Incorporation and modification of dietary fatty acids in gill polar lipids by two bivalve species *Crassostrea gigas* and *Ruditapes philippinarum*

Maryse Delaporte\(^a\), Philippe Soudant\(^b\,*\), Jeanne Moal\(^a\), Edouard Kraffe\(^c\), Yanic Marty\(^c\), Jean-François Samain\(^a\)

\(^a\)Laboratoire de Physiologie des Invertébrés, IFREMER de Brest, 29280 Plouzané, France

\(^b\)UMR CNRS- 6539, IUEM, Université de Bretagne Occidentale, 29280 Plouzané, France

\(^c\)UMR CNRS 6521, Université de Bretagne Occidentale CS93837, 29238 Brest Cedex 3, France

Received 21 October 2004; received in revised form 12 February 2005; accepted 15 February 2005

**Abstract**

Two bivalve species *Crassostrea gigas* and *Ruditapes philippinarum* were fed eight weeks with three mono-specific algae diets: *T-Isochrysis galbana*, *Tetraselmis suecica*, *Chaetoceros calcitrans*, selected on the basis of their polyunsaturated fatty acid (PUFA) composition. The incorporation and the modification of dietary fatty acids in *C. gigas* and *R. philippinarum* gill lipids were analysed and compared. Essential PUFA (20:4n-6, 20:5n-3 and 22:6n-3) and non-methylene interrupted PUFAs (known to be synthesised from monounsaturated precursors) contents of gill polar lipid of both species were greatly influenced by the dietary conditioning. Interestingly, oysters and clams responded differentially to the mono-specific diets. Oysters maintained higher 20:5n-3 level and higher 22:2\(\alpha/22:\beta\) and n-7/n-9 ratio in gill polar lipids than clams. To better discriminate dietary and species influences on the fatty acid composition, a Principal Component Analysis followed by a MANOVA on the two most explicative components was performed. These statistical analyses showed that difference in fatty acid compositions attributable to species were just as significant as the diet inputs. The differences of gill fatty acid compositions between oysters and clams are speculated to result of an intrinsic species characteristic and perhaps of a group characteristic: Fillibranch vs. Eulamellibranch.

© 2005 Elsevier Inc. All rights reserved.

**Keywords:** Bivalves; Clams; Fatty acids; Molluscs; Nutrition; Oysters; Regulation

1. **Introduction**

Several studies about bivalve nutrition have established that the quality of micro-algae diet affects the growth and development of bivalve molluscs such as the King scallop *Pecten maximus* (Delaunay et al., 1993; Soudant et al., 1996a, 1998), the Pacific oyster *Crassostrea gigas* (Knauer and Southgate, 1997; Soudant et al., 1999; 2000; Pennarun et al., 2003), the European flat oyster *Ostrea edulis* (Berntsson et al., 1997), the Chilean scallop *Argopecten purpuratus* (Farias et al., 2003), the Sea scallop *Placopecten magellanicus* (Pernet and Tremblay, 2004), the Carpet shell clam *Ruditapes decussatus* (Albertosa et al., 1996; Fernandez-Reiriz et al., 1998, 1999) and the Manila clam *Ruditapes philippinarum* (Caers et al., 1999a). The nutritive value of microalgae seems to depend at least partially upon the presence of essential polyunsaturated fatty acids (PUFAs) especially arachidonic acid (AA, 20:4n-6), eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3). Indeed, bivalves have a very limited or no capability to synthesise PUFAs (De Moreno et al., 2004). Nevertheless, bivalves have the capabilities of de novo synthesis for some peculiar fatty acids (FAs) called...
non-methylene interrupted fatty acid (NMIs) (Zhukova,
1986, 1991). NMIs can account for up to 15% of fatty
acids in polar lipids of bivalves (Zhukova and Svetashev,
1986), but they were often overlooked in nutrition studies
and occasionally mis-identified. The most abundant NMIs
are those constituted with 22 carbons: the 22:2Δ7,13 (or
22:2i) and 22:2Δ7,15 (or 22:2j) and their functions are not
well understood. It has been suggested that NMIs have a
functional and structural role in membranes (Kraffe De
Laubarede, 2003; Kraffe et al., 2004) and may also
substitute for essential fatty acids such as 20:4n-6, 20:5n-
3 and 22:6n-3 (Ackman and Hooper, 1973; Klingensmith,
1982). Also, as the precursors/intermediates for NMI
synthesis are monoenic acids 16:1, 18:1 and 20:1n-7 for
the j series NMIs and 18:1 and 20:1n-9 for the i series
NMIs (Zhukova, 1986, 1991), one may expect NMI
proportions to depend on the dietary supply of their
precursors, considering that 16:1n-7 and 18:1n-9 are
abundant in diatoms and flagellates, respectively (Volkman
et al., 1989). However, in most bivalve nutrition studies
cited above, dietary effects on NMI FA composition were
not or poorly discussed.

To date, it is unclear whether there are common
responses in bivalves to dietary changes or whether there
are specific responses relating to their feeding behaviour
and/or phylogenetic belonging. Oysters live intertidally,
generally attached on hard substrata and belong to the
Filibranch group. Clams are soft bottom burrowers using
two siphons to feed on and belong to the Eulamellibranch
group (Ruppert and Barnes, 1996). These two groups
developed specific gill structure in relation to their
habitats. These difference of gill structure are likely
associated to specific membrane properties. Phospholipids
are the major component of biological membranes and are!important determinants of membrane properties. Phospho-
lipid composition and metabolism of bivalve gills are
thought to be involved in their adaptation to changes of
environmental factors such as salinity or temperature
(Chapelle, 1987). Gillis and Ballantyne (1999) observed
that subzero thermal acclimation of Crassostrea virginica
and Mercenaria mercenaria resulted in changes of the
phospholipid and phospholipid FA composition of gill
mitochondrial membranes. Interestingly, the FA composi-
tion adjustments of their membrane phospholipids were
different between the two tested species. Modifications of
the FA composition of gill membrane may protect
membrane function from the effects of changes of the
physical properties of water. Indeed in fishes, such
modifications were demonstrated to affect gill activities
(Van Anholt et al., 2004a,b). Thus, dietary changes by
affecting the FA composition of membrane phospholipids
may also result in structural and functional modifications
in bivalve gills.

Because of the gill structure distinction and the specific
environmental adaptation of oysters and clams, it appeared
especially interesting to compare the impact of dietary
changes on their gill phospholipid FA compositions. Our
study aimed to report changes in FA composition of gill
phospholipids of C. gigas and R. philippinarum fed 8
weeks on mono-specific diets (T-Isochrysis galbana,
Tetraselmis suecica, Chaetoceros calcitrans), to compare
retention and modification of 22:6n-3, 20:5n-3, 20:4n-6
and synthesis of NMI fatty acids between the two species
in order to better discriminate species-specific and diet
effects on these FA compositions.

2. Materials and methods

2.1. Dietary conditioning and animal sampling

Juveniles of C. gigas were provided by a French
commercial hatchery (SATMAR, Lannilis, France) and
grown out in Aber Wrach (Finistère, France). Eighteen
month-old oysters reached an average live weight of 20 g.
Clams R. philippinarum were collected in the bay of
Marennes-Oléron (Charente, France) and weighted 10 g
on average. In March 2001, 900 oysters and 300 clams
were maintained in trays which were placed in a 700-L
tank equipped by a air–water lift. They were acclimatized
to experimental temperature 12 days prior to the dietary
conditioning treatment. Temperature was raised 1 °C per
day to a final temperature of 17 °C. During the
acclimatization period, oysters and clams were fed daily
a mixed diet of T-Iso (T-Isochrysis aff. galbana, clone
Tahitian) and C. calcitrans. After the acclimatization
period, oysters and clams were divided randomly and
distributed into three 700-L tanks (290 oysters and 85
clams placed in three trays per tank). Oysters and clams
were fed three mono-algal diets for 2 months. The micro-
algal species were selected on the basis of their
polyunsaturated fatty acid (PUFA) composition: C. calcitrans
which is rich in 20:4n-6 and deficient in 22:6n-3 in comparison with the two other diets, T-Iso
which is rich in 22:6n-3 and deficient in 20:5n-3 and
20:4n-6, and T. suecica which is deficient in 22:6n-3, and
contains only small amounts of 20:5n-3 and 20:4n-6
(Table 1). The daily algal supply was established to
provide an equivalent dry weight (DW) of each alga by
counting algal cell before diet distribution: i.e. 0.6 × 10^9
cells/animal/24 h of C. calcitrans and T-Iso, and
0.06 × 10^9 cells/animal/24 h of T. suecica (i.e. above
6% of algal dry weight per oyster dry weight). The
feeding conditions were maintained constant as total
biomass per tank did not increase during the experiment
(low animal growth and animal number reduction by
sampling). Tanks and animals were cleaned weekly.
Before sampling, animals were maintained unfed for 24
h. This protocol was adopted to provide sufficient time
to remove residual algae from the gills. Three pools of gills
from five animals were used for fatty acid analysis at the
initial point and after 4 weeks and 8 weeks of condition-

M. Delaporte et al. / Comparative Biochemistry and Physiology, Part A 140 (2005) 460–470
Table 1

Fatty acid composition of algae used in the experiment, expressed as weight percentage of total fatty acids in microalgae lipids (n = 3, mean ± S.D.)

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>C. calcitrans</th>
<th>T. suecica</th>
<th>T-Iso</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>23 ± 2.6</td>
<td>0.5 ± 0.1</td>
<td>26.5 ± 3.0</td>
</tr>
<tr>
<td>16:0</td>
<td>5.7 ± 1.5</td>
<td>24.9 ± 3.6</td>
<td>11.2 ± 2.5</td>
</tr>
<tr>
<td>18:0</td>
<td>0.5 ± 0.1</td>
<td>0.6 ± 0.1</td>
<td>0.5 ± 0.2</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>20.6 ± 2.5</td>
<td>0.1 ± 0.3</td>
<td>5.5 ± 1.8</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>0.6 ± 0.1</td>
<td>13.8 ± 7.6</td>
<td>11.2 ± 1.8</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>0.5 ± 0.1</td>
<td>1.5 ± 0.3</td>
<td>0.7 ± 0.2</td>
</tr>
<tr>
<td>16:2n-7</td>
<td>1.9 ± 2.5</td>
<td>0.1 ± 0.2</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>16:3n-4</td>
<td>10 ± 1.7</td>
<td></td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.7 ± 0.1</td>
<td>5.7 ± 1.2</td>
<td>7.1 ± 2.7</td>
</tr>
<tr>
<td>18:3n-3</td>
<td></td>
<td>10.5 ± 0.9</td>
<td>5.7 ± 0.9</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.7 ± 0.2</td>
<td>10.7 ± 3.3</td>
<td>12.5 ± 0.9</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>2 ± 0.5</td>
<td>0.5 ± 0.1</td>
<td>0.1 ± 0.0</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>17.8 ± 2.7</td>
<td>5.4 ± 1.1</td>
<td>0.4 ± 0.1</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>0.1 ± 0.1</td>
<td>1.5 ± 2.0</td>
<td>2.1 ± 0.4</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>0.1 ± 0.0</td>
<td>0.1 ± 0.1</td>
<td>0.1 ± 0.1</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>1.3 ± 0.2</td>
<td>0.1 ± 0.0</td>
<td>7.8 ± 0.9</td>
</tr>
<tr>
<td>TO. SAFA</td>
<td>30.1 ± 3.4</td>
<td>26.2 ± 3.8</td>
<td>39.3 ± 4.8</td>
</tr>
<tr>
<td>TO. MUFA</td>
<td>25.7 ± 2.3</td>
<td>20.1 ± 8.3</td>
<td>18.6 ± 2.9</td>
</tr>
<tr>
<td>TO. PUFA</td>
<td>40.3 ± 4.8</td>
<td>53.3 ± 8.1</td>
<td>41.1 ± 3.2</td>
</tr>
<tr>
<td>n-3n-6</td>
<td>5.6 ± 1.2</td>
<td>4.2 ± 0.6</td>
<td>3.0 ± 1.1</td>
</tr>
</tbody>
</table>

TO. SAFA: total saturated fatty acids; TO. MUFA: total monounsaturated fatty acids; TO. PUFA: total polyunsaturated fatty acids.

ing for both species and each dietary treatment. Gills were dissected, pooled, weighted and frozen in liquid nitrogen for later fatty acid analysis. Fifteen other animals were sampled for flesh and shell wet weight measurements in order to assess oyster and clam condition index at the beginning and at the end of the dietary conditioning. The condition index was calculated according to the formula: (flesh wet weight/shell weight) × 100.

2.2. Lipid extraction of algae and gill for fatty acid analysis

2.2.1. Algae

Algal samples for each diet were filtered onto pre-ignited (450 °C) GF/F filters. The filters were then placed in tubes containing 3 mL of a chloroform-methanol mixture (2:1, v:v) and frozen at −20 °C under nitrogen. Fatty acid analyses were performed on total lipids.

2.2.2. Gills

For the lipid extraction, pooled gills were ground with a Dangoumeau homogeniser at −180 °C. Around 300 mg of the ground tissue was precisely weighted and was transferred to a tube containing 6 mL of chloroform–methanol mixture (2:1, v:v). After centrifugation, lipid extract was transferred into a clean tube, sealed under nitrogen and stored at −20 °C for later lipid analysis.

2.3. Neutral and polar lipid separation

Neutral and polar lipids were separated on a Silica gel micro-column according to Marty et al. (1992). Briefly, an aliquot of gill lipid extract was evaporated to dryness and recovered with three washings of 500 µL each of chloroform–methanol mixture (98:2, v:v). This was placed on top of a silica gel microcolumn [30 × 5 mm I.D. Kieselgel, 70–230 mesh (Merck), previously heated to 450 °C and deactivated with 5 wt.% water]. The neutral lipids were first eluted with 10 mL of chloroform–methanol mixture (98:2, v:v). The polar lipids were then recovered with 15 mL of methanol. A known amount of 23:0 fatty acid, as internal standard, was added in neutral and polar fraction. Both fractions were evaporated to dryness, re-suspended with 1 mL of a chloroform–methanol mixture (2:1, v:v) and stored under nitrogen atmosphere at −20 °C until analysis.

2.4. Fatty acid analysis

After evaporation to dryness, lipid extract or fraction was trans-esterified with 10% BF₃ (w/w) in methanol for 15 min at 95–100 °C (Metcalfe and Schmitz, 1961). After cooling, the fatty acid methyl esters (FAMEs) were extracted with hexane according to the method described by Marty et al. (1992). Separation of FAMEs was carried out on a GC (HP 6890) equipped with a flame ionization detector, an on-column injector and a DBWAX capillary column (J & W, 25 m × 0.32 mm; 0.2 µm film thickness). The column was temperature programmed from 60 to 150 °C at 30 °C/min and 150 to 220 °C at 2 °C/min. Hydrogen was used as the carrier gas at 2.0 mL/min. Identification of FAMEs was based on the comparison of their retention times with those of authentic standards and confirmed by gas liquid chromatograph-mass spectrometry (GC-MS). Non-methylene interrupted (NMI) polyunsaturated fatty acids (PUFAs) 20:2Δ5,11, 22:2Δ7,13, 20:2Δ5,11 and 22:2Δ7,15 were respectively designated 20:2i, 22:2i, 20:2j and 22:2j. Fatty acid peaks were integrated and analysed using HP chemstation software. Total fatty acid content (polar+neutral lipids) per gill was expressed as mg of FAME per g of ground tissue wet weight. Fatty acid composition was expressed as weight percent of the total fatty acids of each fraction.

2.5. Statistical analysis

To compare the differences between species and the dietary conditioning, T-tests and one-way analysis of variance (ANOVA) were respectively performed using Statview (SAS institute Inc., Cary, NC, USA). Percentage data were transformed (arcsin of the square root) before ANOVA and T-test, but are presented in figure and table as untransformed percentage values. Differences were considered statistically significant if p ≤0.05.

Principal Component Analysis (PCA), followed with a MANOVA, was used to characterise relationships between FA variables and to discriminate FA profiles according to dietary conditioning and bivalve species.
3. Results

3.1. Composition of the mono-specific-algal diet

The fatty acid profile of the mono-specific algal diets was determined during the dietary conditioning (Table 1). C. calcitrans was characterised by a high proportion of 14:0 (23%), 16:1n-7 (20.6%) and 20:5n-3 (17.8%) (Table 1). C. calcitrans had also the highest proportion of 20:4n-6 (2%) in comparison with the two other microalgal diets. T. suecica presented a different FA profile with high proportions of 16:0 (24.9%), 18:1n-9 (13.8%), 18:3n-3 (10.5%) and 18:4n-3 (10.7%), small proportions of 20:5n-3 (5.4%) and traces of 20:4n-6 and 22:6n-3 (0.5% and 0.1%, respectively). The third microalgae tested, T-Iso, provided a high proportion of 14:0 (26.5%), 16:0 (11.2%), 18:1n-9 (11.2%), 18:2n-6 (7.1%), 18:4n-3 (12.5%) and 22:5n-6 (2.1%). Its fatty acid profile was also characterised by a high proportion of 22:6n-3 (7.8%) and a very small proportion of 20:5n-3 (0.4%).

3.2. Condition index

After 8 weeks of dietary conditioning, oysters and clams maintained or increased their condition index (CI) when fed on C. calcitrans and T-Iso (p<0.05). CI of oysters fed C. calcitrans increased from 13.2 initially to 18.9, while stayed at the same level for those fed T-Iso (13.8). Similarly, CI of clams increased from 25.8 to 28.8 and 32.6 for C. calcitrans and T-Iso diets, respectively. However, T. suecica feeding...
resulted in a decrease of CI for both bivalve species \((p < 0.001)\). A significant decrease of CI from initial values to 11.1 and 21.6 was observed for oysters and clams, respectively.

3.3. Total fatty acids and polar vs. neutral lipid repartition in gills

At the beginning of the experiment, oysters and clams possess similar contents of fatty acids in their gills: 4 to 5.0 mg of lipids per g of gill tissue (Tables 2 and 3). In both species, polar lipids are the predominant lipid class in the gills. At the beginning of the experiment, oysters and clams have 86.1% and 95.7% of polar lipids, respectively. Although total fatty acid contents tended to be affected by the dietary conditioning, especially for oysters, the proportions of polar lipids, however, remained above 82% in oyster gills and above 94% in clam gills (Tables 2 and 3).

3.4. Impact of the dietary conditioning on the fatty acid composition of oyster and clam gills

The FA composition of polar lipids in oyster and clam gills was greatly influenced by dietary conditioning after 4 weeks and the dietary imprint was accentuated after 8 weeks (Tables 2 and 3). Indeed, most of the individual FAs were significantly affected by the dietary conditioning (ANOVA, \(p < 0.05\)). Among them, some FAs are well known to be important to bivalve development and membrane function.

### Table 3

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Initial</th>
<th>After 4 weeks of dietary conditioning</th>
<th>After 8 weeks of dietary conditioning</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>C. calcitans n=3, mean ± S.D.</td>
<td>T. suecica n=3, mean ± S.D.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>C. calcitans n=3, mean ± S.D.</td>
<td>T. suecica n=3, mean ± S.D.</td>
</tr>
<tr>
<td>20:1n-11</td>
<td>2.6 ± 0.0</td>
<td>4.5 ± 0.4**</td>
<td>3.9 ± 0.2**</td>
</tr>
<tr>
<td>18:1n-9</td>
<td>1.4 ± 0.1</td>
<td>1.1 ± 0.1**</td>
<td>3.0 ± 0.4**</td>
</tr>
<tr>
<td>20:1n-9</td>
<td>2.3 ± 0.1</td>
<td>1.7 ± 0.0**</td>
<td>3.4 ± 0.3**</td>
</tr>
<tr>
<td>20:2i</td>
<td>0.1 ± 0.0</td>
<td>0.3 ± 0.2*</td>
<td>0.2 ± 0.0**</td>
</tr>
<tr>
<td>22:2i</td>
<td>3.1 ± 0.2</td>
<td>2.4 ± 0.2**</td>
<td>4.3 ± 0.1**</td>
</tr>
<tr>
<td>16:1n-7</td>
<td>2.1 ± 0.2</td>
<td>3.6 ± 1.1**</td>
<td>1.5 ± 0.9b</td>
</tr>
<tr>
<td>18:1n-7</td>
<td>1.1 ± 0.0</td>
<td>1.5 ± 0.1a</td>
<td>1.5 ± 0.2a</td>
</tr>
<tr>
<td>20:1n-7</td>
<td>1.7 ± 0.2</td>
<td>2.6 ± 0.1**</td>
<td>1.6 ± 0.3**</td>
</tr>
<tr>
<td>20:2j</td>
<td>0.6 ± 0.2</td>
<td>0.8 ± 0.0**</td>
<td>0.9 ± 0.0**</td>
</tr>
<tr>
<td>22:2j</td>
<td>7.6 ± 0.0</td>
<td>8.4 ± 0.5**</td>
<td>7.9 ± 0.2**</td>
</tr>
<tr>
<td>18:2n-6</td>
<td>0.2 ± 0.0</td>
<td>0.2 ± 0.0**</td>
<td>0.4 ± 0.1**</td>
</tr>
<tr>
<td>18:3n-6</td>
<td>0.1 ± 0.0</td>
<td>0.2 ± 0.0**</td>
<td>0.0 ± 0.0**</td>
</tr>
<tr>
<td>20:2n-6</td>
<td>1.7 ± 0.1</td>
<td>1.6 ± 0.1**</td>
<td>2.2 ± 0.2a</td>
</tr>
<tr>
<td>20:3n-6</td>
<td>0.2 ± 0.0</td>
<td>0.3 ± 0.0**</td>
<td>0.2 ± 0.0**</td>
</tr>
<tr>
<td>20:4n-6</td>
<td>4.8 ± 0.1</td>
<td>6.4 ± 0.1**</td>
<td>5.7 ± 0.2**</td>
</tr>
<tr>
<td>22:4n-6</td>
<td>3.2 ± 0.1</td>
<td>3.7 ± 0.2**</td>
<td>3.6 ± 0.2**</td>
</tr>
<tr>
<td>22:5n-6</td>
<td>2.0 ± 0.1</td>
<td>1.8 ± 0.0**</td>
<td>2.1 ± 0.1**</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>0.3 ± 0.0</td>
<td>0.2 ± 0.0**</td>
<td>1.1 ± 0.4**</td>
</tr>
<tr>
<td>18:4n-3</td>
<td>0.5 ± 0.1</td>
<td>0.3 ± 0.0**</td>
<td>0.6 ± 0.3b</td>
</tr>
<tr>
<td>20:4n-3</td>
<td>0.3 ± 0.0</td>
<td>0.1 ± 0.0**</td>
<td>0.4 ± 0.1b</td>
</tr>
<tr>
<td>20:5n-3</td>
<td>6.2 ± 0.3</td>
<td>9.7 ± 0.3**</td>
<td>6.1 ± 0.77</td>
</tr>
<tr>
<td>22:5n-3</td>
<td>3.2 ± 0.2</td>
<td>2.6 ± 0.1**</td>
<td>2.9 ± 0.1**</td>
</tr>
<tr>
<td>22:6n-3</td>
<td>26.3 ± 0.7</td>
<td>17.4 ± 1.8**</td>
<td>20.7 ± 1.9b</td>
</tr>
<tr>
<td>TO. SAFA</td>
<td>19.8 ± 1.4</td>
<td>20.5 ± 0.4**</td>
<td>18.7 ± 0.5**</td>
</tr>
<tr>
<td>TO. MUFA</td>
<td>15.6 ± 0.6</td>
<td>18.4 ± 1.8**</td>
<td>16.3 ± 1.8**</td>
</tr>
<tr>
<td>TO. PUFA</td>
<td>64.6 ± 1.2</td>
<td>59.4 ± 2.2**</td>
<td>63.0 ± 2.2a</td>
</tr>
<tr>
<td>TO. (n-6)</td>
<td>12.2 ± 0.2</td>
<td>14.1 ± 0.2**</td>
<td>14.1 ± 0.5**</td>
</tr>
<tr>
<td>TO. (n-3)</td>
<td>40.9 ± 0.9</td>
<td>33.1 ± 2.2**</td>
<td>35.4 ± 2.2**</td>
</tr>
<tr>
<td>TO. NMIs</td>
<td>11.3 ± 0.2</td>
<td>11.9 ± 0.8**</td>
<td>13.3 ± 0.1**</td>
</tr>
<tr>
<td>Total FAs</td>
<td>4.2 ± 0.2</td>
<td>4.4 ± 0.2**</td>
<td>4.0 ± 0.3</td>
</tr>
<tr>
<td>% of polar lipids</td>
<td>95.7 ± 0.5</td>
<td>94.2 ± 1.1</td>
<td>95.0 ± 0.6</td>
</tr>
</tbody>
</table>

Different lower-case letters indicate significant differences between dietary treatments (ANOVA \(p < 0.05\)). Asterisks indicate significant differences with the initial composition (\(T_{-}Test\) \(p < 0.05\)).

TO. SAFA: total saturated fatty acids; TO. MUFA: total monounsaturated fatty acids; TO. PUFA: total polyunsaturated fatty acids; TO. NMIs: total non-methylene interrupted fatty acids; 20:2i=20:2Δ5,11; 22:2i=22:2Δ7,13; 20:Δ4=20:2Δ5,13; 22:2i=22:2Δ7,15; WW: wet weight; Branched fatty acids and minor identified fatty acids (16:1n-5, 18:1n-5, 18:2n-4, 18:3n-4, 21:4n-6 and 21:5n-3) are not listed in the table but contribute to the total fatty acid content.
ing and thus more details on their specific incorporation and synthesis according to diet and bivalve species are provided in the following paragraphs: (1) changes in 22:6n-3, 20:5n-3 and 20:4n-6; (2) changes of the 20–22 NMIs and their dietary precursors.

3.4.1. Changes in 22:6n-3, 20:5n-3 and 20:4n-6 in oyster and clam gills

The fatty acid profiles of gill polar lipids of both bivalve species fed C. calcitrans presented the highest content of 20:5n-3 and 20:4n-6 (Tables 2 and 3). Proportions of these two PUFAs were either maintained or greatly increased when compared to their initial values. Concomitantly, the deficiency in 22:6n-3 of C. calcitrans led to a significant decrease (around 50%) of 22:6n-3 in gill polar lipids of both species. Fatty acid profiles of oysters and clams fed T-Iso diet showed an inverse pattern of fatty acid changes. The 22:6n-3 contents of oyster and clam gills were maintained at the initial value whereas T-Iso deficiency in 20:5n-3 and 20:4n-6 led to a significant decrease of these fatty acids in gill polar lipids of both species. The loss of 20:4n-6 in gill polar lipids represented 20% of their initial value for both species while the decrease of 20:5n-3 was more important and represented 70% and 80% of the initial value for oysters and clams, respectively (Tables 2 and 3). Finally, oysters and clams fed T. suecica presented an intermediate level of 20:5n-3, 20:4n-6 and 22:6n-3 content in gill polar lipids.

For the 20–22 carbons with more than 3 double bounds, the most noticeable difference between clams and oysters concerned the 20:5n-3 percentage in gill polar lipids. Regardless of diet and the duration of conditioning, oysters always had a higher 20:5n-3 percentage, ranging from 3.6% to 18.3%, than clams, for which it ranged from 1.4% to 9.7%. Consequently, the 22:6n-3/20:5n-3 ratio was significantly higher for clams than it was in oysters, as both species have similar proportions of 22:6n-3.

3.4.2. Changes in 20–22 NMIs and their dietary precursors in oyster clam gills

20–22 NMIs accounted for a fairly high proportion of the PUFA in gill polar lipids of oysters and clams. When considering all dietary treatments, total NMI was quite stable and ranged from 9.8 to 12.0% in oysters but fluctuated more in clams in which the total NMI varied from 8.6 to 12.8% (Tables 2 and 3). 22:2j and 22:2i were the predominant NMIs in gill polar lipids of both species. Considering 22:2 NMIs isomers individually, dietary conditioning differently affected their relative proportions. Feeding T. suecica and T-Iso resulted in an increase of the 22:2i NMI from 1.5% to above 3.0% in oysters and from 3.1% to 4.8% in clams after 8 weeks of conditioning. In contrast, feeding C. calcitrans led to a decrease of the 22:2i NMI content from 1.5% to 0.6% in oysters and from 3.0% to 1.7% in clams. Meanwhile, oysters fed C. calcitrans for 8 weeks showed a 30% increase of 22:2j (10.6% at the end of the experiment) while clams fed the same diet did not show any changes of 22:2j content. Oysters fed T. suecica and T-Iso maintained their 22:2j content at 7.5% and 6.9% similar to the initial level (7.5%). However, this NMI was observed to decrease by 50% in clams fed T-Iso and was maintained near its initial value when clams were fed T. suecica. Interestingly, 22:2i content was always higher in clams than in oysters regardless of dietary treatment and duration of conditioning while the 22:2j content in oysters was equivalent to that of clams or higher. Consequently, the ratio NMIj / NMIi was consistently higher in oysters than in clams (Fig. 1A).

It is now well established that n-7 FA series (16:1, 18:1, and 20:1) and n-9 FA series (18:1 and 20:1) are the monounsaturated precursors for de novo synthesis of the j and i NMI series, respectively (Zhukova, 1986, 1991). Their proportions changed dramatically between hatchery cultivated micro-algae species. When clams and oysters were fed T-Iso or T. suecica, n-9 rich diets, their content in 18:1n-9 and 20:1n-9 increased by about 1.5–2-fold after 8 weeks of conditioning. In contrast, 16:1n-7, 18:1n-7 and 20:1n-7 contents rose significantly in oysters and clams fed C. calcitrans, an n-7 rich diet. Regardless of dietary condition-
levels of n-7 FA were always higher in oysters than in clams while n-9 FAs were found in higher proportions in clams than in oysters. Thus, the n-7/n-9 ratio was consistently higher in oysters than in clams (Fig. 1B) and paralleled the NMI j/i ratio (Fig. 1A,B).

3.5. Principal Component Analysis (PCA) of FA compositions

As most of the individual FAs were significantly affected (ANOVA, $p < 0.05$) by the dietary conditioning for both species, multivariate analysis was applied to all the FA data to facilitate the interpretation of the results according to dietary composition and bivalve species.

PCA analysis examined 41 cases including FA compositions of gill polar lipids from oysters and clams experiencing dietary treatments for 4 and 8 weeks (24 and 23 cases, respectively) as well as those at the beginning of the experiment (6 cases). The percentage composition of 20 variables was entered into the model: 20:1n-11, 18:1n-9, 20:1n-9, 22:2i, 16:1n-7, 18:1n-7, 20:1n-7, 22:2j, 18:2n-6, 18:3n-6, 20:2n-6, 20:4n-6, 22:4n-6, 22:5n-6, 18:3n-3, 18:4n-3, 20:4n-3, 20:5n-3, 22:5n-3, 22:6n-3.

This analysis generated a small number of linear combinations of the 20 FA variables and extracted only 4 components with eigenvalues higher than 1.0. These components explained 88.3% of the variability in the original data. The first principal component (PC1) explained 47.3% of the combined variance, the second component (PC2) 24.6%, the third 10.7% and the fourth 5.6%. A correlation circle was plotted using PC1 and PC2 as axes in order to separate the individual data according to species and dietary treatments (Fig. 2). This representation showed that the 20 variables are well explained (72%) with these combined variables. When the samples were identified on the biplot of PC1 and PC2 (the most significant combined variables), eight groups can be formed according to the combinations of species and dietary treatment (Fig. 3). This
representation distinguished the two species: oysters on the top left and clams on the bottom right and 4 groups within each species: 3 groups corresponding to the three mono-specific dietary treatments: T-Iso, T. suecica and C. calcitrans and one group corresponding to the FA profile at the initiation of the experiment (named ‘natural’).

As PC1 and 2 represented the strongest correlation (positive and negative) between FA variables, a MANOVA with species and diets as the independent variables and PC1 and PC2 as the dependent variables was applied. The analysis showed that differences in FA profiles attributable to species ($p<0.001$) and diet composition ($p<0.001$) were highly significant (Fig. 4A,B) for both principal components.

4. Discussion

Several studies have reported that dietary quality affects the fatty acid composition of different bivalves, including C. gigas (Pazos et al., 1996; Knauer and Southgate, 1997; Linehan et al., 1999; Soudant et al., 1999), O. edulis (Abad et al., 1995; Berntsson et al., 1997), R. philippinarum (Caers et al., 1999a) and R. decussatus (Albentosa et al., 1996; Fernandez-Reiriz et al., 1998, 1999). However, although these species respond in a similar way to dietary changes, their ability to regulate and modify the FA dietary inputs is difficult to compare. Effectively, various parameters differed among these studies: conditioning temperature, experiment duration, experimental facilities and lipid separation. The present study aimed to examine the changes of gill phospholipid fatty acid compositions in two species C. gigas and R. philippinarum experiencing 3 mono-specific diets (T-Iso, T. suecica, C. calcitrans) under same experimental conditions, and then to compare the retention and modification of dietary and synthesised PUFAs between the two species.

In both species, fatty acids of gills were found predominately (82 to 96%) associated to the polar lipid fraction which is primarily composed of phospholipids in bivalves (Ackman, 1983). These results are in agreement with those obtained by Klingensmith (1982) on M. mercenaria and Chu et al. (2003) on C. virginica. The FA composition of this fraction is known to be tightly regulated when animals experienced different dietary treatments (Marty et al., 1992; Delaunay et al., 1993; Soudant et al., 1996b, 1997, 1998). The 22:6n-3, the 20:5n-3, the 20:4n-6 and the NMI 22 PUFAs are generally found in high proportion in this fraction and were found in this study to be the most strongly regulated.

It is generally agreed that these PUFAs derive mainly from the microalgae diet as bivalves have a limited or total incapability to synthesise them (De Moreno et al., 1976; Waldock and Holland, 1984). Thus, dietary conditioning with mono-specific micro-algae diet allowed for the comparison of the capabilities of oysters and clams to regulate and retain these dietary essential PUFAs and/or modify them (for example: 20:5n-3 and 20:4n-6 into 22:5n-3 and 22:4n-6 by elongation). The observed changes of 22:6n-3, 20:5n-3, and 20:4n-6 contents in gill polar lipids agreed with previous studies conducted on other tissues, as well as larvae and spat (Delaunay et al., 1993; Soudant et al., 1996a,b, 1997, 1999; Berntsson et al., 1997).

Levels of 20:5n-3 in gill polar lipids of animals fed mono-specific dietary treatments appeared to be species specific. Oysters fed C. calcitrans maintained their 20:5n-3 content in their gill polar lipids during the whole experiment (around 18%) while in clams after 4 weeks of dietary conditioning, the 20:5n-3 content reached a maximum value of 10%. This content did not increase further through the duration of the experiment. This suggests that clams can modulate the incorporation of 20:5n-3 even when the dietary supply of 20:5n-3 is high. Probably, the highest proportion of 20:5n-3 in gill clam polar lipids is about 10%. This value is similar to what Porteres (1991) and Caers et al. (1998) found in R. philippinarum spat undergoing dietary treatments. This differential behaviour between clams and oysters maybe also partially due to a lower initial content of
20:5n-3 in clams. When diets were deficient in 20:5n-3, its values fell to as low as 5.6% in oysters and 1.4% in clams fed T-Iso. Overall, oysters always showed a higher 20:5n-3 content than clams. Similarly, Gillis and Ballantyne (1999) also observed that the oyster C. virginica acclimated to either −1 or 12 °C contained two time more 20:5n-3 in their gill mitochondria than the clam M. mercenaria maintained in the same conditions. Such inter-specific differences may reflect specific requirements in relation to their natural biotopes and/or genetic evolutions.

Contrary to 20:5n-3, oysters and clams presented similar levels of 22:6n-3 and 20:4n-6 according to the dietary supply. Indeed when 22:6n-3 was relatively abundant in the diet (T-Iso), this essential PUFA appeared to reach a maximum in the polar lipids and it never went below 10% in oysters or 11% in clams fed C. calcitrans. Similarly, in regard to 20:4n-6 deficiency of T-Iso, both species seemed to maintain their 20:4n-6 content in gill polar lipids (above 4% of the total fatty acids for clams and above 6% for oysters). These PUFA were reported to be generally well preserved when animals are starved or fed a diet deficient in these compounds (Delamay et al., 1993; Coutteau et al., 1996; Soudant et al., 1996a,b).

The NMI fatty acids are the other PUFA found in fairly high proportions in the phospholipids of several bivalve species including oysters and clams and are regularly linked to structural and functional roles in membranes (Kraffe De Laubared, 2003; Kraffe et al., 2004). They are thought to be involved in membrane properties such as phase transition, membrane fluidity or activity of membrane bound proteins (Paradis and Ackman, 1977; Klingensmith, 1982; Kraffe et al., 2004). Interestingly, among organs, the highest proportion of NMI FAs was reported in gills (Klingensmith, 1982; Kraffe et al., 2004). As first proposed by Ackman and Hooper (1973) and demonstrated for the bivalve mollusks Scapharca broughtoni and Mytilus edulis by Zhukova (1986, 1991), NMI FAs are de novo synthesised by bivalves. The major precursors/intermediates for NMI synthesis are monoenoic acids 16:1, 18:1 and 20:1n-7 for the j series NMIs and 18:1 and 20:1n-9 for the i series NMIs (Zhukova, 1986, 1991). A C20 Δ5 desaturation converts the 20:1n-7 and 20:1n-9, respectively, into 20:2j (20:2Δ5,13) and 20:2i (20:2Δ5,11) which are finally elongated, respectively, into 22:2j (22:2Δ7,15) and 22:2i (22:2Δ7,13).

In the present study, results showed that the 22:2 NMI synthesis in both species was affected by the dietary conditioning. Oysters and clams fed C. calcitrans would have more monoenoic n-7 fatty acids available to produce extra 22:2j NMI. On the other hand, animals fed T-Iso would have more monoenoic n-9 fatty acids available in their diet to be converted in 22:2i NMI. It was also interesting to notice that both species differ in their capacity to synthesise NMI. Oysters seemed to use preferentially and/or more efficiently FA n-7 as compared to clams resulting in higher proportions of 20:2j and 22:2j when animals are fed on n-7 rich diets. Opposite trends were observed for the 20:2i and 22:2i as clams maintained a higher percentage of these FA and a lower NMJj/NMJi ratio than oysters. Indeed, feeding clams with T-Iso, a n-9 rich microalgae resulted in an inversion of the 22:2j vs. 22:2i distribution, with the 22:2i becoming the predominant NMI. At this point, it can be stated that NMJj and NMJi synthesis seemed not only to be regulated by the incorporation of their precursor but also to be species specific.

The difference in NMI i and j synthesis and retention capabilities between both species could be the result of a species differentiation of desaturase and elongase enzyme specificity in NMI biosynthesis between the clam R. philippinarum and the oysters C. gigas. Also, it is important to note that n-7/n-9 ratio was systematically higher in oysters than clams for all the dietary treatments. These differences in n-7 vs. n-9 may reflect specific precursor requirements for NMI synthesis. It is especially difficult to explain the physiological consequences of these observations as the functions of NMIs are far from being clearly understood. NMI FAs were supposed to confer resistance in tissues exposed most often to the environmental physico-chemical variations (Klingensmith, 1982) or against attack by microbial lipases (Paradis and Ackman, 1977). The high content of NMI FAs encountered in bivalve gills was also hypothesized to be related to the above functions (Kraffe et al., 2004). These authors also provided first evidence that NMIs were the predominant PUFA of the aminoplasmalegs (alkenyl-acyl-glycerophosphatidylserine and alkenyl-acyl-glycerophosphatidylethanolamine). The selective incorporation of ΣNMIs in aminoplasmalegs led the authors to hypothesize the existence of possible synergistic properties of NMIs and plasmalogen implicating them in biological membrane functions.

To better discriminate the species characteristic (FA requirement and FA metabolism) and dietary impact, a Principal Component Analysis (PCA) was performed. The PCA analysis allowed to obtain a small number of linear combinations of the 20 FA variables and to explain 72% of the variability with two components. The plot of component 1 and 2 weights combined to the application of a MANOVA on these two components clearly showed that difference in FA profiles attributable to species was as significant as the food inputs. This strongly suggests that appropriate statistical analysis is useful for comparisons of FA requirements and metabolism between species. Such multivariate analysis was previously used to describe seasonal changes of the FA profiles in particulate organic matter (Mayzaud et al., 1989; Galois et al., 1996) and to compare the FA profiles of different organs (Caers et al., 1999b) and of phospholipid classes (Duinker et al., 2004). Nevertheless, none of these studies applied MANOVA on the principal components to further evaluate the impact of biotic or abiotic factors on FA profiles. Other advanced statistical analysis such as stepwise multiple regression was also used by Freites et al. (2002) to assess the influence of various environmental factors on the
fatty acid composition of the Blue mussel *Mytilus gallo-provincialis* from sub-tidal or rocky shores.

Such statistical approaches could be of interest to scientists intending to better characterise food inputs of bivalves from natural populations. For example, applying PCA analysis on FA profiles of bivalves may help to determine whether the animal had previously fed on diatoms or flagellates.

In conclusion, when clams and oysters were conditioned with mono-specific diets, variations of their fatty acid composition in gill polar lipid fractions were observed. Although a similar dietary imprint was observed for both species, the FA composition of the gills appeared to be species specific. Oysters always had higher 20:5n-3 content in gill polar lipids and higher 22:2n-11 and n-7/n-9 ratio than clams. These differences in the gill lipid profiles are speculated to be an intrinsic species characteristics and perhaps a phylogenetic group characteristic: Filibranch vs. Eulamllellibranch. Further studies are needed to validate this phylogenetic hypothesis by comparing the lipid compositions of more species belonging to these two groups.

**Acknowledgements**

The authors would like to thank Dr Eric Lund, Virginia Institute of Marine Science, College of William and Mary, for the critical review of this manuscript. This work was supported by a grant from IFREMER (Brest, France) and for the critical review of this manuscript. This work was supported by a grant from IFREMER (Brest, France) and for the critical review of this manuscript.

**References**


