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Enriching *Artemia* nauplii with selenium from different sources and interactions with essential fatty acid incorporation

Authors

Thomas Cavrois-Rogacki[1*], Andrew Rolland[1], Hervé Migaud[1], Andrew Davie[1*], Oscar Monroig[1,2]

Addresses

[1]Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

[2]Instituto de Acuicultura Torre de la Sal, Consejo Superior de Investigaciones Científicas (IATS-CSIC), 12595 Ribera de Cabanes, Castellón, Spain

***Corresponding author**

Andrew Davie

Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, Scotland, UK

E-mail : andrew.davie@stir.ac.uk

Abstract

The production of high-quality marine fish fry is limited by the low survival observed during the larval phase, which is often attributed to dietary deficiencies of the diets at first feeding. Despite progress made with live feed (i.e. rotifers, *Artemia*), enrichments in essential fatty acids for marine fish larvae, little is known on the micronutrient requirements such as selenium (Se). Se is a critical component of several enzymes maintaining important biological functions such as cellular oxidation, and therefore plays a key role in oxidative and stress status of marine larvae. The levels of Se found in the larvae's natural diet (i.e. copepods) is generally higher than those of the enriched live preys used in hatcheries. This study aimed at establishing a protocol to enrich *Artemia* nauplii with Se using different inorganic (sodium selenite) and organic (selenoyeast). Results indicated that the use of dissolved sodium selenite, an alternative inorganic and cheaper form of Se, did not increase the levels of Se in the nauplii. However, the use of selenoyeast (Sel-Plex) confirmed that it is possible to enrich the nauplii with targeted levels of Se, since this process followed a dose-response pattern with Se enrichment ranging from 1.7 to 12.4 mg kg^{-1}. In addition, the supplementation of Sel-Plex to the regular enrichment product did not impact on lipids and fatty acids enrichment irrespective of the dose dispensed. Overall, this study contributes to the refinement of the live prey enrichment protocols that are critical to the success of marine finfish larviculture protocols.

Keywords: *Artemia*; Essential fatty acids; Selenium; Sel-Plex; Sodium selenite.

1. Introduction

Marine finfish larviculture has remarkably improved during the last three decades but it still remains one of the most challenging research areas and commercial bottleneck in marine fish aquaculture. Despite significant advances in the hatcheries' operational procedures, survival rates during the larval phases usually remains low with at best 30 % in well-established species such as gilthead seabream (*Sparus aurata*) (Atalah et al., 2011) and European seabass (*Dicentrachus labrax*) (Villamizar et al., 2009), and <10 % in most other species such as California yellowtail (*Seriola lalandi*) (Hawkyard et al., 2016), Atlantic halibut (*Hippoglossus hippoglossus*) (Bjornsdottir et al., 2009) and ballan wrasse (*Labrus bergylta*) (Øie et al., 2015). Causes accounting for the low survival in marine larviculture are multifactorial but nutrition and feeding have been often regarded as major causal factors (Hamre et al., 2013). While it is fairly well-understood which nutrients are essential in marine fish larvae diets (NRC, 2011), quantitative requirements are largely unknown for most fish species and nutrients. This is mainly due, among other reasons, to the inherent difficulty to manipulate and reliably control dietary content of individual nutrient within live preys (i.e. rotifers, *Artemia*) to match marine finfish larvae requirements (Støttrup, 2000; Conceição et al., 2010; Dhont et al., 2013). Nutrients can be incorporated into live preys through the so-called "bioencapsulation" or "enrichment" protocols (Sorgeloos et al., 2001), however, targeting a recommended level is challenging due primarily to the metabolic activity of live preys towards the enrichment products (Navarro et al., 1999; Reis et al., 2017) and the inherent variability associated with the live prey enrichment process (Navarro et al., 1999; Hamre, 2016). Furthermore, the nauplii's gut can only contain a defined amount of nutrient thus increasing the level of one nutrient (i.e. lipid) can result in the decrease of another nutrient's level (e.g. micromineral).

The brine shrimp *Artemia* sp. is the most commonly used live feed in marine finfish hatcheries (Conceição et al., 2010; Dhont et al., 2013). Unlike copepods, the natural preys of marine fish larvae (Hunter, 1980), *Artemia* have a suboptimal nutritional profile which does not satisfy the requirements of marine fish larvae. It is well-established that *Artemia* is deficient in certain essential lipids for larval stages of fish, particularly phospholipids and long-chain polyunsaturated fatty acids (LC-PUFA) (i.e. arachidonic acid, ARA; eicosapentaenoic acid, EPA; docosahexaenoic acid, DHA) (Navarro et al., 1999; Monroig et al., 2003; Dhont et al., 2013). Along with essential lipids, successful *Artemia* enrichment strategies have also been tested and established for other essential nutrients such as methionine (Tonheim et al., 2000), vitamins A, C and E (Monroig et al., 2007; Adloo, 2012), minerals including cobalt (Fehér et al., 2013), iodine (Moren et al., 2006), manganese (Nguyen et al., 2008) and selenium (Se) (Hamre et al., 2008a,b; Penglase et al., 2011). Importantly, Se is regarded as an essential trace element for virtually all animal species (Hefnawy and Tórtora-Pérez, 2010) and it is a major component of the glutathione peroxidase (GPx), which is involved in the regulation of the antioxidant status in finfish by reducing hydrogen peroxide and hydroperoxides to their base constituents (Lall, 2003; Pacitti et al., 2015). Moreover, Se has also been shown to play a protective role by reducing oxidative stress caused by heavy metals such as copper (Cu), resulting in enhanced immune response in fish (Lin and Shiau, 2007). Studies have shown that dietary deficiencies of Se can result in reduced growth in channel catfish *Ictalurus punctatus* (Wang and Lovell, 1997) and increased mortality in rainbow trout *Oncorhynchus mykiss* (Hilton et al., 1980). The NRC (2011) recommendations for Se dietary requirements in fish vary across species but in average, Se dietary requirements for juvenile and adult stages are ~0.35 mg kg^{-1} (NRC, 2011). Importantly, Se requirements in marine fish larvae are largely unknown despite playing a crucial role as an antioxidant and subsequently reducing stress levels in fragile marine larvae (Saleh et al., 2014). Se levels in enriched *Artemia* nauplii are usually around 2 mg kg^{-1} (Ribeiro et al., 2012a,b), which is at the lower end of wild copepods Se content (i.e. between 2 to 5 mg kg^{-1}) (Hamre et al., 2008b; Mæhre et al., 2013). This may therefore not satisfy the dietary requirements of marine fish larvae (Solbakken et al., 2002; NRC, 2011; Hamre et al., 2013). A study performed in Atlantic cod (*Gadus morhua*) showed that larvae fed rotifers enriched with Se exhibited a higher survival compared to larvae fed the control rotifers (Penglase et al., 2010). In addition, the inclusion of Se in marine fish larval diets has been suggested to potentially offset the high oxidation risk from diets boosted in LC-PUFA (Saleh et al., 2014).

Bioavailability of Se, as per other minerals (NRC, 2011), is greatly dependent upon its form when accumulated in the diet (Pacitti et al., 2015). One of the most common forms of dietary Se is sodium selenite (Na_2SeO_2, hereafter referred to as Na-Se), a highly water-soluble inorganic compound. An alternative Se source is selenoyeast (Se-yeast), an organic source of Se produced by exposing yeast (*Saccharomyces cerevisiae*) to Na-Se (Suhajda et al., 2000), which results in an accumulation of selenomethionine (Se-Met). The latter (organic) form of Se is regarded to be more bioavailable to organisms, thus explaining why it is broadly used as a livestock feed additive (Wang and Lovell, 1997; Rayman, 2004; Thiry et al., 2012). Nevertheless, inappropriate dietary Se levels can induce toxicity effects. Se dietary toxicity levels has been reported in rainbow trout (*Onchorynchus mykiss*) at 13 mg Se kg^{-1} when presented as Na-Se (Hilton et al., 1980) and at levels of 20 mg Se kg^{-1} when presented as Se-Met in hybrid striped bass (*Morone chrysops* × *M. saxatilis*) (Jaramillo et al., 2009). In both studies, Se toxicities resulted in growth impairment and elevated mortality.

Importantly, with a few exceptions (e.g. Penglase et al., 2010), most studies mentioned above were conducted in fish juveniles and information on Se requirements and toxicity levels in fish larvae remain scarce. The present study aimed to compare the effectiveness of different Se enrichment protocols for *Artemia* nauplii using different Se sources and determine how these impact on essential fatty acids enrichment using a commercially available enrichment product. The effects of varying doses of Se-yeast were first tested then the efficiency of different Se sources were studied on *Artemia* nauplii content in Se and essential fatty acids.

2. Materials and Methods

2.1.Artemia hatching and culture

Artemia cysts GSL (EG, Inve, Belgium) were decapsulated and hatched according to Sorgeloos et al. (2001). Newly hatched *Artemia* nauplii were subsequently used for the enrichment experiments. Enrichments were carried in 1-litre Imhoff cones using glass pipes to strongly aerate the cones from the bottom. The cones were subsequently placed in a 28 °C water bath. After the 24 h hatching process, enriched nauplii were collected on a 100 µm sieve, rinsed with freshwater, and distributed in each cone at 300 nauplii ml^{-1} for further enrichment. Artificial seawater (32 ppt) (Instant Ocean, Virginia, USA), disinfected with Pyceze® (0.05 ml l^{-1}), was dispensed in each 1-litre Imhoff cone to provide a final volume of 800 ml after the addition of the enrichment products (see below). The enrichment experiments were run under constant illumination of about 47 klx at the surface of the water.

2.2.Experiment 1: Effects of varying doses of Se-yeast on Se content of Artemia nauplii.

Newly hatched *Artemia* were first enriched with Sel-Plex (SP) (Alltech, Kentucky, USA), during 4 h at different concentrations: 0 mg l^{-1} (Treatment SP0), 12 mg l^{-1} (Treatment SP12), 24 mg l^{-1} (Treatment SP24) and 36 mg l^{-1} (Treatment SP36). Each SP dose was tested in triplicated 1-litre Imhoff cones (i.e. 4 treatments x 3 replicates). After 4 h, Larviva Multigain (MG) (BioMar, Denmark) was added to each of the 12 cones at 0.6 g l^{-1} for a further 24 h. At the end of the MG enrichment period, nauplii were collected on a 100 µm sieve, thoroughly rinsed with freshwater to remove the excess of enrichment product, and gently dried on absorbent paper before transferring them into universal sample tubes. The samples were frozen at -20 ºC before being freeze-dried and stored at -20 ºC for further analysis.

2.3. Experiment 2: Effects of different sources of Se on Artemia nauplii enrichment efficiency.

Experiment 2 investigated the efficiency of two sources of Se to increase the content of Se in *Artemia* nauplii. Four enrichment diets were tested in triplicate: no addition of Se sources (Treatment SP0); SP at 12 mg l^{-1} for 4 h (Treatment SP12); sodium selenite Na-Se (Sigma Aldrich, UK) at the Se equivalent dose (i.e. 24 µg l^{-1}) of Treatment SP12 for 4 h (Treatment NS); soya lecithin emulsion (0.6 g l^{-1}) (Optima Health, UK) containing Na-Se (24 µg l^{-1}) for 4 h (Treatment SL+NS). Treatments SP12, NS and SL+NS contained the same effective dose of Se (24 µg l^{-1}) irrespectively of the form under which Se was presented. For treatment SL+NS, we used a soya lecithin emulsion, potentially creating lipid vesicles (liposomes) that can encapsulate dissolved Se, as this has been proven to be a good strategy to deliver water-soluble nutrients in *Artemia* nauplii (Monroig et al., 2007). The hereby tested sodium selenite was an inorganic, water soluble compound with ≥ 95.0 % purity. During the first 4 h, nothing was added to enrichment medium of Treatment SP0, whereas the other treatments were enriched with Se as indicated above. After the 4 h, Larviva Multigain (0.6 g l^{-1}) was added to all treatments for a further 24 h. Sample collection and storage was done as described in Experiment 1.

2.4. Nutritional analysis

Total lipids (TL) from the enrichment products MG, SL and SP (Table 1), as well as freeze-dried *Artemia* samples collected from Experiments 1 and 2, were extracted according to Folch et al. (1957), with modifications as described by Monroig et al. (2006). Fatty acid methyl esters (FAME) from TL were prepared, extracted and purified according to Christie (2003). Identification and quantification of FAME were carried out using a gas chromatograph coupled with flame ionisation detection as previously described (Houston et al. 2017).

Se concentration was determined after digestion of Sel-Plex, enrichment products and freeze-dried *Artemia* samples in AristAR nitric acid (VWR International, Pennsylvania, USA) in a microwave MARSXpress (CEM, North Carolina, USA) 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, Massachusetts, USA) (Smedley et al., 2016).

2.5.Statistical analysis

All enrichment treatments in both experiments were carried out in triplicate cones (n = 3). Biological and analytical data are expressed as means ± standard deviation (SD). Percentage data were transformed using the arcsine square root function prior to statistical analysis. Difference among treatments for total lipids and fatty acids were analysed by one-way ANOVA followed by a Tukey post-hoc multiple comparison test at a significance level of $P \leq 0.05$ (IBM SPSS Statistics 23, New-York (state), USA).

3. Results

3.1.Experiment 1: Effects of varying doses of Se-yeast on Se content of Artemia nauplii.

The analysis of the unenriched *Artemia* nauplii revealed the presence of Se (1.83 ± 0.18 mg kg^{-1} DW). In the experimental groups, Se concentration in *Artemia* nauplii increased linearly with increasing levels of selenoyeast Sel-Plex (Fig. 1). The equation of the linear regression was:

$$[\mathrm{Se}]_{Artemia} = 3.79 \times [\mathrm{SelPlex}]_{\mathrm{enrichment}} - 2.42 \; (r^2 = 0.97)$$

where $[\mathrm{Se}]_{Artemia}$ is the Se content in the enriched *Artemia* (mg kg^{-1} DW) and $[\mathrm{SelPlex}]_{\mathrm{enrichment}}$ is the dose of Sel-Plex used to prepare enrichment (mg l^{-1}). The Se content in *Artemia* nauplii varied from 1.7 ± 0.1 mg kg^{-1} (Treatment SP0), defined as the basal level of Se found in the non-enriched nauplii, to 12.4 ± 1.0 mg kg^{-1} (Treatment SP36), with significant differences between treatments (P < 0.05) (Fig. 1). Sel-Plex is a yeast-based product that contains lipids (Table 1). However, the dose of Sel-Plex used in Experiment 1 (from 0 to 36 mg l^{-1}) did not affect the TL contents of enriched *Artemia* nauplii (P > 0.05) (Table 2), which ranged between 209.5 ± 9.0 mg g^{-1} (Treatment SP0) and 220.4 ± 22.5 mg g^{-1} (Treatment SP12). Similarly, no statistical differences were observed in the levels of ARA, EPA and DHA, nor DHA/EPA ratios in *Artemia* nauplii enriched with varying doses of Se (Table 2).

3.2. Experiment 2: effects of different sources of Se on Artemia nauplii enrichment efficiency

In Experiment 2, Se levels of *Artemia* from Treatments SP0 and SP12 (1.7 ± 0.1 and 4.3 ± 0.4 mg kg^{-1}, respectively) were consistent with results from Experiment 1 (1.70 and 4.17 mg kg^{-1}, respectively). Moreover, *Artemia* nauplii from NS and SL+NS treatments contained Se levels of 1.7 ± 0.0 and 1.7 ± 0.1 mg kg^{-1}, respectively, very similar to those of the control *Artemia* (Treatment SP0).

The different enrichment regimes resulted in variations in the TL content and fatty acid profiles of *Artemia* nauplii (Table 3). While nauplii from Treatments SP0, SP12 and NS showed similar TL contents (P > 0.05), nauplii from Treatment SL+NS exhibited significantly higher TL content (Table 3). In terms of fatty acid profiles, the nauplii enriched with SL+NS showed a significantly higher n-6 fatty acid content (Table 3), largely due to the contribution of 18:2n-6 (16.2 ± 0.9 %) present in the soybean lecithin (Table 1). Consistently, the n-3/n-6 ratio was significantly lower in SL+NS nauplii (1.7 ± 0.1) compared to that of the other treatments (~3.0). ARA levels were significantly lower in the nauplii SL+NS (1.7 ± 0.2 %) than in the other treatments. The EPA contents of the SP0 nauplii were overall the highest (4.9 ± 0.2 %), but the difference was only significant when compared to the SL+NS nauplii (3.7 ± 0.1 %). DHA contents were significantly higher in SP0 and SP12 nauplii (13.8 ± 0.7 and 13.2

± 0.5 %, respectively) compared to SL+NS (10.8 ± 0.3 %). DHA/EPA ratios were not significantly different between treatments.

4. Discussion

Nutritional deficiencies play an important role in explaining elevated mortalities in marine fish during the early larval stages (Hamre et al., 2013), themselves resulting from a knowledge gap in our understanding of the digestive development and physiology of marine larvae (Rønnestad et al., 2013). Se is an essential trace element required for a variety of biological functions throughout the entire fish life-cycle including early larval stages (Hamre et al., 2008a; Ribeiro et al., 2012a). Copepods, natural preys of marine finfish larvae, contain relatively high levels of Se compared to live preys and it is therefore critical to develop Se enrichment protocols to guarantee that live preys, otherwise deficient in Se, provide adequate Se levels to meet larvae requirements. Importantly, Se enrichment must be achieved along with the provision of essential fatty acids, micronutrients which are typically encapsulated into live preys with commercial products with very low or, even non-exiting, levels of Se.

The effects of varying doses of selenoyeast Sel-Plex, a commercial Se additive used for animal feed, were first tested on the levels of Se and essential fatty acids of *Artemia* nauplii. The control treatment in Exp. 1 (Treatment SP0) without Sel-Plex resulted in *Artemia* containing 1.7 ± 0.1 mg Se kg^{-1}, similar to previously published results (Ribeiro et al., 2012b) despite using a different enrichment protocol (i.e. 3 h instead of 20 h in the current study) and enrichment product (i.e. DHA Selco instead of MG in the current study). In the control treatment, Se concentrations of the nauplii reflected the background level of Se present in unenriched *Artemia*, mainly provided by the enrichment product MG, which contained 2.2 mg Se kg^{-1}. Interestingly, the provision of selenoyeast Sel-Plex into the enrichment medium resulted in increased levels of Se in *Artemia* nauplii. The relationship between Sel-Plex and

Artemia Se was linear thus enabling us to predict Se content in *Artemia* nauplii when enriched with a given Sel-Plex dose. SP12 nauplii showed Se contents (4.2 ± 0.1 mg kg^{-1}) within the upper range of Se concentration reported in wild copepods (i.e. 5 mg kg^{-1}) (Hamre et al., 2008b), whereas SP24 and SP36 nauplii, enriched with Sel-Plex doses of 12 and 24 mg l^{-1}, respectively, contained Se above 10 mg kg^{-1}. Thus, SP12 enrichment treatment resulted in a diet with Se levels well below the potential dietary toxicity threshold observed in rainbow trout and hybrid striped bass (> 10 mg kg^{-1}; Hilton et al., 1980; Jaramillo et al., 2009). However, SP24 and particularly SP36 treatments resulted in nauplii Se contents that could cause toxicity for fish larvae. When compared to other published studies, the efficiency of Se enrichment obtained in Experiment 1 differs from those reported by Ribeiro et al. (2012a). In the latter study, a Sel-Plex dose of 0.6 mg l^{-1} resulted in a Se content in *Artemia* of 3.11 ± 0.27 mg kg^{-1} while in our study, a similar Se content in *Artemia* was obtained using 12 mg l^{-1} of Sel-Plex (4.17 mg kg^{-1}). While the reasons explaining such discrepancy remain unknown, Ribeiro's study lacks details on the enrichment protocol used (e.g. rinsing *Artemia* prior sampling, selenium content of Sel-Plex), which could help explain the differences observed in the efficiency of Se incorporation in nauplii. In addition, the lack of data on the fatty acid composition of the enriched nauplii in Ribeiro et al. (2012a) does not allow us to clarify whether the high Se incorporation correlated with a concomitant increase in essential fatty acids within *Artemia* nauplii.

Results from Exp. 1 clearly showed that simultaneous delivery of Se and essential fatty acids is possible under our enrichment protocol. Nauplii from SP12 treatment contained, in addition to 4.2 mg Se kg^{-1}, markedly higher levels of essential fatty acids such as DHA (18.0 ± 1.1 %). This data is consistent with an *Artemia* enrichment study that showed the high efficiency of Larviva Multigain at supplying DHA, in which levels of 21.8 ± 0.7 % DHA post-enrichment were obtained (Cavrois-Rogacki et al., 2019). Furthermore, none of the treatments with Sel-Plex significantly affected the levels of essential fatty acids (i.e. ARA, EPA and DHA) in the *Artemia* compared to the control, despite the potential dilution effect derived from the inclusion of LC-PUFA free lipids from yeast (Santomartino et al., 2017). Therefore, these results showed that Sel-Plex can be successfully used to enrich *Artemia* in Se while preserving the essential fatty acid contents achieved using commercial enrichment products. Our analyses suggested that Sel-Plex does contain traces of EPA and DHA, although their low levels (<0.2 %) do not appear to have a major contribution to the essential fatty acids of nauplii.

Although it is known that organic Se (e.g. selenomethionine) may be more easily absorbed by living animals compared to inorganic forms (e.g. sodium selenite) (Wang and Lovell, 1997; Izquierdo et al., 2017), high cost of the former can constitute a barrier to their use at a large commercial scale. Cheaper inorganic source of Se have previously been tested on fish larvae fed Se-enriched rotifers (Hamre et al., 2008a) and thus represent potential alternatives. This was investigated in Exp. 2, in which NS and SL+NS treatments consisted of a Se dose of 24 µg l^{-1} (equivalent to Se contained in SP12 treatment with Sel-Plex) supplied as dissolved sodium selenite. Results indicated that neither NS nor SL+NS treatment appeared to be effective ways to enhance Se contents in *Artemia* nauplii since they did not differ from those of control nauplii. While previous studies reported on the low efficiency of delivering dissolved materials into *Artemia* (Tonheim et al., 2000; Monroig et al., 2007), it was somewhat unexpected that delivering the same dose of Se encapsulated into phospholipid vesicles (SL+NS) did not result in any increased Se enrichment efficiency despite *Artemia* being adapted to filtrate discrete particles. The reasons for such a result are unknown but it is reasonable to believe that lipid vesicles produced with the soya lecithin source used in the present study were leaky and did not retain the dissolved Se in the inner aqueous phase of the vesicle. Other highly purified sources of phospholipids, particularly when constituted of more saturated fatty acyl chains, have proven to produce relatively stable vesicles with good efficiency in delivering water soluble compounds into live preys (Hontoria et al., 1994; Monroig et al., 2003, 2007). Thus, in spite of low Se incorporation into *Artemia* nauplii, incorporation of soya lecithin was observed as evidenced by the increased levels of linoleic acid (18:2n-6), its most abundant fatty acid, and the corresponding reduced percentage of other fatty acid contents including EPA, DHA and ARA. The importance of essential fatty acids for marine fish larvae nutrition has been extensively reviewed (Izquierdo, 1996; Tocher 2010, 2015) and it is therefore crucial that the enrichment of live preys with micronutrients is not detrimental to the fatty acids' levels of live feed.

One important aspect of live prey enrichment is its reproducibility and predictability, which is essential in commercial hatcheries to ensure a constant daily production of high-quality live preys. Producing enriched *Artemia* with consistent levels of essential nutrients such as LC-PUFA can be challenging as shown in previous studies (Navarro et al., 1999; Monroig et al., 2006). Importantly, the results from the present study suggests that enrichment of *Artemia* nauplii with selenoyeast Sel-Plex is highly reproducible, according to the consistent levels of Se found in nauplii from the SP12 treatment in two independent experiments (Exp. 1 to 2). While this proves the reproducibility of the method on a small-scale enrichment system, further trials are necessary to confirm the reproducibility at commercial scale.

The study showed that it is possible to enrich *Artemia* with targeted levels of Se using selenoyeast Sel-Plex. Enriching *Artemia* nauplii with 12 mg of Sel-Plex per litre for 4 h prior to a 24 h enrichment with LC-PUFA rich commercial diets produces *Artemia* with Se contents similar to those found in the natural preys (wild zooplankton) of marine fish larvae and high levels of essential fatty acids. The use of inorganic Se was not an effective strategy to enrich *Artemia* nauplii even when it was delivered through phospholipid vesicles. In the case of soya lecithin, the use of low-quality liposomes in the experiment could be a potential cause and should be deeper investigated. Ultimately, these results can be implemented to the *Artemia* enrichment protocols of any marine fish species.

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Table 1. Contents of Selenium (Se), total lipids, and selected fatty acids in enrichment products used in Experiments 1 and 2.

	Larviva Multigain	Soya lecithin	Sel-Plex
Se (mg kg^{-1})	2.2	ND	2000
Total lipids (mg g^{-1})	397.4	808.6	13.4
Fatty acids (% of total)			
14:0	6.1	0.1	0.6
15:0	0.4	ND	0.4
16:0	32.3	19.0	15.3
18:0	0.9	4.2	5.2
Saturates	40.1	24.2	22.2
16:1n-9	0.3	ND	ND
16:1n-7	0.1	0.8	25.1
18:1n-9	1.9	10.2	28.5
18:1n-7	ND	2.0	ND
Monounsaturates	2.3	13.4	54.3
18:2n-6	2.2	54.1	14.7
18:3n-6	0.2	ND	0.1
20:4n-6	1.2	ND	0.1
22:5n-6	14.4	ND	ND
Total n-6	18.4	54.3	15.0
18:3n-3	0.3	6.9	3.9
18:4n-3	0.3	0.1	0.2
20:3n-3	0.1	ND	0.1
20:4n-3	0.7	ND	ND
20:5n-3	0.8	ND	0.1
22:5n-3	0.3	ND	ND
22:6n-3	36.6	ND	0.2
Total n-3	39.0	7.0	4.3

DW, dry weight; ND, not detected.

Table 2. Total lipids and selected fatty acids of *Artemia* nauplii from Experiment 1 enriched for 4 h with different doses of selenoyeast Sel-Plex (SP0: 0 mg l^{-1}; SP12: 12 mg l^{-1}; SP24: 24 mg l^{-1}; SP36: 36 mg l^{-1}) followed by a 24 h enrichment with Larviva Multigain (0.6 g l^{-1}). Data are expressed as means ± standard deviations (n = 3). Differences among treatments were analysed by a one-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$).

Treatment	SP0	SP12	SP24	SP36
Total lipids (mg g^{-1} DW)	209.5 ± 9.0	220.4 ± 22.5	212.5 ± 8.7	211.9 ± 8.9
Fatty acids (% of total)				
14:0	1.6 ± 0.1	1.5 ± 0.0	1.7 ± 0.3	1.7 ± 0.3
15:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	14.6 ± 0.9	14.2 ± 0.3	16 ± 1.9	16.8 ± 3.0
18:0	4.4 ± 0.1	4.3 ± 0.3	4.6 ± 0.3	5.1 ± 1.2
Saturates	21.2 ± 1.1	20.6 ± 0.6	22.8 ± 2.5	24.3 ± 4.6
16:1n-9	0.5 ± 0.0	0.4 ± 0.1	0.5 ± 0.0	0.5 ± 0.2
16:1n-7	1.3 ± 0.1	1.2 ± 0.1	1.5 ± 0.0	1.5 ± 0.6
18:1n-9	13.4 ± 0.4	12.7 ± 0.9	14.6 ± 0.1	15.7 ± 4.8
18:1n-7	4.0 ± 0.1	3.9 ± 0.2	4.3 ± 0.0	4.7 ± 1.3
Monounsaturates	19.7 ± 0.6	18.8 ± 1.4	21.3 ± 0.1	23.0 ± 7.0
18:2n-6	4.7 ± 0.2	4.6 ± 0.1	4.8 ± 0.3	4.8 ± 0.3
18:3n-6	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20:4n-6	2.6 ± 0.1	2.8 ± 0.3	2.1 ± 0.2	2.1 ± 1.5
22:5n-6	6.7 ± 0.2	7.0 ± 0.6	5.6 ± 0.5	4.9 ± 3.0
Total n-6	14.8 ± 0.3	15.4 ± 0.9	13.3 ± 0.5	12.7 ± 4.3
18:3n-3	16.9 ± 0.6	16.7 ± 0.3	18.5 ± 2.8	18.6 ± 2.2
18:4n-3	2.5 ± 0.1	2.4 ± 0.1	2.9 ± 0.6	2.8 ± 0.7
20:3n-3	0.8 ± 0.0	0.8 ± 0.0	0.8 ± 0.1	0.8 ± 0.0
20:4n-3	0.9 ± 0.0	0.9 ± 0.0	0.9 ± 0.1	0.8 ± 0.2
20:5n-3	5.4 ± 0.2	5.9 ± 0.6	4.4 ± 0.4	4.3 ± 2.9
22:5n-3	0.4 ± 0.0	0.3 ± 0.2	0.2 ± 0.2	0.2 ± 0.3
22:6n-3	17.3 ± 0.4	18.0 ± 1.1	14.5 ± 1.3	12.2 ± 6.9
Total n-3	44.1 ± 0.8	45.0 ± 1.2	42.2 ± 2.5	39.7 ± 7.4
n-3/n-6	3.0 ± 0.1	2.9 ± 0.1	3.2 ± 0.2	3.2 ± 0.5
DHA/EPA	3.2 ± 0.0	3.1 ± 0.2	3.3 ± 0.1	3.0 ± 0.4
Total FA (mg g^{-1} DW)	137.6 ± 11.0	136.0 ± 7.4	129.1 ± 2.8	116.5 ± 25.2

DW: dry weight; FA: fatty acids.

Table 3. Total lipids and selected fatty acids in the *Artemia* nauplii from Experiment 2 treated with different enrichment diets (MG: Larviva Multigain; SP: Sel-Plex; NS: sodium selenite; SL: soya lecithin emulsion). Data are expressed as means ± standard deviations (n = 3). Differences in fatty acid contents among treatments were analysed by a one-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). Variables that do not share the same superscript letter within a row are significantly different from each other.

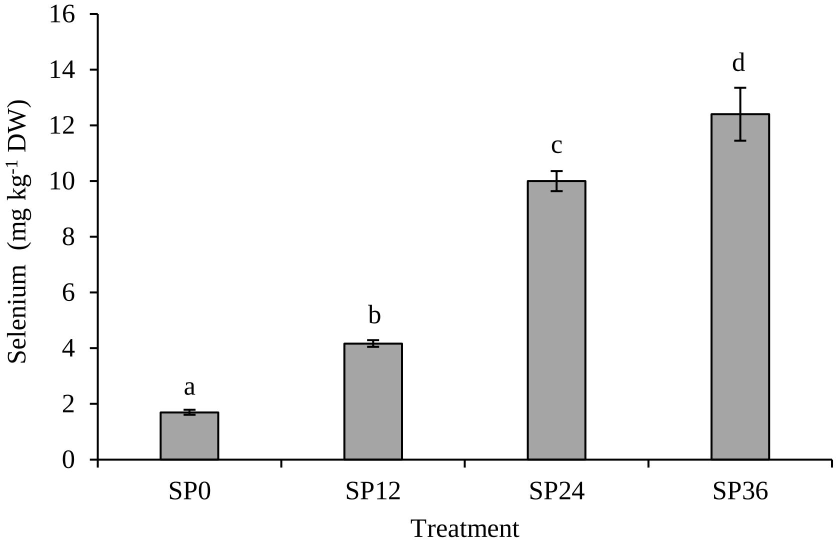
Treatment	SP0	SP12	NS	SL+NS
Total lipids (mg g^{-1} DW)	177.1 ± 8.6^{a}	180.1 ± 2.4^{a}	184.7 ± 2.9^{a}	213.9 ± 7.8^{b}
Fatty acids (% of total)				
14:0	1.3 ± 0.0^{ab}	1.3 ± 0.1^{ab}	1.5 ± 0.0^{b}	1.2 ± 0.1^{a}
15:0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.2 ± 0.0
16:0	13.1 ± 0.5	13.0 ± 0.2	14.8 ± 2.1	13.0 ± 0.3
18:0	4.3 ± 0.2	4.2 ± 0.1	4.7 ± 0.6	4.2 ± 0.1
Saturates	19.3 ± 0.7	18.9 ± 0.3	21.6 ± 2.8	19.0 ± 0.3
16:1n-9	0.6 ± 0.0	0.6 ± 0.0	0.4 ± 0.2	0.5 ± 0.0
16:1n-7	1.1 ± 0.9	1.6 ± 0.0	1.5 ± 0.1	1.3 ± 0.1
18:1n-9	14.8 ± 0.2^{ab}	15.1 ± 0.4^{b}	14.9 ± 0.5^{ab}	13.7 ± 0.6^{a}
18:1n-7	4.4 ± 0.1^{b}	4.5 ± 0.1^{b}	4.5 ± 0.2^{b}	3.8 ± 0.1^{a}
Monounsaturates	21.3 ± 0.6^{b}	22.1 ± 0.4^{b}	21.7 ± 0.7^{b}	19.7 ± 0.7^{a}
18:2n-6	5.2 ± 0.2^{a}	5.3 ± 0.1^{a}	5.2 ± 0.1^{a}	16.2 ± 0.9^{b}
18:3n-6	0.4 ± 0.1	0.4 ± 0.0	0.4 ± 0.0	0.4 ± 0.0
20:4n-6	2.3 ± 0.2^{b}	2.2 ± 0.1^{b}	2.3 ± 0.1^{b}	1.7 ± 0.2^{a}
22:5n-6	5.3 ± 0.1^{b}	4.9 ± 0.2^{b}	4.8 ± 0.4^{ab}	4.3 ± 0.1^{a}
Total n-6	13.7 ± 0.0^{a}	13.2 ± 0.2^{a}	13.1 ± 0.4^{a}	23.0 ± 0.8^{b}
18:3n-3	21.4 ± 0.4^{b}	22.1 ± 0.5^{b}	21.4 ± 1.1^{b}	19.3 ± 0.6^{a}
18:4n-3	3.3 ± 0.1^{b}	3.4 ± 0.1^{b}	3.3 ± 0.1^{b}	2.7 ± 0.2^{a}
20:3n-3	0.8 ± 0.0^{b}	0.8 ± 0.0^{b}	0.8 ± 0.0^{b}	0.7 ± 0.0^{a}
20:4n-3	0.9 ± 0.0^{b}	0.9 ± 0.0^{b}	0.9 ± 0.0^{b}	0.7 ± 0.0^{a}
20:5n-3	4.9 ± 0.2^{b}	4.7 ± 0.1^{ab}	4.5 ± 0.7^{ab}	3.7 ± 0.1^{a}
22:5n-3	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.0	0.1 ± 0.1
22:6n-3	13.8 ± 0.7^{b}	13.2 ± 0.5^{b}	12.0 ± 1.4^{ab}	10.8 ± 0.3^{a}
Total n-3	45.4 ± 1.0^{b}	45.3 ± 0.1^{b}	43.1 ± 2.6^{b}	38.0 ± 0.7^{a}
n-3/n-6	3.3 ± 0.1^{b}	3.4 ± 0.1^{b}	3.3 ± 0.2^{b}	1.7 ± 0.1^{a}
DHA/EPA	2.8 ± 0.3	2.8 ± 0.1	2.7 ± 0.2	2.9 ± 0.1
Total FA (mg g^{-1} DW)	122.1 ± 7.0^{a}	122.7 ± 1.3^{a}	121.4 ± 2.4^{a}	155.8 ± 12.1^{b}

DW: dry weight; FA: fatty acids.

Figure 1. Selenium (Se) concentration of *Artemia* nauplii (µg Se g^{-1} dry weight, "DW") from Experiment 1 enriched 4 h with different dose of selenoyeast Sel-Plex (SP0: 0 mg l^{-1}; SP12: 12 mg l^{-1}; SP24: 24 mg l^{-1}; SP36: 36 mg l^{-1}) followed by a 24 h enrichment with Larviva Multigain (0.6 g l^{-1}). Data are expressed as means ± standard deviations ($n = 3$). Differences in Se contents among treatments were analysed by a one-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). Treatments with different superscripts are significantly different from each other.

Figure 2. Selenium (Se) concentration of *Artemia* nauplii (µg Se g^{-1} dry weight, "DW") from Experiment 2 enriched with different treatments. SP0: SP (0 mg l^{-1}, 4 h); SP12: SP (12 mg l^{-1}, 4 h); NS: NS (24 µg l^{-1}, 4 h); SL+NS: SL emulsion (0.6 mg l^{-1}) + NS (24 µg l^{-1} emulsion). All treatments were followed by a second enrichment with MG (0.6 g l^{-1}, 24 h) Data are expressed as means ± standard deviations ($n = 3$). Differences in fatty acid contents among treatments were analysed by a one-way ANOVA followed by a Tukey post-hoc test ($P \leq 0.05$). Variables that do not share the same superscript letter within a row are significantly different from each other.

Figure 1



Selenium (mg kg-1 DW)
16
14
12
10
8
6
4
2
0
a
b
c
d
SP0
SP12
SP24
SP36
Treatment

514 **Figure 2**