Assessing the dietary sources of two cichlid species in River Nile sub-branches: Stomach contents, fatty acids and stable isotopes analyses

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Abstract We assess the importance of four different food sources as dietary components of Oreochromis niloticus and Sarotherodon galilaeus in Nile sub-branches using stomach contents, fatty acids (FA) and stable isotopes (SI) analyses. Diatoms were the dominant food items, whereas sand and mud constitute a major part of the stomach contents of both cichlids in the northern ElBehery canal. FAs and SI were compared in cichlids and four potential food sources. Carbon isotopes excluded the fresh macrophyte Myriophyllum spicatum and its epiphytes as a potential food source, whereas FA biomarkers indicated that M. spicatum is assimilated in cichlids’ muscles as detrital materials. FA profiles of cichlids’ muscles were highly enriched by live diatom markers whereas decayed diatoms and bacterial markers were partially present. Carbon isotope signatures of cichlids were much close to that of suspended particulate organic matter (SPOM) which elucidated that SPOM was the source of diatoms and bacterial detritus incorporated in cichlids muscles. Cichlids were highly enriched with nitrogen signatures which was a result of increased anthropogenic effects and incorporation of bacterial films. SI and FA analyses precisely indicated that live diatoms and bacteria, detrital macrophytes are the main sources of organic matter incorporated in cichlids muscles.

Introduction

Studying potential food sources and feeding behavior of freshwater fishes is a fundamental issue as it is the starting point for successful fisheries and fish culture (Oronsaye and Nakpodia, 2005). Moreover, understanding carbon flow in food webs and different trophic levels constitutes a very deep need to assess the compositions and functional role of different communities (Pasquaud et al., 2010). Conventional stomach analysis is still successfully used to illustrate fishes’ food and feeding on the short run (Winemiller et al., 2007), but it make it difficult to determine the exactly assimilated organic matter within aggregate pools of different food sources. In contrast to stomach content analysis, fatty acids and stable isotopes reflect assimilated food sources in consumer tissues, which is essential to understand the community behaviors and their position in the food webs (Dalsgaard et al., 2003). Meanwhile, these analytical techniques are used to clarify food web relations and to
what extent different taxa can interact in the aquatic environment (Persic et al., 2004).

Fatty acid biomarker concept in food web is based on the fact that each group of organisms has a specific suite of fatty acid markers (Dalsgaard et al., 2003). Many researchers have used these suites of fatty acids as biomarkers for vascular plants (Wannigama et al., 1981), macroalgae (Khotimchenko and Vaskovsky, 1990), bacteria (Rajendran et al., 1993), dinoflagellates (Parrish et al., 2000), diatoms (Parrish et al., 2000) and zooplankton (Falk-Petersen et al., 2002). When these organisms are eaten, these suites of biomarkers can be used to follow the origin and trajectory of assimilated organic matter by consumers in higher levels (Parrish et al., 2000), particularly most of these markers cannot be synthesized by higher trophic levels organisms and exclusively captured from dietary sources (Olsen, 1999) and can transfer from sources to consumers in different trophic levels without change (Parrish et al., 2000).

Carbon and nitrogen stable isotope ratios have been used since early 1970s to provide a good explanation of the flow of energy through different ecosystems (West et al., 2006). Stable carbon isotope ratio, C13/C12 (δ13C), of the producers and consumers indicate the potential food sources (Fromeman, 2001), whereas stable nitrogen isotopes ratio, N15/N14 (δ15N), exhibit a stepwise enrichment between trophic levels. Stable nitrogen isotope ratios are a powerful tool for estimating trophic position of organisms (Post, 2002). Analysis of stable isotope is based on the fact that predators are enriched in heavy isotope forms compared to their prey in a predictable way. Several authors (DeNiro and Epstein, 1981; Peterson and Fry, 1987) suggest that these enrichments were close to 1% for carbon and 3.4% for nitrogen. Every analytical method has its own disadvantages and a combination of two or more methods is likely to be the most useful tool to study food webs (Budge et al., 2008).

Tilapia is the most commercially exploited fish in Egypt. Tilapias play an important role in transferring energy from the base of the food web to top predators due to their herbivory and detritivory feeding habit (Tadesse, 1997). In this study, we used stable carbon and nitrogen isotopes and fatty acid biomarkers in addition to stomach content analyses to identify the relative contribution of different food sources to the diets of Oreochromis niloticus and Sarotherodon galilaeus.

Materials and methods

Study area

North to Cairo, at Delta Barrage, River Nile bifurcates into two main branches, Damietta and Rosetta, and four sub-branches which are El rayah ElTawilky, El rayah ElMenoufy, El rayah ElBehery and El rayah ElNasery. The four rayahs' (canals) main features, characters and sampling stations (Fig. 1) were previously presented in detail by Abd El-Karim et al. (2016).

Sample collection and processing

Two cichlid species and four different food sources which are surface sediment organic matter (SSOM), suspended particulate organic matter (SPOM), Myriophyllum spicatum, and its epiphytes were collected from 17 stations (Fig. 1) in late winter-2015. SSOM was collected using Ekman grab, where sampling was repeated until undisturbed samples were obtained. Samples of the upper 0.5 cm layer were put in a 100-ml glass bottle. Epiphytic algal communities were collected by cutting three undisturbed bundles of M. spicatum using scissors into a bucket field with filtered river water, and then macrophytes were shocked vigorously in a plastic bottle until all algal communities were de-attached. River water with de-attached algal communities was filtered through 100 μm plankton net to remove large particles and zooplankton. After filtration, one litter of river water with de-attached epiphytes algal communities were taken in plastic bottle. Cleaned shoots of M. spicatum were put in a plastic page. SPOM was collected using 20 μm plankton net, which was blocked after a few minutes due to the water contents of mud and organic particles.

SPOM was filtered and collected in one-liter plastic bottles as discussed for epiphytic communities. Samples were stored in ice box until returned to the laboratory. In the laboratory, macrophyte samples were washed several times with tape water and finally with distilled water and muscle samples of fishes were removed from the dorsal musculature. All Samples these were stored at −80 °C.

Stomach content analysis

The weight of each specimen was taken using a top loading Metler balance to the nearest 0.1 g after draining excess water with a pile of filter paper while standard length was measured in centimeter using a measuring board. In the laboratory, specimens were dissected and the gut taken out and were identified to the highest possible taxonomic separation. Sorting of different categories was occurred by frequency of occurrence method according to Hyslop (1980).

Fatty acids analysis

For FAs analysis, sub-samples of cichlids’ muscles and the four different food sources were lyophilized and ground to a fine (< 120 μm) homogenized powder using mortar and pestle. Extraction, methylation and quantification of samples were done using modified Folch method (Taiple et al., 2011 and Galloway et al., 2013) as described in detail by Abd El-Karim et al. (2016). Fatty acids were measured by gas chromatography–mass spectrometry (GC–MS) using Agilent 7890 Series GC system interfaced to 5975 inert MSD with triple-axis detector MS. Temperature and pressures program of GC–MS spectrometry were described in detail by Abd El-Karim et al. (2016). Then Fatty acid profile of fishes was compared with those of food sources to identify what fishes ate.

Stable isotope analysis (SIA)

For SIA, sub-samples of cichlids’ muscles and the four different food sources were dried in an air-circulating oven at 60 °C, ground to a fine (< 120 μm) homogenized powder using mortar and pestle and stored in 2 ml glass tubes. Isotope analyses
and measurements were performed at Colorado Plateau Stable Isotope Laboratory, University of Northern Arizona, USA. Carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) isotopes were measured by Thermo-Electron Delta V Advantage IRMS configured through a Finnigan CONFLO III for automated continuous-flow analysis of $\delta^{15}N$ and $\delta^{13}C$, using Carlo Erba NC2100 elemental analyzer for combustion and separation of C and N. Carbon and nitrogen isotopic composition ($\delta^{13}C$ and $\delta^{15}N$) was expressed as the relative difference between isotopic ratios in the sample and in conventional standards (Vienna Pee Dee Belemnite for carbon and atmospheric N$_2$ for nitrogen), using the standard equation:

$$\delta^{13}C \text{ or } \delta^{15}N(\%) = \left[ \frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right] \times 1000$$

where $R$ is $^{13}C/^{12}C$ or $^{15}N/^{14}N$.

More positive values for N and less negative values for C ($\delta$) indicate that the sample is enriched in the heavy $^{13}C$ or $^{15}N$ isotope (Fry, 2006). Comparing stable isotopes of consumers with those of their prey, SI concentration of consumers’ muscles must be enriched more than their prey with values close to 1% for carbon and 3.4% for nitrogen (DeNiro and Epstein, 1981; Peterson and Fry, 1987).

### Trophic level estimation

The trophic levels of consumers were calculated according to Le Loc’h et al. (2008):

$$\text{Trophic level} = \left( \frac{\delta^{15}N_{\text{consumer}} - \delta^{15}N_{\text{producer}}}{3.4} \right) + 1$$

where 3.4‰ is the $^{15}N$ trophic enrichment factor for each trophic level (Minagawa and Wada, 1984). $\delta^{15}N_{\text{consumer}}$ is the $\delta^{15}N$ value for the fish species, $\delta^{15}N_{\text{producer}}$ is the $\delta^{15}N$ value of the producer and 1 assigned for the first trophic level of the primary producer. In the present work, the available organic material for fish species is the SPOM, as will be illustrated hereafter, therefore SPOM is used as the first trophic level as reported by Le Loc’h et al. (2008).

### Statistical analysis

Differences and correlation between fatty acid biomarker and stable isotopes in both producers and body consumers among canals were tested using a one-way ANOVA and Spearman’s correlation analyses using SPSS 20.0 package.

### Results

#### Stomach contents

Gut content analysis of *O. niloticus* ($n = 41$) and *S. galilaeus* ($n = 32$) indicated that the diet of both fishes composed mainly of phytoplankton which appear with their organic material contents and marginally of zooplankton and silt. About 20 genera belonging to diatoms, green algae and cyanobacteria were identified in the diet of both fishes. Common diatoms were represented by *Cyclotella* spp followed by *Synedra* spp and *Aulacoseira* spp, whereas epipelic taxa as *Nitzschia* spp and *Navicula* spp were scarcely and sporadically present. Among cyanobacteria, *Microcystis* spp and *Merismopedia* spp were common, whereas *Oscillatoria* spp were rarely present. The greens *Scenedesmus* spp, *Crucigenia* spp and *Coelastrium* spp were the common. Sand and silt constituted a large proportion of both fishes’ diet and gave a characteristic muddy appearance of the food in the northern ELBehery canal, whereas plant fragments were insignificantly present.

Zooplankton sharing to stomach content was inconsiderable especially at the northern stations of the canals; generally its abundance did not exceed 5.0% of total stomach contents of fish specimens. The highest abundance of zooplankton was found before the river bifurcation and in the southern area...
of the canals. Zooplankton consisted mainly of cladocera and copepod before the river bifurcation, rotifers in the northern of the canals, while the three groups shared at the rest of the area of study. Cladocera represented mainly by Alona sp., Bosmina sp. and Cladoceran sp. Among copepod, Copepodite cyclopoid and Mesocyclops sp. were the most common, whereas rotifers were mainly represented by Keratella cochlearis, Anuraeopsis fissa and Philodina sp.

**Fatty acid profiles of food sources**

GC–MS identified thirty-four FAs in the tissues of both consumers and the four potential food sources then classified to definite biomarkers as illustrated in Table 1 and used for comparing FA profiles of consumers with those of food sources. FA profiles of *M. spicatum* were clearly distinguished from other primary producers (*Table* 2), with representation of 18:3ω3 and the saturated long chain fatty acids (LCFAs), 24:0, with mean concentrations of 2.5% and 4.1%, respectively. *M. spicatum* samples were characterized by the saturated short chain FA 6:0 (caproic acid) with a mean percentage of 3.44% of the total fatty acids (*Table* 2). 6:0 nearly disappeared from other food sources, so it can be considered as a FA biomarker of *M. spicatum* in the studied area. Spatial variations of *M. spicatum* FAs were significantly differed (*P* < 0.002). SPOM presented high proportion of diatom markers (mean = 18.0%), including large quantities of 16:1ω7c (mean = 10.08%), 14:0 (mean = 6.7%) and 20:5ω3 (mean = 12.1%). Concentrations of typical bacterial FA biomarkers were high (mean = 20.12%) in SPOM, particularly those of 17:1ω7 (mean = 19.91%) and odd numbered saturated FAs (13:0, 15:0, 17 and 23:0) (mean = 3.21%). Zooplankton biomarkers were partially identified in the samples of SPOM, which are expressed by the low contributions of 18:1ω9 (mean = 0.7%), 20:4ω6 (mean = 1.06) and low ratio of 20:4ω6 (Arachidonic, ARA) to 20:5ω3 (cis-5,8,11,14, 17-Eicosapentenoic, EPA) (ARA/EPA, mean ratio = 0.55). Epiphytic communities were less enriched with diatoms FAs biomarkers (mean = 11.02%) compared with SPOM. The diatom biomarker 16:1ω7c was slightly contributing with a mean of 5.67%, the mean of 14:0 was 3.64%, whereas 20:5ω3 had a mean of 1.7%. Concentrations of epiphytic bacterial FA biomarkers were comparable (mean = 18.79%) with those of SPOM. Bacterial biomarker 17:1ω7 was 15.18%, whereas odd numbered saturated FAs had a mean of 3.61%, FA composition in SSOM was characterized by a considerable proportion of diatom markers (10.75%), including 16:1ω7c (6.93%), 20:5ω3 (1.34%) and 14:0 (2.48%), with a clear spatial significant difference (*P* < 0.001). SSOM had a higher proportion of bacterial biomarker FAs (28.42%) compared to SPOM and Epiphytic communities. Bacterial biomarker 17:1ω7 had the highest proportion with a mean of 25.29% whereas odd numbered saturated FAs had a mean of 2.48%. Macrophyte biomarkers were considerably presented in SSOM with percentage to the total fatty acids of 4.17%, with a higher mean contribution of LCFAs of 3.1% and a lower proportion of 18:3ω3 (mean = 1.07%).

**Fatty acid profiles of consumers**

Results from fatty acid profiles of *O. niloticus* and *S. galilaeus* revealed the presence of biomarkers from different food items with different proportions (*Table* 2). The two species of cichlids were non-significantly differed in their food source biomarkers (*P* = 0.15). Both species were highly enriched with diatom markers (mean = 20.69%) followed by bacterial markers (mean = 8.61%). Vascular plant markers had lower proportions with an average of 4.9% to the total FAs, whereas zooplankton biomarkers were very low, had an average of 1.32% of total FAs. The diatom marker 16:1ω7c had an obvious increase in *S. galilaeus* tissues (mean = 10.4%) compared to the tissues of *O. niloticus* (mean = 7.25%). Among bacterial markers, 17:1ω7 was higher (mean = 8.39%) in *O. niloticus* tissue whereas odd numbered saturated fatty acids (mean = 3.74%) were more contributors in *S. galilaeus* tissues. Vascular plant markers, LCFAs and 18:3ω3, non-significantly differed between cichlids, whereas FA 6:0 biomarker nearly disappeared from cichlids’ tissues with a mean percentage of 0.42%. The FA profile of both cichlids revealed high ω3/ω6 ratios of 7.38 and 8.91 for *O. niloticus* and *S. galilaeus*, respectively, which indicate high herbivory feeding habits. Also, low contribution of 20:4ω6 (ARA) combined with 20:5ω3 (EPA) reflects lower animal origin of fish dietary component. ARA/EPA ratios were on average 0.1 and 0.03 for *O. niloticus* and *S. galilaeus*, respectively, which also reflects the low carnivory feeding habits of both species.

**Isotopes composition of food sources**

The δ13C in food sources in the four canals ranged from −12.09‰ for SSOM to −29.04‰ for *M. spicatum* (*Table* 3).

<table>
<thead>
<tr>
<th>Trophic markers</th>
<th>References</th>
<th>Trophic markers</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diatoms</td>
<td>14:0</td>
<td>(1, 9)</td>
<td>T. plant</td>
</tr>
<tr>
<td></td>
<td>16:1ω7c</td>
<td>(2, 9)</td>
<td>LCFAs</td>
</tr>
<tr>
<td></td>
<td>20:5ω3</td>
<td>(6)</td>
<td>Zoo</td>
</tr>
<tr>
<td>Bacteria</td>
<td>17:1ω7</td>
<td>(3, 11)</td>
<td>18:1ω9</td>
</tr>
<tr>
<td>Odd FAs</td>
<td>(13:0, 15:0, 17:0 and 23:0)</td>
<td>(3, 7, 11)</td>
<td>20:4ω6</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ARA/EPA</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ω3/ω6</td>
</tr>
</tbody>
</table>

References: (1) Sargent and Whittle (1981); (2) Berge et al. (1995); (3) Rajendran et al. (1993); (4) Wannigama et al. (1981); (5) Kharlamenko et al. (2001); (6) Budge and Parrish (1998); (7) Sargent et al. (1987); (8) Viso et al. (1993); (9) Dunstan et al. (1994); (10) Ramos et al. (2003); (11) Volkman et al. (1980).
Table 2  Fatty acid trophic marker ratios to the total fatty acids concentration in different food sources and consumers.

<table>
<thead>
<tr>
<th>Fatty Acid</th>
<th>O. n.</th>
<th>S. g.</th>
<th>SPOM</th>
<th>SSOM</th>
<th>Epiphytes</th>
<th>M. spicatum</th>
</tr>
</thead>
</table>
| 14:0       | 5.5  | 3.1 | 3.5 | 3.5 | 2.9 | ns | 4.6  | 6.8 | 3.8 | 3.8 | 10.5 | 4.7 | 5.9 | 7.1 | 10.1 | 6.8 | 2.3 | 2.1 | 2.1 | 1.8 | 5.0  | 4.2 | 3.6 | 4.0 | 2.6 | 4.2  | 3.4 | 3.4 | 4.7 | 3.2 | 2.0  
| 16:1n7c    | 11.9 | 7.3 | 9.2 | 8.5 | 3.5 | ns | 12.7 | 14.5 | 10.4 | 8.9 | 7.3 | 9.6 | 11.6 | 15.0 | 17.5 | 6.4 | 6.3 | 5.3 | 5.1 | 10.0 | 3.4 | 2.4 | 9.2 | 6.0 | 2.7  | 3.9 | 4.4 | 2.3 | 2.2 | 2.0  
| 20:5n3     | 10.1 | 4.2 | 10.1 | 10.2 | 9.1 | ns | 8.5  | 7.3 | 7.1 | 0.9 | 1.4 | 1.1 | 1.2 | 1.2 | 1.2 | 1.3 | 1.5 | 1.4 | 1.2 | 1.3  | 1.9 | 1.6 | 1.6 | 1.8 | 3.3  | 3.9 | 7.0 | 1.7 | 3.0 | 2.0  
| Sum        | 27.6 | 14.7 | 22.8 | 22.2 | 15.5 | ns | 25.8 | 28.5 | 21.3 | 20.3 | 13.3 | 16.7 | 19.9 | 26.2 | 25.5 | 10.0 | 9.9 | 8.8 | 8.1 | 16.2 | 9.5 | 7.5 | 14.9 | 10.4 | 10.1 | 11.1 | 14.9 | 8.8 | 8.4  
| 20:5n3     | 10.1 | 4.2 | 10.1 | 10.2 | 9.1 | ns | 8.5  | 7.3 | 7.1 | 0.9 | 1.4 | 1.1 | 1.2 | 1.2 | 1.2 | 1.3 | 1.5 | 1.4 | 1.2 | 1.3  | 1.9 | 1.6 | 1.6 | 1.8 | 3.3  | 3.9 | 7.0 | 1.7 | 3.0 | 2.0  
| Sum        | 27.6 | 14.7 | 22.8 | 22.2 | 15.5 | ns | 25.8 | 28.5 | 21.3 | 20.3 | 13.3 | 16.7 | 19.9 | 26.2 | 25.5 | 10.0 | 9.9 | 8.8 | 8.1 | 16.2 | 9.5 | 7.5 | 14.9 | 10.4 | 10.1 | 11.1 | 14.9 | 8.8 | 8.4  
| 17:1n9     | 0.5  | 29.4 | 3.9 | 6.9 | 1.0 | ns | 0.5  | 0.6 | 4.7 | 16.6 | 22.5 | 23.7 | 12.4 | 8.3 | 2.0 | 29.4 | 18.9 | 28.3 | 34.6 | 27.1 | 22.0 | 16.2 | 13.5 | 7.7 | 1.5  | 3.9 | 1.5 | 1.6 | 1.8 | 2.0  
| Odd        | 2.2  | 2.0 | 2.1 | 1.9 | 3.5 | ns | 2.0  | 1.9 | 5.6 | 3.5 | 3.2 | 3.0 | 3.5 | 4.4 | 2.9 | 3.1 | 2.9 | 2.9 | 3.3 | 3.4 | 3.6 | 3.5 | 4.4 | 4.9 | 4.2 | 4.7 | 5.0 | 5.1 | 5.5 | 2.0  
| Sum        | 2.8  | 31.4 | 6.0 | 8.8 | 4.5 | ns | 2.5  | 2.5 | 10.3 | 20.1 | 25.7 | 26.8 | 15.9 | 12.7 | 4.9 | 32.5 | 21.8 | 31.2 | 38.0 | 30.6 | 25.6 | 19.8 | 17.9 | 12.6 | 5.7 | 8.6 | 6.5 | 6.7 | 7.4 | 2.0  
| 18:3n3     | 1.4  | 1.3 | 2.7 | 2.7 | 3.3 | ns | 1.5  | 1.4 | 2.8 | 1.1 | 1.5 | 1.1 | 1.3 | 1.3 | 1.0 | 1.0 | 1.3 | 1.1 | 0.9 | 1.0 | 1.6 | 1.7 | 1.7 | 1.7 | 0.2 | 4.9 | 2.3 | 0.3 | 3.7 | 2.0  
| LCFAs      | 0.9  | 1.1 | 1.0 | 0.9 | 2.6 | ns | 0.8  | 0.6 | 4.4 | 1.8 | 2.5 | 2.1 | 2.3 | 1.8 | 2.7 | 3.0 | 3.4 | 2.6 | 3.0 | 2.1 | 2.6 | 2.8 | 2.6 | 3.8 | 3.7 | 3.3 | 4.5 | 4.5 | 4.5 | 2.0  
| 6:0        | 0.0  | 0.0 | 0.0 | 0.0 | 0.4 | ns | 0.0  | 0.0 | 1.5 | 0.1 | 0.3 | 0.6 | 0.2 | 0.4 | 0.2 | 0.3 | 0.3 | 0.2 | 0.1 | 0.0 | 0.5 | 0.3 | 0.1 | 0.0 | 10.1 | 1.7 | 1.7 | 1.8 | 1.9 | 2.0  
| Sum        | 2.3  | 2.4 | 3.7 | 3.5 | 6.3 | ns | 2.3  | 2.0 | 8.7 | 3.0 | 4.3 | 3.8 | 3.8 | 3.5 | 3.9 | 4.3 | 5.0 | 4.0 | 4.0 | 3.3 | 4.6 | 4.7 | 4.4 | 5.5 | 14.1 | 9.9 | 8.5 | 6.6 | 10.0 | 2.0  
| 18:1n9     | 0.7  | 0.7 | 0.5 | 0.5 | 0.3 | ns | 0.4  | 1.0 | 0.5 | 0.5 | 0.8 | 0.6 | 0.6 | 0.6 | 0.7 | 0.7 | 0.8 | 0.7 | 0.6 | 0.7 | 0.8 | 0.8 | 0.8 | 1.0 | 0.3 | 2.7 | 0.5 | 0.3 | 3.9 | 2.0  
| 20:4n6     | 0.7  | 0.6 | 0.8 | 0.6 | 2.0 | ns | 0.5  | 0.7 | 0.3 | 0.6 | 1.0 | 0.8 | 0.9 | 1.7 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.4 | 0.6 | 0.5 | 0.6 | 0.3 | 0.5 | 1.4 | 0.6 | 0.4 | 2.0  
| ARA/EPA    | 0.0  | 0.2 | 0.0 | 0.0 | 0.1 | ns | 0.0  | 0.0 | 0.0 | 0.0 | 0.5 | 0.5 | 0.5 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.6 | 0.4 | 0.6 | 0.5 | 0.6 | 0.3 | 0.5 | 1.4 | 0.6 | 0.4 | 2.0  
| Sum        | 1.4  | 1.3 | 1.3 | 1.1 | 2.4 | ns | 0.9  | 1.6 | 0.6 | 1.1 | 1.8 | 1.4 | 1.6 | 2.3 | 0.6 | 0.7 | 0.8 | 0.7 | 0.6 | 1.7 | 2.1 | 2.3 | 2.3 | 2.4 | 2.8 | 3.8 | 1.3 | 3.0 | 6.8 | 2.0  

Assessing the dietary sources of two cichlid species
SPOM was always the most $\delta^{13}C$-depleted (mean = $-26.09\%e$) and was significantly different from other food sources, whereas SSOM was the most enriched food source (mean = $-19.67\%e$). $\delta^{13}C$ of different food sources at RN station showed non-significant difference with other stations in the four canals. ElBehery canal was highly depleted in both SPOM (mean = $-27.41\%e$) and the macrophyte shoots (mean = $-27.87\%e$), whereas ElMenoufy canal was highly depleted in both epiphytic communities (mean = $-23.78\%e$) and SSOM (mean = $-22.23\%e$). Mean $\delta^{15}N$ of food sources ranged from 2.86 for $M. spicatum$ in the north of ElNasery canal to 9.22‰ for epiphytic communities in the south of the canals. On average, different food sources were highly enriched with $\delta^{15}N$ at RN station, except for SPOM, compared with different canals. On spatial mean basis, epiphytic communities were the most $\delta^{15}N$-enriched (7.7%), whereas $M. spicatum$ was the most $\delta^{15}N$-depleted (6.42%).

**Isotope composition of consumers**

Carbon isotope composition of consumers revealed that the cichlids use SPOM as a unique food source but feed on different prey items (Table 3). Moreover, we observed a high enrichment of the $\delta^{15}N$ signatures in cichlids’ tissues. Cichlids species were highly $\delta^{13}C$ depleted with a mean value of $-26.8\%e$ and $-26.6\%e$ for $O. niloticus$ and $S. galilaeus$, respectively (Table 3). On spatial mean basis, $O. niloticus$ was $\delta^{13}C$ enriched at RN station and ElTawfikey canal, whereas $S. galilaeus$ was $\delta^{13}C$-enriched at the other canals, although both species showed non-significant spatial variations. $\delta^{15}N$ of $O. niloticus$ was more enriched with a mean $\delta^{15}N$ value of 12.12‰, while mean $\delta^{15}N$ value of $S. galilaeus$ was 10.9‰. On spatial mean basis, $O. niloticus$ was $\delta^{15}N$-enriched at RN station and different canals. Our results revealed no correlation between $\delta^{13}C$ of consumers and different food sources except between $S. galilaeus$ and SSOM ($r = 0.57$, $P < 0.05$). In the same context, SSOM $\delta^{15}N$ had a high correlation with $O. niloticus$ ($r = 0.67$, $P < 0.05$) and $S. galilaeus$ ($r = 0.71$, $P < 0.05$). $\delta^{15}N$ signature reflected the enrichment factor of 5.22 and 4.92 for $O. niloticus$ and $S. galilaeus$, respectively, in relation to different food sources.

Table 3  Mean $\delta^{13}C$ (SD ± 1.58) and $\delta^{15}N$ (SD ± 1.18) values of producers and consumers (fish species) collected in Nile sub-branches (NS, no samples collected).

<table>
<thead>
<tr>
<th></th>
<th>RN</th>
<th>ElTawfikey</th>
<th>ElMenoufy</th>
<th>ElBehery</th>
<th>ElNasery</th>
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<td><strong>Producers</strong></td>
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<tr>
<td>SPOM</td>
<td>-24.7</td>
<td>-25.6</td>
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In general, trophic level of both cichlids were non-significantly differed ($P = 0.19$) and both showed a non-significant regional variation ($P = 0.23$). Trophic level of $S. galilaeus$ showed a wider ring (1.48–2.82) of variation compared to $O. niloticus$ (1.95–3.08). Trophic level of $S. galilaeus$ was highest in the northern area of ElMenoufy and ElTawfikey canals, whereas that of $O. niloticus$ was highest before the river bifurcation and in the north of ElNasery and ElBehery canals. The mean TL of $O. niloticus$ was slightly higher (2.57) than the mean trophic level of $S. galilaeus$ (2.29).

**Discussion**

In the studied area, stomach contents of Nile tilapias specimens comprised natural food materials such as phytoplankton, detritus, few zooplankton species and mud and sand grains as main food items in the north of the four canals. Abayomi (1986) and Getachew (1993) confirmed the presence of phytoplankton, detritus, macrophytes and zooplankton in the stomach of $S. galilaeus$ and $O. niloticus$. The sand grains consumed along with mud by Nile tilapias were also recorded as a part of food items for tilapia species in Lake Victoria, Kenya (Njiru et al., 2004). Mud and sand grains fed upon by tilapias in the northern of the canals were mainly due to the reduction in the pelagic productivity which is mirrored by low chlorophyll a concentrations ($<30 \mu g L^{-1}$) compared to RN station and the south of the canals (about 200 $\mu g L^{-1}$). Detritus and silt are less nutritious than phytoplankton to support growth and maintenance of the fish but still a compartment of their diets probably due to low productivity of lake Chamo, Ethiopia (Tadesse, 1999).

Food sources were much better characterized by FA profiles and $\delta^{13}C$ than by their $\delta^{15}N$ values that largely overlapped. The fatty acid biomarkers used in this study successfully reflected the dietary composition of the body tissue of both fishes as reported by previous studies (Jobling et al., 2002; Izquierdo et al., 2003). $\delta^{13}C$ signature within the body tissue of both consumers give an indication of food sources as reported by Froneman (2001). The fatty acid anal-

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Table 3

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yses strongly argued that different food sources significantly varied in their contribution to tilapia diets and the food web in the Nile canals is based mainly on SPOM. The high proportion of diatom markers in cichlid FA profiles (mean = 20.7%) and the $\delta^{13}$C close to SPOM allows thus to determine that planktonic live diatoms are the main contributors to diet of both cichlids. FA profiles indicate that SPOM was composed of fresh and decaying diatoms associated with bacteria. There are relatively high levels of 20:5($n$−3) and 16:1($n$−7) (total mean = 11.26% of SPOM), which are markers of living diatoms (Dunstan et al., 1994) compared to 14:0 (mean = 6.7% of SPOM), which represents the decaying process of diatoms (Cammel and Martens, 1996). Living diatom biomarkers were highly contributed to the cichlids’ diets (total mean = 16.9% of both consumers profile), whereas decaying diatom biomarker was lower contributor (mean = 3.77% of both consumers profile). These results showed that diatoms are the main trophic resource in tilapias diets due to their high biomass, constant availability and their high nutritional quality (Lebreton et al., 2011). Nevertheless fatty acid profile of epiphytic diatoms was similar to that of cichlids but it was excluded as a potential food source because the carbon signature of epiphytic diatoms ($\delta^{13}$C) was much enriched (mean = $-21.95\%\circ$) compared to $\delta^{13}$C of cichlids (mean = $-26.68\%\circ$). Also, none of the species characterize the epiphytic diatoms as Cymbella spp and Gomphonema spp were recorded in the stomach of the consumers, which argued that epiphytic diatoms are not a potential dietary source of both cichlids.

The incorporation of bacterial fatty acids in tilapia tissues (mean of 8.61% of total fatty acids) indicated that bacteria partially contributed to their diets. Sakdullah and Tsuchiya (2009) indicated that tilapias not only prefer more palatable organic food sources such as bacterioplankton but also have the ability to ingest and assimilate them in the aquatic environment (Beveridge et al., 1989). The results of Sanderson et al. (1996) enforced the fact that tilapia is a suspension feeding animal that can ingest and assimilate bacteria. SPOM, epiphytic communities and SSOM are considered as contributors of the assimilated bacteria as they have higher levels of bacterial markers (mean of 20.12%, 18.79% and 28.42% of total fatty acids, respectively). The epiphytic communities were excluded as a source of the assimilated bacteria as they are not incorporated into the cichlids’ tissue as mentioned above. SPOM and/or SSOM are likely a potential source of the assimilated bacteria. Even though the isotopic $\delta^{13}$C enrichment from food source to consumers is 0.8% (Vander Zanden and Rasmussen, 2001), SPOM was slightly enriched (mean $\delta^{13}$C = $-26.09\%\circ$) compared with the isotopic $\delta^{13}$C signature of their consumers (mean = $-26.68\%\circ$). These results indicated that SPOM is composed of not only phytoplanktonic-origin particles but also include, in part, particles from more enriched particles (mean $\delta^{13}$C = $-19.67\%\circ$) that may resuspend in the water column as well. Navicula spp and Nitzschia spp were found in the stomach of both consumers, which enforced the fact that bacterial FAs detected in the tilapia tissue may originate, in a part, from $\delta^{13}$C enriched SSOM that resuspend in the water column due to human and navigation activities in this area. In this context, Le Loc’h et al. (2008) reported that bottom SPOM is composed of resuspension of sedimented organic matter and POM of planktonic origin. The detection of 14:0 in the tissue of cichlids indicted that bacterial cells can be incorporated as a film associated with the decayed diatom cells as previously investigated (Dijkstra et al., 2008).

According to the enrichment value of 0.8% (Vander Zanden and Rasmussen, 2001) from producers to consumers, stable carbon isotope results indicated that fresh M. spicatum (mean of $\delta^{13}$C = $-20.55\%\circ$) could be excluded as a potential food source for cichlids (mean of $\delta^{13}$C = $-26.7\%\circ$). On the other side, the low percentages of bacterial FAs and LCFAs (2.02%) and FA 6:0 (mean < 0.5%) suggest that detritus of M. spicatum was partially utilized via bacteria mediation, which corroborates the previous study of Lebreton et al. (2011). When M. spicatum decomposed, LCFAs remain unchanged in planktonic and deposition detritus for more than 4 months, or even for years, whereas PUFAs including 18:3o3 are rapidly decomposed (Milingle et al., 2003). The high percentage of 18:3o3 in cichlids’ tissue over its percentage in M. spicatum indicated that this FA may have an alternative origin rather than vascular plant markers (Budge and Parrish, 1998).

Many previous investigators (Li et al., 2002 and Prato et al., 2012) reported that 18:3o3 can contribute to green algae as a biomarker. Polyunsaturated FAs (PUFAs) of green algae predominantly contain 18:3o3 which is similar to that of vascular plants since they have common ancestors (Harwood and Russell, 1984). So, the presence of 18:3o3 in tilapia’s tissues can be attributed as a contributor of green microalgae rather than a contributor to vascular plants. These results indicated that fresh vascular plant was not a likely food source for tilapia diets in River Nile canals, but partially as suspended detritus. These results were supported by similar findings of Sakdullah and Tsuchiya (2009) in mangrove food web. The presence of some green species in the stomach of both consumers and the absence of fresh plant fragments support these findings. Many investigators have demonstrated that fresh macrophyte materials are not consumed by most consumers (Jaschinski et al., 2008). The low utilization of macrophytes by consumers may be owing to their low nutritional quality (Cebrian, 1999), high levels of lignin and tannins and high phenolic compounds which consumers generally avoid (Harrison, 1982).

The high enrichment factor of 5.22 and 4.92 for O. niloticus and S. galiliacus, respectively, was an indication of the incorporation of bacterial biofilm which increases the $\delta^{15}$N concentration (Dijkstra et al., 2008) and also the effect of increased anthropogenic nitrogen loading in the surrounding media (Costanzo et al., 2003 and NIOF, 2015). In this study, the trophic levels of both cichlids, in average, appear much closed although they partially differed between stations, which revealed that they compete for the same food sources. O. niloticus had a slightly higher trophic position that is considerably due to its higher affinity toward carnivory that was reflected by higher zooplankton FA biomarker, higher ARA/EPA ratio (affinity toward carnivory) and lower o3o6o9 (affinity toward herbivory). A similar trophic position was reported for cichlids by Faye et al. (2011) and for suspension feeders by Le Loc’h et al. (2008).

**Conclusion**

Fatty acid biomarkers precisely indicated that mainly live diatoms and partially bacteria associated with decayed materials...
were the main dietary sources of Oreochromis niloticus and Sarotherodon galilaeus. δ¹⁵N successfully determined that the source of diatoms and detritus material associated with bacteria was the suspended particulate organic matter. The carbon isotope signature (δ¹³C) elucidated that surface sediment organic matter partially contributed as a food source of the consumers after resuspension where δ¹³C of consumers were intermediate between suspended particulate organic matter and surface sediment organic matter but was very close to the suspended particulate organic matter. δ¹⁵N signature reflected the enrichment factor of 5.22 and 4.92 for Oreochromis niloticus and Sarotherodon galilaeus, respectively, which revealed that bacterial incorporation and anthropogenic activities affect the cichlids' enrichment of nitrogen. Even though this study investigated simple, short and linear food web, these techniques need to be tested in more complex and longer food webs in Egypt.

Conflict of interest

No conflict of interest.

Acknowledgement

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