Changes in body condition and fatty acid composition of wild Mediterranean horse mackerel
(*Trachurus mediterraneus*, Steindachner, 1868) associated to sea cage fish farms

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Abstract

Net-cage fish farms attract a great number of wild fishes, altering their behaviour and possibly their physiology. Wild Mediterranean horse mackerel (*Trachurus mediterraneus*), sampled from populations aggregated around two Mediterranean fish farms and from two natural control populations, were analyzed for differences in body condition, stomach content and fatty acid composition. Pellets used to feed caged fish in both farms were also analyzed to identify their relationship with the fatty acid composition of tissue of wild fish. *T. mediterraneus* aggregated around the farms throughout the year although large seasonal changes in abundance and biomass occurred. Wild fish aggregated at farms mainly ate food pellets while control fish fed principally on juvenile fish and cephalopods. Wild fish that fed around the cages had a significantly higher body fat content than the control fish (7.30 ± 1.8% and 2.36 ± 0.7%, respectively). The fatty acid composition also differed between farm-associated and control fish, principally because of the significantly increased levels of linoleic

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(C18:2ω6) and oleic (C18:1ω9) acids and decreased docosahexaenoic acid (C22:6ω3) in farm-associated fish. The increased condition of wild fish associated with farms could increase the spawning ability of coastal fish populations, if wild fish are protected from fishing while they are present at farms. The fatty acids compositions could also serve as biomarkers to infer the influence of a fish farm on the local fish community, helping to better describe the environmental impact of fish farming.

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1. Introduction

The presence of wild fish beneath sea-cage fish farms has often been noted, and may affect the presence, abundance and residence times of fishes in a given area (Carss, 1990; Bjordal and Skar, 1992). Dempster et al. (2002) demonstrated that sea-cage fish farms act as ‘super-FADs’ (FAD: fish attraction device) in the south-western Mediterranean Sea, attracting large multi-species schools of pelagic fish. However, the potential ecological and biological effects on wild fish populations have rarely been tested despite the present high level of public interest in the issue in many countries. As production from sea-cages in coastal regions continues to expand rapidly (FAO, 2003, Federation of European Aquaculture Producers, www.feap.info/feap), information on such effects will become increasingly important for managing the interactions between aquaculture and wild fish populations.

Marine fish, especially carnivores, have a natural diet rich in highly unsaturated ω3 fatty acids. As a consequence, long-chain ω3 polyunsaturated fatty acids occur in higher concentrations in marine fish muscle (Ackman, 1967). The production of fish, such as seabream (Sparus aurata), and sea-bass (Dicentrarchus labrax) in coastal fish farms is carried out using food pellets composed in part with vegetable oils. The corn or soya used in food production gives a high concentration of oleic acid (18:1ω9), linoleic acid (18:2ω6) and ω-linolenic acid (18:3ω3). The introduction of this source of food to the marine environment could modify the fatty acid (FA) composition of wild fish that feed on the lost pellets as well as their total fat content due to the high availability of food.

In this study, we tested if Mediterranean horse mackerel (Trachurus mediterraneus), a carnivorous species of commercial interest in the Mediterranean Sea, aggregate around fish farms throughout the year using visual counts. Further, we tested whether the diet of T. mediterraneus differed when it was aggregated around fish farms compared to natural control locations and if this led to a modification of its muscle nutritional condition and FA composition. Finally, we attempted to identify if fatty acid compositions could function as a ‘physiological tag’ that could be useful in evaluating the impact of the lost feed from farms on marine trophic nets.

2. Materials and methods

T. mediterraneus aggregated around two fish farms separated by 50 km in the south-east of Spain were spear-fished (Fig. 1). The farm at Campello was 3.2 km off the coast at an
average depth of 28.6 m, with 12 cages of 17 m diameter and 17 m deep producing 300 t yr$^{-1}$ of fish. The farm at Guardamar was 3.7 km from shore at a depth of 22.6 m, with 24 cages of 19 m diameter and 15 m deep producing 1000 t yr$^{-1}$. Both farms reared sea-bass (*Dicentrarchus labrax*) and sea-bream (*Sparus aurata*). Individuals from natural control populations were obtained from commercial, bottom-trawling fisheries in two locations at least 10 km away from the nearest fish farm (Villajoyosa and Alicante). Ten fish from each locality were used to carry out the analysis. Farm-associated and control fishes were caught on 3 different random days but during the same period (July 2005) to avoid seasonal variation in their chemical composition (e.g. Bandarra et al., 2001). Farm-associated fish ranged from 179 to 410 mm total length (TL) while control fish ranged from 227 to 315 mm TL. Commercial food pellets used to feed the crop were also analyzed; two different types of pellets were used in Campello (Types I and II) while only one was used in Guardamar (Type III).

To estimate the abundance of *T. mediterraneus* aggregated around the cages, we conducted rapid visual counts (RVCs; Kingsford and Battershill, 1998) using SCUBA. Full details of the count methodology are given in Dempster et al. (2002). At each farm, fish were counted at 3 random times over a period of 2 months for every season from winter 2004 until the fish sampling time in summer 2005 (January and February for winter, April and May for spring, August and September for summer and October and November for autumn). Six 5-min rapid visual counts were conducted each time within the farm complex. Each count covered a volume of approximately 11,250 m$^3$ (15 m wide$\times$15 m deep$\times$50 long). During counts, the average total length (TL) of each group was noted. Count data were entered into the ecoCEN program (Bayle-Sempere et al., 2002), where conversions to biomass were made using ecoCEN based on a published length–weight relationship for *T. mediterraneus*. Counts and sampling by spear-fishing were conducted on separate days.

Fish were weighed after emptying the stomach content and measured. After dissection, the liver was weighed and stomach contents were analyzed to determine the dietary
composition of farm-associated and control fish. Gut contents were extracted and prey items were identified under the microscope. The percentage of fish containing each kind of prey item was calculated.

All fish were iced after landing. In the laboratory, a part of the anterior-dorsal white muscle (approximately 6 g) was removed, frozen at −18 °C and analyzed within one week. Tissue composition was determined after homogenisation as follows: protein (Kjeldahl method with a 6.25 nitrogen to protein conversion factor), fat (ethyl-ether extraction using a SOXTEC System HT6 extractor), moisture (drying to constant weight in an oven at 105 ± 1 °C), total ash (incineration to constant weight in a muffle oven at 450 ± 2 °C) and diet NFE (nitrogen-free extract) was calculated by subtracting the other components from 100.

After individual tissue homogenisation, the fatty acid composition of the total lipid fraction was determined by fat extraction following the method of Folch et al. (1957), with a mixture of chloroform and methanol (1:1 proportion for the first extraction and 2:1 proportion for the second one). Fatty acid methyl esters (FAME) samples were analyzed according to the method of Stoffel et al. (1959) by gas-liquid chromatography using a SP™ 2560 flexible fused silica capillary column (100-m length and 0.25-mm internal diameter and 0.20 μm of film thickness; SUPELCO) in a Hewlett-Packard 5890 gas chromatograph. The oven temperature was programmed for 5 min at an initial temperature of 140 °C, was increased at a rate of 4 °C per min to 230 °C, was further increased at a rate of 1 °C per min to 240 °C and then held at that temperature for 6 min. The injector and flame ionization detector were set at 250 °C. Helium was used as a carrier gas at a pressure of 290 kPa, and peaks were identified by comparison of their retention times with appropriate FAME standards purchased from Sigma Chemical Company (St. Louis, MO, USA). Individual FA concentrations were expressed as percentages of the total content.

Fulton’s Condition Factor Index \( CI = \left[100,000 \times \frac{W}{L^3}\right]\), where \( W \) = weight and \( L \) = length), and Liver-somatic Index (LSI = 100 × [liver weight/total weight]) were calculated as indicators of fish condition.

Non-parametric multivariate techniques were used to compare FA compositions of fish and pellets. All multivariate analyses were performed using the PRIMER statistical package. Triangular similarity matrices were calculated using the Bray–Curtis similarity coefficient (Clarke and Warwick, 1994). Non-metric multidimensional scaling (nMDS) was used as the ordination method. Variables that had more influence on similarities within groups and dissimilarities among groups of locations were calculated using the SIMPER (similarity percentages) procedure (Warwick et al., 1990; Clarke, 1993). A permutation test (PERMANOVA) was used to assess the significance of the overall fatty acid composition among the considered sources of variation (Clarke, 1993; Anderson, 2004).

To test whether fat content and the main FAs varied among the two farms and two control sites, we used an analysis of variance (ANOVA) which incorporated the factors – feeding (fixed) with two treatments (pellets and natural diet) and locations (random and nested in feeding) with two random localities; Campello and Guardamar for pellets and Villajoyosa and Alicante for natural diet.

Prior to ANOVA, heterogeneity of variance was tested with Cochran’s C-test. As data were percentages, they were transformed with arcsen \((x + 1)\). ANOVA is robust to heterogeneity of variances, particularly when experiments are large and balanced (Underwood, 1997).
3. Results

3.1. Visual counts

Rapid visual counts indicated that *T. mediterraneus* aggregated around farms throughout the study period at both Campello and Guardamar, except for winter 2005 at Guardamar. However, the size of aggregations (abundance and biomass) differed greatly among times. At Campello, abundance and biomass peaked during autumn 2004 (289.9 ± 122.3 ind./11,250 m³ and 68.8 ± 26.2 kg/11,250 m³) and large aggregations also occurred in spring and summer 2005 (Fig. 2). Lowest abundances at this farm occurred in spring of 2004 (8–10 ind./11,250 m³ and 1–2 kg/11,250 m³). At Guardamar, variation in abundances was also high; the population remained at low levels until spring and summer of 2005 when abundances reached 110–313 ind./11,250 m³ and biomasses were between 30 and 37 kg/11,250 m³.

![Fig. 2](image-url). Number of individuals and biomass of *Trachurus mediterraneus* aggregated around the 2 fish farms. Bars represent the average of 18 censuses made during 3 sampling days (6 censuses per day). Error bars indicate ±SE.
3.2. Stomach contents

Fish caught by local commercial fisheries far from the farms used a variety of food sources, principally juvenile fish, crustaceans and cephalopods (Fig. 3). Diet composition differed between the two natural control populations; cephalopods were most important at Villajoyosa while juvenile fish dominated the stomach contents of the fish from Alicante. In contrast, food pellets were the main item found in the stomach of *Trachurus mediterraneus* aggregated around both the Guardamar and Campello farms. Other prey items, such as juvenile fish, made up only a small proportion of the diet.

Fig. 3. Stomach contents of *Trachurus mediterraneus* aggregated around farms (Campello and Guardamar) and non-aggregated (Alicante and Villajoyosa). Values are percentages of wet weight.

Fig. 4. Individual total fish fat content (percentage of total body composition) and total length (mm) in farms and control sites.
3.3. Body condition

Farm-associated fish had on average 3.5 times higher body fat content than control fish (7.30 ± 1.8% vs. 2.36 ± 0.7%). Fat content levels were considerably more variable in the farm-associated fish (Fig. 4), ranging from 1.5% to 13% in contrast to the fat levels of Table 1.

Table 1
Composition of the three kinds of food pellets used to feed farmed fish; values represent percentages as mean ± standard deviation

<table>
<thead>
<tr>
<th></th>
<th>Campello Type I</th>
<th>Campello Type II</th>
<th>Guardamar Type III</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fat</td>
<td>22.57 ± 0.61</td>
<td>17.73 ± 0.06</td>
<td>24.04 ± 0.01</td>
</tr>
<tr>
<td>Protein</td>
<td>42.62 ± 0.15</td>
<td>44.50 ± 0.31</td>
<td>42.49 ± 0.29</td>
</tr>
<tr>
<td>Moisture</td>
<td>6.81 ± 0.20</td>
<td>10.51 ± 4.05</td>
<td>5.80 ± 0.15</td>
</tr>
<tr>
<td>Ash</td>
<td>7.36 ± 0.24</td>
<td>7.00 ± 0.06</td>
<td>8.70 ± 0.02</td>
</tr>
<tr>
<td>NFE</td>
<td>20.65 ± 0.72</td>
<td>20.26 ± 3.62</td>
<td>18.98 ± 0.15</td>
</tr>
<tr>
<td>C12:0</td>
<td>0.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>C14:0</td>
<td>6.81</td>
<td>3.38</td>
<td>3.03</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.97</td>
<td>0.49</td>
<td>0.63</td>
</tr>
<tr>
<td>C15:1o5</td>
<td>0.16</td>
<td>0.45</td>
<td>0.5</td>
</tr>
<tr>
<td>C16:0</td>
<td>18.17</td>
<td>20.48</td>
<td>17.53</td>
</tr>
<tr>
<td>C16:1o7</td>
<td>5.8</td>
<td>7.07</td>
<td>3.23</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.52</td>
<td>0.45</td>
<td>0.5</td>
</tr>
<tr>
<td>C17:1o7</td>
<td>0.77</td>
<td>0.16</td>
<td>0.2</td>
</tr>
<tr>
<td>C18:0</td>
<td>3.98</td>
<td>6.19</td>
<td>4.8</td>
</tr>
<tr>
<td>C18:1o9</td>
<td>10.39</td>
<td>14.57</td>
<td>16.18</td>
</tr>
<tr>
<td>C18:1o7</td>
<td>2.31</td>
<td>3.41</td>
<td>2.16</td>
</tr>
<tr>
<td>C18:2o6</td>
<td>14.78</td>
<td>8.7</td>
<td>23.35</td>
</tr>
<tr>
<td>C20:0</td>
<td>0.3</td>
<td>0.26</td>
<td>0.35</td>
</tr>
<tr>
<td>C18:3o6</td>
<td>1.84</td>
<td>0.28</td>
<td>2.21</td>
</tr>
<tr>
<td>C20:1o9</td>
<td>0.87</td>
<td>4.57</td>
<td>1.67</td>
</tr>
<tr>
<td>C18:3o3</td>
<td>1.87</td>
<td>1.55</td>
<td>2.65</td>
</tr>
<tr>
<td>C18:4o3</td>
<td>1.87</td>
<td>1.43</td>
<td>0.81</td>
</tr>
<tr>
<td>C20:2o6</td>
<td>0.73</td>
<td>0.64</td>
<td>0.67</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.26</td>
<td>0.11</td>
<td>0.26</td>
</tr>
<tr>
<td>C20:3o6</td>
<td>0</td>
<td>0.38</td>
<td>0.45</td>
</tr>
<tr>
<td>C22:1o9</td>
<td>0</td>
<td>1.18</td>
<td>1.12</td>
</tr>
<tr>
<td>C20:3o3</td>
<td>0</td>
<td>0.31</td>
<td>0</td>
</tr>
<tr>
<td>C20:4o6</td>
<td>0.64</td>
<td>0.68</td>
<td>1.14</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.22</td>
<td>0.36</td>
<td>0.2</td>
</tr>
<tr>
<td>C20:5o3</td>
<td>13.47</td>
<td>6.64</td>
<td>5.59</td>
</tr>
<tr>
<td>C24:1o9</td>
<td>0.33</td>
<td>1.1</td>
<td>0.49</td>
</tr>
<tr>
<td>C22:4o6</td>
<td>0</td>
<td>0.08</td>
<td>0</td>
</tr>
<tr>
<td>C22:5o3</td>
<td>1.1</td>
<td>1.2</td>
<td>0.76</td>
</tr>
<tr>
<td>C22:6o3</td>
<td>11.52</td>
<td>14.68</td>
<td>10.03</td>
</tr>
<tr>
<td>ω3</td>
<td>29.83</td>
<td>25.81</td>
<td>19.84</td>
</tr>
<tr>
<td>ω6</td>
<td>18</td>
<td>10.78</td>
<td>27.82</td>
</tr>
<tr>
<td>ω5</td>
<td>0.16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>ω7</td>
<td>8.11</td>
<td>10.48</td>
<td>5.39</td>
</tr>
<tr>
<td>ω9</td>
<td>11.6</td>
<td>21.42</td>
<td>19.45</td>
</tr>
<tr>
<td>Saturated</td>
<td>31.53</td>
<td>31.35</td>
<td>27.3</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>20.64</td>
<td>32.06</td>
<td>25.04</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>47.83</td>
<td>36.59</td>
<td>47.66</td>
</tr>
</tbody>
</table>

NFE: Nitrogen free extract.
Table 2
Fulton’s Condition Index (CI) and Liver Somatic Index (LSI) for the fish (n = 10) from the four localities

<table>
<thead>
<tr>
<th>Location</th>
<th>Length (cm)</th>
<th>Weight (g)</th>
<th>CI</th>
<th>LSI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Villajoyosa Control</td>
<td>291.7 ± 27.9</td>
<td>222.9 ± 87.7</td>
<td>0.86 ± 0.06</td>
<td>1.32 ± 0.50</td>
</tr>
<tr>
<td>Alicante Control</td>
<td>250.3 ± 15.2</td>
<td>134.4 ± 20.3</td>
<td>0.85 ± 0.04</td>
<td>1.60 ± 0.61</td>
</tr>
<tr>
<td>Campello Farm</td>
<td>378.7 ± 20.6</td>
<td>570.9 ± 107.5</td>
<td>1.04 ± 0.11</td>
<td>1.02 ± 0.34</td>
</tr>
<tr>
<td>Guardamar Farm</td>
<td>302.9 ± 30.9</td>
<td>275.7 ± 63.0</td>
<td>1.02 ± 0.24</td>
<td>1.11 ± 0.47</td>
</tr>
</tbody>
</table>

Values represent means ± standard deviation.

Table 3
Fatty acid composition of associated and non-associated Trachurus mediterraneus at farms and control sites (n = 10, % of total fatty acids)

<table>
<thead>
<tr>
<th>Name</th>
<th>Villajoyosa Control</th>
<th>Alicante Control</th>
<th>Campello Farm</th>
<th>Guardamar Farm</th>
</tr>
</thead>
<tbody>
<tr>
<td>C12:0</td>
<td>0.25 ± 0.51</td>
<td>0.16 ± 0.24</td>
<td>0.05 ± 0.08</td>
<td>0.14 ± 0.15</td>
</tr>
<tr>
<td>C13:0</td>
<td>1.02 ± 3.23</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.03 ± 0.42</td>
<td>2.53 ± 1.09</td>
<td>3.58 ± 0.55</td>
<td>3.32 ± 0.69</td>
</tr>
<tr>
<td>C14:1</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.04 ± 0.12</td>
</tr>
<tr>
<td>C15:0</td>
<td>0.46 ± 0.35</td>
<td>0.57 ± 0.36</td>
<td>0.35 ± 0.19</td>
<td>0.49 ± 0.22</td>
</tr>
<tr>
<td>C15:1</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.02 ± 0.06</td>
</tr>
<tr>
<td>C16:0</td>
<td>21.84 ± 1.94</td>
<td>23.39 ± 3.11</td>
<td>19.73 ± 1.79</td>
<td>18.81 ± 2.74</td>
</tr>
<tr>
<td>C16:1</td>
<td>1.97 ± 0.4</td>
<td>2.66 ± 1.28</td>
<td>4.49 ± 0.62</td>
<td>4.63 ± 1.83</td>
</tr>
<tr>
<td>C17:0</td>
<td>0.66 ± 0.24</td>
<td>0.55 ± 0.3</td>
<td>0.48 ± 0.21</td>
<td>0.67 ± 0.29</td>
</tr>
<tr>
<td>C17:1</td>
<td>0.24 ± 0.27</td>
<td>0.27 ± 0.22</td>
<td>0.21 ± 0.16</td>
<td>0.31 ± 0.28</td>
</tr>
<tr>
<td>C18:0</td>
<td>8.63 ± 2.67</td>
<td>7.85 ± 1.2</td>
<td>7.28 ± 2.88</td>
<td>7.66 ± 3.69</td>
</tr>
<tr>
<td>C18:1</td>
<td>9.30 ± 1.63</td>
<td>11.34 ± 2.45</td>
<td>18.81 ± 2.68</td>
<td>20.60 ± 9.09</td>
</tr>
<tr>
<td>C18:2</td>
<td>1.65 ± 0.2</td>
<td>1.73 ± 0.99</td>
<td>2.69 ± 0.23</td>
<td>2.70 ± 0.69</td>
</tr>
<tr>
<td>C18:3</td>
<td>2.69 ± 1.22</td>
<td>2.77 ± 2.88</td>
<td>14.26 ± 2.11</td>
<td>12.02 ± 5.59</td>
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<tr>
<td>C20:0</td>
<td>0.15 ± 0.2</td>
<td>0.20 ± 0.17</td>
<td>0.23 ± 0.18</td>
<td>0.29 ± 0.14</td>
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<tr>
<td>C20:1</td>
<td>0.59 ± 1.08</td>
<td>0.40 ± 0.62</td>
<td>0.66 ± 1.11</td>
<td>1.81 ± 3.22</td>
</tr>
<tr>
<td>C20:2</td>
<td>0.45 ± 0.33</td>
<td>0.98 ± 1.31</td>
<td>1.90 ± 0.82</td>
<td>1.93 ± 1.16</td>
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<tr>
<td>C20:3</td>
<td>0.24 ± 0.27</td>
<td>0.51 ± 0.71</td>
<td>1.60 ± 0.24</td>
<td>1.29 ± 0.61</td>
</tr>
<tr>
<td>C20:4</td>
<td>0.07 ± 0.12</td>
<td>0.19 ± 0.25</td>
<td>0.50 ± 0.31</td>
<td>0.57 ± 0.43</td>
</tr>
<tr>
<td>C20:5</td>
<td>0.21 ± 0.27</td>
<td>0.44 ± 0.33</td>
<td>0.56 ± 0.4</td>
<td>0.64 ± 0.26</td>
</tr>
<tr>
<td>C22:0</td>
<td>0.35 ± 0.31</td>
<td>0.33 ± 0.22</td>
<td>0.18 ± 0.17</td>
<td>0.19 ± 0.21</td>
</tr>
<tr>
<td>C22:1</td>
<td>0.18 ± 0.31</td>
<td>0.13 ± 0.31</td>
<td>0.23 ± 0.39</td>
<td>0.78 ± 1.32</td>
</tr>
<tr>
<td>C22:2</td>
<td>0.02 ± 0.06</td>
<td>0.12 ± 0.28</td>
<td>0.28 ± 0.25</td>
<td>0.20 ± 0.28</td>
</tr>
<tr>
<td>C22:3</td>
<td>0.00 ± 0</td>
<td>0.06 ± 0.17</td>
<td>0.05 ± 0.09</td>
<td>0.04 ± 0.09</td>
</tr>
<tr>
<td>C22:4</td>
<td>1.45 ± 0.25</td>
<td>1.42 ± 0.36</td>
<td>0.68 ± 0.41</td>
<td>0.68 ± 0.21</td>
</tr>
<tr>
<td>C23:0</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.15 ± 0.48</td>
</tr>
<tr>
<td>C24:0</td>
<td>0.72 ± 0.3</td>
<td>0.46 ± 0.31</td>
<td>0.14 ± 0.17</td>
<td>0.18 ± 0.24</td>
</tr>
<tr>
<td>C20:5o3</td>
<td>5.83 ± 1.09</td>
<td>6.18 ± 1.61</td>
<td>6.04 ± 1.67</td>
<td>4.53 ± 1.74</td>
</tr>
<tr>
<td>C24:1o9</td>
<td>0.98 ± 0.37</td>
<td>0.90 ± 0.37</td>
<td>0.40 ± 0.23</td>
<td>0.51 ± 0.2</td>
</tr>
<tr>
<td>C22:4o6</td>
<td>0.05 ± 0.1</td>
<td>0.02 ± 0.07</td>
<td>0.05 ± 0.08</td>
<td>0.02 ± 0.05</td>
</tr>
<tr>
<td>C22:5o3</td>
<td>1.36 ± 0.53</td>
<td>1.29 ± 0.56</td>
<td>1.39 ± 0.24</td>
<td>1.11 ± 0.43</td>
</tr>
<tr>
<td>C22:6o3</td>
<td>36.61 ± 4.01</td>
<td>32.56 ± 6.55</td>
<td>13.19 ± 2.63</td>
<td>13.64 ± 3.72</td>
</tr>
<tr>
<td>e3</td>
<td>44.11 ± 5.2</td>
<td>40.79 ± 6.99</td>
<td>22.76 ± 4.31</td>
<td>21.19 ± 4.63</td>
</tr>
<tr>
<td>e6</td>
<td>5.17 ± 1.82</td>
<td>5.19 ± 2.82</td>
<td>16.43 ± 2.93</td>
<td>15.95 ± 5.27</td>
</tr>
<tr>
<td>e5</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.00 ± 0</td>
<td>0.06 ± 0.13</td>
</tr>
<tr>
<td>e7</td>
<td>3.62 ± 0.57</td>
<td>4.38 ± 2.11</td>
<td>7.18 ± 0.82</td>
<td>7.33 ± 2.47</td>
</tr>
<tr>
<td>e9</td>
<td>10.75 ± 1.51</td>
<td>13.34 ± 3.75</td>
<td>21.40 ± 3.15</td>
<td>23.25 ± 9.01</td>
</tr>
<tr>
<td>Saturated</td>
<td>36.10 ± 4.77</td>
<td>36.04 ± 3.16</td>
<td>32.02 ± 4.18</td>
<td>31.91 ± 6.81</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>14.62 ± 1.88</td>
<td>17.99 ± 5.45</td>
<td>28.79 ± 3.16</td>
<td>30.95 ± 10.86</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>49.28 ± 5.22</td>
<td>45.98 ± 6.44</td>
<td>39.19 ± 4.17</td>
<td>37.14 ± 7.81</td>
</tr>
</tbody>
</table>

Data are expressed as mean ± standard deviation.
control fish which remained stable within a narrow range (mainly 1–4%). The total lipid amount in farm-associated fish was significantly higher ($p < 0.01$) than that in control fish, which is a common difference between cultivated and wild fish. Total protein content, however, did not differ between farm-associated and control fish. In spite of the bigger average size of farm-associated fish, total fat content was not correlated with fish size from either farm-associated or control fish.

The protein and fat content of food pellets were similar for Guardamar and ‘Campello Type I’, but ‘Campello Type II’ contained lower levels of fat and higher levels of protein (Table 1). Significant differences existed in the Condition and Liver Somatic indexes between the farm-associated and control fish. Farm-associated fish had a higher Condition Index (1.02–1.04) than found in control fish (0.85–0.86, $p < 0.01$, Table 2). In contrast, the Liver Somatic Index was higher in control fish (1.32–1.60) than in farm-associated fish (1.02–1.11, $p = 0.1$).

3.4. Fatty acid composition

The total FA composition differed significantly between control and farm-associated fish (Table 3). The two-dimensional MDS plot (Fig. 5) based on relative presence of different FAs revealed a clear separation of the two groups (farm-associated and control horse mackerel) with a low stress value (0.08). Further, the separation of the groups among localities was clearer in farm-associated fish. The FA composition of food pellets presented a similar distribution to the farm-associated fish. The composition of pellet Type II, used to feed the caged fish in Campello, appeared particularly close to the fatty acid composition of wild *T. mediterraneus* aggregated there. Such a close match between pellet and wild fish composition, however, did not occur for Type I pellets at Campello or Type III pellets at Guardamar.

PERMANOVA analysis showed that the differences in FA composition between treatments were significant ($p < 0.001$). SIMPER analysis indicated that docosahexaenoic acid (C22:6ω3) was the main differentiating FA (32.6% contribution to dissimilarity) due to its elevated presence in control *T. mediterraneus* (Table 4). The two other main differentiating
Variables were linoleic acid (C18:2\(\text{\textomega}6\); 16.2% contribution) and oleic acid (C18:1\(\text{\textomega}9\); 14.7% contribution). Both of the latter two fatty acids were present in high levels in the commercial pellet food.

Levels of \(\text{\textomega}3\) were lower in farm-associated fish at both farms than in control fish, being lowest at Guardamar (Fig. 6). Docosahexaenoic acid (C22:6\(\text{\textomega}3\)) was the main \(\text{\textomega}3\) FA present in all fish, and was significantly higher (\(p < 0.01\)) in control fish. In contrast, \(\alpha\)-linolenic acid (C18:3\(\text{\textomega}3\)) was significantly lower (\(p < 0.05\)) in control fish (Table 5). However, there were no significant differences in eicosapentaenoic acid (C20:5\(\text{\textomega}3\)) between farm-associated and control fish.

![Table 4](image)

**Table 4** Contributions of the main fatty acids to overall dissimilarities between associated and non-associated *T. mediterraneus* and percentage contributions to the cumulative dissimilarity

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Non-associated</th>
<th>Associated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average abundance</td>
<td>Average dissimilarity</td>
</tr>
<tr>
<td>C22:6(\text{\textomega}3)</td>
<td>34.58</td>
<td>13.41</td>
</tr>
<tr>
<td>C18:2(\text{\textomega}6)</td>
<td>2.73</td>
<td>13.14</td>
</tr>
<tr>
<td>C18:1(\text{\textomega}9)</td>
<td>10.32</td>
<td>19.71</td>
</tr>
<tr>
<td>C16:0</td>
<td>22.61</td>
<td>19.27</td>
</tr>
<tr>
<td>C18:0</td>
<td>8.24</td>
<td>7.47</td>
</tr>
<tr>
<td>C16:1(\text{\textomega}7)</td>
<td>2.31</td>
<td>4.56</td>
</tr>
<tr>
<td>C20:5(\text{\textomega}3)</td>
<td>6.01</td>
<td>5.29</td>
</tr>
<tr>
<td>C20:4(\text{\textomega}6)</td>
<td>0.71</td>
<td>1.92</td>
</tr>
<tr>
<td>C18:3(\text{\textomega}6)</td>
<td>0.49</td>
<td>1.23</td>
</tr>
<tr>
<td>C14:0</td>
<td>2.28</td>
<td>3.45</td>
</tr>
<tr>
<td>C18:3(\text{\textomega}3)</td>
<td>0.38</td>
<td>1.44</td>
</tr>
<tr>
<td>C18:1(\text{\textomega}7)</td>
<td>1.69</td>
<td>2.7</td>
</tr>
</tbody>
</table>

![Fig. 6](image)

Fig. 6. Fish muscle values of the main fatty acids in the four localities and in the three kinds of food pellets. Control sites are Villajoyosa and Alicante, Farms are Campello and Guardamar. Food pellets Types I and II were used in Campello; Type III in Guardamar. Error bars show ± SE.
The percentage of ω6 FA was higher in farm-associated fish. Nevertheless, concentrations of arachidonic acid (C20:4ω6) were significantly (p < 0.01) higher in control fish, but linoleic acid (C18:2ω6) showed significantly higher values (p < 0.01) in farm-associated fish (Fig. 6). In the same way, ω7 FAs were more elevated in farm-associated individuals (Table 3).

Markedly higher monounsaturated and ω9 FAs, such as oleic acid (C18:1ω9, Table 3), were recorded from tissues of farm-associated fish (p < 0.05). Control fish tissues presented the opposite pattern, with more total saturated FAs, although differences were not significant.

The three types of food pellets had similar fatty acid compositions, with high concentrations of palmitic (C16:0), linoleic (C18:2ω6, especially high in the food pellets Type III), oleic (C18:1ω9), eicosapentaenoic (C20:5ω3) and decosahexaenoic (C22:6ω3) acids (Table 1). Food pellets used to feed the fish at Campello had more ω3 FA content than the fish food at Guardamar, but had a higher content of ω6 FAs (27.82%). Levels of monounsaturated acids in farm-associated fish exceeded those found in pellets, total saturated acids approached those found in pellets and total polyunsaturated acids occurred in lower levels than in pellets. This was due to the low amount of docosahexaenoic acid (C22:6ω3) in

---

### Table 5
Analysis of variance (ANOVAS) of the most important fatty acids, total ω3 FAs, ω3/ω6 ratio and total fat content

<table>
<thead>
<tr>
<th></th>
<th>Palmitic 16:0</th>
<th>Oleic 18:1ω9</th>
<th>TOTAL n3</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>DF</td>
<td>MS</td>
<td>P</td>
</tr>
<tr>
<td>Af</td>
<td>1</td>
<td>55.6398</td>
<td>0.0642</td>
</tr>
<tr>
<td>Lo(Af)</td>
<td>2</td>
<td>3.9462</td>
<td>0.2675</td>
</tr>
<tr>
<td>RES</td>
<td>36</td>
<td>2.8845</td>
<td>0.3685</td>
</tr>
<tr>
<td>C test</td>
<td>39</td>
<td></td>
<td>0.3695</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Linoleic 18:2ω6</th>
<th>ω-linolenic 18:3ω3</th>
<th>18:3ω6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>P</td>
<td>MS</td>
</tr>
<tr>
<td>Af</td>
<td>1</td>
<td>1413.7398</td>
<td>0.0104</td>
</tr>
<tr>
<td>Lo(Af)</td>
<td>2</td>
<td>14.9759</td>
<td>0.3338</td>
</tr>
<tr>
<td>RES</td>
<td>36</td>
<td>13.2388</td>
<td>0.5511*</td>
</tr>
<tr>
<td>C test</td>
<td>39</td>
<td></td>
<td>0.4720</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Arachidonic 20:4ω6</th>
<th>EPA 20:5ω3</th>
<th>DHA 22:6ω3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>P</td>
<td>MS</td>
</tr>
<tr>
<td>Af</td>
<td>1</td>
<td>58.2873</td>
<td>0.0096</td>
</tr>
<tr>
<td>Lo(Af)</td>
<td>2</td>
<td>0.5671</td>
<td>0.7244</td>
</tr>
<tr>
<td>RES</td>
<td>36</td>
<td>1.7428</td>
<td>0.7570**</td>
</tr>
<tr>
<td>C test</td>
<td>39</td>
<td></td>
<td>0.4540</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Total fat</th>
<th>ω3/ω6</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MS</td>
<td>P</td>
</tr>
<tr>
<td>Af</td>
<td>1</td>
<td>372.4215</td>
</tr>
<tr>
<td>Lo(Af)</td>
<td>2</td>
<td>43.9167</td>
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<tr>
<td>RES</td>
<td>36</td>
<td>11.4920</td>
</tr>
<tr>
<td>C test</td>
<td>39</td>
<td></td>
</tr>
</tbody>
</table>

Af: Artificial feeding; Lo: Locality; Cochran’s C-test significances: *: significant p < 0.05. **: significant p < 0.01. All data were transformed arcsen (x + 1).
farm-associated fish tissues, since eicosapentaenoic acid (C20:5ω3) levels remained constantly below those of the pellets.

4. Discussion

Rearing of fish in coastal sea-cages using food pellets containing terrestrial plant products impacts wild fish that aggregate around farms by altering their natural diet in a way that leads to a change in body condition and FA composition. These changes make their body composition more similar to the cultivated fish.

Populations of wild *T. mediterraneus* occurred at farms throughout the year although with large scale differences in abundance between times. As seen in Dempster et al. (2002), large numbers of this species were seen at eight of nine farms investigated along the Spanish Mediterranean coastline, appearing as an important part of the fish assemblage at many farms.

*T. mediterraneus* was not seen in visual counts in winter 2005 at Guardamar. This suggests that the individuals sampled at Guardamar aggregated after the visual counts made in winter (March at the earliest), pointing out that they could only have been resident for a maximum of three to four months prior to our sampling in July. This was also likely to have occurred in Campello, since populations decreased markedly at the same time. This period of time is likely to be sufficient to change the fish FA composition (Izquierdo et al., 2005).

Juvenile fish and cephalopods were the main items found in control fish stomachs, but there was a drastic change in the feeding behaviour of *T. mediterraneus* around the cages. As expected, since samples were taken during feeding hours, the vast majority of fish had pellets in their guts. As a consequence of the decrease in other natural food items, it is possible to assert that *T. mediterraneus* strongly changes its feeding behaviour while it is associated with fish farms. Although no quantitative data is available for comparison, direct feeding of *T. mediterraneus* on pellets lost from farms has been observed previously (Dempster et al., 2002).

Farm-associated fish had markedly higher muscle fat content and condition than control fish. Similarly, Skog et al. (2003) found that wild saithe associated with a single fjord-based farm in Norway had higher condition than control fish taken from within the same fjord. In contrast to condition and muscle fat content, Liver Somatic Index was higher in control *T. mediterraneus* than farm-associated fish. Abnormal LSI index values could be caused by ingestion of hormonally active compounds in the lost fish food beneath farms, which may be capable of modifying liver weight due to the activation of metabolising enzymes (Sloof et al., 1983). Further research into the physiological effects on wild fish caused by aggregation at farms should target the mechanisms driving the changes we have observed.

Tzikas et al. (2005), found seasonal variations in the muscle lipid content of *T. mediterraneus* off the coast of Greece; the mean lipid content during August was 0.8%, a value considerably lower than the mean content found in the present study for control specimens (2.19%) and much lower than the value obtained for associated fish (6.37%). Differences between the results of the two studies could be due to natural variation in the populations, although farm-associated fish showed much more lipid content than control fish from the coast of Spain and the Greek populations. This fact could lead to substantial physiological changes, since better body condition increases the spawning success of fish (Izquierdo et al.
From a previous study carried out at nine farms along the SE coast of Spain (including those in this paper) it is known that 85% of farm-associated fish are of adult size (Dempster et al., 2002). Therefore, we hypothesise that the better condition these adults gain while associated with farms enables greater production of eggs and sperm, which translates to greater spawning success. *T. mediterraneus* is known to spawn in summer and autumn in the Mediterranean (Ragonese et al., 2004) precisely at this time when we observed their condition to be enhanced by farms. Further research is required to test the quality of eggs and larvae. This important source of spawners of adult size could function as both spawning product and biomass exporters in the same way as the numerous large adult fish protected within Marine Protected Areas (Gell and Roberts, 2003; García-Chartón et al., 2004). Subsequently, our results add weight to the management concept of protecting wild fish assemblages around fish farms, when they are possibly more susceptible to capture due to their aggregating behaviour. Furthermore, the fact that wild fish help to remove up to 80% of wastes that a farm produces (Vita et al., 2004) supports the idea of protecting the farm-associated fauna following the dual objectives of enhancing local fishing and reducing the environmental impacts caused by farm wastes (Dempster et al., 2005).

The high variation in fat contents in farm-associated fish could be due to differing residence times of individuals around the farms before capture or even due to feeding on different kinds of food, natural and pellets, during short migrations. Different individuals likely consumed pellets over different periods of time; less fatty natural food for longer periods prior to sampling in some fish was probably responsible for the variability we observed (e.g. Deudero and Morales-Nin, 2001). Fat levels in control fish were relatively stable among individuals, suggesting they had similar dietary profiles rich in low-fat natural items. The total lipid amount in cultivated fish is usually higher than that of wild fish, a feature common in studies that compare reared and wild fish of the same species (Chanmugam et al., 1986; Nettleton et al., 2000; Saglik et al., 2003). However, little is known about the effect on wild fishes that aggregate around fish farms and feed on lost pellets. Fish aggregate around sea-cages mainly due to the availability of food (Tuya et al., 2006). We have demonstrated that if wild fish that occur beneath farms change their natural feeding patterns to consume food pellets, a modification in their physiology will occur.

Ackman and Takeuchi (1986) reported that the percentage of ω3 PUFA in farmed marine fish is usually lower than in their wild relatives, presumably because of the lack of lipids originating from algae and marine phytoplankton (Nichols et al., 1989; Muje et al., 1989). In this study, we have demonstrated that this also happens in wild *T. mediterraneus* that aggregate and feed around fish farms. Skog et al. (2003) found that wild saithe (*Pollachius virens*) feeding around a salmon farm in a Norwegian fjord had similar fatty acid profiles to the food pellets used at the farm, with increased levels of oleic and ω-linolenic acids as well as a comparatively low ratio of ω3/ω6 which reflected that in pellets. They also detected an increase in palmitic acid in the flesh of fish from reference areas, as we detected with *T. mediterraneus*, pointing out de novo synthesis of these acids (Henderson and Sargent, 1985).

The increased levels of ω6 acids in farm-associated fish may have been due to the linoleic acid (C18:2ω6) present in pellets. It seems that unsaturated acids are to some degree regulated by the fish, leading to a change in the FAs of farm-associated fish with respect to pellet composition, especially in linoleic acid (e.g. Linko et al., 1992).
The clear differences in FA composition were mainly due to docosahexaenoic acid (C22:6ω3), because of the low values for farm-associated fish, and linoleic acid (C18:2ω6), which was found in higher levels than in the control *T. mediterraneus* because of their presence in the commercial food pellets. The several-fold decrease in docosahexaenoic acid content in farm-associated fish may be due to their changed feeding behaviour, since natural food is rich in ω3 FAs (e.g. Shirai et al., 2002).

The MDS analysis (Fig. 5) showed that the FA composition of *T. mediterraneus* aggregated around the Guardamar farm was more variable than that of the fish aggregated at the Campello farm. This could be related to the increased abundances around the farm prior to the capture of samples in summer 2005, indicating the recent arrival of new individuals at the farm; the differing influence of artificial (pellets) and natural food items would explain the different muscle FA composition. The visual count data suggest that the observed physiological changes in wild *T. mediterraneus* condition, body fat content and FA composition occurred over a maximum time scale of three to four months.

The ω3/ω6 ratio is generally lower in cultivated than in wild fish (Van Vliet and Katan, 1990). Despite the higher relative quantity of ω6 in farm-associated fish, the total content of ω3 was also higher because of their elevated fat contents. However, the relationship between ω3/ω6 was significantly lower. Rueda et al. (2001), nevertheless, found higher levels of ω3 FAs in cultivated *Diplodus puntazzo*, an omnivorous species, with a slightly non-significant increment of ω3 FAs in their wild counterparts, however, linoleic acid (C18:2ω6) was much higher in cultivated fish.

The fatty acids 20:1ω9 and 22:1ω11 have been found to be possible biomarkers of cod-farms (*Gadus morhua*) since they are abundant in the feed and are not well digested by fish so they can be detected in the dispersed wastes (Van Biesen and Parris, 2005). However, 22:1ω11 was not a component in the pellets used to feed sea bass and sea bream in the farms tested here. We suggest that it may also be possible to detect the influence that farms have on wild fish in an area by analyzing the FA profile of the local ichthyofauna (e.g. Skog et al., 2003). FAs are generally taken in by tissues, and it is possible to distinguish between dietary and non-dietary components. Some fatty acids are deposited in adipose tissue with little modification and in a predictable way (Iverson et al., 2004). The specific FA patterns are passed from prey to predator near the bottom of the food web (Sargent et al., 1988; Fraser et al., 1989; Graeve et al., 1994: Navarro et al., 1995; St. John and Lund, 1996; Kirsch et al., 1998), determining the FA composition of higher predator levels (Hooper et al., 1973) and indicating the presence of specific prey in predator diets (Colby et al., 1993; Pond et al., 1995; Raclot et al., 1998). Tracking of dietary components through the food web cannot be entirely achieved using other methods such as stomach contents, which are modified by digestion, or stable isotopes, which are useful in estimating the trophic level of a predator but cannot determine the species composition of the diet (Hobson, 1993; Gilmore et al., 1995; Koch et al., 1995).

FA could, therefore, be used as biomarkers in the study of the structure and dynamics of fish food webs around fish farms, as alternatives to direct or indirect methods that provide only a snapshot of the most recent meal and may not be representative of the longer term diet. Their qualitative use has inferred trophic levels and spatial and temporal differences in diets both within and among species (Kakela et al., 1993; Smith et al., 1996: Smith et al., 1997; Iverson et al., 1997a,b). Similar methods could provide quantitative estimates of predator diets in fish assemblages aggregated around cages. To do this requires understanding the FA dynamics of each species. This study is the first contribution in that direc-
tion to the knowledge of trophodynamics of fish assemblages around cages in the Mediterranean Sea.

To develop the concept of using fatty acids as biomarkers, it is necessary to undertake laboratory and field experiments to measure the accumulation times of FAs in tissue. Further, the residence times of the aggregated species around cages and the magnitude of their migrations is important information to determine the extent to which pellet-originated FAs are transported over a broader scale than the immediate vicinity of the cages. Such a method would be a key tool to infer the scale and magnitude of the influence of a net-cage fish farm on the local fauna, not only on fish that directly feed on lost food pellets but on the whole food web.

5. Conclusion

*T. mediterraneus* aggregated around sea-cage fish farms underwent ecological and physiological changes. Their diet differed from control fish, which affected both their body condition and fatty acid composition. Differences in residence times at farms or short migrations from individual fish may explain the large differences in fat content among the aggregated fish. The ω3/ω6 ratio and 22:6ω3 level were significantly lower in farm-associated fish, reflecting the food pellet composition, while control fish showed significantly lower levels of ω6 fatty acids. Increased levels of 18:2ω6 and 18:1ω9 and decreased levels of 22:6ω3 are promising candidates for biomarkers to study the influence of fish farms on the local food web.

Acknowledgements

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