Accepted refereed manuscript of: Cavrois Rogacki T, Davie A, King E, Esnault S, Migaud H & Monroig O (2019) Short-term lecithin enrichments can enhance the phospholipid and DHA contents of the polar lipid fraction of Artemia nauplii. *Aquaculture*, 510, pp. 122-130. DOI: https://doi.org/10.1016/j.aquaculture.2019.05.041

Short-term lecithin enrichments can enhance the phospholipid and DHA contents of the polar lipid fraction of *Artemia* nauplii

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Abstract

Wild copepods are the main natural diet of marine finfish and they meet the larvae's requirements in phospholipids and essential fatty acids (EFA). While *Artemia* nauplii are an easier and more reliable live feed to produce in hatcheries for marine fish larvae than wild zooplankton, enrichment products commercially used lack phospholipids and essential long-chain polyunsaturated fatty acids (LC-PUFA). This is particularly true for docosahexaenoic acid (DHA) within their polar lipid fraction (PL_{DHA}), which is critical to the survival and good development of the larvae. In this study, we showed that it is possible to increase the levels of phospholipids and DHA within the PL fraction of *Artemia* nauplii using marine lecithin through a process referred to as "boosting". A cheaper alternative to marine lecithin, soya lecithin, was also tested but resulted only in a significant increase of the phospholipid content of the nauplii with no positive effect on the essential LC-PUFA levels, due to the absence of LC-PUFA in the soya lecithin. This study also showed that the levels of PL_{DHA} in the *Artemia* boosted with marine lecithin did not reflect the levels of PL_{DHA} in the lecithin, highlighting there the complexity of the boosting process. Finally, chilling enriched *Artemia* nauplii at 5 ºC for up to 10 h did not impact on their nutritional quality post-enrichment. Ultimately, this study proposes innovative and sound enrichment strategies to produce *Artemia* nauplii rich in EFA and/or PL, similarly to that of the wild copepods' lipid profile.

Keywords: *Artemia*, DHA, enrichment, lecithin, polar lipids.

Introduction

The emergence of new marine fish species in aquaculture is leading to an increase in the demand for high quality fish larvae. The production of marine fish larvae is generally hampered by low survival, poor growth and high occurrence of deformities (Hamre et al., 2013; Holt, 2011). The underlying causes of poor performance in marine fish larvae is multifactorial, however nutrition plays a critical role to ensure normal development of fish larvae (Hamre et al., 2013; Rønnestad et al., 2013). Even though considerable progress has been made on the formulation of artificial larval feeds, most marine hatcheries still rely on the production of live preys used to feed larval stages of marine fish (Conceição et al., 2010). Among those live preys, wild copepods are the natural diet of marine fish larvae (Hunter, 1980) and they exhibit optimal nutritional profiles (Ajiboye et al., 2011; van der Meeren et al., 2008; Støttrup, 2000). However, wild copepods are rarely used in hatcheries as the production is highly seasonal, lacks reliability and presents an elevated risk of introducing pathogens into the hatchery systems (Støttrup, 2000). Although farmed copepods constitute an alternative to their wild counterparts, they are challenging to produce, and the limited supply of high-quality cysts cannot meet the demand of the rapidly expanding marine finfish hatcheries. Therefore, the traditional use of live preys including rotifers and *Artemia* nauplii remains the most common choice to feed larval stages of any established marine finfish species such as Atlantic cod *Gadus morhua* (Hamre et al., 2008; Rocha et al., 2017) and Atlantic halibut *Hippoglossus hippoglossus* (Evjemo et al., 2003) as well as new emerging species like Amberjack *Seriola dumerili* (Papandroulakis et al., 2005; Yamamoto et al., 2008) and ballan wrasse *Labrus bergylta* (Øie et al., 2015).

Arguably, the main disadvantage of using *Artemia* is their poor nutritional profile compared to that of copepods. This is particularly true with regards to essential lipids for marine fish larvae such as the eicosapentaenoic acid (EPA, 20:5n-3) and docosahexaenoic acid (DHA, 22:6n-3) (Hamre et al., 2013; Izquierdo et al., 2000; Tocher, 2010). DHA is of particular

65 importance during early stages of development and it is very abundant in the cell membranes
66 of rapidly forming neural tissues (i.e. eye, brain) (Tocher and Harvie, 1988). EPA is a precursor
67 of 3-series antagonistic prostaglandins that provide a low-inflammatory response (Tocher,
68 2003). The analysis of marine finfish eggs is considered a good proxy of the lipid requirements
69 of the fish. For instance, Atlantic cod and Atlantic halibut eggs contain around 14 % and 28 %
70 of EPA and DHA, respectively (Bell et al., 2003) and copepods contained similar levels of 19
71 % and 29 %, respectively (Hamre, 2016). On the contrary, *Artemia* contain low levels of EPA
72 (< 5 %) and are devoid of DHA (Dhont et al., 2013; Navarro et al., 1999). It has clearly been
73 demonstrated that the DHA/EPA ratio in the *Artemia* should be higher than 2 to fulfil the
74 marine larvae's requirements (Izquierdo et al., 2000; Sargent et al., 1999). Furthermore, the
75 lipid fraction within copepods is characterised by high contents of polar lipids (PL) rich in n-3
76 long-chain ($\geq C_{20}$) polyunsaturated fatty acids (LC-PUFA) including EPA and DHA, with
77 levels of 24 and 41 % of the total fatty acids, respectively (Bell et al., 2003). On the other hand,
78 *Artemia* are naturally rich in neutral lipids (NL) (e.g. triacylglycerol) and therefore have low
79 levels of PL_{EPA} (11.5-14.9 %) and PL_{DHA} (0.6-2.4 %) (Bell et al., 2003). Some of these
80 nutritional deficiencies, particularly in EFA, can be addressed to a certain extent through the
81 so-called "bioencapsulation" or "enrichment", which consists of exposing live preys to a
82 culture medium containing essential nutrients that are passively filtered and incorporated into
83 the digestive track of the live preys prior to their use as feed items (Monroig et al., 2003).
84 Standard enrichment protocols have been successfully developed to enhance the LC-PUFA
85 levels of live preys but boosting of PL contents in live preys has been proven to be more elusive
86 (Guinot et al., 2013a; Monroig, 2003). Furthermore, it has been shown that LC-PUFA are more
87 beneficial to the larvae when presented as PL (Cahu et al., 2003b; Gisbert et al., 2005; Kjørsvik
88 et al., 2009; Rainuzzo et al., 1994). However, *Artemia*, rather than simply being passive carriers
89 of enrichment products, can metabolise the PL contained in the enrichment diet into other lipid

90 classes, particularly triacyglycerides (TAG), subsequently metabolising EFA from the PL
91 fraction into the NL fraction (Guinot et al., 2013a; Guinot et al., 2013b; Monroig et al., 2006a,
92 2003; Navarro et al., 1999). The supplementation of the standard enrichment diets with
93 products rich in phospholipids (often referred to as "lecithins") can therefore compensate for
94 the inefficient bioencapsulation of PL within live preys (Guinot et al., 2013a; Monroig et al.,
95 2006a). Lecithins are typically composed of phosphatidylcholine (PC) as the most abundant
96 phospholipid class, and the fatty acid composition varies depending on their origin and whether
97 it is marine (e.g. krill lecithin) or terrestrial (e.g. soya lecithin). Finally, to avoid the loss of the
98 *Artemia* nutritional value post-enrichment, hatcheries cold store the enriched nauplii at 5 to 10
99 ºC. However, there is a lack of evidence on the fate of the essential lipids (i.e. EFA, PL)
100 contained in the enriched nauplii following a short-term cold storage.

101 This study aimed to develop enrichment protocols that enable the marine fish hatcheries
102 to produce high quality *Artemia* nauplii that have enhanced levels of PL, while still
103 guaranteeing the provision of EFA. First, an experiment was carried out to benchmark four
104 commercially available enrichment products commonly used for *Artemia* enrichment in marine
105 fish hatcheries (Exp. 1). Based on their enrichment efficiencies, the best performing enrichment
106 product in terms of DHA content was subsequently used in a second experiment aiming at
107 enhancing the PL content of enriched *Artemia* via a method referred to as "boosting" (Barr et
108 al., 2005) involving a short-term incubation with phospholipid sources conducted after the
109 standard enrichment process (Exp. 2). Finally, the effects of short-term cold storage on *Artemia*
110 LC-PUFA content were also investigated in order to determine if a common practice in marine
111 finfish hatcheries can impact on the nutritional profile of enriched *Artemia*.

112

Materials and Methods

Artemia hatching and culture

Artemia cysts GSL (EG, Inve, Belgium) were decapsulated and hatched according to Sorgeloos et al. (2001). Enrichments were performed in 1-litre Imhoff cones with vigorous aeration from the bottom and constant light (light intensity at water surface: 47,000 lux, 1.78 W m^{-2}). The cones were filled with 32 ppt artificial seawater (Instant Ocean, USA) previously disinfected with Pyceze® (0.05 ml l^{-1}) and placed in a 28 °C water bath. After the 24 h hatching process, the nauplii were collected on a 100 µm sieve, rinsed with freshwater, and distributed in each cone at 300 nauplii ml^{-1} for further enrichment.

Experiment 1: Benchmarking of Artemia enrichment products

The four commercial enrichment products, commonly used in marine finfish hatcheries, were Larviva Multigain (MG, BioMar, Denmark), Ori-Go (OG, Skretting, Norway), Red Pepper (RP, Bernaqua, France) and Easy DHA Selco (SEL, Inve, Belgium). Enrichment diets stocks were prepared by emulsifying the required quantity of enrichment products in 1 litre of artificial seawater for 3 min using a domestic blender. The required volume of enrichment was then distributed to each 1-litre Imhoff cone filled with 800 ml of artificial seawater, resulting in concentrations of 0.6 g l^{-1} (MG, SEL and OG) and 1.5 g l^{-1} (RP), which was chosen based on ranging studies to keep the enrichment level within context of commercial guidelines but also to ensure comparable total lipid loading within enriched *Artemia* (Tables 1 and 3). Newly hatched *Artemia* nauplii were stocked in each cone at a density of 300 nauplii ml^{-1}. Samples of enriched nauplii (24 h) were collected by concentrating them on a 100 µm sieve, thoroughly rinsed with freshwater to remove the excess of enrichment and gently dried on absorbent tissue and transferred to 15 ml plastic tubes. All samples were immediately frozen at -20 °C upon collection and subsequently freeze-dried and stored at -20 ºC for further analysis.

Experiment 2: Boosting Artemia nauplii with phospholipids

The enrichment product that showed the highest level of DHA and DHA/EPA ratio in *Artemia* nauplii in Exp. 1 (i.e. Larviva Multigain) was selected for Exp. 2, which aimed at increasing the PL contents of *Artemia* nauplii. Newly hatched *Artemia* nauplii were initially enriched with MG (0.6 g l^{-1}) for 22 h in nine 1-litre Imhoff cones at a nauplii density of 300 nauplii ml^{-1} (Table 1). Three cones were left under the same conditions for 2 h, thus resulting in 24 h enrichment with MG. Nauplii from six other cones (two triplicate treatments) were individually collected on a 100 µm sieve, rinsed with freshwater and placed back in the same Imhoff cones containing 800 ml of fresh artificial seawater. Nauplii from three of those cones were then subjected to a short-term (2 h) enrichment ("boosting") with soya lecithin (Optima, UK) (Treatment MG+SL). The three others were boosted with marine lecithin (supplied by BioMar, UK) (Treatment MG+ML). Both soya and marine lecithins were supplied at 0.6 g l^{-1} in the Imhoff cones, after emulsification of the boosting material in 1 litre of artificial seawater for 3 min. At 24 h, *Artemia* samples (approximately half of the population in each cone) from MG and boosting (MG+SL and MG+ML) treatments were collected for analysis as explained above. The other half of the enriched nauplii population was maintained at 5 ºC for 10 h in gently aerated seawater ("chilling") prior to sample collection. This procedure aimed to simulate the standard production practice in marine finfish hatcheries whereby enriched *Artemia* are chilled below 10 °C to preserve their nutritional quality prior to being offered later in the daily feeding schedule. Overall, final sample set from Experiment 2 included enriched nauplii before boosting (22 h), enriched nauplii after boosting (24 h) and chilled nauplii (34 h). All samples were immediately frozen at -20 °C upon collection and subsequently freeze-dried and stored at -20 ºC until further analysis.

Nutritional analysis

Total lipids (TL) from enrichment products and lecithins, as well as freeze-dried *Artemia* samples collected from Exp. 1 and 2 were extracted according to Folch et al. (1957), with modifications as described by Monroig et al. (2006a). In order to analyse the fatty acid profiles of total polar lipids (PL) and total neutral lipids (NL) in Larviva Multigain, lecithins and *Artemia* nauplii in Exp. 2, these fractions were separated by loading 300 µl of TL solutions (*circa* 3 mg) onto a 20 x 20 cm silica gel thin-layer chromatography plate (Merck, Germany). The plate was run with a solvent mixture made of isohexane:diethylether:acetic acid (80/20/1, v/v/v) and subsequently sprayed with 2,7-dichlorofluorescein (0.1 %) dissolved in aqueous methanol (97 %, v/v). PL and NL were visualised under UV light, scrapped off the plate and transferred to separate test tubes. Fatty acid methyl esters (FAME) from TL, PL and NL were prepared, extracted and purified according to (Christie, 2003). Identification and quantification was carried out using a gas chromatograph (Thermo Trace GC Ultra, Thermo Electron Corporation, USA) as described by Houston et al. (2017).

To calculate the percentage of PL and NL of the samples, lipid class analysis was conducted according to Henderson and Tocher (1992). Lipid classes were separated by double-development, high-performance thin-layer chromatography (HPTLC). Total lipid samples (1-2 µg) were applied and the plates developed in methyl acetate/isopropanol/chloroform/methanol/0.25 % aqueous KCl (25:25:25:10:9, v/v). Excess solvent was evaporated via air drying and vacuum desiccation and plates developed using a solvent mixture containing isohexane/diethyl ether/acetic acid (85:15:1.5, v/v). Lipid classes were visualised by spraying with 3 % (w/v) aqueous cupric acetate containing 8 % (v/v) phosphoric acid and charring plates at 160 °C for 25 min. Lipid classes were quantified by densitometry using a CAMAG-3 TLC scanner (version Firmware 1.14.16; CAMAG, Muttenz, Switzerland).

To estimate total phospholipid content (Guinot et al., 2013b), total phosphorus was determined after digestion of freeze-dried *Artemia* samples in nitric acid (69 %) in a microwave (MARSXpress, CEM) for 40 min (20 min ramping to 120 ºC and 20 min holding that temperature). Digests were transferred into a volumetric flask and made up into x 25 dilutions with distilled water. Samples were analysed by Inductively Coupled Plasma Mass Spectrometry (ICP-MS, Thermo Scientific Model X Series 2, USA) as described by Smedley et al. (2016).

Statistical analysis

Enrichment diets used in Exp. 1 were analysed in technical duplicates and are expressed as means ± standard deviations (SD) (n = 2). All other data are expressed as means ± SD (n = 3). Percentage data were transformed using the arcsine square root function. Normality and homogeneity of variance in the data were confirmed using Shapiro-Wilk and Levene's tests, respectively. Data were analysed by one or two-way ANOVA followed by a Tukey's post-hoc test at a significance level of $P < 0.05$. Individual fatty acid absolute values (i.e. mg FA g^{-1}) are not presented in this paper but where nevertheless analysed similarly to that of relative values (i.e. % FA). Statistical differences for both values were the same unless specified otherwise. Phosphorus levels at 22 h and 24 h for each treatment were compared with a paired t-test ($T <$ 0.05). All data were analysed using SPSS (IBM SPSS Statistics 23, NY, USA).

Results

Experiment 1: benchmarking of Artemia enrichment products

The total lipid content of the enrichment products greatly varied, ranging from 147.6 ± 12.0 (RP) to 589.9 ± 2.0 mg g^{-1} (OG) (Table 2). The total fatty acid contents also greatly varied, ranging from 139.1 ± 21.7 (RP) to 376.2 ± 16.8 mg g^{-1} (SEL). In terms of EFA, the levels of

ARA were similar across products, ranging from 1.2 ± 0.0 (MG) to 1.9 ± 0.0 % (RP). The levels of both EPA and DHA considerably varied, ranging from 0.8 ± 0.0 % (MG) to 7.5 ± 0.1 % (SEL) and from 21.3 ± 0.1 % (SEL) to 36.6 ± 0.3 % (MG), respectively (Table 2).

The total lipid content of the 24 h enriched nauplii did not vary significantly between the commercial enrichment products tested, ranging between 264.3 ± 19.4 (RP) and 287.5 ± 15.0 mg g^{-1} (SEL) (Table 3). The polar lipid fraction was significantly lower in the nauplii enriched with OG (19.7 ± 2.3 %) while it was comparable between the other enrichments ranging between 31.0 ± 1.3 % (MG) and 34.7 ± 2.6 % (SEL). In terms of EFA, there were significant differences in the levels of ARA, EPA and DHA in relation to the enrichment products (Table 3). Nauplii enriched with MG showed the highest levels of DHA (21.8 ± 0.7 %), which were 25, 59 and 102 % higher than in the nauplii RP, OG and SEL, respectively. Interestingly, these DHA levels in the nauplii reflected the ones found in the corresponding enrichment products (Table 2). Furthermore, DHA/EPA ratios were all above 2, except for SEL (1.3 ± 0.0), and nauplii enriched with MG exhibited a significantly higher DHA/EPA ratio (3.8 ± 0.1). Regarding the other EFA, ARA levels were lowest for nauplii enriched with OG and SEL (1.5 ± 0.1 and 1.5 ± 0.0 %, respectively), then MG (2.9 ± 0.0 %) and lastly RP (3.1 ± 0.0 %). EPA levels were highest in SEL (8.1 ± 0.3 %) and lowest in MG (5.8 ± 0.2 %).

Experiment 2: Boosting Artemia nauplii with phospholipids

Prior to their use to boost phospholipid contents in *Artemia*, we analysed the two sources of phospholipids used in the present study, namely soya lecithin (SL) and marine lecithin (ML) (Tables 2 and 4). The total lipid content of both soya and marine lecithins varied from 801.3 ± 10.1 (ML) to 864.1 ± 4.9 mg g^{-1} (SL) (Table 2). The PL fraction of the soya lecithin accounted for more than 86.2 ± 3.1 % of the total and in the case of the marine lecithin for 37.7 ± 1.1 % (Table 4). In terms of EFA, soya lecithin is devoid of ARA, EPA and DHA whereas the levels

found in ML were 0.8 ± 0.0, 10.0 ± 0.4 and 19.1 ± 1.0 %, respectively, of total fatty acids (Table 2). When determining the location of EFA within NL or PL fractions of ML, ARA was found in both fractions (1.1 ± 0.2 and 0.6 ± 0.0 % for PL and NL, respectively). EPA levels were also found in both fractions of ML at levels of 9.8 %, while DHA was exclusively located in the PL fraction of ML (30.9 ± 0.3 %). On the contrary, DHA was mostly present in the NL fraction of MG (7.6 ± 0.2 and 43.8 ± 0.2 % for PL and NL, respectively) (Table 4).

Total phosphorus (P), showed a significant increase in all three treatment groups between prior (22 h) and post (24 h) the boosting (Fig. 1). The change in P content pre- and post-boosting, calculated as a percentage increase, was higher in the two treatments with lecithin sources such as soya lecithin (MG+SL, 16.7 % increase) and marine lecithin (MG+ML, 11.2 %). When 24 h nauplii from all three treatments were compared, P content was significantly higher in the *Artemia* nauplii enriched with soya lecithin (MG+SL) in comparison to the nauplii enriched with just MG (Fig. 1).

Total lipid contents of *Artemia* nauplii enriched for 24 h in Exp. 2 were not affected by either the chilling or the enrichment while total fatty acids were noted to significantly reduce in the MG+ML treatment in response to the 10hr chilling (Table 5). EPA, ARA and DHA levels were not significantly different in relation to enrichment protocols or in response to the chilling process. Equally, DHA/EPA ratios were not affected by either the chilling or the enrichment, ranging between 1.9 and 2.4.

The inclusion of marine lecithin significantly decreased the PL_{ARA} in the nauplii (2.5 ± 0.3 %) compared to that of the nauplii enriched with only MG (3.2 ± 0.2 %) (Table 6). However, the same marine lecithin significantly increased the PL_{EPA} of the nauplii (8.0 ± 0.2 %) compared to that of the nauplii MG (6.7 ± 0.5 %) or MG+SL (5.9 ± 0.1 %) as well as the PL_{DHA} in the nauplii enriched with MG+ML (10.6 ± 1.3 %) compared to MG (3.4 ± 0.7 %) or MG+SL

256 (3.3 ± 0.4 %) (Table 6). Furthermore, the proportion of DHA in the PL and NL fractions (i.e.
257 PL_{DHA}/NL_{DHA} ratio) was more than 5 times higher in the nauplii MG+ML (1.63) compared to
258 that of the nauplii MG (0.24) and MG+SL (0.30). As for chilling, the levels of PL_{ARA}, PL_{EPA}
259 and PL_{n-3} were significantly increased after the chilling phase in all treatments while PL_{DHA}
260 remained unchanged. With regards to the NL fraction, the enrichments did not significantly
261 affect the levels of the EFA within the neutral lipids. However, NL_{EPA} and NL_{DHA} of nauplii
262 enriched with MG+ML increased significantly (>two-fold) following chilling (Table 6).

Discussion

Marine finfish larvae production is constrained by low survival, developmental impairment and deformities that are often attributed to deficiencies in essential lipids such as phospholipids and LC-PUFA in larval diets (Cahu et al., 2003a; Hamre et al., 2013; Izquierdo et al., 2000). Based on the lipid composition of wild copepods, dietary provision of EFA, particularly DHA in the form of PL, would be critical to normal development and survival of marine fish larvae (Gisbert et al., 2005; Tocher et al., 2008). The benefits of feeding marine fish larvae with copepods compared to other live preys (i.e. rotifers, *Artemia*) have been extensively reported (Karlsen et al., 2015; Øie et al., 2015; Støttrup, 2000). However availability of wild copepods for marine fish hatcheries is very limited and seasonal (Støttrup, 2000). Consequently, it is crucial to develop sound enrichment strategies for live preys such as *Artemia* resulting in EFA-rich PL, thus mimicking the copepod's lipid profile. The copepod's lipid composition is mainly characterised by high contents of PL including high levels of DHA (van der Meeren et al., 2008). Increasing PL_{DHA} can be achieved in microdiets by including marine ingredients (Gisbert et al., 2005) but it has been proven difficult in live preys such as *Artemia* due to the limited efficiency of the enrichment process, along with the occurrence of undesired metabolic conversions that live preys exert on enrichment products (Ando et al., 2004; Ando and Narukawa, 2002; Ando and Oomi, 2001; Navarro et al., 1999; Shiozaki and Ando, 2005). The present study showed that boosting a commercial *Artemia* enrichment using soya lecithin can increase the nauplii's phospholipids level while boosting with marine lecithin results in nauplii presenting similar EFA and/or PL profiles to that of copepods.

The importance of EFA including ARA, EPA and DHA on growth and development of marine fish larvae has been previously reported (Bell et al., 1986; Rainuzzo et al., 1997; Tocher, 2010). DHA is very important during the early developmental stages since cell membranes of rapidly forming neural tissues (i.e. eye, brain) are particularly rich in DHA (Tocher and Harvie,

1988). This is especially true for marine fish larvae, compared to freshwater species, which generally have a low capacity for *de novo* synthesis of DHA (Castro et al., 2016) and therefore are almost exclusively dependent upon dietary DHA input from live preys. The first experiment of the present research consisted in a benchmark study of commercially available enrichment products for *Artemia* which are commonly used in marine finfish hatcheries. Our results showed that following correction to comparable levels of total lipid enrichment, *Artemia* enriched with MG exhibited the highest DHA content (i.e. 21.8 ± 0.7 %), reaching comparable levels as those found by Boglino et al. (2012) (i.e. 16.9 ± 2.0 %) where a similar *Artemia* enrichment protocol using MG was used. Interestingly, the observed DHA contents in *Artemia* nauplii were twice as high as those reported in the literature when using DHA-rich enrichment products (Viciano et al., 2015). This reflects the high DHA content of MG compared to other enrichments used in this study or in the literature. These results confirmed that DHA content is highly variable between enrichment products (Monroig et al., 2006b; Sorgeloos et al., 2001). Interestingly, the levels of DHA in the nauplii did not reflect those of the enrichment products themselves and where lower in all enriched nauplii compared to the respective enrichment product. An increase in EPA was also found in *Artemia* nauplii enriched with MG (5.8 ± 0.2 %), despite a low EPA content in MG (0.8 ± 0.0 %). This was very likely due to the "retroconversion", a metabolic process demonstrated in *Artemia* by which DHA is converted into EPA (Guinot et al., 2013a; Han et al., 2001; Navarro et al., 1999; Viciano et al., 2017). However, the MG enrichment product still delivered the highest DHA/EPA ratio (i.e. 3.8 ± 0.1 %) of all treatments. It should be noted that while results are presented in terms of % total lipid, the total lipid and fatty acid contents in the enriched nauplii were comparable across treatments thus if the results are considered in an absolute basis (i.e. mg FA g^{-1}) the same conclusion is found (data not shown). Finally, the PL content in the nauplii MG was among the highest ones

312 (31.0 ± 1.3 %), including the nauplii RP and SEL. Based on these results, MG was selected as
313 the enrichment product for Exp. 2.

314 Exp. 2 aimed to boost *Artemia* nauplii in PL after standard MG enrichment and compare
315 the efficiency of marine (37.7 ± 1.1 % PL and 30.9 ± 0.3 % PL_{DHA}) vs. soya (86.2 ± 3.1 % PL
316 and no PL_{DHA}) lecithins. As opposed to the studies by Guinot et al. (2013a, 2013b) and Monroig
317 et al. (2003, 2006a, 2006b), which adopted the use of liposomes to deliver phospholipids, this
318 current study trialled a technically simpler alternative method to boost phospholipids and
319 essential fatty acids in *Artemia* nauplii. Our results showed that soya lecithin was able to
320 significantly enhance the PL content of *Artemia* compared to MG. Nevertheless, the PL levels
321 in the MG+ML enriched *Artemia* appeared to be higher than nauplii MG, although not
322 significantly. This is in agreement with findings from previously published studies using
323 slightly different enrichment protocols (Guinot et al., 2013a; Guinot et al., 2013b; Rainuzzo et
324 al., 1994). The present results confirm that the "boosting" first described by Barr et al. (2005)
325 is not only an effective way to increase PL contents in live preys enriched using oil emulsion-
326 based products (e.g. Guinot et al., 2013a,b), but also with spray-dried algae cell products such
327 as Larviva Multigain used in the present study. Clearly, soya lecithin was, as discussed above,
328 an efficient product to enhance the total PL content of MG+SL nauplii but the PL_{DHA} was not
329 increased compared to the MG. On the contrary, the marine lecithin, while not being such an
330 efficient product to enhance total PL in *Artemia* as SL, did significantly increase the PL_{DHA},
331 reaching levels three-fold over those of MG. This work clearly demonstrates that marine
332 lecthins are required to effectively boost DHA within polar lipids, which are regarded as the
333 more bioavailable molecular form to present this EFA to marine larvae and as found in the wild
334 copepods (Bell et al., 2003; Gisbert et al., 2005; Tocher, 2010). Nevertheless, soya lecithin
335 contains good phospholipids (mainly PC) irrespective of EFA, and is 10 to 30 times cheaper
336 than marine lecithin, which may still be of benefit to the hatcheries. Ultimately, the hereby

337 presented enrichment boosting strategies should be implemented in a fish trial to qualify the
338 benefits of the EFA-rich PL *Artemia* on the survival and growth of the larvae.

339 The development of marine fish larviculture depends upon the quality and consistency
340 of the nutritional value of live feeds, which themselves are dependent upon the enrichment
341 protocols. As marine fish larvae are fed throughout the day this requires that hatcheries employ
342 strategies to preserve the nutritional quality of the *Artemia* up to 10 h post enrichment while
343 also assuring that the quality of the enriched live preys is constant from one day to another. In
344 order to achieve this, hatchery procedures often involve cold storage of the enriched live preys
345 to preserve their nutritional value. Results from the present study showed that the cold storage
346 (5 ºC) of enriched *Artemia* for up to 10 h did not have a major effect on the total lipid and EFA
347 composition of the nauplii. However, variations were observed within the polar and neutral
348 lipid fractions. For instance, PL_{EPA} in all treatments increased in the region of 19-29 % after
349 chilling. Although this could have been attributed to the retroconversion of DHA into EPA
350 taking place in the nauplii (Navarro et al., 1999), the levels of PL_{DHA} remained constant after
351 chilling. Another variation was that NL_{EPA} and NL_{DHA} in the nauplii MG+ML increased by
352 more than two fold after chilling with a similar, though not significant, pattern being observed
353 in the nauplii MG and MG+SL. Since it has been shown that good marine larvae growth and
354 survival greatly relies on *Artemia* nauplii containing high levels of PL_{DHA} (Gisbert at al., 2005),
355 attention should be put into methods to compensate or inhibit the relocation of PL_{DHA} into NL
356 (e.g. TAG) in order to preserve the quality of the enriched *Artemia*. While the drivers of the
357 observed change are not fully understood at present, it can be concluded that chilling is an
358 efficient way to preserve the nutritional quality of the nauplii at a total lipid level. However,
359 the differences observed at the polar/neutral lipid levels require further investigation in order
360 to conclude at this level. With regards to the nutritional quality of the live preys from one day
361 to another, an important aspect of the study was that in the *Artemia* nauplii from Exp. 2, the

levels of DHA were remarkably lower (*circa* 50% reduction) compared to the nauplii enriched with MG in Exp. 1, while the same enrichment product and protocol was used, in both experiments. This shows the high variability in *Artemia* enrichment even in a small-scale, highly controlled experimental system. While the enrichment protocol is standardised, other factors such as aeration, water flows in the culture and enrichment cones are more challenging to standardise and could be associated with such variation (Monroig et al., 2006a; Navarro et al., 1999). *Artemia* nauplii intake the enrichment product by filtration, therefore slight changes in the enrichment parameters may affect the naupliar filtering capacity, ultimately affecting the composition of the enriched *Artemia* nauplii. This variability in the enrichment of live feed can be even greater in large-scale systems and this constitutes a major challenge for commercial hatcheries. In order to guarantee the supply of high quality enriched *Artemia*, all aspects of the enrichment protocol must be standardised at all times to maintain optimal live prey nutritional quality in tune with the species nutritional requirements.

In conclusion, when comparing four commercial enrichment products Larviva Multigain produced enriched *Artemia* with the highest levels of DHA, an EFA particularly important for larval stages of marine finfish. When this enrichment is further boosted with either marine or soya lecithin products, the polar lipid fraction is notably increased in the enriched *Artemia*. However, while marine lecithin is a good candidate to boost *Artemia* nauplii enrichment in both PL and EFA with notable elevation of PL_{DHA}, soya lecithin did not increase the EFA content of the PL fraction in comparison to standard enrichment. Overall, this study has demonstrated a technically simple means to significantly enhance phospholipid and/or EFA content of enriched *Artemia* that could benefit marine finfish larviculture.

Acknowledgments

The project and T. Cavrois Rogacki PhD studentship were co-funded by the Scottish Aquaculture Innovation Centre (SAIC) and University of Stirling. The authors are grateful to Daniel Leeming from BioMar for providing the marine lecithin.

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Table 1. Summary of the different treatments tested in Exp. 1 (benchmarking of commercial *Artemia* enrichment products) and Exp. 2 (boosting a commercial *Artemia* enrichment with phospholipids).

	Enrichment	Dose ($g\ l^{-1}$)	Enrichment duration (h)	Enrichment density ($nauplii\ ml^{-1}$)
Experiment 1	Larviva Multigain (MG)	$0.6\ g\ l^{-1}$	24 h	300
	Ori-Go (OG)	$0.6\ g\ l^{-1}$		
	Red Pepper (RP)	$1.5\ g\ l^{-1}$		
	Easy DHA Selco (SEL)	$0.6\ g\ l^{-1}$		
Experiment 2	Larviva Multigain	$0.6\ g\ l^{-1}$	24 h	300
	Larviva Multigain + soya lecithin (MG+SL)	MG $0.6\ g\ l^{-1}$ SL $0.6\ g\ l^{-1}$	22 h 2 h	
	Larviva Multigain + marine lecithin (MG+ML)	MG $0.6\ g\ l^{-1}$ ML $0.6\ g\ l^{-1}$	22 h 2 h	

Table 2. Total lipids and selected fatty acids in the enrichment products (Larviva Multigain, Ori-Go, Red Pepper and Easy DHA Selco) and the lecithins (soya or marine lecithin) used in the enrichment diet preparation for Exp. 1 and 2.

	Enrichment product				Lecithins	
	MG	OG	RP	SEL	SL	ML
Total lipids (mg g^{-1} DW)	397.4 ± 12.8	589.9 ± 2.0	147.6 ± 12.0	352.4 ± 18.7	864.1 ± 4.9	801.5 ± 10.1
Total FA (mg g^{-1} DW)	276.9 ± 6.7	178.2 ± 0.7	139.1 ± 21.7	376.2 ± 16.8	405.9 ± 3.6	459.1 ± 32.7
% of total FA						
14:0	6.1 ± 0.1	1.8 ± 0.1	8.5 ± 0.1	2.9 ± 0.0	0.1 ± 0.0	4.4 ± 0.2
15:0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.7 ± 0.0	ND	0.6 ± 0.0
16:0	32.3 ± 0.4	19.6 ± 0.2	36.7 ± 0.6	17.7 ± 0.0	19.3 ± 0.1	21.8 ± 0.6
18:0	0.9 ± 0.0	5.2 ± 0.2	1.2 ± 0.0	4.8 ± 0.0	3.9 ± 0.0	4.0 ± 0.1
Saturates	40.1 ± 0.5	27.9 ± 0.1	47.3 ± 0.8	26.7 ± 0.0	24.3 ± 0.1	31.3 ± 0.9
16:1n-9	0.3 ± 0.0	2.9 ± 0.3	ND	4.6 ± 0.1	ND	5.5 ± 0.2
16:1n-7	0.1 ± 0.0	0.1 ± 0.0	1.3 ± 0.0	0.2 ± 0.0	ND	0.3 ± 0.0
18:1n-9	1.9 ± 0.0	15.9 ± 0.2	2.2 ± 0.0	19.2 ± 0.1	8.5 ± 0.1	16.0 ± 0.4
18:1n-7	ND	2.1 ± 0.4	0.5 ± 0.0	2.9 ± 0.1	1.4 ± 0.0	2.8 ± 0.1
Monounsaturates	2.3 ± 0.0	23.1 ± 1.1	4.1 ± 0.1	28.7 ± 0.3	10.2 ± 0.1	30.5 ± 0.8
18:2n-6	2.2 ± 0.0	13.5 ± 0.4	4.5 ± 0.1	5.7 ± 0.1	58.1 ± 0.1	1.2 ± 0.0
18:3n-6	0.2 ± 0.0	0.3 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	ND	0.1 ± 0.0
20:4n-6 (ARA)	1.2 ± 0.0	1.4 ± 0.1	1.9 ± 0.0	1.6 ± 0.1	ND	0.8 ± 0.0
22:5n-6	14.4 ± 0.2	1.5 ± 0.1	10.0 ± 0.2	1.2 ± 0.1	ND	0.2 ± 0.0
Total n-6	18.4 ± 0.2	17.3 ± 0.6	16.9 ± 0.3	9.4 ± 0.3	58.2 ± 0.1	2.9 ± 0.1
18:3n-3	0.3 ± 0.0	2.5 ± 0.1	0.3 ± 0.0	1.6 ± 0.0	7.1 ± 0.0	1.1 ± 0.0
18:4n-3	0.3 ± 0.0	0.5 ± 0.3	0.4 ± 0.0	1.0 ± 0.0	ND	2.2 ± 0.0
20:3n-3	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	ND	0.1 ± 0.0
20:4n-3	0.7 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	0.5 ± 0.0	ND	0.7 ± 0.0
20:5n-3 (EPA)	0.8 ± 0.0	4.6 ± 0.9	2.2 ± 0.0	7.5 ± 0.1	ND	10.0 ± 0.4
22:5n-3	0.3 ± 0.0	1.2 ± 0.1	0.4 ± 0.0	1.8 ± 0.0	ND	1.2 ± 0.1
22:6n-3 (DHA)	36.6 ± 0.3	21.6 ± 1.0	28.1 ± 0.1	21.3 ± 0.1	ND	19.1 ± 1.0
Total n-3	39.0 ± 0.4	30.8 ± 0.6	32.1 ± 0.1	33.8 ± 0.0	7.3 ± 0.0	34.4 ± 1.6

DW: dry weight; FA: fatty acids; MG: Larviva Multigain; ML: marine lecithin; ND: not detected; OG: Ori-Go; RP: Red Pepper; SEL: Easy DHA Selco; SL: soya lecithin.

Table 3. Total lipids, polar lipids, neutral lipids and fatty acid profiles in the *Artemia* nauplii from Exp. 1 enriched 24 h with four commercial enrichment products. Data are expressed as means ± standard deviations (n = 3). Superscripts denote significant differences between treatments (one-way ANOVA and Tukey's test, $P < 0.05$).

	MG	OG	RP	SEL
Total lipids (mg g^{-1} DW)	285.1 ± 1.5	278.7 ± 11.1	264.3 ± 19.4	287.5 ± 15.0
Polar lipids (% of TL)	31.0 ± 1.3^{b}	19.7 ± 2.3^{a}	33.8 ± 1.2^{b}	34.7 ± 2.6^{b}
Neutral lipids (% of TL)	69.0 ± 1.3^{a}	80.3 ± 2.3^{b}	66.2 ± 1.2^{a}	65.3 ± 2.6^{a}
Total FA (mg g^{-1} DW)	188.3 ± 13.7	187.9 ± 33.5	184.5 ± 2.4	193.2 ± 17.7
% of total FA				
14:0	1.7 ± 0.1^{c}	0.7 ± 0.0^{a}	2.5 ± 0.1^{d}	1.1 ± 0.0^{b}
15:0	0.2 ± 0.0^{a}	0.2 ± 0.0^{b}	0.2 ± 0.0^{b}	0.3 ± 0.0^{c}
16:0	14.1 ± 0.7^{b}	10.4 ± 0.3^{a}	15.7 ± 0.2^{c}	11.3 ± 0.4^{a}
18:0	3.8 ± 0.1^{a}	4.4 ± 0.2^{bc}	4.0 ± 0.1^{ab}	4.6 ± 0.2^{c}
Saturates	20.0 ± 1.1^{b}	16 ± 0.6^{a}	22.7 ± 0.2^{c}	17.7 ± 0.7^{a}
16:1n-9	0.9 ± 0.0	1.2 ± 1.0	0.4 ± 0.0	2.5 ± 1.6
16:1n-7	ND	1.5 ± 1.3	1.5 ± 0.0	1.1 ± 1.9
18:1n-9	10.9 ± 0.5^{a}	18.4 ± 0.7^{b}	10.4 ± 0.1^{a}	21.9 ± 0.6^{c}
18:1n-7	3.1 ± 0.1^{a}	4.3 ± 0.3^{b}	3.3 ± 0.0^{a}	5.3 ± 0.1^{c}
Monounsaturates	15.3 ± 0.3^{a}	26.5 ± 0.6^{b}	16.1 ± 0.1^{a}	32.1 ± 0.3^{c}
18:2n-6	4.9 ± 0.1^{a}	12.0 ± 0.2^{c}	6.6 ± 0.2^{b}	6.5 ± 0.1^{b}
18:3n-6	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20:4n-6 (ARA)	2.9 ± 0.0^{b}	1.5 ± 0.1^{a}	3.1 ± 0.0^{c}	1.5 ± 0.0^{a}
22:5n-6	8.1 ± 0.3^{d}	1.0 ± 0.2^{b}	5.8 ± 0.0^{c}	0.6 ± 0.0^{a}
Total n-6	16.7 ± 0.2^{c}	15.5 ± 0.2^{b}	16.4 ± 0.2^{c}	9.4 ± 0.1^{a}
18:3n-3	15.5 ± 0.7	16.2 ± 0.9	15.5 ± 0.0	15.7 ± 0.5
18:4n-3	2.4 ± 0.1	2.4 ± 0.1	2.5 ± 0.1	2.4 ± 0.1
20:3n-3	0.7 ± 0.0	0.7 ± 0.0	0.8 ± 0.0	0.7 ± 0.0
20:4n-3	1.0 ± 0.0^{d}	0.7 ± 0.0^{a}	0.9 ± 0.0^{c}	0.8 ± 0.0^{b}
20:5n-3 (EPA)	5.8 ± 0.2^{a}	6.5 ± 0.5^{ab}	6.6 ± 0.1^{b}	8.1 ± 0.3^{c}
22:5n-3	0.4 ± 0.0^{a}	0.9 ± 0.1^{b}	0.4 ± 0.0^{a}	1.1 ± 0.0^{c}
22:6n-3 (DHA)	21.8 ± 0.7^{d}	13.7 ± 1.6^{b}	17.5 ± 0.1^{c}	10.8 ± 0.7^{a}
Total n-3	47.6 ± 0.9^{c}	41.2 ± 1.1^{a}	44.1 ± 0.1^{b}	39.7 ± 0.5^{a}
DHA/EPA	3.8 ± 0.1^{d}	2.1 ± 0.1^{b}	2.7 ± 0.0^{c}	1.3 ± 0.0^{a}

DHA/EPA: docosahexaenoic and eicosapentaenoic fatty acid ratio; DW: dry weight; FA: fatty acids; MG: Larviva Multigain; ND: not detected; OG: Ori-Go; RP: Red Pepper; SEL: Easy DHA.

Table 4. Selected fatty acids in the polar lipid (PL) and neutral lipid (NL) fractions of the Larviva Multigain (MG), soya lecithin and marine lecithin used in Exp. 2.

Products	MG		Soya lecithin		Marine lecithin	
Lipid fraction	PL	NL	PL	NL	PL	NL
% of total lipids	28.6 ± 0.0	71.4 ± 0.0	86.2 ± 3.1	13.8 ± 3.1	37.7 ± 1.1	62.3 ± 1.1
% of total FA						
14:0	0.5 ± 0.0	6.3 ± 0.0	0.1 ± 0.1	2.3 ± 0.0	1.4 ± 0.0	4.5 ± 0.1
15:0	0.2 ± 0.0	0.3 ± 0.0	0.1 ± 0.0	ND	0.4 ± 0.0	0.6 ± 0.0
16:0	56.2 ± 0.1	27.6 ± 0.2	21.1 ± 0.1	25.7 ± 0.1	19.9 ± 0.0	20.2 ± 0.0
18:0	1.9 ± 0.0	0.7 ± 0.1	4.4 ± 0.1	9.6 ± 0.0	5.0 ± 0.1	3.5 ± 0.1
Saturates	59.0 ± 0.1	35.1 ± 0.1	26.7 ± 0.0	37.6 ± 0.1	28.0 ± 0.0	42.8 ± 0.0
16:1n-9	ND	ND	ND	5.3 ± 0.1	2.9 ± 0.0	0.3 ± 0.1
16:1n-7	0.5 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	ND	2.5 ± 0.1	5.8 ± 0.0
18:1n-9	11.8 ± 0.0	0.4 ± 0.0	8.9 ± 0.1	15.2 ± 0.0	11.0 ± 0.0	16.6 ± 0.1
18:1n-7	0.5 ± 0.1	0.1 ± 0.0	1.5 ± 0.1	2.0 ± 0.1	3.1 ± 0.1	2.5 ± 0.0
Monounsaturates	12.9 ± 0.1	0.7 ± 0.1	10.7 ± 0.1	22.6 ± 0.1	30.9 ± 0.0	32.9 ± 0.1
18:2n-6	14.4 ± 0.0	0.5 ± 0.1	55.9 ± 0.0	30.9 ± 0.0	1.0 ± 0.0	1.1 ± 0.1
18:3n-6	ND	0.2 ± 0.0	ND	ND	ND	0.1 ± 0.0
20:4n-6 (ARA)	ND	1.3 ± 0.1	ND	ND	1.1 ± 0.2	0.6 ± 0.0
22:5n-6	1.6 ± 0.0	10.1 ± 0.1	ND	ND	ND	0.2 ± 0.1
Total n-6	16.1 ± 0.1	12.6 ± 0.1	55.9 ± 0.2	33.0 ± 0.0	2.1 ± 0.0.	2.3 ± 0.0
18:3n-3	1.2 ± 0.0	0.1 ± 0.1	6.6 ± 0.2	3.6 ± 0.0	0.5 ± 0.0	1.1 ± 0.0
18:4n-3	0.3 ± 0.1	0.2 ± 0.1	ND	3.2 ± 0.2	0.5 ± 0.0	2.6 ± 0.0
20:3n-3	ND	0.1 ± 0.0	ND	ND	ND	ND
20:4n-3	ND	0.8 ± 0.0	ND	ND	0.4 ± 0.2	0.8 ± 0.1
20:5n-3 (EPA)	0.4 ± 0.1	0.8 ± 0.1	ND	ND	9.8 ± 0.4	9.8 ± 0.0
22:5n-3	ND	0.3 ± 0.0	ND	ND	1.0 ± 0.2	1.3 ± 0.2
22:6n-3 (DHA)	7.6 ± 0.2	41.2 ± 0.1	ND	ND	30.9 ± 0.3	ND
Total n-3	9.7 ± 0.1	43.8 ± 0.2	6.6 ± 0.2	6.8 ± 0.3	43.4 ± 0.1	15.8 ± 0.1

DW: dry weight; FA: fatty acids; ND: not detected.

Table 5. Total lipids and selected fatty acids in the *Artemia* nauplii from Exp. 2 enriched with Larviva Multigain (24 h, MG), Larviva Multigain (22 h) boosted with soya lecithin (2 h) (MG+SL) and Larviva Multigain (22 h) boosted with marine lecithin (2 h) (MG+ML). Lipid and fatty acid profiles from *Artemia* nauplii maintained at 5 °C for 10 h (Chilled) post enrichment are also shown. Data are expressed as means ± standard deviations (n = 3). Data that do not share the same letter among enrichments in the same phase differ significantly and * denotes a significant difference within a same treatment between 24 h and Chilled (two-way ANOVA and Tukey's test, $P < 0.05$).

Phase	24 h			Chilled		
Enrichment	MG	MG+SL	MG+ML	MG	MG+SL	MG+ML
Total lipid (mg g^{-1} DW)	199.7 ± 19.7	277.2 ± 66.8	266.5 ± 26.5	193.0 ± 13.6	197.1 ± 17.1	244.4 ± 57.4
Total fatty acids (mg g^{-1} DW)	128.7 ± 11.9	197.7 ± 71.3	173.0 ± 7.3*	121.3 ± 17.4	122.5 ± 13.4	135.9 ± 26.2*
% of total FA						
14:0	1.5 ± 0.1	1.7 ± 0.6	2.0 ± 0.5	1.4 ± 0.0	1.3 ± 0.0	1.8 ± 0.5
15:0	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.2	0.8 ± 0.1	0.9 ± 0.1
16:0	15.2 ± 1.1	16 ± 1.1	15.5 ± 1.6	14.1 ± 1.2	14.1 ± 0.8	14.7 ± 1.8
18:0	4.7 ± 0.6	4.8 ± 0.4	4.3 ± 0.3	4.8 ± 0.6	4.8 ± 0.4	4.5 ± 0.3
Saturates	22.7 ± 1.7	23.9 ± 1.8	23.1 ± 2.4	21.5 ± 2.1	21.4 ± 1.3	22.4 ± 2.6
16:1n-9	0.5 ± 0.1	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.1	0.5 ± 0.0	0.6 ± 0.0
16:1n-7	1.3 ± 0.2	1.7 ± 1.0	2.0 ± 0.9	1.3 ± 0.3	1.2 ± 0.1	2.1 ± 0.6
18:1n-9	13.2 ± 1.5	14.1 ± 1.5	13.4 ± 1.6	13.7 ± 1.9	13.3 ± 0.6	14.8 ± 1.3
18:1n-7	4.1 ± 0.5	4.0 ± 0.2	3.7 ± 0.2	4.2 ± 0.7	4.0 ± 0.2	4.1 ± 0.5
Monounsaturates	20.1 ± 2.4	21.8 ± 3.8	21.4 ± 3.8	20.7 ± 3.0	20.0 ± 0.9	23.3 ± 2.6
18:2n-6	4.9 ± 0.2	8.7 ± 4.2	5.8 ± 2.9	5.1 ± 0.3^{a}	9.6 ± 2.1^{b}	4.4 ± 0.7^{a}
18:3n-6	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.1
20:4n-6 (ARA)	2.5 ± 0.2	2.1 ± 0.3	2.2 ± 0.3	2.7 ± 0.3	2.5 ± 0.2	2.3 ± 0.3
22:5n-6	5.0 ± 1.6	3.5 ± 1.0	4.6 ± 1.4	4.9 ± 1.7	4.3 ± 1.0	4.0 ± 1.4
Total n-6	13.5 ± 1.7	15.2 ± 5.0	13.5 ± 4.3	13.8 ± 1.9^{ab}	17.4 ± 0.9^{b}	11.7 ± 2.3^{a}
18:3n-3	20.2 ± 2.0	18.1 ± 2.8	16.4 ± 1.8	20.5 ± 2.0	19.5 ± 1.0	17.1 ± 3.2
18:4n-3	2.9 ± 0.2	2.6 ± 0.0	2.6 ± 0.1	2.9 ± 0.3^{b}	2.6 ± 0.1^{a}	2.7 ± 0.2^{ab}
20:3n-3	0.9 ± 0.0^{b}	0.8 ± 0.1^{ab}	0.7 ± 0.1^{a}	0.9 ± 0.0	0.8 ± 0.0	0.8 ± 0.1
20:4n-3	0.9 ± 0.1	0.8 ± 0.0	0.9 ± 0.1	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1
20:5n-3 (EPA)	5.0 ± 0.4	5.1 ± 1.2	6.0 ± 0.8	5.5 ± 0.7	5.2 ± 0.6	6.7 ± 0.8
22:5n-3	0.3 ± 0.1	0.4 ± 0.2	0.5 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.5 ± 0.1
22:6n-3 (DHA)	12.1 ± 4.2	10.1 ± 2.1	13.9 ± 2.0	11.8 ± 4.6	10.5 ± 3.0	12.8 ± 2.8
Total n-3	42.4 ± 2.5	37.9 ± 1.6	40.9 ± 2.7	42.8 ± 3.2	39.8 ± 2.7	41.4 ± 2.8
DHA/EPA	2.4 ± 0.7	2.0 ± 0.3	2.4 ± 0.5	2.1 ± 0.6	2.0 ± 0.4	1.9 ± 0.5

DHA/EPA: docosahexaenoic and eicosapentaenoic fatty acid ratio; DW: dry weight; FA: fatty acids; TL: total lipids.

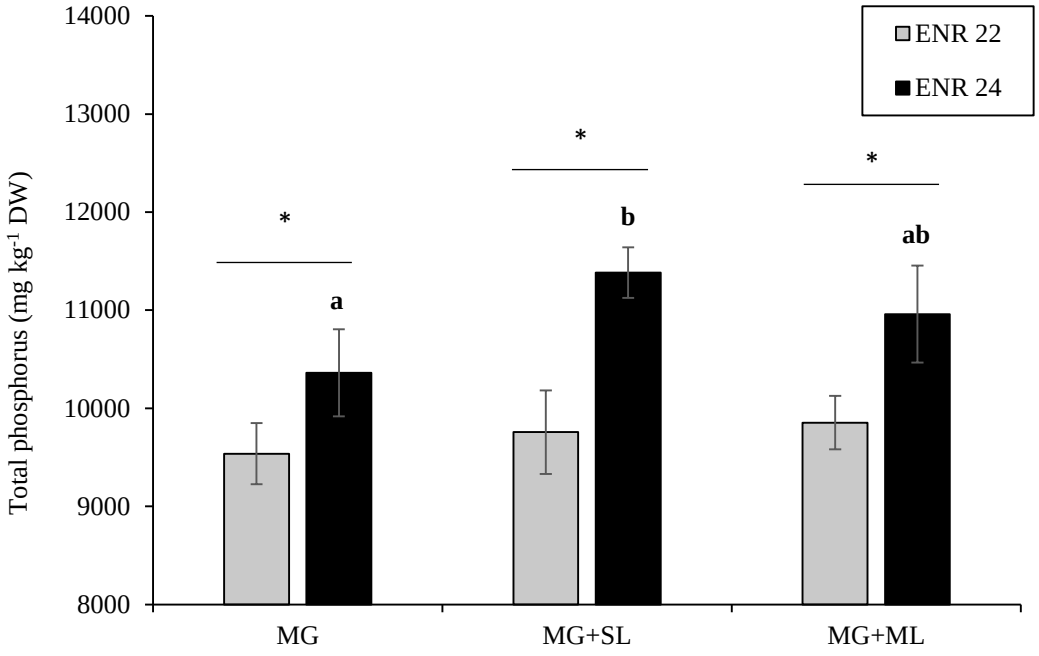
Table 6. Fatty acid levels of total polar lipids (PL) and total neutral lipids (NL) of *Artemia* nauplii from Exp. 2 enriched with Larviva Multigain (24 h, MG), Larviva Multigain (22 h) boosted with soya lecithin (2 h) (MG+SL) and Larviva Multigain (22 h) boosted with marine lecithin (2 h) (MG+ML). Data are expressed as means ± standard deviations (n = 3). Data that do not share the same letter among enrichments in the same phase (24 h or Chilled) differ significantly and * denotes a significant difference within a same treatment between 24 h and Chilled (two-way ANOVA and Tukey's test, $P < 0.05$).

Lipid fraction	PL						NL					
Phase	24 h			Chilled			24 h			Chilled		
Enrichment	MG	MG+SL	MG+ML	MG	MG+SL	MG+ML	MG	MG+SL	MG+ML	MG	MG+SL	MG+ML
% of total FA												
14:0	1.4 ± 0.3	1.3 ± 0.4	1.8 ± 0.2	1.1 ± 0.2	1.0 ± 0.1	1.3 ± 0.2	1.8 ± 0.0^{a}	1.8 ± 0.1^{a}	3.0 ± 0.5^{b}	1.4 ± 0.0	1.3 ± 0.0	1.7 ± 0.5
15:0	0.2 ± 0.0^{ab}	0.2 ± 0.0^{a}	0.3 ± 0.0^{b}	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.1	0.3 ± 0.0^{a}	0.3 ± 0.0^{a}	0.4 ± 0.1^{b}	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.1
16:0	14.8 ± 0.4^{a}	14.7 ± 0.5^{a}	16.4 ± 0.6^{b}	12.2 ± 0.1	12.2 ± 0.4	13.4 ± 1.9	16.7 ± 1.6	17.8 ± 1.7	20.4 ± 1.7	14.9 ± 1.4	14.6 ± 1.3	14.9 ± 1.8
18:0	10.3 ± 0.2	12.2 ± 4.7	8.4 ± 0.2	9.2 ± 0.3	9.0 ± 0.3	8.2 ± 0.8	3.5 ± 0.4	4.0 ± 0.4	4.3 ± 0.3	3.5 ± 0.4	3.4 ± 0.3	3.3 ± 0.2
Saturates	27.5 ± 0.4	29.2 ± 5.7	27.6 ± 0.5	23.5 ± 0.1	23.1 ± 0.6	23.9 ± 1.5	22.6 ± 2.1	24.3 ± 2.1	28.5 ± 2.4	20.3 ± 1.9	19.8 ± 1.7	20.5 ± 2.6
16:1n-9	1.2 ± 0.1^{ab}	0.7 ± 0.5^{a}	2.1 ± 0.2^{b}	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	1.5 ± 0.3	0.9 ± 0.4	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0.0	0.6 ± 0.0
16:1n-7	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.0	1.1 ± 0.0^{a}	1.0 ± 0.1^{a}	1.8 ± 0.5^{b}	0.1 ± 0.0^{a}	0.2 ± 0.0^{a}	3.4 ± 0.8^{b}	1.5 ± 0.3	1.3 ± 0.1	2.4 ± 0.8
18:1n-9	19.4 ± 0.7	14.3 ± 6.2	17.7 ± 0.2	18.7 ± 0.9	17.6 ± 0.7	18.1 ± 1.8	12.4 ± 1.7^{a}	13.5 ± 1.3^{ab}	16.7 ± 1.0^{b}	12.5 ± 1.9	11.9 ± 0.7	13.9 ± 1.5
18:1n-7	8.1 ± 0.3	9.0 ± 2.8	6.8 ± 0.2	7.7 ± 0.1	7.4 ± 0.2	7.2 ± 1.0	3.5 ± 0.6	3.6 ± 0.4	4.1 ± 0.1	3.6 ± 0.6	3.3 ± 0.3	3.6 ± 0.3
Monounsaturates	29.7 ± 0.9^{b}	24.9 ± 2.8^{a}	27.8 ± 0.3^{ab}	28.9 ± 1.1	27.3 ± 1.1	28.8 ± 2.1	18.1 ± 2.7^{a}	18.7 ± 1.8^{a}	27.1 ± 2.6^{b}	18.7 ± 2.9	17.6 ± 1.2	22.0 ± 3.2
18:2n-6	6.2 ± 0.0	8.7 ± 7.2	4.7 ± 0.4	6.0 ± 0.1	10.6 ± 0.6	5.2 ± 1.0	4.9 ± 0.3^{a}	9.9 ± 2.6^{b}	5.0 ± 1.1^{a}	5.0 ± 0.4^{a}	9.8 ± 2.3^{b}	4.3 ± 0.5^{a}
18:3n-6	0.2 ± 0.2	0.2 ± 0.2	0.1 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.4 ± 0.1	0.5 ± 0.0^{b}	0.2 ± 0.2^{a}	0.1 ± 0.0^{a}	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
20:4n-6 (ARA)	3.2 ± 0.2^{b}	2.8 ± 0.1^{ab}	2.5 ± 0.3^{a}	3.7 ± 0.3	3.4 ± 0.2	2.9 ± 0.5	2.2 ± 0.3	2.0 ± 0.3	2.1 ± 0.5	2.6 ± 0.4	2.5 ± 0.3	2.3 ± 0.3
22:5n-6	1.5 ± 0.4	1.4 ± 0.2	1.2 ± 0.3	1.7 ± 0.4	1.5 ± 0.4	1.4 ± 0.5	5.7 ± 1.8	4.6 ± 1.5	4.9 ± 2.1	6.0 ± 2.1	5.4 ± 1.3	5.0 ± 1.4
Total n-6	11.6 ± 0.8	13.6 ± 7.1	9.0 ± 1.0	12.4 ± 0.8	16.5 ± 0.5	10.4 ± 2.0	13.9 ± 1.9	17.1 ± 1.3	12.7 ± 3.8	14.7 ± 2.2^{ab}	18.7 ± 1.1^{b}	12.6 ± 2.0^{a}
18:3n-3	15.9 ± 1.1^{b}	15.4 ± 1.4^{b}	12.2 ± 0.9^{a}	17.0 ± 1.1	16.3 ± 0.4	14.1 ± 3.3	21.6 ± 2.4	20.3 ± 0.7	18.6 ± 2.1	21.4 ± 3.2	20.7 ± 1.2	18.4 ± 2.9
18:4n-3	2.8 ± 0.2^{b}	2.4 ± 0.2^{ab}	2.4 ± 0.1^{a}	2.9 ± 0.3	2.6 ± 0.0	2.6 ± 0.4	3.0 ± 0.1^{b}	2.4 ± 0.1^{ab}	1.1 ± 0.9^{a}	2.9 ± 0.4	2.8 ± 0.2	2.8 ± 0.2
20:3n-3	1.3 ± 0.0^{b}	1.2 ± 0.0^{b}	0.9 ± 0.1^{a}	1.4 ± 0.0	1.3 ± 0.0	1.1 ± 0.2	0.7 ± 0.0	0.7 ± 0.0	0.7 ± 0.1	0.8 ± 0.0	0.8 ± 0.0	0.7 ± 0.1
20:4n-3	0.4 ± 0.0^{b}	0.4 ± 0.0^{a}	0.5 ± 0.0^{c}	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	1.0 ± 0.1	0.9 ± 0.1	1.0 ± 0.0	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.0
20:5n-3 (EPA)	6.7 ± 0.5^{b*}	5.9 ± 0.1^{a*}	8.0 ± 0.2^{c*}	8.3 ± 0.7^{ab*}	7.6 ± 0.3^{a*}	9.5 ± 0.9^{b*}	4.3 ± 0.6	3.8 ± 0.7	2.9 ± 2.0*	4.8 ± 0.7	4.7 ± 0.7	6.2 ± 0.4*
22:5n-3	ND	ND	0.4 ± 0.1	0.1 ± 0.0	0.1 ± 0.0	0.2 ± 0.2	0.4 ± 0.1	0.3 ± 0.1	0.4 ± 0.2	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.4
22:6n-3 (DHA)	3.4 ± 0.7^{a}	3.3 ± 0.4^{a}	10.6 ± 1.3^{b}	4.2 ± 1.0	3.9 ± 1.0	8.4 ± 5.4	13.8 ± 4.9	10.9 ± 3.9	6.5 ± 3.2*	14.4 ± 5.6	13.0 ± 3.5	14.7 ± 3.2*
Total n-3	30.6 ± 0.2^{b*}	28.6 ± 1.2^{a*}	35.0 ± 0.4^{c*}	34.3 ± 0.3*	32.3 ± 1.4*	36.5 ± 2.6*	44.7 ± 3.1^{b}	39.3 ± 4.1^{ab}	31 ± 4.8^{a}	45.7 ± 2.8	43.3 ± 3.2	44.4 ± 3.6
DHA/EPA	0.5 ± 0.1^{a}	0.6 ± 0.1^{a}	1.4 ± 0.2^{b}	0.5 ± 0.1	0.5 ± 0.1	0.9 ± 0.5	3.4 ± 0.8	3.1 ± 0.5	2.8 ± 0.9	2.9 ± 0.8	2.7 ± 0.4	2.4 ± 0.6

DHA/EPA: docosahexaenoic and eicosapentaenoic fatty acid ratio; DW: dry weight; FA: fatty acids; ND: not detected; TL: total lipids.

Figure 1. Total phosphorus contents in *Artemia* nauplii from Exp. 2 before (ENR 22 h) and after (ENR 24 h) the phospholipid source enrichment ("boosting"). Data represent means ± standard deviations (n = 3). ***** indicates a statistical difference between time samples within the same diet (paired t-test, $T \leq 0.05$). Superscripts denote significant differences between treatments (one-way ANOVA and Tukey's post-hoc test, $P < 0.05$). MG: Larviva Multigain; SL: soya lecithin; ML: marine lecithin.

Figure 1.


14000
13000
12000
11000
10000
9000
8000
Total phosphorus (mg kg-1 DW)
ENR 22
ENR 24
*
*
*
a
b
ab
MG
MG+SL
MG+ML