Determination of Fatty Acid Compositions as Biomarkers in the Diet of *Turbo coronatus* in Chabahar Bay

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Received: March 2011 Accepted: July 2011

Abstract

The aim of this study was to obtain a basic knowledge of the fatty acid compositions and consequently, determination of diet for *Turbo coronatus* by means of fatty acids biomarkers. Samples were collected during a year (April, July, October 2007 and February 2008) in coastal areas of Chabahar Bay in Oman Sea. Biomarker fatty acids revealed that diet of *Turbo coronatus* included phytoplankton, zooplankton, detritus, bacteria, diatoms and red and brown algae. Thirteen fatty acids were identified by using GC/MS. Saturated fatty acids were the most abundant compounds of total lipids. Monounsaturated fatty acids of 16:1n-9, 18:1n-9 and 20:1n-11 were also observed in all samples. No significant differences were observed between total contents of saturated as well as total contents unsaturated fatty acids in different seasons (P>0.05). This suggests similar sources of diet, but in different combination for the mollusc in different seasons.

Keywords: Diet composition, *Turbo coronatus*, Fatty acids, Oman Sea

1. Introduction

The phylum Mollusca is the second largest phylum of animals. Diversity of molluscs is enormous, and most of them are marine. The phylum Mollusca is divided into seven classes of which Polyplacophora, Gastropoda, Bivalvia and Cephalopoda are the largest and exhibit a diversity of lipid and fatty acid components in both freshwater and marine species (Ackman, 1989; Joseph, 1989, Misra et al., 2002).

Lipid biochemistry of many gastropods, bivalves and cephalopods has been described in details. The pioneering studies on the fatty acids of molluscs, mostly of bivalves and gastropods, were carried out in the 1970s (Gardner and Riley, 1972; Ackman and Hooper, 1973; Ackman et al., 1974). Lipid and fatty acid compositions of different classes of Molluscs have been reviewed (Joseph, 2003) and their gut contents analysis in polyplacophora have been studied (Latsyhev, 2004). Different fatty acids have been found or mollusc species. These are influenced by taxonomic relations, environmental conditions, nutrient habits, food availability and also physiological conditions (Khan and Parrish, 2006; Sajjadi et al., 2009).

Various techniques are available to investigate diets, including gut content analyses, fatty acid profiling and stable isotope applications. Gut content analyses are relatively easy and inexpensive to conduct, but only
provide information on ingested food and not the assimilated ones. Conversely, fatty acid profiling and stable isotope applications record the assimilated food types, but fail to identify specific food items consumed. Stable isotopes are especially problematic when used to identify diets at higher trophic levels or for organisms that feed on a wide variety of food types. Fatty acid profiles may provide useful dietary information for species with known lipid biomarkers, but these are not available for many taxa. In light of these methodological strengths and limitations, a combination of two or more techniques is preferred when investigating food diets.

Fatty acids are carbon-rich compounds that are ubiquitous in all organisms and relatively easy to metabolize. Once incorporated in the organism, their biological specificity and the fact that they are transferred from primary producers to higher trophic levels without changing in characteristics, make fatty acids suitable for use as biomarkers. However, some fatty acid markers may be metabolized and transformed while consumed, only absolute amounts may be measured (Alfaro, 2008).

*Turbo coronatus* (Mollusca: Gastropoda) occurs naturally in Chabahar bay in northern Indian Ocean. This eutrophic area, which has one of the highest known rates of primary productivity in the world due to seasonal upwellings (Barlow and Mantoura 1999, Johnson et al., 2002), provides diverse and unique dietary items such as algae with high contents of fatty acids for consumption by molluscs. The purpose of this research was to investigate fatty acids composition of *Turbo coronatus* and determine its diet by means of fatty acids as biomarkers in a habitat with diverse food sources.

2. Materials and Methods

Intertidal areas of Chabahar bay was monitored for diversity and density of molluscs and specimens within the same size group were collected selectively from the designated stations (Fig. 1).

The stations were Konarak (208), Tis beach (209), Kolbe ghavasi (210) and Tis khor (211). Adult *T. coronatus* (6-7 cm) were collected in the rocky shores of the bay because of their dominancy in this area (60° 37’ 45” longitude and 27° 15’ 45” latitude), along the coast of Sistan province (southeast of Iran, northern part of Oman Sea) once in every seasons in April, July, October 2007 and February 2008.

Whole body tissues of fifteen oysters from each sampling were dissected from the shells (about 2
weeks old). Wet fresh weight of the standard sample was 7 g. Five g of each sample was taken, extracted using a homogenizer (Wagtech T1813) with a solvent mixture of chloroform / methanol 2:1 (v/v) and volume to weight ratio of 20:1. About 50 ppm of BHT (Butylated Hydroxy Toluene) was added as an antioxidant into the mixture (Jones et al., 1972). The total extract was filtered under vacuum using glass fiber filter (Whatman, S&S, GF6) and 0.5% NaCl (0.2 vol. of the extract) was added. The aqueous layer was re-extracted with chloroform. The combined organic layers were evaporated to about 3-5 Cm3 and then hydrolyzed with 5% aqueous KOH (20 Cm3) and methanol (100 Cm3) for 2-3 hours at reflux temperature. After cooling, water (50 Cm3) was added and the basic solution was extracted twice with n-heptane / diethyl ether 1:1 (v/v). The aqueous methanolic layer was acidified to pH=2 and the fatty acids were extracted with n-heptane / diethyl ether 1:1 (v/v, 3 x 100 Cm3). Then, acids were dried over anhydrous MgSO4 and filtered. The filtrate was concentrated to 2-3 cm3 (Johns et al., 1980). The fatty acids were esterified with BF3-methanol (Morrison and Smith, 1964) and heated in boiling water for 5 minutes. After cooling, 1 mL water and 2 mL pentane were added, vortexed (Stuart SA8) for 1 minute and centrifuged (Heraeus Biofuge). Then, the upper phase was collected. Pentane was evaporated and the residue dissolved immediately in 50-100 µL of n-hexane for injection to the gas chromatograph. Triplicate samples were treated and analyzed.

Samples were injected into GC/Mass and the separation of the fatty acids methyl esters was performed using a Gas Chromatograph (Agilent Technologies, 6890) with a mass selective detector (6973N). GC/Mass analysis was done with an electron impact (EI) mode of 70 ev as ionization source and quadruple mass filter with Chemstation data analysis system. The capillary column used was HP-5 (5% diphenyl 95% dimethyl siloxane copolymer) with 30m length, 320 µm internal decimeter and 1 µm film thickness. The carrier gas was Helium (purity, 99.999%). 0.5 µL of the extract containing the fatty acids methyl esters was injected into the injector using split mode with 50:1 split ratio. The injector temperature was 200 °C, the detector temperature was 280 °C and the oven temperature was programmed from 75 °C/min to 270 °C at 30 °C/min, while the final temperature was held for 7 min (Casado et al., 1998). To ensure that all the components were detected, the final temperature was held for 20 min in the replicated test runs. Triplicate samples were treated and analyzed. Seawater conditions (temperature and salinity) were monitored by CTD (Idronaut Ocean Seven) during sampling.

Statistical analysis of the data was carried out using SPSS V16. The normality of the data was evaluated by Kolmogorov-Smirnov test. For assessing changes of fatty acids in different seasons, ANOVA and Tukey comparisons were used (P>0.05). Regression analysis of fatty acids and temperature was performed as well.

3. Results and Discussion

The results are summarized in Tables 1-3. By winter, an increase in the temperature was observed until it reached the highest in the beginning of July 2007. The minimum temperature was recorded in February 2008 (Table 1).

<table>
<thead>
<tr>
<th>Month</th>
<th>Temperature (°C)</th>
<th>Salinity (PSU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>March</td>
<td>27.04±1.35</td>
<td>36.89±1.84</td>
</tr>
<tr>
<td>April</td>
<td>30.79±1.54</td>
<td>37.01±1.85</td>
</tr>
<tr>
<td>May</td>
<td>32.74±1.64</td>
<td>32.74±1.64</td>
</tr>
<tr>
<td>June</td>
<td>33.00±1.65</td>
<td>36.68±1.83</td>
</tr>
<tr>
<td>July</td>
<td>33.07±1.65</td>
<td>36.65±1.83</td>
</tr>
<tr>
<td>August</td>
<td>31.20±1.56</td>
<td>36.84±1.84</td>
</tr>
<tr>
<td>September</td>
<td>27.62±1.38</td>
<td>36.87±1.84</td>
</tr>
<tr>
<td>October</td>
<td>26.00±1.30</td>
<td>36.43±1.82</td>
</tr>
<tr>
<td>November</td>
<td>24.94±1.25</td>
<td>36.91±1.85</td>
</tr>
<tr>
<td>December</td>
<td>23.67±1.18</td>
<td>36.86±1.84</td>
</tr>
<tr>
<td>January</td>
<td>22.73±1.14</td>
<td>36.72±1.84</td>
</tr>
<tr>
<td>February</td>
<td>21±1.05</td>
<td>36.60±1.83</td>
</tr>
<tr>
<td>Spring</td>
<td>30.19±1.51</td>
<td>35.54±1.78</td>
</tr>
<tr>
<td>Summer</td>
<td>32.42±1.62</td>
<td>36.72±1.84</td>
</tr>
<tr>
<td>Fall</td>
<td>26.18±1.31</td>
<td>36.73±1.84</td>
</tr>
<tr>
<td>Winter</td>
<td>22.46±1.12</td>
<td>36.72±1.84</td>
</tr>
<tr>
<td>Annual average</td>
<td>27.81±1.39</td>
<td>36.42±1.82</td>
</tr>
</tbody>
</table>

Table 1. Variations of temperature (a) and salinity (b) during a year at Chabahar bay
The highest salinity was observed in April 2007 until it reached the lowest in October 2007 (Table 1). No significant differences (P>0.05) were observed between salinity changes between seasons.

Total saturated fatty acids for spring, summer, fall and winter samplings were: 69.23, 55.49, 78.13 and 67.83 (mean ± SD, %), and total unsaturated fatty acids for spring, summer, fall and winter were: 30.77, 44.51, 21.87 and 32.17 (mean ± SD, %), respectively (Table 2).

| Fatty Acid Compositions (%) of *Turbo coronatus* in different seasons |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| % Fatty Acids   | Spring STDV     | Summer STDV     | Fall STDV       | Winter STDV     |
| 14:0            | 2.84 ± 0.63     | 2.94 ± 0.61     | 2.88 ± 0.50     | 2.86 ± 0.72     |
| 4,8,12-tri Me-13:0 | 14:0            | 15:0            | 16:1-9          | 16:2           |
| 15:0            | 1.20 ± 0.72     | 1.23 ± 0.74     | 1.23 ± 0.63     | 1.24 ± 0.58     |
| 16:0            | 53.51 ± 4.72    | 53.09 ± 4.25    | 53.09 ± 4.25    | 53.09 ± 4.25    |
| 17:0            | 1.69 ± 0.81     | 1.69 ± 0.81     | 1.69 ± 0.81     | 1.69 ± 0.81     |
| 18:0            | 13.27 ± 6.48    | 13.27 ± 6.48    | 13.27 ± 6.48    | 13.27 ± 6.48    |
| 20:4 n-6        | 3.27 ± 0.60     | 3.27 ± 0.60     | 3.27 ± 0.60     | 3.27 ± 0.60     |
| 20:5n-3         | 6.12 ± 3.02     | 6.12 ± 3.02     | 6.12 ± 3.02     | 6.12 ± 3.02     |
| 20:1n-11        | 7.50 ± 3.74     | 7.50 ± 3.74     | 7.50 ± 3.74     | 7.50 ± 3.74     |
| Total Saturated | 67.67 ± 5.46    | 67.67 ± 5.46    | 67.67 ± 5.46    | 67.67 ± 5.46    |
| Total Saturated/Total Unsaturated | 2.25 ± 0.86 | 2.25 ± 0.86 | 2.25 ± 0.86 | 2.25 ± 0.86 |
| Total Saturated/Total | 2.25 ± 0.86 | 2.25 ± 0.86 | 2.25 ± 0.86 | 2.25 ± 0.86 |
| Total Unsaturated | 32.33 ± 15.63 | 32.33 ± 15.63 | 32.33 ± 15.63 | 32.33 ± 15.63 |

Maximum total saturated and unsaturated fatty acids were recorded in fall and maximum summer, respectively. Major saturated fatty acids were: Tri-Methyl-tridecanoic acid (14:0), Pentadecanoic acid (15:0), Palmitic acid (16:0), Heptadecanoic acid (17:0), Methyl-heptadecanoic acid (Me-17:0) and Stearic acid (18:0). Palmitic acid (36.10-53.52%) and Stearic acid (2.39-13.27%) were generally the most abundant saturated fatty acids in all seasons. Three monounsaturated fatty acids were observed in all samples: Palmitoleic acid (16:1n-9), Oleic acid (18:1n-9) and Gadoleic acid (20:1n-11). Oleic acid ranged from 14.15% in spring to 4.01% in fall without any significant differences (P>0.05). Gadoleic acid also varied with no significant differences from 7.50% in summer to 1.55% in spring (P>0.05). Only one di-unsaturated fatty acid (DUFA) was observed. Linoleic acid (9, 12-18:2n-6) contents ranged from 7.51% in spring to 5.22% in fall with a significant differences (P>0.05) between them. Also, two PUFAs, Arachidonic acid (20:4 n-6) and eicosapentaenoic acid (20:5n-3) were found. The former ranged from 3.27% in spring to 6.82% in summer and latter ranged from 0.48% in fall to 6.12% in summer (Table 2). There were no significant differences (P>0.05) between total saturated and unsaturated fatty acids in different seasons (Table 2).

Statistical analysis showed significant correlations between Palmitic acid (C:16) and EPA (C20:n-11) with salinity, but not with temperature (Table 3).
Studies have shown significant correlations between some fatty acids and temperature on other molluscs (Sajjadi et al., 2009; Eghtesadi and Sajjadi, 2010). Although lipids and fatty acids of marine molluscs including *Turbo coronatus* have been extensively studied, (Freije and Awadh, 2010), this is the first report on fatty acid composition of *Turbo coronatus* from the Iranian waters in the Oman Sea.

The lipid levels in the body composition of marine invertebrates vary with temperature, diet and annual reproductive cycle (Simpson, 1982; Navaro et al., 2000, Pernet and Tremblay, 2004). According to previous reports, unsaturation of fatty acids in the tissues of marine organisms increases with low temperatures while saturation, increases with high temperatures (Lewis, 1962), which is not consistent with results of this study. Besides, fatty acids profiles of molluscs usually contain about 30–40% saturated fatty acids (SFA) (Phleger et al., 2001). This is not in agreement with findings of this study (55.49-78.13%) and the saturated fatty acids were dominated over unsaturated ones, which could be due to environmental conditions such warm temperatures throughout the year. According to Gabbott (1983), palmitic acid is the major end -product of the fatty acid synthesis in animal tissues and is the precursor for de novo synthesis of long-chain saturated and unsaturated fatty acids and so, it can be considered the best biomarker. Initial levels of C16:0 (36.10-53.52%) was greater compared to other saturated fatty acids in this species.

Nearly one-third of all fatty acids in the total lipids of molluscs are constituted from monounsaturated fatty acids (Gardner and Riley, 1972; Johns et al., 1980; Feuntes et al., 2009). Some far-eastern species of bivalves contain lower (less than 20% of the total acids) concentrations of these acids (Zhukova and Svetashev, 1986). In this study, concentration of monounsaturated fatty acids was less than 20% of the total acids, which is consistent with previous reports.

Poly unsaturated fatty acids (PUFAs) play important role in animals which are generally derived either directly from the diet or following conversion of dietary components. PUFAs are considered as the main constituents of molluscan lipids, accounting for one-third to half of total fatty acids (Johns et al., 1980; Zhukova and Svetashev, 1986). In present study, we found only 20:5n-3 of n-3 PUFAs in *Turbo coronatus* which ranged from 0.48 to 6.12%. We suggest that probably more than this level of n-3 is not necessary for survival of this mollusc in this subtropical region.

It has been shown that marine detritus contains significant quantities of saturated fatty acids between C14:0 and C18:0 carbons (Ackman and Tocher, 1968; Perry et al., 1979). This result may be related to the higher amounts of saturated fatty acids found in bivalves, residing in organic detritus-rich environments with a rich bacterial load (Galap et al., 1999). Thus, in this study, the occurrence of high levels of C16:0 probably indicates detritus food.

As reported earlier (Sargent and Bell, 1990), photosynthetic organisms biosynthesize both n-3 and n-6 PUFAs, initially by converting newly biosynthesised 16:0 and 18:0 to 16:1n-7 and 18:1n-9, respectively. Unlike animals, plants can continue the further desaturation of the 18:1n-9 to 18:2n-6 and 18:3n-3 fatty acids. Therefore, in this study, the occurrence of 18:1n-9 (4.01-14.15%) fatty acid probably indicates food of plant origin such as phytoplankton or macroalgae.

Falk-Petersen and Dahl ( 2002) showed that the lipids of copepods contained 20:1n-9 and 20:1n-11, which together constituted 60% of their total fatty acids. When ingested by predators, the long-chain monoenes partially accumulate in the predators' tissue lipids (Raclot, Groscolas et al., 1998). In this study, the concentration of the fatty acid 20:1n-11 in the tissues of *Turbo coronatus* (1.55-7.50% of total fatty acids) suggests that *T. coronatus* may consume considerable quantities of zooplanktons. The use of zooplanktons as food, has also been reported in the oyster *Ostrea edulis* (Knox,1986) and other molluscs (Brett and Navarra, 1997).
Because of the fact that marine molluscs cannot synthesize essential fatty acids de novo, the quality and the quantity of algal lipids is very important in the diet of marine animals and algae are the main sources of these fatty acids (Sukenik, 1993; Zmora et al. 1993). Diatoms (Bacillariophycea) are rich sources of EPA, ARA and to a lesser extent of C16 PUFA. Dinoflagellates (Dinophycea) are rich in DHA. Green algae (Chlorophycea) tend to be rich in C16 and C18 (n-3) PUFA, especially 18:4(n-3) and deficient in both C20 and C22 PUFAs. Red algae (Rhodophycea) have (n-3) PUFA, mainly EPA and considerable amounts of ARA and finally, brown algae tend to be rich in ARA and EPA and significant levels of C18 (n-3) PUFA are also found. Thus, in this study, the occurrence of the fatty acids 20:5n-3 and 20:4 n-6 might indicate diatoms, red algae and brown algae (macroalgae) in food webs of T. coronatus.

Some bacteria are capable of producing 20:4(n-6) and other PUFA (Russell and Nichols, 1999). It is possible that bacterial input to the diet may also be responsible for the high levels of 20:4 (n-6) in T. coronatus. Furthermore, the ratio of omega-6 to omega-3 EFA is an important determinant of health. Many of the chronic conditions, cardiovascular disease, diabetes, cancer, obesity, autoimmune diseases, rheumatoid arthritis, asthma and depression, are associated with n-6/n-3 ratio as one of the determining factors. Amounts of dietary omega-6 and omega-3 fatty acids at a ratio of about 1:2/1 are recommended for dietary intakes in human (Simopoulos, 2002). In this study, the n-6/n-3 ratio found in this species was quite high (0.95 - 8.33%) suggesting that the meat quality of Turbo coronatus is not suitable for human consumption in autumn and spring (Table 2).

4. Conclusion

The data acquired in this study has shed some light on diet of Turbo coronatus in the study area where photosynthetic organisms (macroalgae and phytoplankton), zooplankton or detritus food occurred in abundance due to the availability of nutrients in this highly productive area. Besides, high n-6/n-3 ratio of meat quality in some seasons, indicated consuming of this species unsuitable throughout the year. The information on seasonal variations of fatty acids obtained from three other dominant molluscs (data in press) showed similar trends for molluscs in this region.

Acknowledgement

This article was a part of a report on the research project granted by Iranian National Institute for Oceanography (INIO), grant number 385-0111 to the authors.

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