to the eggs. The authors further noted that the transfer of maternally derived contaminants dramatically varies from species to species. Henriksen et al. (1996) investigated how the mobilization of lipids associated with breeding influences PCBs concentrations and compositions in kittiwakes (*Rissa tridactyla*). The authors noted that the mean concentration of PCBs in the brain tissue approximately quadrupled during the reproduction. The authors attributed this increase to the redistribution of PCBs from the utilized depot fat to the metabolizing organs. Jørgensen et al. (2006) reported that winter

fasting and emaciation, which are common among Arctic charr living in the high latitudes, resulted in the redistribution of the lipophilic PCBs from lipid-storing tissue such as the muscle, to the vital and sensitive organs like liver and brain. Weak correlations between lipids and contaminants observed in the present study can also be explained by the possibility that some bluefin tuna may have been foraging in the less contaminated areas. This scenario may be somewhat less likely as the differences in average PCBs, DDTs and chlordanes in bluefin tuna from different locations were insignificant. It seems likely that bluefin tuna in the northwestern Atlantic share prey resources that are overall contaminated to the approximately similar extent. The individual components in a group of contaminants appeared to vary between the locations; however the group averages appeared statistically similar.

Based on the results of our prior study on bluefish (*Pomatomus saltatrix*) (Deshpande et al. 2002), we were not surprised that all contaminant groups correlated fairly with each other (Figure 2). Thus, it may be possible to predict concentration of one group of contaminants if concentration of other contaminant group is known. For example, we will be able estimate the concentrations of DDTs in the northwestern Atlantic bluefin tuna if we knew the concentrations of PCBs or chlordanes.

Ratio Plots in the Assignment of Origin of Bluefin Tuna

We previously demonstrated that trans-nonachlor/PCB 153 ratios in the bluefin tuna of eastern origin are low compared to the corresponding ratios in the northwestern bluefin

tuna (Dickhut et al. 2009). As the Mediterranean bluefin tuna migrate to the northwestern Atlantic, these ratios gradually turnover due to the foraging in the northwestern Atlantic, and coincident with the accumulation of biomass tagged with higher trans-nonachlor/PCB 153 ratios. Origin of a given bluefin tuna specimen was determined as the Mediterranean if the trans-nonachlor/PCB 153 ratio was less than 0.164 (Dickhut et al. 2009). The value of 0.164 is 90% of the lowest measured trans-nonachlor/PCB 153 ratio of 0.182 in YOY bluefin tuna from the U.S. Mid Atlantic Bight in the Dickhut et al. (2009) study. Examination of this ratio guideline suggested that one juvenile bluefin tuna from the offshore Virginia and one large bluefin tuna from the Gulf of Maine possibly originated from the feeding grounds in the Mediterranean Sea. The remainder of all bluefin tuna was determined to be spawned either in Gulf of Mexico or the trans-nonachlor/PCB 153 ratios in the putative Mediterranean migrants were completed turned over to the ratios akin to the northwestern Atlantic to tell the difference.

Our previous study (Dickhut et al. 2009) indicated that the turnover time for trans-nonachlor/PCB 153 ratio in the Mediterranean bluefin tuna, that arrive in the northwestern Atlantic at age 1, is approximately 10 months and it increases to approximately 1.6 years in those arriving at age 2. If this ratio information can be extrapolated to the trans-nonachlor/PCB 153 ratios for bluefin tunas in the present study, then the length of residence of juvenile bluefin tuna in offshore Virginia can be estimated to be between 0.83 to 1.6 years. As the growth rate of larger bluefin tuna is different and smaller than the growth rate of juvenile bluefin tuna (Santamaria et al. 2009), estimation of length of residence of larger bluefin tuna in the northwest Atlantic was not straight

forward. However, if it is assumed that the turnover time for the trans-nonachlor/PCB 153 ratio continues to increase with age, as suggested by Dickhut et al. (2009), then the larger bluefin tuna can be speculated to have resided in the northwest Atlantic for a period greater than 1.6 years. We can also infer that the two bluefin tuna that were assigned the Mediterranean origin most possibly made the trans-Atlantic migrations from the Mediterranean to northwestern Atlantic less than 10 months before being captured. Additional supporting information, such as the data on $\delta^{18}O$ levels in otoliths, would be helpful in the future studies in understanding the feeding grounds of individual bluefin tuna specimens. Nevertheless, the foraging patterns in the northwestern Atlantic clearly appeared to be sufficiently prolonged to sustain stable and unique ratios of certain PCB congeners and pesticides. The results of migratory behaviors of juvenile bluefin tuna from the offshore Virginia appeared different than the results of our previous study (Dickhut et al. 2009). We previously reported the presence of 2 of 6 small school sized bluefin tuna from the Mediterranean in the sampling along the Virginia coast, and the presence of 10 of 12 small school sized bluefin tuna from the Mediterranean in the sampling along the New Jersey coast. Additional studies are needed to examine if the differences in the results were due to the random seasonal variability in the bluefin tuna behavior, distribution, or something else.

Tagging Studies to Examine the Movement of Bluefin Tuna

In a tagging study using long-term, pop-up archival tags, Lutcavage et al. (2001) reported that that the giant bluefin tuna left New England feeding grounds within 10 to 30 days

and traveled to the low latitudes past North Carolina. The authors also reported that the majority of tagged bluefin tuna released from North Carolina fishery and recaptured in New England/Canada spent most of their time in the northwestern Atlantic except for the three migrants to the Mediterranean Sea. Block et al. (2001) reported that the most tagged bluefin tuna remained in the vicinity of release off the North Carolina coast during winter, and they proceeded offshore along the Gulf Stream in the early spring either east toward Bermuda or southeast toward the Bahamas. The authors reported that the majority of bluefin tuna displayed the northwestern residency track year after their release, moving from the Carolinas along the Gulf Stream northern edge in spring and toward the New England and Canadian shelf in early summer. Bluefin tuna in this study remained on the continental shelf through the autumn and returned to the Carolinas or Bahamas by winter. Block et al. (2005) reported that bluefin tuna that had been electronically tagged in the northwestern Atlantic resided in the northwestern Atlantic foraging grounds for 0.5 to 3 years before migrating to the Mediterranean Sea. The authors hypothesized that a proportion of bluefin tuna electronically tagged in the northwestern Atlantic were of eastern stock origin and that they seemed to be returning to their natal spawning areas. Behaviors of these tagged bluefin tunas to mostly reside in the northwestern Atlantic foraging grounds agreed with our observation of mostly northwestern Atlantic residence of large bluefin tuna, which was based on the use of unique trans-nonachlor/PCB 153 ratios in their east/west designations.

Fromentin and Powers (2005) discussed that the major difficulty with the electronic tagging is the representativeness of sampled specimens to the Atlantic bluefin tuna

population of several million. The authors stated that even with the large number of electronic tags used to date, it is unclear if all of the modes of bluefin tuna behavior are represented in the sample, and that the population migration rates are still difficult to quantify. The authors discussed that the electronic tagging method does not provide the location of birth of the migrating fish which is the key information needed for the understanding of population structure. The authors suggested the use of naturally embedded chemical signatures in the hard structures, for example, measuring the elements Li, Mg, Ca, Mn, Sr, and Ba (Rooker et al. 2003) or $\delta^{18}O$ (Rooker and Secor 2004) in the otoliths of bluefin tuna. In case where otoliths are not available or a non-lethal sampling is preferred, then the results of the present study suggest that the fingerprints of PCBs and organochlorine pesticides in the muscle tissue can serve as the low-cost alternatives for the naturally fortified chemical tracer tags in the assessment of foraging grounds of bluefin tuna. A possibility of non-lethal biopsy sampling also permits multiple sampling possibilities along the multiple spatial and temporal axes.

Fatty Acids as a Proxy for the PCB Signature Turnover

In a study to understand the rate at which fatty acids seen in the predator will mimic the fatty acids seen in the prey, Kirsch et al. (1998) fed adult Atlantic cod (*Gadus morhua*) with two prey items that differed significantly in the fat content and fatty acid compositions. The authors first fed the Atlantic cod with squid (*Illex illecebrosus*, 2.0% fat) for 6 weeks, and it was subsequently followed by Atlantic mackerel (*Scomber scombrus*, 15.7% fat) for 8 weeks. The authors reported that despite being on a low-fat

squid diet, the fatty acid signatures in the Atlantic cod changed significantly within just 3 weeks to reflect the patterns found in squid and that these signatures did not further change by week 6. After the diet was changed from squid to mackerel, the Atlantic cods were sampled at week 5 and week 8. The authors reported that the fatty acid signature of Atlantic cod changed significantly toward the fatty acid signatures of mackerel at week 5. The authors hypothesized that the most changes in the fatty acid signatures in the both feeding experiments had taken place within the first 3-5 weeks and that subsequent changes over the next few weeks were not detectable. In an analogous feeding study but at a much earlier life history scale, Turner and Rooker (2005) reported that cobia (*Rachycentron canadum*) larvae underwent significant dietary modification of polyunsaturated fatty acids (PUFAs) after 24 h and conserved >90% of dietary PUFAs after an average of 6 days. The authors reported that the significant dietary modification of PUFAs in the juvenile cobia took place after 3 days and >90% were conserved after an average of 12 days. The results of the feeding studies of Kirsch et al. (1998) and Turner and Rooker (2005) suggest that the turnover of fatty acid signature was life history stage dependent. As only a single species was used in each study, it is not possible to understand the role of the species variable in the turnover rates of fatty acid signatures. As the diet plays an important role in the bioaccumulation of PCBs and pesticides, we hypothesize that fatty acids in the prey may serve as the proxies for PCBs and pesticides in the prey, and that the turnover rates of the two signatures are somehow related. It is not clear if the turnover time of 3-5 weeks as described by Kirsch et al. (1998) for the adult Atlantic cod can be directly extrapolated to the bluefin tuna due to their size and species differences, but the residence of bluefin tuna in the respective areas of capture certainly

appeared to be sufficiently long to sustain stable PCB signatures that allowed their geographical groupings.

The turnover of fatty acid signatures probably takes place at a subtle level, and therefore it is rather quick. This may be due the fact that the component fatty acids are already present in fish at some levels, in some relative ratios, and a period of 3-5 weeks was apparently sufficient to change these relative ratios to match the signature of a new prey species. Likewise, the PCB congeners are already present in fish at some levels and in some relative ratios. If the fatty acids can be used as a proxy for the PCB congeners, then the turnover time for PCBs can also be approximated to be 3-5 weeks. The tagging studies of Lutcavage (2001) as described above do suggest that the bluefin tuna reside in a particular are for a period of 10-30 days, which appears to roughly overlap the estimated 3-5 week turnover period for the PCB signatures. In contrast to the relatively quick turnover of fatty acid or PCB congener signatures, the turnover rate for trans-nonachlor/PCB ratio from the Mediterranean to northwestern Atlantic appears to be quite slow. Dickhut et al. (2009) estimated that a period of 8-16 months is required to turn over the trans-nonachlor/PCB 153 ratio when a bluefin tuna from the Mediterranean migrates to the northwestern Atlantic. A slow turnover rate is possibly related to the large differences in the relative amounts of trans-nonachlor and PCBs in marine organisms on the two sides of the Atlantic (Dickhut et al. 2009). Thus, trans-nonachlor is present at low concentrations in the Mediterranean bluefin tuna and at relatively high concentrations in the northwestern Atlantic bluefin tuna. A westward migrating Mediterranean bluefin tuna therefore has to turnover from the low to high concentrations by virtue of gradual

addition of biomass and chemicals tags characteristic to the northwestern Atlantic. Longer turnover time for trans-nonachlor also results in longer turnover time for the trans-nonachlor/PCB 153 ratio.

The Concept of Metapopulation of Bluefin Tuna

Kritzer and Sales (2004) discussed that just the presence-absence resolution is far too coarse to understand the stock dynamics in a meaningful way. Fromentin and Powers (2005) discussed the concept of metapopulation in which the Atlantic bluefin tuna would be seen as a collection of discrete local populations, occupying distinct and patchy suitable habitats, displaying their own dynamics, but with a degree of demographic influence from the other local populations through dispersal. The authors discussed that within each local population, the individuals would display similar migration or habitat uses, resulting from a common spatial learning among the individuals. Newlands et al. (2012) discussed spatial ecology and school aggregation behavior of bluefin tuna. The authors reported that bluefin tuna aggregated where the prey species were abundant and estimated aggregation range was approximately 40 km with 27% seasonal variation in the aggregation strength. The authors reported that at spatial ranges of < 5 km, separately resolved groups of schools were also identified in the observed school distribution.

As the results of the studies so far using the physical or chemical tags are limited to east or west designation of bluefin tuna, we were further interested in utilizing the organic chemical tags to examine if any geographical groupings or metapopulation schools

689 existed within the northwestern Atlantic bluefin tuna specimens. We examined the
690 possibility of this fine-scale resolution by performing DFA analyses of the total PCB
691 normalized signatures of PCB congeners. We were surprised that the DFA plot showed
692 three clearly separated subgroups of bluefin tuna corresponding to the three
693 geographically different capture locations within the northwest Atlantic Ocean (Figure 5).
694 Additional analyses of DFA results showed that the predicted post-hoc classification of
695 each bluefin tuna to the known respective geographical location was 100% accurate.
696 Distinct DFA groupings also suggested that bluefin tuna captured from a particular area
697 bioaccumulated unique and stable PCB congener signatures by virtue of demographic
698 connectivity of foraging on uniquely similar prey assemblages for an extended period of
699 time. Samples of larger bluefin tuna from the Gulf of Maine and Nova Scotia were
700 obtained in 2006 either from specimens caught by purse seine or hook-and-line. These
701 specimens were originally caught from June to October in 2004 and from June and July
702 in 2005. Despite their collection at different time intervals over a period of about 4
703 months, the resulting PCB fingerprints appeared stable for a given capture area and the
704 related DFA showed tight grouping or metapopulation schools. This was remarkable
705 considering the highly migratory trait of bluefin tuna. Despite their highly migratory trait,
706 the random movements and mixing behavior between the different distant locations also
707 did not appear to be significantly extensive in these bluefin tuna specimens.
708
709 Golet et al. (2013) hypothesized that the dispersal patterns of bluefin tuna shift to the
710 regions with higher prey abundance or profitability. The authors discussed that since the
711 Atlantic bluefin tuna forage disproportionately on the Atlantic herring (*Clupea*

harengus), the changes in herring dynamics will trigger and impact bluefin tuna dynamics in the Gulf of Maine. Chase (2002) discussed that spatial variation in prey populations is probably the primary influence on the annual aggregations of bluefin tuna. The author reported that the fish prey species such the Atlantic herring, sand lance (*Ammodytes* spp.), Atlantic mackerel (*Scomber scombrus*), and bluefish (*Pomatomus saltatrix*), in the decreasing frequency of occurrence, represented a large majority of diet of bluefin tuna, while the cephalopods, primarily the squid, were the important secondary prey. Logan et al. (2011) reported that the young Atlantic bluefin tuna feed mainly on the zooplanktivorous fishes and crustaceans on the foraging grounds in both eastern and northwestern Atlantic Ocean. The authors reported that bluefin tuna diet in the Mid-Atlantic Bight was mainly sand lance. Logan et al. (2014) reported that Atlantic herring were the primary forage for bluefin tuna in the Gulf of Maine. Three distinct groupings in DFA plot of total PCB normalized PCB signatures possibly arising from unique prey consumptions in different areas supported the concept of prey density driven metapopulation behavior of bluefin tuna.

Butler et al. (2015) discussed that bluefin tuna in the western Atlantic Ocean migrate annually between the known spring spawning grounds in the Gulf of Mexico and the summer and winter feeding grounds in the northwest Atlantic Ocean. The authors noted that bluefin tuna meet their high energetic requirements by consuming diets that are frequently dominated by a single, lipid-rich, schooling teleost species such as Atlantic herring (*Clupea harengus*) or Atlantic menhaden (*Brevoortia tyrannus*). The authors noted that although several recent studies have suggested that bluefin tuna may utilize the

Gulf of Mexico as a feeding ground, no prior works have assessed the diet on the spawning grounds, and fasting during the spawning period has been previously assumed. In their own assessment of the diet of bluefin tuna in the Gulf of Mexico, Butler et al. (2015) observed that the two most important prey items were a colonial pelagic tunicate (*Pyrosoma atlanticum*) and the longnose lancetfish (*Alepisaurus ferox*). The authors noted that both these prey species are energetically inferior to the typical bluefin tuna prey on the northern feeding grounds. For example, the tunicate is composed of about 94% water and has a mean energy content of 0·3 kJ g−1 (Davenport and Balazs, 1991), which is about 30 times less than that of herring or menhaden (Steimle & Terranova, 1985). Similarly, the authors noted that the longnose lancetfish muscle tissue has lower ΔC:N values (0·1±0·2) than the whole herring (3·3±2·0) or menhaden (2·3±1·2). The low lipid contents of the primary Gulf of Mexico prey species indicated that the energetic demands of bluefin tuna are not fully met. The authors suggested that the low liver lipid stores in the summer Gulf of Maine and Gulf of Mexico bluefin tuna were probably due to the feeding on energetically inferior prey species and feeding less frequently during their migrations than the bluefin tuna that are already established on the northern feeding grounds. The authors noted that the summer Gulf of Maine and Gulf of Mexico bluefin tuna would also experience additional energetic stress from the earlier long distance migrations. Lower lipid contents of the important prey species in the Gulf of Mexico and less feeding during migration may probably result in lesser exposure to PCBs and other contaminants during these times. Therefore, it can be argued that the contribution to the PCB congener signatures in bluefin tuna is predominantly modulated by the PCB congener signatures characteristic to their northern feeding grounds. The prey driven

distinct PCB signatures in the different metapopulation schools of bluefin tuna therefore also appears to be influenced mainly by the PCB congener patterns in the respective northern feeding grounds.

Conclusions

In conclusion, the unique signatures of the naturally bioaccumulated PCBs and organochlorine pesticides like chlordanes provided a relatively low-cost (compared to the electronic data recording tags, Block et al. 2005; Musyl et al. 2011) exploratory method for understanding the migratory trends of bluefin tuna, mixing, and population dynamics. Similar to what we reported before (Dickhut et al. 2009), the relatively high PCBs in conjunction with the significantly low chlordanes suggested Mediterranean Sea as the origin or primary foraging ground of the bluefin tuna. As a corollary, the higher chlordanes are unique chemical tags for bluefin tuna feeding in the northwestern Atlantic. Utilization of the differences between the trans-nonachlor to PCB ratios on the two sides of the Atlantic thus allowed the east/west discrimination between the feeding grounds of bluefin tuna. The DFA plot of unique PCB signatures in each area of capture suggested the fidelity of bluefin tuna to a particular foraging ground for an extended period, which in turn supported the metapopulation theory of spatial ecology of bluefin tuna comprising of discrete aggregates of local populations with the limited interpopulation exchanges. Advancement in the scientific knowledge of the bluefin tuna population dynamics beyond the traditional discussions of east/west designation and trans-Atlantic migration to the metapopulation hypothesis provides the researchers a novel and low-cost

781 exploratory tool for the stock assessment and management of this prized species. It is
782 recognized that the additional analyses are warranted to further proof the hypothesis of
783 metapopulation by sampling several different putative metapopulation schools of bluefin
784 tuna. As the muscle tissues can be sampled in a non-lethal manner, the repeated
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786
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Figure 1. Locations of bluefin tuna collections in Gulf of Maine, Nova Scotia and offshore Virginia

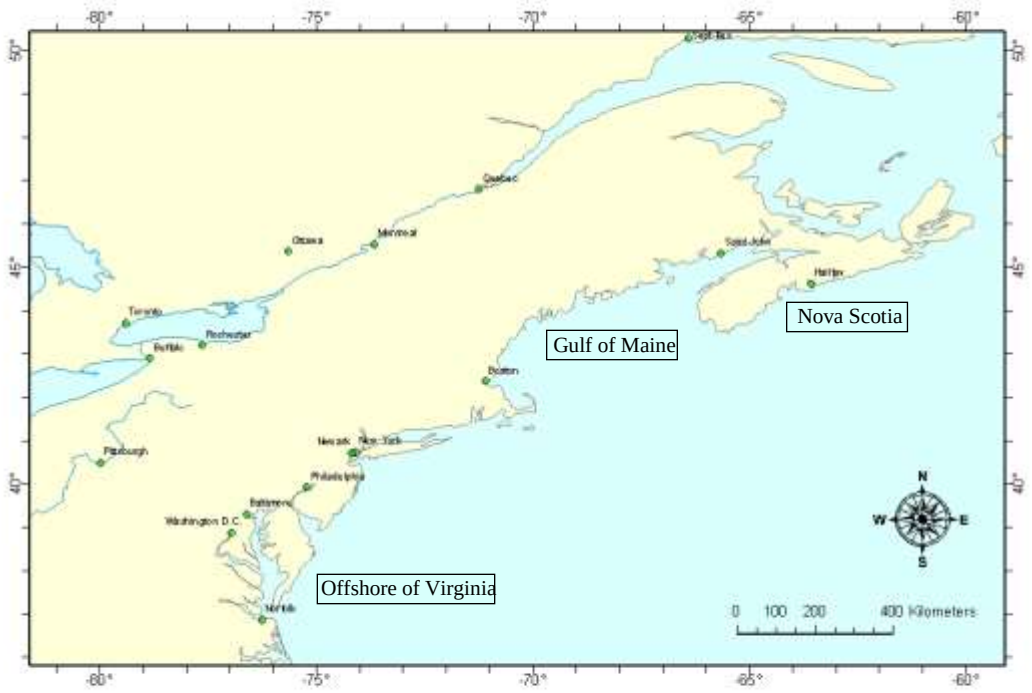
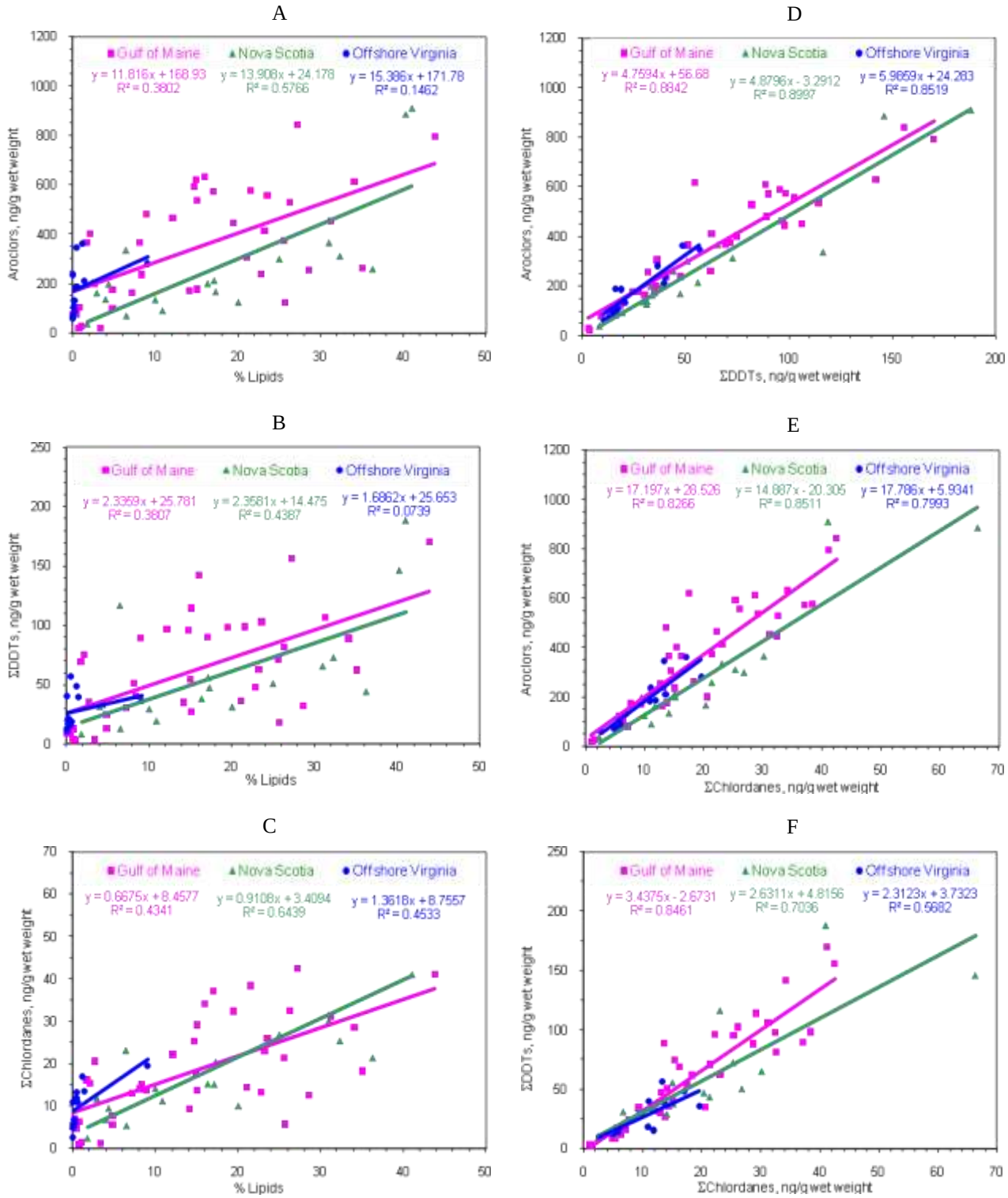


Figure 2. Different correlation graphs for liver samples of bluefin tuna from offshore Virginia and muscle samples of bluefin tuna from Gulf of Maine and Nova Scotia. Figures in the left Column: correlations between % lipids and PCBs (A); % lipids and DDTs (B); and % lipids and chlordanes (C). Figures in the right Column: correlations between PCBs and DDTs (D); PCBs and chlordanes (E); and DDTs and chlordanes.



1049 Figure 3. Average concentrations of total PCBs (as Aroclors), total DDTs and total

1050 chlordanes in bluefin tuna.

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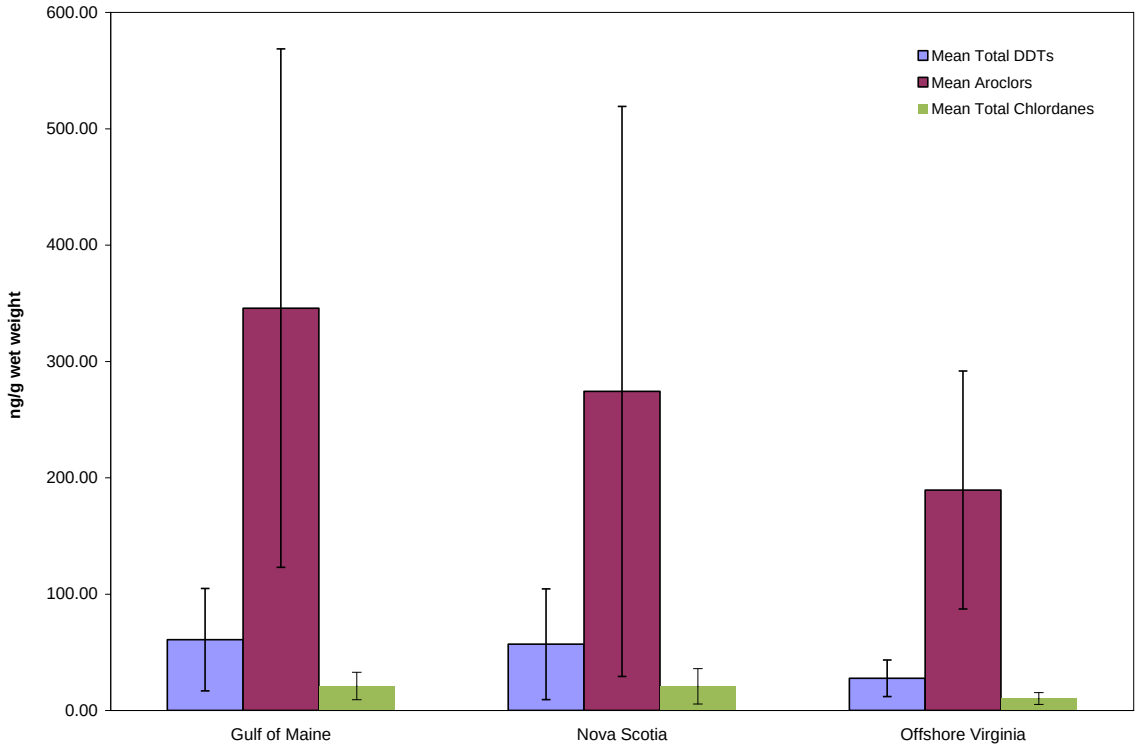
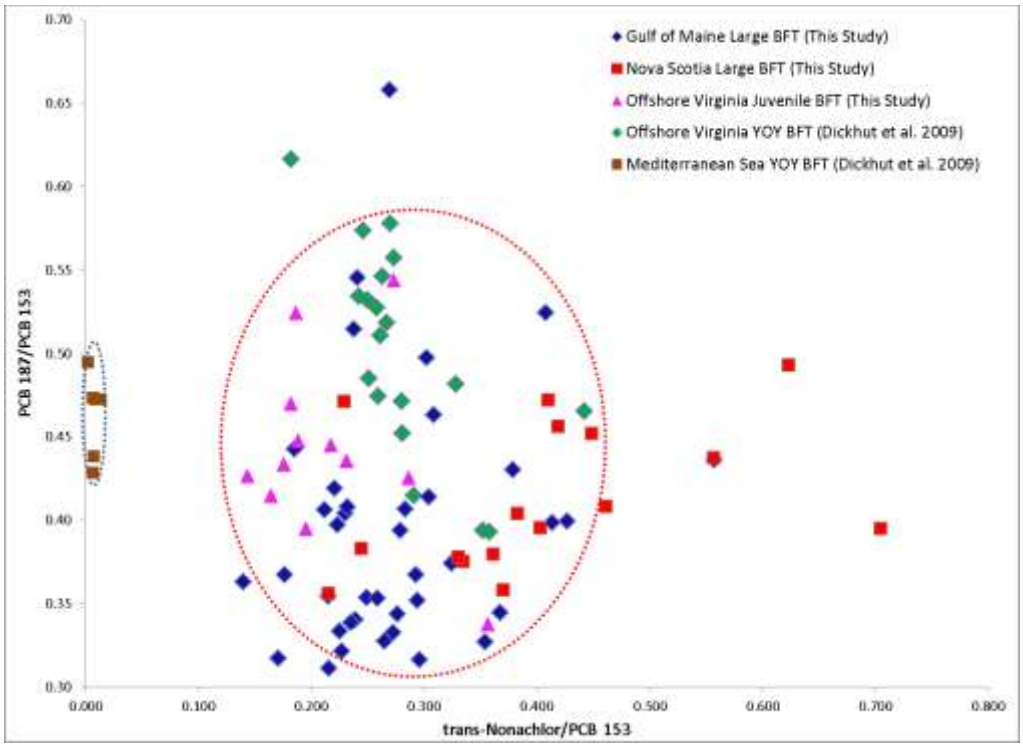


Figure 4. A scatterplot of ratios of trans-nonachlor/PCB 153 against ratios of PCB 187/PCB 153 for juvenile bluefin tuna from offshore of Virginia, larger bluefin tuna from Gulf of Maine and Nova Scotia, and young of year bluefin tuna from offshore of Virginia and Mediterranean Sea.



1059 Figure 5. Discriminant function analysis (DFA) plot of total PCB normalized

1060 concentrations of PCB congeners.

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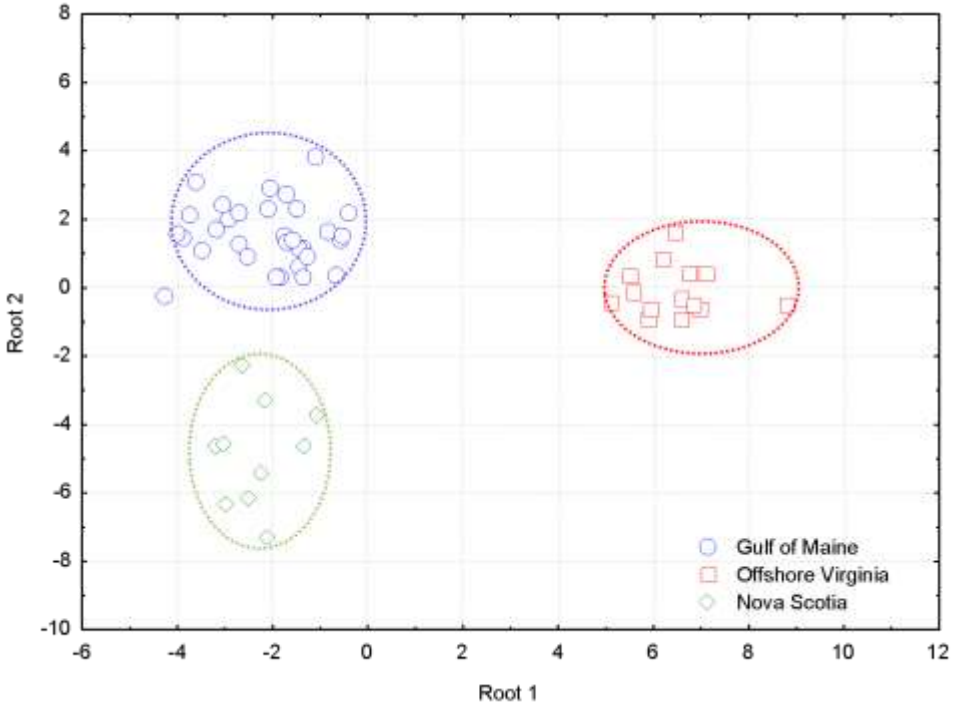

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Table 1. Lipids, PCBs (as Aroclors), DDTs and chlordanes in the liver tissue of bluefin tuna from offshore of Virginia.

No.	% Lipids (wet weight basis)	Aroclors (ng/g wet tissue)	Total DDTs (ng/g wet tissue)	Total Chlordanes (ng/g wet tissue)
1	1.18	361.23	48.69	17.00
2	0.02	61.26	10.37	2.72
3	9.09	280.83	36.25	19.58
4	0.18	132.64	20.59	6.81
5	1.43	211.18	39.47	13.55
6	0.06	71.93	12.78	5.04
7	0.41	188.60	15.73	11.88
8	0.53	185.28	18.65	10.96
9	0.10	106.19	16.67	5.80
10	0.20	90.94	14.85	5.96
11	0.05	237.01	40.22	11.10
12	0.49	345.68	56.74	13.38
Min	**0.02**	**61.26**	**10.37**	**2.72**
Max	**9.09**	**361**	**56.74**	**19.58**
Mean	**1.15**	**189**	**27.58**	**10.32**
SD	**2.54**	**102**	**15.77**	**5.14**

Table 2. Lipids, PCBs (as Aroclors), DDTs and chlordanes in the muscle tissue of bluefin tuna from the Gulf of Maine. First sample in the table represents average values for a duplicate sample.

No.	% Lipids (wet weight basis)	Aroclors (ng/g wet tissue)	Total DDTs (ng/g wet tissue)	Total Chlordanes (ng/g wet tissue)
1*	15.96	631.20	142.25	34.19
2	8.34	237.53	40.45	15.08
3	27.23	841.82	155.91	42.49
4	21.56	575.75	98.50	38.44
5	12.11	465.32	96.76	22.20
6	4.84	177.17	24.25	7.66
7	14.92	619.07	54.41	17.56
8	19.42	446.22	97.99	32.41
9	15.06	538.03	114.39	29.18
10	16.99	573.71	89.88	37.17
11	15.02	178.33	26.76	13.75
12	14.69	592.51	95.45	25.38
13	8.11	366.38	51.10	14.12
14	7.16	165.00	30.06	12.97
15	23.22	412.93	62.42	23.14
16	26.24	529.57	81.90	32.59
17	23.54	557.53	102.65	26.07
18	21.08	306.63	36.04	14.49
19	34.08	612.74	88.62	28.66
20	28.57	256.03	31.66	12.63
21	0.25	83.43	16.03	7.08
22	2.10	401.93	74.85	15.41
23	1.72	367.47	69.11	16.19
24	8.96	482.09	88.95	13.70
25	31.25	453.10	106.36	31.25
26	35.05	262.29	62.04	18.29
27	43.83	795.27	170.20	41.18
28	22.83	239.08	47.49	13.21
29	25.56	376.00	71.25	21.38
30	14.13	173.78	34.79	9.34
31	25.66	124.01	17.91	5.76
32	3.41	23.38	3.28	1.16
33	0.78	22.50	3.41	1.01
34	1.07	30.07	2.95	1.45
35	0.49	77.66	9.02	4.84
36	0.05	78.19	9.00	5.28
37	0.78	105.23	12.45	6.40
38	2.67	202.54	35.26	20.60
39	4.83	102.31	12.85	5.65
Min	**0.05**	**22.50**	**2.95**	**1.01**

Max	**43.83**	**841.82**	**170.20**	**42.49**
Mean	**14.96**	**345.74**	**60.73**	**18.45**
SD	**11.63**	**222.81**	**44.02**	**11.78**

Table 3. Lipids, PCBs (as Aroclors), DDTs and chlordanes in the muscle tissue of bluefin tuna from Nova Scotia. First two samples in the table represent average values for two separate triplicate samples.

No.	% Lipids (wet weight basis)	Aroclors (ng/g wet tissue)	Total DDTs (ng/g wet tissue)	Total Chlordanes (ng/g wet tissue)
1*	1.80	39.14	8.21	2.38
2*	30.99	367.00	65.45	30.19
3	6.44	337.45	116.44	23.13
4	41.02	910.81	187.98	41.03
5	4.00	138.66	31.33	6.77
6	17.12	214.68	55.86	15.10
7	20.03	127.02	31.07	10.00
8	32.30	313.28	72.65	25.36
9	16.34	202.43	38.03	15.24
10	6.52	72.10	12.82	5.36
11	10.88	92.90	19.32	11.18
12	17.30	169.17	47.37	20.39
13	36.26	260.85	43.97	21.38
14	40.27	886.22	145.97	66.34
15	10.00	137.09	29.44	14.15
16	2.94	164.51	33.81	11.75
17	4.37	200.07	33.04	9.48
18	24.99	301.64	50.74	26.84
Min	**1.80**	**39.14**	**8.21**	**2.38**
Max	**41.02**	**910.81**	**187.98**	**66.34**
Mean	**17.98**	**274.17**	**56.86**	**19.78**
SD	**13.37**	**244.94**	**47.61**	**15.18**