Seasonal habitat and length influence on the trophic niche of co-occurring tropical tunas in the eastern Atlantic Ocean

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Abstract:

In the Gulf of Guinea, bigeye Thunnus obesus (BET) and yellowfin tuna Thunnus albacares (YFT) are important for commercial fisheries and play a prominent ecological role as top predators. Using fatty acid profiles and carbon and nitrogen stable isotopes we examined their niche partitioning in this understudied region. Niche overlap was high (>70%), similar to percentages in other ocean basins. BET occupied a higher trophic position than YFT and fed on deeper prey (high δ15N values and high proportions of mono-unsaturated fatty acids). The trophic position of YFT decreased slightly in the last 15 years (δ15N values decrease ~0.5 ‰) suggesting a change in epipelagic communities, as observed in the eastern Pacific Ocean. Ontogenic changes were limited to BET. For both species, the dietary proportion of the diatoms marker (20:5n-3) increased in the seasonal upwelling area, highlighting the seasonal habitat influence on tunas diet. The relatively lipid-rich muscle (~6 % dry weight) of Atlantic tropical tunas suggested a richer diet in this region than for Indian Ocean tropical tunas and/or differences in energy allocation strategies.

Keywords: niche overlap, fatty acids, stable isotopes, body condition, pelagic predators
1. INTRODUCTION

Bigeye *Thunnus obesus* (BET) and yellowfin tuna *Thunnus albacares* (YFT) are oceanic predators co-occurring in tropical waters worldwide. In the eastern Atlantic Ocean, around 200,000 tons of BET and YFT are caught each year, leading to an overexploitation of these species (ICCAT 2015). Tunas occupy high trophic positions in pelagic habitats and their biomass reduction induced by fishing raises concerns about the health of both tuna populations and pelagic ecosystems, especially in a global change context (Chust et al. 2014, Duffy et al. 2017, Hobday et al. 2017). This is a topical issue within the Gulf of Guinea, a productive ecosystem that supports complex food webs, which has been subject to increased pressure from commercial fisheries, human population growth and pollution from domestic and industrial sources in the adjacent countries (Aryeetey 2002, Ukwe et al. 2006).

Information on the trophic ecology of tuna in the Gulf of Guinea is limited, especially those relating to ecological tracers (Olson et al. 2016). Ecological tracers are biochemically stable compounds within organisms, including stable isotopes (SI) of carbon and nitrogen and fatty acids (FA). They provide time-integrated information on food assimilated in consumers’ tissues (Ramos and González-Solís 2012) over a period of several weeks to years in fish (Iverson et al. 2004, Madigan et al. 2012). Predictable increase from prey to predator in the nitrogen isotope ratio ($^{15}$N/$^{14}$N expressed as $\delta^{15}$N values) allows the determination of trophic position (Vander Zanden et al. 1997). Changes in the carbon isotope ratio ($^{13}$C/$^{12}$C expressed as $\delta^{13}$C values) are linked to modifications of the forage habitat (i.e., coastal vs. open ocean) (France 1995). FA are lipid constituents necessary for physiological functions, some of which cannot be readily synthesized by all consumers and are transferred conservatively in food webs. Some ‘essential’ FA, such as docosahexaenoic acid (22:6n-3), eicosapentaenoic acid (20:5n-3) and arachidonic acid (20:4n-6) are best preserved during trophic transfer (Tocher 2003). The profile of the FA transferred from prey to predator may inform on the forage taxa
(Dalsgaard et al. 2003, Budge et al. 2012). For example, in the pelagic environment, 22:6n-3 is generally a marker for dinoflagellates, 20:5n-3 is a marker for diatoms (Dalsgaard et al. 2003) and 18:1n-9 is a marker for deep-copepods (Teuber et al. 2014). In the highest trophic levels, transferred FA are better preserved in storage lipids than in structural lipids (Robin et al. 2003, Budge et al. 2012). The quantification of these ecological tracers also allows the estimation of feeding niche extent (niche space) and provides insights into resource partitioning (niche overlap) between co-occurring species (Jackson et al. 2011, Layman et al. 2012) including tunas (Teffer et al. 2015, Sardenne et al. 2016).

Given their bioenergetics (i.e. regional heterotherms, fast swim), tunas have adapted a generalist foraging strategy in oligotrophic waters worldwide, consuming small fishes, crustaceans, and cephalopods (Olson et al. 2016). In the eastern Atlantic Ocean, more than 160 prey taxa have been identified in their stomach content (Dragovich 1970, Dragovich and Potthoff 1972) indicating that diet variability is linked to environmental conditions (e.g. sea surface temperature and mixed-layer depth) (Weng et al. 2009, Parrish et al. 2015, Duffy et al. 2017). In the Atlantic Ocean, tropical tunas take advantage of prey aggregations, such as the mesopelagics lightfish *Vinciguerria nimbaria* (Ménard and Marchal 2003) and the cigarfish *Cubiceps pauciradiatus* (Ménard et al. 2000, Bard et al. 2002). The different tuna species share a common habitat and often form schools, but BET feeds more on vertically migrating Ommastrephidae squids than YFT (Cherel et al. 2007, Young et al. 2010b, Logan and Lutcavage 2013). Resource partitioning between BET and YFT is however not quantified for tuna populations from the eastern Atlantic. Olson et al. (2016) provided the first bulk isotopic values for tropical tunas in this area, suggesting a slightly higher trophic level of BET compared to other species (i.e., $\delta^{15}$N of 12.7±0.9 ‰ for BET and of 12.2±1.0 ‰ for YFT, between 2000 and 2004). They suggested that these values were affected by a size sampling bias or unaccounted for habitat effects.
To build upon this research, we aimed to (i) determine whether seasonal upwelling, length and sex (male vs. female) have an effect on BET and YFT diet using SI and FA; and (ii) quantify feeding niches and partitioning of tunas (i.e. niches extent and overlap).

**2. MATERIAL & METHODS**

**2.1. Tunas and tissue sampling**

BET and YFT were caught by purse-seiners operating in the Gulf of Guinea, eastern equatorial Atlantic Ocean, between July 2013 and September 2014. Samples were collected at the landing site (fishing port for BET and “Pêche et froid” cannery for YFT) in Abidjan Port, Ivory Coast (Fig. 1). Fishing dates (Julian days) and coordinates of fishing locations were recovered from vessel logbooks, corresponding to the catch location or catch estimates. Catch estimates are mean data computed from logbooks when anglers have grouped fish from several fishing activities in the brine freezing wells of purse-seiners (maximum uncertainty of 33 days and 7.4° square). Catch dates covered July-September and January-February, i.e. seasonal upwelling and non-upwelling periods, respectively. Fork length (FL measured with calliper to the nearest 0.1 cm), sex and macroscopic identification of gonad maturity (visual examination according to Diaha et al. 2016) were determined for each fish. Forty-five BET (22 females, 23 males) and 50 YFT (19 females, 31 males) were selected. Length ranges were 111.3±25.8 cm FL for BET and 124.6±22.6 cm FL for YFT and included only developing fish (male and female of gonad macroscopic stage 1–2) as tunas diet may change during the spawning process (Zudaire et al. 2015). Around 2g of front dorsal white muscle tissue and liver were sampled on each fish and stored frozen prior to analyses (-20°C during four months after sampling then -80°C for two years).

**2.2. Ecological tracers analysis**

SI analyses were performed on white muscle, the less variable tissue and the most extensively used in fish ecology (Pinnegar and Polunin 1999), while FA profiles were examined from
liver tissue, the richest tissue in storage lipids in tropical tunas (Sardenne et al. 2017). In addition, we recorded the muscle lipid content as a proxy of fish body condition (Tocher 2003), thereafter included in the term ‘ecological tracers’.

2.2.1. Stable isotope analysis and lipid content determination

Carbon and nitrogen SI and lipid content were analyzed on 92 freeze-dried muscle samples of tunas (45 BET and 47 YFT, three YFT samples have been lost during lab analysis). Samples were ground up to a fine homogeneous powder with a ball mill MM200 (Retsch). They were then treated with dichloromethane using a Dionex Accelerated Solvent Extractor (ASE 200), as described by Bodin et al. (2009) to remove naturally $^{13}$C-depleted lipids that affect $\delta^{13}$C values (Sardenne et al. 2015). Lipid extracts were dried to a constant weight and weighed to the nearest 0.1 mg to determine total lipid content (TLC, in % of dry weight (dw) sample). Lipid-free powders were analyzed for SI using an Elemental Analyser (Flash EA 1112; Thermo Scientific) coupled to an Isotope Ratio Mass Spectrometer (Delta V Advantage with a Conflo IV interface; Thermo Scientific) at the LIENSs Stable Isotope facility (La Rochelle, France). Results were reported in the $\delta$ unit notation and expressed as parts per thousand (‰) relative to international standards (atmospheric N$_2$ for nitrogen and Vienna-Pee Dee Belemnite for carbon). Calibration was completed using reference materials (IAEA-N$_2$, -NO$_3$, -600 for nitrogen; USGS-24, IAEA-CHE, -600 for carbon). Analytical precision based on replicate measurements of internal laboratory standard (acetanilide, Thermo Scientific) was <0.15 ‰ for both $\delta^{15}$N and $\delta^{13}$C. The effectiveness of the chemical extraction of the lipid-free samples was checked by examining the C:N ratio from the percent element weight (C:N < 3.5; Post et al. 2007).

2.2.2. Fatty acid analysis

FA were determined on total lipid extracts of fresh liver samples (45 BET and 50 YFT). Around 165±35 mg of tissue were extracted using a mixture of dichloromethane and methanol.
(2:1, v/v) with a potter homogenizer (glass/teflon). A known amount of 23:0 fatty acid was added as an internal standard, and extracts were then trans-esterified with sulphuric acid (3.8 % in methanol) at 100°C for 10 min. After addition of 800 µL of hexane and three washes with hexane-saturated distilled water, the fatty acid methyl esters (FAME) were separated and quantified on a Varian CP8400 gas chromatography equipped with a Zebron ZB-WAX column (30 m in length, 0.25 mm internal diameter, 0.25 µm film thickness; Phenomenex) and a flame ionisation detector at the LEMAR Lipidocean facility (Brest, France). Samples were injected in splitless mode at 280°C and carried by hydrogen gas. The oven temperature was raised from 60°C to 150 °C at 50°C/min, to 170 °C at 3.5 °C/min, to 185 °C at 1.5 °C/min, to 225 °C at 2.4 °C/min and then to 250°C at 5.5°C/min. FAMEs were identified by comparing sample retention times to those of a commercial standard mixture (37-components FAME Mix; Sigma) and lab-made standards using Galaxie 1.9.3.2 software (Varian). Individual FA results were expressed as percentage of the total identified FA. Finally, only FA accounting for >1% in at least two samples (n=20) were kept for statistical analyses (Table 1).

2.3. Data analysis

We used linear regressions or multivariate analyses to examine the relative importance of spatiotemporal (fishing longitude and date) and biological variables (species, length, and sex) on ecological tracers (δ¹⁵N and δ¹³C, TLC and FA profile). There was no collinearity among the five factors (variance inflation factors ranking 1.1 to 4.3). We added to our models the interaction of species*length interaction to detect inter-specific differences in diet shift. For isotopic data and TLC, covariate selection was based on the Akaïke Information Criterion (AIC). ANOVA on multiple regressions and post-hoc t-tests (with t the test value) were then applied on scaled data, and normality of the residuals checked on Q-Q plots (based on the Q-Q plot, one TLC outlier of 53.5% was removed from the dataset). For the multivariate FA
data, we tested for the equality of variances (using the function *Betadisper*, an analogous to Levene’s test available in the R package *vegan*). We then used Permutational multivariate analysis of variance (PERMANOVA; non-parametric) based on the Bray-Curtis distance matrix of the untransformed % FA to test the effect of candidate variables. Untransformed % FA were used to avoid giving artificial weight to FA present in small quantities (Kelly and Scheibling 2012). Differences in individual FA were assessed using Wilcoxon’s tests (with W the test value) for categorical variables (i.e., species and sex) and linear regressions for continuous ones (i.e., longitude, date, and length).

Then, we compared tunas’ feeding niches using the R package *SIBER* (Stable Isotope Bayesian Ellipses in R) (Jackson et al. 2011) on biplots of $\delta^{13}$C and $\delta^{15}$N values and of individual coordinates from dimension 1 and 2 of the principal components analysis (PCA) for the untransformed % FA. For each species, we computed standard ellipses encompassing 95% of the data and calculated the Bayesian standard ellipse areas (SEA$_B$) and their credible intervals (50%, 75% and 95%). The SEA$_B$ is used as a proxy of the extent of the feeding niche, parallel to the isotopic niche of Layman et al. (2012) and the credible intervals represent the uncertainty surrounding the metric. The SEA$_B$ quantifies the feeding niche occupied by each species (i.e., intra-specific) while the overlap between SEA$_B$ quantify the feeding niche share by the two species (i.e., inter-specific). We computed a Bayesian estimate for the overlap between the two SEA$_B$ and an overlap coefficient (OC, express in %) to quantify the proportion of feeding niche overlap. OC is the ratio between the Bayesian estimates for the overlap and the minimal SEA$_B$ filled by a species (similar to the Jaccard similarity coefficient). OC of 0% corresponds to no overlap and of 100% to a full SEA$_B$ overlap. All statistical analyses were performed with R 3.0.2 software.

### 3. RESULTS

#### 3.1. Inter- and intra-specific variability of ecological tracers
Muscle $\delta^{15}$N values were more variable than $\delta^{13}$C values for both species (BET $\delta^{15}$N: min–max=11.6–14.2 ‰, Coefficient of variation CV=5.0 ‰; $\delta^{13}$C: -17.6–16.5 ‰; CV=1.1 ‰ and YFT $\delta^{15}$N: 10.1–13.2 ‰, CV= 7.8 ‰; $\delta^{13}$C: -17.8–16.4 ‰; CV=1.7 ‰). The total lipid content in muscle showed large variations in BET (1.6–36.2% dw, CV=123 ‰) and YFT (0.5–18.2 ‰ dw, CV=82.6 ‰). In liver tissue, the three most abundant FA in both BET and YFT were the saturated FA (SFA) 16:0 (BET: 17.4–31.7 ‰; YFT: 18.0–32.4 ‰), the polyunsaturated FA (PUFA) 22:6nf3 (BET: 15.9–29.8 ‰; YFT: 12.9–32.0 ‰) and the monounsaturated FA (MUFA) 18:1nf9 (BET: 6.9–25.4 ‰; YFT: 4.4–24.2 ‰). Majors FA in the two species also included PUFA 20:5n-3 (BET: 3.3–8.8 ‰; YFT: 3.4–10.4%) and 20:4n-6 (BET: 2.3–8.3 ‰; YFT: 2.0–6.7 ‰); MUFA 18:1n-7 (BET: 0.8–4.1 ‰; YFT: 1.2–4.0%) and 16:1n-7 (BET: 1.2–4.1 ‰; YFT: 1.1–3.8%); SFA 18:0 (BET: 6.1–11.7 ‰; YFT: 7.9–13.9%) and 17:0 (BET: 1.5–3.0 ‰; YFT: 1.0–2.0%). Overall, elevated proportions of PUFA n-3 (>30 %) compared to PUFA n-6 (<10 %) were found for both species (Table 1).

‘Species’ and to a lesser extent ‘length’ had the largest influence on the ecological tracers, especially on $\delta^{15}$N values, TLC and FA profiles (highest F and Pseudo-F; Table 2). The spatiotemporal variables ‘longitude’ affected only FA while ‘fishing date’ has no influence on ecological tracers. First, inter-specific differences were noted with higher $\delta^{15}$N values in BET than YFT (mean±SD = 12.8±0.7 ‰ vs. 11.7±0.9 ‰; t = -7.8, p < 0.001) while a larger intra-specific variability was found in YFT’s values (CV = 5 ‰ for BET vs. 8 ‰ for YFT). TLC was also higher for BET (t = 2.0, p < 0.05) despite a larger intra-specific variability (CV = 133 ‰ for BET vs. 83 ‰ for YFT). Overall, FA profiles and levels of several individual FA were generally different between species (Pseudo-F = 10.5, p < 0.001 and Table 1). Main differences concerned 18:1n-9 (ca. 14±5 ‰ for BET vs. 11±6 ‰ for YFT), 18:0 (ca. 9±1 ‰ for BET vs. 11±2 ‰ for YFT) and 20:4n-6 (ca. 5±2 ‰ for BET vs. 4±1 ‰ for YFT). In contrast, no inter-specific differences were observed for the two other essential FA,
i.e. 22:6n-3 (W = 873, p = 0.06) and 20:5n-3 (W = 1090, p = 0.80). Regarding the main FA classes, SFA were in higher proportions in YFT than BET (ca. 39±3 % vs. 36±5 %; W = 662, p < 0.001), MUFA were higher in BET than YFT (ca. 23±6 % vs. 18±7 %; W = 1625, p < 0.001) and no difference was observed for PUFA (40±6 %; W = 882, p = 0.07) (Table 1).

Second, BET length explained 33% and 19% of the variance in δ¹⁵N values and TLC, respectively. The δ¹⁵N values linearly increased with length in BET from 12.7±0.4 ‰ under 80 cm to 13.5±0.3 ‰ above 130 cm but no significant increase was observed for YFT (Fig. 2a). TLC and its intra-specific variability also increased with length in BET, from 2.5±0.8 % dw (CV = 30 %) for individuals under 80 cm to 13.1±12.6 % dw (CV = 96 %) above 130 cm, while it remained constant in YFT (around 4.5±3.7 % dw; Fig. 2c). Length influenced FA profiles (PERMANOVA, Pseudo-F = 5, p < 0.05) for both species, but results were different for each individual FA and generally unclear because of large variability within species. For example, the proportion of essential n-3 PUFA 20:5n-3 increased with length in BET, from 5.0±1.5 % of total FA under 80 cm to 7.7±1.2 % above 130 cm but remained constant in YFT (6.2±1.6 %; CV = 26 %) (Fig. 3a). In contrast, the proportion of essential n-6 PUFA 20:4n-6 tended to decrease with length in both species, from 4.9±1.5 % under 80 cm to 3.9±1.1 % above 130 cm (mean±SD for the two species) but the relationship was poor (r² = 0.12) (Fig. 3b). For some other important FA trophic markers, such as 22:6n-3 and 18:1n-9, no relationship with ‘length’ was detected (Fig. 3c and 3d).

Finally, longitude influenced the FA profiles (Table 2). The addition of ‘longitude’ as an illustrative variable on PCA indicated an increasing proportion of 20:5n-3, 20:4n-3 and 18:2n-6 and a decreasing proportion of 20:4n-6 in tunas’ liver with the longitude value. Between 17.6°W and 4°E, the proportion of 20:5n-3 increased similarly in both species from 5.2±0.8% to 6.7±1.6% (mean±SD for the two species; Fig. 4a) and the 20:4n-3 proportion increased from 0.3±0.1% to 0.2±0.6% (Fig 4b). Within this longitude range, the increase of the 18:2n-6
proportion was more restricted (mean ranging from 0.8 to 1%; p < 0.05, r²=0.06). A large
decrease in the 20:4n-6 proportion was observed in BET only, (mean ranging from 6.7% to
3.8%; p < 0.001, r²=0.28; Fig. 4b). Carbon isotopic values were unaffected by the five tested
factors (Table 2). Fish ‘sex’ affected only TLC, females being leaner than males (4.6±5.5%
vs. 7.1±8.0%; t=2.7, p < 0.01). ‘Fishing date’ had no effect on any of the ecological tracers
analyzed.

3.2. Comparison of tropical tuna feeding niches

First, feeding niches of BET and YFT mostly overlapped according to both isotopic and FA
data. Despite the slightly higher δ¹⁵N values of BET, the isotopic feeding niches of the two
species overlapped of OC = 76.8±13.7 % (Fig. 5a). This result was in accordance with FA
feeding niches of the species, that also had an important overlap (OC = 70.2±8.2 %; Fig. 5b).
FA feeding niches were only discriminated on the PCA-Dimension 2 which explained 21.3%
of the total variability in FA profiles, and was mainly driven by 20:5n-3 and by minors FA
such 24:1n-9, 17:0 18:2n-6 and 22:4n-6 and to a lesser extent by 20:1n-9 and 18:1n-9 (Fig.
5b). Second, the extent of tuna feeding niches (proxy of the intra-specific variability) varied
across tracers. The isotopic niche extent was smaller for BET than for YFT (t=−192, p < 0.001
with all posterior estimates smaller for BET than for YFT; Fig. 5a) while the FA niche extent
was larger for BET than for YFT (t=74, p < 0.001 with 89 % of the posterior estimates larger
for BET than for YFT; Fig. 5b). Finally, the variability in FA profiles was mainly explained
by individual variability among all fish rather than the difference between species (31.1 % of
the explained variability was observed on PCA-Dimension 1 while the two species were
discriminated on the PCA-Dimension 2; Fig. 5b).

4. DISCUSSION

Using SI and FA ecological tracers, we quantified for the first time the feeding niches of BET
and YFT and their overlap in the eastern Atlantic Ocean. Overall, there was a large overlap in
the feeding niches of tropical tunas, despite BET occupying a slightly higher trophic position (higher δ¹⁵N values). The fish length appeared to be the most influencing factor for ecological tracers in BET. Spatiotemporal variables had no influence on SI values, while higher proportions of diatoms’ FA marker (20:5n-3) were observed in the liver tissue of tunas caught beyond 5°E during the seasonal upwelling. Essential FA such as 20:5n-3 could therefore be interesting tracers for the monitoring of tuna’s trophic ecology over large spatial scales. Large datasets of SI and FA should allow future studies to consider the effect of other biological and environmental factors (e.g. tuna maturity, sea surface temperature, oxygen conditions) and their interactions that we could not integrate here.

4.1. Trophic position of yellowfin decreases in the Atlantic Ocean

Spatial changes in baseline nitrogen composition preclude any direct comparison of tuna isotopic values among oceans (Lorrain et al. 2015). In the Indian and Pacific Oceans, the baseline δ¹⁵N values change with latitude, probably related to denitrification process in reduced oxygen conditions (Ménard et al. 2007, Lorrain et al. 2015) or diazotrophy in highly oligotrophic areas (Houssard et al. 2017). In the tropical Atlantic Ocean, denitrification occurs in the Caribbean Sea (Gruber and Sarmiento 1997) and upwelling occurs on the African coast, suggesting possible baseline differences between the eastern and western Atlantic Ocean. However, in the Gulf of Guinea, we assumed that the isotopic baseline is similar for these two co-occurring tunas as fishing date and longitude had no effect on tuna isotopic values in our study. BET and YFT are closely related (same genus; Dickson 1996), and because selected individuals were at similar maturity stage and of similar length, we presume that differences in δ¹⁵N values did not resulted from physiological specificities. We concluded that higher δ¹⁵N values for BET indicate its slightly higher trophic position than YFT (difference of ca. 1.1 ‰), with less than one trophic level between the two species (i.e. difference < 3.4 ‰; Post 2002). Olson et al. (2016) obtained a smaller difference between the two species in the same
region (i.e. 0.5 ‰) due to higher δ¹⁵N values for YFT in the past (12.2±1.0 ‰ in 2000-2004 vs. 11.7±0.9 ‰ here in 2013-2014). Regardless of possible fish length differences between studies (no length effect detected for YFT here), it appears that the trophic level of YFT has been decreasing over the last ten years. This suggests that either (i) YFT has a high trophic plasticity related to a flexible and opportunistic diet all year round, and thus it is affected by the random sampling; or (ii) as in the eastern Pacific Ocean, a decadal diet shift occurs in YFT with changes in mid-trophic level communities, from large epipelagic fish (0-200 m) to smaller mesopelagic species (200-1000 m), especially crustaceans (Olson et al. 2014). BET feeds at greater depth on mesopelagic species and its trophic level has remained similar across the two studies (δ¹⁵N values of 12.7±0.9 ‰ in 2000-2004 vs. 12.8±0.6 ‰ here) suggesting a decline in the trophic level of YFT prey over the last 15 years in the epipelagic ecosystem of the Gulf of Guinea. Changes in the epipelagic communities can have a broader implication on food web balance and stability and should be monitored in the Atlantic Ocean. For example, it may increase predation on mesopelagic communities and favor competition among large predators already suspected to be less resilient to climate change than previously assumed (Lefort et al. 2015, Del Raye and Weng 2015). It might also affect tuna diving behaviour as BET seems to dive according to the food availability in the upper layers (Arrizabalaga et al. 2008) and to the thermocline depth (Houssard et al. 2017).

Unfortunately, δ¹³C values could not be compared with bulk values of Olson et al. (2016) because lipid correction models require bulk C:N ratios (Logan et al. 2008) but changes in carbon sources (forage habitats and phytoplankton taxa) during the last decades should be explored. In the eastern Atlantic Ocean, a reduction of phytoplankton biomass might indeed propagate into the food web through a bottom-up control by the end of the 21st century (Chust et al. 2014). Here, we detected changes in the phytoplankton taxa with an increase of 20:5n-3 proportions in tunas collected after the upwelling period started (~ August) between 0° and
10°E. This change is consistent with the 20:5n-3-rich diatoms development favored by cool upwelling waters (20–25 °C) in the eastern Gulf of Guinea (Wiafe et al. 2016). FA might thus be an efficient tool to monitor forage taxa of tuna across seasons and years.

4.2. Large overlap in the tropical tuna feeding niches

Both SI and FA detected large overlaps (>70 %) in tuna feeding niches. Trophic position derived from δ¹⁵N values was the main source of difference in tuna feeding niche (see section 4.1). The highest trophic position of BET coincides with the δ¹⁵N values from the Indian Ocean: ca. 1 % higher in BET than in YFT (Sardenne et al. 2016). Stomach content analyses confirm this trend and shows that BET feeds more on high trophic level prey, such as squids, than YFT (Cherel et al. 2007, Logan and Lutcavage 2013).

In contrast, δ¹³C values were similar between species, probably because phytoplankton is the major source of primary production in the pelagic systems (absence of coastal macroalgae or plants with different isotopic carbon values). Large overlap (ca. 70%) in FA profiles indicated that BET and YFT feed on similar prey species but in different proportions. The higher MUFA proportion for BET compared to YFT (ca. 23±6 % vs. 18±7 %), especially in 18:1n-9, suggest a higher proportion of mesopelagic prey in the diet. In the tropical eastern Atlantic Ocean, MUFA- and 18:1n-9-rich copepods such as *Megacalanus princeps* are found between 400 and 1000 m deep (Teuber et al. 2014) and in the stomachs of small fish ingested by tropical tunas (Dragovich 1970). As a consequence, MUFA are also in great proportion in small fish feeding on mesopelagic copepods such as the myctophids (Saito and Murata 1998) and the cigarfish *Cubiceps pauciradiatus* (Young et al. 2010a), which are typical tuna prey (Bard et al. 2002, Zudaire et al. 2015). In the Indian Ocean, MUFA are also present in great proportion in tropical tunas >100 cm (Sardenne et al. 2016) which have deep dive capacities and commonly swim down to 900 m depth (Schaefer et al. 2011). The tuna FA profiles confirmed that BET generally feed at greater depth than YFT in the Gulf of Guinea, which is

The feeding niche overlaps observed here are larger than those observed in large tunas from the Indian Ocean (no overlap for SI feeding niche and 53 % for FA feeding niche in the Indian Ocean using bootstrapped convex hulls; Sardenne et al. 2016) although difference in metrics may explain these dissimilarities (convex hulls vs. SIBER). Convex hulls would provide lower overlaps here (see Fig. 3) but SIBER was preferred to reduce metrics bias related to sample size (Jackson et al. 2011).

4.3. Ontogenic changes are limited

Diet change during ontogeny was relatively limited in the present study for tuna ranging 64–174 cm FL. Only the trophic level of BET (through δ¹⁵N values) increased linearly with length, but this was not associated with noticeable changes in prey taxa according to FA profiles. No ontogenic change was observed for YFT. Our sampling lacked, however, a robust representation across length classes to detect such early changes. Stomach content analyzes of YFT from the western Atlantic Ocean showed an increase in the proportion of small fish prey in individuals between 70 and 90 cm FL (Vaske et al. 2003). In the Pacific and Indian Oceans, diet changes with length were detected through a fast increase of δ¹⁵N values for tunas between 25–55 cm FL (Graham et al. 2006, Sardenne et al. 2016). In the Indian Ocean, δ¹⁵N values slowly increased with length in both BET and YFT after 60 cm (ca. 1 ‰ between 60–130 cm) (Ménard et al. 2007, Sardenne et al. 2016) as observed here for BET (Fig. 2a). Due to their opportunistic behavior, tunas continue to feed on small prey when larger/adults (Vaske et al. 2003, Ménard et al. 2006), which have lower δ¹⁵N values than larger prey (Logan and Lutcavage 2013, Ménard et al. 2014), resulting in intermediate δ¹⁵N values for large tunas and limited changes in trophic level across length classes. Most FA proportions did not change with tuna length, contrary to the observations from the Indian Ocean for 18:1n-9 and 22:6n-3.
(Sardenne et al. 2016). Future work should focus on small and large-sized tuna (20-60 cm and 180 cm) to investigate ontogenic changes of foraging strategies in these species.

4.4. Body condition of tropical tuna

Lipids are the primary energy storage form in fish. The lipid content in an individual generally indicates the energy available for vital functions and is therefore a good proxy of fish global condition (Tocher 2003). Tropical tunas face limited seasonal changes in comparison with their temperate counterparts and consequently store fewer lipids in muscle (e.g. TLC~20 % dw in muscle tissue of bluefin tuna *T. thynnus*; Mourente et al. 2001). Yet, muscle lipid content can provide valuable indication to compare general condition of tropical tuna among regions. Here, the total lipid content of white muscle remained constant among seasons (no influence of fishing date). Total lipid content in the Gulf of Guinea is similar to TLC in the western central Pacific (TLC ~ 6.8±4.8 % dw, n=43; Lydie Couturier, unpubl. data) and higher than in the western Indian Ocean (TLC ~ 2.3±1.1 % dw for both species, n=111; Sardenne et al. 2016) although data from the three basins were collected across different seasons. This suggests two non-exclusive assumptions: (i) BET and YFT food sources are richer in the Atlantic and Pacific Oceans (in quality and/or quantity) which favors energy storage in muscle, or (ii) tunas have different energy allocation strategies among world regions. Tuna condition may indeed depend on prey quality, and seasonal upwelling favor the biomass production from phytoplankton to small fishes which might benefit tunas (Champalbert et al. 2008). Extended studies on the energy allocation strategies of tunas and on lipid content in their prey in relation to the environmental condition (e.g. upwelling intensity) are required to elucidate this point and assess its ecological implications (e.g. vulnerability of populations). Reproduction should be further considered as it seasonally affects lipid content (Mourente et al. 2001, Zudaire et al. 2014) and any decrease in energy allocation to the reproductive process might influence tuna demography.
4.5. Future directions

Information about the trophic ecology of skipjack tuna *Katsuwonus pelamis* (over 50% of total catch in the Eastern Atlantic Ocean; ICCAT 2015) sharing schools with small BET and YFT, would improve our understanding of competition among co-occurring species and ontogenic stages. In addition, skipjack tuna and small BET and YFT occur mostly in epipelagic waters and can therefore be an interesting mid-trophic level ‘sampler’ in this stratum (e.g. Cherel et al. 2007). The epipelagic stratum should be more extensively monitored and the use of ecological tracers can be a powerful tool in this context.

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Fig. 1. Location of bigeye (BET; n=45) and yellowfin tuna (YFT; n=50) caught by purse-seiners in the Gulf of Guinea between July 2013 and September 2014. Major seasonal upwelling (grey area) develops in boreal summer (July–September).
Fig. 2. Length influence on (a) nitrogen stable isotopic values ($\delta^{15}$N), (b) carbon stable isotopic values ($\delta^{13}$C) and (c) total lipid content (TLC) in muscle tissue of bigeye (BET) and yellowfin tuna (YFT) collected from the Gulf of Guinea. Linear regressions with confidence intervals are plotted when the relationship is significant.
Fig. 3. Length influence on proportions of common fatty acids trophic markers in pelagic environment: (a) 20:5n-3, (b) 20:4n-6, (c) 18:1n-9 and (d) 22:6n-3, in the liver tissue of bigeye (BET) and yellowfin tuna (YFT) collected from the Gulf of Guinea. Simple linear regressions with confidence intervals are plotted when length influence is detected. The black regression in (b) is adjusted on both BET and YFT data (similar length influence for BET & YFT).
Fig. 4. Longitude influence on the three most affected fatty acids according to PCA: (a) 20:5n-3, (b) 20:4n-3 and (c) 20:4n-6, in liver tissue of bigeye (BET) and yellowfin tuna (YFT) collected from the Gulf of Guinea. Linear regressions with confidence interval are adjusted on both BET and YFT data (no species difference) (a & b) and for BET only (c). The intensity of the grey in background indicates the seasonal upwelling influence.
Fig. 5. Feeding niches of bigeye (BET; n=45) and yellowfin tuna (YFT; n=50) from the Gulf of Guinea using SIBER on (a) δ¹³C and δ¹⁵N values of muscle, and on (b) Principal component analyze (PCA) dimensions of fatty acids (FA) profiles of liver. FA most influential in PCA (cos²>0.35) are superimposed. Ellipses areas contain 95% of the data; Boxes represent the credible intervals (95, 75 and 50%) for the Bayesian standard ellipses areas and their overlaps (in green).
Table 1. Characteristics of fish, stable isotopes (δ\textsubscript{13}C and δ\textsubscript{15}N values in ‰) and total lipid content (TLC in % dry weigh) of muscle and fatty acid (FA) distribution (in % of total FA) of liver for bigeye (BET) and yellowfin tuna (YFT) from the Gulf of Guinea. Data are mean ± SD. n denotes sample numbers analyzed. * denotes significant differences between BET and YFT (post-hoc t-tests for stable isotopes and TLC and Wilcoxon tests for fatty acids; \( p < 0.05 \)). SFA: Saturated FA; MUFA: Mono-unsaturated FA; PUFA: Poly-unsaturated FA.

<table>
<thead>
<tr>
<th></th>
<th>BET</th>
<th>YFT</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size (cm FL)</td>
<td>111.3 ± 25.8</td>
<td>124.6 ± 22.6</td>
</tr>
<tr>
<td>Sex ratio (♀ : ♂)</td>
<td>1 : 1.05</td>
<td>1 : 1.63</td>
</tr>
<tr>
<td><strong>Muscle</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>45</td>
<td>47</td>
</tr>
<tr>
<td>δ\textsubscript{13}C (‰)</td>
<td>-17.1 ± 0.2</td>
<td>-17.1 ± 0.3</td>
</tr>
<tr>
<td>δ\textsubscript{15}N (‰)</td>
<td>12.8 ± 0.6</td>
<td>11.7 ± 0.9 *</td>
</tr>
<tr>
<td>TLC (% dw)</td>
<td>7.6 ± 9.3</td>
<td>4.5 ± 3.7 *</td>
</tr>
<tr>
<td><strong>Liver</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>45</td>
<td>50</td>
</tr>
<tr>
<td>14:0</td>
<td>0.8 ± 0.2</td>
<td>0.7 ± 0.2 *</td>
</tr>
<tr>
<td>15:0</td>
<td>0.6 ± 0.2</td>
<td>0.6 ± 0.2</td>
</tr>
<tr>
<td>16:0</td>
<td>23.3 ± 4.1</td>
<td>25.0 ± 2.9 *</td>
</tr>
<tr>
<td>17:0</td>
<td>2.0 ± 0.4</td>
<td>1.5 ± 0.2 *</td>
</tr>
<tr>
<td>18:0</td>
<td>8.7 ± 1.2</td>
<td>11.0 ± 1.5 *</td>
</tr>
<tr>
<td>Σ SFA</td>
<td>35.5 ± 4.7</td>
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<tr>
<td>16:1n-7</td>
<td>2.4 ± 0.7</td>
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<tr>
<td>17:1n-7</td>
<td>1.0 ± 0.3</td>
<td>1.2 ± 0.2 *</td>
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<tr>
<td>18:1n-9</td>
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<td>11.0 ± 5.9 *</td>
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<td>18:1n-7</td>
<td>2.7 ± 0.8</td>
<td>2.3 ± 0.7 *</td>
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<td>20:1n-9</td>
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<td>0.9 ± 0.4 *</td>
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<tr>
<td>20:1n-7</td>
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<td>0.1 ± 0.1 *</td>
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<tr>
<td>24:1n-9</td>
<td>0.8 ± 0.6</td>
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<tr>
<td>Σ MUFA</td>
<td>22.7 ± 6.3</td>
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<tr>
<td>18:2n-6</td>
<td>0.9 ± 0.2</td>
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<tr>
<td>20:4n-6 (ARA)</td>
<td>5.2 ± 1.7</td>
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<tr>
<td>22:4n-6</td>
<td>0.4 ± 0.2</td>
<td>0.8 ± 0.4 *</td>
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<td>22:5n-6</td>
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<td>1.4 ± 0.4</td>
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<td>20:5n-3 (EPA)</td>
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<td>1.7 ± 0.9</td>
<td>1.7 ± 0.7</td>
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<tr>
<td>22:6n-3 (DHA)</td>
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<td>7.5 ± 1.7</td>
</tr>
<tr>
<td>Σ n-3</td>
<td>30.2 ± 3.9</td>
<td>31.8 ± 5.9</td>
</tr>
<tr>
<td>Σ PUFA</td>
<td>38.0 ± 4.8</td>
<td>39.3 ± 7.2</td>
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Table 2. Summary of factors influencing the ecological tracers of bigeye (BET) and yellowfin tuna (YFT) from the Gulf of Guinea according to ANOVA (after an AIC-based selection, _ denotes the unselected factors) for stable isotopes values ($\delta^{13}C$ and $\delta^{15}N$) and total lipid content (TLC), and PERMANOVA (based on Bray-Curtis distance matrix and 1000 permutations) for fatty acids profile. P-values in bold denote factors with significant influence on the ecological tracers.

<table>
<thead>
<tr>
<th>Factors</th>
<th>$\delta^{13}C$</th>
<th>$\delta^{15}N$</th>
<th>TLC</th>
<th>FATTY ACIDS</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>df</td>
<td>F</td>
<td>p-value</td>
<td>df</td>
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<tr>
<td>Fishing longitude</td>
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<td>3.6</td>
<td>0.060</td>
<td>_</td>
</tr>
<tr>
<td>Fishing date</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
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<td>Species</td>
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<td>1</td>
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<tr>
<td>Size</td>
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<td>0.4</td>
<td>0.511</td>
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<tr>
<td>Sex</td>
<td>_</td>
<td>_</td>
<td>_</td>
<td>_</td>
</tr>
<tr>
<td>Species*Size</td>
<td>1</td>
<td>3.8</td>
<td>0.054</td>
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