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ORIGINAL PAPER

Variations in the diet and stable isotope ratios during the ovarian development of female yellowfin tuna (*Thunnus albacares*) in the Western Indian Ocean

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Abstract The feeding strategy of female yellowfin tuna (*Thunnus albacares*) during their reproductive cycle was investigated using a combination of different trophic tracers, i.e., stomach contents and dual stable isotope analysis, along with an assessment of ovarian development based on a histological analysis. To complete these analyses, we collected 215 female yellowfin from the Western Indian Ocean in 2009 and 2010. From these fish, we noted the ovarian development and analyzed the contents of 166 non-empty stomachs and 104 liver and muscle tissue samples. Stomach content analysis identified a large variety of prey species (45 prey families), key groups including crustaceans dominated by the swimming crab *Charybdis smithii* and crustacean larvae; fish dominated by the cigarfish *Cubiceps pauciradiatus*; and cephalopods dominated by ommastrephids *Sthenoteuthis oualaniensis* and *Ornithoteuthis volatilis*. Individuals capable of spawning appeared to feed intensively, particularly on cigarfish, during the reproductive period. From the mean reconstituted weight values of the preys, our results indicated that this intensive feeding led to increased amounts of acquired energy. The results of the stable isotope analyses, carried out on the muscle and liver tissues, indicated a clear decrease in values from north to south. These analyses also showed that liver $\delta^{15}N$ values in spawning females were significantly lower than those in immature and developing individuals. This latter observation highlights the differences in metabolic processes that occur between tissues during ovarian development and underlines the importance of the liver in energy acquisition and mobilization in female yellowfin tuna during reproduction.

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Introduction

Analyzing feeding strategies is key to understanding the mechanisms driving survival, growth, and reproduction (Stearns 1992; Wootton 1998). For fish, reproduction has been identified as the most metabolically demanding process during which high amounts of nutrients and energy are required for the production of hormones and gametes, the development of secondary sexual characteristics and reproductive physiology (Potts and Wootton 1984). The manner in which energy is allocated for these reproductive processes has been shown to depend on the breeding strategy used (Stearns 1992; Wootton 1998). This in turn affects the feeding strategy (i.e., the process of energy acquisition and storage) prior to and/or during reproduction. Two main breeding strategies have been defined (Drent and Daan 1980; Stearns 1992): capital breeders, who rely on stored energetic resources related to their synchronous oocyte development, and income breeders, who have asynchronous oocyte development that depends on concurrent food

intake (Stearns 1992; McBride et al. 2013). Some organisms use a mixed capital–income breeding strategy, which can change in response to internal and external conditions (McBride et al. 2013).

Traditionally, dietary studies have relied on stomach content analysis (SCA) (Hyslop et al. 1980) to provide valuable quantitative and qualitative information on the most recent meal of an individual, which then provides information on predator–prey interactions (e.g., Gislason and Helgason 1985). However, as this analysis does not adequately consider spatial and temporal variations when drawing dietary inferences, it can give a biased perception of a species' diet composition (Cortés 1997; Ménard et al. 2007). In contrast, stable isotope analysis (SIA) provides a longer, integrated measurement of feeding relationships and dietary preferences. Indeed, an organism's energy sources and trophic position can be inferred from isotope tracers (Post 2002). In fish, tissues such as muscle that have a slow isotopic turnover provide information on feeding at a medium-term scale (i.e., months), while tissues with faster metabolic rates (e.g., liver tissue) provide information at a shorter timescale (i.e., several days to several weeks) (Tieszen et al. 1983; Madigan et al. 2012). However, the variability in trophic fractionation and the lack of accurate information on species- and tissue-specific isotopic turnover rates may complicate the interpretation of isotopic values (Jennings and van der Molen 2015). Despite the limitations of each method, SCA and SIA have been widely used to elucidate questions regarding the feeding ecology, predator–prey interactions and niche overlaps of large pelagic fish species (Roger 1994; Potier et al. 2004, 2007; Ménard et al. 2007; Olson et al. 2010).

Yellowfin tuna (*Thunnus albacares*) have a high metabolic rate that consequently requires large energy supplies to fulfill the high bioenergetics requirements of their migration, growth, and reproduction (Olson and Boggs 1986). Globally, yellowfin tuna have been characterized as a generalist predator, feeding on small pelagic fishes, crustaceans, and cephalopods (Dragovich and Potthoff 1972; Potier et al. 2007); however, variations in prey composition have previously been associated with fish size (Graham et al. 2007), aggregating behaviors (Jaquemet et al. 2011) and in particular, opportunistic feeding behaviors (Ménard et al. 2006). Yellowfin are multiple batch spawners (Schaefer 1998) with an indeterminate oocyte recruitment process (Zudaire et al. 2013a). Itano (2001) suggested that they might support their high reproductive effort during the spawning season by using a concurrent energy income gained from feeding. Recent studies looking at the variability of body condition and tissue lipid composition during the reproductive cycle suggest that this species use a mixed income–capital breeding strategy (Zudaire et al. 2014). Thus, the cost of spawning is mainly fulfilled by the instant energy gained from feeding, but can also be sourced from lipid energy previously stored in the liver (Zudaire et al. 2014). Using SCA, Potier et al. (2007) suggested that ovarian development could affect yellowfin diet; however, to our knowledge, there are no studies that have investigated the particular feeding strategies of mature females. Nevertheless, this information is essential to understanding the energy allocations associated with ovarian development and female reproductive strategies.

Here, we investigated the feeding ecology of female yellowfin tuna from the Western Indian Ocean during their reproductive cycle using a combined approach of SCA and SIA of dual stable isotopes (i.e., carbon C and nitrogen N) in muscle and liver tissues. In particular, we addressed the following questions: (1) does the diet composition of female yellowfin vary during their maturation cycle?; (2) is there any relationship between diet composition and reproductive capacity?; and (3) are the identified diet variations related to fish length, environmental conditions (i.e., fishing location and season) or school type association, i.e., fish caught in free-swimming schools (FREE) versus fish caught around fish aggregating devices (FADs)?

Materials and methods

Field sampling

A total of 215 female yellowfin tuna, ranging in size from 49 to 153 cm fork length (L_F), were sampled by scientific observers during two surveys in 2009 (January 21–March 23 and June 5–July 25) and one survey in 2010 (April 3–May 21), all of which were conducted onboard a commercial purse seiner in the Western Indian Ocean (Fig. 1). In total, 46 sets were performed: 37 sets occurred under FADs, which provided 162 females while nine sets were performed in association with FREEs and resulted in 53 females. For each fish, L_F was measured to the nearest cm, the body was weighed to the nearest 0.1 kg and liver, gonad and stomach weights were recorded to the nearest gram. A 4- to 5-cm cross section of the gonad was cut from between the middle-end part of the right or left lobe and preserved in a solution of 4 % buffered formaldehyde for subsequent histological analysis. For the SIA, samples of liver tissue (±10 g) and white muscle (taken from the dorsal part of the fish between the head and the first dorsal fin; ±10 g) were collected and stored at −20 °C. The gonadosomatic index (GSI) was estimated for each female as the ratio between the gonad weight (g) and the gonad-free body weight (g).

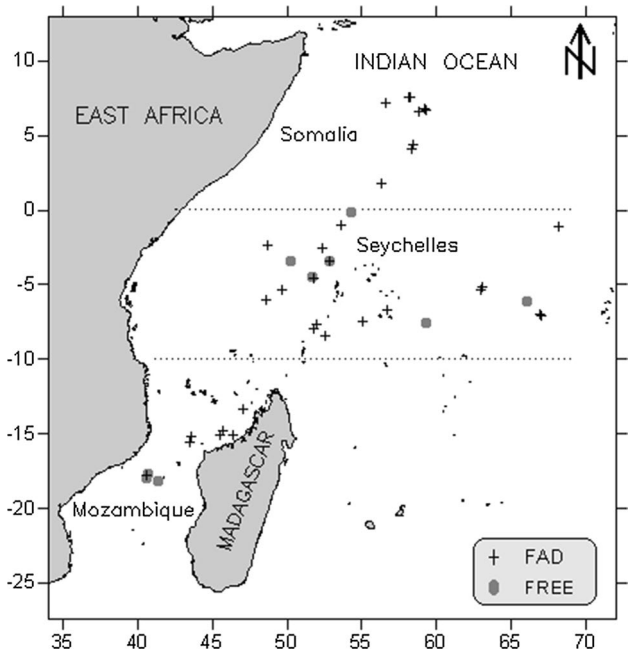

Fig. 1 Locations where female yellowfin tuna (*Thunnus albacares*) used in analyses in this study were caught, according to school type [Fish Aggregating Devices (FADs; *plus symbol*) and free-swimming schools (FREE; *filled circle*].The boundaries of the three defined regions (i.e., Somalia, Seychelles, and Mozambique) are indicated by *dashed lines*

Histological analysis

The histological classification of yellowfin tuna ovaries followed the criteria described by Zudaire et al. (2013a). The ovaries were classified according to the most advanced oocyte stage present in the ovary sample: (1) immature phase (P_I), which includes oocytes in the primary growth stage; (2) developing phase (P_D), which includes oocytes in the stages of cortical alveoli and primary and secondary vitellogenesis; (3) spawning-capable phase (P_{SC}), which includes oocytes in the stages of tertiary vitellogenesis, and germinal vesicle migration and hydration; and (4) regenerating phase (P_R), which is characterized by the presence of late-stage atresia and a thicker ovarian wall than seen in immature fish.

Stomach content analysis

The stomachs of each female were sampled, stored at −20 °C immediately after sampling and then thawed and drained in the laboratory. Stomach contents were removed, and after rinsing and wiping, the stomach wall was weighed to the nearest gram. The total food wet mass (M_W) for each stomach was estimated as the difference between the total stomach weight and the weight of the emptied stomach. The contents of the stomach were sorted into five main categories (i.e., cephalopod, fish, crustacean, non-cephalopod mollusk and other), and the M_W of each of these categories was recorded. For each category, each identifiable item was sorted into two groups: (1) fresh prey (defined as whole individuals), parts of individuals and/or hard parts (e.g., otoliths, bones, cephalopod beaks) in good condition, which corresponded to the last feeding event; and (2) accumulated prey, defined as damaged hard parts (e.g., cephalopod beaks without meat, eroded otoliths, fish bones without flesh), which corresponded to earlier feeding events. This latter group was excluded from the analysis as they could lead to overestimations of the importance of some prey items in the recent diet (Potier et al. 2007). Each fresh prey item was sorted and the number of individuals was noted using the identifiable body parts found in the stomach. Each prey item was identified to the lowest possible taxon using keys and descriptions found in the literature (Crosnier and Forest 1973; Tregouboff and Rose 1978; Clarke 1986; Smith and Heemstra 1986; Nesis et al. 1987; Smale et al. 1995; Potier et al. 2007) and by comparing them with material from our reference collection (Potier et al. 2007).

Morphometric measurements to the nearest mm were taken for prey items in good condition: L_F and standard length (L_S) for fishes, dorsal mantle length (L_{DM}) for cephalopods, and total length (L_T) and carapace width (W_C) for crustaceans. The reconstituted mass (M_R) of each fresh prey item was estimated using allometric equations found in the literature and our own equations that relate the morphometric measurements of identifiable hard parts (i.e., otoliths and dental records for fish, beaks for cephalopods and telsons for crustaceans) to the prey weight (Potier et al. 2007). Similarly, the size of the digested prey items was estimated using allometric equations which relate the size of different hard parts to the overall size of the prey. Firstly, indices were calculated using the non-empty stomachs to describe the overall diet composition of yellowfin tuna, namely, the frequency of occurrence (F_O), i.e., the number of occurrence of a prey species divided by the number of non-empty stomachs, the average percent of prey items in the stomach by number (P_N), i.e., the number of each species divided by the total number of preys found in the individual stomach and computing the mean proportion found in all stomachs, and average percent of prey by reconstituted mass (P_M), i.e., reconstituted mass of a prey species divided by the total reconstituted mass in the individual stomach and computing the mean proportion found in all stomachs (Potier et al. 2007). Secondly, the total food wet mass (M_W) of the stomach contents, the total number of prey (N_P) recently ingested, and the total reconstituted mass (M_R) of the consumed prey were used to investigate the variability in the diet of female yellowfin (see "Statistical analysis" section). Although these three indices are

Table 1 Distribution of female yellowfin tuna (*Thunnus albacares*) stomachs collected in the Western Indian Ocean according to their ovarian development phase (D) (P_I = immature phase, P_D = developing phase, P_{SC} = spawning-capable phase, P_R = regenerating phase) with associated fork length range (L_F; cm), sorted by region and school type [fish aggregating device (FAD) or free-swimming school (FREE)]

D	L_F range	Somalia		Seychelles		Mozambique	
		FAD	FREE	FAD	FREE	FAD	FREE
P_I	48–112	32 (16)	–	30 (7)	–	16 (1)	12 (0)
P_D	50–148	32 (6)	–	41 (17)	13 (0)	1 (0)	2 (0)
P_{SC}	88–153	–	–	5 (0)	22 (0)	–	–
P_R	104–120	–	–	–	–	5 (1)	4 (0)

The number of empty stomachs is specified in brackets

partially correlated, they provide different but complementary information about the feeding strategy.

Stable isotopic analysis

A total of 104 liver and muscle tissue samples were selected from the 215 individuals, based on the four ovarian developmental phases previously identified. Each sample was cryogrinded using a mixer mill MM400 Retsch® (Verder, France). A subsample (~2 ± 0.1 g) of the frozen homogenized powder was then freeze-dried, and the water content (%) was estimated based on the subsequent weight loss. Lipids were removed from each sample by using dichloromethane on an accelerated solvent extraction system (ASE®, Dionex), as described by Bodin et al. (2009). The $^{13}C/^{12}C$ and $^{15}N/^{14}N$ ratios were determined using an elemental analyzer (Flash EA 1112, Thermo Scientific, Milan, Italy) coupled to an isotope ratio mass spectrometer (Delta V Advantage with a Conflo IV interface, Thermo Scientific, Bremen, Germany). The isotopic ratios are expressed in the conventional δ notation as parts per thousand (‰) relative to the international standard for Peedee Belemnite Carbonate (C) and atmospheric nitrogen (N). Analytical precision, based on the isotope values of the acetanilide (Thermo Scientific) used to estimate the C and N content for each sample series, was found to be <0.1 %.

Statistical analysis

We used linear regression models to test for the effects of region (R), season (M), school type association (S), fish length (F_L), and ovarian development phase (D) on the individual diet indices (M_W, N_P, M_R) and the isotopic ratios of C ($\delta^{13}C$) and N ($\delta^{15}N$). Distinct models were considered for the isotopic ratios in each tissue type, as they result from different metabolic processes that occur at different temporal scales. Differences in the isotopic ratios between muscle and liver tissues ($\Delta\delta^{13}C_{M-L}$ and $\Delta\delta^{15}N_{M-L}$) were also used to describe the temporal changes in diet due to assimilation. The diet indices were log-transformed prior to analysis to remove the skew and minimize the large standard deviations in the data. Three regions, extending along a latitudinal gradient from 10°N to 20°S, were considered to represent the distinct habitats in which prey availability changes: Somalia (10°N–0°), Seychelles (0°–10°S), and Mozambique (10°S–20°S) (Fig. 1). Monsoons have been shown to have a major influence on the oceanographic features and biological productivity of the Indian Ocean (Schott et al. 2009), with effects on tuna prey propagating through the food web (Romanov et al. 2009; Vipin et al. 2012). Consequently, we modeled three distinct seasons, i.e., the Northeast (NE) monsoon (January–March), the spring inter-monsoon (SI; April–May), and the Southwest (SW) monsoon (June–July) as factors to account for any seasonal effects on the relationship between the reproductive status of female yellowfin and their trophic ecology. The type of school association, i.e., FAD or FREE, was included in the model as a factor to describe the local environment of the fish at the moment of capture. When possible, interaction between school type and region (S:R) was considered in the model. No interaction was considered between season and region and between season and school type in the model because the sampling design appeared to be unbalanced (Table 1) due to the seasonal patterns in purse seine fishing in the Indian Ocean (Kaplan et al. 2014). The following model was considered for each tissue-specific isotopic ratio and diet index:

$$Y_i = R + M + L_{Fi} + S + D_i + S:R + \varepsilon_i$$

where Y is the variable of interest available for the fish i, R is the region, M is the monsoon season, L_F is the fork length, S is the school type, D is the ovarian development phase, and S:R was the interaction between school type and region. ε was modeled as an independent, normally distributed random variable with mean zero and constant variance. The residuals were checked to support assumptions

of homoscedasticity and Gaussian error. Models were evaluated using the Akaike information criterion (AIC).

Results

Yellowfin diet in the Western Indian Ocean

The diet of female yellowfin from the Western Indian Ocean was found to be highly diverse and highly variable between individuals. A total of 2608 prey items from 45 distinct families were recovered from the sampled stomachs. Overall, the diversity of prey items appeared larger in individuals caught under FADs (39 families) versus those swimming in unassociated schools (25 families). Detailed information on the frequency of prey occurrence in the stomachs and the average proportions of prey reconstitute mass for each group and item are given in Table 2. Crustaceans were the dominant prey group in terms of occurrence ($F_O = 64.5$ %), while the two other main prey groups showed very similar levels of occurrence, i.e., $F_O = 53.6$ % and $F_O = 52.4$ % for fish and cephalopods, respectively. On average, crustaceans contributed 40.8 % (SD = 41 %) of the total number of prey items found in each stomach and 35.9 % (SD = 44 %) of the total reconstituted mass of prey ingested. The main crustacean species was the swimming crab *Charybdis smithii*, followed by undefined crustacean larvae and stomatopoda larvae *Odontodactylus scyllarus*. Fishes dominated the mass of prey consumed ($P_M = 37.3$ %), with cigarfish (*Cubiceps pauciradiatus*) as main species. The cephalopod group contributed approximately 25 % to the diet and was dominated by two species of Ommastrephidae, *Sthenoteuthis oualaniensis* and *Ornithoteuhis volatilis*. The main difference in diet composition between the two school types was the presence of cigarfish (Pearson's Chi-squared test, $p < 0.001$). This small pelagic species was found in the stomachs of 21.1 % of FREE-associated females and composed about 50 % of their diet (48.2 % P_N and 47.8 % P_M). However, it only occurred in 1.3 % of the stomachs of FAD-associated females, making up a very small contribution to overall diet (1 % P_N and 0.9 % P_M) (Table 3).

The composition of prey species varied between regions and seasons. *S. oualaniensis* was found significantly more frequent during the NE monsoon than the SW monsoon in the Seychelles and Somalia regions (Seychelles: 33.3 and 10.6 %, respectively; and Somalia: 41.9 and 9.1 %, respectively; Pearson's Chi squared tests, $p < 0.001$). The highest F_O for this cephalopod species was found in Mozambique during the SI monsoon (54.1 %); however, it was absent in the stomachs of females caught in the Seychelles during the same period. Other main prey species such as cigarfish, swimming crab, and crustacean larvae presented more restricted spatial distributions, and their presence in the different regions was related to season. For example, the cigarfish was found mainly in the Seychelles, with occurrence increasing significantly from the NE monsoon ($F_O = 25$ %) to the SW monsoon ($F_O = 51.1$ %) (Pearson's Chi-squared test, $p < 0.001$). This pelagic species was also found in Mozambique during the SI monsoon but at lower values ($F_O = 13.5$ %). Swimming crabs were found in stomachs of yellowfin from the Somalia region in both seasons, but a fourfold significant increase in F_O was observed during the SW monsoon ($F_O = 100$ %), as compared to the NE monsoon ($F_O = 25$ %) (Pearson's Chi-squared test, $p < 0.001$). In the Seychelles region, swimming crabs were only recorded during the SW monsoon ($F_O = 42.6$ %) and were absent in Mozambique during the SI monsoon. Stomatopoda larvae were found in 40.5 % of the stomachs sampled in the Mozambique region during the SI monsoon, but this prey group occurred in much lower numbers in the Seychelles during the same period (25 %) (Pearson's Chi-squared test, $p = 0.03$).

Diet composition and reproduction in the Seychelles region

Our analysis of the Seychelles region revealed major differences in the diet of female yellowfin according to season and school type association. The F_O of the main prey species (i.e., cigarfish, swimming crab and crustacean larvae) varied considerably depending on school type and season (Table 4). Cigarfish were found in 100 and 92.3 % of FREE-associated individuals caught during the NE and SW monsoons, respectively, while they were absent from FAD-associated individuals. In contrast, crustacean larvae were present in 66.7 % of FAD-associated individuals during the NE monsoon, but absent from those captured in FREE-associated individuals during the same period. This trend changed during the SW monsoon when crustacean larvae were present in 11.5 % of FREE-associated tuna, but absent from FAD-associated tuna. Finally, swimming crabs were only recorded during the SW monsoon, with significantly higher occurrence in FAD-associated individuals ($F_O = 52.4$ %) than in FREE-associated individuals ($F_O = 34.6$ %) (Pearson's Chi-squared test, $p = 0.02$).

In the Seychelles region, fish prey species with a $P_M > 80$ % were more prominent in females who were in the spawning-capable phase during the NE and SW monsoons ($P_M = 80.3 \pm 32.7$ and 91.5 ± 24.6 %, respectively). These high values coincided with the highest GSI values (GSI = 1.6 ± 0.6 and 1.2 ± 0.5, respectively) (Table 5). Fish species were not common in the stomachs of developing females, but showed a slight increase in P_M as the ovaries developed during both seasons. Moreover, the diet of females in P_I and P_D phases caught in the Seychelles

Table 2 Diet composition of yellowfin tuna (*Thunnus albacares*) by school type [fish aggregating device (FAD) or free-swimming school (FREE)]: the number of stomachs collected (*n*), frequency of occurrence of each prey(F_O), average percent of prey type by number (P_N) and average percent of the reconstituted mass (P_M) for each prey group, species or category where Und. = undetermined

Prey group		F_O				$P_N \pm SD$		$P_M \pm SD$	
		n	%						
Fishes		89	53.61			31.79 ± 40.26		37.33 ± 44.62	
Crustaceans		107	64.46			40.78 ± 41.04		35.94 ± 44.14	
Cephalopods		87	52.41			24.56 ± 33.62		25.80 ± 37.58	
Other preys		16	9.64			2.87 ± 15.04		0.93 ± 8.16	
	Prey item	FAD		FREE		FAD	FREE	FAD	FREE
		F_O				$P_N \pm SD$	$P_N \pm SD$	$P_M \pm SD$	$P_M \pm SD$
		n	%	*n*	%				
Fishes									
Acanthuridae	*Naso brevirostris*	1	0.6	–	–	0.08 ± 0.86	–	0.17 ± 1.82	–
Balistidae	*Canthidermis maculata*	2	1.2	2	1.2	1.05 ± 9.55	0.24 ± 1.21	0.97 ± 9.44	0.44 ± 2.96
Bramidae	*Brama sp*	1	0.6	–	–	0.88 ± 9.41	–	0.88 ± 9.41	–
Carangidae	Und. Carangid	1	0.6	–	–	0.09 ± 0.94	–	0.83 ± 8.81	–
	Decapterus sp	1	0.6	1	0.6	0.88 ± 9.41	0.08 ± 0.58	0.88 ± 9.41	0.41 ± 2.99
	Decapterus macarellus	4	2.4	–	–	0.56 ± 3.21	–	1.55 ± 8.74	–
	Decapterus macrosoma	5	3.0	–	–	2.38 ± 13.76	–	3.60 ± 17.52	–
Dactylopteridae	*Dactyloptena orientalis*	–	–	5	3.0	–	1.35 ± 7.00	–	3.39 ± 14.50
Diretmidae	*Diretmus argenteus*	1	0.6	1	0.6	0.10 ± 1.05	0.02 ± 0.14	0.01 ± 0.07	0.01 ± 0.07
Echeneidae	*Remora brachyptera*	1	0.6	–	–	0.29 ± 3.14	–	0.32 ± 3.45	–
Exocoetidae	*Exocoetus volitans*	6	3.6	–	–	1.65 ± 10.06	–	3.01 ± 14.99	–
Hemiramphidae	*Oxyporhamphus micropterus*	–	–	1	0.6	–	0.05 ± 0.34	–	0.11 ± 0.82
Holocentridae	*Sargocentron sp*	1	0.6	2	1.2	0.09 ± 0.94	0.29 ± 1.55	0.09 ± 0.96	0.23 ± 1.20
Monacanthidae	Und. Monacanthids	–	–	1	0.6	–	0.67 ± 4.91	–	0.21 ± 1.52
Myctophidae	Und. Myctophids	–	–	1	0.6	–	0.09 ± 0.67	–	0.03 ± 0.22
	Diaphus sp	3	1.8	4	2.4	1.11 ± 7.23	1.79 ± 7.01	1.59 ± 9.86	0.71 ± 3.15
	Myctophum sp	4	2.4	1	0.6	0.57 ± 3.23	0.04 ± 0.32	0.45 ± 2.75	0.01 ± 0.09
Nomeidae	*Cubiceps pauciradiatus*	3	1.8	35	21.1	1.05 ± 9.47	48.17 ± 45.15	0.97 ± 9.43	47.84 ± 47.02
	Psenes arafurensis	2	1.2			1.59 ± 11.99	–	1.01 ± 9.49	–
Ostraciidae	*Ostracion cubicus*	–	–	1	0.6	–	0.19 ± 1.37	–	0.01 ± 0.04
Paralepididae	*Paralepis sp*	1	0.6	–	–	0.88 ± 9.41	–	0.88 ± 9.41	–
Scaridae	Und.Scarids	–	–	1	0.6	–	0.15 ± 1.06	–	0.01 ± 0.05
Scombridae	Und.Scombrids	4	2.4	–	–	1.65 ± 10.49	–	3.41 ± 17.93	–
	Katsuwonus pelamis	5	3.0	–	–	1.88 ± 11.53	–	3.81 ± 18.05	–
Scopelarchidae	*Scopelarchus analis*	1	0.6	–	–	0.44 ± 4.70	–	0.73 ± 7.76	–
Unknown fish		5	3.0	1	0.6	2.11 ± 12.04	0.13 ± 0.92	2.11 ± 13.53	0.84 ± 6.10
Fish larvae	Fish larvae	4	2.4	3	1.8	1.85 ± 13.25	1.14 ± 5.22	1.81 ± 13.25	0.62 ± 3.17
Crustaceans									
Brachyscelidae	*Brachyscelus sp*	2	1.2	–	–	0.41 ± 3.34	–	0.21 ± 2.05	–
Cyphocaridae	*Cyphocaris faurei*	1	0.6	–	–	0.06 ± 0.59	–	0.00 ± 0.02	–
Enoplometopidae	*Enoplometopus sp*	3	1.8	–	–	0.32 ± 2.23	–	0.02 ± 0.17	–
Larves crustacés		27	16.3	11	6.6	8.99 ± 20.38	3.26 ± 8.03	3.00 ± 13.03	0.80 ± 2.78
Lysiosquillidae	*Lysiosquilla tredecimdentata*	11	6.6	14	8.4	2.79 ± 9.67	7.54 ± 18.86	2.40 ± 10.65	6.05 ± 19.09
Odontodactylidae	*Odontodactylus scyllarus*	21	12.7	11	6.6	5.45 ± 14.14	4.37 ± 15.86	4.97 ± 14.65	3.93 ± 15.93
Oplophoridae	Und. Oplophorids	1	0.6	–	–	0.10 ± 1.05	–	0.32 ± 3.37	–
	Oplophorus spinosus	1	0.6	–	–	0.09 ± 0.94	–	0.02 ± 0.19	–

Table 2 continued

	Prey item	FAD		FREE		FAD	FREE	FAD	FREE
		F_O				$P_N \pm SD$	$P_N \pm SD$	$P_M \pm SD$	$P_M \pm SD$
		n	%	*n*	%				
Phrosinidae	*Phrosina semilunata*	1	0.6	–	–	0.18 ± 1.88	–	0.02 ± 0.26	–
Platyscelidae	*Platyscelus ovoides*	3	1.8	3	1.8	0.33 ± 2.22	0.27 ± 1.22	0.02 ± 0.13	0.01 ± 0.06
Portunidae	*Charybdis smithii*	31	18.7	9	5.4	21.30 ± 38.88	14.18 ± 32.10	24.55 ± 41.86	15.71 ± 35.20
Amphipoda		2	1.2	–	–	0.90 ± 9.41	–	0.89 ± 9.41	–
Caridea		1	0.6	–	–	0.10 ± 1.05	–	0.01 ± 0.12	–
Mysidacea		2	1.2	–	–	0.63 ± 4.70	–	0.19 ± 1.42	–
Und. Srhimps		1	0.6	3	1.8	0.29 ± 3.14	1.88 ± 8.38	0.21 ± 2.26	0.68 ± 2.93
Stomatopoda		1	0.6			0.29 ± 3.14	–	0.45 ± 4.78	–
Und. Crustacea	Unknown crustacea	7	4.2	1	0.6	2.82 ± 11.77	0.18 ± 1.33	2.74 ± 14.82	0.03 ± 0.21
Cephalopods									
Bolitaenidae	*Japetella diaphana*	4	2.4	1	0.6	0.59 ± 3.55	0.02 ± 0.18	0.98 ± 7.09	0.06 ± 0.43
Chiroteuthidae	*Chiroteuthis sp*	8	4.8	6	3.6	1.56 ± 6.87	1.10 ± 5.09	0.63 ± 3.45	0.84 ± 4.10
Halloposidae	*Haliphron atlanticus*	4	2.4	1	0.6	0.94 ± 7.00	0.02 ± 0.18	0.81 ± 7.81	0.06 ± 0.43
Octopoteuthidae	Und. Octopoteuthids	–	–	1	0.6	–	0.05 ± 0.37	–	0.32 ± 2.33
	Octopoteuthis sp	1	0.6	–	–	0.18 ± 1.88	–	0.00 ± 0.04	–
	Taningia danae	–	–	1	0.6	–	0.03 ± 0.22	–	0.00 ± 0.03
Ommastrephidae	*Ornithoteuthis volatilis*	16	9.6	12	7.2	4.70 ± 16.36	2.38 ± 6.82	4.79 ± 17.31	2.26 ± 5.77
	Sthenoteuthis oualaniensis	32	19.3	19	11.4	13.16 ± 26.74	8.13 ± 18.56	13.62 ± 28.98	11.25 ± 25.45
Onychoteuthidae	*Moroteuthis lonnbergii*	8	4.8	1	0.6	1.97 ± 8.54	0.47 ± 3.43	0.87 ± 4.87	1.06 ± 7.71
	Onychoteuthis banksi	18	10.8	6	3.6	4.57 ± 15.12	0.95 ± 5.44	4.67 ± 17.92	1.51 ± 8.85
	Walvisteuthis rancureli	1	0.6	–	–	0.27 ± 2.82	–	0.56 ± 5.95	–
Pholidoteuthidae	*Pholidoteuthis boschmai*	1	0.6	–	–	0.10 ± 1.05	–	0.05 ± 0.57	–
Thysanoteuthidae	*Thysanoteuthis rhombus*	3	1.8	1	0.6	0.35 ± 2.16	0.05 ± 0.39	0.90 ± 9.20	0.40 ± 2.90
Enoploteuthidae	Und. Enoploteuthids	–	–	1	0.6		0.04 ± 0.27		0.08 ± 0.61
Oegopsidae	Und. Oegopsids	2	1.2	–	–	0.21 ± 1.57	–	0.34 ± 3.11	–
Und. cephalopod	Unknown cephalopod	3	1.8	–	–	1.29 ± 9.95	–	1.29 ± 10.33	–
Other preys									
Cavoliniidae	*Cavolinia sp.*	1	0.6	–	–	0.18 ± 1.88	–	0.00 ± 0.02	–
Gastropods		3	1.8	–	–	0.62 ± 4.35	–	0.02 ± 0.11	–
Salpidae		7	4.2	5	3.0	3.10 ± 13.37	0.67 ± 2.20	1.31 ± 9.77	0.09 ± 0.33

during the NE monsoon were characterized by similar P_M of fish, crustaceans, and cephalopods (Table 5). Conversely, during the SW monsoon, individuals in the P_I and P_D development phases showed higher P_M for crustaceans ($P_I = 62.5 \pm 51.8$ %, and $P_D = 53.2 \pm 46.5$ %, respectively) as compared to the other prey categories. This increase was mainly driven by the presence of the swimming crab.

Modeling variability in yellowfin diet in the Western Indian Ocean

The recent diet of female yellowfin was found to significantly vary between region, school type, and size. No effect of female reproductive status was detected for the three diet indices. L_F was found to be the biggest explanatory factor of observed variance in the total quantity of recently ingested prey (Table 3). Linear regression models showed a significant increase in the total wet mass of stomach contents, the total number of prey items, and the total reconstituted mass of ingested prey ($p < 0.001$) with L_F. The mean M_W of the stomach contents was 87.3 ± 156.01 g (ranging from <0.1 g to 1126.2 g). Despite a high variability observed in the stomach contents, a positive relationship between M_W and L_F was found by the model. Although region did not explain much variability in the total wet mass of the prey consumed (<7 %), M_W in the Somalia region was significantly higher ($p = 0.002$) than in the Seychelles and Mozambique regions. No empty stomachs were observed in the FREE-associated samples ($n = 53$;

Table 3 Parametric coefficients for the best models identified for modeling the variability in total food wet mass (M_W), total number of prey items (N_P), and total reconstituted mass (M_R) of recently ingested prey derived from the SCA of female yellowfin tuna (*Thunnus albacares*), as determined by the smallest AIC (Akaike information criterion)

Response variable	Best model	Covariate	*Df*	*F* value	Pr(>*F*)	AIC	ΔAIC	% variance explained
M_W	$\sim R + L_F$					−33.76	0	54.4
		Region *R*	2	7.33	<0.001	−32.92	0.84	4.2
		Fork length L_F	1	144.06	<0.001	24.60	58.36	50.2
N_P	$\sim R + S + L_F$					−1.41	0	44.4
		Region *R*	2	20.25	<0.001	2.96	4.37	14.0
		School association *S*	1	40.92	<0.001	34.18	35.59	14.1
		Fork length L_F	1	47.07	<0.001	2.66	4.07	16.3
M_R	$\sim R + L_F$					218.21	0	32.7
		Region *R*	2	8.17	<0.001	223.37	5.16	6.8
		Fork length L_F	1	62.49	<0.001	248.15	29.94	25.9

The three response variables were analyzed as a function of region (*R*), season (*M*), school association (*S*), fish length (L_F), ovarian development phase (*D*) and the interaction between school type and region (*S:R*)

Table 4 Frequency of occurrence (F_O) of the main prey species of female yellowfin tuna (*Thunnus albacares*) in the Seychelles region by season (northeast monsoon (NE), southwest monsoon (SW), spring inter-monsoon (SI)) and school type (fish aggregating device (FAD), free-swimming school (FREE)) where n indicates the number of stomachs analyzed

Species	NE		SW		SI
	FAD ($n = 9$)	FREE ($n = 27$)	FAD ($n = 21$)	FREE ($n = 26$)	FAD ($n = 4$)
Charybdis smithii	0.0	0.0	52.4	34.6	0.0
Cubiceps pauciradiatus	0.0	100.0	0.0	92.3	0.0
Crustacean larvae	66.7	0.0	0.0	11.5	0.0
Odontodactylus scyllarus	37.0	33.3	0.0	0.0	25.0
Ornithoteuthis volatilis	22.2	22.2	14.3	15.4	25.0
Sthenoteuthis oualaniensis	25.9	55.6	4.8	15.4	0.0

Table 5 Range of fish fork length (L_F, cm), the gonadosomatic index (GSI, mean ± SD), the total reconstituted mass of prey items (M_R, g) and the average percentage of reconstituted mass per prey category (P_M) according to the season [northeast monsoon (NE), southwest monsoon (SW), spring inter-monsoon (SI)] and ovarian development phase *D* (immature phase (P_I), developing phase (P_D), and spawning-capable phase (P_{SC})) for yellowfin tuna females (*Thunnus albacares*) in the Seychelles region

Season	*D*	*n*	L_F	GSI	M_R	P_M				
						CU	CE	F	M	O
NE monsoon	P_I	13	49–87	0.16 ± 0.10	24.6 ± 32.3	34.4 ± 42.6	38.2 ± 45.5	26.0 ± 43.4	0.0 ± 0.2	1.4 ± 5.1
	P_D	11	50–110	0.35 ± 0.15	66.7 ± 133.1	32.7 ± 42.7	30.7 ± 40.9	36.6 ± 45.6	0.0 ± 0.0	0.0 ± 0.0
	P_{SC}	12	88–153	1.59 ± 0.60	131.5 ± 82.5	0.2 ± 0.6	19.4 ± 32.3	80.3 ± 32.7	0.0 ± 0.0	0.1 ± 0.2
SW monsoon	P_I	8	56–104	0.24 ± 0.08	15.9 ± 10.1	62.5 ± 51.8	14.7 ± 35.0	22.8 ± 42.5	0.0 ± 0.0	0.0 ± 0.0
	P_D	24	61–148	0.52 ± 0.29	174.2 ± 195.4	53.2 ± 46.5	7.9 ± 21.2	38.5 ± 46.5	0.0 ± 0.0	0.3 ± 1.1
	P_{SC}	15	107–146	1.19 ± 0.51	152.7 ± 104.7	6.6 ± 25.0	1.9 ± 2.7	91.5 ± 24.6	0.0 ± 0.0	0.1 ± 0.2
SI monsoon	P_I	2	54–112	0.22 ± 0.16	7.92 ± 3.6	7.0 ± 5.9	7.9 ± 1.1	84.7 ± 4.2	0.4 ± 0.5	0.0 ± 0.0
	P_D	2	57–113	0.4 ± 0.16	25.71 ± 17.4	0.0 ± 0.0	51.5 ± 68.6	48.5 ± 68.6	0.0 ± 0.0	0.0 ± 0.0

n number of individuals with non-empty stomachs, *CU* crustacean, *CE* cephalopod, *F* fish, *M* mollusks other than cephalopods, *O* other

$M_W = 128.5 \pm 128.3$ g), but they represented 30 % of the FAD-associated stomachs sampled ($M_W = 67.8 \pm 164.4$ g). The number of prey items found in the stomachs was significantly affected by region ($p < 0.001$), L_F ($p < 0.001$) and school type ($p < 0.001$). Females caught in FREEs showed significantly higher N_P values ($p < 0.001$) than females caught in association with FADs, especially in the Seychelles region ($p = 0.015$).

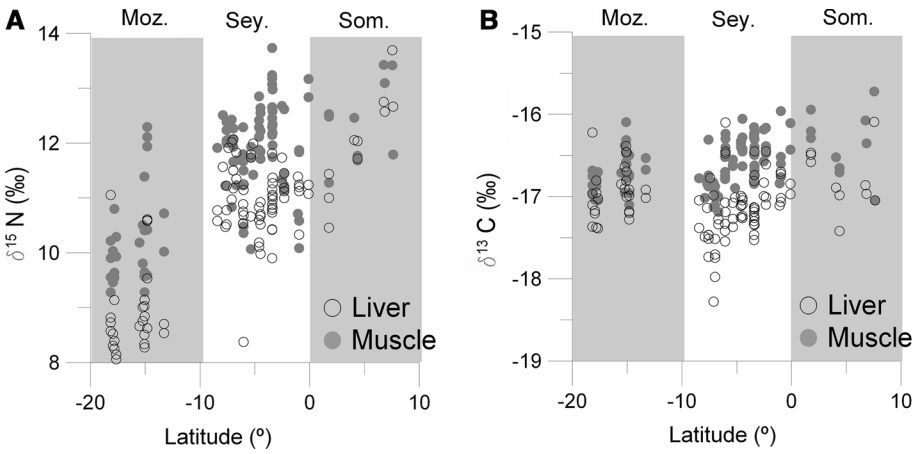

Fig. 2 Values of $\delta^{15}N$ (**a**) and $\delta^{13}C$ (**b**) in muscle tissue (*filled circle*) and liver tissue (*circle*) of female yellowfin tuna (*Thunnus albacares*) plotted versus latitude in the Western Indian Ocean

Stable isotopic ratios of yellowfin in the Western Indian Ocean

The isotopic values of carbon and nitrogen in both liver and muscle tissues strongly differed between regions and seasons, reflecting large spatiotemporal variations in the available energy sources. Raw values of muscle $\delta^{15}N$ ranged from 9.3 to 13.7 ‰ (mean ± SD = 11.5 ± 1.1 ‰), showing a decreasing trend along a north-to-south latitudinal gradient from Somalia to Mozambique with a variation range of ~2.5 ‰ (Fig. 2). The linear model selected for muscle $\delta^{15}N$ showed that the region effect was highly significant ($p < 0.001$) and explained the majority of the variance observed (>50 %) (Table 6). Significant differences in muscle $\delta^{15}N$ values were also found for L_F and season, but these variables explained a small amount of variance (i.e., 1.7 and 3.4 %, respectively). FREE-associated females, especially those caught in the Seychelles region, showed significantly higher muscle $\delta^{15}N$ values ($p < 0.001$) than females caught in FAD-associated schools. Muscle $\delta^{13}C$ values varied from −17.2 to −15.7 ‰ (mean ± SD = −16.6 ± 0.3 ‰) and were characterized by a decrease from north to south (~1.3 ‰) (Table 7). Muscle $\delta^{13}C$ values were significantly affected by region, season, F_L, and school type (Table 6). The season effect explained 28 % of the total variance in muscle $\delta^{13}C$ values and appeared to be a more important factor than the muscle $\delta^{15}N$ ratio. Larger females were described by higher muscle $\delta^{13}C$ values. Significantly lower muscle $\delta^{13}C$ values were found for FREE-associated individuals ($p = 0.004$) and those caught during the NE monsoon season ($p < 0.001$) than individuals caught in association with FADs or during the SI and SW monsoons.

Liver $\delta^{15}N$ values ranged from 8.1 to 13.7 ‰ (mean ± SD = 10.5 ± 1.2 ‰) and showed a decreasing trend along a latitudinal gradient from Somalia to Mozambique, with a variation range of 4 ‰. This figure is similar to the range observed for muscle $\delta^{15}N$ values (Fig. 2). Liver $\delta^{15}N$ was significantly affected by region ($p < 0.0001$), with this variable explaining 68 % of the total variance. Season was also found to significantly affect $\delta^{15}N$ in the liver: females caught during the NE monsoon showed higher liver $\delta^{15}N$ values than individuals caught during the two other seasons. Liver $\delta^{15}N$ values were also found to vary significantly with ovarian development ($p < 0.0001$). Females who were in their spawning-capable phase had significantly lower ($p < 0.001$) liver $\delta^{15}N$ values than those in the other development phases. As with muscle, liver $\delta^{13}C$ values were significantly affected by region ($p < 0.0001$), season ($p < 0.0001$), school type ($p < 0.0001$), and L_F. Values varied from −18.3 to −16.1 ‰ (mean ± SD = −17.1 ± 0.4 ‰), and similar to the other stable isotope values, a decreasing trend with latitude was observed. Significantly lower values of liver $\delta^{13}C$ were found in females caught during the NE monsoon as compared to the SI and SW monsoon seasons ($p = 0.0001$). FREE-associated females caught in the Seychelles had significantly lower liver $\delta^{13}C$ values than FAD-associated females caught in the same region.

Differences in the nitrogen isotopic ratios between muscle and liver ($\Delta\delta^{15}N_{M-L}$) were found to vary significantly between region, season, and school type (Table 6). The significant interaction ($p < 0.001$) between region and school type indicated that the difference in $\delta^{15}N$ decreased between FAD-associated and FREE-associated individuals in the Mozambique region, but increased in the Seychelles region. Overall, the highest differences in $\delta^{15}N$ between muscle and liver tissues were observed in FREE-associated individuals in the Seychelles during the SW and SI monsoons. Differences in the carbon isotopic ratios between muscle and liver ($\Delta\delta^{13}C_{M-L}$) were mainly explained by region (>20 % of the variance) (Table 6). As with nitrogen, $\Delta\delta^{13}C_{M-L}$ differed significantly according to school type ($p < 0.001$): Little difference was observed in $\delta^{13}C$ (−0.013) for FREE-associated females caught in the Mozambique region, but a heightened effect (+0.29) was noted for $\delta^{13}C$ in the Seychelles region.

Table 6 Parametric coefficients for the best models identified for modeling the $\delta^{13}C$ and $\delta^{15}N$ in muscle and liver tissues and the $\Delta\delta^{13}C_{M-L}$ and $\Delta\delta^{15}N_{M-L}$ ratios (i.e., the differences in $\delta^{13}C$ and $\delta^{15}N$ values between the two tissue types) as determined by smallest AIC (Akaike information criterion)

Response variable	Best model	Covariate	*df*	*F* value	Pr(>*F*)	AIC	ΔAIC	% variance explained
$\delta^{15}N$ in Muscle	$\sim R + M + L_F + R{:}S$					−65.35	0	63.3
		Region *R*	2	67.00	<0.001	–	–	51.8
		Season *M*	2	4.35	0.015	−65.29	0.06	3.4
		Fork length L_F	1	4.36	0.039	−64.88	0.47	1.7
		Region/School association *R:S*	1	8.31	<0.001	−51.76	13.56	6.4
$\delta^{13}C$ in Muscle	$\sim R + M + S + L_F$					−316.04	0	54.3
		Region *R*	2	12.28	<0.001	−287.22	28.82	11.7
		Season *M*	2	29.66	<0.001	−295.25	20.75	28.3
		School association *S*	1	8.93	0.003	−308.88	7.16	4.3
		Fork length L_F	1	21.11	<0.001	−289.99	26.05	10.1
$\delta^{15}N$ in Liver	$\sim R + M + D$					−92.79	0	76.9
		Region *R*	2	138.29	<0.001	−71.19	21.60	68.0
		Season *M*	2	6.99	0.001	−80.39	12.40	3.4
		Development phase *D*	3	7.33	<0.001	−77.36	15.43	5.4
$\delta^{13}C$ in Liver	$\sim R + M + S + L_F$					−246.97	0	45.1
		Region *R*	2	10.42	<0.001	−227.44	19.52	12.0
		Season *M*	2	19.76	<0.001	−227.95	19.02	22.8
		School association *S*	1	7.16	<0.001	−232.93	14.04	4.1
		Fork length F_L	1	10.65	0.001	−238.14	8.83	6.1
$\Delta\delta^{15}N_{M-L}$	$\sim R + M + S + R{:}S$					−70.54	0	43.5
		Region *R*	2	6.77	0.002	–	–	8.1
		Season *M*	2	10.79	<0.001	−65.23	5.31	13.0
		School association *S*	3	20.94	<0.001	–	–	12.6
		Region/school association *R:S*	1	16.44	<0.001	−56.26	14.28	9.9
$\Delta\delta^{13}C_{M-L}$	$\sim R + S + R{:}S$					−248.80	0	32.9
		Region *R*	2	16.01	<0.001	–	–	22.4
		School association *S*	1	10.22	<0.001	–	–	7.1
		Region/school association *R:S*	1	4.78	<0.001	−245.88	2.92	3.3

Values were derived from SIA of female yellowfin tuna (*Thunnus albacares*) as a function of region (*R*), season (*M*), school type (*S*), fish length (L_F), ovarian development phase (*D*) and the interaction between school type and region (*S:R*)

Table 7 Mean ± SD of $\delta^{13}C$ and $\delta^{15}N$ values (‰) measured in white muscle (M) and liver (L) tissues of female yellowfin tuna (*Thunnus albacares*) collected in three regions in the Western Indian Ocean (Somalia, Seychelles and Mozambique) during the northeast monsoon (NE; Jan–Mar), the southwest monsoon (SW; June–July), and the spring inter-monsoon (SI; April–May) seasons

Region	Monsoon	*n*	L_F	Liver		Muscle		$\Delta\delta^{13}C_{M-L}$	$\Delta\delta^{15}N_{M-L}$
				$\delta^{13}C$	$\delta^{15}N$	$\delta^{13}C$	$\delta^{15}N$		
Somalia	NE	7	77.3 ± 25.5	−16.9 ± 0.4	12.5 ± 0.7	−16.4 ± 0.4	12.5 ± 0.8	0.5 ± 0.3	0.0 ± 0.6
	SW	3	77.0 ± 21.2	−16.5 ± 0.5	11.0 ± 0.5	−16.1 ± 0.2	12.1 ± 0.7	0.4 ± 0.2	1.1 ± 0.4
Seychelles	NE	24	95.4 ± 34.0	−17.4 ± 0.3	11.0 ± 0.5	−16.8 ± 0.2	11.8 ± 0.5	0.6 ± 0.3	0.5 ± 0.7
	SW	39	125.5 ± 19.9	−17.1 ± 0.3	10.9 ± 0.5	−16.4 ± 0.2	12.2 ± 0.8	0.6 ± 0.3	1.3 ± 0.9
	SIM	4	109.2 ± 3.2	−16.6 ± 0.5	10.4 ± 1.4	−16.4 ± 0.1	11.0 ± 0.7	0.2 ± 0.5	0.6 ± 0.9
Mozambique	SIM	27	97.3 ± 18.8	−16.9 ± 0.3	8.9 ± 0.8	−16.7 ± 0.2	10.2 ± 0.8	0.2 ± 0.3	1.3 ± 0.8

The number of individuals (*n*) and fork length (L_F, cm ± SD) are included. $\Delta\delta^{13}C_{M-L}$ and $\Delta\delta^{15}N_{M-L}$ ratios (i.e., the differences in $\delta^{13}C$ and $\delta^{15}N$ values between the two tissue types)

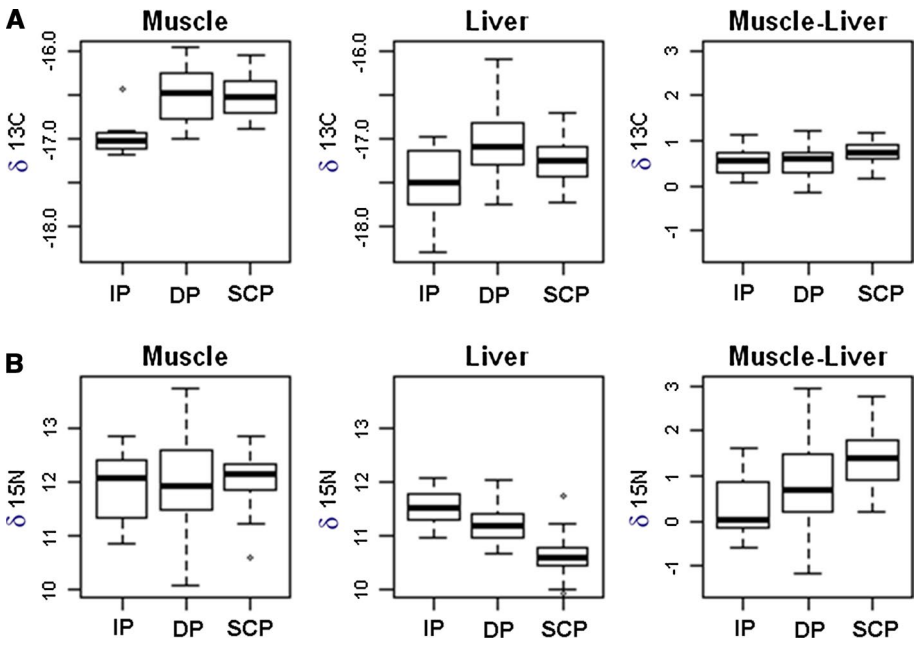

Fig. 3 Box plots of $\delta^{13}C$ in muscle and liver tissues (**a**), $\delta^{15}N$ in muscle and liver tissues (**b**), and differences of isotopic ratios between tissues ($\Delta\delta^{13}C_{M-L}$ and $\Delta\delta^{15}N_{M-L}$) (**c**) in female yellowfin tuna (*Thunnus albacares*) caught in the Seychelles region according to their reproduction phase (P_I = immature phase; P_D = developing phase; P_S = spawning-capable phase)

Stable isotopic ratios and yellowfin tuna reproduction in the Seychelles region

For the Seychelles region, the observed liver $\delta^{15}N$ values were significantly affected by ovarian development ($p < 0.0001$) (Table 6). In the P_I and P_D phases, liver $\delta^{15}N$ values were similar (11.6 ± 0.5 and 11.5 ± 0.4 ‰, respectively), but in the P_{SC} phase they decreased significantly (10.8 ± 0.4 ‰) ($p = 0.0002$). In contrast, no significant differences were observed in muscle $\delta^{15}N$, liver $\delta^{13}C$ or muscle $\delta^{13}C$ values relative to ovarian development (Fig. 3). The $\Delta\delta^{15}N_{M-L}$ increased with ovarian development from 0 in P_I females to 1.1 ‰ in P_{SC} females, but no significant effect was found.

Discussion

Diet composition of yellowfin tuna in the Western Indian Ocean

Our results suggest that the females considered in this study used an opportunistic feeding strategy, an outcome which aligns with previous observations for the Western Indian Ocean (Roger 1994; Potier et al. 2004, 2007; Jaquemet et al. 2011). FREE-associated yellowfin caught by purse seiners were characterized by low diet diversity, feeding mainly on mono-specific aggregations of prey species in both the Indian (Potier et al. 2004) and Atlantic Oceans (Bard et al. 2002). In this study, the females sampled were found to prey on a wide range of species (45 families), a result that is similar to observations made for deep swimming yellowfin caught by longline fisheries (Potier et al. 2007). However, most of the females we analyzed were caught around FADs, explaining this difference. Our results showed that crustaceans, fish, and cephalopods made almost equal contributions (P_N and P_M) to the diet of female yellowfin tuna with four key species (swimming crab, crustacean larvae, cigarfish, and *S. oualaniensis*) contributing the majority of the diet. With the exception of *S. oualaniensis*, each prey species characterized a region and a season (Potier et al. 2007). For example, cigarfish and swimming crab characterize the Seychelles and Somalia regions, where they can form large shoals during the NE and SW monsoons (Schott et al. 2002; Potier et al. 2008; Romanov et al. 2009). In previous studies, a similar aggregation pattern was observed for the stomatopod (*Natosquilla investigatoris*), described as the main prey species of surface-dwelling yellowfin tuna in the Western Indian Ocean (Potier et al. 2004, 2007). The absence of this species in the stomachs analyzed here could reflect inter-annual changes in oceanic productivity in the region and the replacement of this stomatopod species by swimming crab (Romanov et al. 2009).

Diet composition in relation to yellowfin tuna reproduction in the Seychelles region

In the Seychelles region, the prey mass (P_M) of fish showed an increasing trend as the ovaries developed but decreased for crustaceans and cephalopods. We observed that females in the spawning-capable phase fed almost exclusively on cigarfish but that this species was scarce

in stomachs of individuals in the P_D phase and absent for individuals in the P_I phase. In contrast, crustaceans (i.e., swimming crab) were predominantly found in the stomachs of females in the P_I and P_D phases, but their proportion was much lower in the P_{SC} period. The dominance of fish species, and in particular the abundance of cigarfish, in the diet of spawning females could be related to the specific energy content of this species group and the high energy levels required during the spawning phase (McBride et al. 2013). The observation that schools of fish species such as cigarfish are associated with large concentrations of tuna has previously been reported in the Western Indian Ocean (Fonteneau et al. 2008), the Atlantic Ocean (Bard et al. 2002) and the Pacific Ocean (Flynn and Paxton 2013). It is related to either the feeding aggregation behavior of tuna (i.e., targeting a prey concentration) or a spawning aggregation prompted by favorable environmental conditions (Bard et al. 2002; Fonteneau et al. 2008). Yellowfin have a high metabolic rate which requires large amounts of energy due to early maturation, rapid growth and high reproductive outputs (Juan-Jordá et al. 2013). This species has been described as an income–capital breeder (Zudaire et al. 2014), suggesting that while female reproductive costs are mainly fulfilled by energy acquired through current feeding activities previously accumulated resources in the liver also make a contribution. This statement is supported by the fact that females are known to feed intensively during the P_{SC} phase. As a multiple batch spawner (Schaefer 1998) with an indeterminate fecundity regulation strategy (Zudaire et al. 2013a), yellowfin need to continuously acquire energy to ensure that they fulfill the cost of gamete development (Zudaire et al. 2014). The increase in acquired prey weight that we observed in P_{SC} females could be related to the need to meet these heightened energy demands for reproduction. Thus, seasonal fish aggregations provide an important energy resource for ovarian development. Similar dietary specificity (i.e., cigarfish) in female yellowfin during the P_{SC} phase has also been reported in the Atlantic Ocean (i.e., cigarfish; Bard et al. 2002) and the Pacific Ocean (i.e., lanternfish *Diaphus danae* and anchovy *Ecrasicholina punctifer*; Itano 2001; Flynn and Paxton 2013). Variations in fish diets, in relation to seasonal or inter-annual changes in oceanic productivity, can affect the reproductive potential of those species (McBride et al. 2013). Thus, female yellowfin seem to be able to rapidly adjust (i.e., within days to weeks) their reproductive investment depending on food availability (Margulies 2007), and moreover, to take advantage of areas of high food abundance to increase their reproductive capacity (Itano 2001). In the Western Indian Ocean, cigarfish appear to play a central role in these dynamics.

FAD versus FREE school effects on feeding and reproductive success

In recent years, the fishing industry has increasingly used FADs to optimize their catch of target species. This strategy has led to the hypothesis that the intensive use of FADs could modify tuna behavior, trapping them in substandard areas with fewer feeding opportunities, with associated negative impacts on their health (Hallier and Gaertner 2008; Jaquemet et al. 2011). In this study, we found that irrespective of fish size, 32 % of the females caught under FADs in the Seychelles region had empty stomachs. However, in previous studies, the occurrence of empty stomachs has been linked to fish size (Graham et al. 2007; Jaquemet et al. 2011). According to these studies, small tunas are more influenced by FADs than larger individuals due to their more restricted hunting abilities. In this study, the largest differences in the occurrence of the main prey species were noted between school type: cigarfish were almost absent ($F_O = 1.8$ %) from the stomachs of FAD-associated females, but constituted the most important prey species ($F_O = 21.1$ %) for FREE-associated individuals during the same seasons. Females in the P_{SC} phase collected from both school types were characterized by a higher percentage of fish prey (P_M), as compared with females in the P_I and P_D phases. However, this increase was lower in FAD-associated females than FREE-associated females (data not shown). Previous studies on tagged tunas have estimated a residence time of 3 to 8 days for FAD-associated tuna (Dagorn et al. 2007; Schaefer and Fuller 2010) during which time the absence of a fatty prey species like cigarfish (lipid composition of a comparative species, *Cubiceps gracilis*, is 18.9 % fat in flesh (Hayashi et al. 1978)) could negatively influence the capacity of females to acquire energy. In the Seychelles region, Zudaire et al. (2014) reported lower lipid contents in the liver and gonads of spawning females caught under FADs as compared with FREE-associated females. Such results may suggest that FAD-driven aggregations are negatively impacting the feeding success of female yellowfin, leading to a reduction in the consumption of energy-rich prey species (e.g., pelagic fish) and an associated decrease in the amounts of overall energy required for gonad development in the P_{SC} phase. However, to further inform this hypothesis, we need more information on the residency times of mature females under FADs (Zudaire et al. 2014).

Stable isotopic ratios of yellowfin tuna in the Western Indian Ocean

A strong latitudinal effect, characterized by a decreasing pattern from north to south, was observed in the isotopic ratios, especially $\delta^{15}N$. This pattern has been previously

described for yellowfin tuna in the Indian Ocean (Ménard et al. 2007) and the Pacific Ocean (Popp et al. 2007; Olson et al. 2010), and is mainly related to differences in N_2 dynamics at the base of the food webs. In the Western Indian Ocean, the Somalia region is affected by an intense denitrification process which occurs in the oxygen-poor oceanic region of the Arabian Sea. Here, the accumulation of isotopically enriched nitrate in subsurface waters increases $\delta^{15}N$ values (Naqvi et al. 2006). In contrast, the waters of the Mozambique region are affected by the subtropical gyre and are thus, characterized by its prevailing process of N_2 fixation (Davis 2005), known to be responsible for lower $\delta^{15}N$ values at the base of its food webs (Gruber and Sarmiento 1997). The variation in muscle $\delta^{15}N$ values recorded in this study (2.5 ‰) was twice as high than the variation observed by Ménard et al. (2007), and could be related to annual variability in individual feeding activities.

The change along the latitudinal gradient in $\delta^{15}N$ values measured in the muscle and liver tissues were consistent with the propagation of the baseline isotopic characteristics of the relevant food webs (Olson et al. 2010). This isotopic pattern also suggests that certain individuals were displaying a resident behavior (Ménard et al. 2007; Popp et al. 2007) at the temporal scale of their tissue isotopic turnover rate. This seasonality was reflected by the results of the SCA, in particular through the spatial and temporal distribution patterns of the main prey species (Bard et al. 2002; Potier et al. 2008; Romanov et al. 2009). For example, although cigarfish were found all year round in the Western Indian Ocean, their abundance was significantly higher during the NE monsoon in the Seychelles region (Potier et al. 2008). Similarly, the abundance of swimming crabs was linked to seasonal upwelling in the Somalia region during the SW monsoon (Romanov et al. 2009). During the intermonsoon period, the area north of 10ºS is characterized by low biological activity (Wieggert et al. 2006), while the Mozambique Channel supports an important feeding area (Roger 1994) for tuna, as evidenced by fisheries activities (IOTC 2012). Thus, the resident behavior of certain yellowfin suggested by Ménard et al. (2007) might follow these monsoon-induced intra-annual productivity patterns in the Western Indian Ocean.

Stable isotopic ratios and yellowfin tuna reproduction in the Seychelles region

Nitrogen stable isotopes constitute good tracers of metabolic fluxes in marine organisms, which in turn help to understand energy allocation processes (Lorrain et al. 2002). In this study, we found that the liver $\delta^{15}N$ values decreased with ovarian development, resulting in lower ratio during the P_{SC} phase than during the P_I and P_D phases. A similar pattern was described for liver $\delta^{15}N$ values in mature skipjack tunas (Grande 2013) and $\delta^{15}N$ values in the digestive gland of crustaceans (Schmidt et al. 2004). Collectively, these results reflect the role of metabolically active tissues, such as the liver in fish and the digestive gland in crustaceans, during reproduction. The high production of yolk proteins in fish livers, associated with the process of vitellogenesis in spawning-capable females, could be related to a lower ratio of protein degradation-to-protein synthesis, which in turn can lead to a decrease in $\delta^{15}N$ values in the liver (Schmidt et al. 2004). The increased synthesis of proteins in spawning-capable yellowfin tuna could also explain the increases in liver mass observed by Zudaire et al. (2013b). In contrast, muscle $\delta^{15}N$ values were stable throughout the reproductive cycle.

Because the turnover rates of stable isotopes vary according to tissue metabolic activity, the isotopic measurements of various tissues from the same fish population can provide dietary information over the short term and long term (Tieszen et al. 1983). Thus, the isotopic composition of proteins in fish livers reflects the integration of recent dietary inputs (Martínez del Rio et al. 2009). These dietary proteins have a regulatory effect on protein synthesis and degradation (Lobley 2003). An increase in the incorporation of dietary nutrients, i.e., protein intake, has been suggested to intensify protein synthesis (Martínez del Rio et al. 2009). In this study, the diet of females during the P_{SC} phase was characterized by an increased intake of fish species (as represented by higher P_M), especially the cigarfish. The high protein intake at this phase could act as precursor to an increase in yolk protein synthesis in the liver (for reproduction) and thus could explain the decrease in liver $\delta^{15}N$ values that we observed. Further research on the tissue-specific protein composition in mature females is required to better understand the mechanisms of energy allocation for reproduction in this species. The seasonal availability and quality of available prey (i.e., cigarfish versus swimming crab) could significantly affect the amounts of energy acquired for female yellowfin reproduction in an environment where food is scarce and patchily distributed.

Conclusions

Despite a fishery-dependent sampling strategy, the combination of different trophic tracers (i.e., stomach contents and multi-tissue dual stable isotopes) with histological data provided valuable information on the feeding behavior of female yellowfin during their reproductive cycle. Females mainly prey on fish species during their spawning-capable phase, with cigarfish being a key prey species for FREE-associated females. To meet their reproductive costs, females adapt their reproductive behavior to monsoon-induced productivity

patterns in the Western Indian Ocean. Our analysis of $\delta^{15}N$ levels in the liver tissue confirmed the major role that this organ takes in the synthesis of essential compounds for reproduction (i.e., yolk protein). However, further research is required to assess the effects of interannual variations on SCA and SIA patterns during ovary maturation and to better understand all the energy allocation mechanisms occurring during the reproductive cycle of this highly exploited tropical large pelagic species.

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