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Abstract

Lipids play a crucial role in polar regions and are of particular importance in early life stages of Antarctic fish. This work presents the significance of lipids and fatty acids (FA) in the early life stages of the icefish *Chionodraco hamatus*. Analysis of lipid classes (Polar lipids, LP; Cholesterol, Chol; and Triacyglycerol, TAG) suggested different energy allocation strategies in preflexion (<25 mm) and postflexion larvae (>25 mm). Structural PL dominated lipid dynamics for preflexion larvae, indicating that small individuals allocate the majority of energy toward somatic growth. Conversely, postflexion larvae appear able to switch between growth (contribution of PL) and energy storage (contribution of TAG) strategies. The condition index

ratio TAG:Chol varied from 0.2 to <2 with no differences between the two larval stages. Further, FA composition of the TAG and PL fractions suggests that both developmental stages share the same carnivorous diet and that *C. hamatus* relies on a few key prey items.

Key-words: Larvae, fatty acids, nutritional condition, diet, Antarctica

Introduction

In the pelagic ecosystem of the Southern Ocean, icefish (Channichthyidae, 'white-blooded' fish) form a family within the perciform suborder Notothenioidei, the most predominant suborder of demersal Antarctic fishes (Kock 2005). Early life stages of most species of channichthyids have been described (Efremenko 1987; Kellermann and Schadwinkel 1991) and their distribution and relative abundance reviewed elsewhere (La Mesa and Ashford 2008; Loeb et al. 1993). At hatching, larvae of channichthyids are relatively large (13–17 mm), suggesting greater likelihood of survival compared to smaller larvae of other Antarctic fishes (Kock 2005). In addition, larval channichthyids exhibit relatively fast growth rates and feed primarily on young stages of euphausiids and larval nototheniids (Kellermann 1990; La Mesa et al. 2011). Although most channichthyids are demersal-benthic as adults, larvae and postlarvae are typically pelagic. The most common channichthyid within the pelagic communities of the Ross and Dumont D'Urville Sea are larval and juvenile *Chionodraco sp.* (Granata et al. 2002; North 1988). The genus *Chionodraco* includes *C. hamatus*, *C. myersi*, and *C. rastrospinosus*, with *C. hamatus* and *C. myersi* previously identified from the Dumont d'Urville Sea (Koubbi et al. 2010). As the taxonomic identification of larval stages of *Chionodraco sp*. is still unclear (Kellermann 1990), most authors base larval identification on the co-occurrence of adults in the

same area (Hubold 1990; Moteki and Ishimaru 2008). In the Dumont d'Urville Sea, adult *C. hamatus* are overwhelmingly more common than adult *C. myersi* (Koubbi et al. 2009), and some barcoded larvae have been identified as *C. hamatus* (Dettai, pers. comm.). Therefore, larvae of the genus *Chionodraco* are referred to as *C. hamatus* in this study.

The life history and biology for early life stages of *C. hamatus* remain poorly understood. This species has been reported to spawn during summer in the Ross Sea (La Mesa et al. 2003; Vacchi et al. 1996) and demonstrates nesting behavior (Ferrando et al. 2014). Hatching time is not known, although presumably occurs in summer/autumn because few reproductively active females have been observed in the coastal zone near the Dumont d'Urville station in January (Koubbi, pers. comm.).

In polar regions, lipids and their constituent FA play major roles in growth, movement, buoyancy, and reproduction, and also represent the main energy reserves of many polar species (Sargent et al. 2002). Lipid class dynamics reflect the ability of fish larvae to withstand starvation events and can be used to determine a lipid-based condition index (Fraser 1989; Giraldo et al. 2012; Håkanson 1989). The lipid condition index is based on the principal assumption that larval condition in many marine organisms is dependent upon lipid energy reserves, which typically correspond to triacylglycerol (TAG) content. TAG content is dependent on larval size or body mass and must be standardized by cholesterol (Chol) content; Chol is a membrane lipid that is correlated with larval size but is independent of nutritional condition because it is not catabolized during starvation (Fraser 1989).

The relationship between the storage-class lipids (TAG) and the structural membrane lipids (polar lipids, PL) to total lipids (TL) also reflects the main metabolic strategy of larvae and the tradeoff between energy allocation toward growth and/or lipid storage. Further, FA

composition of the TAG fraction directly reflects FA composition of the diet, and differences in FA composition between different organisms or developmental stages therefore reflect dietary similarities/differences (Dalsgaard et al. 2003). In contrast, FA composition of the PL fraction is under genetic control (i.e. independent of diet) and can be used to determine ontogenetic changes within an organism (Giraldo et al. 2015a).

In this study, the lipid composition and dynamics of *C. hamatus* are analyzed to (1) determine lipid composition and the role of lipids in early life strategies of *C. hamatus*, and (2) provide an overview of the lipid class-specific FA composition of *C. hamatus* that can be used to determine trophic pathways in the East-Antarctic pelagic ecosystem.

Materials and Methods

a. Sampling

Field sampling was conducted in the Dumont d'Urville Sea (East Antarctica) during the austral summer 2010-2011 as part of the French IPEV-ICO²TA program (Integrated Coastal Ocean Observations in Terre Adélie) (Koubbi et al. 2011). Larval fish samples were collected from the RV *"L'Astrolabe"* using an Isaacs-Kidd midwater trawl (IKMT) at four stations along transects oriented from nearshore to across the continental shelf located from the Mertz Glacier Tongue to the Adelie Bank. All samples (n =26) were immediately frozen in liquid nitrogen (-196°C) and stored at -80°C until further analysis. Back to our laboratory, samples were thawed on ice, weighted (degree of precision 0.01 mg) and measured to the nearest 0.1 mm with a digital caliper (standard length, SL) before analysis. Two larval developmental stages were differentiated based on flexion and corresponded to specimens smaller (pre-flexion, n = 16) and larger (post-flextion, n = 10) than 25mm. Preflexion larvae were defined as having a straight notochord with depleted yolk reserves. The second group corresponded to postflexion larvae and was characterized by an

angled notochord with partially developed anal and dorsal fins. These two distinct larval stages were subsequently separated for further lipid analysis.

b. Lipid analysis

Lipid extraction followed the method of Bligh and Dyer (1959) as modified by Mayzaud et al. (2007). Samples were mechanically homogenized and twice-extracted with a one-phase solvent mixture of methanol:chloroform:water (2:1:0.8 v/v/v). Phases were separated overnight by addition of chloroform and NaCl 0.7 % (w/v) to a final solvent ratio of 2:2:1.8 methanol:chloroform:water (v/v/v). The total extract was vacuum concentrated using a rotary evaporator and stored under nitrogen at –80°C in the laboratory.

TL content was determined gravimetrically. Lipid classes were quantified using a chromatographic separation coupled with FID (Flame Photometry Detection) detection on an Iatroscan MK V TH 10. TL extracts were applied to SIII chromarods using a SAS A4100 autospotter programmed to deliver 1 µl of chloroform extract per rod. Analyses were performed in triplicate. Lipid classes were separated by chromatography using a double development procedure with the following solvent systems: n-hexane:benzene:formic acid 80:20:1 (v/v/v) followed by n-hexane:diethyl ether:formic acid 97:3:1.5 (v/v/v). The FID was calibrated for each compound class using commercial standards. For FA analysis, lipid classes were isolated by thin layer chromatography with the hexane:diethyl ether:acetic acid 170:30:2.5 (v/v/v), and the band of PL was then scraped off from the origin and eluted. Lipid classes were visualized using dichlorofluorescein and identification was achieved by comparison with standard mixtures. Following identification, the TAG fraction was then scraped off and eluted. FA from PL and TAG were subsequently converted into methyl esters with 7% boron trifluoride in methanol

(Morrison & Smith 1964). A control thin layer chromatography was performed to verify the efficiency of the trans methylation procedure.

Gas chromatography (GC) of all FA methyl esters (FAME) was carried out on a 30 m (length) x 0.32 mm (width) internal diameter quartz capillary column coated with Famewax (Restek) in a Perkin-Elmer XL Autolab GC equipped with FID. The column was operated isothermally at 185°C for FAME. Helium was used as carrier gas at 7 psig. Injector and detector were maintained at 250°C. Individual components were identified by comparing retention time data with those obtained from laboratory standards (capelin:menhaden oils, 50:50). In addition, FAME samples were hydrogenated to confirm FA determination. The level of accuracy was ±3% for major components, 1–9% for intermediate components, and up to ±25% for minor components (<0.5% of total FA). In order to have enough material for GC analysis, two to three preflexion larvae were pooled together according to their TAG:Chol ratio. Postflexion larvae were analyzed individually.

c. Statistics

Linear regression of log-transformed values was used to assess the relationship between TL, WW and SL. Contribution of main lipid classes (i.e. Chol, PL and TAG) to TL was assessed using linear regression on standardized values (µg lipid class per mg WW). Correspondence analysis (CA) (Benzécri et al. 1973) has been widely applied to analyze ecological data (Giraldo et al. 2015b) and was chosen here to describe the total inertia in the multi-dimensional FA data as a sample of fewer dimensions that best visualizes patterns in the data. The FA matrix was transformed to relative frequencies and scaled such that each row (or column) can be viewed as a row (or column) of conditional probability distribution. Distances between profiles were

computed with X^2 metrics. This distance gives symmetry to the 2 sets of data such that each factorial axis of the cloud of variables corresponds to a factorial axis of the cloud of observations. Thus, it was possible to represent both descriptors and observations on the plane defined by the factorial axes. The percent variance explained is given for each analysis. Symbol size is proportional to the cosine^2, illustrating the quality of representation for each point. Computations were performed in the statistical software package R ver. 3.1.0 (R Team Core 2012). Data were transformed to normalize (Zar 1999).

Results

Size, weight and lipid components

The SL of *C. hamatus* (n = 26) ranged from 19.3 to 35.1 mm with WW from 31 to 249 mg. The regression between WW and SL was described by a log-log function (Eq. 1):

$$(1)\ log\ (WW) = -2.1 + 2.9 * log\ (SL),\quad F = 100.4,\ df = 24\ p < 0.001,\ R^2 = 0.80$$

TL content varied from 1.1 to 2.1% (of WW) and was positive correlated with WW and SL, as described by the following equations (Eq. 2 & Eq. 3):

$$(2)\ log\ (TL) = -2.0 + 1.1 * log\ (WW),\ F = 584.4,\ df = 24,\ p < 0.001,\ R^2 = 0.96$$

$$(3)\ log\ (TL) = -4.3 + 3.2 * log\ (SL),\ F = 76.2,\ df = 24,\ p < 0.001,\ R^2 = 0.75$$

Chol and PL content were used to indicate the mass of an individual. A relatively good linear fit (Fig. 1) shows that these lipid components are indeed indicative of larval weight (Eq. 4 & Eq. 5):

$$(4)\ log\ (Chol) = -1.70 + 1.59 * log\ (WW),\ F = 69.1,\ df = 24,\ p < 0.001,\ R^2 = 0.73$$

$$(5)\ log\ (LP) = 0.36 + 1.39 * log\ (WW),\ F = 205.8,\ df = 24,\ p < 0.001,\ R2 = 0.89$$

PL were the major constituent in both larval stages (96.2 ± 1.7% and 94.1% ± 2.5% for pre-flexion and post-flexion, respectively) followed by Chol (1.96 ± 0.6 % and 2.70 ± 1.1%) and TAG (1.5 ± 0.2 % and 3.0 ± 1.5%). In some samples free FA and diacylglycerols were identified but represented less than 1% of TL. Wax esters were not present.

Lipid dynamics

The contribution of the main lipid classes (i.e. PL, Chol and TG) to TL content was analyzed by standardizing lipids (µg) by WW (mg). In preflexion larvae, PL and Chol were the dominant constituent influencing changes in TL (*Linear regression F = 24470 and 30.84, respectively; df =14, p < 0.001*), while TAG levels were low relative to other lipid fractions and did not contribute significantly to TL content (*Linear regression, F = 0.55, df =14, p = 0.46*) (Fig. 2). Similarly, PL dominated TL in postflexion larvae, but all lipid classes contributed significantly to changes in TL (*Linear regression F = 2039, 37.15, 34.6 for PL, Chol and TAG respectively, df =8, p < 0.001*) (Fig. 3).

Condition index TG:Chol

Although there was a slightly better fit between PL and WW than between Chol and WW, Chol was used to standardize TAG content because PL can be mobilized during starvation and might therefore be less stable. The TAG:Chol ratio varied between 0.18 to 1.74 in preflexion larvae and from 0.22 to 2.20 in postflexion larvae. No significant differences were detected for TAG:Chol ratios (*Wilcoxon test, W= 45, p > 0.05*) between the two larval stages.

Fatty acid signature

Polar lipids

Overall, saturated (~26%) and polyunsaturated FA (PUFA, ~45%) dominated the FA composition of the PL fraction of each larval stage (Table 1). For all developmental stages, saturated FAs were dominated by palmitic acid (16:0) and, to a lesser extent, by stearic acid (18:0). Monoenoic acids (~23%) were dominated by oleic (18:1n-9), vaccenic (18:1n-7) and palmitoleic (16:1n-7), while PUFA were dominated by EPA (20:5n-3) and DHA (22:6n-3). Correspondence analysis explained 52% of the variance between the FA of preflexion and postflexion larvae. Individuals were distributed around the centroid of the analysis with no clear differences between the two stages (Fig 4).

Triacylglycerols

Monoenoic (~28%) and PUFA (~36%) dominated the TAG fraction of both preflexion and postflexion larvae (Table 1). For all developmental stages, saturated FAs were dominated by palmitic acid (16:0), monoenoic acids were dominated by oleic (18:1n-9), palmitoleic (16:1n-7) and vaccenic acid (18:1n-7), and PUFA were dominated by EPA (20:5n-3) and DHA (22:6n-3). Correspondence analysis explained 73.49% of variance in FA signature of the TAG fraction of preflexion and postflexion larvae. However, all samples fell within the centroid of the analysis, indicating no marked differences in FA composition between the two larval stages.

Discussion

Larval development in many marine organisms is largely dependent upon energy reserves, which typically correspond with TAG content. Starvation and predation represent major causes of mortality in larval fishes, particularly once yolk reserves are depleted and larvae must switch to deriving energy from exogenous sources (Leggett and Deblois 1994). Predation risk is lower in individuals with larger body size, and fish larvae are therefore confronted with a

tradeoff between allocating energy reserves to optimize growth, reduce predation risk and increase the opportunity to consume larger prey items, versus storing TAG to increase the likelihood of survival during starvation events. In this study, the dominance of polar structural lipids (PL) compared to TAG content in preflexion larvae (< 25 mm) of *C. hamatus* indicates that small individuals primarily allocate energy reserves toward somatic growth. Conversely, the important contribution of both TAG and PL to TL dynamics of older individuals (> 25 mm, postflexion larvae) suggests an ability to allocate energy toward both growth and lipid storage reserves because the benefits of allocating energy toward growth decrease with increase in body size. Overall, the results of this study highlight a marked ontogeny of energy allocation that likely decreases predation risk for small larvae and favors lipid storage in postlarval *C. hamatus*.

Ratios of TAG:Chol suggested no significant differences in condition factor of pre- and postflexion larvae, indicating that both stages were in good condition and that prey availability during the summer 2011 was sufficient to meet the basal requirements of *C. hamatus* individuals from this study.

PL are known to be strongly regulated and appear to be directly related to membrane requirements and specific energetic pathways (reviewed by Sargent et al., 2002 and Dalsgaard et al., 2003). Although the lipid class dynamics of preflexion and postflexion *C. hamatus* larvae were different, neither of these developmental stages exhibited any detectable ontogeny in the FA pattern of PL and TAG fractions. High levels of DHA with a mean EPA: DHA ratio of 0.6 ± 0.05 reflects the role of polyunsaturated n-3 FA in the maintenance of biological membranes in *C. hamatus* larvae. In contrast to PL, the FA composition of TAG is related to trophic interactions in marine organisms (Dalsgaard et al. 2003; Mayzaud et al. 2011). In this study, there were no significant differences between the pattern of FA trophic markers for pre- and

post- larval *C. hamatus*, indicating similar diet across larval stages. Specifically, early life stages of *C. hamatus* were characterized by low contribution of *Calanus*-type copepod markers (ΣC20:1, C22:1) and a relatively high value of the ratio C18:1n-9/C18:1n-7, a typical carnivory index in marine trophic ecology (Hagen et al. 2000). This finding is consistent with previous work demonstrating that young stages of euphausiids (furcilia to juveniles) and fish larvae constitute the most important food source for channichthyids larvae (Kock 2005).

Early life stages of the pelagic fish, *Pleuragramma antarctica* (Antarctic Silverfish), one of the most important key species of Antarctica (along with the krill *Euphausia superba*) (Corsolini et al. 2002) may be of particular importance in the diet of *C. hamatus*. Early life stages of *C. hamatus* have been previously reported as specialized predators in the Ross Sea, feeding on a relatively narrow niche width, with *P. antarctica* larvae as their overwhelmingly dominant prey (La Mesa et al. 2011). Compared to the carnivory index previously described for *P. antarctica* larvae (1.34 and 1.06) (Mayzaud et al. 2011, Tavernier et al. 2012), the relative high value (1.93) of the ratio C18:1n-9/C18:1n-7 reported for *C. hamatus* is in good agreement with previous studies. Moreover, stable nitrogen signatures ($\delta^{15}N$), mainly used to establish trophic relationships, have been reported as 10.5 ‰ for *C. hamatus* larvae (SL= 31-38 mm) (Cherel et al. 2011) and 6.7 ‰ for larval *P. antarctica* (Giraldo et al. 2011). Considering that, on average, a ~3‰ enrichment in $\delta^{15}N$ values accompanies each trophic step, these results also support the hypothesis that larval *P. antarctica* could be the main prey item for *C. hamatus* larvae. The hatching time of *C. hamatus* and *P. antarctica* are thought to coincide. Further, *C. hamatus* and *P. antarctica* co-occur spatially and have a similar geographic distribution in the western Ross Sea (La Mesa et al. 2010) and offshore of the Antarctic Peninsula (White and North 1987). Future studies could use FA as dietary biomarkers to test the hypothesis that survival of larval *C.*

hamatus is closely linked to spatial and temporal dynamics of young stages of euphausiids and *P. antarctica* larvae.

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Figure 1: Linear regression on log-transformed membrane lipids (Chol: cholesterol, PL: polar lipids, µg) and wet weight (WW).

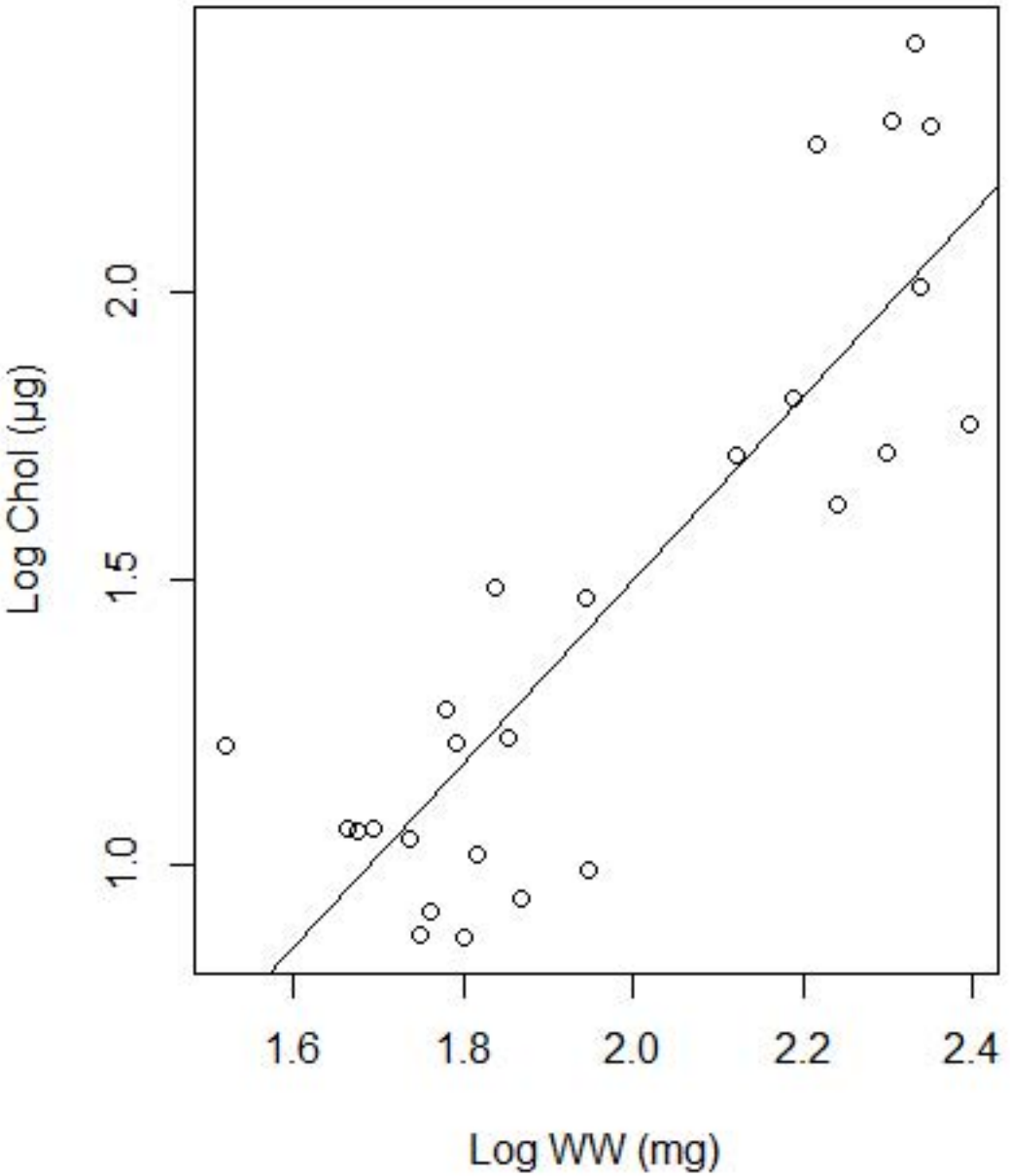
Figure 2: Contribution of the main lipid classes (PL: Polar Lipids, TAG: Triacylglycerol, Chol: Cholesterol) to the total lipid (TL) content of preflexion larvae *C. hamatus*.

Figure 3: Contribution of the main lipid classes (PL: Polar Lipids, TAG: Triacylglycerol, Chol: Cholesterol) to the total lipid (TL) content of postflexion larvae of *C. hamatus*.

Figure 4: Correspondence Analysis of the fatty acid signature of the polar lipid fraction for preflexion larvae (L1-6) and postflexion lavae (P1-10) of *C. hamatus*.

Table 1: Fatty acid composition of the triacylglycerol and polar lipid fraction in pre-flexion and post-flexion larvae. Minor fatty acids (<1%) common for all individuals are not shown. Data is reported as means ± standard deviation.

	Triacylglycerol		Polar Lipids	
Fatty acid/Stage	**Pre - flexion**	**Post - flexion**	**Pre - flexion**	**Post - flexion**
C14	2.49 ± 0.64	2.60 ± 0.54	0.96 ± 0.19	0.97 ± 0.19
C15	0.47 ± 0.22	0.30 ± 0.04	0.18 ± 0.02	0.18 ± 0.01
ISO17	0.76 ± 0.12	0.84 ± 0.11	0.53 ± 0.08	0.55 ± 0.07
ANT17	0.20 ± 0.06	0.21 ± 0.06	0.14 ± 0.05	0.10 ± 0.05
C16	12.88 ± 0.77	11.82 ± 0.37	20.39 ± 0.49	20.21 ± 1.37
C17	2.27 ± 0.36	2.88 ± 0.23	0.26 ± 0.03	0.25 ± 0.04
C18	3.11 ± 0.34	2.79 ± 0.22	3.70 ± 0.25	3.72 ± 0.29
Σ Saturates	23.17 ± 1.27	22.15 ± 0.86	26.43 ± 0.43	26.27 ± 1.62
C16:1n7	7.32 ± 0.56	7.02 ± 0.47	3.91 ± 0.16	3.95 ± 0.35
C16:1n5	0.81 ± 0.15	0.85 ± 0.15	1.03 ± 0.11	1.00 ± 0.10
C18:1n9	13.98 ± 1.28	14.11 ± 1.29	7.21 ± 0.24	7.92 ± 0.71
C18:1n7	7.75 ± 1.33	7.28 ± 0.95	8.08 ± 0.50	7.83 ± 1.01
C18:1n5	0.95 ± 0.03	0.97 ± 0.19	0.75 ± 0.05	0.79 ± 0.08
C20:1n9	1.09 ± 0.24	0.90 ± 0.32	1.10 ± 0.11	1.09 ± 0.11
C20:1n7	0.36 ± 0.42	0.21 ± 0.03	0.16 ± 0.03	0.17 ± 0.03
C24:1n9	0.23 ± 0.10	0.20 ± 0.09	0.16 ± 0.03	0.21 ± 0.05
Σ Monoenes	33.72 ± 1.78	32.56 ± 1.61	22.73 ± 0.28	23.29 ± 1.57
C18:2n7	0.30 ± 0.24	0.37 ± 0.29	0.11 ± 0.03	0.13 ± 0.03
C18:2n6	2.28 ± 0.22	2.42 ± 0.10	1.15 ± 0.11	1.34 ± 0.13
Σ Dienes	2.79 ± 0.13	3.06 ± 0.39	1.51 ± 0.15	1.76 ± 0.20
C16:3n6	0.54 ± 0.17	0.64 ± 0.11	0.32 ± 0.06	0.38 ± 0.07
C16:3n3	0.43 ± 0.04	0.42 ± 0.02	0.25 ± 0.05	0.27 ± 0.05
C18:3n6	1.41 ± 0.78	0.57 ± 0.13	0.17 ± 0.06	0.14 ± 0.07
C18:3n3	0.98 ± 0.37	1.23 ± 0.13	0.40 ± 0.13	0.37 ± 0.14
Σ Trienes	3.46 ± 0.76	2.97 ± 0.26	1.22 ± 0.22	1.21 ± 0.21
C16:4n3	0.37 ± 0.08	0.36 ± 0.11	0.36 ± 0.05	0.37 ± 0.06
C18:4n3	1.93 ± 0.66	2.77 ± 0.35	0.49 ± 0.14	0.58 ± 0.09
C20:4n6	0.62 ± 0.11	0.63 ± 0.09	1.36 ± 0.14	1.35 ± 0.10
Σ Tetraene	3.36 ± 0.69	4.14 ± 0.36	2.41 ± 0.08	2.44 ± 0.11
C20:5n3	13.6 ± 0.69	14.85 ± 0.59	16.77 ± 0.89	16.26 ± 1.23
C22:5n3	0.71 ± 0.06	0.68 ± 0.08	0.76 ± 0.07	0.60 ± 0.04
C22:6n3	18.58 ± 1.03	19.24 ± 1.34	28.02 ± 1.09	28.03 ± 2.16
Σ PUFA	33.51 ± 1.90	35.12 ± 1.74	45.55 ± 0.64	44.89 ± 2.97


Log Chol (µg)
1.0
1.5
2.0
1.6
1.8
2.0
2.2
2.4
Log WW (mg)

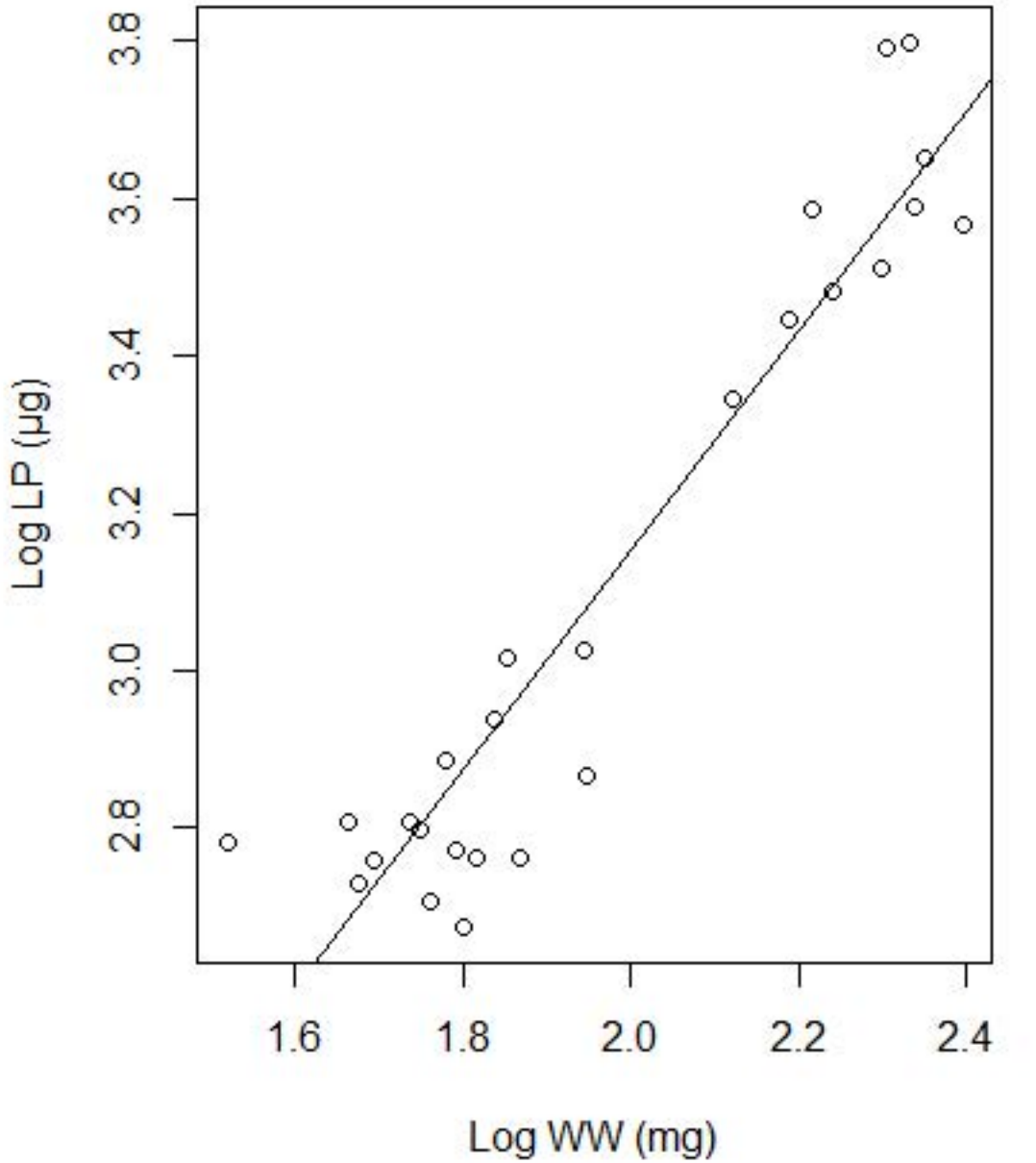

Log LP (µg)
2.8
3.0
3.2
3.4
3.6
3.8
1.6
1.8
2.0
2.2
2.4
Log WW (mg)

Figure 1

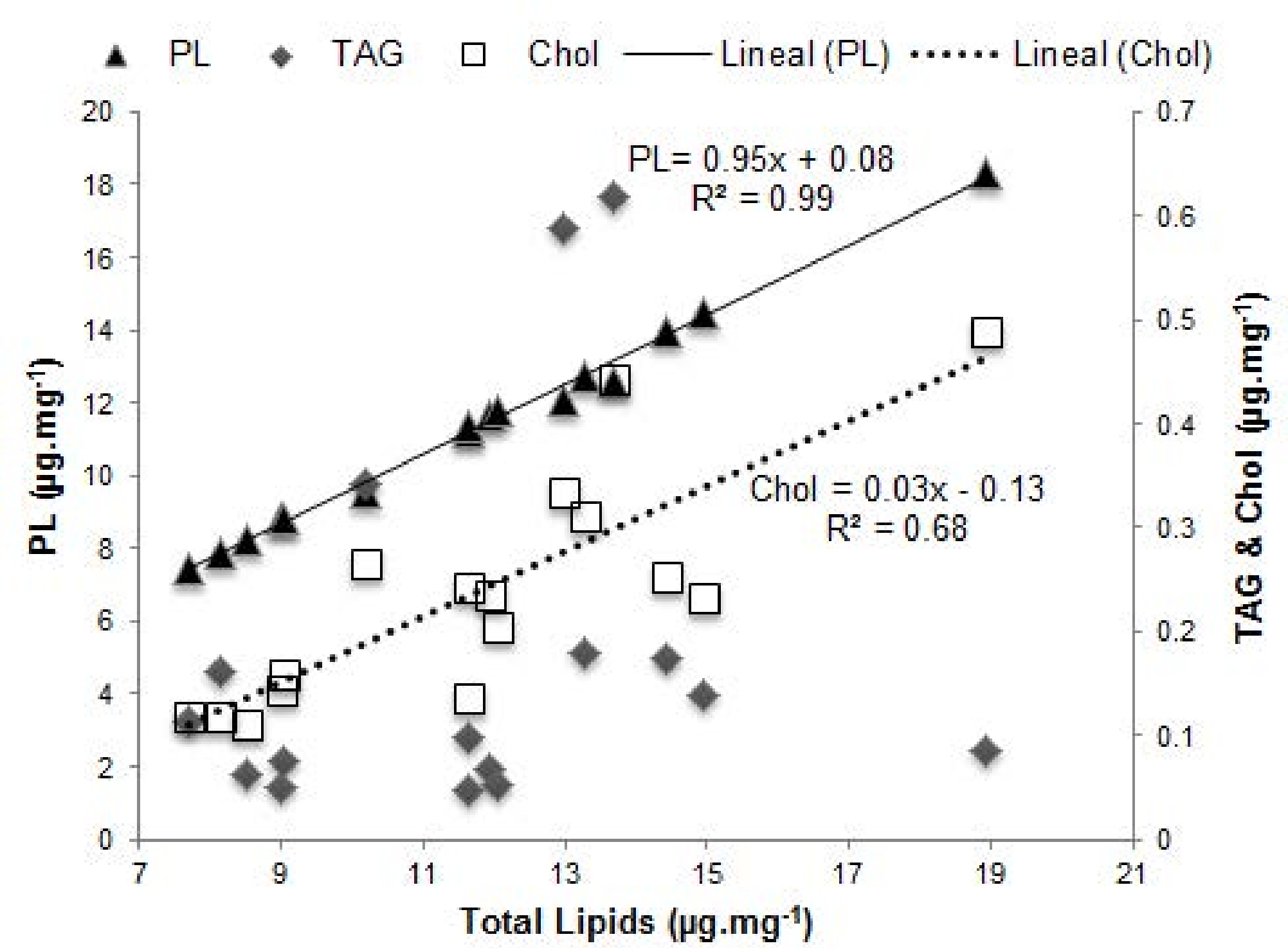

PL
TAG
Chol
Lineal (PL)
Lineal (Chol)
PL= 0.95x + 0.08
R² = 0.99
Chol = 0.03x - 0.13
R² = 0.68
PL (µg.mg-1)
TAG & Chol (µg.mg-1)
Total Lipids (µg.mg-1)
0
2
4
6
8
10
12
14
16
18
20
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
7
9
11
13
15
17
19
21

Figure 2

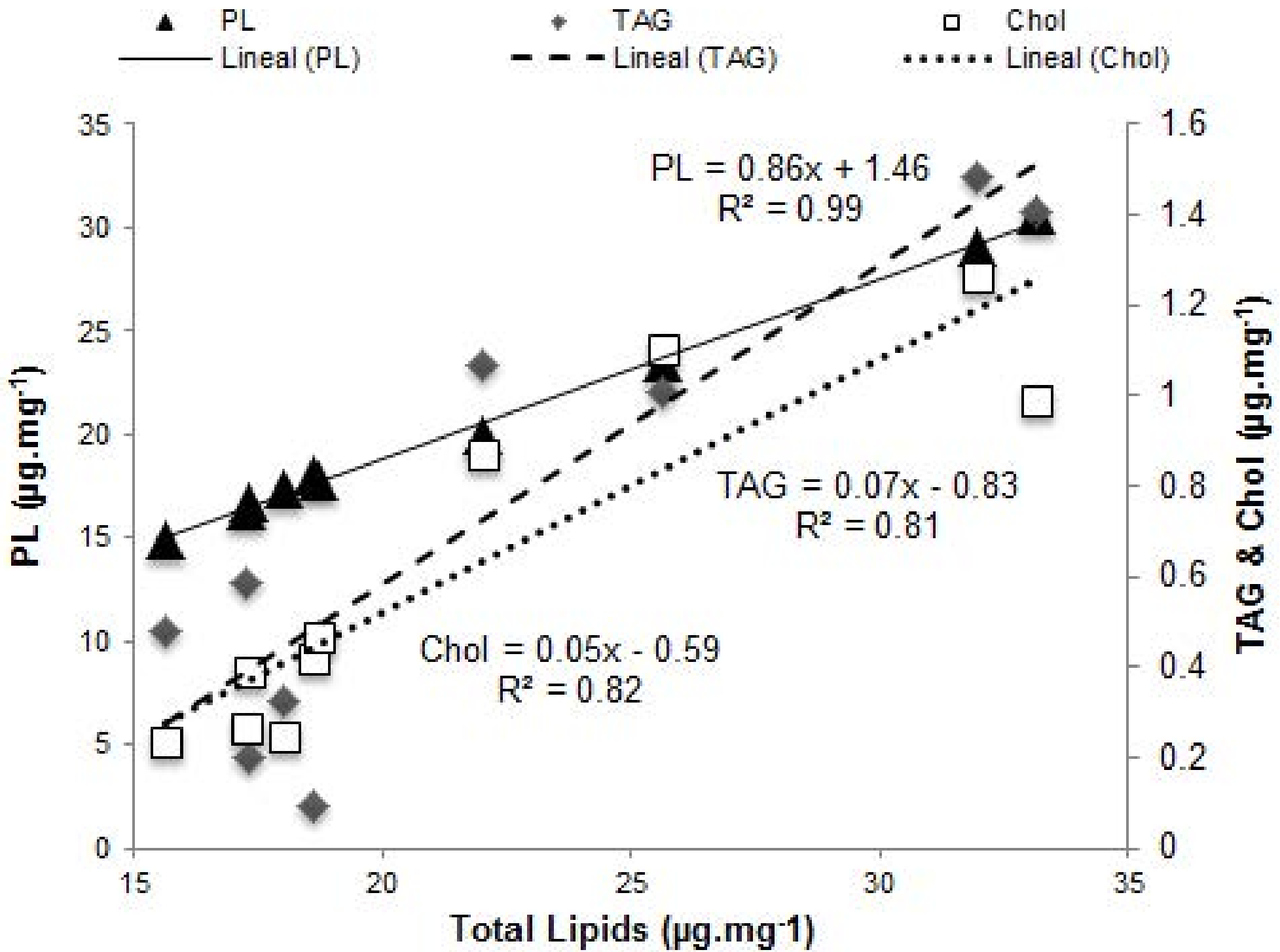

PL
TAG
Chol
Lineal (PL)
Lineal (TAG)
Lineal (Chol)
PL = 0.86x + 1.46
R² = 0.99
TAG = 0.07x - 0.83
R² = 0.81
Chol = 0.05x - 0.59
R² = 0.82
PL (µg.mg-1)
TAG & Chol (µg.mg-1)
Total Lipids (µg.mg-1)
0
5
10
15
20
25
30
35
0
0.2
0.4
0.6
0.8
1
1.2
1.4
1.6
15
20
25
30
35

Figure 3

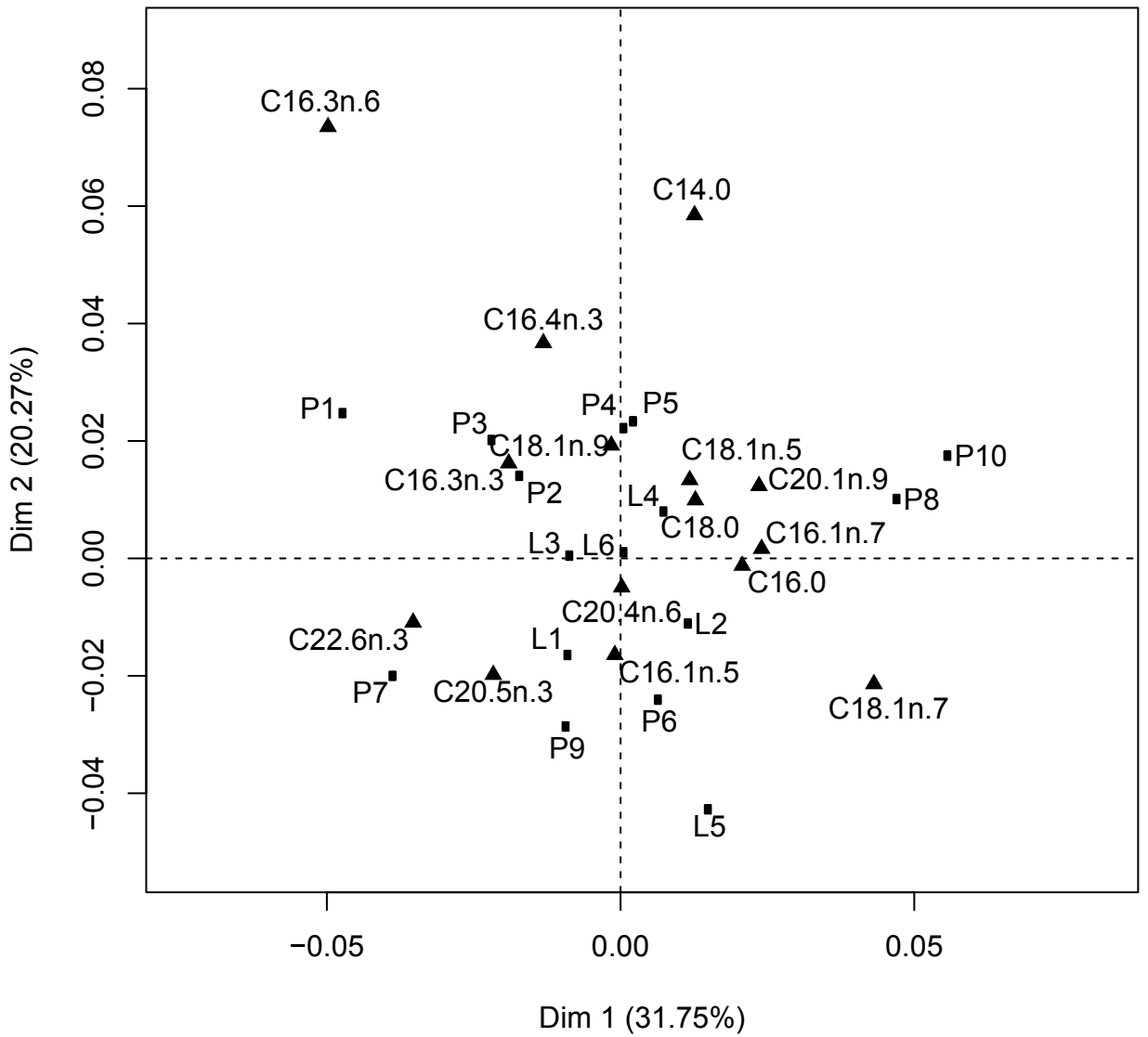

C16.3n.6
C14.0
C16.4n.3
P1
P4
P5
P3
C18.1n.9
C18.1n.5
P10
C16.3n.3
P2
C20.1n.9
L4
P8
C18.0
C16.1n.7
L3
L6
C16.0
C20.4n.6
L2
C22.6n.3
L1
C16.1n.5
P7
C20.5n.3
P6
C18.1n.7
P9
L5
0.08
0.06
0.04
0.02
0.00
−0.02
−0.04
Dim 2 (20.27%)
−0.05
0.00
0.05
Dim 1 (31.75%)

Figure 4