Deliverable Report

Deliverable No:	D11.1	**Delivery Month:**	24
Deliverable Title	Report on the nutrient profile of *Artemia* nauplii and on-grown *Artemia*		
WP No:	11	**WP Lead beneficiary:**	P17. NIFES
WP Title:	Nutrition – Atlantic halibut		
Task No:	11.2	**Task Lead beneficiary:**	P7. IMR
Task Title:	Development of a production strategy for on-grown Artemia		
Other beneficiaries:	P22. SWH		
Status:	Delivered	**Expected month:**	24
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Other Scientists participating: Harboe, T. (IMR) Erstad, B., de Kok, J. (SWH)

Objective: This Deliverable reports on the nutrient profile of *Artemia* and on-grown *Artemia* produced with the protocol described in ***Deliverable 17.1 Production protocol for on-grown Artemia***.

Introduction

Atlantic halibut (*Hippoglossus hippoglossus*) larvae are approximately 12 mm in standard length (SL) at first-feeding and, because of their relatively large larval size, they are first fed on *Artemia*. The main constrains for Atlantic halibut hatcheries are (1) slow growth during the late larval stages, (2) high mortalities caused by opportunistic bacteria and (3) slow growth after weaning.

A strategy to alleviate the slow growth of later stage Atlantic halibut larvae is to feed them on-grown *Artemia*. On-grown *Artemia* are larger, contain more protein and phospholipids and have different micronutrient status from *Artemia* nauplii (Hamre and Harboe, unpublished results). Because of the larger size, they will probably also have a lower shell-to-soft tissue ratio. These differences may explain why Atlantic halibut fed on-grown *Artemia* grow faster and develop into juveniles with better pigmentation and eye migration than larvae fed *Artemia* nauplii (Olsen *et al.*, 1999; Hamre and Harboe, unpublished).

Two experiments were performed at P7. IMR. In experiment 1, *Artemia* was grown for 4 days and sampled for analyses of macronutrients, fatty acid and amino acid each day, to obtain information on the optimal production period. In experiment 2, *Artemia* nauplii and *Artemia* grown for three days according to results from experiment 1, before and after enrichment, were sampled and analyzed for macronutrients, amino acids, fatty acids, lipid classes, vitamins and minerals, to document differences in nutrient profile. Similar types of *Artemia* were also produced at the halibut hatchery of SWH and subjected to whole nutrient profile analyses.

Materials and methods

At IMR (P7), *Artemia* cysts (EG, INVE Aquaculture) were hatched in a separate tank, held for 24 h from incubation, and then transferred to either short-term enrichment or on-growing tanks. Conical 300-l

fiberglass tanks were used both for hatching, short-term enrichment and on-grown *Artemia*. All tanks were equipped with temperature (500 W, and Carlo Gavazzi 600+ temperature regulator) and oxygen control systems (Ocea). Hatching and short-term enrichment was performed at stagnant conditions, while on-growing tanks had a flow-through system (**Fig. 1**).

Seawater was pumped from 160 m depth. For hatching and short-term enrichment, the water was treated with chlorine and thereafter thio-sulphuric acid for at least 18 hours. For the on-growing tanks the 160 m depth water was only filtered down to 5 µm before being connected to the tanks. Flowrate was 15 l h^{-1} for the entire period. 100 g of the disinfectant Sanocare ACE (INVE Aquaculture, Belgium) was mixed with 1 l of freshwater using a blender (Hamilton Beach commercial) for 2 min and added to the tanks daily.

OriGreen from Skretting AS (Stavanger, Norway) was used for grow-out of *Artemia* nauplii. In a pilot trial, *Artemia* was fed using a belt feeder, but due to variation in how the feed dispersed in the *Artemia* on-growing tanks, the feed was mixed with 1 l of freshwater using a blender (Hamilton Beach commercial) for 2 min and added to the tanks twice a day. The *Artemia* were fed 20 g of OriGreen in each meal.

LARVIVA MULTIGAIN (Biomar, Denmark) was used for short-term enrichment of both nauplii and on-grown *Artemia,* using the manufacturer's standard procedure for short-term enrichment of *Artemia*. Enrichment period was 12 hours and density of *Artemia* was 200 ind ml^{-1}.

Sampling was performed by siphoning *Artemia* onto a 250-µm plankton screen. *Artemia* for nutrient analyses was washed in freshwater and the screen was dried thoroughly from underneath with a paper towel. The samples were then frozen in at -80˚C and transported to NIFES on dry ice, where they were stored again at -80˚C until analysis. For measuring *Artemia* size, live *Artemia* were photographed using a dissecting microscope.

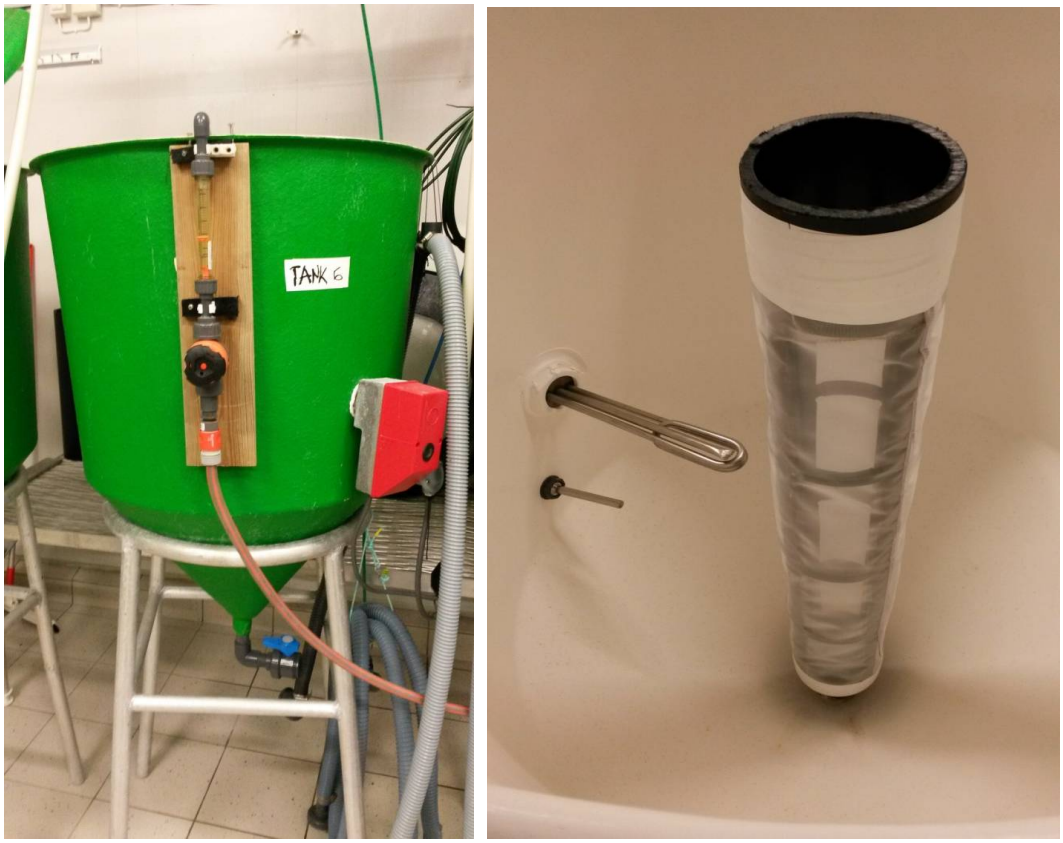

Figure 1. *Artemia* tank and outlet sieve.

Experiment 1. Determining the culture period (IMR)

This experiment lasted for 4 days and was performed to decide the optimal culture period for on-grown *Artemia*, starting at a density of 100 to 110 ind ml^{-1}. *Artemia* were not enriched and samples for size determination and nutrient analyses were taken daily at 13.00. The experiment was performed in triplicate tanks.

Experiment 2. Culture and enrichment of on-grown Artemia (IMR)

Artemia were hatched and the nauplii transferred either directly to enrichment or to on-growing for three days and then to enrichment, as described. On-growing and enrichment were performed in triplicate. After enrichment, the *Artemia* were pumped from the tanks to a 70 l washing tank with a 250-µm plankton mesh and heavy aeration to prevent clogging to the sieve. Thereafter *Artemia* was flushed with warm seawater (22°C, chlorine and thiosulphuric acid treated) at 35 l h^{-1} for 10 min and then freshwater until the salinity reached 0.5 ppt. *Artemia* was held below this salinity for another 5 min by continued flushing with freshwater. Samples were taken of unenriched and enriched naulpii and from unenriched and enriched on-grown *Artemia*, however, the unenriched on-grown *Artemia* were not analysed in triplicate, due to a limited amount of material. Samples of enriched *Artemia* were taken after completion of the washing procedure to mimic *Artemia* fed to halibut larvae.

Experiment 3. Culture and enrichment of on-grown *Artemia (SWH)*

Artemia (SepArt EG cysts>240000 npl/g) were obtained from INVE Aquacultuture Inc., hatched and grown on OriOne (Skretting, Norway) for 3 days, using the procedure described above and removing excess feed every day before feeding. The seawater for the hatchery was pumped from 150 m depth, sand-filtered and treated with ozone and UV light. The *Artemia* tanks were conical 250 l cylinders, supplied at 20 l h^{-1} with seawater heated to 22.5˚C and aerated in a separate 2700 l silo. Both inlet and outlet of water were mounted at the water surface in the tanks. The tanks were supplied with both oxygen and air. An outlet for debris was mounted at the bottom of the cone. Oxygen saturation was highly variable, between less than 50 and more than 250 % saturation and also varied between the replicates. The temperature varied between 20 and 22 ˚C and pH between 7 and 8.

At day 3, samples of unenriched *Artemia* were first taken. Before sampling, *Artemia* were transferred to a washer and flushed with seawater until the water became clear. A 5 l sample was taken and sieved through plankton mesh, which was then dried from underneath with a paper towel. The *Artemia* were then enriched with 0.5 g LARVIVA MULTIGAIN and 0.01 g thiamine per million individuals for 15 min. The culture was then washed with freshwater until the salinity reached less than 5 ppt and kept there for 10 min. Thereafter the salinity was taken back to >31 ppt by flushing with seawater and the samples of enriched *Artemia* were taken as explained. The samples were frozen flat in plastic bags in liquid nitrogen and transported to NIFES, where they were kept at -80˚C until analysis.

Measurements of growth and survival.

The length of *Artemia* was measured as shown in **Fig. 2** and survival was calculated from counts of *Artemia* in 3 samples of 200 µl per tank (P7. IMR) or 7 samples of 100 µl per tank (SWH) at start and end of the *Artemia* culture period.

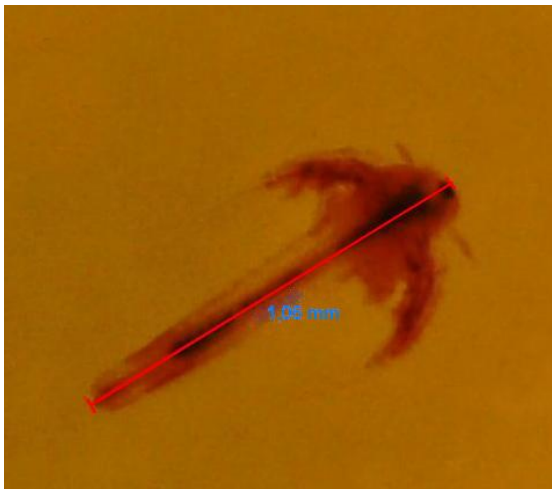

Figure 2. Length measurement of *Artemia.*

Nutrient analyses

The nutrient composition of *Artemia* was measured by ISO certified routine methods at NIFES. **Table 1** presents an overview over the biochemical methods with analysis principles and references. Protein is given as Nx5.30, which has been specifically calculated for *Artemia* (Hamre *et al.*, 2013).

Statistics

Statistica (ver11, Statsoft Inc., Tusla, OK) was used for the statistical treatment.

In experiment 1, data on nutrient composition of *Artemia* cultured for an increasing number of days was subjected to one-way ANOVA analysis, after use of Levenes test for check of homogenous variances.

Table 1. Analytical methods for the different nutrients.

Analyte	Principle	Reference
Dry matter	Gravimetric after freeze drying	(Hamre and Mangor-Jensen, 2006)
Protein	N x 6.25 Leco N Analyzer	(Hamre and Mangor-Jensen, 2006)
Total amino acids	Hydrolyses, derivatization and HPLC analyses	(Espe *et al.*, 2006)
Free amino acids	HPLC and post column derivatization	(Srivastava *et al.*, 2006)
Total lipids	Gravimetric after acid hydrolyses	EU directive 84/4 1983
Fatty acids	Transmethylation extraction and GC/FID	(Lie and Lambertsen, 1991)
Lipid classes	HPTLC	(Jordal *et al.*, 2007)
Glycogen	Hydrolysis and spectrometric detection	(Hemre *et al.*, 1989)
Thiamine	HPLC	(CEN, 2003b)
Vitamin C	HPLC	(Mæland and Waagbø, 1998)
Vitamin A	HPLC	(Moren *et al.*, 2002)
Vitamin D	HPLC	(CEN, 1999)
Vitamin E	HPLC	(Hamre *et al.*, 2010)
Sum vitamin K^3	HPLC	(CEN, 2003a)
Microminerals	ICP-MS	(Julshamn *et al.*, 2004)
Iodine	ICPMS	(Julshamn *et al.*, 2001)

Variables with significant results in Levenes test were Box-Cox transformed. Differences between days were determined using Tukeys HSD test.

Variances of data on the nutrient composition of enriched *Artemia* nauplii and on-grown *Artemia* in experiment 2 and 3 were homogenous (Levenes tests) and the data were analyzed with t-tests. In experiment 2, samples of unenriched nauplii and unenriched on-grown *Artemia* were not replicated and could not be included in the statistical analyses. In experiment 3, only one sample of the on-grown unenriched *Artemia* and two samples of unenriched nauplii were taken, therefore these samples were not compared by statistical analyses. Differences between nauplii and on-grown *Artemia* were tested by combining unenriched and enriched specimens in the two groups. Differences between enriched and unenriched on-grown *Artemia* were analyzed separately. Means were assumed to be different at $p<0.05$ (labeled in red).

Results

Growth and survival experiment 1 and 3 (IMR and SWH)

Figures 3 and **4** show *Artemia* of different stages. **Figure 5** shows the growth of *Artemia* over four days at both hatcheries. The nauplii used by IMR were smaller than those at SWH (n.s. for SWH1 and $p=0.001$ for SWH2), but the growth and the resulting final weight were higher ($p<0.001$). In experiment 2, on-grown *Artemia* was produced every day for 15 days at IMR. During this period, survival of *Artemia*, from start of incubation in on-growing tanks on day 1 until harvest on day 3, varied from 45 to 95% (**Fig. 5**). The survival of *Artemia* grown at SWH was 68±30%, the large variation was probably due to flooding in two of the tanks when the outlet sieve became clogged.

Nutrient composition Experiment 1

Artemia was cultured for four days, samples were taken for analyses each day and the results are given in **Fig. 6**. The protein concentration was measured by two different methods, total hydrolyzed amino acids (TAA) and N*5.30. The TAA method does not detect cysteine, cystine and tryptophan, and therefore underestimates the protein. In animal protein, protein concentration can generally be estimated as N*6.25, while previous results have found the protein to N factor in *Artemia* to be 5.30 (Hamre *et al.*, 2013).

For the N*5.30 method, the protein concentration was higher on day 3 and 4 than on day 1 and 2, while the TAA method showed no differences between days, due to a higher variation. Taurine increased gradually from day 1 to 4, while methionine was similar between days. Lipid was analyzed by acid hydrolyses and as total fatty acids (TFA). TFA decreased gradually from day 2 until day 4, while lipid was lowered on day 4 compared to day 1-3. Arachidonic acid (ARA) decreased gradually from day 2 until day 4, while DHA increased in the same period and reached 2-3% of TFA. EPA increased over the entire culture period from approximately 3 to 9% of TFA (Results in **Fig. 6**). Based on these results and considering the work load, a culture period of 3 days was chosen.

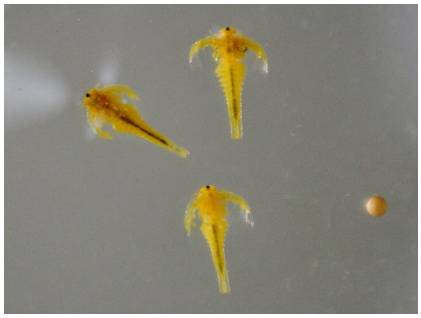

Figure 3. *Artemia* grown for 3 days from nauplii compared to an *Artemia* cyst.

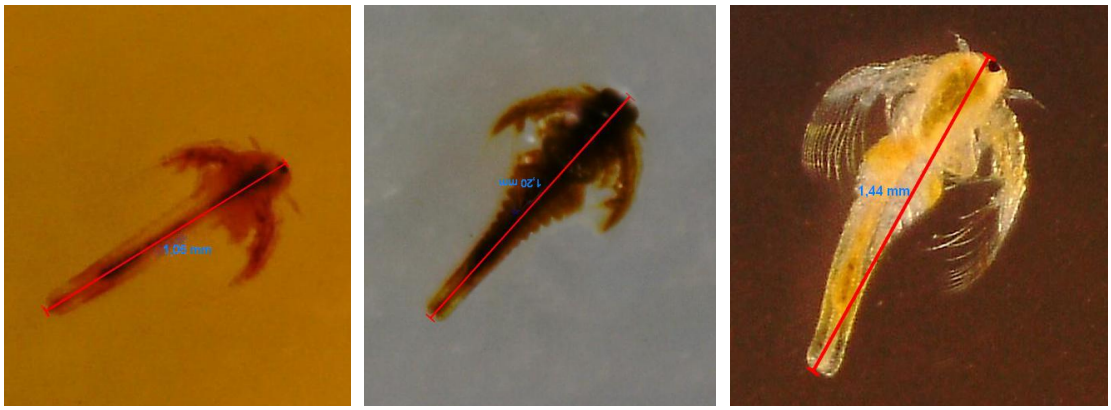

Figure 4. *Artemia* grown from nauplii for 2, 3 and 4 days. Length: 1,06, 1,2 and 1,4 mm respectively.

Nutrient composition experiment 2 and 3

Artemia were grown for three days from nauplii at both IMR and SWH. The protein and TAA concentrations were significantly increased in *on-grow*n *Artemia* compared to nauplii, 10% at IMR (**Table 2**) and 12-22% at SWH (**Table 3**). The protein to N factor was similar in Day 1 and day 3 *Artemia* (**Table 2**). FAA concentrations increased by 30% at IMR and 60% at SWH and taurine concentrations with 25 and 17%, respectively. Glycogen decreased to less than 50% at both hatcheries (**Tables 2 and 3**).

Lipid was generally higher in the *Artemia* from SWH than in those from IMR (24 and 17% of DM in enriched nauplii, respectively) and decreased to between 50 and 70% of the nauplii concentration, to 17 and 11% of DM, respectively, after on-growing for 3 days. TFA again showed lower values but similar trends as lipid analyzed by acid hydrolyses (**Tables 2 and 3**). The level of PL increased after on-growing from 25 to 33 or from 24 to 34 % of TL at SWH and IMR, respectively. There were differences in fatty acid composition of *Artemia* between the two hatcheries. At IMR ARA decreased (from 2.4 to 2.1 % of TFA) and EPA and DHA increased (4.1-6.0 % TFA and 5.9-17%TFA, respectively; **Table 2**). At SWH, ARA increased (2.6-3.2%TFA), there was an increase in EPA (5.6-6.9 %TFA), while DHA was higher in enriched nauplii than in enriched orngrown *Artemia* (19 and 9.4%TFA, respectively; **Table 3**).

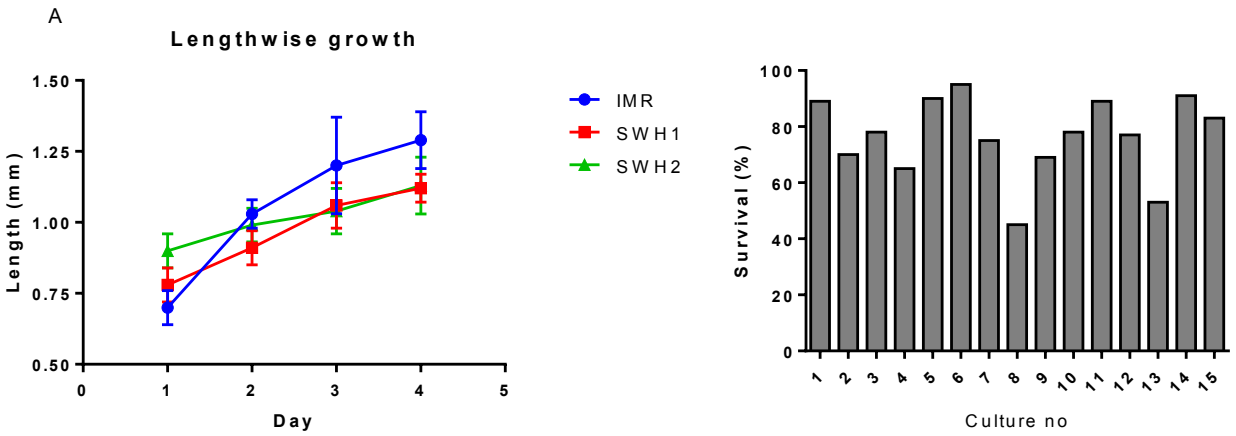

Figure 5. A. Growth of *Artemia* cultured for four days at IMR and SWH (mean±SD). B. Survival in 15 batches of on-grown *Artemia* cultured for three days at IMR. Numbers are based on numbers of *Artemia* ml^{-1} at start and end of the 3-day culture period.

For the vitamin and mineral levels we refer to **Tables 2** and **3**. Enriched nauplii and on-grown *Artemia* did not seem to be deficient in micronutrients when compared to copepod levels and the requirement in fish as given by (NRC, 2011) (**Table 4**).

Conclusion

Artemia grown for 3 days on OriGreen and OriOne and enriched with LARVIVA MULTIGAIN, obtained an improved nutrient profile in many aspects. The protein, free amino acid, and taurine contents increased, lipid and glycogen decreased, while the ratio of phospholipid to total lipid increased. The fatty acid composition improved at IMR, but not at SWH. The micronutrient profiles did not seem to be negatively affected by culture of *Artemia* on OriGreen and OriOne.

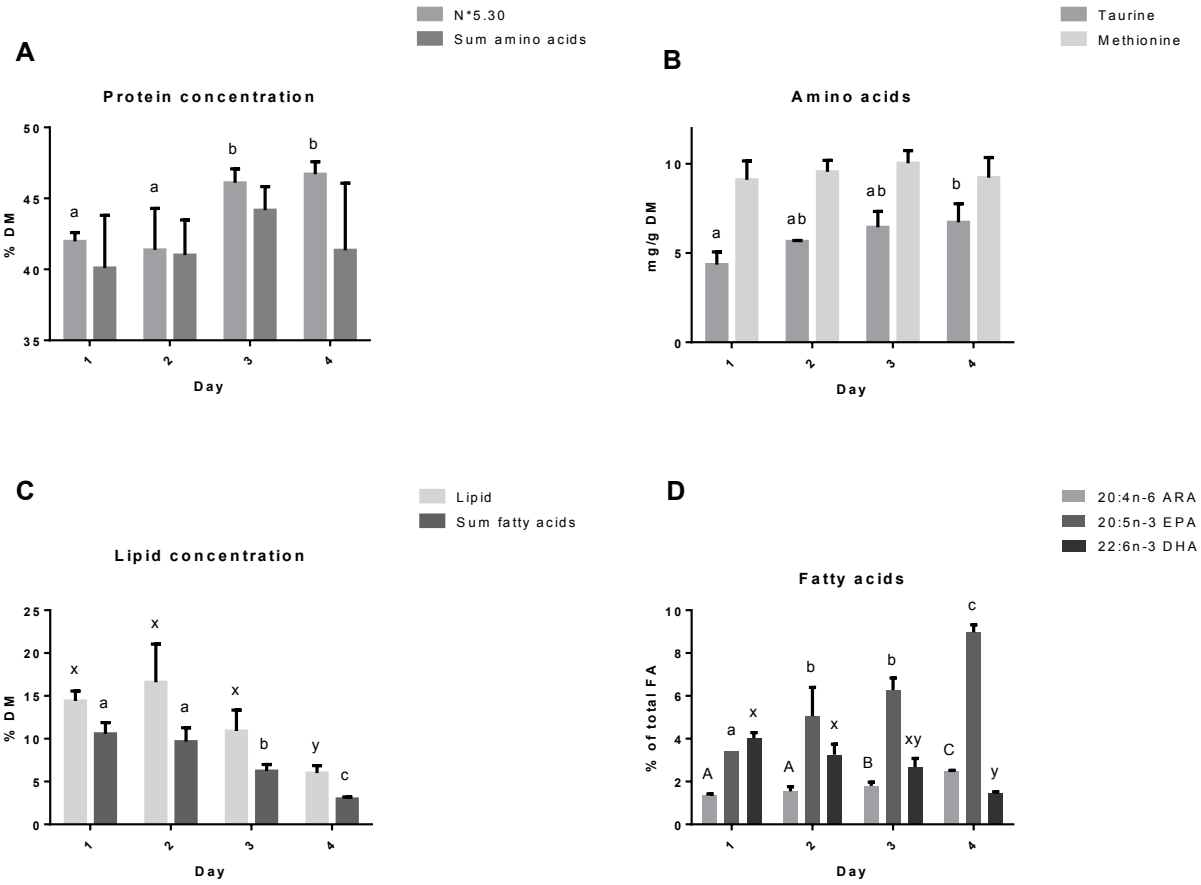

Figure 6. Nutrient concentrations in *Artemia* grown for four days on OriGreen in Experiment 1. A) Protein concentration (% of dry matter) expressed as the sum of amino acids, cysteine and cysteine excluded, or as nitrogen (N) * 5.30 (the calculated protein to nitrogen factor for *Artemia* according to Hamre et al., 2013). B) The amino acid methionine and the aminosulfonic acid taurine (mg g^{-1} dry matter). C) Lipid concentration measured as the sum of fatty acids or as total lipid after acid hydrolyses. D) Arachidonic (ARA), eicopentaenoic acid (EPA) and docosahexaenoic acid (DHA) in % of total fatty acids. Data were analyzed with one-way ANOVA and differences between days are indicated with different letters ($p<0.05$).

Table 2. Nutrient composition of *Artemia* from IMR; nauplii, on-grown, enriched nauplii and enriched on-grown *Artemia*. Data are on dry matter. *Artemia* were grown on OriGreen for three days in triplicate and both nauplii and on-grown *Artemia* were enriched with Multigain. Only one sample of the unenriched *Artemia* types were taken, therefore these samples were not included in the statistical analyses. Differences between enriched nauplii and on-grown *Artemia* (Day 1-3) were analyzed by t-tests. Means were assumed to be different at $p<0.05$ (labeled in red).

	Unit	Nauplii unenriched	On-grown unenriched	Nauplii enriched	On-grown enriched	*P* Day 1-3
Dry matter	g $100g^{-1}$	10.3	10.2	8.4±0.6	8.3±0.4	0.884
Protein Nx5.30	g $100g^{-1}$	53	50	46±1	51±2	0.026
TAA	g kg^{-1}	443.8	-	411±10	452±10	0.008
Protein/TAA		0.119	-	0.11±0.01	0.11±0.004	0.908
FAA	g kg^{-1}	62	-	70±4	92±13	0.044
Taurine	g kg^{-1}	4.15	-	4.4±0.2	5.5±0.6	0.040
Glycogen	g kg^{-1}	-	-	25±3	7.1±3.2	0.002
Lipid	g $100g^{-1}$	20	-	17±1	11±1	0.004
TFA	g kg^{-1}	168	93	147±6	75±8	0.000
PL	% TL	25.2	21	24±3	34±3	0.013
ARA	% TFA	1.6	1.8	2.4±0.1	2.1±0.1	0.016
EPA	% TFA	1.5	6.3	4.1±0.2	6.0±0.7	0.010
DHA	% TFA	<0.1	2.7	5.9±0.6	17±2	0.001
Thiamine	mg kg^{-1}	10	12	10.8±0.8	12.5±1.1	0.096
Vitamin C	mg kg^{-1}	824	307	1037±336	1401±|66	0.168
Vitamin D3	mg kg^{-1}	0.10	0.29	0.12±0.01	0.24±0.01	0.000
Vitamin E	mg kg^{-1}	129	775	580±27	890±224	0.076
MK4	µg kg^{-1}	1.1	-	1040±137	102±37	0.000
Phylloquinone (K1)	µg kg^{-1}	7.6	-	13±1	281±131	0.024
MK6	µg kg^{-1}	0.0	-	nd	15±7	0.024
MK7	µg kg^{-1}	4.8	-	6.7±0.7	75±37	0.033
MK8	µg kg^{-1}	0.0	-	nd	242±111	0.020
MK9	µg kg^{-1}	0.0	-	nd	22±11	0.026
MK10	µg kg^{-1}	0.0	-	nd	41±22	0.031
Total vitamin K	µg kg^{-1}	13.5	-	1073±124	778±340	0.231
Iodine	mg kg^{-1}	2.2	3.1	5.2±0.5	8.2±0.5	0.002
Ca	g kg^{-1}	2.3	3.9	3.4±0.5	3.1±0.5	0.460
K	g kg^{-1}	14.6	12.7	15±1	14±0.1	0.152
Mg	g kg^{-1}	6.6	7.7	8.2±0.8	7.1±0.9	0.165
P	g kg^{-1}	12.6	9.8	11.1±0.9	10.9±0.4	0.420

TAA, total amino acids, cysteine, cystine and tryptophan are not included; FAA, free amino acids; TFA, total fatty acids; TL, total lipid; PL, Phospholipids; ARA, arachidonic acid 20:4n-6; EPA, Eicosapentaenoic acid 20:5n-3; DHA, docosahexaenoic acid 22:6n-3; MK, menaquinone (vitamin K forms), number of isoprenoid units in the tail is indicated; – not analyzed due to shortage of sample; nd, not detected.

Table 3. Nutrient composition of *Artemia* from SWH; nauplii, on-grown, enriched nauplii and enriched on-grown *Artemia*. Data are on dry matter. *Artemia* were grown for three days in triplicate one OriOne and both nauplii and on-grown *Artemia* were enriched with LARVIVA MULTIGAIN. Only one sample of the on-grown unenriched *Artemia* and two samples of unenriched nauplii were taken, therefore these samples were not subjected to statistical analyses. Data were analyzed by t-tests. Differences between nauplii and on-grown *Artemia* (Day 1-3) were tested by combining unenriched and enriched specimens in the two groups. Differences between enriched and unenriched on-grown *Artemia* was analyzed separately (Enrichment on-grown). Means were assumed to be different at $p<0.05$ (labeled in red).

	Unit	Nauplii unenriched	On-grown unenriched	Nauplii enriched	On-grown enriched	*P* Day 1-3	*P* Enrichment on-grown
Dry matter	g 100g^{-1}	13	7.87±0.32	9.0±0.1	7.9±0.2	0.000	0.863
Protein N*5.30	g 100g^{-1}	55	55±1	49±2	55±4	0.006	0.914
TAA	g kg^{-1}	441	476±22	397±7	477±15	0.000	0.895
FAA	g kg^{-1}	55	117±13	79±3	121±12	0.000	0.659
Taurine	g kg^{-1}	4.2	5.1±0.3	4.7±0.0	5.3±0.3	0.022	0.455
Glycogen	g kg^{-1}	122	10.8±1.6	24±5	10.3±2.1	0.000	0.746
Lipid	g 100g^{-1}	17	14±1	27±3	17±1	0.000	0.010
PL	% TL	32	38±1	25±1	33±1	0.000	0.001
ARA	% TFA	1.5	3.6±0.3	2.8±0.2	3.2±0.2	0.015	0.033
EPA	% TFA	2.3	8.0±0.2	5.9±0.4	6.9±0.3	0.005	0.002
DHA	% TFA	<0,1	3.8±0.3	18±2	9.4±0.9	0.000	0.000
TFA	g kg^{-1}	149	107±7	216±9	126±8	0.000	0.024
Thiamine	mg kg^{-1}	9.2	18±2	22±1	20±2	0.009	0.323
Vitamin C	mg kg^{-1}	495	460±53	920±127	786±254	0.098	0.086
Vitamin D3	mg kg^{-1}	0.15	0.21±0.08	0.28±0.08	0.25±0.01	0.544	0.436
MK4	µg kg^{-1}	6.1	178±14	2776±214	246±28	0.000	0.012
K1	µg kg^{-1}	5.7	776±42	14±1	536±101	0.000	0.012
MK6	µg kg^{-1}	nd	50±19	nd	29±17	0.013	0.171
MK7	µg kg^{-1}	nd	145±8	nd	105±28	0.000	0.060
MK8	µg kg^{-1}	nd	243±33	nd	140±38	0.001	0.014
MK9	µg kg^{-1}	nd	77±14	nd	46±14	0.001	0.038
MK10	µg kg^{-1}	nd	60±10	nd	36±13	0.002	0.044
Sum vitamin K	µg kg^{-1}	12	1529±94	2790±215	1137±209	0.000	0.031
Vitamin E	mg kg^{-1}	141	792±55	743±18	869±81	0.072	0.222
Iodine	mg kg^{-1}	-	1.02±0.06	5.8±1.9	7.5±1.1	0.388	0.000
Mn	mg kg^{-1}	12	5.7±0.6	3.7±0.1	4.1±0.3	0.321	0.008
Fe	mg kg^{-1}	1154	233±15	123±33	187±13	0.399	0.007
Co	mg kg^{-1}	0.32	0.41±0.06	0.25±0.02	0.37±0.03	0.001	0.306
Cu	mg kg^{-1}	7.08	24±12	10.6±2.3	21±8	0.071	0.730
Zn	mg kg^{-1}	108	178±19	184±22	177±11	0.641	0.984
Se	mg kg^{-1}	2.5	1.12±0.09	1.7±0.1	1.06±0.17	0.000	0.600

TAA, total amino acids, cysteine, cystine and tryptophan are not included; FAA, free amino acids; TFA, total fatty acids; TL, total lipid; PL, Phospholipids; ARA, arachidonic acid 20:4n-6; EPA, Eicosapentaenoic acid 20:5n-3; DHA, docosahexaenoic acid 22:6n-3; MK, menaquinone (vitamin K forms), number of isoprenoid units in the tail is indicated; nd, not detected.

Table 4. Nutrient composition of copepods (Hamre *et al.*, 2013; Karlsen *et al.*, 2015) and nutrient requirements in fish according to (NRC, 2011) (nd, not determined).

Macronutrients (g kg-1 DM)	TAA	P/N factor	FAA	Lipid (TL)	PL(% TL)	Glycogen
Copepods	634±89	5.30±0.44	79±11	156±31	50±12	5±2
Vitamins (mg kg-1 DM)	Thiamine	C	E	K	D3	
Copepods	13-23	500	110	0.21	nd	
NRC (2011)	1	50	50	0.5-2	0.01-0.06	
Macrominerals (g kg-1 DM)	P	Ca	Mg			
Copepods	12.4-15.0	1.1-2.4	2.4-3.1			
NRC (2011)	3-8	nd	0.4-0.6			
Microminerals (mg kg-1 DM)	Iodine	Manganese	Copper	Zinc	Selenium	Iron
Copepods	50-350	8-25	12-38	340-570	03.mai	85-371
NRC (2011)	0.6-1.1	2-12	3-5	15-37	0.15-0.25	30-150

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