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# Lipid classes and fatty acid composition in two parasitic copepods *Peroderma cylindricum* and *Lernaeocera lusci* and their respective fish hosts *Sardina pilchardus* and *Merluccius merluccius* from the Tunisian waters

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**SUMMARY:** The present study investigates the detailed lipid classes and their fatty acid (FA) compositions from two parasitic copepods *Lernaeocera lusci* and *Peroderma cylindricum* and their respective fish host species *Merluccius merluccius* and *Sardina pilchardus*. The lipid classes, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylserine (PS), phosphatidylinositol (PI), triacylglycerol (TAG), wax ester/cholesterol ester (WE/CE), mono-diacylglycerol (MDG), and free fatty acids (FFA) were separated by thin layer chromatography. The results revealed that TAG and PC were the major lipid classes in parasites; while WE/CE and PS were the most abundant in hosts. As for FA composition, C16:0, C18:0, C18:1n-9, C20:5n-3, and C22:6n-3 were recurrently found to be dominant in all lipid classes of the different organisms studied. However, some differences concerning the abundance and the distribution of several FAs were observed. Overall, the obtained results highlighted that despite the quite strong trophic connection between the parasites and their respective hosts, the parasites could be distinguished by specific lipid profiles.

***KEYWORDS:*** *Copepods; Fatty acid; Hake; Lipid classes; Parasite; Sardine.*

***RESUMEN: Clases de lípidos y composición de ácidos grasos en dos copépodos parásitos* Peroderma cylindricum *y* Lernaeocera lusci *y sus respectivos peces hospedadores* Sardina pilchardus *y* Merluccius merluccius *de aguas tunecinas.*** El presente estudio investiga en detalle las clases de lípidos y sus composiciones de ácidos grasos (AG) de dos copépodos parásitos *Lernaeocera lusci* y *Peroderma cylindricum* y sus respectivas especies de peces hospedadores *Merluccius merluccius* y *Sardina pilchardus*. Las clases de lípidos incluyen fosfatidilcolina (FC), fosfatidiletanolamina (FE), fosfatidilserina (FS), fosfatidilinositol (FI), triacilgliceroles (TAG), ceras/ésteres de colesterol (C/EC), mono-diacilglicerol (MDG) y ácidos grasos libres (AGL), que fueron separados mediante cromatografía en capa fina. Los resultados mostraron que TAG y FC eran las principales clases de lípidos en los parásitos, mientras que C/EC y FS eran las más abundantes en los hospedadores. En cuanto a la composición de AG, se encontró de forma recurrente que C16:0, C18:0, C18:1n-9, C20:5n-3 y C22:6n-3 eran dominantes en todas las clases de lípidos de los diferentes organismos estudiados. Sin embargo, se observaron algunas diferencias en cuanto a la abundancia y distribución de varios AGs. En general, los resultados obtenidos destacaron que a pesar de la fuerte conexión trófica entre los parásitos y sus respectivos hospedadores, los parásitos podían distinguirse por perfiles de lípidos específicos.

***PALABRAS CLAVE:*** *Ácidos grasos; Clases de lípidos; Copépodos; Merluza; Parásito; Sardina.*

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## 1. INTRODUCTION

*Peroderma cylindricum* (Heller, 1865) and *Lernaeocera lusci* (Bassett-Smith, 1896) are two common parasitic copepods belonging to the Pennellidae family which infect several marine fish. These parasites have complex and heteroxenous cycles that comprise different larval stages (Brooker *et al*., 2007). While the life cycle of *L. lusci* is well established, that of *P. cylindricum* remains only partially elucidated. The typical host species of *L. lusci* in the Tunisian coastal area are the sole *Solea solea* (intermediate host) and the European hake *Merluccius merluccius* is the definitive host (Kabata, 1979). Once passing through copepodid and chalimus developmental stages, *L. lusci* males and females attain maturity on the gills of their intermediate host. After copulation, the females leave the sole and swim actively to infect the definitive host where they embed deeply into the gill arches and continue to develop (Kabata, 1979). As for *P. cylindricum,* only the adult metamorphosed female embedded into pilchard *Sardina pilchardus* (definitive host) is known to date. It was reported that *P. cylindricum* inserts its holdfast into the pilchard's kidney and spine while its genital segment, producing two egg strings, emerges on the exterior (Becheikh *et al*., 1997). As a result of their attachment and feeding, these two hematophagous parasites *P. cylindricum* and *L. lusci* can affect the survival, physiology and fitness of their hosts (van Damme *et al*., 1994; Hajji *et al.*, 1998). Particularly, these parasites were found to be able to inflect substantial alteration in their host's lipids (Hajji *et al*., 2015; Telahigue *et al.*, 2017; Telahigue *et al.*, 2019). However, little is known about their own lipid profiles.

Lipids and their building block fatty acids are fundamental components for animal health and survival. They are involved in a wide range of biological functions due to their complexity and structural diversity (Tracey *et al*., 2018). Neutral lipids, predominantly constituted by triacylglycerols (TAG) and wax esters (WE), have the primary function of energy storage and are widely related to the physiological status of the organism (Şen Özdemir *et al*., 2019). However, phospholipids and sterols serve as the principal structural constituents of cell membranes (Dufourc, 2008). They may also play significant roles in cell signaling pathways and serve as precursors to bioactive compounds such as steroid hormones (Tocher, 2003). The common phospholipid classes including phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylcholine (PC), and phosphatidylserine (PS), possess significant structural diversity and complexity which mainly depend on the type of fatty acid (length of the fatty acid chain and number of double bonds) which is incorporated (Şen Özdemir *et al*., 2019; Zhou *et al*., 2020).

Owing to their involvement in these diverse cellular and physiological processes, lipids are attractive targets for parasites and are recognized as key players in host-parasite interactions (Vallochi *et al*., 2018). It was reported that various pathogens such as obligate hematophagous and intracellular parasites are able to hijack host's lipids to their own benefit in order to modulate and escape the host response and to complete their own life cycle (O'Neal1 *et al*., 2020). Although some authors have reported that the fatty acid composition of parasites may largely reflect that of their hosts (Tocher *et al*., 2010), others have highlighted that several parasites may have their own specific fatty acid fingerprints (Taraschewski *et al*., 1995).

The main objective of this study was to illustrate the lipid profiles of two common parasitic copepods from the Mediterranean waters: *L. lusci* and *P. cylindricum*, and to investigate whether these parasites share common patterns with their respective hosts or whether they have their own specific profiles. For this purpose, the lipid class fatty acid (FA) compositions of the two parasitic copepods as well as those of their respective host fish were studied. For more relevance, the specific fixation sites of these parasites (i.e. *M. merluccius* Gills for *L. lusci* and *S. pilchardus* kidney for *P. cylindricum*) were considered in this work. The obtained results will help to better elucidate the trophic connections in the two studied parasite-host systems and to bring new comprehensive knowledges about some biochemical aspects of *L. lusci* and *P. cylindricum*, which remain hitherto poorly understood.

## 2. MATERIAL AND METHODS

### 2.1. Sample collection

A total of 243 specimens of *Sardina pilhcardus* (16.5±1.5 cm) and 276 specimens of *Merluccius*

*merluccius* (18.3±2.2 cm) were purchased from fishermen at the port of Bizerte (Northeast of Tunisia). The samples were directly transported to the laboratory in polystyrene ice-cooled boxes. All specimens were carefully examined for the presence of *P. cylindricum* and *L. lusci*. Specimens from the two parasite species (adult females with egg strings) as well as *M. merluccius* gills and *S. pilhcardus* kidney tissues were collected, weighed, and stored at -20 °C until analysis.

### 2.2. Total lipid extraction and lipid classes' separation

Total lipids from the whole body (including egg strings) of both *P. cylindricum* and *L. lusci* and their respective hosts' tissues were extracted according to the method of Folch *et al*. (1957) using a mixture of the chloroform-methanol (2:1 V/V) solvent containing 0.01% butylated hydroxytoluene (BHT) as an antioxidant. An analysis of lipid classes was performed by one-dimensional double development high performance thin layer chromatography (HPTLC) as described by Olsen and Henderson (1996). Briefly, aliquots of 500 µl of lipid extracts were spotted onto a TLC plate (20x20 cm, silica gel 60, Merck, Germany), and hexane/diethyl ether/glacial acetic acid (80: 20: 2. V/V) was used as developing solvent system for the neutral lipid classes and methyl acetate/isopropanol/chloroform/methanol/0.25% KCl (25: 25: 25: 10: 9. V/V), for the polar lipid separation. Lipid fractions were visualized under UV light after spraying with 0.1% 2'-7' dichloro-fluorescein in absolute methanol. Eight lipid classes, including phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), triacylglycerol (TAG), wax ester/cholesterol ester (WE/CE), mono-diacylglycerol (MDG), and free fatty acids (FFA) were identified by comparison with known standards. Each lipid class band was then scraped from the TLC plates and subjected to transmethylation to yield fatty acid methyl esters (FAME) as given by Cecchi *et al*. (1985).

### 2.3. Fatty acid analysis

FAMEs were analyzed on a HP 6890 gas chromatograph with a split/splitless injector equipped with a flame ionization detector at 275 °C, and a 30 m HP Innowax capillary column with an internal diameter of 250 µm and a film thickness of 0.25 µm. The injector temperature was held at 250 °C. The oven temperature was programmed from 50 to 180 °C at a rate of 40 °C/min, then from 180 to 220 °C at 1.33 °C/min and to stabilize at 220 °C for 7 min. Nitrogen was the carrier gas. Methyl nonadecanoate 19:0 (Sigma) was added as internal standard. The identification of FAMEs was based on the comparison of their retention times with those of a mixture of methyl esters (SUPELCO PUFA-3 and Supelco 37 component FAME). Fatty acid peaks were integrated and analyzed using HP chemstation software.

### 2.4. Statistical analysis

The R software version 4.0.2. (R Core Team, 2020) was used to carry out statistical analyses. The normality of data distribution and homogeneity of variance were evaluated using the Shapiro-Wilk test and Levene's test, respectively. One-way analysis of variance (ANOVA) followed by the Bonferroni test were performed to check the significant differences between fatty acid amounts of lipid classes from the two studied parasites. The Bonferroni conservative correction method adjusts p values because of the increased risk in a type I error (significance level). Additionally, the hierarchical clustering analysis (HCA) was made by "FactoMineR" R package (Zhao *et al*., 2014) using Ward's method.

## 3. RESULTS

### 3.1. Lipid classes and their fatty acid composition from *Lernaeocera lusci* and its host *Merluccius merluccius*

The percentages of each lipid class in relation to total lipid content in the whole body of *L. lusci* and in the gills of *M. merluccius* are presented in Figures 1 and 2, respectively. TAG was the major lipid class in *L. lusci*, constituting ~46% of total lipids followed by PC (with ~26%) and PE (with ~11%). WE/CE was found to represent around 7%, while PI and MDG were found in lesser amounts (~1.5%) (Figure 1). As for the host, it was found that WE/CE was by far the most dominant lipid class (up to 40%) followed by TAG and PS, which constituted around 16% of the total lipids. However, PC and FFA were found to be minor constituents, each representing around 3% of the TL in *M. merluccius* gill tissue (Figure 2).

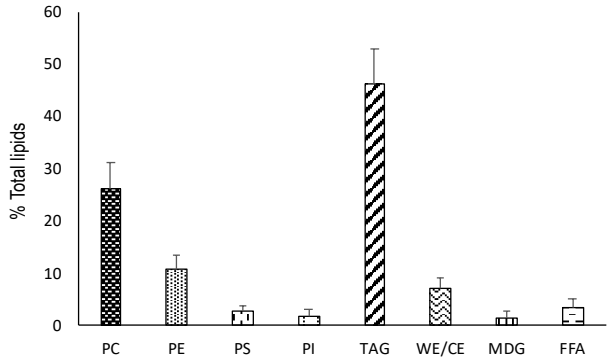


Figure 1. Lipid class composition (% of total lipids) of *Lernaeocera lusci.* The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

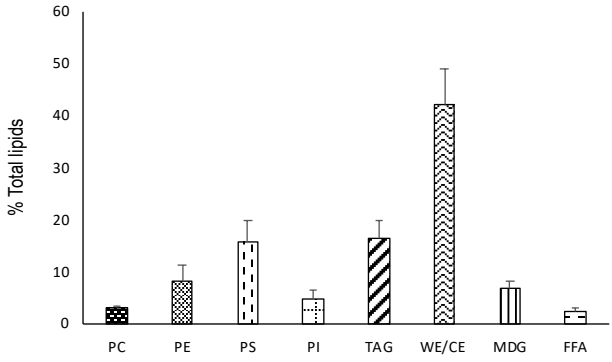


Figure 2. Lipid class composition (% of total lipids) of *Merluccius merluccius* gill tissue. The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

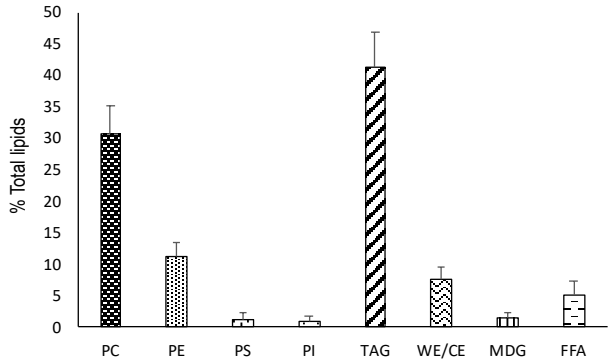


Figure 3. Lipid class composition (% of total lipids) of *Peroderma cylindricum.* The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

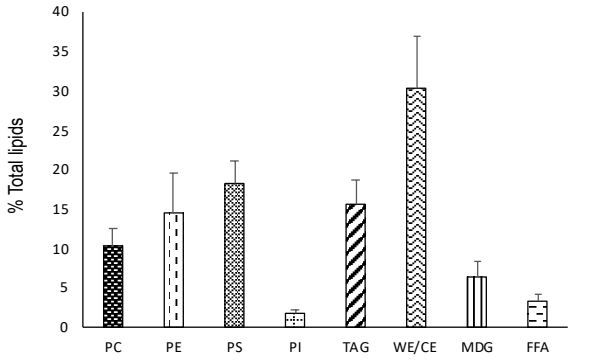


Figure 4. Lipid class composition (% of total lipids) of *Sardina pilchardus* kidney. The results were expressed as the means with error bar of triplicate analyses (n=3) performed on the pool of 9 samples. PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

The fatty acid composition of the various lipid classes isolated from *L. lusci* and its host *M. merluccius* are respectively given in Tables 1 and 2. Overall, our results revealed that all *L. lusci* lipid classes were dominated by saturated fatty acids (SFA), which accounted for about 50% of the total FA followed by polyunsaturated fatty acids (PUFA), ranging from 28.41 to 39.93% and monounsaturated fatty acids (MUFA) by about 15% (Figure 2). Among the 33 FA species identified, palmitic (C16:0), stearic acid (C18:0), oleic acid (C18:1n-9), eicosapentaenoic acid (C20:5n-3, EPA) and docosahexaenoic acid (C22:6n-3, DHA) were recurrently found to be major in all lipid classes. Among the phospholipid classes, PC had significantly ($p < 0.05$) higher proportions of C14:0 (9.97%) than the other lipid classes. Significantly high amounts of DHA were also recorded in PC and PI fractions. In addition, substantial amounts of C16:0, reaching 30.81 and 32.83% of the total FA were recorded for PI and PS ($p > 0.05$). As for neutral lipids, TAG and FFA appeared to be richer in PUFA, mainly in terms of EPA and DHA when compared to the other lipid classes. The WE/CE fraction was characterized by a higher amount

Table 1. Fatty acid composition of phospholipid and neutral lipid classes from the parasitic copepod *Lernaeocera lusci*. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

| %FA | Polar lipids | | | | Neutral lipids | | | |
|---|---|---|---|---|---|---|---|---|
| | PC | PE | PI | PS | TAG | WE/CE | MDG | FFA |
| C14:0 | 9.97±1.12$^{a}$ | 4.49±0.55$^{b}$ | 2.81±0.33$^{b}$ | 6.65±0.67$^{c}$ | 6.46±0.65$^{a}$ | 8.29±0.75$^{b}$ | 5.01±0.63$^{a}$ | 5.20±0.52$^{a}$ |
| C15:0 | 2.09±0.43$^{a}$ | 1.41±0.25$^{a}$ | 1.51±0.22$^{a}$ | 0.09±0.02$^{b}$ | 1.49±0.18$^{a}$ | 1.80±0.42$^{a}$ | 1.79±0.38$^{a}$ | 0.42±0.05$^{b}$ |
| C16:0 | 27.21±2.24$^{a}$ | 27.45±1.88$^{a}$ | 30.81±2.30$^{a}$ | 32.83±2.01$^{a}$ | 21.46±1.95$^{a}$ | 19.43±1.64$^{a}$ | 27.57±2.08$^{b}$ | 22.83±2.16$^{ab}$ |
| C17:0 | 0.84±0.11$^{a}$ | 2.56±0.44$^{b}$ | 2.12±0.25$^{bc}$ | 3.76±0.42$^{d}$ | 2.47±0.20$^{a}$ | 2.33±0.22$^{a}$ | 3.20±0.31$^{b}$ | 0.03±0.00$^{c}$ |
| C18:0 | 10.89±1.02$^{a}$ | 9.61±1.02$^{a}$ | 11.41±0.88$^{a}$ | 10.01±0.95$^{a}$ | 13.77±0.15$^{a}$ | 14.71±1.68$^{a}$ | 8.26±0.62$^{b}$ | 16.14±1.15$^{a}$ |
| C20:0 | 0.27±0.04$^{ab}$ | 0.17±0.03$^{b}$ | 0.36±0.05$^{ac}$ | 0.18±0.03$^{b}$ | 0.52±0.07$^{a}$ | 0.17±0.04$^{b}$ | 3.39±0.25$^{c}$ | 0.13±0.03$^{b}$ |
| C22:0 | 0.02±0.00$^{a}$ | 0.15±0.04$^{b}$ | 0.26±0.04$^{c}$ | 0.37±0.05$^{d}$ | 0.02±0.01$^{a}$ | 0.09±0.03$^{a}$ | 1.22±0.18$^{b}$ | 0.04±0.02$^{a}$ |
| C24:0 | 0.05±0.01$^{a}$ | 0.37±0.07$^{b}$ | 0.34±0.04$^{b}$ | 1.17±0.15$^{c}$ | 0.88±0.08$^{a}$ | 0.01±0.00$^{b}$ | 0.10±0.03$^{b}$ | 0.01±0.00$^{b}$ |
| **ΣSFA** | **51.34± 6.29$^{a}$** | **46.21±4.95$^{a}$** | **49.64±5.55$^{a}$** | **55.06±6.33$^{a}$** | **47.08±4.11$^{a}$** | **46.82±5.15$^{a}$** | **50.53±4.88$^{a}$** | **44.81±5.27$^{a}$** |
| C14:1 | 0.31±0.04$^{a}$ | 0.51±0.08$^{b}$ | 0.23±0.03$^{a}$ | 0.38±0.05$^{ab}$ | 1.39±0.21$^{a}$ | 1.51±0.18$^{a}$ | 0.26±0.04$^{b}$ | 0.88±0.07$^{c}$ |
| C15:1 | 0.37±0.05$^{a}$ | 0.83±0.09$^{b}$ | 0.42±0.06$^{a}$ | 0.77±0.08$^{b}$ | 1.40±0.22$^{ac}$ | 4.33±0.55$^{b}$ | 2.08±0.19$^{a}$ | 0.87±0.09$^{c}$ |
| C16:1n-9 | 2.38±0.82$^{a}$ | 2.64±0.35$^{a}$ | 1.02±0.08$^{b}$ | 1.81±0.22$^{a}$ | 3.57±0.33$^{a}$ | 2.42±0.24$^{b}$ | 2.65±0.20$^{b}$ | 4.37±0.52$^{a}$ |
| C16:1n-7 | 0.40±0.05$^{a}$ | 1.24±0.21$^{b}$ | 0.25±0.03$^{a}$ | 3.09±0.31$^{c}$ | 0.60±0.04$^{a}$ | 1.05±0.15$^{a}$ | 2.67±0.25$^{b}$ | 1.90±0.21$^{c}$ |
| C18:1n-9 | 6.04±0.77$^{a}$ | 7.49±0.66 $^{ab}$ | 7.70±0.68$^{ab}$ | 8.08±0.75$^{b}$ | 5.32±0.63$^{a}$ | 6.14±0.66$^{a}$ | 5.17±0.54$^{a}$ | 6.09±0.58$^{a}$ |
| C18:1n-7 | 2.12±0.42$^{ab}$ | 2.94±0.33$^{a}$ | 2.41±0.32$^{ab}$ | 1.52±0.20$^{b}$ | 1.40±0.24$^{a}$ | 2.43±0.21$^{b}$ | 1.13±0.15$^{ac}$ | 0.78±0.08$^{c}$ |
| C20:1 | 0.11±0.08$^{a}$ | 0.31±0.06$^{b}$ | 0.00$^{a}$ | 0.81±0.07$^{c}$ | 0.14±0.03$^{a}$ | 0.01±0.00$^{b}$ | 0.65±0.06$^{c}$ | 0.37±0.05$^{d}$ |
| C22:1 | 0.31±0.04$^{a}$ | 0.05±0.002$^{b}$ | 0.06±0.02$^{b}$ | 0.07±0.02$^{b}$ | 0.02±0.00$^{a}$ | 0.00$^{a}$ | 0.17±0.04$^{a}$ | 0.01±0.00$^{a}$ |
| **ΣMUFA** | **12.04±3.09$^{a}$** | **16.01±2.25$^{a}$** | **12.08±1.92$^{a}$** | **16.53±2.86$^{a}$** | **13.82±1.04$^{a}$** | **17.89±2.12$^{b}$** | **14.79±1.75$^{ab}$** | **15.26±2.96$^{ab}$** |
| C16:2n-4 | 3.17±0.75$^{a}$ | 2.28±0.28$^{a}$ | 2.75±0.04$^{a}$ | 2.90±0.33$^{a}$ | 3.44±0.51$^{ab}$ | 2.77±0.28$^{b}$ | 4.23±0.51$^{ac}$ | 4.86±0.49$^{c}$ |
| C16:3n-4 | 0.91±0.08$^{a}$ | 0.51±0.08$^{b}$ | 0.81±0.07$^{a}$ | 0.94±0.07$^{a}$ | 1.79±0.20$^{ab}$ | 2.31±0.31$^{bc}$ | 2.61±0.30$^{c}$ | 1.14±0.18$^{a}$ |
| C16:4 | 0.01±0.00$^{a}$ | 0.55±0.07$^{b}$ | 0.43±0.05$^{b}$ | 1.50±0.22$^{c}$ | 1.62±0.16$^{a}$ | 2.10±0.25$^{b}$ | 1.09±0.10$^{c}$ | 0.21±0.04$^{d}$ |
| C18:2n-6 | 3.22±0.35$^{a}$ | 4.86±0.49$^{b}$ | 1.40±0.21$^{c}$ | 1.24±0.25$^{c}$ | 1.06±0.15$^{a}$ | 0.40±0.08$^{b}$ | 1.66±0.22$^{c}$ | 1.00±0.11$^{a}$ |
| C18:3n-6 | 0.11±0.03$^{a}$ | 0.37±0.04$^{b}$ | 0.39±0.04$^{b}$ | 0.21±0.04$^{a}$ | 0.42±0.05$^{a}$ | 0.03±0.01$^{b}$ | 0.27±0.05$^{c}$ | 0.09±0.03$^{b}$ |
| C18:3n-3 | 0.61±0.07$^{a}$ | 0.45±0.05$^{b}$ | 0.39±0.03$^{b}$ | 0.33±0.05$^{b}$ | 0.56±0.07$^{a}$ | 0.14±0.03$^{b}$ | 0.41±0.06$^{c}$ | 0.72±0.06$^{d}$ |
| C18:4n-3 | 0.75±0.08$^{a}$ | 0.68±0.00$^{a}$ | 0.18±0.03$^{b}$ | 1.33±0.23$^{c}$ | 2.01±0.24$^{ac}$ | 3.11±0.25$^{bc}$ | 2.56±0.26$^{c}$ | 3.55±0.31$^{b}$ |
| C20:2n-6 | 0.58±0.06$^{a}$ | 0.47±0.04$^{a}$ | 0.54±0.06$^{a}$ | 0.48±0.06$^{a}$ | 0.82±0.09$^{a}$ | 0.20±0.04$^{b}$ | 0.47±0.05$^{c}$ | 0.39±0.06$^{c}$ |
| C20:3n-6 | 0.13±0.04$^{a}$ | 0.31±0.05$^{b}$ | 0.14±0.04$^{a}$ | 0.13±0.04$^{a}$ | 0.46±0.05$^{a}$ | 0.43±0.07$^{a}$ | 0.06±0.01$^{b}$ | 1.19±0.20$^{c}$ |
| C20:4n-6 | 3.44±0.32$^{ab}$ | 2.63±0.32$^{b}$ | 3.89±0.44$^{a}$ | 4.24±0.43$^{a}$ | 1.09±0.11$^{a}$ | 1.16±0.21$^{a}$ | 3.35±0.31$^{b}$ | 3.51±0.34$^{b}$ |
| C20:3n-3 | 0.02±0.00$^{a}$ | 0.10±0.02$^{b}$ | 0.08±0.02$^{b}$ | 0.02±0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.01±0.00$^{a}$ |
| C20:4n-3 | 1.01±0.22$^{a}$ | 1.90±0.31$^{b}$ | 2.28±0.33$^{b}$ | 0.25±0.04$^{c}$ | 2.65±0.23$^{a}$ | 2.51±0.20$^{a}$ | 2.67±0.22$^{a}$ | 3.02±0.29$^{a}$ |
| C20:5n-3 | 5.96±0.92$^{ab}$ | 7.22±0.85$^{ab}$ | 7.99±0.85$^{a}$ | 5.51±0.45$^{b}$ | 8.09±0.66$^{ab}$ | 7.42±0.52$^{bc}$ | 6.14±0.52$^{c}$ | 9.43±0.82$^{a}$ |
| C22:2n-6 | 0.27±0.05$^{a}$ | 0.71±0.16$^{b}$ | 0.57±0.06$^{b}$ | 0.55±0.06$^{b}$ | 1.24±0.22$^{a}$ | 1.53±0.24$^{a}$ | 1.33±0.19$^{a}$ | 1.47±0.25$^{a}$ |
| C22:5n-6 | 0.05±0.02$^{a}$ | 0.58±0.07$^{a}$ | 0.50±0.05$^{a}$ | 0.03±0.00$^{a}$ | 0.05±0.02$^{a}$ | 0.01±0.00$^{a}$ | 0.21±0.04$^{a}$ | 0.01±0.00$^{a}$ |
| C22:5n-3 | 3.32±0.41$^{a}$ | 5.91±0.64$^{b}$ | 3.47±0.51$^{a}$ | 2.71±0.29$^{a}$ | 7.70±0.53$^{a}$ | 9.14±0.75$^{a}$ | 4.59±0.67$^{b}$ | 5.06±0.62$^{b}$ |
| C22:6n-3 | 13.06±1.15$^{a}$ | 8.25±0.95$^{b}$ | 12.45±1.05$^{a}$ | 6.01±0.41$^{b}$ | 5.08±0.62$^{a}$ | 2.02±0.18$^{b}$ | 3.01±0.24$^{b}$ | 4.30±0.33$^{a}$ |
| **ΣPUFA** | **36.62±3.75$^{a}$** | **37.78±4.08$^{a}$** | **38.28±3.44$^{a}$** | **28.41±2.35$^{b}$** | **39.10±4.52$^{a}$** | **35.28±3.27$^{a}$** | **34.68±3.08$^{a}$** | **39.93±4.04$^{a}$** |

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

of C15:1 (4.33%, $p < 0.05$) and C22:5n-3 (9.14%), while MDG showed significantly elevated amounts of SFA, mainly C16:0, with 27.57%.

A quite different pattern was observed for the host, where the PUFA group was found to be dominant mainly in the phospholipid classes (with around 50%), followed by SFA (varying from 28.86% in PI to 37.73% in PE) and MUFA (ranging from 14.07 to 21.20% in PE and PI, respectively). Within saturates, C16:0 was the major FA in all lipid classes, exhibiting significantly highest levels in PS (27.16%) and FFA (29.9%) fractions

Table 2. Fatty acid composition of phospholipid and neutral lipid classes from the gills of *Merluccius merluccius*. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

| %FA | Polar lipids | | | | Neutral lipids | | | |
|---|---|---|---|---|---|---|---|---|
| | PC | PE | PI | PS | TAG | WE/CE | MDG | FFA |
| C14:0 | 0.53±0.08$^{ac}$ | 2.95±0.13$^{b}$ | 1.24±0.48$^{a}$ | 0.22±0.02$^{c}$ | 1.87±0.19$^{a}$ | 3.77±0.42$^{b}$ | 1.66±0.13$^{a}$ | 4.83±0.66$^{b}$ |
| C15:0 | 0.49±0.07$^{ac}$ | 3.56±0.78$^{b}$ | 1.42±0.15$^{a}$ | 0.27±0.02$^{c}$ | 3.74±0.36$^{a}$ | 2.00±0.83$^{b}$ | 1.56±0.20$^{b}$ | 1.71±0.26$^{b}$ |
| C16:0 | 21.61±3.67$^{ab}$ | 16.16±2.15$^{b}$ | 12.66±1.07$^{b}$ | 27.16±4.50$^{a}$ | 17.06±2.44$^{a}$ | 21.11±4.60$^{ab}$ | 25.89±3.45$^{ab}$ | 29.90±3.60$^{b}$ |
| C17:0 | 2.87±0.41$^{ac}$ | 7.72±0.37$^{b}$ | 3.47±0.76$^{c}$ | 1.99±0.14$^{a}$ | 3.69±0.36$^{a}$ | 1.83±0.85$^{b}$ | 1.61±0.12$^{b}$ | 0.83±0.09$^{b}$ |
| C18:0 | 7.98±2.60$^{a}$ | 6.92±3.03$^{a}$ | 8.74±3.52$^{a}$ | 5.98±2.30$^{a}$ | 10.97±1.84$^{a}$ | 8.29±2.57$^{a}$ | 8.46±1.19$^{a}$ | 7.60±0.96$^{a}$ |
| C20:0 | 0.20±0.03$^{a}$ | 0.41±0.05$^{b}$ | 0.51±0.06$^{b}$ | 1.07±0.09$^{c}$ | 2.47±0.25$^{a}$ | 0.15±0.01$^{b}$ | 0.78±0.11$^{c}$ | 0.15±0.03$^{b}$ |
| C22:0 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.81±0.02$^{b}$ | 0.00±0.00$^{a}$ | 0.08±0.02$^{a}$ | 0.09±0.02$^{a}$ | 0.02±0.00$^{a}$ | 0.44±0.06$^{b}$ |
| C24:0 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.02±0.00$^{b}$ | 0.12±0.01$^{c}$ | 0.02±0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.70±0.05$^{b}$ | 0.50±0.04$^{c}$ |
| **ΣSFA** | **33.68±3.69$^{ab}$** | **37.73±4.07$^{a}$** | **28.86±2.82$^{b}$** | **36.82±3.07$^{a}$** | **39.90±3.11$^{a}$** | **37.26±4.10$^{a}$** | **40.69±5.37$^{a}$** | **45.96±4.06$^{a}$** |
| C14:1 | 0.69±0.10$^{ac}$ | 1.80±0.22$^{b}$ | 0.38±0.11$^{c}$ | 0.89±0.06$^{a}$ | 2.51±0.25$^{a}$ | 4.76±0.66$^{b}$ | 4.40±0.24$^{b}$ | 0.52±0.06$^{c}$ |
| C15:1 | 0.16±0.02$^{a}$ | 2.38±0.15$^{b}$ | 0.81±0.09$^{c}$ | 0.08±0.01$^{a}$ | 1.21±0.41$^{a}$ | 0.47±0.07$^{b}$ | 1.17±0.09$^{a}$ | 0.21±0.03$^{b}$ |
| C16:1n-9 | 4.34±0.69$^{a}$ | 2.65±0.43$^{b}$ | 1.54±0.66$^{b}$ | 1.37±0.10$^{b}$ | 2.99±0.56$^{a}$ | 3.89±0.36$^{a}$ | 2.90±0.60$^{a}$ | 7.18±0.88$^{b}$ |
| C16:1n-7 | 9.97±1.28$^{a}$ | 0.62±0.11$^{b}$ | 1.65±0.90$^{b}$ | 0.23±0.02$^{b}$ | 1.13±0.10$^{a}$ | 5.41±0.13$^{b}$ | 2.44±0.56$^{c}$ | 4.36±0.62$^{b}$ |
| C18:1n-9 | 3.76±0.24$^{a}$ | 5.34±1.05$^{ab}$ | 12.09±2.37$^{c}$ | 8.96±1.55$^{bc}$ | 6.92±0.39$^{a}$ | 9.11±1.56$^{a}$ | 9.01±2.08$^{a}$ | 8.19±0.95$^{a}$ |
| C18:1n-7 | 0.01±0.00$^{a}$ | 0.90±0.01$^{b}$ | 1.62±0.22$^{c}$ | 3.26±0.23$^{d}$ | 2.41±0.26$^{a}$ | 0.96±0.33$^{b}$ | 0.77±0.17$^{b}$ | 1.73±0.15$^{c}$ |
| C20:1 | 1.76±0.31$^{a}$ | 0.39±0.02$^{b}$ | 1.35±0.15$^{ac}$ | 1.14±0.05$^{c}$ | 2.04±0.22$^{a}$ | 0.64±0.08$^{b}$ | 0.77±0.06$^{b}$ | 0.48±0.05$^{b}$ |
| C22:1 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 1.78±0.44$^{b}$ | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.07±0.02$^{a}$ | 0.41±0.07$^{b}$ | 0.49±0.06$^{b}$ |
| **ΣMUFA** | **20.70±2.10$^{a}$** | **14.07±1.53$^{b}$** | **21.20±2.39$^{a}$** | **15.93±1.20$^{b}$** | **19.22±1.77$^{a}$** | **25.30±2.61$^{b}$** | **21.88±2.05$^{ab}$** | **23.15±2.08$^{b}$** |
| C16:2n-4 | 4.93±0.28$^{a}$ | 4.13±0.55$^{ab}$ | 2.00±0.10$^{c}$ | 3.30±0.23$^{b}$ | 2.28±0.24$^{ab}$ | 2.56±0.41$^{a}$ | 1.59±0.12$^{b}$ | 0.52±0.07$^{c}$ |
| C16:3n-4 | 0.14±0.02$^{a}$ | 0.50±0.06$^{b}$ | 1.36±0.14$^{c}$ | 0.91±0.05$^{d}$ | 2.21±0.40$^{a}$ | 1.76±0.49$^{a}$ | 1.05±0.77$^{a}$ | 0.90±0.08$^{a}$ |
| C16:4 | 0.11±0.02$^{a}$ | 1.90±0.72$^{b}$ | 2.40±0.81$^{b}$ | 0.03±0.00$^{a}$ | 0.67±0.09$^{a}$ | 0.42±0.08$^{b}$ | 0.18±0.06$^{c}$ | 0.12±0.03$^{c}$ |
| C18:2n-6 | 0.18±0.03$^{a}$ | 0.50±0.09$^{a}$ | 4.05±0.99$^{b}$ | 0.42±0.03$^{a}$ | 10.03±2.80$^{a}$ | 10.78±1.36$^{a}$ | 2.46±0.04$^{b}$ | 2.21±0.25$^{b}$ |
| C18:3n-6 | 0.01±0.00$^{ab}$ | 0.10±0.00$^{b}$ | 0.88±0.07$^{c}$ | 0.00±0.00$^{a}$ | 0.24±0.01$^{a}$ | 0.78±0.09$^{b}$ | 0.52±0.04$^{c}$ | 0.08±0.02$^{d}$ |
| C18:3n-3 | 0.21±0.03$^{a}$ | 1.64±0.82$^{b}$ | 0.27±0.02$^{a}$ | 3.58±0.25$^{c}$ | 0.38±0.03$^{a}$ | 1.60±0.58$^{b}$ | 1.15±0.42$^{ab}$ | 0.53±0.05$^{a}$ |
| C18:4n-3 | 1.16±0.14$^{a}$ | 0.39±0.01$^{b}$ | 0.74±0.02$^{c}$ | 0.31±0.02$^{b}$ | 0.40±0.03$^{a}$ | 2.87±0.61$^{b}$ | 1.69±0.33$^{c}$ | 1.88±0.23$^{bc}$ |
| C20:2n-6 | 0.56±0.08$^{a}$ | 0.67±0.05$^{a}$ | 2.93±0.58$^{b}$ | 2.50±0.17$^{b}$ | 1.21±0.13$^{a}$ | 0.16±0.02$^{b}$ | 1.32±0.10$^{a}$ | 0.26±0.04$^{b}$ |
| C20:3n-6 | 0.31±0.04$^{a}$ | 0.79±0.10$^{b}$ | 0.97±0.09$^{b}$ | 0.59±0.25$^{ab}$ | 1.30±0.14$^{a}$ | 0.10±0.04$^{b}$ | 0.31±0.06$^{b}$ | 0.96±0.11$^{c}$ |
| C20:4n-6 | 5.57±0.66$^{a}$ | 4.48±0.41$^{a}$ | 2.21±0.77$^{b}$ | 4.01±0.83$^{ab}$ | 1.73±0.17$^{a}$ | 0.40±0.12$^{b}$ | 2.02±0.30$^{a}$ | 0.55±0.06$^{b}$ |
| C20:3n-3 | 0.20±0.03$^{a}$ | 0.22±0.01$^{a}$ | 0.53±0.10$^{b}$ | 0.27±0.07$^{a}$ | 0.06±0.01$^{a}$ | 0.07±0.03$^{a}$ | 0.24±0.07$^{b}$ | 0.14±0.03$^{ab}$ |
| C20:4n-3 | 4.53±0.65$^{a}$ | 4.40±0.94$^{a}$ | 3.23±0.97$^{a}$ | 3.04±0.49$^{a}$ | 2.39±0.20$^{a}$ | 0.17±0.07$^{b}$ | 3.34±0.26$^{c}$ | 0.04±0.01$^{b}$ |
| C20:5n-3 | 6.20±1.03$^{ab}$ | 5.32±0.02$^{a}$ | 8.22±1.39$^{b}$ | 6.89±0.68$^{ab}$ | 0.27±0.04$^{a}$ | 2.57±0.68$^{b}$ | 0.38±0.03$^{a}$ | 5.43±0.55$^{c}$ |
| C22:2n-6 | 0.81±0.12$^{ab}$ | 1.22±0.61$^{a}$ | 0.19±0.02$^{b}$ | 0.33±0.11$^{ab}$ | 0.79±0.09$^{a}$ | 0.12±0.05$^{b}$ | 1.10±0.30$^{a}$ | 0.07±0.02$^{b}$ |
| C22:5n-6 | 0.00±0.00$^{a}$ | 0.00±0.00$^{a}$ | 0.73±0.05$^{b}$ | 0.01±0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.06±0.03$^{a}$ | 0.89±0.13$^{b}$ | 0.56±0.22$^{b}$ |
| C22:5n-3 | 2.58±0.78$^{a}$ | 1.21±0.24$^{b}$ | 1.06±0.05$^{b}$ | 0.54±0.13$^{b}$ | 0.76±0.06$^{a}$ | 0.34±0.09$^{b}$ | 1.00±0.17$^{a}$ | 2.03±0.04$^{c}$ |
| C22:6n-3 | 18.13±2.75$^{a}$ | 20.72±0.70$^{a}$ | 18.15±2.08$^{a}$ | 19.53±2.13$^{a}$ | 16.17±3.64$^{a}$ | 12.67±1.74$^{a}$ | 18.20±1.34$^{a}$ | 14.58±1.74$^{a}$ |
| **ΣPUFA** | **45.61±4.35$^{a}$** | **48.20±3.71$^{a}$** | **49.93±4.19$^{a}$** | **47.25±4.88$^{a}$** | **40.88±4.04$^{a}$** | **37.44±3.09$^{a}$** | **37.43±3.20$^{a}$** | **30.89±2.85$^{b}$** |

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

($p < 0.05$). Along with C16:0, C18:0 was also found to be abundant in all analyzed polar and neutral lipid fractions with a percentage exceeding 5% of total FAs ($p > 0.05$). Within the MUFA group, we particularly noticed significantly high amounts of C16:1n-7 in PC (~10%) and C18:1n-9 in PI, reaching 12.09% of total FAs. The latter FA was also found to be dominant in all neutral lipid classes ($p > 0.05$). With respect to PUFA, DHA was by far the most dominant FA in all lipid classes, varying from 14.58% in FFA to 20.72% in PE. In addition to DHA, EPA was also found at quite important

Table 3. Fatty acid composition of phospholipid and neutral lipid classes from the parasitic copepod Peroderma cylindricum. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

| %FA | Polar lipids | | | | Neutral lipids | | | |
|---|---|---|---|---|---|---|---|---|
| | PC | PE | PI | PS | TAG | WE/CE | MDG | FFA |
| C14:0 | 2.73±0.31$^{a}$ | 2.53±0.22$^{ab}$ | 1.67±0.25$^{b}$ | 3.19±0.45$^{a}$ | 4.07±0.45$^{a}$ | 4.65±0.52$^{a}$ | 4.17±0.55$^{a}$ | 3.70±0.44$^{a}$ |
| C15:0 | 1.46±0.18$^{ab}$ | 1.65±0.18$^{ab}$ | 2.14±0.23$^{a}$ | 1.19±0.21$^{b}$ | 2.11±0.22$^{ac}$ | 1.11±0.20$^{b}$ | 1.82±0.39$^{a}$ | 2.57±0.37$^{c}$ |
| C16:0 | 25.83±2.25$^{a}$ | 26.08±2.44$^{ab}$ | 33.65±2.95$^{b}$ | 32.85±3.05$^{ab}$ | 33.04±2.75$^{a}$ | 29.58±1.98$^{a}$ | 30.07±2.45$^{a}$ | 29.75±2.66$^{a}$ |
| C17:0 | 2.95±0.30$^{a}$ | 2.23±0.25$^{b}$ | 1.84±0.22$^{b}$ | 2.25±0.22$^{ab}$ | 2.48±0.25$^{a}$ | 1.01±0.12$^{b}$ | 2.21±0.20$^{b}$ | 2.13±0.17$^{ab}$ |
| C18:0 | 8.96±0.85$^{a}$ | 7.54±0.66$^{a}$ | 12.19±0.19$^{b}$ | 12.47±0.15$^{b}$ | 6.32±0.82$^{a}$ | 8.79±0.93$^{b}$ | 8.74±0.83$^{b}$ | 7.35±0.65$^{ab}$ |
| C22:0 | 0.03±0.0$^{ab}$ | 0.01±0.00$^{b}$ | 0.09±0.03$^{b}$ | 0.07±0.02$^{ac}$ | 0.28±0.04$^{a}$ | 0.14±0.03$^{b}$ | 0.13±0.03$^{b}$ | 0.22±0.04$^{ab}$ |
| **ΣSFA** | **41.96±3.82$^{a}$** | **40.04±3.88$^{a}$** | **51.58±4.75$^{b}$** | **52.02±4.58$^{b}$** | **48.30±4.95$^{a}$** | **45.28±3.11$^{a}$** | **47.14±4.79$^{a}$** | **45.72±5.87$^{a}$** |
| C22:1 | 0.05±0.02$^{a}$ | 0.02±0.00$^{b}$ | 0.01±0.00$^{b}$ | 0.00$^{b}$ | 0.11±0.03$^{a}$ | 0.03±0.01$^{b}$ | 0.01±0.00$^{b}$ | 0.04±0.01$^{b}$ |
| C14:1 | 0.39±0.04$^{a}$ | 0.38±0.05$^{a}$ | 0.83±0.07$^{b}$ | 0.46±0.05$^{a}$ | 0.15±0.04$^{a}$ | 1.47±0.18$^{b}$ | 0.55±0.05$^{c}$ | 1.54±0.19$^{b}$ |
| C15:1 | 0.18±0.03$^{a}$ | 0.09±0.02$^{b}$ | 0.14±0.03$^{ab}$ | 0.08±0.04$^{b}$ | 0.50±0.05$^{ab}$ | 0.39±0.06$^{a}$ | 0.48±0.04$^{ab}$ | 0.59±0.07$^{b}$ |
| C16:1n-9 | 2.49±0.28$^{a}$ | 2.16±0.22$^{a}$ | 3.55±0.32$^{b}$ | 1.26±0.18$^{c}$ | 2.11±0.19$^{a}$ | 2.82±0.23$^{b}$ | 3.66±0.31$^{c}$ | 3.50±0.33$^{bc}$ |
| C16:1n-7 | 0.30±0.01$^{a}$ | 1.67±0.17$^{b}$ | 0.59±0.08$^{ac}$ | 0.72±0.09$^{c}$ | 3.33±0.41$^{a}$ | 2.45±0.31$^{a}$ | 2.83±0.25$^{a}$ | 3.34±0.34$^{a}$ |
| C18:1n-9 | 4.93±0.45$^{a}$ | 6.95±0.55$^{b}$ | 1.63±0.19$^{c}$ | 8.73±0.85$^{d}$ | 8.95±0.58$^{a}$ | 9.86±0.95$^{a}$ | 10.96±0.95$^{a}$ | 9.53±0.84$^{a}$ |
| C18:1n-7 | 1.86±0.21$^{a}$ | 2.61±0.21$^{b}$ | 1.16±0.15$^{c}$ | 1.11±0.23$^{c}$ | 1.50±0.11$^{a}$ | 1.49±0.17$^{a}$ | 1.83±0.22$^{a}$ | 0.41±0.05$^{b}$ |
| C20:1 | 0.03±0.01$^{a}$ | 0.22±0.07$^{b}$ | 0.03±0.02$^{a}$ | 0.07±0.03$^{a}$ | 0.04±0.02$^{a}$ | 0.55±0.06$^{b}$ | 0.24±0.04$^{a}$ | 1.57±0.17$^{c}$ |
| **ΣMUFA** | **10.23±1.82$^{ab}$** | **14.10±2.91$^{a}$** | **7.94±1.77$^{b}$** | **12.43±1.58$^{a}$** | **16.69±2.64$^{a}$** | **19.06±2.17$^{a}$** | **20.56±2.55$^{a}$** | **20.52±3.06$^{a}$** |
| C16:2n-4 | 2.77±0.25$^{a}$ | 3.20±0.35$^{a}$ | 2.54±0.39$^{a}$ | 3.22±0.33$^{a}$ | 1.56±0.18$^{a}$ | 2.11±0.22$^{a}$ | 3.76±0.33$^{b}$ | 2.03±0.23$^{a}$ |
| C16:3n-4 | 0.69±0.08$^{ac}$ | 1.23±0.15$^{b}$ | 0.84±0.08$^{a}$ | 0.46±0.05$^{c}$ | 0.95±0.08$^{a}$ | 0.68±0.07$^{b}$ | 0.50±0.06$^{bc}$ | 0.47±0.06$^{c}$ |
| C18:2n-6 | 2.78±0.30$^{a}$ | 1.11±0.28$^{b}$ | 3.19±0.25$^{a}$ | 1.12±0.16$^{b}$ | 0.75±0.09$^{ab}$ | 1.03±0.13$^{a}$ | 0.71±0.08$^{b}$ | 0.98±0.11$^{ab}$ |
| C18:3n-4 | 0.34±0.07$^{a}$ | 0.23±0.06$^{ac}$ | 0.07±0.03$^{b}$ | 0.10±0.04$^{bc}$ | 0.18±0.03$^{a}$ | 1.23±0.16$^{b}$ | 0.48±0.7$^{ab}$ | 0.07±0.02$^{a}$ |
| C18:3n-3 | 0.38±0.04$^{a}$ | 0.34±0.05$^{ab}$ | 0.39±0.05$^{a}$ | 0.22±0.04$^{b}$ | 0.39±0.05$^{a}$ | 0.43±0.05$^{a}$ | 0.02±0.00$^{b}$ | 0.09±0.02$^{b}$ |
| C20:2n-6 | 0.41±0.05$^{a}$ | 0.44±0.06$^{a}$ | 0.23±0.04$^{b}$ | 0.34±0.05$^{ab}$ | 0.31±0.04$^{a}$ | 0.22±0.04$^{a}$ | 0.31±0.04$^{a}$ | 0.62±0.03$^{b}$ |
| C20:4n-6 | 2.80±0.25$^{ab}$ | 1.94±0.21$^{a}$ | 3.57±0.66$^{b}$ | 3.24±0.44$^{b}$ | 2.53±0.21$^{a}$ | 2.88±0.25$^{a}$ | 2.38±0.25$^{a}$ | 1.62±0.21$^{b}$ |
| C20:3n-3 | 0.05±0.02$^{a}$ | 0.08±0.03$^{a}$ | 0.08±0.02$^{a}$ | 0.33±0.06$^{b}$ | 0.08±0.02$^{ab}$ | 0.10±0.03$^{b}$ | 0.01±0.00$^{a}$ | 0.22±0.04$^{c}$ |
| C20:4n-3 | 2.98±0.31$^{a}$ | 2.06±0.25$^{b}$ | 2.17±0.22$^{b}$ | 1.64±0.18$^{b}$ | 0.15±0.04$^{a}$ | 0.78±0.08$^{b}$ | 1.32±0.11$^{c}$ | 1.71±0.18$^{d}$ |
| C20:5n-3 | 11.05±0.85$^{a}$ | 10.83±0.85$^{a}$ | 8.86±0.75$^{a}$ | 11.24±0.95$^{a}$ | 8.72±0.65$^{a}$ | 6.95±0.66$^{b}$ | 5.93±0.66$^{bc}$ | 4.71±0.45$^{c}$ |
| C22:3n-3 | 1.48±0.18$^{a}$ | 0.55±0.07$^{b}$ | 1.24±0.26$^{a}$ | 0.07±0.02$^{c}$ | 0.42±0.05$^{a}$ | 0.37±0.05$^{a}$ | 2.21±0.26$^{b}$ | 0.34±0.06$^{a}$ |
| C22:5n-6 | 0.57±0.08$^{a}$ | 0.69±0.08$^{a}$ | 0.27±0.04$^{b}$ | 0.76±0.07$^{a}$ | 0.02±0.00$^{a}$ | 0.12±0.03$^{a}$ | 0.03±0.00$^{a}$ | 0.82±0.07$^{b}$ |
| C22:5n-3 | 2.49±0.28$^{a}$ | 2.94±0.33$^{a}$ | 4.15±0.55$^{b}$ | 1.37±0.21$^{c}$ | 0.71±0.08$^{a}$ | 2.33±0.22$^{b}$ | 2.05±0.19$^{b}$ | 3.35±0.28$^{c}$ |
| C22:6n-3 | 19.02±1.88$^{a}$ | 20.22±2.15$^{a}$ | 12.88±1.05$^{b}$ | 11.44±1.95$^{b}$ | 18.24±1.75$^{a}$ | 16.43±1.66$^{a}$ | 12.59±0.96$^{b}$ | 16.73±0.88$^{a}$ |
| **ΣPUFA** | **47.81±5.18$^{a}$** | **45.86±4.13$^{a}$** | **40.48±4.66$^{ab}$** | **35.55± 3.78$^{b}$** | **35.01±5.54$^{a}$** | **35.66±3.96$^{a}$** | **32.30±3.77$^{a}$** | **33.76±4.15$^{a}$** |

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

percentages (up to 5%), mainly in phospholipid and FFA fractions. Another FA, C18:2n-6, was also found at significantly high amounts (around 10% of TFA) in both TAG and WE/CE fractions ($p < 0.05$).

### 3.2. Lipid classes and their fatty acid composition from *Peroderma cylindiricum* and its host *Sardina pilchardus*

PC and PE were the major lipid classes in *P. cylindricum* polar lipids, representing 30.63%, and 11.21% of TL, respectively. Among neutral lipids, a high proportion of TAG (41.38%) was found while WE/CE and FFA represented 7.75 and 5.20%, respectively. The remaining lipid classes (i.e. PI, PS, and MDG) occurred in smaller proportions (ranging from ~1 to 1.6%) (Figure 3). Regarding the host, we found that the lipid fraction of the *S. pilchardus* kidney was characterized by the dominance of the WE/CE, which accounted for over 30% of TL followed by PS (18.16%), TAG (15.62%), PE (14.42%), and

Table 4. Fatty acid composition of phospholipid and neutral lipid classes from the kidney of *Sardina pilchardus*. FA: fatty acid; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids.

| %FA | Polar lipids | | | | Neutral lipids | | | |
|---|---|---|---|---|---|---|---|---|
| | PC | PE | PI | PS | TAG | WE/CE | MDG | FFA |
| C14:0 | 3.67±0.35$^{a}$ | 3.75±0.38$^{a}$ | 1.16±0.12$^{b}$ | 1.06±0.21$^{b}$ | 7.92±0.85$^{a}$ | 6.07±0.55$^{a}$ | 3.80±0.39$^{b}$ | 6.14±0.74$^{a}$ |
| C15:0 | 0.97±0.09$^{a}$ | 1.32±0.12$^{b}$ | 1.29±0.15$^{ab}$ | 0.97±0.11$^{a}$ | 0.77±0.08$^{a}$ | 0.58±0.06$^{a}$ | 2.82±0.32$^{b}$ | 3.82±0.41$^{c}$ |
| C16:0 | 23.55±2.55$^{ab}$ | 19.53±2.78$^{a}$ | 25.18±2.66$^{ab}$ | 29.13±3.16$^{b}$ | 26.18±2.87$^{a}$ | 33.05±3.56$^{a}$ | 32.46±3.09$^{a}$ | 29.08±2.98$^{a}$ |
| C17:0 | 1.16±0.14$^{a}$ | 1.13±0.15$^{a}$ | 0.62±0.07$^{b}$ | 1.63±0.21$^{c}$ | 0.30±0.04$^{a}$ | 0.00$^{b}$ | 0.55±0.06$^{c}$ | 0.44±0.05$^{c}$ |
| C18:0 | 7.57±0.84$^{a}$ | 10.65±1.42$^{ab}$ | 11.60±1.68$^{b}$ | 9.45±0.95$^{ab}$ | 17.14±1.94$^{a}$ | 13.27±1.66$^{ab}$ | 11.30±1.45$^{b}$ | 14.30±1.86$^{ab}$ |
| C20:0 | 0.00$^{a}$ | 0.01±0.00$^{ab}$ | 0.07±0.02$^{c}$ | 0.04±0.01$^{bc}$ | 0.00$^{a}$ | 0.03±0.01$^{a}$ | 0.13±0.03$^{b}$ | 0.01±0.00$^{a}$ |
| C22:0 | 0.02±0.01$^{a}$ | 0.05±0.02$^{a}$ | 0.13±0.03$^{b}$ | 0.00$^{a}$ | 0.07±0.02$^{a}$ | 0.07±0.03$^{a}$ | 0.73±0.08$^{b}$ | 0.12±0.20$^{a}$ |
| **ΣSFA** | **36.94±3.55$^{a}$** | **36.44±3.05$^{a}$** | **40.04±4.08$^{a}$** | **42.28±3.96$^{a}$** | **52.38±4.95$^{a}$** | **53.07±4.15$^{a}$** | **51.79±5.22$^{a}$** | **53.91±4.76$^{a}$** |
| C14:1 | 0.35±0.04$^{a}$ | 0.25±0.03$^{a}$ | 0.63±0.07$^{b}$ | 0.64±0.06$^{b}$ | 0.82±0.09$^{a}$ | 0.68±0.07$^{a}$ | 1.80±0.21$^{b}$ | 2.00±0.23$^{b}$ |
| C15:1 | 0.31±0.03$^{a}$ | 0.26±0.04$^{a}$ | 0.44±0.05$^{b}$ | 0.14±0.03$^{c}$ | 0.66±0.07$^{a}$ | 0.09±0.02$^{b}$ | 0.91±0.08$^{c}$ | 1.11±0.12$^{c}$ |
| C16:1n-9 | 0.41±0.05$^{a}$ | 0.89±0.11$^{b}$ | 1.19±0.21$^{b}$ | 0.93±0.08$^{b}$ | 0.45±0.05$^{a}$ | 4.01±0.52$^{b}$ | 4.22±0.46$^{b}$ | 2.37±0.25$^{c}$ |
| C16:1n-7 | 0.35±0.04$^{ab}$ | 0.27±0.03$^{a}$ | 0.67±0.07$^{a}$ | 1.11±0.21$^{c}$ | 3.82±0.41$^{ab}$ | 4.49±0.53$^{a}$ | 3.70±0.44$^{ab}$ | 2.98±0.29$^{b}$ |
| C18:1n-9 | 7.37±0.75$^{a}$ | 8.08±0.68$^{a}$ | 9.30±0.82$^{a}$ | 14.44±1.87$^{b}$ | 10.99±1.55$^{a}$ | 12.01±1.74$^{a}$ | 10.04±1.35$^{a}$ | 5.42±0.73$^{b}$ |
| C18:1n-7 | 1.22±0.11$^{a}$ | 3.84±0.35$^{b}$ | 1.51±0.22$^{a}$ | 2.56±0.31$^{c}$ | 1.86±0.17$^{a}$ | 2.43±0.32$^{ab}$ | 3.15±0.38$^{b}$ | 0.98±0.08$^{c}$ |
| C20:1 | 0.11±0.03$^{a}$ | 0.72±0.08$^{b}$ | 0.16±0.03$^{a}$ | 0.25±0.04$^{c}$ | 0.46±0.05$^{a}$ | 0.45±0.06$^{a}$ | 0.81±0.09$^{b}$ | 0.93±0.11$^{b}$ |
| C22:1 | 0.08±0.02$^{a}$ | 0.10±0.00$^{a}$ | 0.08±0.02$^{a}$ | 0.06±0.03$^{a}$ | 0.90±0.12$^{a}$ | 0.17±0.03$^{b}$ | 0.51±0.06$^{c}$ | 0.10±0.02$^{d}$ |
| **ΣMUFA** | **10.20±1.55$^{a}$** | **14.41±1.78$^{a}$** | **13.97±1.65$^{a}$** | **20.13±2.06$^{b}$** | **19.95±2.12$^{a}$** | **24.33±2.85$^{ab}$** | **25.14±2.55$^{b}$** | **15.89±1.44$^{c}$** |
| C16:2n-4 | 2.02±0.21$^{a}$ | 1.17±0.14$^{b}$ | 1.44±0.16$^{b}$ | 2.01±0.24$^{a}$ | 0.83±0.07$^{a}$ | 0.30±0.03$^{b}$ | 1.01±0.15$^{a}$ | 2.20±0.24$^{c}$ |
| C16:3n-4 | 0.50±0.06$^{a}$ | 0.74±0.08$^{b}$ | 0.46±0.05$^{ac}$ | 0.32±0.04$^{c}$ | 0.39±0.04$^{a}$ | 0.46±0.06$^{a}$ | 0.71±0.08$^{ab}$ | 1.07±0.23$^{b}$ |
| C18:2n-6 | 7.16±0.82$^{ac}$ | 4.66±0.53$^{b}$ | 5.32±0.46$^{ab}$ | 8.01±0.92$^{c}$ | 7.02±0.84$^{a}$ | 9.30±0.78$^{b}$ | 4.44±0.56$^{c}$ | 6.75±0.71$^{a}$ |
| C18:3n-6 | 0.02±0.01$^{a}$ | 0.01±0.00$^{a}$ | 0.00$^{a}$ | 0.34±0.05$^{b}$ | 0.87±0.09$^{a}$ | 0.42±0.05$^{b}$ | 0.11±0.03$^{c}$ | 0.00$^{c}$ |
| C18:3n-3 | 0.30±0.04$^{a}$ | 1.01±0.12$^{b}$ | 0.42±0.06$^{ac}$ | 0.61±0.07$^{c}$ | 0.67±0.08$^{a}$ | 1.52±0.22$^{b}$ | 0.23±0.03$^{c}$ | 0.21±0.04$^{c}$ |
| C18:4n-3 | 0.15±0.03$^{a}$ | 0.52±0.05$^{b}$ | 0.23±0.03$^{a}$ | 0.24±0.04$^{a}$ | 0.92±0.08$^{a}$ | 0.73±0.08$^{b}$ | 0.09±0.02$^{c}$ | 0.31±0.04$^{d}$ |
| C20:2n-6 | 0.81±0.07$^{a}$ | 1.32±0.11$^{b}$ | 0.63±0.05$^{ac}$ | 0.58±0.06$^{c}$ | 4.15±0.42$^{a}$ | 0.35±0.04$^{b}$ | 0.25±0.03$^{b}$ | 0.47±0.05$^{b}$ |
| C20:3n-6 | 0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.10±0.02$^{b}$ | 0.00$^{a}$ |
| C20:4n-6 | 0.11±0.03$^{a}$ | 0.27±0.04$^{b}$ | 0.14±0.03$^{a}$ | 0.27±0.04$^{b}$ | 0.00$^{a}$ | 0.00$^{a}$ | 0.01±0.00$^{a}$ | 0.19±0.02$^{b}$ |
| C20:3n-3 | 3.63±0.35$^{a}$ | 2.28±0.22$^{b}$ | 1.09±0.1$^{c}$ | 3.03±0.34$^{ab}$ | 1.78±0.25$^{a}$ | 0.24±0.03$^{b}$ | 1.26±0.18$^{c}$ | 1.05±0.17$^{c}$ |
| C20:4n-3 | 1.72±0.22$^{a}$ | 1.66±0.18$^{a}$ | 0.45±0.05$^{b}$ | 1.13±0.15$^{c}$ | 0.11±0.03$^{a}$ | 0.31±0.04$^{b}$ | 0.02±0.01$^{a}$ | 0.32±0.04$^{b}$ |
| C20:5n-3 | 11.61±1.76$^{ab}$ | 15.91±1.84$^{a}$ | 14.41±2.02$^{ab}$ | 10.07±1.13$^{b}$ | 4.66±0.56$^{a}$ | 4.34±0.61$^{a}$ | 7.66±0.82$^{b}$ | 6.70±0.72$^{b}$ |
| C22:3n-3 | 0.89±0.11$^{a}$ | 0.22±0.03$^{b}$ | 0.90±0.08$^{a}$ | 0.09±0.02$^{b}$ | 0.07±0.02$^{a}$ | 0.12±0.03$^{a}$ | 0.12±0.04$^{a}$ | 0.34±0.05$^{b}$ |
| C22:5n-6 | 0.43±0.04$^{a}$ | 0.59±0.06$^{ab}$ | 0.63±0.07$^{b}$ | 0.83±0.09$^{c}$ | 0.13±0.02$^{a}$ | 1.34±0.15$^{bc}$ | 1.74±0.19$^{c}$ | 1.20±0.16$^{b}$ |
| C22:5n-3 | 3.12±0.45$^{a}$ | 2.14±0.26$^{b}$ | 3.25±0.36$^{a}$ | 2.67±0.27$^{ab}$ | 0.45±0.05$^{a}$ | 0.81±0.07$^{b}$ | 1.24±0.15$^{c}$ | 1.62±0.17$^{d}$ |
| C22:6n-3 | 18.39±1.77$^{a}$ | 16.64±1.85$^{a}$ | 15.62±2.03$^{a}$ | 9.05±0.85$^{b}$ | 5.62±0.7$^{a}$ | 2.35±0.25$^{b}$ | 4.08±0.52$^{ab}$ | 7.77±0.83$^{c}$ |
| **ΣPUFA** | **42.86±3.65$^{ab}$** | **49.15±5.08$^{a}$** | **45.99±4.92$^{a}$** | **37.59±3.77$^{b}$** | **27.67±2.88$^{ab}$** | **22.60±2.75$^{a}$** | **23.07±2.81$^{a}$** | **30.20±3.07$^{b}$** |

All values are averages of triplicate analyses (n=3) performed on the pool of 9 samples. Values labelled with different letters in the same line by considering polar and neutral lipids separately, are significantly different (by the Bonferroni test at the 5% probability level). SFA (saturated fatty acids that have no double bonds), MUFA (monounsaturated fatty acids that contain one double bond), PUFA (polyunsaturated fatty acids that contain two or more double bonds).

PC (10.26%). The other lipid classes, including MDG, FFA and PI were found to be minor components with proportions ranging from 6.27 to 3.30% and to 1.65% of TL, respectively (Figure 4).

The fatty acid compositions of the different P. cylindricum lipid classes are presented in Table 3. The obtained results showed that almost all lipid classes contained higher proportions of SFA than MUFA and PUFA, except for PC and PE fractions, where PUFA constituted the major group with 47.81 and 45.86%, respectively. Among the total of 28 identified FA, five (i.e. C16:0, C18:0, C18:1n-9, EPA and DHA) appeared to be dominant in all lipid fractions. Within phospholipids, PC and PE showed quite sim-

ilar compositions and exhibited the highest level of DHA, which constitutes almost 20% of total FAs ($p < 0.05$). C16:0 and C18:0 were mostly found in PI and PS (~33%). As for neutral lipid classes, large proportions of C16:0 (33.04%) and DHA (18.24%) were recorded in the TAG fraction. Furthermore, substantial amounts of DHA were also recorded in WE/CE, FFA, and MDG.

The fatty acid profiles of polar and neutral lipid classes extracted from the host kidney are shown in Table 4. All neutral lipids were dominated by SFA (up to 50%), followed by PUFA and MUFA. C14:0, C16:0 and C18:0 were the major FAs of SFA group, while C18:1n-9 was the most abundant MUFA. In the PUFA group, C18:2n-6, EPA and DHA tended to be dominant, mainly in the FFA fraction. Regarding the polar lipid classes, PUFA percentages were higher than SFA and MUFA except for PS, where SFA dominated (by representing 42.28% of total FA). C16:0 and C18:1n-9 were the most abundant SFA and MUFA, culminating to 29.13 and 14.44% ($p < 0.05$) in PS, respectively. EPA and DHA were the major FA within the PUFA group with significantly higher levels of DHA in PC, PE, and PI. In addition, C18:2n-6 was found in relatively important amounts (up to 5%) in all phospholipid fractions.

### 3.3. Multivariate analysis

To provide an overview of similarities and discrepancies within each studied host-parasite system, hierarchical clustering analysis (HCA) was applied to the whole FA data set (Figure 5). Figure 5a presents the dendrogram of HCA, giving information about *L. lusci* and its host *M. merluccius.* Two major groups, comprising two clusters each, were separated out in the resulting dendrogram. The first group was constituted by the hosta' TAG and PL classes, while the second group involved the remaining data. Interestingly, we found that among the second group, almost all neutral lipid classes (including FFA, WE/CE, and MDG) of the host clustered with the parasite phospholipid. Similarly, the dendrogram generated from *P. cylindricum* and *S. pilchardus* data revealed two major groups. The first one constituted two clusters related to the host neutral lipid moieties. The second group was composed of two distinct clusters: Cluster I comprised the polar lipids of the host; while cluster II grouped all lipid classes of *P. cylindricum*.

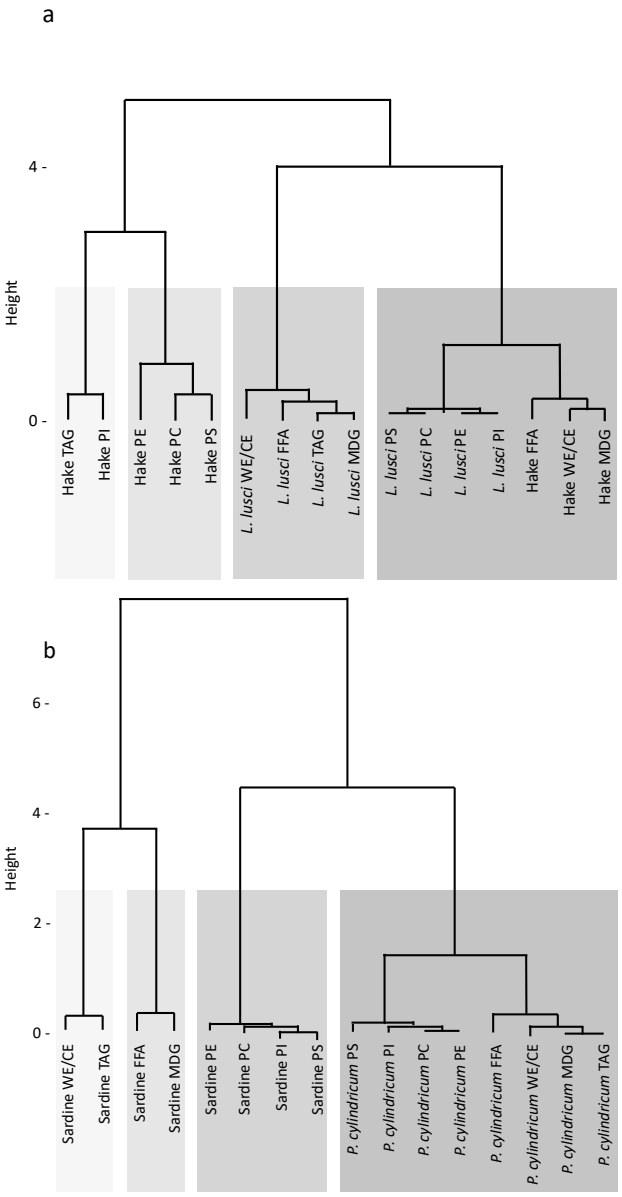


FIGURE 5. Hierarchical clustering analysis (Euclidien distance, Ward's clustering) of the whole fatty acid data set obtained from the two studied host-parasite systems. (a): Dendrogram obtained for *Lernaeocera lusci* and its host *Merluccius merluccius.* (b): Dendrogram obtained for *Peroderma cylindricum* and its host *Sardina pilchardus.* PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; PC: phosphatidylcholine; PE: phosphatidylethanolamine; PI: phosphatidylinositol; PS: phosphatidylserine; TAG: triacylglycerol; WE/CE: wax ester/cholesterol ester; MDG: mono-diacylglycerol; FFA: free fatty acids; MDG: mono-diacylglycerol; FFA: free fatty acids.

## 4. DISCUSSION

It is well established that lipid content and composition in copepods are diverse and vary depend-

ing upon several parameters such as species, type of food, latitude, season, developmental stage, and life cycle strategy (van der Meeren *et al*., 2008). Although a substantial amount of literature exists on free-forms, information on the lipid composition of parasitic forms is still limited. Thus, this study was conducted to explore similarities and differences among the lipid fractions and their fatty acid compositions in *L. lusci* and *P. cylindricum,* and their respective hosts.

According to the obtained results, clear differences in the relative percentages of the different lipid classes were noticed between parasites and their respective hosts. Indeed, TAG turned out to be the most predominant neutral lipid class in both *L. lusci* and *P. cylindricum*, while WE/CE prevailed in fish tissues. These results are in accordance with those of Tocher *et al*. (2010) and Kotani (2006), who reported that parasitic and opportunistic feeder copepods store their lipids mainly as TAGs. However, it is worth noting that by feeding regularly on their host's blood, the parasitic copepod females are able to maintain a sufficient lipid content to sustain their own survival and to fuel maturation and egg production as recently reported in *L. lusci* by Hajji *et al*. (2021). Thus, it is thought that TAG is rather stored in lipovitellin as a main source of energy for the next stages (e.g. nauplii and infective copepodids) to ensure development, basic metabolism, swimming activity and infectivity. In this context Tocher *et al*. (2010) demonstrated that the eggs of parasitic copepods contain significantly higher proportions of TAG than adulate females. The same authors reported that, furthermore, due to the short time period between fish host infections, long-term energy deposits such as WEs are not required for parasitic copepods (Tocher *et al*., 2010). In the other hand, the high proportions of WE/CE recorded in the host's tissues (i.e. gills and kidney) could be explained by the essential role played by cholesterol in the physiological regulation of the physical properties of cell membranes (Díaz *et al*., 2016). Differences in the proportion of polar lipid classes were herein observed between the two studied parasites and their respective hosts. Although PC, and to a lesser extent, PE were found to be the major phospholipid classes in parasites, PS appeared among the most dominant phospholipid classes in host tissues. In line with this, Tocher *et al*. (2010) also reported that PC and PE were the major phospholipid classes in both the females and egg strings of parasitic caligid copepods belonging to the genus *Lepeophtheirus.* These key components of biological membranes are known for their functional and structural roles. Moreover, it was reported that PC is the principal lipid component in crustacean lipovitellin and may serve as metabolic energy storage for reproduction and embryonic development (Lee *et al*., 2006).

Regarding the fatty acid composition of the different lipid classes, a general pattern characterized by the dominance of five FAs comprising C16:0, C18:0, C18:1n-9, C20:5n-3 and C22:6n-3 was herein clearly observed in both copepods and fish. Such findings corroborate previous studies which emphasized that the FA profile of the parasite can largely reflect that of its host (Tocher *et al*., 2010; Telahigue *et al*., 2017; Hajji *et al*., 2021). However, some dissimilarities were noticed as for the abundance and the distribution of some fatty acids. For instance, SFA was found to be the major FA group in almost all lipid classes of the two parasites *L. lusci* and *P. cylindricum,* mainly due to the substantial level of palmitic acid (C16:0). Although C16:0 was also found in a considerable amount in the host's tissues, their lipid fractions (mainly polar ones) tended to be more unsaturated and affected by the high DHA and EPA percentages. Remarkably high proportions of saturated fatty acids were also recorded in the phospholipid molecular species of other parasites such as *Isoparorchis hypselobagri* which infect the catfish *Wallago attuand* (Mondal and Dey, 2013) and *Paratenuisentis ambiguous,* an intestinal helminth parasite in eels (Aitzetmüller *et al*., 1994). Next to SFA, relatively high proportions of PUFA were also recorded in all lipid molecular species of the two studied parasitic copepods. Interestingly, it was observed that *L. lusci* neutral lipid classes, mainly TAG and FFA were particularly rich in PUFA (~40%), mostly in terms of long-chain FA such as C20:5n-3, C22:5n-3 and C22:6n-3. This may reflect a direct diversion of these essential FA from the host by the adult female and their probable incorporation in their egg strings. In fact, these fatty acids are known for their key roles in the reproductive success, development, and somatic growth of copepods as reported by Arendt *et al.* (2005). It is worth noting that the high unsaturation level in FFA could also be considered as an indicator of a good conser-

vation of the acyl lipids during the storage and/or extraction process. High proportions of PUFA (up to 40% of the total FAs) with substantial amounts of DHA and EPA were also recorded in *P. cylindricum* phospholipid classes, chiefly in PC and PE. This pattern, reflecting the importance of PUFAs as essential structural components of the cell membrane phospholipids, was also reported by Tocher *et al*. (2010) in the parasitic female lice and its egg strings. According to several authors, these components are involved in various physiological functions such as the modulation of physicochemical properties of the membrane, ion exchange and transport, and cell signaling (Tocher, 2003). Furthermore, it has been reported that some C20-PUFAs such as ARA and EPA, recognized as main precursor molecules for eicosanoids, are thought to be involved in the modulation/ suppression of the host's immune responses, which is crucial for parasite survival (Fast *et al*., 2004; Tocher *et al*., 2010).

The scarce available literature on the lipid profile of parasitic copepods has pointed out that neutral lipids (principally TAG) are characterized by high levels of MUFA (Lee, 1975; Tocher *et al*., 2010). This pattern contrasts with that observed in our study where monoenes were found to not exceed 20% of the total FAs in all lipid classes of both *L. lusci* and *P. cylindricum*. This seems to be reflective of the host's lipid profiles, where MUFAs were found to compose approximately 20% of the total FAs across almost all the analyzed lipid classes. According to our results, the MUFA group was mainly comprised of oleic acid (C18:1n-9). This FA, known as a potential metabolic energy source (Tocher, 2003), has been found to occur in high proportions in several free and parasitic forms of marine copepods (Lee, 1975; Tocher *et al*., 2010; Escribano and Pérez, 2010). Furthermore, this FA is generally considered a trophic marker for omnivorous and carnivorous copepods (Dalsgaard *et al*., 2003). Although the main source of oleic acid is food intake, its synthesis *de novo* or by conversion of 18:0 was also reported in copepods (Kattner *et al*., 1994).

To better visualize the trophic connection within each parasite-host system, hierarchical clustering analysis was applied. It was found that *L. lusci* lipid classes clustered with the neutral lipids of its host *M. merluccius,* which suggested that they were closely related. However, the lipid classes of *P. cylindricum* linked more with the phospholipids of its host *S. pilchardus*. These observations could be attributed to several parameters such as the host/parasite species, the fixation site, and the ability of each parasite to modulate its FA composition.

Overall, our data revealed that although the two studied host-parasite systems exhibited differences concerning the abundance of lipid classes as well as the distribution of some of their fatty acids, they appeared to share general similar FA patterns due to the strong trophic connection between them. Yet, to be more conclusive, further investigations (taking apart the eggs from the adult females) are needed to go deeper into the knowledge of their molecular biology and biochemistry in order to better understand host-parasite interactions.

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## REFERENCES

Aitzetmüller K, Taraschewski H, Filipponi C, Werner G, Weber N. 1994. Lipids of fish parasites and their hosts: fatty acids of phospholipids of *Paratenuisentis ambiguus* and its host eel (*Anguilla anguilla*). *Comp. Biochem. Physiol*. **109**, 383–389. https://doi.org/10.1016/0305-0491(94)90021-3.

Arendt KE, Jonasdottir SH, Hansen PJ, Gartner S. 2005. Effects of dietary fatty acids on the reproductive success of the calanoid copepod *Temora longicornis*. *Mar. Biol*. **146**, 513–530. https://doi.org/10.1007/s00227-004-1457-9

Becheikh S , Rousset V, Maamouri F, Ben Hassine OK, Raibaut A. 1997. Pathological effects of *Peroderma cylindricum* (Copepoda: Pennellidae) on the kidneys of its pilchard host, *Sardina pilchardus* (Osteichthyes: Clupeidae), from Tunisian coasts. *Dis. Aquat. Org*. **28**, 51–59. https://doi.org/10.3354/dao028051

Brooker AJ, Shinn AP, Bron JE. 2007. A review of the biology of the parasitic copepod *Lernaeocera branchialis* (L. 1767) (Copepoda: Pennellidae). *Adv. Parasitol*. **65**, 297–341. https://doi.org/10.1016/S0065-308X(07)65005-2

Cecchi G, Basini S, Castano C. 1985. Méthanolyse rapide des huiles en solvant. *Rev. Fr .Corps Gras* **4**, 163–164.

Dalsgaard J, John M, Kattner G, Mueller-Navarra D, Hagen W. 2003. Fatty acid trophic markers in the pelagic marine environment. *Adv. Mar. Biol*. **46**, 225–340. https://doi.org/10.1016/S0065-2881(03)46005-7.

Dufourc EJ. 2008. Sterols and membrane dynamics. *J. Chem. Biol*. **1**, 63–77. https://doi.org/10.1007/s12154-008-0010-6

Díaz M, Dópido R, Gómez T, Rodríguez C. 2016. Membrane lipid microenvironment modulates thermodynamic properties of the Na+-K+-ATPase in branchial and intestinal epithelia in euryhaline fish in vivo. *Front. Physiol*. **7**, 589. https://doi: 10.3389/fphys.2016.00589.

Escribano R, Pérez C. 2010. Variability in fatty acids of two marine copepods upon changing food supply in the coastal upwelling zone off Chile: Importance of the picoplankton and nanoplankton fractions. *J. MAR. BIOL. ASSN. UK*. **90**, 301–313. https://doi.org/10.1017/S002531540999083X.

Fast MD, Ross NW, Craft CA, Locke SJ, Mackinnon SL, Johnson SC. 2004. *Lepeophtheirus salmonis*: characterization of prostaglandin E2 in secretory products of the salmon louse by RP-HPLC and mass spectrometry. *Exp. Parasitol*. **107**, 5–13. https://doi.org/10.1016/j.exppara.2004.04.001

Folch J, Lees M, Sloane-Stanley GA. 1957. A simple method for the isolation and purification of total lipids from animal tissues. *J. Biol. Chem*. **226**, 497–509.

Hajji T, Ben Hassine OK, Farrugio H. 1998. Impact du copépode parasite *Peroderma cylindricum* Heller, 1868 sur la croissance et la fécondité des stocks exploités sur la sardine *Sardina pilchardus* (Walbaum, 1792). *Cah. Options Mediterr.* **35**, 79–86.

Hajji T, Telahigue K, Bennour S, Gharbi M, El Cafsi M. 2015. Impact of *Peroderma cylindricum* (Copepoda: Pennellidae) infection on fatty acid composition and lipid quality of Sardine (*Sardina pilchardus*). *J. Parasitol*. **101**, 682–686. https://doi.org/10.1645/15-777.

Hajji T, Telahigue K, Rabeh I, Ben Ammar R, Mdaini Z, El Cafsi M, Ghali R. 2021. Polar and neutral lipid composition of the copepod *Lernaeocera lusci* and its host *Merluccius merluccius* in relationship with the parasite intensity. *Parasitol. Res.* **120**, 1979-1991. http://doi: 10.1007/s00436-021-07182-z. Epub 2021 May 14. PMID: 33987737.

Kabata Z. 1979. *Parasitic Copepoda of British fishes*. The Ray Society, London.

Kattner G, Graeve M, Hagen W. 1994. Ontogenetic and seasonal changes in lipid and fatty acid/alcohol compositions of the dominant Antarctic copepods *Calanus propinquus*, *Calanoides acutus* and *Rhincalanus gigas*. *Mar. Biol*. **118**, 637–644. https://doi.org/10.1007/BF00347511

Kotani Y. 2006. Lipid content and composition of dominant copepods in the Oyashio waters analyzed by the thin layer chromatography flame ionization detection method. *Plankton Benthos Res*. **1**, 85–90. https://doi.org/10.3800/pbr.1.85

Lee RF. 1975. Lipids of parasitic copepods associated with marine fish. *Comp. Biochem.Physiol*. **52**, 363–364. https://doi.org/10.1016/0305-0491(75)90146-7

Lee RF, Hagen W, Kattner G. 2006. Lipid storage in marine zooplankton. *Mar. Ecol. Prog. Ser*. **307**, 273–306. https://doi.org/10.3354/meps307273

Mondal J, Dey C. 2013. Lipid and fatty acid compositions of a trematode, *Isoparorchis hypselobagri* Billet, 1898 (Digenea: Isoparorchiidae) infecting swim bladder of *Wallago attu* in the district North 24-Parganas of West Bengal. *J. Parasit. Dis.***39,** 67–72. https://doi.org/10.1007/s12639-013-0283-8

O'Neal AJ, Butler LR, Rolandelli A, Gilk SD, Pedra JH. 2020. Lipid hijacking: a unifying theme in vector-borne diseases. *ELife*, ***9***, e61675. https://doi.org/10.7554/eLife.61675

Olsen RE, Henderson RJ. 1996. The rapid analysis of neutral and polar marine lipids using double-developed HPTLC and scanning densitometry. *J. Exp. Marine Biol. Ecol*. **129**, 189–197. https://doi.org/10.1016/0022-0981(89)90056-7

R Core Team. 2020. A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing. Retrieved from https://www.R-project.org/

Şen Özdemir N, Parrish CC, Parzanini C, Mercier A. 2019. Neutral and polar lipid fatty acids in five families of demersal and pelagic fish from the deep Northwest Atlantic. *ICES J. Mar. Sci.* **76**, 1807–1815. https://doi:10.1093/icesjms/fsz054.

Taraschewski H, Aitzetmüller K, Werner G, Kühs K. 1995. Lipids of fish parasites and their hosts: fatty acid fingerprints of four species of acanthocephalans and of their hosts' intestinal tissues. *Parasitol. Res.* **81,** 522–526. https://doi.org/10.1007/BF00931796

Telahigue K, Hajji T, Gharbi M, Cherif A, El Cafsi M. 2017. The parasitic copepod *Peroderma cylindricum* Heller, 1865 (Copepoda: Pennellidae) and its host *Sardina pilchardus* (Walbaum, 1792): trophic relationships as revealed by fatty acid profiles. *J. Crustacean Biol.* **37**, 453–457. https://doi.org/10.1093/jcbiol/rux054

Telahigue K, Rabeh I, Chetoui I, Bejaoui S, El Cafsi M, Hajji T. 2019. To what extent are hake fat and its oil quality affected by the parasite *Lernaeocera lusci*? *Grasas y Aceites* **70**, e297. https://doi.org/10.3989/gya.0697181

Tocher DR. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Rev. Fisheries Sci.* **11**, 107–184. https://doi.org/10.1080/713610925

Tocher JA, Dick JR, Bron JE, Shinn AP, Tocher DR. 2010. Lipid and fatty acid composition of parasitic caligid copepods belonging to the genus *Lepeophtheirus*. *Comp. Biochem. Physiol*. **156**, 107–114. https://doi.org/10.1016/j.cbpb. 2010.02.010

Tracey TJ, Steyn FJ, Wolvetang EJ, Ngo ST. 2018. Neuronal lipid metabolism: Multiple pathways driving functional outcomes in health and disease. *Front. Mol. Neurosci*. **11**, 10. https://doi.org/10.3389/fnmol.2018.00010

Vallochi AL, Teixeira L, Oliveira KDS, Maya-Monteiro CM, Bozza PT. 2018. Lipid Droplet, a Key Player in Host-Parasite Interactions. *Front. Immunol*. **9**, 1022. https://doi.org/10.3389/fimmu.2018.01022

van der Meeren T, Olsen R, Hamre K, Fyhn H. 2008. Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish. *Aquaculture* **274**, 375–397. https://doi.org/10.1016/j.aquaculture.2007.11.041

van Damme PA, Ollevier F, Hamerlynck O. 1994. Pathogenicity of *Lernaeocera lusci* and *L. branchialis* in bib and whiting in the North Sea. *Dis. Aquat. Organ*. **19**, 61–65. http://dx.doi.org/10.3354/dao019061

Zhao S, Guo Y, Sheng Q, Shyr Y. 2014. Heatmap3: an improved heatmap package with more powerful and convenient features. *BMC Bioinformatics* **15**, 16.

Zhou L, Yang F, Zhao M, Zhang M, Liu J, Marchioni E. 2020. Determination and comparison of phospholipid profiles in eggs from seven different species using UHPLC-ESI-Triple TOF-MS. *Food Chem*. **339**, 127856. https://doi.org/10.1016/j.foodchem.2020.127856.