Artemia Enrichment Model - How to Keep Them Small, Rich and Alive?

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Abstract

Artemia nauplii are among the most commonly used prey in aquaculture, but lack some essential fatty acids, particularly DHA. While enrichment can improve prey nutritional profile, enrichment procedures cause undesired effects such as mortality and growth (which is a problem for larvae with small mouth gape). In this study we tested the effect of salinity (3-33), temperature (16-28ºC) and enrichment time (0-24 h) on survival, total length and fatty acid profile of Artemia nauplii using a factorial design. Results were utilized to construct an Artemia nauplii enrichment model. Temperature was the most important forcing function for the processes of mortality, growth and fatty acid incorporation; an increase in temperature causes greater mortality, growth and fatty acid incorporation. Salinity affected primarily growth and ARA incorporation; lower salinities reduce growth and maintain ARA levels higher. The model allows us to test different combinations of temperature and salinity, predict their outcomes, and consequently, to choose the optimal combination of these abiotic factors and enrichment time to produce a prey with the desired properties (a specific total length and fatty acid profile), while minimizing mortality.

Keywords: Artemia; enrichment; model; optimisation; growth; fatty acid; survival.

1. Introduction

Aquaculture is developing, expanding and intensifying in most regions of the world, and is probably the fastest growing food-producing sector. In 2004, aquaculture produced 59.4 millions tonnes of animals and plants, mainly fish and crustaceans (FAO, 2006). One of the major constraints in marine aquaculture is the larval culture (Rainuzzo et al., 1997), since marine larvae are generally small and underdeveloped (Sargent et al., 1997). Most marine larvae require live prey as they do not have sufficient enzymes to digest the prey (Kmulu and Jones, 1995a; Kmulu and Jones, 1995b). The use of wild plankton as larval diet in captivity is ethically questionable, costly and unreliable, therefore live prey need to be cultured. Prey items used in aquaculture must have adequate size, nutritional profile, swimming behaviour, availability in the water column, and ability to survive in the target species media (McConaugha, 1985; Beck and Turingan, 2007). The most common prey used are Artemia nauplli and rotifers since they swim slowly (which turns them easy to be captured by the larvae) and can be reliably cultured at high densities (Rainuzzo et al., 1997; Narciso, 2000; Sorgeloos et al., 2001; Zmora and Shpigel, 2006; Beck and Turingan, 2007). However, these animals are not natural prey in the wild and have an unsatisfactory nutritional profile, particularly lack the essential polyunsaturated fatty acids (Léger et al., 1987; Tocher et al., 1997; Narciso and Morais, 2001; Sorgeloos et al., 2001). Lipids are very important to growth, development and survival of marine larvae (Harrison, 1990; Anger, 1998): phospholipids are structurally bound in membranes where they fulfil crucial physiological functions; triacylglycerides constitute a major energy reserve that can be rapidly mobilized during periods of nutritional, thermal or osmotic stress; sterols are precursors of hormones; long-chain polyunsaturated fatty acids (polyunsaturated fatty acid with ≥ 20 C atoms, PUFA) such as eicosapentaenoic (EPA, 20:5*n*-3) and docosahexaenoic (DHA, 22:6*n*-3) acid are particularly important for larval growth but cannot be synthesized de novo, and therefore, need to be taken up from food (Bergé and Barnathan, 2005). A low level of essential PUFA in the diet reduces larvae physiological condition, and therefore, their chances of survival and growth (Anger, 1998). Efforts have been made to find a protocol to raise other prey more nutritionally adequate for the larvae such as copepods, which are natural prey and display a better fatty acid profile (Shields et al., 1999; Payne and Rippingale, 2000; Chen et al., 2006; Peck and Holste, 2006; Rajkumar and Kumaraguru, 2006; Jepsen et al., 2007; Sørensen et al., 2007). However, their culture is still unreliable and/or cannot be raised at high densities to support an aquaculture industry. Therefore, Artemia nauplii and rotifers are still the most commonly used prey in aquaculture (Rainuzzo et al., 1997; Narciso, 2000; Sorgeloos et al., 2001).

Both rotifers and Artemia are non-selective and continuous filter feeders that consume particles in suspension (Narciso, 2000). These characteristics permit one to improve their nutritional profile by placing them in a solution rich in the nutrients that they are lacking, such as DHA. Thus, the desired nutrients are accumulated in the digestive tract and become available to the larvae when they consume both prey and the nutrients contained in the digestive tract (Sorgeloos et al., 2001). The enrichment can be made with natural emulsions such as rice, corn, soy, algae or with inert commercial enrichment products like Selco® and AlgaMac 2000® (Southgate and Lou, 1995; Tinh et al., 1999; Narciso, 2000). It is believed that a balanced larval diet fro marine species should have a DHA/EPA ratio of 2 or higher (Sorgeloos et al., 2001). Since Artemia nauplii have low DHA/high EPA content (Narciso and Morais, 2001), the enrichment product should have a high DHA/ low EPA content. Linolenic acid (18:3n-3) competitively suppresses the conversion of EPA (20:5n-3) to DHA (22:6n-3) (Buzzi et al., 1996; Sargent et al., 1997), and should not be incorporated in the enrichment solution.

The prey enrichment contributed to several breakthroughs in the culture of several species (Sorgeloos et al., 2001; Olivotto et al., 2006). A total of 442 species were reported to have been cultured at least once between 1950 and 2004 (FAO, 2006). The use of Artemia as live feed for fish and crustacean larvae was during a long time restricted to the fist nauplii stage (Vanhaecke and Sorgeloos, 1980), since Artemia nauplii use the majority of their reserves during the first nauplii stage (Sorgeloos et al., 2001). Therefore, the enrichment also allows the use of Artemia in more advanced nauplii stages (greater in size) as larval diet. Several studies have already shown that as the larvae grow, they will display better survival and growth rates if fed with larger prey (Pryor and Epifanio, 1993; Mookerji and Rao, 1994; Mayer and Wahl, 1997; Narciso, 2000; Ritar et al., 2003).

However, the enrichment process can have a negative side. As the prey is enriched and its fatty acid profile is improved, Artemia nauplii are also growing (Sorgeloos et al., 2001). This can be a problem for species with mouth gape limitations such as fish (Krebs and Turingan, 2003; Turingan et al., 2005). Besides, we also have to take into account that there is Artemia nauplii mortality associated with the enrichment process (Narciso, 2001); Harel et al. (2002) reported 18% mortality after 16 h of enrichment at salinity of 20 and temperature of 28ºC. Recent studies on enrichment process have been mainly focusing on developing more suitable enrichment products (Southgate and Lou, 1995; McEvoy et al., 1996; Tocher et al., 1997; Thinh et al., 1999), and less attention has been paid to the abiotic conditions during the enrichment process. Several studies have addressed the effect of temperature, salinity and enrichment time on survival, growth and fatty acid profile (Narciso, 2000; Han et al., 2001; Ritar et al., 2004). However, to our knowledge, none has examined all factors simultaneously using a factorial design to understand their interactions.

Integration of ecological and experimental data through computer modeling allows synthesis of data and simulations to be performed, facilitating the design and understanding of the system structure and its relationships (Nunes and Parsons, 2006). Models have traditionally been used to address multiple ecological systems such as prediction of the impacts of disturbances in a certain ecosystem, allowing ecosystem managers to more wisely decide on the policies that should be applied (Miller, 2001; McCarthy et al., 2007). Models have also been increasingly applied in aquaculture (Nunes and Parsons, 2006) to describe and predict the effects of biological and environmental conditions on survival, growth, production, profitability and economic feasibility of aquaculture operations (Hanson et al., 1985; Yi, 1998; Zhu et al., 1998; Hernández et al., 2003; Christensen et al., 2004; Forsberg and Guttormsen, 2005; Halachmi et al., 2005; Figueiredo and Narciso, 2006; Halachmi, 2006; Yu et al., 2006; Grant et al., 2007; Penha-Lopes et al., 2007; Figueiredo et al., 2008). The implementation of predictive models to the culture of a wider range of species would improve aquaculture efficiency and profitability and therefore, protect the environment by minimizing wild harvesting (Figueiredo et al., 2008).

Our objective was to construct a model for the enrichment of Artemia nauplii that would allow us to predict the optimal temperature, salinity and enrichment duration to improve Artemia nauplii profile, control or minimize their growth (if the prey size is a problem for the target species) and reduce mortality during the enrichment process.

2. Materials and Methods

2.1. Experimental design and data analysis

Artemia franciscana cysts (Unibest™ 020730, marine strain) were hatched under standard conditions (Sorgeloos et al., 1986; Sorgeloos et al., 2001): cysts were decapsulated and subsequently incubated for 24 h in 6 L cylindrical tanks with water at 28ºC and salinity 28, strong bottom aeration (near saturated oxygen levels) and light (2000 lux) at a maximum density of 2 g/L. The number of newly hatched Artemia nauplii obtained was estimated by sub-sampling (3 replicates of 1 mL subsamples).

Artemia nauplii were stocked at a density of 50 nauplii.mL^{-1} in 5 L cylindrical tanks with strong bottom aeration (near saturated oxygen levels) and light (2000 lux), and enriched with 0.2 g.L^{-1} of AlgaMac 2000® (Aquafauna – Biomarine Inc.) (as recommended by the manufacturer). According to the manufacturer, this product has 27 DHA: 0.54 EPA ratio. The effects of temperature (16, 20, 24 and 28ºC), salinity (3, 13, 23 and 33) and enrichment time (6, 12, 18 and 24 h) on Artemia nauplii survival, total length and fatty acid profile during enrichment were tested using a factorial design.

Three replicates were used for each combination. Temperature was controlled through the use of demersible thermostats. The different salinities were used by dilution of seawater. All water used was sterilized through ultraviolet radiation.

The enriched Artemia survival in each replicate of a treatment was estimated by counting the number of live nauplii in ten 1mL sub-samples. Percent survival was then estimated using average number of live nauplii counted in the ten sub-samples.

For each treatment, 30 nauplii per replicate were haphazardly chosen, fixed with a solution of iodine (2%) for total length (TL) measurement under a stereomicroscope (Olympus™, model SZ6045TR) with a calibrated micrometer eyepiece to the nearest 0.001 mm. The TL of newly hatched Artemia nauplii was measured in triplicate.

To determine the fatty acid profile of Artemia nauplii enriched under the different combinations of salinity, temperature and enrichment time, enriched nauplii were rinsed in freshwater and sampled (one sample per replicate, 3 replicates for each treatment). Each sample weighted 1.06 -1.75 g. Newly hatched Artemia nauplii were also sampled in triplicate. After freeze-dried, samples were ground in a Potter homogenizer with chloroform-methanol-water (2:2:1.8) (Bligh and Dyer, 1959). An internal standard fatty acid (19:0) was added to the extracts. After saponification and esterification of the lipid extracts (Metcalfe and Schmitz, 1961), the fatty acid methyl esters (FAME) were injected into capillary columns (30 m fused silica, 0.32 I.D.) installed in a Varian Star 3400CX gas-liquid chromatograph (GLC). Helium was used as carrier gas at a flow rate of 1mL.min^{-1}; oven temperature was 180ºC for 7 min, then 200ºC (with a temperature gradient of 4ºC.min^{-1}) over a period of 71 min. Both the injector and the FID detector were set at 250ºC. GLC data acquisition and handling were performed using a Varian integrator 4290 connected to the GLC. Peak quantification was carried out with a Star Chromatography workstation. Peak identification was performed using well-characterised cod liver oil chromatograms as a reference.

Knowing the average TL of the Artemia nauplii in each sample, individual Artemia nauplii dry weight for each sample was calculated using the equation:

Artemia nauplii dry weight= $1.7065\ e^{0.7446\ TL}$ (R^2=0.98), where dry weight (DW, µg) and TL (mm) , obtained by conjunction of data published by Reeve (1963) and Narciso (2000). Knowing the fatty acid (FA) dry weight per unit of dry weight in each sample and individual Artemia nauplii dry weight for all combinations of the factorial design, the fatty acid content of each Artemia nauplii was estimated.

This is important since DW of FA per DW of sample might lead to erroneous conclusions (Rønnestad, 1995). For example, a newly hatched Artemia nauplii (0.455mm, 2.4 µg=2400 ng) has 0.1 ng FA X.ng dw^{-1}, but when reaches nauplii stage V (1.4mm, 4.8 µg=4800 ng) has 0.05 ng FA X.ng dw^{-1}. At first it appears the FA X was consumed through development. However, in fact, it was conserved since both newly hatched nauplii and nauplii stage V have 240 ng FA X.Artemia $nauplii^{-1}$.

Response surface regression were used to analyze interactive, polynomial and interactive by polynomial effects of the continuous predictors (temperature, salinity and enrichment time) on the survival, total length and fatty acid profile of the Artemia nauplii. A backward stepwise method was used in these regressions to eliminate the predictors that do not significantly affect survival, total length and fatty acid profile of the Artemia nauplii (Kuehl, 1994). Analyses were performed in Statistica 7.0 at a level of significance of 0.05.

2.2 Enrichment model

2.2.1 State variables, forcing functions and processes

The initial values of the state variables (Artemia percent survival, total length and fatty acid composition) were the ones presented by the newly hatched Artemia nauplii (enrichment time=0). To build a model to predict Artemia survival, length and fatty acid profile (state variables), it is important to understand how the forcing functions temperature, salinity and enrichment time affect the most important processes occurring during enrichment (Table I). The forcing functions may accelerate, delay or not affect the different processes. The equations of the processes were obtained differentiating the response surface regressions for survival, total length and fatty acid composition (for the most important fatty acids and groups of fatty acids) in order to enrichment time.

Table I – Notation, value, unit and interpretation of forcing functions, state variables and parameters of the Artemia enrichment model (* user defined, - not applied)

	Notation	Value	Unit	Interpretation
Forcing functions	T	*	ºC	Temperature
	S	*	-	Salinity
	ET	*	h	Enrichment time
State variables	ARA	-	ng.Artemia nauplii^{-1}	Arachidonic acid (20:4n-6) content
	EPA	-	ng.Artemia nauplii^{-1}	Eicosapentaenoic acid (20:5n-3) content
	DHA	-	ng.Artemia nauplii^{-1}	Docosahexaenoic acid (22:6n-3) content
	LA	-	ng.Artemia nauplii^{-1}	Linoleic acid (18:2n-6) content
	ALA	-	ng.Artemia nauplii^{-1}	Linolenic acid (18:3n-3) content
	SFA	-	ng.Artemia nauplii^{-1}	Saturated fatty acids content
	BFA	-	ng.Artemia nauplii^{-1}	Branched fatty acids content
	MUFA	-	ng.Artemia nauplii^{-1}	Monounsaturated fatty acids content
	PUFA	-	ng.Artemia nauplii^{-1}	Polyunsaturated fatty acids content
	Survival%	-	%	Artemia nauplii percent survival
	TL	-	mm	Artemia nauplii total lenght
Parameters	a	0.005527	ng.h^{-1}	ARA incorporation rate
	b	-9.3x10^{-5}	ng.h^{-1}	Effect of S on ARA incorporation rate
	c	2.60007	ng.h^{-1}	EPA incorporation rate
	d	-0.17662	ng.h^{-2}	Effect of ET on EPA incorporation rate
	e	-1.1898	ng.h^{-1}	DHA incorporation rate
	f	0.103962	ng.ºC.h^{-1}	Effect of T on DHA incorporation rate
	g	1.04863	ng.h^{-1}	LA incorporation rate
	h	-0.05854	ng.h^{-2}	Effect of ET on LA incorporation rate
	i	-0.01071	ng.ºC.h^{-1}	Effect of T on LA incorporation rate
	j	0.63024	ng.h^{-1}	ALA incorporation rate
	k	-0.0383	ng.h^{-2}	Effect of ET on ALA incorporation rate
	l	-0.00515	ng.ºC.h^{-1}	Effect of T on ALA incorporation rate
	m	3.36076	ng.h^{-1}	SFA incorporation rate
	n	-0.26914	ng.h^{-2}	Effect of ET on SFA incorporation rate
	o	0.110064	ng.ºC.h^{-1}	Effect of T on SFA incorporation rate
	p	0.590757	ng.h^{-1}	BFA incorporation rate
	q	-0.03338	ng.h^{-2}	Effect of ET on BFA incorporation rate
	r	-0.00704	ng.ºC.h^{-1}	Effect of T on BFA incorporation rate
	s	11.4903	ng.h^{-1}	MUFA incorporation rate
	t	-0.73634	ng.h^{-2}	Effect of ET on MUFA incorporation rate
	u	5.54718	ng.h^{-1}	PUFA incorporation rate
	v	-0.37766	ng.h^{-2}	Effect of ET on PUFA incorporation rate
	w	-0.073906	ng.ºC.h^{-1}	Effect of T on PUFA incorporation rate
	x	0.79353	%.h^{-1}	Mortality rate
	y	-0.094576	%.h^{-2}	Effect of ET on mortality rate
	z	0.03273	%.ºC.h^{-1}	Effect of T on mortality rate
	a2	-0.00516	mm.h^{-1}	Growth rate
	a3	-0.00023	mm.h^{-2}	Effect of ET on growth rate
	a4	0.000774	mm.ºC.h^{-1}	Effect of T on growth rate
	a5	2.59x10^{-5}	mm.h^{-1}	Effect of S on growth rate

As mentioned in section 2.2, the response surface regressions were obtained through a back ward stepwise method that eliminates the effect of the forcing functions (temperature, salinity and enrichment time) which do not significantly affect the processes. Percent Survival decreases over enrichment time (ET) through the process of Mortality. Artemia nauplii total length increases due to growth process. The FA composition of the nauplii changes over enrichment time due to processes of FA ingestion, catabolism and synthesis. The ideal enrichment model would take into account the concentration of each fatty acid in the enrichment product and its oxidation rate (McEvoy et al., 1995), as well as the filtration rate of the Artemia nauplii (that would probably vary over development), and fatty acid conversions (synthesis and catabolism) (Navarro et al., 1999). This approach is, however, too complex to access with exactness since it is very difficult to know what is happening in the nauplii and, even more difficult, to distinguish between the processes of ingestion, excretion, synthesis and catabolism. Therefore, the enrichment model developed used a process that we named FA incorporation which entails all of these processes without differentiating them. The differential equations that represent the change in each state variable over enrichment time (due to the processes of FA incorporation, mortality and growth) are:

$$\frac{dARA}{dET} = + \text{Incorporation of ARA}$$

$$\frac{dEPA}{dET} = + \text{Incorporation of EPA}$$

$$\frac{dDHA}{dET} = + \text{Incorporation of DHA}$$

$$\frac{dLA}{dET} = + \text{Incorporation of LA}$$

$$\frac{dALA}{dET} = + \text{Incorporation of ALA}$$

$$\frac{dSFA}{dET} = + \text{Incorporation of SFA}$$

$$\frac{dBFA}{dET} = + \text{Incorporation of BFA}$$

$$\frac{dMUFA}{dET} = + \text{Incorporation of MUFA}$$

$$\frac{dPUFA}{dET} = + \text{ Incorporation of PUFA}$$

$$\frac{dSurvival\%}{dET} = - \text{ Mortality}$$

$$\frac{dTL}{dET} = + \text{ Growth}$$

where ALA is linolenic acid, LA is linoleic acid, SFA is saturated fatty acids, BFA is branched fatty acids and MUFA is monounsaturated fatty acids.

The model was developed in STELLA 9.0.3.

2.2.2 Sensitivity analysis

Sensitivity analyses were carried out to determine which inputs in the model contributed most for the output variability. The analysis was conducted by means of successive simulations, varying each parameter and forcing function included in the model 10% up and down of their initial baseline values (keeping the others equal to their baseline) and recording the corresponding change in the state variables. Thus, the sensitivity, *S*, of a parameter, *P*, is defined as:

$$S = \frac{\delta\psi / \psi}{\delta P / P}$$

where Ψ is the state variable under consideration (Jørgensen and Bendoricchio, 2001). Sensitivity analysis was performed in STELLA 9.0.3.

2.2.3 Calibration

The model was calibrated by simulating Artemia nauplii percent survival, total length and fatty acid profile, and comparing it with the original data. All combinations of temperature, salinity and enrichment time tested (see section 2.1) were compared with simulated data to check for fitness. Regression analyses between observed and expected values (R^2) were used to test the data adjustment to the model.

2.2.4 Models predictions

To exemplify the application and usefulness of the model, predictions of Artemia survival, total length and DHA content were done and compared for two scenarios: (1) temperature of 28ºC, and (2) temperature of 16ºC.

Model was also used to determine the optimal combination of temperature, salinity and enrichment time to produce a prey with TL ≤ 0.65 mm (nauplii stage II) with maximized DHA content, more suitable DHA: EPA: ARA ratio and minimal mortality through enrichment. To do so, model was run with possible combinations of the forcing functions (temperature, salinity and enrichment time), and TL, percent survival and DHA content of the prey were predicted. Of all the enrichment protocols that enabled the production of a prey with the desired size, it was chosen the one that maximized DHA content, improved DHA: EPA: ARA ratio and minimized mortality.

3. Results

3.1 Experimental data

Artemia survival for all combinations of salinity, temperature and enrichment time are presented in Table II (N=192). During enrichment, nauplii mortality occurred. The response surface regression revealed that salinity and enrichment time have a quadratic effect on survival and that enrichment time interacts with temperature affecting survival (Table III).

Newly hatched nauplii measured 0.455 ± 0.005 mm (N=90). Artemia total length for all combinations of salinity, temperature and enrichment time are presented on II (N=5760). The response surface regression revealed that all independent variables have a quadratic effect on survival, and that the there is interaction between enrichment time and the other two variables (Table III).

Table II – Survival and total length (average ± standard error) of Artemia nauplii enriched under different conditions of salinity (S), temperature (T) and enrichment time (ET).

S	T	ET	Survival		TL	
			AVG	S.E.	AVG	S.E.
3	16	6	95.56	2.50	0.498	0.006
3	16	12	80.89	3.23	0.486	0.007
3	16	18	97.11	2.26	0.553	0.008
3	16	24	85.78	0.89	0.544	0.007
3	20	6	90.22	1.82	0.500	0.006
3	20	12	83.56	0.22	0.589	0.008
3	20	18	94.00	3.91	0.615	0.006
3	20	24	94.67	2.04	0.656	0.006
3	24	6	81.11	5.58	0.551	0.008
3	24	12	88.00	5.75	0.619	0.006
3	24	18	98.89	1.11	0.677	0.006
3	24	24	93.78	3.32	0.722	0.007
3	28	6	83.78	5.47	0.578	0.008
3	28	12	92.89	1.35	0.646	0.006
3	28	18	67.56	3.45	0.707	0.006
3	28	24	63.78	5.17	0.759	0.006
13	16	6	91.33	0.77	0.477	0.007
13	16	12	87.56	5.31	0.503	0.007
13	16	18	92.22	6.49	0.516	0.007
13	16	24	85.78	8.16	0.540	0.007
13	20	6	89.33	1.68	0.507	0.007
13	20	12	86.22	3.23	0.570	0.007
13	20	18	95.56	2.35	0.611	0.006
13	20	24	88.89	3.78	0.669	0.007
13	24	6	93.33	4.06	0.506	0.007
13	24	12	87.33	0.38	0.648	0.005
13	24	18	90.89	5.84	0.684	0.005
13	24	24	89.33	4.68	0.766	0.005
13	28	6	80.67	6.57	0.563	0.009
13	28	12	92.44	6.58	0.655	0.005
13	28	18	92.00	3.85	0.709	0.006
13	28	24	83.56	4.64	0.784	0.007
23	16	6	93.56	0.44	0.450	0.004
23	16	12	86.89	5.76	0.531	0.007
23	16	18	92.89	1.74	0.559	0.007
23	16	24	94.22	2.32	0.554	0.007
23	20	6	84.67	4.54	0.502	0.007
23	20	12	98.89	1.11	0.573	0.008
23	20	18	99.11	0.89	0.619	0.008
23	20	24	90.22	6.47	0.656	0.006
23	24	6	91.78	4.15	0.551	0.009
23	24	12	82.67	4.67	0.623	0.005
23	24	18	89.33	2.67	0.659	0.006
23	24	24	82.44	1.82	0.736	0.006
23	28	6	84.00	2.40	0.556	0.008
23	28	12	89.56	9.14	0.632	0.005
23	28	18	88.44	1.46	0.747	0.006
23	28	24	91.11	5.41	0.765	0.007
33	16	6	86.67	1.92	0.454	0.004
33	16	12	92.00	5.55	0.505	0.006
33	16	18	78.00	1.39	0.555	0.007
33	16	24	91.56	3.20	0.536	0.007
33	20	6	83.78	8.28	0.498	0.007
33	20	12	87.11	3.20	0.553	0.007
33	20	18	92.44	3.49	0.610	0.006
33	20	24	96.67	3.33	0.650	0.006
33	24	6	83.11	5.61	0.499	0.007
33	24	12	78.00	4.67	0.607	0.005
33	24	18	71.78	2.70	0.662	0.007
33	24	24	85.11	1.74	0.733	0.005
33	28	6	76.22	2.89	0.557	0.007
33	28	12	78.22	1.46	0.634	0.005
33	28	18	95.11	1.24	0.755	0.006
33	28	24	78.22	1.46	0.793	0.007

Table III – Response surface regression (and respective R^2) of Survival (%) and Total length (TL, mm) of enriched Artemia nauplii (S – salinity; T – temperature; ET – Enrichment time)

	Response surface regression (backward stepwise method)	R^2
Survival	$95.99+0.5S-0.02S^2-0.79ET+0.05ET^2-0.03TxET$	0.28
TL	$0.24+0.02T-4.06x10^{-4}T^2-1.28x10^{-5}S^2-5.16x10^{-3}ET-1.16x10^{-4}ET^2+7.74x10^{-4}TxET+2.59x10^{-5}SxET$	0.72

The fatty acid profile of newly hatched Artemia nauplii is presented in Table IV.

Table IV – Fatty acid composition (ng.Artemia nauplii^{-1}) of newly hatched Artemia nauplii (N=3)

FA	Newly hatched *Artemia* nauplii	
	AVG	S.E.
14:0	5.93	0.33
16:0	47.47	3.31
17:0	1.97	0.15
18:0	14.65	1.14
SFA	72.76	5.12
Anteiso 15:0	2.68	0.15
Iso 17:0	1.52	0.12
Anteiso 17:0	2.39	0.19
BFA	9.97	0.68
15:1	2.90	0.18
16:1n-7	73.61	4.94
17:1n-8	4.00	0.30
18:1n-7	38.20	2.54
18:1n-9	57.02	4.88
19:1n-8	2.53	0.21
20:1n-9	1.98	0.14
MUFA	183.21	13.38
16:3n-4	8.12	0.64
18:2n-6	14.07	1.13
18:3n-3	8.23	0.69
18:4n-3	3.59	0.26
20:4n-6	0.32	0.00
20:4n-3	10.35	0.86
20:5n-3	51.93	3.86
22:4n-6	0.06	0.00
22:5n-6	0.01	0.01
22:5n-3	0.50	0.08
22:6n-3	1.09	0.08
PUFA	100.87	7.90
TFA	381.55	28.15

The fatty acid profile of Artemia nauplii enriched under different temperature, salinity and enrichment time are presented in Tables V-X (N=192). Temperature, salinity and enrichment time significantly affected Artemia FA profile. The response surface regressions for each fatty acid and groups of fatty acids are presented in Table XI.

Table V – Saturated fatty acids (14:0, 16:0, 17:0, and 18:0) content of Artemia nauplii enriched under different combinations of salinity (S), temperature (T) and enrichment time (ET) (fatty acids which content was < 2ng were excluded)

S	T	ET	14:0		16:0		17:0		18:0	
			AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.
3	16	6	9.03	0.35	71.90	2.82	2.89	0.10	23.60	0.88
3	16	12	9.72	0.66	71.82	1.64	2.66	0.14	22.84	0.69
3	16	18	8.65	0.19	67.88	1.35	2.77	0.05	21.83	0.43
3	16	24	8.40	0.22	65.50	1.40	2.65	0.07	20.88	0.42
3	20	6	9.34	0.15	72.75	1.00	2.78	0.03	24.44	0.36
3	20	12	9.59	0.06	73.58	1.18	2.73	0.06	24.56	0.61
3	20	18	9.84	0.11	71.33	0.59	2.60	0.08	23.21	0.22
3	20	24	9.70	0.71	69.89	3.65	2.66	0.14	22.58	1.11
3	24	6	12.63	0.71	78.64	2.10	2.53	0.05	26.06	0.50
3	24	12	9.54	0.24	69.08	2.04	2.44	0.07	22.58	0.64
3	24	18	12.75	2.95	79.07	7.89	2.77	0.09	25.04	1.05
3	24	24	10.17	0.60	74.00	3.30	2.66	0.07	24.71	0.92
3	28	6	11.65	0.86	77.93	4.40	3.26	0.72	26.90	2.36
3	28	12	10.04	0.44	69.69	2.53	2.21	0.08	22.69	0.82
3	28	18	10.44	0.53	71.62	1.30	2.38	0.06	23.51	0.30
3	28	24	10.59	0.07	72.64	1.65	2.69	0.20	23.71	0.49
13	16	6	8.29	0.45	64.41	3.28	2.44	0.14	20.72	1.05
13	16	12	9.13	0.19	68.12	1.53	2.67	0.08	21.82	0.52
13	16	18	9.18	0.18	68.22	1.89	2.67	0.12	21.89	0.64
13	16	24	9.16	0.64	68.25	4.36	2.50	0.20	22.48	1.26
13	20	6	12.99	0.58	80.59	2.80	2.52	0.06	26.80	0.92
13	20	12	12.11	0.54	73.15	4.78	2.49	0.19	24.26	1.44
13	20	18	11.96	0.01	75.33	1.88	2.41	0.19	24.59	0.49
13	20	24	11.96	0.01	75.33	1.88	2.41	0.19	24.59	0.49
13	24	6	15.34	0.60	81.75	1.16	2.05	0.16	26.93	0.74
13	24	12	18.04	0.91	84.48	1.31	1.79	0.09	25.94	0.49
13	24	18	16.94	0.84	79.07	4.77	1.77	0.12	24.14	1.28
13	24	24	17.67	0.31	85.07	1.88	3.22	0.87	26.64	0.43
13	28	6	16.07	1.77	86.01	4.32	2.08	0.27	30.01	1.18
13	28	12	22.50	1.77	91.04	3.16	1.80	0.11	27.35	0.60
13	28	18	20.99	0.48	92.03	0.33	2.12	0.13	28.61	0.38
13	28	24	21.66	1.19	89.63	2.79	1.70	0.15	25.97	0.29
23	16	6	10.47	0.43	73.24	2.60	2.46	0.21	23.65	0.92
23	16	12	11.44	0.89	72.71	1.32	2.52	0.03	22.84	0.27
23	16	18	10.37	0.31	72.20	0.08	2.65	0.02	23.25	0.07
23	16	24	10.28	0.37	76.39	1.87	2.83	0.07	24.73	0.60
23	20	6	13.50	0.88	84.06	4.02	2.57	0.20	27.54	1.34
23	20	12	15.66	0.87	88.61	1.75	2.79	0.05	28.52	0.33
23	20	18	15.45	0.93	79.49	1.40	2.25	0.07	24.12	0.38
23	20	24	14.34	0.42	80.10	3.21	2.18	0.10	24.71	0.65
23	24	6	12.93	0.44	83.33	2.67	1.97	0.16	30.70	1.62
23	24	12	21.12	0.63	92.11	3.07	2.62	0.47	28.92	1.22
23	24	18	16.71	1.34	83.12	1.57	2.00	0.04	27.63	0.77
23	24	24	23.64	1.35	93.07	2.75	1.78	0.11	28.06	0.89
23	28	6	15.19	0.35	81.97	4.06	2.14	0.49	31.28	0.68
23	28	12	18.03	1.76	84.27	7.63	1.55	0.14	27.83	2.04
23	28	18	21.75	1.25	91.92	2.78	2.16	0.40	30.03	1.01
23	28	24	21.57	1.01	96.54	2.73	1.50	0.15	29.53	1.81
33	16	6	11.31	0.97	78.48	5.39	2.72	0.30	25.47	1.96
33	16	12	11.85	0.26	79.73	0.46	2.74	0.01	26.00	0.18
33	16	18	7.87	1.21	44.58	8.71	1.53	0.29	13.78	3.09
33	16	24	8.28	0.31	52.48	1.09	1.89	0.05	16.59	0.37
33	20	6	17.73	0.85	89.25	2.83	2.23	0.20	28.32	0.97
33	20	12	19.12	0.94	92.04	2.74	2.55	0.05	28.57	0.46
33	20	18	15.85	0.44	79.56	0.48	2.16	0.08	25.08	0.19
33	20	24	16.70	2.75	93.75	15.95	2.42	0.38	28.41	4.09
33	24	6	17.29	1.25	90.46	2.86	1.65	0.22	32.53	0.94
33	24	12	22.94	1.66	101.35	6.47	2.82	0.49	33.37	2.12
33	24	18	16.93	1.66	66.27	7.94	1.53	0.20	21.06	2.94
33	24	24	17.25	1.55	72.77	0.67	1.45	0.06	22.46	0.30
33	28	6	19.32	1.81	93.28	1.69	1.56	0.05	35.98	0.99
33	28	12	21.60	0.79	92.83	1.31	1.38	0.05	34.57	0.96
33	28	18	21.70	0.37	93.08	1.39	1.50	0.14	32.52	0.08
33	28	24	17.27	1.12	75.98	8.46	1.27	0.13	26.45	3.73

Table VI – Branched fatty acids (Anteiso 15:0, Iso 17:0, and Anteiso 17:0) content of Artemia nauplii enriched under different combinations of salinity (S), temperature (T) and enrichment time (ET) (fatty acids which content was < 2ng were excluded)

S	T	ET	Anteiso 15:0		Iso 17:0		Anteiso 17:0	
			AVG	S.E.	AVG	S.E.	AVG	S.E.
3	16	6	4.08	0.20	2.47	0.06	3.63	0.14
3	16	12	4.23	0.31	2.30	0.06	3.35	0.14
3	16	18	3.91	0.09	2.18	0.05	3.44	0.09
3	16	24	3.79	0.17	2.11	0.08	3.27	0.10
3	20	6	3.95	0.15	2.28	0.07	3.58	0.08
3	20	12	3.84	0.17	2.35	0.03	3.56	0.03
3	20	18	3.53	0.16	2.18	0.04	3.26	0.04
3	20	24	3.65	0.30	2.17	0.09	3.35	0.17
3	24	6	3.74	0.15	2.31	0.03	3.45	0.04
3	24	12	3.96	0.15	2.27	0.12	3.25	0.12
3	24	18	3.80	0.19	2.26	0.10	3.36	0.13
3	24	24	3.84	0.27	2.28	0.12	3.35	0.21
3	28	6	4.08	0.44	2.07	0.11	3.64	0.34
3	28	12	3.78	0.21	2.21	0.06	3.08	0.08
3	28	18	3.57	0.22	2.26	0.07	3.22	0.02
3	28	24	3.52	0.04	1.97	0.28	3.23	0.09
13	16	6	3.65	0.31	2.13	0.12	3.17	0.18
13	16	12	3.83	0.22	2.19	0.04	3.37	0.08
13	16	18	4.00	0.13	2.24	0.06	3.38	0.15
13	16	24	3.61	0.26	2.15	0.16	3.13	0.24
13	20	6	3.70	0.06	2.32	0.07	3.34	0.12
13	20	12	3.23	0.13	2.11	0.14	3.03	0.25
13	20	18	3.62	0.17	2.21	0.08	3.16	0.18
13	20	24	3.62	0.17	2.21	0.08	3.16	0.18
13	24	6	3.62	0.14	2.20	0.08	2.99	0.20
13	24	12	3.59	0.11	2.17	0.09	2.78	0.12
13	24	18	3.22	0.25	2.02	0.14	2.64	0.16
13	24	24	3.42	0.07	2.22	0.04	3.02	0.06
13	28	6	3.59	0.10	2.35	0.09	3.08	0.19
13	28	12	2.99	0.12	2.12	0.07	2.73	0.13
13	28	18	3.30	0.29	2.19	0.09	2.83	0.06
13	28	24	2.89	0.21	2.02	0.08	2.62	0.22
23	16	6	3.83	0.12	2.26	0.10	3.18	0.18
23	16	12	3.71	0.04	2.19	0.02	3.26	0.03
23	16	18	3.76	0.01	2.21	0.04	3.33	0.03
23	16	24	4.04	0.14	2.40	0.08	3.56	0.12
23	20	6	3.98	0.15	2.42	0.10	3.37	0.13
23	20	12	4.29	0.08	2.51	0.03	3.70	0.05
23	20	18	3.59	0.10	2.12	0.08	3.05	0.08
23	20	24	3.73	0.16	2.22	0.10	2.97	0.08
23	24	6	3.41	0.14	2.29	0.07	3.05	0.19
23	24	12	3.07	0.19	2.19	0.07	2.95	0.06
23	24	18	3.27	0.22	2.15	0.06	2.91	0.06
23	24	24	2.87	0.16	2.01	0.05	2.70	0.12
23	28	6	2.67	0.07	2.06	0.06	2.47	0.13
23	28	12	3.12	0.24	2.06	0.16	2.53	0.20
23	28	18	3.23	0.22	2.21	0.05	2.69	0.11
23	28	24	2.89	0.13	2.13	0.03	2.42	0.11
33	16	6	3.98	0.26	2.33	0.16	3.44	0.34
33	16	12	4.14	0.01	2.46	0.03	3.54	0.04
33	16	18	2.28	0.39	1.28	0.28	1.95	0.38
33	16	24	2.74	0.05	1.56	0.03	2.42	0.05
33	20	6	3.87	0.12	2.30	0.05	3.14	0.17
33	20	12	3.99	0.03	2.36	0.02	3.40	0.06
33	20	18	3.47	0.09	2.10	0.04	3.01	0.09
33	20	24	3.72	0.56	2.31	0.33	3.28	0.50
33	24	6	3.78	0.24	2.38	0.02	2.80	0.22
33	24	12	3.94	0.26	2.48	0.18	3.29	0.20
33	24	18	2.49	0.24	1.56	0.20	2.07	0.24
33	24	24	2.63	0.03	1.63	0.00	2.20	0.03
33	28	6	3.25	0.23	2.39	0.13	2.78	0.08
33	28	12	2.82	0.15	2.10	0.05	2.42	0.04
33	28	18	2.75	0.08	2.18	0.04	2.64	0.04
33	28	24	2.72	0.29	1.72	0.23	2.07	0.23

Table VII – Mono-unsaturated fatty acids (15:1, 16:1n-7, 17:1n-8, 18:1n-9, 18:1n-7, 19:1n-8, and 20:1n-9) content of <u>Artemia</u> nauplii enriched under different combinations of salinity (S), temperature (T) and enrichment time (ET) (fatty acids which content was < 2ng were excluded)

ET	15:1		16:1n-7		17:1n-8		18:1n-9		18:1n-7		19:1n-8		20:1n-9	
	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.
6	4.18	0.17	110.37	4.40	6.21	0.25	90.80	3.82	59.05	1.75	3.76	0.22	3.04	0.13
12	4.04	0.10	105.20	3.47	6.05	0.18	84.87	2.43	59.24	2.30	3.73	0.25	3.15	0.21
18	3.91	0.09	103.93	2.19	5.75	0.13	85.00	1.80	54.83	1.21	3.71	0.19	2.67	0.06
24	3.81	0.11	98.79	2.30	5.59	0.16	80.93	2.51	52.75	0.64	2.96	0.07	2.57	0.05
6	4.09	0.04	110.59	1.29	6.15	0.09	90.15	1.92	62.09	0.63	3.82	0.06	2.98	0.03
12	4.11	0.04	111.42	1.82	6.17	0.10	91.63	1.85	62.38	1.43	3.66	0.19	2.99	0.07
18	3.93	0.06	104.08	1.07	5.85	0.05	84.28	1.17	60.36	0.19	3.39	0.27	2.86	0.05
24	3.85	0.19	103.27	5.06	5.71	0.28	84.88	5.00	57.40	2.05	3.62	0.19	2.77	0.13
6	4.11	0.08	109.90	1.78	6.24	0.12	88.23	1.47	68.43	0.95	3.79	0.05	3.13	0.09
12	3.89	0.11	100.97	3.07	5.84	0.18	83.31	2.71	57.98	1.30	3.31	0.20	2.94	0.03
18	4.09	0.23	107.97	5.60	6.07	0.27	87.39	3.60	64.98	3.63	3.58	0.22	2.93	0.12
24	4.07	0.20	105.76	4.83	6.14	0.28	86.28	4.86	64.05	1.86	3.34	0.22	2.88	0.13
6	4.01	0.21	113.38	7.89	5.86	0.29	94.82	8.00	62.47	2.33	3.30	0.31	3.26	0.21
12	3.93	0.16	98.58	3.24	5.77	0.20	82.00	3.08	57.36	1.69	2.74	0.03	2.81	0.09
18	3.89	0.16	100.42	1.89	5.81	0.17	84.77	2.01	59.54	0.36	3.14	0.13	2.84	0.06
24	3.79	0.09	100.95	2.28	5.63	0.16	85.05	3.06	60.05	1.22	3.25	0.03	2.85	0.10
6	3.75	0.18	96.65	4.97	5.51	0.29	78.29	4.57	53.26	2.28	2.67	0.18	2.56	0.12
12	3.95	0.08	101.77	2.41	5.80	0.15	82.92	2.71	55.53	1.20	2.92	0.08	2.67	0.07
18	3.97	0.12	101.73	3.41	5.82	0.20	83.49	2.99	55.50	1.57	2.88	0.16	2.68	0.10
24	3.86	0.27	99.90	6.23	5.70	0.38	81.32	5.77	56.86	3.34	3.14	0.63	2.77	0.15
6	4.06	0.12	108.96	3.81	6.24	0.21	86.94	3.14	67.75	2.33	3.44	0.49	3.54	0.62
12	3.67	0.26	98.33	7.42	5.61	0.42	78.31	6.41	61.16	4.13	3.05	0.25	2.73	0.18
18	3.85	0.15	102.68	4.56	5.85	0.21	83.09	3.87	62.80	1.62	2.97	0.24	2.79	0.09
24	3.85	0.15	102.68	4.56	5.85	0.21	83.09	3.87	62.80	1.62	2.97	0.24	2.79	0.09
6	3.91	0.14	100.77	3.86	5.93	0.22	80.78	3.86	67.06	1.68	2.67	0.27	2.95	0.09
12	3.95	0.09	97.79	3.03	5.74	0.17	77.93	2.70	65.21	1.52	2.27	0.16	2.94	0.03
18	3.55	0.22	89.80	5.15	5.28	0.32	70.99	4.40	61.21	3.21	2.42	0.36	2.60	0.19
24	3.90	0.07	100.22	2.02	5.80	0.09	80.16	1.77	65.90	1.20	3.13	0.18	2.79	0.06
6	3.91	0.12	101.30	3.97	6.15	0.19	83.28	3.06	72.16	2.22	2.75	0.27	3.03	0.06
12	3.78	0.06	95.73	2.65	5.58	0.10	76.09	2.23	69.45	1.24	2.56	0.28	2.76	0.02
18	3.88	0.15	99.34	3.46	5.82	0.17	78.37	2.77	71.94	1.65	2.68	0.04	2.86	0.11
24	3.81	0.16	90.98	3.08	5.30	0.17	70.85	4.04	65.84	1.08	2.16	0.47	2.65	0.07
6	4.13	0.20	104.72	3.81	6.00	0.22	85.09	4.05	59.62	1.52	2.79	0.34	2.94	0.15
12	3.89	0.05	101.87	1.21	5.78	0.07	82.94	1.28	57.75	0.65	2.91	0.07	2.72	0.07
18	3.97	0.02	104.66	0.40	5.96	0.04	84.63	0.94	59.26	0.30	3.19	0.03	2.78	0.04
24	4.25	0.10	112.01	2.95	6.43	0.14	90.63	2.71	64.13	1.13	3.39	0.09	2.99	0.06
6	4.33	0.21	112.17	4.24	6.43	0.26	89.57	3.39	68.88	2.69	2.82	0.23	3.13	0.13
12	4.52	0.06	117.52	1.84	6.81	0.08	94.84	1.34	72.01	0.84	3.41	0.05	3.14	0.04
18	3.88	0.06	99.91	1.34	5.75	0.10	78.79	1.83	61.23	0.71	2.75	0.08	2.61	0.06
24	4.08	0.11	103.17	1.64	5.91	0.20	80.61	2.16	63.58	1.74	2.78	0.38	2.96	0.11
6	3.91	0.13	102.27	4.72	6.28	0.32	82.15	3.58	72.04	3.13	2.75	0.32	3.05	0.12
12	3.88	0.08	101.62	1.68	5.82	0.14	79.19	1.41	70.62	1.33	2.82	0.13	2.78	0.07
18	3.69	0.07	97.30	0.93	5.71	0.16	77.45	2.93	67.59	0.78	2.84	0.15	2.72	0.04
24	3.69	0.05	96.63	2.48	5.46	0.14	75.09	2.45	69.61	2.27	2.34	0.26	2.67	0.06
6	3.25	0.08	86.63	3.13	5.45	0.19	70.74	1.55	71.28	1.52	2.04	0.34	2.70	0.10
12	3.61	0.27	90.05	5.84	5.46	0.42	72.35	5.05	68.88	4.07	2.10	0.27	2.69	0.20
18	3.74	0.16	97.56	3.88	5.72	0.20	77.21	3.07	74.39	2.02	2.46	0.16	2.84	0.10
24	3.66	0.05	92.71	3.35	5.54	0.12	72.18	2.55	72.59	4.10	1.90	0.33	2.83	0.10
6	4.25	0.25	111.44	7.65	6.36	0.44	89.00	7.29	64.78	3.92	3.24	0.37	3.13	0.15
12	4.34	0.01	113.77	0.30	6.55	0.04	93.30	0.74	64.52	0.30	3.19	0.04	3.12	0.01
18	2.50	0.44	63.78	11.96	3.44	0.73	46.43	10.49	36.16	7.61	2.09	0.43	1.74	0.39
24	2.98	0.07	76.70	1.59	4.20	0.09	57.43	1.23	43.29	1.04	2.57	0.08	2.12	0.05
6	4.26	0.09	107.87	3.17	6.29	0.17	85.00	2.57	68.27	2.15	2.73	0.45	3.03	0.08
12	4.35	0.05	113.43	1.82	6.46	0.06	89.46	0.78	70.33	0.80	3.06	0.06	2.99	0.02
18	3.84	0.06	99.17	1.22	5.78	0.09	79.25	1.10	61.85	0.88	2.64	0.05	2.72	0.06
24	4.18	0.62	108.02	15.15	6.38	0.93	86.76	12.66	69.00	9.28	2.98	0.41	2.96	0.38
6	3.97	0.06	102.11	4.50	6.23	0.18	81.65	3.61	74.66	3.03	2.11	0.53	3.34	0.11
12	4.33	0.28	115.36	7.34	6.61	0.46	90.16	6.42	78.96	4.56	3.01	0.24	3.07	0.15
18	2.89	0.29	75.44	8.47	4.17	0.52	55.93	7.88	52.27	6.72	1.88	0.26	2.07	0.31
24	3.10	0.00	76.45	0.98	4.40	0.00	58.09	0.89	54.78	1.23	1.75	0.10	2.21	0.01
6	3.57	0.08	97.51	2.31	6.07	0.19	79.35	2.75	80.61	2.18	2.38	0.10	3.02	0.11
12	3.39	0.05	90.99	0.56	5.50	0.08	75.23	1.19	78.14	1.17	1.92	0.21	2.71	0.07
18	3.50	0.08	92.84	4.69	5.70	0.01	77.93	1.32	75.97	2.06	2.42	0.17	2.79	0.08
24	2.99	0.33	76.67	8.73	4.54	0.58	59.39	8.33	60.45	7.59	1.42	0.08	2.29	0.43

Table VIII – Polyunsaturated fatty acids (16:3n-4, 18:2n-6, 18:3n-3, 18:4n-3, 20:4n-3, and 22:4n-6) content of Artemia nauplii enriched under different combinations of salinity (S), temperature (T) and enrichment time (ET) (fatty acids which content was < 2ng were excluded)

S	T	ET	16:3n-4		18:2n-6		18:3n-3		18:4n-3		20:4n-3		22:4n-6	
			AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.
3	16	6	12.39	0.55	21.64	0.74	12.38	0.50	5.95	0.19	16.45	0.60	0.29	0.04
3	16	12	11.84	0.41	20.90	1.12	12.53	0.63	6.05	0.29	14.92	0.97	0.75	0.36
3	16	18	11.70	0.28	20.37	0.45	12.35	0.29	5.74	0.09	14.91	0.36	0.32	0.01
3	16	24	11.22	0.33	19.29	0.39	11.71	0.32	5.53	0.15	14.58	0.39	0.47	0.06
3	20	6	12.23	0.19	22.06	0.41	13.21	0.26	6.09	0.10	16.35	0.33	0.73	0.08
3	20	12	12.21	0.18	21.87	0.65	12.73	0.46	5.85	0.18	15.71	0.56	0.98	0.06
3	20	18	11.41	0.12	20.30	0.42	11.85	0.36	5.49	0.28	15.20	0.68	1.40	0.27
3	20	24	11.40	0.57	20.58	1.10	12.16	0.65	5.75	0.28	15.57	0.73	1.24	0.09
3	24	6	11.96	0.17	21.83	0.14	13.41	0.03	6.41	0.29	17.93	0.71	4.23	0.53
3	24	12	11.34	0.26	19.51	0.57	11.81	0.58	5.38	0.40	14.89	0.56	1.35	0.15
3	24	18	11.76	0.53	21.35	0.98	12.34	0.78	5.84	0.14	17.40	0.90	3.42	1.49
3	24	24	11.80	0.59	20.86	0.86	12.59	0.31	5.70	0.25	16.58	0.79	1.91	0.12
3	28	6	11.64	0.64	22.19	1.96	11.39	0.85	6.23	0.78	15.60	1.41	1.97	0.13
3	28	12	10.77	0.32	17.76	0.56	9.99	0.17	4.31	0.25	12.22	0.65	1.37	0.14
3	28	18	11.12	0.21	19.56	0.35	11.36	0.33	5.26	0.27	14.83	0.52	1.65	0.09
3	28	24	10.96	0.29	20.21	0.49	11.55	0.09	5.53	0.17	15.10	0.45	1.68	0.07
13	16	6	10.94	0.58	18.20	1.04	10.94	0.68	4.97	0.32	13.15	0.87	0.64	0.10
13	16	12	11.56	0.29	19.58	0.52	11.98	0.35	5.53	0.20	14.72	0.44	1.00	0.07
13	16	18	11.68	0.43	19.57	0.79	11.95	0.65	5.68	0.30	14.92	0.71	0.91	0.10
13	16	24	11.01	0.75	18.76	1.74	11.01	1.18	4.96	0.92	13.73	1.99	1.12	0.06
13	20	6	11.57	0.40	21.15	0.92	12.70	0.66	6.00	0.34	17.60	1.23	4.54	0.25
13	20	12	10.42	0.81	19.14	1.56	11.35	1.05	5.16	0.35	15.96	1.12	4.26	0.20
13	20	18	11.14	0.61	20.13	1.10	11.80	0.66	5.40	0.41	16.20	0.71	3.20	0.38
13	20	24	11.14	0.61	20.13	1.10	11.80	0.66	5.40	0.41	16.20	0.71	3.20	0.38
13	24	6	10.63	0.50	18.51	1.53	10.72	1.22	4.90	0.45	15.84	2.10	5.67	0.94
13	24	12	10.04	0.41	16.31	0.86	8.79	0.61	4.13	0.19	12.55	0.77	4.83	0.29
13	24	18	9.36	0.57	16.47	1.36	9.66	1.13	4.17	0.49	14.34	2.14	6.47	0.93
13	24	24	10.60	0.21	19.49	0.42	11.72	0.28	5.03	0.14	16.87	0.44	6.92	0.47
13	28	6	10.83	0.48	19.15	1.26	11.29	0.84	5.15	0.47	17.86	1.44	6.57	1.78
13	28	12	9.83	0.28	17.82	1.21	10.67	1.07	4.62	0.38	16.75	1.76	9.44	1.12
13	28	18	10.28	0.35	18.63	0.54	11.06	0.48	4.84	0.30	17.72	1.22	8.80	1.01
13	28	24	9.12	0.61	15.92	2.11	9.21	1.72	3.66	0.67	15.02	2.58	9.02	0.68
23	16	6	11.41	0.49	18.91	1.88	10.71	1.61	4.77	1.00	13.54	2.23	1.77	0.33
23	16	12	11.19	0.11	19.67	0.09	11.55	0.21	5.32	0.20	14.74	0.45	2.07	0.28
23	16	18	11.62	0.08	20.40	0.11	12.13	0.05	5.39	0.22	15.66	0.17	1.77	0.14
23	16	24	12.55	0.34	22.01	0.58	13.07	0.32	5.89	0.26	16.54	0.49	1.32	0.05
23	20	6	11.86	0.42	20.55	1.04	12.20	0.79	5.00	0.61	15.76	1.63	4.05	1.06
23	20	12	12.92	0.17	22.82	0.30	13.83	0.14	6.45	0.35	18.95	0.24	4.20	0.16
23	20	18	10.80	0.25	18.79	0.45	11.46	0.25	4.87	0.14	15.55	0.30	4.99	0.71
23	20	24	10.99	0.29	18.49	0.85	11.11	0.79	4.77	0.58	14.47	1.65	3.50	0.59
23	24	6	10.71	0.58	19.08	1.48	10.85	1.00	4.15	0.71	17.99	2.15	7.24	1.04
23	24	12	10.45	0.21	19.65	0.32	11.77	0.06	5.05	0.61	18.78	0.33	9.73	0.73
23	24	18	10.22	0.29	18.64	0.66	11.25	0.65	4.11	0.43	17.24	0.40	9.13	3.32
23	24	24	9.60	0.34	17.45	1.06	10.45	0.60	3.50	0.58	16.16	1.77	11.14	1.29
23	28	6	8.88	0.25	16.23	1.12	9.32	0.96	2.92	0.34	18.33	2.42	11.19	2.02
23	28	12	9.33	0.67	16.23	1.27	9.46	0.84	3.06	0.29	15.54	1.85	7.03	1.04
23	28	18	9.96	0.44	17.61	0.75	10.17	0.45	3.29	0.19	17.39	1.34	15.21	5.44
23	28	24	9.11	0.33	15.41	1.56	8.79	0.96	2.58	0.32	15.46	2.48	13.00	4.52
33	16	6	12.23	0.96	21.10	2.28	12.31	1.64	5.21	0.99	16.18	2.29	2.04	0.39
33	16	12	12.50	0.05	21.82	0.17	13.01	0.15	5.08	0.12	15.97	0.20	2.13	0.13
33	16	18	6.66	1.36	11.83	2.53	7.18	1.51	3.02	0.58	9.40	2.03	1.58	0.38
33	16	24	8.15	0.16	14.46	0.38	8.83	0.25	3.67	0.13	11.33	0.40	1.76	0.18
33	20	6	11.33	0.41	19.06	1.68	12.22	0.33	3.59	0.57	18.51	1.13	6.35	1.33
33	20	12	12.05	0.12	21.46	0.19	12.89	0.15	5.23	0.12	17.65	0.52	6.90	0.73
33	20	18	10.71	0.23	18.59	0.47	11.22	0.61	3.94	0.43	14.95	0.49	5.46	0.32
33	20	24	11.60	1.62	20.75	3.09	12.41	2.02	4.80	0.88	18.33	3.08	6.74	2.07
33	24	6	10.31	0.59	16.78	2.52	9.32	1.69	2.75	0.63	8.59	3.70	6.81	1.67
33	24	12	11.78	0.84	21.32	1.65	12.65	1.07	4.80	0.69	19.31	1.31	10.50	0.78
33	24	18	7.50	0.94	13.92	1.88	8.31	1.10	2.66	0.37	12.68	1.78	7.42	1.26
33	24	24	7.64	0.08	14.14	0.30	8.43	0.25	2.61	0.13	13.01	0.61	7.01	1.33
33	28	6	9.94	0.30	18.91	0.66	11.66	0.76	3.15	0.06	21.81	0.02	17.78	3.96
33	28	12	9.14	0.15	17.26	0.98	10.49	0.95	2.58	0.22	18.59	1.76	12.94	1.99
33	28	18	9.65	0.31	18.47	0.44	10.68	0.14	3.03	0.15	18.26	0.22	10.63	0.51
33	28	24	7.67	0.92	13.34	1.13	7.91	0.50	2.28	0.12	13.59	1.53	9.40	1.32

Table IX – Polyunsaturated fatty acids (20:4n-6, 20:5n-3, 22:5n-3, 22:5n-6, 22:6n-3) content of Artemia nauplii enriched under different combinations of salinity (S), temperature (T) and enrichment time (ET) (fatty acids which content was < 2ng were excluded, except ARA, 20:4n-6)

S	T	ET	20:4n-6		20:5n-3		22:5n-3		22:5n-6		22:6n-3	
			AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.
3	16	6	0.39	0.00	76.51	2.43	3.80	0.00	1.99	0.15	2.87	0.74
3	16	12	0.52	0.19	70.07	5.21	4.27	0.74	2.09	0.26	4.71	0.74
3	16	18	0.32	0.01	73.02	2.72	1.90	0.89	1.28	0.38	1.51	0.26
3	16	24	0.30	0.01	70.89	3.33	2.07	0.96	1.45	0.49	2.77	0.56
3	20	6	0.37	0.01	75.04	1.77	1.52	1.27	1.44	0.60	2.52	0.27
3	20	12	0.37	0.01	71.52	2.52	5.75	0.69	3.09	0.24	3.43	0.16
3	20	18	0.35	0.03	67.65	3.34	3.53	0.55	2.20	0.21	5.23	0.93
3	20	24	0.31	0.03	71.96	3.63	3.23	0.20	1.99	0.07	4.61	0.26
3	24	6	0.45	0.04	79.10	2.20	7.46	0.82	3.79	0.43	14.94	1.59
3	24	12	0.35	0.06	65.45	3.62	3.51	0.52	2.48	0.16	4.42	0.74
3	24	18	0.37	0.01	78.05	3.61	5.31	0.75	3.55	0.35	11.15	4.58
3	24	24	0.36	0.01	75.49	3.22	4.36	0.38	2.92	0.21	7.05	0.16
3	28	6	0.48	0.10	72.41	9.24	5.49	1.17	3.44	0.33	6.70	0.33
3	28	12	0.38	0.04	51.41	2.65	3.38	0.79	2.76	0.15	4.25	0.49
3	28	18	0.35	0.01	66.76	3.32	4.08	1.08	2.79	0.35	5.94	0.51
3	28	24	0.37	0.01	69.19	2.13	5.59	1.06	3.52	0.40	5.66	0.32
13	16	6	0.29	0.01	60.04	4.38	3.10	0.20	3.22	0.11	2.94	0.40
13	16	12	0.31	0.01	68.08	2.25	2.66	0.34	2.80	0.23	4.28	0.35
13	16	18	0.29	0.02	70.48	5.34	2.11	0.23	3.44	0.39	3.25	0.34
13	16	24	0.34	0.03	64.58	10.62	2.59	0.28	3.38	0.39	5.09	0.15
13	20	6	0.37	0.01	77.03	6.14	2.21	0.91	1.78	0.40	16.57	0.63
13	20	12	0.42	0.02	72.57	5.13	2.52	1.63	2.02	0.86	14.66	0.97
13	20	18	0.35	0.01	70.54	4.50	3.06	0.03	3.09	0.02	10.65	1.09
13	20	24	0.35	0.01	70.54	4.50	3.06	0.03	3.09	0.02	10.65	1.09
13	24	6	0.39	0.01	69.43	6.68	3.27	0.23	3.33	0.30	20.52	1.56
13	24	12	0.38	0.01	57.88	3.82	2.47	0.38	2.80	0.10	18.53	0.35
13	24	18	0.36	0.03	57.56	9.16	3.98	0.66	5.50	0.52	19.82	2.99
13	24	24	0.51	0.04	73.88	5.21	2.41	0.23	4.93	0.34	21.07	0.96
13	28	6	0.45	0.03	78.54	7.98	3.56	0.48	3.13	0.42	24.76	4.45
13	28	12	0.40	0.00	65.93	7.06	5.93	0.66	4.65	0.39	29.84	2.68
13	28	18	0.43	0.02	76.60	2.10	2.02	0.34	3.40	0.16	29.67	2.58
13	28	24	0.37	0.01	59.47	12.88	3.54	0.48	4.98	0.31	29.58	2.01
23	16	6	0.31	0.02	61.49	10.45	2.05	0.10	3.74	0.39	7.10	1.00
23	16	12	0.31	0.00	46.60	20.09	2.46	0.33	3.81	0.37	8.25	1.17
23	16	18	0.25	0.05	72.75	1.37	1.76	0.13	0.31	0.31	8.04	0.91
23	16	24	0.32	0.01	76.47	2.46	1.08	0.10	0.00	0.00	7.56	0.60
23	20	6	0.37	0.02	69.26	7.01	1.91	1.15	3.54	1.46	13.70	3.82
23	20	12	0.39	0.00	86.71	3.04	1.95	0.92	3.55	1.44	13.82	0.04
23	20	18	0.32	0.01	68.98	2.31	2.09	0.18	3.35	0.23	17.43	1.92
23	20	24	0.23	0.08	67.30	9.77	1.54	0.45	2.74	0.79	10.36	5.05
23	24	6	0.40	0.03	72.51	7.86	3.05	1.00	6.30	1.46	21.19	2.39
23	24	12	0.47	0.10	78.21	3.54	2.94	0.57	4.73	0.85	31.84	2.36
23	24	18	0.38	0.02	73.57	1.72	1.82	0.67	3.16	1.06	25.08	6.32
23	24	24	0.37	0.04	63.97	7.72	3.79	0.45	6.36	0.76	35.55	5.12
23	28	6	0.44	0.07	66.43	10.31	4.36	0.10	7.33	0.33	34.21	5.72
23	28	12	0.37	0.06	61.74	5.94	2.36	0.94	5.22	0.86	22.99	2.52
23	28	18	0.47	0.07	68.72	3.22	1.00	0.29	2.18	0.43	36.00	7.77
23	28	24	0.38	0.05	54.65	9.85	3.40	1.04	4.50	1.24	33.89	8.01
33	16	6	0.34	0.00	76.89	8.84	2.16	0.51	0.22	0.19	11.59	1.19
33	16	12	0.32	0.00	72.46	1.92	0.83	0.20	0.05	0.00	8.47	1.18
33	16	18	0.30	0.03	48.06	9.37	0.63	0.17	0.06	0.03	8.36	2.46
33	16	24	0.38	0.02	56.92	2.41	0.73	0.06	0.01	0.01	7.58	1.85
33	20	6	0.37	0.07	72.82	9.43	0.78	0.40	0.16	0.10	21.40	4.85
33	20	12	0.36	0.01	76.72	2.11	1.43	0.09	0.10	0.10	24.24	1.52
33	20	18	0.30	0.01	64.95	2.60	0.92	0.09	0.11	0.05	18.24	0.72
33	20	24	0.34	0.05	76.59	12.25	1.18	0.35	0.22	0.13	21.82	6.41
33	24	6	0.37	0.08	58.73	17.10	1.20	0.30	0.48	0.24	26.09	6.28
33	24	12	0.48	0.07	81.64	6.44	1.25	0.35	0.30	0.14	36.34	0.74
33	24	18	0.30	0.06	51.70	7.44	0.77	0.19	0.10	0.02	25.15	4.77
33	24	24	0.36	0.01	51.24	2.58	1.06	0.17	0.07	0.01	22.30	4.68
33	28	6	0.41	0.01	77.39	0.50	1.20	0.47	0.38	0.19	44.64	5.79
33	28	12	0.37	0.04	63.78	7.08	1.98	0.70	0.83	0.42	42.37	2.41
33	28	18	0.45	0.01	68.51	2.21	1.49	0.04	0.10	0.01	35.84	3.33
33	28	24	0.29	0.02	48.51	4.84	1.15	0.32	0.06	0.03	34.48	2.23

Table X – Groups/Families of fatty acids (SFA, BFA, MUFA, PUFA) and total fatty acid (TFA) content of Artemia nauplii enriched under different combinations of salinity (S), temperature (T) and enrichment time (ET)

S	T	ET	SFA		BFA		MUFA		PUFA		TFA	
			AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.	AVG	S.E.
3	16	6	111.07	4.21	14.55	0.60	281.70	10.80	158.12	4.70	589.62	17.97
3	16	12	110.09	2.61	13.81	0.56	271.12	9.52	152.63	8.87	574.64	20.34
3	16	18	104.36	2.06	13.51	0.32	264.35	5.41	146.79	2.77	551.28	7.54
3	16	24	100.51	2.27	12.96	0.44	252.09	6.48	143.27	2.79	534.88	12.09
3	20	6	112.77	1.36	13.98	0.37	283.65	3.99	154.87	5.09	588.46	7.23
3	20	12	114.07	1.96	13.92	0.25	286.05	5.42	156.46	5.38	598.55	12.52
3	20	18	110.49	0.83	12.79	0.23	269.14	2.81	147.78	6.33	574.93	9.99
3	20	24	108.28	5.67	12.96	0.72	265.67	13.06	151.98	7.47	565.63	25.86
3	24	6	123.76	3.57	13.53	0.22	288.25	4.71	185.07	4.31	638.17	15.25
3	24	12	107.21	2.89	13.32	0.47	262.67	7.14	144.06	7.11	552.42	14.65
3	24	18	123.04	11.98	13.37	0.58	281.50	13.70	174.74	13.97	621.14	41.72
3	24	24	115.31	5.02	13.51	0.85	276.84	12.75	163.00	6.11	597.93	19.75
3	28	6	123.73	8.88	13.82	0.70	291.41	19.00	160.65	13.89	620.75	42.43
3	28	12	108.13	4.07	12.86	0.43	257.20	8.81	121.33	5.20	523.61	16.14
3	28	18	111.60	2.14	12.81	0.36	264.59	4.22	146.71	6.54	562.82	6.64
3	28	24	113.29	2.14	12.53	0.35	265.91	7.16	152.17	4.23	574.56	14.64
13	16	6	98.90	5.00	12.73	0.84	246.23	12.56	131.15	8.58	517.21	25.96
13	16	12	105.10	2.30	13.45	0.34	259.58	6.65	145.52	3.05	551.90	12.58
13	16	18	105.24	2.86	13.58	0.47	260.17	8.61	147.42	7.77	549.87	16.02
13	16	24	105.63	6.23	12.63	0.95	257.39	16.46	139.48	17.65	538.13	36.21
13	20	6	126.72	4.36	13.41	0.31	284.64	10.42	174.99	8.11	633.05	17.48
13	20	12	115.70	7.15	11.99	0.71	257.00	19.37	161.65	12.85	573.85	43.86
13	20	18	117.39	2.60	12.79	0.63	267.94	10.80	158.68	6.76	589.80	21.31
13	20	24	117.39	2.60	12.79	0.63	267.94	10.80	158.68	6.76	589.80	21.31
13	24	6	129.61	1.91	12.54	0.58	268.09	10.25	166.29	14.07	611.67	20.96
13	24	12	133.72	2.28	12.07	0.43	259.69	7.68	141.11	6.97	581.04	15.79
13	24	18	125.17	7.05	11.12	0.74	239.40	13.84	150.43	19.76	555.11	30.21
13	24	24	136.02	2.16	12.17	0.25	266.02	5.46	176.56	7.24	621.68	15.40
13	28	6	138.05	7.45	12.90	0.53	276.57	9.79	184.39	15.23	645.80	33.35
13	28	12	146.25	5.68	11.20	0.37	259.79	6.42	178.75	14.49	624.51	24.86
13	28	18	147.51	0.74	11.92	0.47	268.78	7.84	186.85	7.91	650.90	6.84
13	28	24	142.38	3.65	10.71	0.64	245.02	8.59	162.74	22.43	595.07	24.56
23	16	6	113.35	4.18	13.21	0.44	269.63	10.16	138.72	18.81	564.86	28.26
23	16	12	112.85	2.00	12.95	0.22	261.87	3.18	128.92	18.35	541.45	15.91
23	16	18	111.71	0.33	13.22	0.03	268.52	1.25	153.34	1.37	670.72	92.51
23	16	24	117.45	3.02	14.20	0.48	288.09	7.26	160.16	3.93	619.15	12.50
23	20	6	131.27	6.50	13.91	0.54	291.22	11.44	161.62	18.35	643.14	41.71
23	20	12	139.15	3.12	14.85	0.20	306.83	4.27	189.81	2.16	678.94	11.23
23	20	18	124.53	2.02	12.33	0.34	258.49	4.20	161.64	1.43	593.20	3.12
23	20	24	125.13	3.90	12.79	0.22	267.34	5.18	148.67	14.74	590.37	16.99
23	24	6	132.61	4.19	12.44	0.55	276.61	12.55	177.02	20.23	641.21	33.04
23	24	12	148.45	5.61	11.70	0.18	270.62	4.73	197.26	6.23	661.14	11.65
23	24	18	132.89	2.47	11.77	0.46	261.35	4.84	177.70	4.35	620.80	6.69
23	24	24	150.25	4.90	10.77	0.40	259.37	7.64	181.36	18.67	633.12	32.16
23	28	6	134.29	4.53	10.17	0.40	245.97	6.66	182.79	23.28	614.29	32.19
23	28	12	135.19	11.68	10.88	0.85	249.05	16.19	155.98	15.97	598.79	47.72
23	28	18	149.60	5.06	11.49	0.44	268.10	8.76	185.33	18.54	660.07	27.73
23	28	24	153.11	5.02	10.51	0.04	255.41	10.35	164.00	27.17	631.53	43.05
33	16	6	121.65	8.92	13.93	1.13	286.61	20.43	163.61	17.17	623.05	41.25
33	16	12	124.06	0.90	14.38	0.06	293.39	1.16	155.92	3.13	619.39	7.55
33	16	18	70.28	13.79	8.08	1.50	158.62	32.63	99.39	20.68	349.74	72.79
33	16	24	82.21	1.91	10.06	0.08	192.37	4.19	116.52	5.36	419.26	12.81
33	20	6	141.28	5.04	13.08	0.38	281.43	8.81	169.71	18.85	641.36	32.77
33	20	12	145.96	4.16	13.77	0.12	294.32	3.24	182.49	5.36	670.21	11.72
33	20	18	125.96	0.69	12.16	0.34	259.01	3.37	152.27	4.09	584.37	8.39
33	20	24	144.91	23.64	13.12	1.92	284.57	40.39	178.47	32.25	656.19	100.96
33	24	6	145.84	5.22	12.63	0.35	278.49	11.95	144.40	28.40	620.26	44.01
33	24	12	164.64	10.10	13.66	0.84	306.10	19.63	204.29	12.40	725.47	40.77
33	24	18	109.06	13.16	8.79	0.92	197.29	24.84	133.35	19.38	477.53	61.96
33	24	24	117.47	2.60	9.25	0.08	203.63	3.22	130.55	10.28	496.42	12.21
33	28	6	154.14	3.58	11.80	0.53	276.84	7.75	211.13	9.15	692.27	13.09
33	28	12	154.31	1.82	10.30	0.23	262.02	2.51	183.54	15.03	666.37	22.38
33	28	18	152.78	2.28	10.69	0.18	265.50	8.82	180.72	7.51	648.35	19.63
33	28	24	124.65	13.70	9.15	1.02	211.11	26.47	141.55	11.65	521.35	55.05

Table XI – Response surface regression (and respective R^2) of each fatty acid, group/family of fatty acids, and total fatty acid of enriched Artemia nauplii (S – salinity; T – temperature; ET – Enrichment time)

FA	Response surface regression (backward stepwise method)	R^2
14:0	$-16.47+2.14T-0.05T^2-1.35x10^{-3}S^2-1.15x10^{-2}ET^2+3.12x10^{-2}TxET+4.96x10^{-3}SxET$	0.81
16:0	$-2.23+4.96T-0.11T^2+2.44ET-9.02x10^{-2}ET^2-6.12x10^{-3}TxS+5.45x10^{-2}TxET$	0.73
17:0	$2.13+0.15ET-3.66x10^{-3}ET^2-2.20x10^{-4}TxS-3.32x10^{-3}TxET$	0.37
18:0	$15.57+0.19S+0.57ET-2.69x10^{-2}ET^2-9.97x10^{-3}TxS+3.23x10^{-2}TxET-5.15x10^{-3}SxET$	0.78
SFA	$-19.44+8.45T-0.18T^2+0.78S+3.36ET-0.13ET^2-4.46x10^{-2}TxS+0.11TxET$	0.77
Anteiso 15:0	$3.00+0.20ET-5.68x10^{-3}ET^2-5.08x10^{-4}TxS-2.11x10^{-3}TxET$	0.48
Iso 17:0	$1.71+0.09ET-3.06x10^{-3}ET^2-3.41x10^{-4}TxS$	0.53
Anteiso 17:0	$2.64+0.17ET-4.66x10^{-3}ET^2-3.93x10^{-4}TxS-2.45x10^{-3}TxET$	0.48
BFA	$10.9+60.59ET-1.67x10^{-2}ET^2-1.68x10-3TxS-7.04x10^{-3}TxET$	0.46
15:1	$3.13+0.18ET-5.25x10^{-3}ET^2-3.88x10^{-4}TxS-1.21x10^{-3}TxET$	0.52
16:1n-7	$80.80+4.97ET-0.15ET^2-1.22x10^{-2}TxS-3.34x10^{-2}TxET$	0.54
17:1n-8	$4.49+0.26ET-8.64x10^{-3}ET^2-8.63x10^{-4}TxS$	0.56
18:1n-9	$64.03+4.40ET-0.12ET^2-1.15x10^{-2}TxS-3.82x10^{-2}TxET$	0.51
18:1n-7	$1.83+3.46T-0.07T^2+0.47S+1.99ET-7.33x10^{-2}T^2+2.89x10^{-2}xS+.35x10^{-2}TxET$	0.77
19:1n-8	$5.29-0.24T+0.01T^2+0.18ET-4.19x10^{-3}ET^2-3.76x10^{-3}TxET-4.46x10^{-4}SxET$	0.45
20:1n-9	$2.27+2.44x10^{-4}S+20.12ET-3.67x10^{-3}ET^2-8.59x10^{-4}TxS$	0.56
MUFA	$204.14+11.49ET-0.37ET^2-3.70x10^{-2}TxS$	0.57
16:3n-4	$8.98+0.59ET-1.68x10^{-2}ET^2-1.35x10^{-3}TxS-6.28x10^{-3}TxET$	0.49
18:2n-6	$15.45+1.05ET-2.93x10^{-2}ET^2-2.24x10^{-3}TxS-1.07x10^{-2}TxET$	0.45
18:3n-3	$9.00+0.63ET-1.91x10^{-2}ET^2-1.21x10^{-3}TxS-5.05x10^{-3}TxET$	0.42
18:4n-3	$3.76+0.49ET-1.01x10^{-2}ET^2-1.05x10^{-2}TxET-8.91x10^{-4}SxET$	0.56
20:4n-6	$0.31+0.01ET-9.32x10^{-5}SxET$	0.20
20:4n-3	$11.22+0.66ET-1.83x10^{-2}ET^2-1.45x10^{-3}TxS$	0.41
20:5n-3	$56.16+2.60ET-8.83x10^{-2}ET^2-8.47x10^{-3}TxS$	0.27
22:4n-6	$-15.55+1.49T-0.03T^2-0.42ET+3.60x10^{-2}TxET$	0.75
22:5n-6	$-25.02+2.44T-0.06T^2+0.41ET-5.54x10^{-3}ET^2-7.04x10^{-3}TxET$	0.61
22:5n-3	$0.88-1.41x10^{-2}S+0.53ET-4.87x10^{-3}ET^2-1.42x10^{-2}TxET$	0.57
22:6n-3	$-39.50+3.82T-0.09T^2-1.19ET+0.10TxET$	0.83
PUFA	$109.96+5.55ET-0.19ET^2-1.80x10^{-2}TxS+7.39x10^{-2}TxET$	0.54
TFA	$109.51+26.83T-0.56T^2+26.92ET-0.76ET^2-6.56x10^{-2}TxS$	0.63

3.2 Enrichment model

3.2.1 Initial values of the state variables and processes

The initial value of the state variable Percent Survival is 100%. The initial value of the state variable TL is 0.455mm. The initial values of all the FA state variables are listed in Table IV (newly hatched Artemia nauplii FA profile.

The forcing functions temperature, salinity and enrichment time affected some of the processes (causing its acceleration or delay), while did not affect others:

Incorporation of ARA = $a + b$ x Salinity

Incorporation of EPA = $c + d$ x Enrichment time

Incorporation of DHA = $e + f$ x Temperature

Incorporation of LA = $g + h$ x Enrichment time + i x Temperature

Incorporation of ALA = $j + k$ x Enrichment time + l x Temperature

Incorporation of SFA = $m + n$ x Enrichment time + o x Temperature

Incorporation of BFA = $p + q$ x Enrichment time + r x Temperature

Incorporation of MUFA = $s + t$ x Enrichment time

Incorporation of PUFA = $u + v$ x Enrichment time + w x Temperature

Mortality = $x + y$ x Enrichment time + z x Temperature

Growth = $a2 + a3$ x Enrichment time + $a4$ x Temperature + $a5$ x Salinity

Parameters values (*a* to *z*, *a2*, *a3* and *a4*) are listed in Table I. This model can be used for conditions of constant temperature and salinity, but it can also be used with varying temperatures and salinities over enrichment time.

3.2.2 Sensitivity analysis

Sensitivity analysis allowed the determination of the parameters with a major influence on disturbances of our predictions. In this model, the forcing function with higher sensitivity was temperature, particularly for the state variable DHA (2.93). State variables were not very sensitive to changes in salinity. The sensitivity of the parameters was generally low, with the exception of the parameters *e* (associated with change over enrichment time) and *f* (associated with the effect of temperature) which had respectively a sensitivity of -2.09 and 2.92 for the state variable DHA (Table XII).

Table XII – Sensitivity of the forcing functions and parameters for all state variables

	State variables										
	ARA	**EPA**	**DHA**	**LA**	**ALA**	**SFA**	**BFA**	**MUFA**	**PUFA**	**%S**	**TL**
Forcing function											
Temperature	0.00	0.00	2.93	-0.09	-0.13	0.17	-0.09	0.00	0.08	-0.09	0.27
Salinity	-0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01
Parameter											
a	0.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
b	-0.04	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
c	0.00	0.40	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
d	0.00	-0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
e	0.00	0.00	-2.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
f	0.00	0.00	2.92	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
g	0.00	0.00	0.00	0.56	0.00	0.00	0.00	0.00	0.00	0.00	0.00
h	0.00	0.00	0.00	-0.16	0.00	0.00	0.00	0.00	0.00	0.00	0.00
i	0.00	0.00	0.00	-0.09	0.00	0.00	0.00	0.00	0.00	0.00	0.00
j	0.00	0.00	0.00	0.00	0.59	0.00	0.00	0.00	0.00	0.00	0.00
k	0.00	0.00	0.00	0.00	-0.17	0.00	0.00	0.00	0.00	0.00	0.00
l	0.00	0.00	0.00	0.00	-0.13	0.00	0.00	0.00	0.00	0.00	0.00
m	0.00	0.00	0.00	0.00	0.00	0.32	0.00	0.00	0.00	0.00	0.00
n	0.00	0.00	0.00	0.00	0.00	-0.18	0.00	0.00	0.00	0.00	0.00
o	0.00	0.00	0.00	0.00	0.00	0.17	0.00	0.00	0.00	0.00	0.00
p	0.00	0.00	0.00	0.00	0.00	0.00	0.48	0.00	0.00	0.00	0.00
q	0.00	0.00	0.00	0.00	0.00	0.00	-0.12	0.00	0.00	0.00	0.00
r	0.00	0.00	0.00	0.00	0.00	0.00	-0.09	0.00	0.00	0.00	0.00
s	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.46	0.00	0.00	0.00
t	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.18	0.00	0.00	0.00
u	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.39	0.00	0.00
v	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.19	0.00	0.00
w	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00	0.00
x	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.10	0.00
y	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.08	0.00
z	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.09	0.00
a2	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.11
a3	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	-0.03
a4	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.27
a5	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.01

3.2.3 Calibration

The model predictions for TL, TFA, DHA, SFA, MUFA and PUFA adjust relatively well to the data (R^2=0.71, 0.60, 0.81, 0.75, 0.50 and 0.52, respectively), while the model predictions for Survival, ARA, EPA, BFA, ALA and LA (R^2=0.25, 0.21, 0.21, 0.26, 0.14 and 0.33, respectively) were not so precise (Figures 1-4). However, standardized residuals for all state variables were distributed randomly around zero indicating that the model predictions closely reflect the data (Figure 1-4) and mostly inside de 95% confidence level [-1.96;1.96].

Figure 1 – Model calibration/validation: standard residuals and observed vs. expected values of survival, total length, total FA content (TFA).

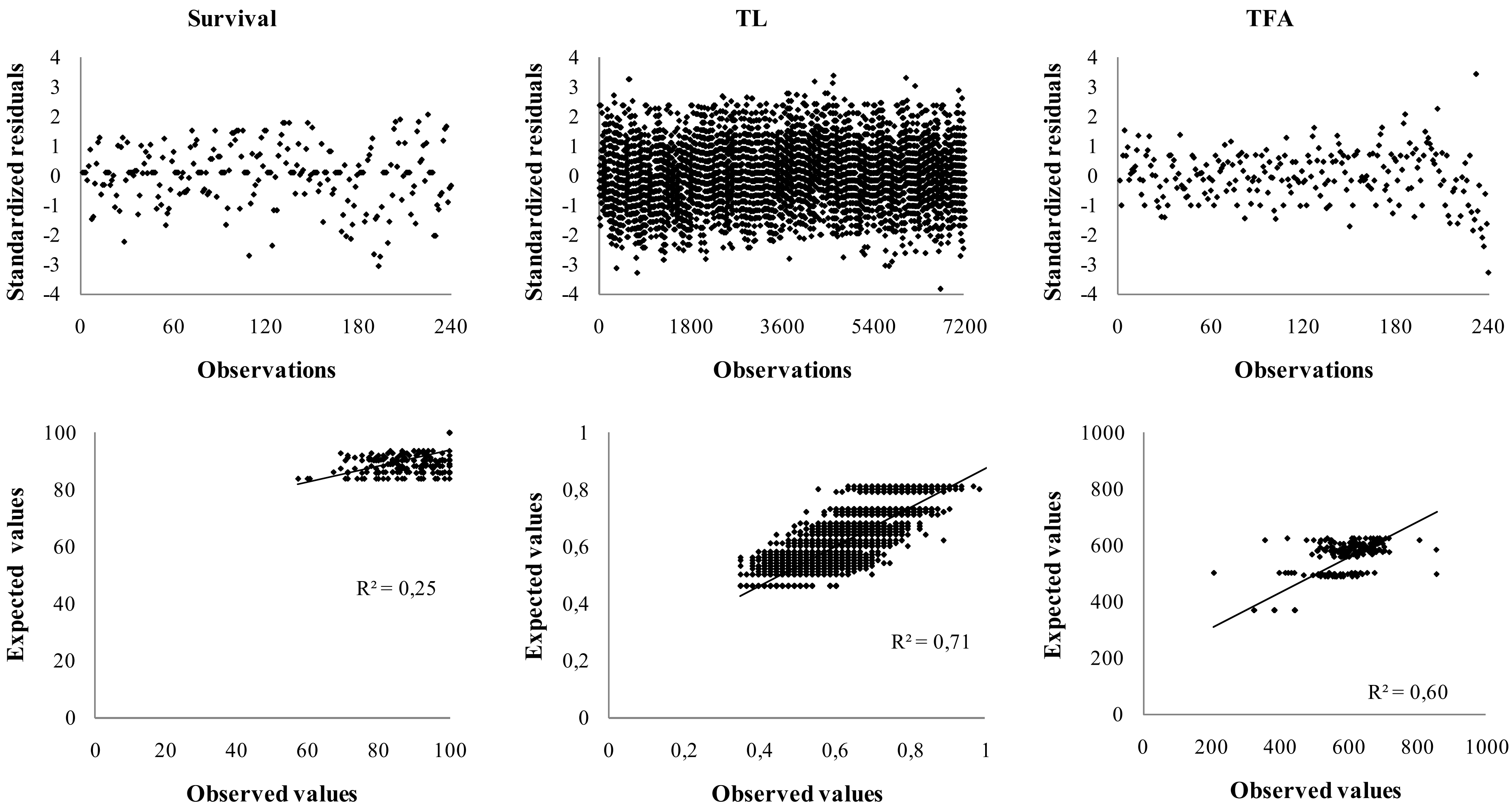


Figure 2 – Model calibration/validation: standard residuals and observed vs. expected values of the essential fatty acids (ARA, EPA and DHA).

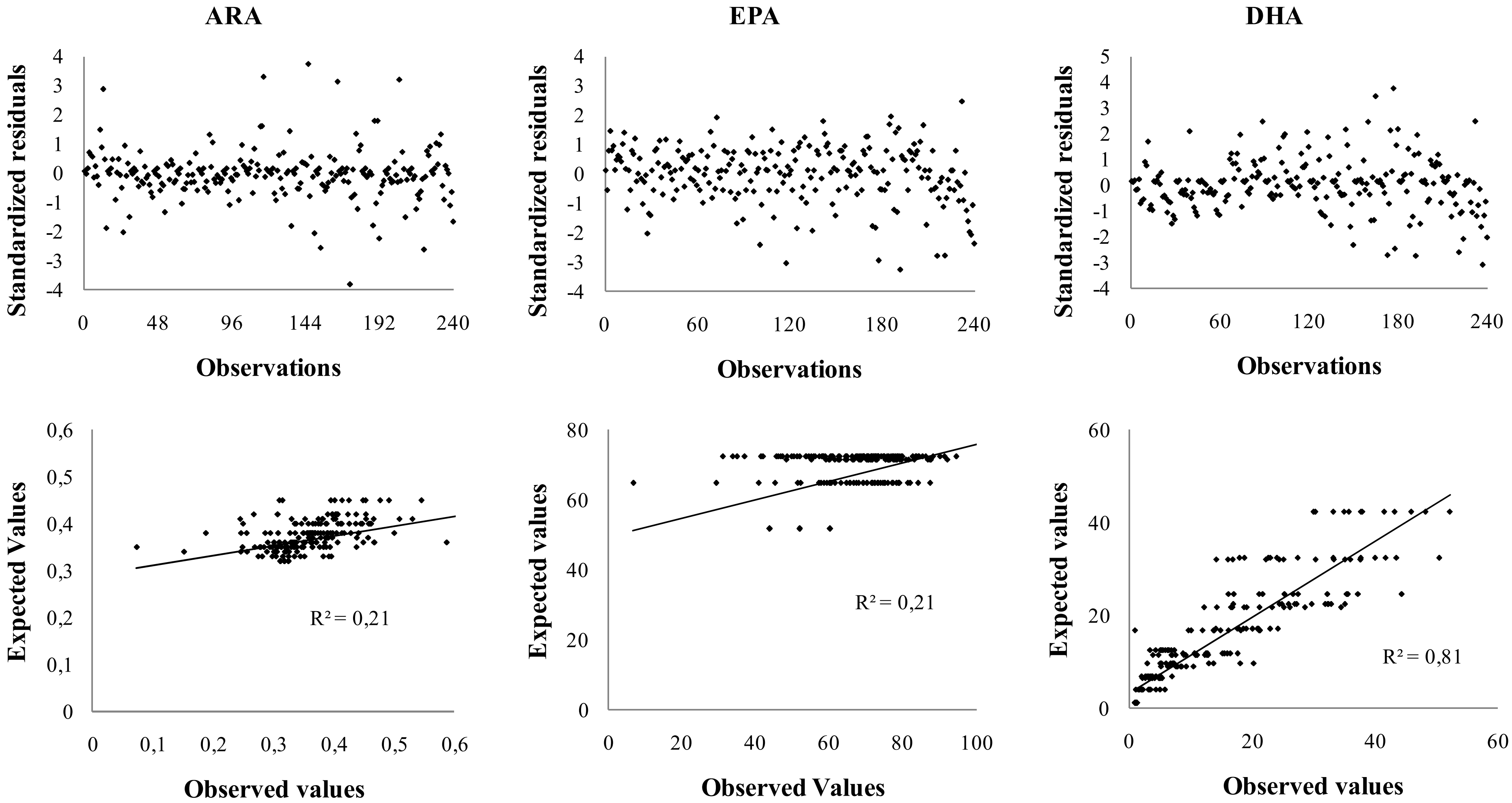


Figure 3 – Model calibration/validation: standard residuals and observed vs. expected values of linolenic (ALA) and linoleic acid (LA) and SFA.

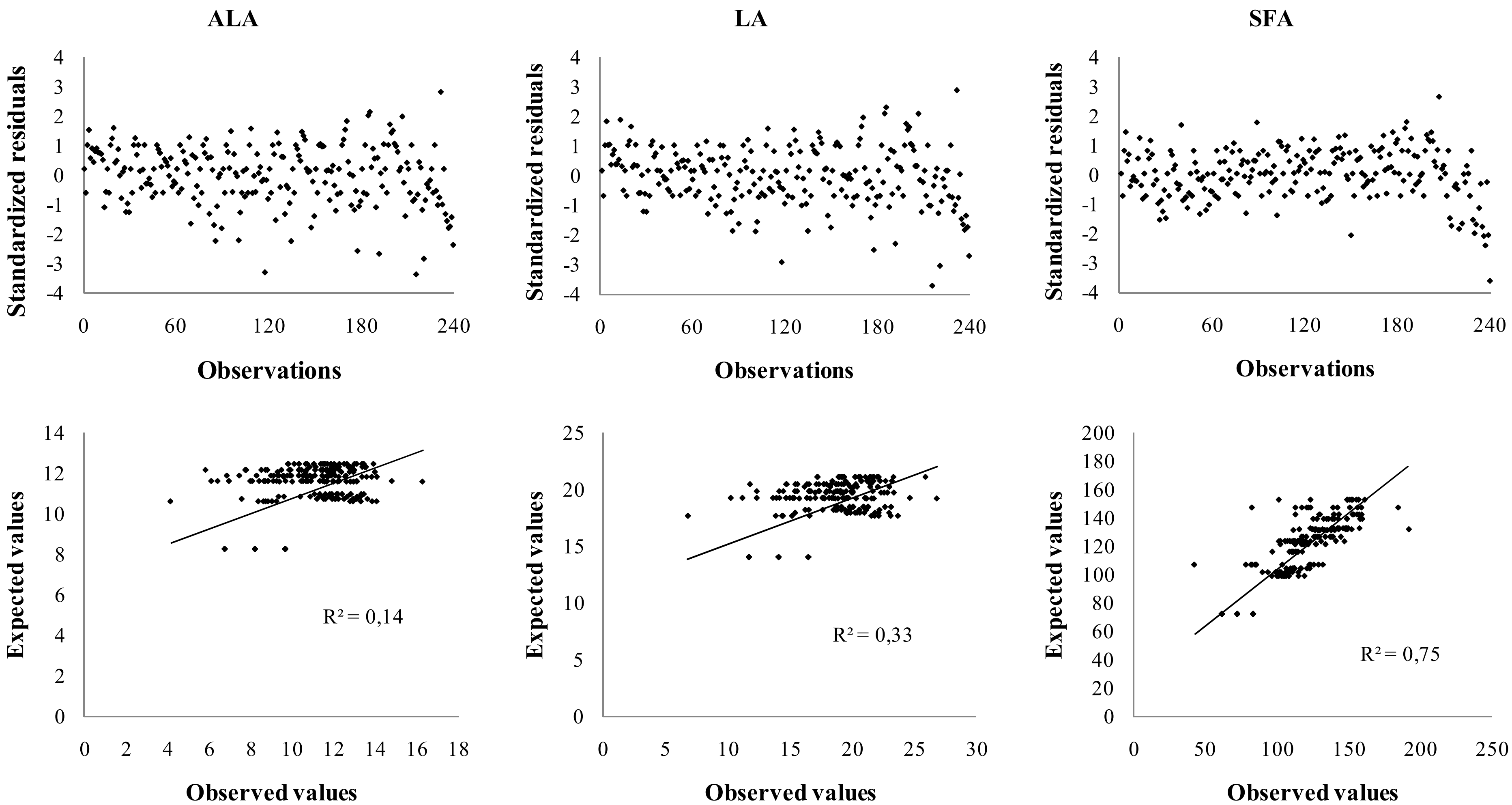
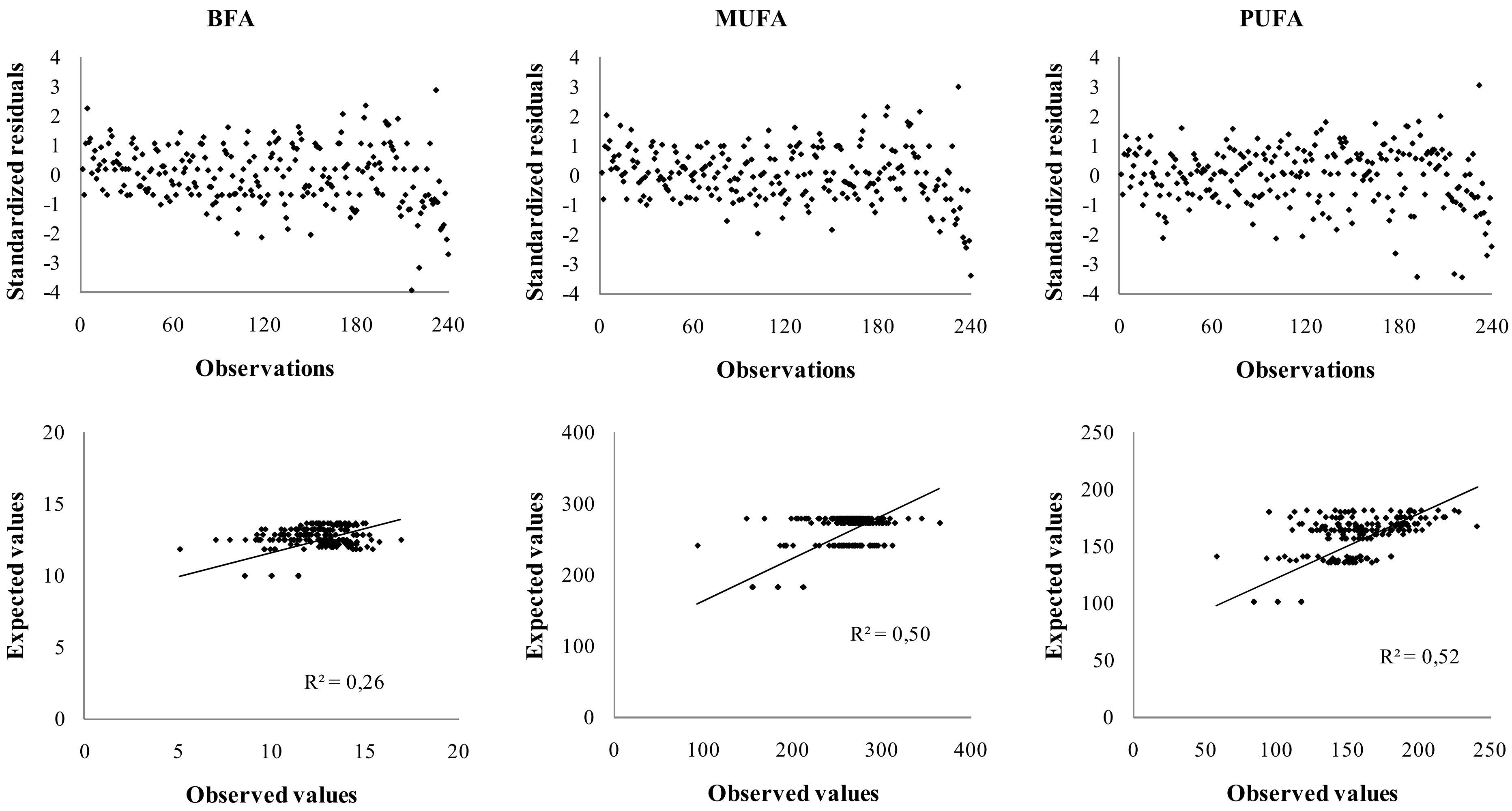


Figure 4 – Model calibration/validation: standard residuals and observed vs. expected values of BFA, MUFA and PUFA.



3.2.4 Model predictions

The predictions of percent survival, total length and DHA content of the preys enriched under two scenarios of temperature (16 and 28ºC) are presented in Figure 5; since salinity does not influence DHA incorporation, a salinity of 3 was used during enrichment to reduce mortality and growth in both scenarios. Figure 5 shows that in scenario 1 (28ºC) DHA incorporation is maximized, nauplii grow faster (larger prey size) and survival is lowered. In scenario 2, growth and mortality are reduced, but its DHA content drastically lowers.

The enrichment protocols that could produce a nauplii with the desired size (TL ≤ 0.65 mm, nauplii stage II) are presented in Table XIII. DHA content, Survival (%) and DHA: EPA ratio are optimised/maximized with the combination 21ºC, salinity 3 and 23 h of enrichment time (Table XIII).

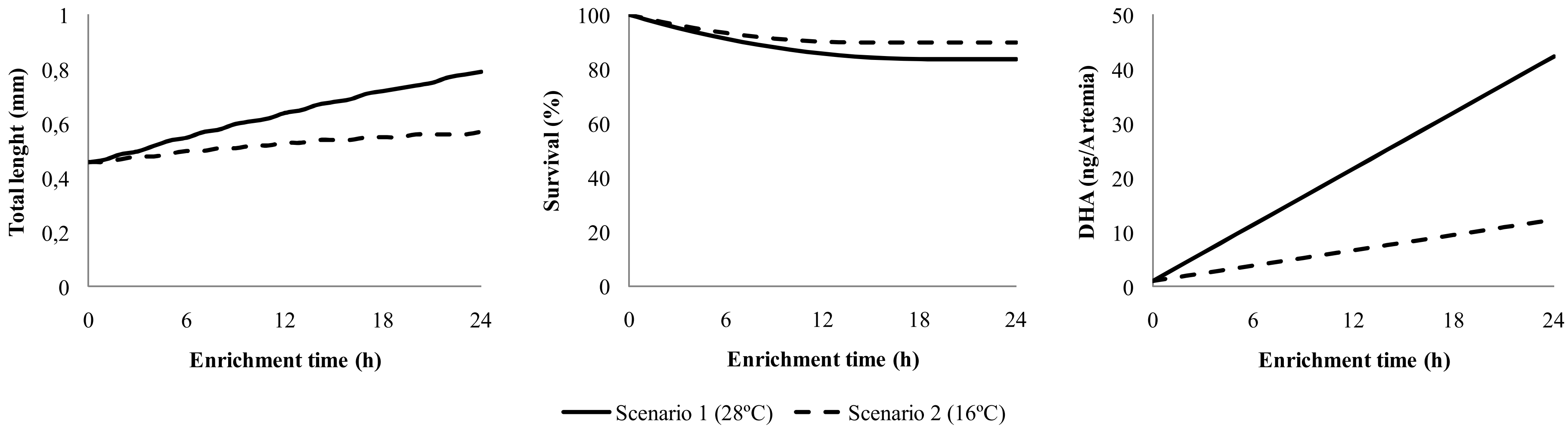
Table XIII – Possible enrichment protocols (combinations of temperature and enrichment time) to produce an enriched Artemia nauplii with TL ≤ 0.65 mm, and respective DHA content and Survival (%) estimated by the model

Temperature (ºC)	Salinity	Enrichment time (h)	TL (mm)	Survival (%)	DHA (ng.Artemia^{-1})	DHA:EPA:ARA ratio	DHA:EPA ratio
16	3	24	0.57	90.2	12.46	27.7:160.9:1	0.17:1
17	3	24	0.58	89.7	14.95	33.2:160.9:1	0.21:1
18	3	24	0.6	89.2	17.45	38.8:160.9:1	0.24:1
19	3	24	0.62	88.7	19.94	44.3:160.9:1	0.28:1
20	3	24	0.64	88.2	22.44	49.9;160.9:1	0.31:1
21	3	23	0.65	87.7	23.94	54.4:164.5:1	0.33:1
22	3	20	0.65	87.1	23.04	54.9.172.4:1	0.32:1
23	3	18	0.65	86.6	22.71	55.4:176.6:1	0.31:1
24	3	17	0.65	86.0	23.28	56.8:176.6:1	0.32:1
25	3	15	0.64	85.8	22.23	55.6:181.0:1	0.31:1
26	3	14	0.64	85.6	22.27	57.1:185.3:1	0.31:1
27	3	13	0.64	85.6	22.11	56.7:184.5:1	0.31:1
28	3	13	0.65	85.2	23.46	60.2:184.5:1	0.33:1

4. Discussion

According to the model, Artemia nauplii survival during the enrichment was not significantly affected by salinity which can be explained by the fact that Artemia is a euryhaline species (Narciso, 2000) with the most efficient osmoregulatory system of the animal kingdom (Croghan, 1958). Artemia has never been found in the sea or estuaries since it does not possess anatomic defences against predation and it is an easy prey for

Figure 5 – Total length (mm), Survival (%) and DHA content (ng.Artemia nauplii^{-1}) for Scenario 1 (28ºC) and 2 (16ºC).



carnivorous species. In nature, it is only found in hypersaline environments where the salinity is above the upper limit of its predators (Narciso, 2000). Artemia mortality increased with enrichment time; mortality rate was hastened in higher temperatures. This can be explained by an acceleration of the metabolic process in higher temperatures (Hochachka and Somero, 1984; Prosser, 1986), which directly influences survival rates (Sastry, 1977; Anger, 2001).

Temperature as been recognised as a key environmental factor influencing crustacean development and growth (Abele, 1982; Hartnoll, 1982; Verhoef et al., 1998; Anger, 2001). Artemia nauplii length significantly increased over the enrichment process. The growth rate increased with temperature, which may be explained by an acceleration of the metabolism and consequently greater moult frequency (Hochachka and Somero, 1984; Prosser, 1986). Lower temperatures caused a reduced final total length (as it has been already described by Narciso (2000) and Reeve (1963)). While several authors state that an increase in salinity reduced growth rate (Koraay, 1958; Gilchrist, 1960; Baid, 1963; Baid, 1964), in this study the opposite was obseved: growth rate was greater at higher salinities, which had been also found by Narciso (2000). Nevertheless, all literature agrees that temperature is a greater deterministic factor for growth than salinity (Narciso, 2000).

The fatty acid content, particularly essential fatty acid, of the prey is a determinant for larval culture success (Monroig et al., 2006). The level of each fatty acid in enriched Artemia nauplii varies as a function of the enrichment protocol (Han et al., 2000; Han et al., 2001). Newly hatched Artemia had a relatively high content in EPA (0.02 DHA: 1 EPA), but a low DHA and ARA content (3.41 DHA: 162.28 EPA: 1 ARA, Table IV), similar to what has already been described by several authors (Coutteau and Mourente, 1997; Han et al., 2001; Narciso and Morais, 2001). Enrichment intends to improve and balance the fatty acid profile, particularly increasing DHA content, to attain the optimal essential fatty acid ratio of 10 DHA: 5 EPA: 1 ARA suggested by Sargent et al. (1999) and Castell et al. (2001). AlgaMac 2000® composition, according to the manufacturers (Bio-Marine, Inc.), is 27% DHA and 0.54% EPA in TFA, i.e., 50 DHA: 1 EPA. Artemia nauplii enriched with AlgaMac 2000® had its EPA content increasing, but DHA content and DHA: EPA ratio also increased (Table IX).

Higher temperatures cause an increase of the metabolic rates which is then reflected in a negative effect of temperature in ALA and LA levels (higher temperatures reduce the ALA and LA contents). This is a good outcome since high concentrations of ALA and LA competitively suppress the conversion of EPA into DHA; enzyme Δ-6 desaturase used in the catabolism of ALA and LA is also used in the synthesis reactions to convert EPA to DHA (Voss et al., 1991; Teshima et al., 1992; Buzzi et al., 1996; Sargent et al., 1997). The BFA and PUFA content were also lower at higher temperatures. Lower salinities promoted higher ARA incorporation, which is similar to what Vanhaecke and Sorgeloos (1980) previously described: lower salinities (5) produce nauplii displaying a higher energetic value. Higher temperatures generally cause an increase of the metabolic rate, and consequently may enhance ingestion rate, increase the DHA incorporation and hasten the catabolism of other FA. The enrichment process in Artemia is generally regarded as a "bioencapsulation" process whereby the Artemia ingest the enricher particles until the gut is full. Larger individuals, with larger digestive tract, will also have, for instance, a higher content in DHA. Thus, the conditions that promote greater length of the nauplii (such as higher temperatures) may also promote higher DHA content. Feeding on larger nauplii with greater energetic content is advised since the predator will spend less energy capturing a smaller number of prey to fulfill its energetic requirements, unless that interferes with the feeding processes of the predators (Narciso, 2000). This is a problem when the larvae we desire to culture feed by swallowing prey and their mouth is gape limited; if the prey is too large, the success capture of the prey is jeopardized. In this case, we have to choose feeding them with smaller nauplii with an inherent increment of capture energetic effort.

Model predictions (scenarios 1 and 2) show us that temperature produces antagonist effects on the objectives of enrichment: promotes the production of a prey with higher fatty acid content, particularly DHA (which is the essential fatty acid that lacks in newly hatched Artemia nauplii), but promotes large size prey and increases mortality during the enrichment process. Scenario 1 (higher temperature) would be ideal in cases when larvae do not have mouth gape limitations. But how can the model help us to find the optimal combinations of temperature, salinity and enrichment time to produce a suitable when we have larvae with mouth gape limitations? The example developed on Table XIII illustrates how the model can help in this situation. Utilizing the model, we find to produce a nauplii with the desired size, DHA content, Survival

(%) and DHA: EPA ratio are optimised/maximized with the combination 21ºC, salinity 3 and 23 h of enrichment time (Table XIII). Against what has been previously suggested by other authors (Sorgeloos et al., 2001), the ideal salinity during enrichment is a low salinity since it reduces growth (Reeve, 1963), increases ARA incorporation and has no effect on DHA incorporation.

An Artemia enrichment model can be a helpful tool to culture culture commercial species whose larvae have specific demands on prey size (e.g. fish prey are conditioned by gape height) and nutritional profile (particularly in some essential fatty acid such as EPA, DHA and ARA). The model predicts the optimal combination of temperature, salinity and enrichment time to minimize mortality, control length and improve fatty acid profile of Artemia nauplii.

6. Conclusion

Artemia nauplii are the most commonly used prey in the culture of fish and crustacean larvae. However, a great number of species cannot be successfully raised with newly hatched Artemia nauplii due to low content of certain essential fatty acids, particularly, DHA. Since Artemia is a non-selective continuous filter-feeder, its nauplii can be encapsulated with enrichment products rich in EFA. The possibility of modifying Artemia nauplii fatty acid profile through the use of different enrichment product allowed researchers and aquaculturists to work with a more balanced larval diet (Sorgeloos and Léger, 1992; Narciso, 2000). The enrichment procedure, however, has disadvantages since it causes Artemia nauplii mortality and growth, which can be a limiting factor for its successful capture by mouth gape limited larvae. Temperature, salinity and enrichment time can be manipulated to optimise the final product (enriched Artemia nauplii). In this study we examined all these factors simultaneously using a factorial design to understand their interactions.

Temperature seems to be the forcing function that most influences Artemia percent survival, total length and fatty acid profile; high temperatures favour growth and fatty acid incorporation, but increases mortality. Salinity is more important for growth, and ARA incorporation. The best use of the enrichment model is to find the optimal combination of temperature, salinity and enrichment time to improve nauplii FA profile, while minimizing growth and mortality during enrichment, when we have specific limitations in regards to prey size and fatty acid profile. This model was designed for the enrichment of Artemia franciscana (marine strain) with AlgaMac 2000®, and therefore will need to be adapted to predict results for enrichment of other Artemia strains and/or other enrichment products.

A possible and important follow-up for this study (and model) would be to predict survival, total length and fatty acid content after the enrichment, i.e., once the prey is moved to the larval tank to feed the larvae, the Artemia nauplii become expose to starvation. It will be important to take into consideration the change in the fatty acid content of each nauplii, the undesired growth of the prey and the mortality due to starvation during this period. Several authors reported that enriched Artemia nauplii subject to starvation see their FA content decrease, particularly DHA which is converted to EPA (Dhert et al., 1993; Danielsen et al., 1995; Triantaphyllidis et al., 1995; Evjemo et al., 1997; Estévez et al., 1998; Navarro et al., 1999; Han et al., 2001). It will be important to know for how long does the prey remain suitable, i.e., maintains a suitable fatty acid profile and a size that does not diminish the chances of successful capture. This timing will determine the frequency of prey replacement. Temperature is also known to be an important aspect during this period; the de-enrichment rate, as well as the mortality rate, is known to be reduced at lower temperatures (Evjemo et al., 1997; Evjemo et al., 2001). Thus, when culturing cold water species, the nauplii starvation might not have such bad consequences to the Artemia nauplii nutritional profile as when culturing tropical species (Estévez et al., 1998; Evjemo et al., 2001).

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References

Abele, L.G., 1982. Larval Morphology and Diversity. In: Vernberg, F.J., Vernberg, W.B. (Eds.) The Biology of Crustacea, vol. 2. Academic Press, New York, USA. pp. 43-100.

Anger, K., 1998. Patterns of growth and chemical composition in decapod crustacean larvae. Invertebr. Repr. Dev. 33, 159-176.

Anger, K., 2001. The biology of decapod crustacean larvae. In: Vonk, R. (Ed) Crustacean Issues, vol.14. A. A. Balkema Publishers, Lisse, 419 pp.

Baid, I.C., 1963. The effect of salinity on growth and form of Artemia salina. J. Exp. Zool. 153, 279-283.

Baid, I.C., 1964. Neoteny in the genus Artemia. Acta Zool. 45, 167-177.

Beck, J.L., Turingan, R., 2007. The effects of zooplankton swimming behavior on prey-capture kinematics of red drum larvae, Sciaenops ocellatus. Mar. Biol. 151, 1463-1470.

Bergé, J.P., Barnathan, G., 2005. Fatty acids from lipids of marine organisms: molecular biodiversity, roles as biomarkers, biologically active compounds, and economical aspects. Adv. Biochem. Engin/Biotechnol. 96, 49-105.

Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipids extraction and purification. Can. J. Biochem. Physiol. 37, 911-917.

Buzzi, M., Henderson, R.J., Sargent, J.R., 1996. The desaturation and elongation of linolenic acid and eicosapentaenoic by hepatocytes and liver microsomes from rainbow trout (Oncorhyncus mykiss) fed diets containing fish oil or olive oil. Biochim. Biophys. Acta 1299, 235-244.

Castell, J.D., Blair, T., Neil, S., Howes, K., Mercer, S., Reid, J., Young-Lai, W., Gullison, B., Dhert, P., Sorgeloos, P., 2001. The effect of different HUFA enrichment emulsion on the nutritional value of rotifers (Brachionus plicatilis) to larval haddock (Melanogrammus aeglefinus). In: Hendry, C.I., Van Stappen, G., Wille, M., Sorgeloos, P. (Eds.), Larvi'01 – Fish and Shellfish Larviculture

Symposium, European Aquaculture Society, Special Publication, vol. 30. Oostende, Belgium, pp. 111-114.

Chen, Q., Sheng, J., Lin, Q., Gao, Y., Lv, J., 2006. Effect of salinity on reproduction and survival of the copepod Pseudodiaptomus annandalei Sewell, 1919. Aquaculture 258, 575-582.

Christensen, S.M., Macintosh, D.J., Phuong, N.T., 2004. Pond production of the mud crabs Scylla paramamosain (Estampador) and S. olivacea (Herbst) in the Mekong Delta, Vietnam, using two different supplementary diets. Aquacult. Res. 35, 1013-1024.

Coutteau, P., Mourente, G., 1997. Lipid classes and their content of n-3 highly unsaturated fatty acids (HUFA) in Artemia franciscana after hatching, HUFA-enrichment and subsequent starvation. Mar. Biol. 130, 81-91.

Croghan, P.C., 1958. The osmotic and ionic regulation of Artemia salina. J. Exp. Biol. 35, 213-218.

Danielsen, T.L., Evjemo, J.O., Olsen, Y., 1995. Stability of short term enriched n-3 fatty acids in Artemia during starvation at different temperatures. European Aquaculture Society Special Publication 24, 128-131.

Dhert, P., Sorgeloos, P., Devresse, B., 1993. Contributions towards a specific DHA enrichment in the live food Brachionus plicatilis and Artemia sp. In: Reinertsen, H., Dahle, L., Jorgensen, L., Tvinnereims, K. (eds.) Fish Farming Technology, Rotterdam, 482 pp.

Estévez, A., McEvoy, L.A., Bell, J.G., Sargent, J.R., 1998. Effects of temperature and starvation time on the pattern and rate loss of essential fatty acids in Artemia nauplii previously enriched using arachidonic acid and eicosapentaenoic acid-rich emulsions. Aquaculture 165, 295-311.

Evjemo, J.O., Coutteau, P., Olsen, Y., Sorgeloos, P., 1997. The stability of docosahaexaenoic acid in two Artemia species following enrichment and subsequent starvation. Aquaculture 155, 135-148.

Evjemo, J.O., Danielsen, T.L., Olsen, Y., 2001. Losses of lipid, protein and n-3 fatty acids in enriched Artemia franciscana starved at different temperatures. Aquaculture 193, 65-80.

FAO. 2006. State of world aquaculture. FAO Fisheries Technical Paper. No. 500. Rome, FAO. 2006. 134p.

Figueiredo, J., Narciso, L., 2006. Productivity improvement of Lysmata seticaudata larval rearing protocol through modelling. Aquaculture 261, 1249 – 1258.

Figueiredo, J., Penha-Lopes, G., Lin, J., Narciso, L., 2008. Productivity and profitability of Mithraculus forceps aquaculture. Aquaculture 283, 43-49.

Forsberg, O.I., Guttormsen, A.G., 2005. A pigmentation model for farmed Atlantic salmon: Non linear regression analysis of published experimental data. Aquaculture 253, 415-420.

Gilchrist, B. Y., 1960. Growth and form of the brine shrimp Artemia salina (L.) Proc. Zool. Soc. Lond. 134, 221-235.

Grant, J., Curran, K.J., Guyondet, T.L., Tita, G., Bacher, C., Koutitonsky, V., Dowd, M., 2007. A box model of carrying capacity for suspended mussel aquaculture in Lagune de la Grande-Entrée, Iles-de-la-Madeleine, Québec. Ecol. Model. 200, 193-206.

Halachmi, I., 2006. Systems engineering for ornamental fish production in a recirculating aquaculture system. Aquaculture 259, 300–314.

Halachmi, I., Simon, Y., Guetta, R., Hallerman, E.M., 2005. A novel computer simulation model for design and management of recirculating aquaculture systems. Aquac. Eng. 32, 443–464.

Han, K., Geurden, I., Sorgeloos, P., 2000. Enrichment strategies for Artemia using emulsions providing different levels of n-3 highly unsaturated fatty acids. Aquaculture 183, 335-347.

Han, K., Geurden, I., Sorgeloos, P., 2001. Fatty acid changes in enriched and subsequently starved Artemia franciscana nauplii enriched with different essential fatty acids. Aquaculture 199, 93-105.

Hanson, J.S., Griffin, W.L., Richardson, J.W., Nixon, C.J., 1985. Economic feasibility of shrimp farming in Texas: an investment analysis for semi-intensive pond grow-out. J. World Aquac. Soc.16, 129–150.

Harel, M., Koven, W., Lein, I., Bar, Y., Behrens, P., Stubblefield, J., Zohar, Y., Place, A.R., 2002. Advanced DHA, EPA, and ArA enrichment materials for marine aquaculture using single cell heterotrophs. Aquaculture 213, 347-362.

Harrison, K.E., 1990. The role of nutrition in maturation, reproduction and embryonic development of decapod crustaceans: a review. J. Shellfish Res. 9, 1-28.

Hartnoll, R. G. 1982. Growth. In: Bliss, D.E., Abele, L.G. (Eds.), The Biology of Crustacea, vol. 2. Academic Press, New York, USA. pp. 111-196.

Hernández, J.M., Gasca-Leyva, E., León, C.J., Vergara, J.M., 2003. A growth model for gilthead seabream (Sparus aurata). Ecol. Model. 165, 265-283.

Hochachka, P. W., Somero, G. N. 1984. Temperature adaptation. In: Hochachka, P.W., Somero, G.N. (Eds.) Biochemical adaptation. University Press, Princeton, New Jersey, USA. pp. 355-449.

Jepsen, P.M., Andersen, N., Holm, T., Jørgensen, A.T.; Højgaard, J.K., Hansen, B.W., 2007. Effects of adult stocking density on egg production and viability in cultures of the calanoid copepod Acartia tonsa (Dana). Aquacult. Res. 38, 764-772.

Jørgensen, S.E., Bendoricchio, G., 2001. Fundamentals of Ecological Modelling, 3rd ed. Developments in Environmental Modelling 21.Elsevier, Amsterdam. 530 pp.

Koray, T., 1958. The effect of salinity on the growth of Izmir Calmati Saltern Artemia. E.U. Faculty of Science Journal 6B, 67-75.

Krebs, J. M., Turingan, R.G., 2003. Intraspecific variation in gape-prey size relationships and feeding success during early ontogeny in red drum, Sciaenops ocellatus. Environ. Biol. Fish 66, 75-84.

Kuehl, R.O., 1994. Statistical Principles of the Research Design and Analysis. Duxbury Press. Wadsworth Publishing Company, Belmont, California, USA. 686pp.

Kumlu, M., Jones, D.A., 1995a. Feeding and digestion in the caridean shrimp larva of Palaemon elegans Rathke and Macrobrachium rosenbergii (De Man) (Crustacea: Palaemonidae) on live and artificial diets. Aquacult. Nutr. 1, 3-12.

Kumlu, M, Jones, D.A., 1995b. The effect of live and artificial diets on growth, survival, and trypsin activity in larvae of Penaeus indicus. J. World Aquacult. Soc. 26(4), 406-415.

Léger, P., Bengtson, D.A., Sorgeloos, P., Simpson, K.L., Beck, A.D., 1987. The nutritional value of Artemia: a review. In: Sogeloos, P., Bengtson, D.A., Decleir, W., Jaspers, E. (Eds.), Artemia Research and its Applications. Ecology, Culturing, Use in Aquaculture. Universa Press, Wetteren, pp. 357-372.

Mayer, C.M., Wahl, D.H., 1997. The relationship between prey selectivity and growth and survival in larval fish. Can. J. Fish. Aquat. Sci. 54, 1504-1512.

McCarthy, A. M., Bales, J.D., Cope, W.G., Damian Shea, D., 2007. Modeling pesticide fate in a small tidal estuary. Ecol. Model. 200, 149–159.

McConaugha, J. R., 1985. Nutrition and larval growth. In: Larval Growth - Crustacean Issues, vol.2 (Ed. Wenner, A. M.). AA Balkema Publishers, Rotterdam, Netherlands. pp. 127-154.

McEvoy, L.A., Navarro, J.C., Amat, F., Sargent, J.R., 1995. Autoxidation of oil emulsions during the Artemia enrichment process. Aquaculture 134, 101-112.

McEvoy, L.A., Navarro, J.C., Hontoria, F., Amat, F., Sargent, J.R., 1996. Two novel Artemia enrichment diets containing polar lipid. Aquaculture 144, 339-352.

Metcalfe, L.D., Schmitz, A.A., 1961. The rapid preparation of fatty acids esters for gas chromatography analysis. Anal. Chem. 33, 363-364.

Miller, T.J., 2001. Matrix-Based Modelling of Blue Crab Population Dynamics with Applications to the Chesapeake Bay. Estuaries 24 (4), 535-544.

Monroig, O., Navarro, J.C., Amat, F., González, P., Bermejo, A., Hontoria, F., 2006. Enrichment of Artemia nauplii in essential fatty acids with different types of liposomes and their use in the rearing of gilthead sea bream (Sparus aurata) larvae. Aquaculture 257, 382-392.

Mookerji, N., Rao, T. R., 1994. Influence of ontogenic changes in prey selection on the survival and growth of rohu, Labeo rohita and singhi, Heteropneustes fossilis larvae. J. Fish Biol. 44, 479-490.

Narciso, L. 2000. Biologia e Cultivo de Artemia sp. (Crustacea, Branchiopoda): sua Utilização em Aquacultura. Prémio do Mar Rei D. Carlos 1998. Câmara Municipal de Cascais. 94pp.

Narciso, L., Morais, S., 2001. Fatty acid profile of Palaemon serratus (Palaemonidae) eggs and larvae during embryonic and larval development using different live diets. J. Crust. Biol. 21, 566-574.

Navarro, J.C., Henderson, R.J., McEvoy, L.A., Bell, M.V., Amat, F., 1999. Lipid conversions during enrichment of Artemia. Aquaculture 174, 155-166.

Nunes, A.J.P., Parsons. G.J., 2006. A computer-based statistical model of the food and feeding patterns of the Southern brown shrimp Farfantepenaeus subtilis under culture conditions. Aquaculture 252, 534-544.

Olivotto, I., Rollo, A., Sulpizio, R., Avella, M., Tosti, L., Carnevali, O., 2006. Breeding and rearing the Sunrise Dottyback Pseudochromis flavivertex: the importance of live prey enrichment during larval development. Aquaculture 255, 480-487.

Payne, M. F., Rippingale, R. J., 2000. Evaluation of diets for culture of the calanoid copepod Gladioferens imparipes. Aquaculture 187, 85-96.

Peck, M.A., Holste, L., 2006. Effects of salinity, photoperiod and adult stocking density on egg production and egg hatching success in Acartia tonsa (Calanoida: Copepoda): Optimizing intensive cultures. Aquaculture 255, 341-350.

Penha-Lopes, G., Figueiredo, J., Narciso, L., 2007. Modelling survival and growth of larvae and juveniles of Mithraculus forceps (A. Milne Edwards, 1875) (Decapoda: Brachyura: Majidae) in aquaculture. Aquaculture 264, 285-296.

Prosser, C. L. 1986. Adaptational biology: Molecules to organisms. John Wiley and Sons, Inc., New York, USA.

Pryor, V. K., Epifanio, C.E., 1993. Prey selection by larval weakfish (Cynoscion regalis): the effects of prey size, speed, and abundance. Marine Biology 116, 31-37.

Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155, 103-115.

Rajkumar, M., Kumaraguru K.P., 2006. Suitability of the copepod, Acartia clausi as a live feed for Seabass larvae (Lates calcarifer Bloch): Compared to traditional live-food organisms with special emphasis on the nutritional value. Aquaculture 261, 649-658.

Reeve, M. R., 1963. Growth Efficiency in Artemia under laboratory conditions. Biological Bulletin 125, 133-145.

Ritar, A. J., Smith, G.G., Dunstan, G.A., Brown, M.R., Hart, P.R., 2003. Artemia prey size and mode of presentation: effects on the survival and growth of phyllosoma larvae of southern rock lobster (Jasus edwardsii). Aquacult. Int. 11, 163-180.

Ritar, A.J., Dunstan, G.A., Nelson, M.M.,Brown, M.R., Nichols, PD., Thomas, C.W., Smith, E.G., Crear, B.J., Kolkovski, S. 2004. Nutritional and bacterial profiles of juvenile Artemia fed enrichments and during starvation. Aquaculture 239, 351-373.

Rønnestad, I., 1995. Interpretation of ontogenic changes in composition studies of fish embryos and larva – presenting proportional data can lead to erroneous conclusions. Aquacult. Nutr. 1, 199.

Sargent, J.R, McEvoy, L.A., Bell, J.G., 1997. Requirements, presentation and sources of polyunsaturated fatty acids in marine fish larval feeds. Aquaculture 155, 117-127.

Sargent, J., Bell, G., McEvoy, Tocher, D. L., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191-199.

Sastry, A. N., 1977. The larval development of the rock crab, Cancer irroratus Say, 1817. under laboratory conditions (Decapoda Brachyura). Crustaceana 32, 155-168.

Shields, R.J., Bell, G., Luizi, F.S., Gara, B., Bromage, N.R., Sargent, J.R., 1999. Natural copepods are superior to enriched Artemia nauplii as feeds for halibut larvae (Hippoglossus hippoglossus) in terms of survival, pigmentation, and retinal morphology: relation to dietary essential fatty acids. J. Nutr. 129, 1186-1194.

Sørensen, T.F., Drillet, G., Engell-Sørensen, K., Hansen, B. W., Ramløv, H., 2007. Production and biochemical composition of eggs from neritic calanoid copepods reared in large outdoor tanks (Limfjord, Denmark). Aquaculture 263, 84–96.

Sorgeloos, P., Léger, P., 1992. Improved larviculture outputs of marine fish, shrimp and prawn. J. World Aquacult. Soc. 23(4), 251-264.

Sorgeloos, P., Lavens, Ph., Léger, P., Tackaert, W., Versichele, D., 1986. Production of Artemia nauplii and decapsulated cysts for use as food source in the hatchery production of fish and crustaceans. FAO Manual for the Culture of Brine Shrimp Artemia in Aquaculture. University of Ghent, Ghent, Belgium, pp. 69-117.

Sorgeloos, P., Dhert, P., Candreva, P., 2001, Use of the brine shrimp, Artemia spp., in marine fish larviculture. Aquaculture 200, 147-159.

Southgate, P.C., Lou, D. C., 1995. Improving the *n*-3 HUFA composition of Artemia using microcapsules containing marine oils. Aquaculture 134, 91-99.

Teshima, S.I., Kanazawa, A., Koshio, S., 1992. Ability for bioconversion of n-3 fatty acids in fish and crustaceans. Océanis 18, 67-75.

Thinh, L., Renaud, S.M., Parry, D.L., 1999. Evaluation of recently isolated Australian tropical microalgae for the enrichment of the dietary value of brine-shrimp, Artemia nauplii. Aquaculture 170, 161-173.

Tocher, D.R., Mourente, G., Sargent, J.R., 1997. The use of silages prepared from fish neural tissues as enrichers for rotifers (Brachionus plicatilis) and Artemia in the nutrition of larval marine fish. Aquaculture 148, 213-231.

Triantaphyllidis, G.V., Coutteau, P., Sorgeloos, P., 1995. The stability of n-3 HUFA in various Artemia populations following enrichment and subsequent starvation. European Aquaculture Society Special Publication 24, 149-153.

Turingan, R. G., Beck, J.L., Krebs, J.M., Licamele, J. D., 2005. Development of feeding mechanisms in marine fish larvae and the swimming behavior of zooplankton prey: implications for rearing marine fishes. In: Lee C.S., O'Brien P.J., Marcus N. M. (eds.) Copepods in aquaculture. Blackwell, Ames, pp 119-132.

Vanhaecke, P., Sorgeloos, P., 1980. International Study on Artemia. . In: Persoone, G., Sorgeloos, P., Roels, O., Jaspers, E. (eds.) IV The biometrics of Artemia strains from different geographical origin. Universia Press, Wetteren, Belgium, pp. 393-405.

Verhoef, G. D., Austin, C. M., Jones, P. L., Stagnitti, F. 1998. Effect of temperature on molt increment and intermolt period of a juvenile australian fresh-water crayfish, Cherax destructor. J. Crust. Biol. 18 (4), 673-679.

Voss, A., Reinhart, M., Sankarappa, S., Sprecher, H., 1991. The metabolism of 7, 10, 13, 16, 19-docosapentaenoic acid to 4, 7, 10, 13, 16, 19-docosahexaenoic acid in rate liver is independent of a 4-desaturase. J. Biol. Chem. 266, 19995-20000.

Yi, Y., 1998. A bioenergetics growth model for Nile tilapia (Oreochromis niloticus) based on limiting nutrients and fish standing crop in fertilized ponds. Aquac. Eng. 18, 157– 173.

Yu, R., Leung, P., Bienfang, P., 2006. Optimal production schedule in commercial shrimp culture. Aquaculture 254, 426-441.

Zhu, S., Deltour, J., Wang, S., 1998. Modeling the thermal characteristics of greenhouse pond systems. Aquac. Eng. 18, 201– 217.

Zmora, O., Shpigel, M., 2006. Intensive mass production of Artemia in a recirculated system. Aquaculture 255, 488-494.