International Journal of Agricultural Technology 2017 Vol. 13(1): 59-72
Available online http://www.ijat-aatsea.com
ISSN 2630-0192 (Online)

Effects of Arachidonic Acid Supplementation in Maternal Diet on Low-Salinity Tolerance of Newly Hatched Larvae of Giant Freshwater Prawn (*Macrobrachium rosenbergii* De Man)

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Kangpanich, C., Pratoomyot, J., Siranonthana, N. and Senanan, W. (2017). Effects of arachidonic acid supplementation in maternal diet on low-salinity tolerance of newly hatched larvae of giant freshwater prawn (*Macrobrachium rosenbergii* De Man). International Journal of Agricultural Technology 13(1):59-72.

Abstract Arachidonic acid (20:4n-6, ARA) is an important fatty acid in giant river prawn reproduction. Improving larval quality may be possible through improved nutrition of maternal diets. The present study evaluated the effects of ARA supplementation in maternal diets on survival rates of newly hatched larvae exposed to six salinity levels (0, 2, 4, 6, 8, and 14 ppt) for 24 hours. Six experimental diet consisted of two base diets differing in fish oil (FO)/soy oil (SO) blends (A and B) and three ARA supplementation levels 0, 0.4 and 0.8%. The two diets diffred in the proportional contents of linoleic acid (18:2n-6, LOA, B>A) and docosahexaenoic acid (22:6n-3, DHA, A>B). At 2, 4 and 6 ppt, the survival rates at 24 hours of newly hatched larvae from females fed the diet with 0.8% ARA supplementation and higher LOA contents from SO (diet B2) were highest followed by those fed the B1 diet (0.4% supplemented ARA) ($p<0.05$) (B2=87.04, 93.32 and 98.88%; B1=79.23, 91.84 and 97.03 % for 2, 4 and 6 ppt respectively). Similarly, 24-hour LD_{50} values were lowest for the B2 treatment (0.80±0.02), suggesting their high tolerance to extremely low salinity levels. Our results have a great implication for an inland hatchery operation where seawater is a scarce resource.

Keywords: giant freshwater prawn, *Macrobrachium rosenbergii*, Arachidonic acid, ARA, maternal diet, salinity test

Introduction

The giant freshwater prawn, *Macrobrachium rosenbergii*, is an economically important species in the Indo-Pacific region (New, 2005). In 2013, the global production of *M. rosenbergii* was 203,028 tons, making it the 7th

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largest crustacean aquaculture industry (FAO, 2016). Thailand is among the major prodcucers of this species although its *M. rosenbergii* production in recently years has been steadily declined to 18,168 tons in 2013 (FAO, 2016). In Thailand, *M. rosenbergii* farming has been well-established expanded across all geographic regions, especially central Thailand, since late 1990s. As a consequence, the aquaculture production of this species had continuously increased from 2,200 tons in 1998 to 36,200 tons in 2008. However, the 2009 production of *M. rosenbergii* had dropped to 27, 500 tons as a consequence of disease outbreaks, a shortage of broodstock, and the unavailability of high quality post settlement juveniles (Department of Fisheries, 2007). Since then, the production has been on a declining trend to the level below 20,000 tons in 2013.

One approach to improve quality of hatchery-produced larvae is through improved maternal diets. Essential fatty acids (EFAs) are extremely important for prawn maturation and reproduction because they are precursors to prostaglandins (PGs), a group of biologically active lipid compound that controls a wide-range of relevant reproductive functions (Lytle *et al*., 1990). Like other crustaceans, *M. rosenbergii* is not able to synthesize EFA or elongate short chain n-3 and n-6 polysaturated fatty acid (C18, PUFA) to longer chain PUFA (C≥20, PUFA). Relevant EFA for prawn reproduction include linoleic acid (18:2n-6, LOA, Cavalli *et al*., 1999; Kangpanich *et al*. 2016), arachidonic acid (20:4n-6, ARA, Kangpanich *et al*. 2016), and n-3 long-chain polyunsaturated fatty acids (n-3 lcPUFA), such as EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (D'Abramo and Sheen, 1993; Cavalli *et al.*, 1999). Laval quality (e.g. tolerance to suboptimal condition) is partially controlled by maternal diets (e.g., De Caluwe, Lavens, and Sorgeloos, 1995; Cavalli *et al.*, 1999) and therefore may be improved upon EFA supplementation in maturation diets. This is particularly important for the newly hatched larvae as they are still dependent on yolk for nutrients.

ARA is an important precursor of prostaglandin E2 (PGE2) and F2α (PGF2α). It is one of the fatty acids preferentially retained in the ovaries of crustaceans (Kumar, 2013; Cavalli *et al.*, 2001). In *M. rosenbergii*, Kangpanich *et al.* (2016) suggested that both ARA and LOA are essential for ovarian maturation and embryonic development. In addition, broodstock diets containing high level of LOA and overall n-3 PUFA enhanced fecundity of females and improve offspring quality compared to that containing low LOA and n-3 PUFA levels (Cavalli *et al.*, 1999).

M. rosenbergii is a diadromous species. During its life cycle, *M. rosenbergii* typically exposes to a wide range of salinities (0-18 ppt) (Cheng *et al*., 2003). Juveniles and subadults live and grow in freshwater environment and mature adults migrate to a brackish environment to reproduce. The newly

hatched larvae, therefore require immediate exposure to brackish water for survival and growth for at least 1-2 days (Nandlal, 2005) after which they can be acclimatized to fresh water. An optimal salinity for larval hatchery production ranges from 12 to 16 ppt (New, 2003). For juveniles and subadults, Singh (1980) demonstrated that in aquaculture settings they can grow in salinity up to 17 ppt with highest growth achieved at salinity between 0 ppt and 2 ppt. Chand *et al*., 2015 also stated that juvenile of *M. rosenbergii* are grew and survived satisfactorily at 0-15 ppt salinities.

For this study, we aimed to determine the effect of supplemented dietary ARA and different SO/FO blends in maternal diets on the quality of newly hatched larvae giant freshwater prawn, *M. rosenbergii*. These oil blends in diets represent a different fatty acid profile, especially the proportional contents of LOA and n-3 lcPUFA. We are particularly interested in the larval tolerace to extremely low salinity, a production trait that may help lower operation costs for hatchery production.

Materials and methods

Experimental design and broodstock prawns

We used a 6 × 3 completely randomized design (CRD) in this experiment, with six dietary treatments performed in triplicate. Each experimental unit was sub-divided into fifteen 18 x 13 x 12 cm compartments, each of which contained one individual female. Each unit received one of the six diet formulations. All females (270 individuals) were raised in a 3 x 3 x 1.2 m recirculating concrete tanks. Males were kept separately until mating in a two communal 1 x 3 x 1 m concrete tanks. There was no unit partion in the male tank.

Approximately four-month-old *M. rosenbergii* adults were obtained from a commercial prawn farm in Chachoengchao Province, Thailand. Individuals were acclimatized to 28 ^{o}C in a freshwater recirculation system at Rajamangala University of Technology Tawan-ok and fed a commercial prawn diet (Charoen Pokphand Foods, 40% protein and 8 % lipid) for seven days. The initial weight and total length of females and males were 15.22 ± 0.13 g and 11.12 ± 0.09 cm, and 17.68 ± 0.22 g and 11.44 ± 0.09 cm, respectively. Only females received the experimental diets; the males were fed the commercial prawn diet (Charoen Pokphand Foods, 38% protein). Experimental animals were fed twice daily (07.00 and 18.00) at approximately 5% of body weight.

Experimental animals were maintained at an optimal water quality. During the experimental period, the temperature ranged from 26.4-28.3^{o}C, dissolved oxygen from 7.12-7.78 mgL^{-1}, pH from 8.19-8.38, alkalinity from 178.42-242.33

mgL^{-1}, hardness from 129.42-146.33 mgL^{-1}, ammonia nitrogen, NO_2-N and NO_3-N were 0.01-0.06, 0.01-0.04 and 0.05-0.08 mgL^{-1}. The water quality was consistent across all treatments during the experiment.

Experimental diets

Experimental diets were formulated to meet the nutritional requirements of adult *M. rosenbergii* recommended by Somsueb (2009) and Cavalli *et al*. (1999) (Table 1). The six experimental isonitrogenous and isolipidic diets contained approximately 42% protein and 9% lipid by weight. Each diet contained one of the two base compositions (A or B) and one of the three ARA supplementation levels (0, 0.4 or 0.8% by ingredient weight). The ARA used in this study was derived from *Mortierella alpine*, containing 40% ARA. One of the base diets (base diet A) contained 2% of FO and 2% SO by ingredient weight (A0, A1, A2) and the other (base diet B) contained 1.5% FO and 2.5% SO (diets B0, B1, B2). These diets were identical to those described in Kangpanich *et al*. (2016) (Tables 1 and 2).

The diets contained similar proximate compositions and energy contents (Table 1). The digestible energy content of each diet was between 320 and 325 Kcal $100g^{-1}$. The base diets A and B mainly differed in their total monenes, saturates, LOA, EPA and DHA contents. The base diet B contained higher monoenes (22.91 ± 0.45% to 25.03 ± 0.62% of total fatty acids) and LOA (20.39 ± 1.45% to 22.49 ± 0.55% of total fatty acids) while the diet A contained higher saturates (32.01± 1.52 to 36.46 ± 1.33%), EPA (4.54 ± 0.17 to 5.58 ± 0.11%) and DHA (9.15 ± 0.05 to 10.47 ± 0.26%). ARA supplementation differentiated diets A1, A2 from A0 and B1, B2 from B0 (Table 2, Figure 1).

Table 1. Ingredients (% dry weight) and proximate composition (%) of the experimental diets.

Ingredients	A0	A1	A2	B0	B1	B2
	2%FO+2%SO (Base diet A)			1.5%FO+2.5%SO (Base diet B)		
	0%ARA	0.4%ARA	0.8%ARA	0%ARA	0.4%ARA	0.8%ARA
Fish meal[1]	35.0	35.0	35.0	35.0	35.0	35.0
Soybean meal	25.0	25.0	25.0	25.0	25.0	25.0
Shrimp shell meal	14.0	14.0	14.0	14.0	14.0	14.0
Corn grain	5.0	5.0	5.0	5.0	5.0	5.0
Wheat meal	5.0	4.0	3.0	5.0	4.0	3.0
Rice bran	10.0	10.0	10.0	10.0	10.0	10.0
Fish oil	2.0	2.0	2.0	1.50	1.50	1.50
Soy oil	2.0	2.0	2.0	2.50	2.50	2.50
ARA(40%)[2]	0	0.4	0.8	0	0.4	0.8
Binder[3]	1.0	1.0	1.0	1.0	1.0	1.0
Vitamin premix[4]	1.0	1.0	1.0	1.0	1.0	1.0
Proximate composition						
Moisture	6.74	6.88	5.77	6.09	5.86	6.17
Protein	42.19	42.60	41.70	41.53	42.53	42.04
Lipid	9.41	9.36	9.17	9.27	9.47	9.65
Ash	16.70	16.90	17.73	16.78	16.21	15.95
*NFE	24.96	24.26	25.63	26.33	25.93	26.19
Digestible energy (Kcal 100g-[1])	321.32	319.17	318.50	320.89	324.88	324.82

*NFE=nitrogen free extract + fiber

[1]Mix of marine fish containing 55% protein from Siam fish meal Lp.

[2]Arachidonic acid 40% made from *Mortierella alpine* (single cell oil). from Anhui Minmetals development I/E Co., Ltd.

[3] α-starch from Mario bio products., Co. Ltd.

[4] premix prawn from Planet Aquatic Chemical Co., Ltd.

Table 2. Major classes of fatty acids (% of total fatty acids) of the experimental diets (modified from Kangpanich *et al*. (2016)).

Fatty acids	A0	A1	A2	B0	B1	B2
	2%FO+2%SO (Base diet A)			1.5%FO+2.5%SO (Base diet B)		
	0%ARA	0.4%ARA	0.8%ARA	0%ARA	0.4%ARA	0.8%ARA
18:2n6(LOA)	17.23 ± 0.96	14.85 ± 1.15	13.35 ± 0.74	20.39 ± 1.45	21.78 ± 0.38	22.49 ± 0.55
20:4n6(ARA)	2.77 ± 0.05	6.17 ± 0.26	9.43 ± 0.28	3.08 ± 0.08	5.74 ± 0.12	8.33 ± 0.04
18:3n3(LNA)	6.27 ± 1.93	7.66 ± 1.03	6.77 ± 0.83	7.82 ± 0.56	6.89 ± 0.38	7.31 ± 0.18
20:5n3(EPA)	4.54 ± 0.17	5.09 ± 0.16	5.58 ± 0.11	4.74 ± 0.04	4.43 ± 0.13	4.05 ± 0.09
22:6n3(DHA)	10.47 ± 0.26	9.99 ± 0.45	9.15 ± 0.05	7.41 ± 1.50	6.12 ± 0.30	6.78 ± 0.63
∑saturates	36.46 ± 1.33	34.47 ± 0.38	32.01 ± 1.52	31.59 ± 0.88	30.01 ± 0.37	28.13 ± 0.32
∑monoenes	22.27 ± 1.88	21.77 ± 1.01	24.37 ± 0.54	24.97 ± 0.48	25.03 ± 0.62	22.91 ± 0.45
∑n-6PUFA	19.99 ± 0.93	21.02 ± 1.06	22.77 ± 1.03	23.47 ± 1.41	27.52 ± 0.50	30.82 ± 0.54
∑n-3PUFA	21.27 ± 1.02	22.74 ± 0.86	21.51 ± 0.89	19.97 ± 2.05	17.44 ± 0.24	18.14 ± 0.61
∑ n-3lcPUFA	15.01 ± 0.40	15.08 ± 0.60	14.73 ± 0.10	12.15 ± 1.49	10.56 ± 0.25	10.83 ± 0.71
∑n-3/∑n-6	1.06 ± 0.08	1.09 ± 0.08	0.95 ± 0.08	0.85 ± 0.13	0.64 ± 0.02	0.59 ± 0.03

Values are given as the mean ± standard deviation (n = 3).
∑saturates = 14:0, 15:0, 16:0, 17:0, 18:0.
∑monoenes = 16:1, 17:1, 18:1n9.
∑n-6PUFA = 18:2n-6, 20:4n-6.
∑n-3PUFA = 18:3n-3, 20:5n-3, 22:6-n-3.
∑n-3lcPUFA = 20:5n-3, 22:6-n-3.

Mating and larval preparation

For mating, we paired a mature female to a male at a 1:1 ratio. A mature male from the male tank was manually placed in a compartment with a freshly molted gravid female bearing a stage V ovary. After the eggs were fertilized, the male was removed from the cage; each male was used only once. Freshly fertilized eggs (orange eggs, OE, Habayashy *et al.*, 2012) migrated to the female's brood chamber in the abdominal area and became visible at approximately eight hours after mating.

At approximately 9-10 days after OE became visible, three gravid females bearing brownish eggs (midstage to advanced embryos) from each experimental unit of the dietary treatments were weighed and transferred into an aerated 20-L tank containing 14 ppt water. We then assigned their newly hatched

larvae to tanks contained water with one of the six salinity levels. We, therefore, obtained nine observations of the larval survival rates for each dietary treatment for each tested salinity level (described below).

Determination of larval quality: low-salinity tolerance

We tested the tolerance to low salinity of newly hatched larvae of the experimental females. The larvae from each dietary treatment were exposed to one of the six salinity levels, namely 0, 2, 4, 6, 8, 14 ppt for 24 hours. Each experimental unit for the salinity treatments contained 30 individual larvae. A group raised in 14 ppt water was treated as a control. We recorded the survival for each treatment at 3, 6, 9, 12 and 24 hours. We also estimated LD_{50}, a salinity level led to 50% mortality of experimental animals at a given time, based on probit analysis implemented in StatPlus Professional (Analystsoft, 2008).

Statistical analysis

To evaluate the variation of fatty acid profiles among experimental diets, we performed principal component analysis based on percentages of 13 fatty acids detected (only major fatty acids are reported in Table 2). Total saturates and monoenes were treated as a supplementary variable on a principal component analysis (PCA) biplot for a better understanding of the ordination based on the 13 active quantitative variables; it did not interfere with the ordination. To simplify the multidimensional data (13 dimensions in our case), this multivariate approach reduced the dimensions to two dimensions in our case. The first few principal components typically captured most of the variation in the data set. PCA was performed using algorithms implemented in the FactoMineR package (Lê, Josse & Husson, 2008) and the biplot was created using factoextra package (Kassambara, 2015), developed in the R statistical language (R Development Core Team, 2015).

We determined the differences among dietary treatments in the LD_{50} values at 24 hours using one-way analysis of variance (ANOVA). The significance of the differences in means was determined by Duncan's new multiple range test at a p value < 0.05. ANOVA was executed with SPSS version 17 for Windows (SPSS, Inc.). Because larval survival data were not normally distributed, we performed Kruskal-Wallis rank tests to determine statistical differences among treatments and subsequent post-hoc multiple comparisons. These nonparametric tests and post-hoc comparisons were performed with the statistical software R, version 3.3.1 for Windows (http://www.r-project.org, R Development Core Team, 2015).

Results

Variation in fatty acid profiles among experimental diets

Principal component analysis revealed distinct fatty acid profiles based on diet type and ARA treatment (Figure 1). The first two components explained approximately 58% of total variation, with the first and second dimensions explaining 38.33% and 19.62% respectively. Diets A and B differed in their LOA, EPA and DHA proportional contents in the fatty acid profiles (dimension 1). The fatty acid composition of each diet generally reflects the ARA treatments. The dietary ARA contents were diferent between diets with and without ARA supplementation (A0, B0 vs. A1, A2, B1 and B2, dimension 2) (Figure 1).

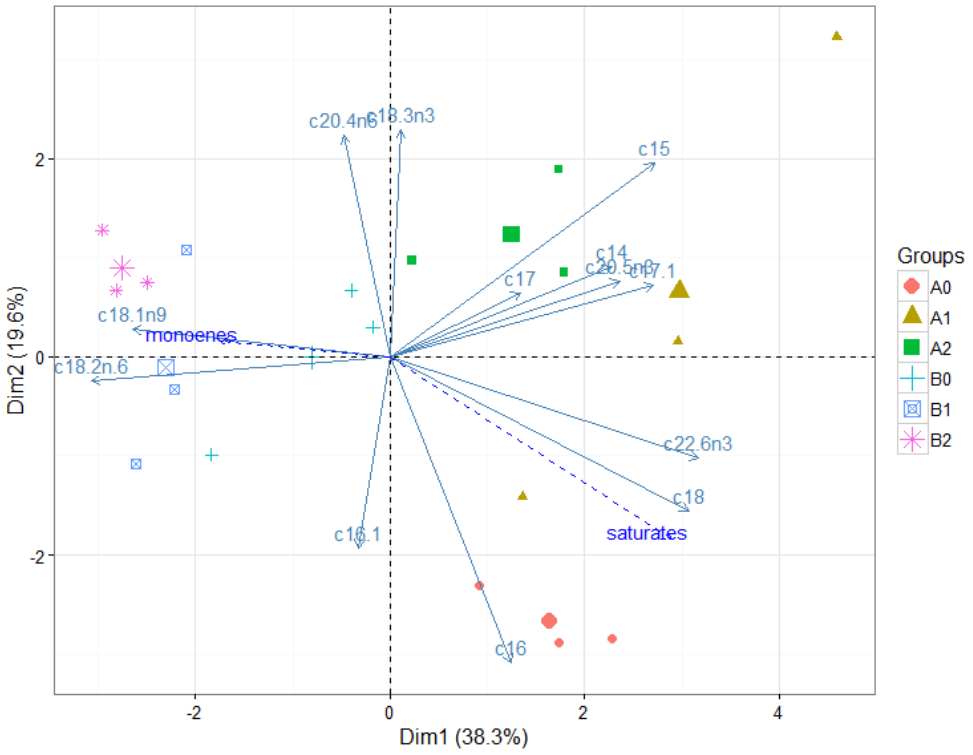

Figure1. Principal component analysis (PCA) biplot based on proportional contents of 13 fatty acids detected in the experimental diets.

Survival of larvae exposed to various salinity levels

At 24 hours after exposure, we observed 0% and 100% survival of larvae exposed to 0 ppt and 14 ppt for all dietary treatments (Table 3). The survival for all salinity levles, larvae of females fed diet B2 had the highest survival rates ($p<0.05$) followed by those in the treatment B1. At 2 ppt, the larval survival ranged from 62.22 (A0) to 87.04 % (B2). At 4 ppt, the survival ranged from 87.39 (A0) to 93.32% (B2). At 6 and 8 ppt, the survival ranges from 93.32 (A0) and 98.88% (B2) and 98.14 (A0) to 99.99 (B2) respectively. As the salinity level approached 14 ppt, the differences among dietary treatments became less noticeable.

The pattern of the differences in survival rates of larvae among dietary treatments was consistent across all observation periods at low salinity levels (Figure 2). Larvae in treatment B2 generally had the highest survival rates at all observation periods for all salinity levels, followed by treatment B1. At 2, 4 and 6 ppt, we began to observe statistical differences in the larval survival rates among treatments at 6 hours after exposure onward with larvae from treatment B2 having the highest survival ($p<0.05$) followed by those from treatment B1. At 8 ppt, the differences among treatments were apparent after 9 hours with the treatment B0 having the lowest survival ($p<0.05$); survival rates were not different among the remaining treatments.

Table 3. Survival rates of *M. rosenbergii* larvae exposed to different salinity for 24 hours.

Salinity (ppt)	A0	A1	A2	B0	B1	B2
	2%FO+2%SO (Base diet A)			1.5%FO+2.5%SO (Base diet B)		
	0%ARA	0.4%ARA	0.8%ARA	0%ARA	0.4%ARA	0.8%ARA
0	0	0	0	0	0	0
2	62.22 ± 9.43^{c}	65.93 ± 16.39^{bc}	62.22 ± 6.45^{c}	56.67 ± 14.43^{c}	79.23 ± 4.65^{b}	87.04 ± 3.89^{a}
4	87.39 ± 5.95^{b}	89.25 ± 2.77^{b}	88.51 ± 4.11^{b}	79.99 ± 6.23^{b}	91.84 ± 2.42^{ab}	93.32 ± 2.35^{a}
6	93.32 ±2.88^{b}	94.43 ± 1.67^{b}	95.92 ± 2.22^{b}	94.06 ± 2.77^{b}	97.03 ± 2.00^{ab}	98.88 ± 1.67^{a}
8	98.14 ± 2.42^{a}	99.25 ± 1.47^{a}	98.88 ± 1.67^{a}	96.65 ± 1.67^{b}	99.62 ± 1.11^{a}	99.99 ± 0.01^{a}
14	100	100	100	100	100	100

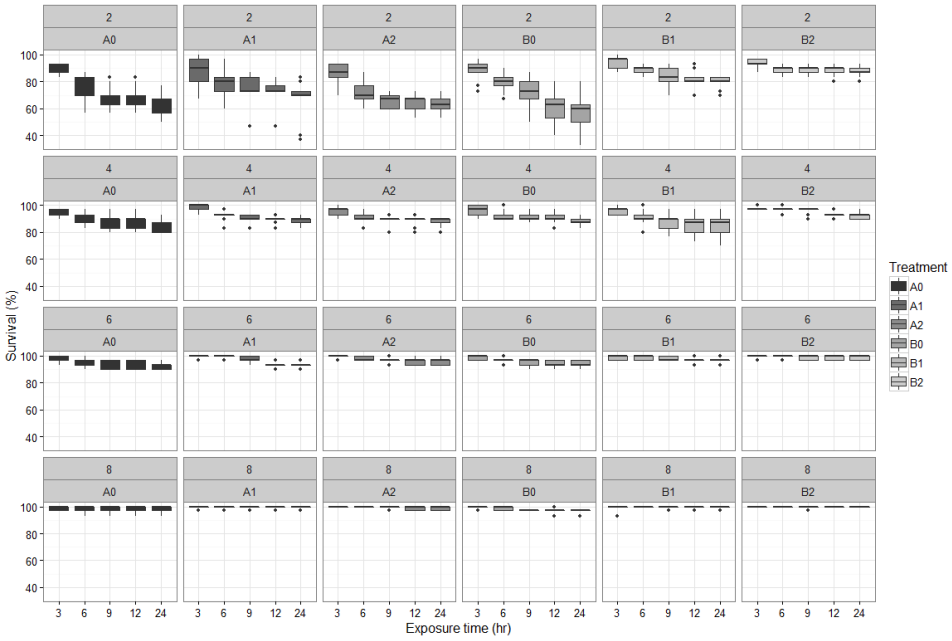

Figure 2. Cumulative survival of newly hatched larvae of experimental female *M. rosenbergii* at 2, 4, 6, 8 ppt during 3, 6, 9, 12 and 24 hours after the exposure.

The 24-hour LD_{50} values significantly differed among dietary treatments. Treatment B2 had the lowest 24-hour LD_{50} estimate (0.81±0.02) followed by the treatment B1 while treatments A0 and B0 had highest values (1.66±0.27 and 1.67±0.43) ($p<0.05$). The values for treatments A1, A2 and B1 were not statistically different ($p>0.05$). The results suggested that larvae from the treatments B2 could tolerate much lower salinity level than those from other treatments. This observation was consistent with the overall survival pattern.

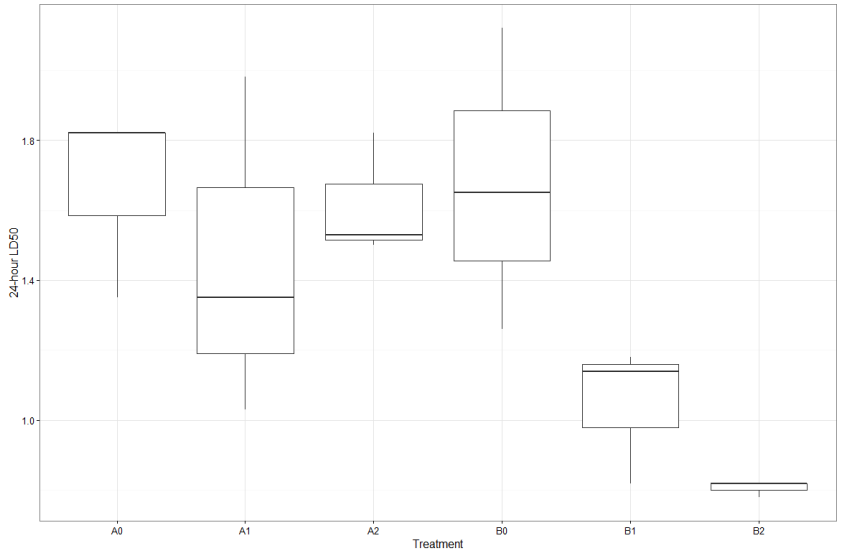

Figure 3. LD_{50} values estimated at 24 hours after an exposure to salinity levels, ranging from 0 to 14 ppt, of newly hatched larvae of the experimental female *M. rosenbergii*.

Discussion

Maternal diets have greatly impacted the quality of larvae, especially at a yolk-sac stage where their physiology is affected by the endogenous supply of essential fatty acids. Our study suggested that higher proportional contents of ARA and LOA in maternal diets improved the larval ability to tolerate extremely low salinity. Larvae from the dietary treatments with high levels of ARA and LOA (diets B1 and B2) were more tolerant to low salinity compared to the remaining treatments. A combination of enhanced ARA and LOA in diets, especially in the B2 treatment, may enhance the ability of the larvae to osmoregulate in suboptimal conditions, low salinity in this case (2 ppt). Our results were consistent with Cavalli *et al*. (1999) who demonstrated that high levels of LOA (approximately 13 mg g^{-1} DW, 27.08% of total dietary fatty acids) and high levels of n-3 lcPUFA (approximately 15 mg g^{-1} DW, 31.25% of total dietary fatty acids) in maternal diets improved larval tolerance to ammonia stress (100 mgL^{-1} total ammonia nitrogen, TAN). Both fatty acids may play a complementary role in larval development and their ability to regulate ion balance. ARA is a precursor of PGE2, important signalling molecules in prawn reproduction (Sumpownon *et al.*, 2015) and possibly larval development

(Kanpanich *et al.*, 2016). LOA may provide additional energy required during larval development and osmoregulation.

The regulation of ion balance may be particularly important for diadromous species, such as *M. rosenbergii*, where the majority of life is spent in freshwater systems and reproduction occurs for a relatively short time in brackish water (approximately 14 ppt). For these species, juveniles eventually migrate back to freshwater to grow. In other aquatic species, essential fatty acid composition in gill tissues change as environmental ionic concentrations change (reviewed in Glencross, 2009). Glencross (2009) hypothesized that essential fatty acids, especially lcPUFA, may be involved in ionic regulation in two ways, by (1) changing cell membrane fluidity and membrane permeability and (2) providing suitable substrate for the production of prostaglandins, which may be involved in active transport of ions across the gill membranes. ARA seems to be an important fatty acid to be retained in the gill tissue (Chand *et al*., 2015). Bell *et al*. (1985) showed that ARA is a preferred substrate for the production of PGE2 over EPA in gill tissue of turbot (*Scophthalmus maximus*). In *Oreochromis mossambicus*, a euryhaline fish species, dietary supplementation of ARA (3% ARA of total fatty acid) altered ARA levels in gill and kidney tissues and helped mitigate the effects of environmental ionic imbalance through the production of PGE2 or other ARA-induced stress hormones (Van Anholt *et al*., 2012). For crustacean larvae, there is no clear example to specifically illustrate the role of ARA on osmoregulation, but supplementing dietary lcPUFA tends to enhance osmoregulatory mechanisms in gill tissue, mainly Na+/K+ ATPase pump and carbonic anhydrase activity, to cope with an osmotic shock. In *P. vannamei* post larvae exposed to low salinity (10 ppt), groups fed lcPUFA-enriched Artimia sp. nauplii had higher enzymatic activities and retained higher levels of lcPUFA in gills than those fed with low lcPUFA (Palacios *et al.*, 2004). Gill areas were also enlarged in groups fed high lcPUFA, suggesting the use of lcPUFA for tissue formation.

Even though ARA is important for the reproduction of *M. rosenbergii* (Kangpanich *et al*., 2016) and possibly for osmoregulation capability of newly hatched larvae, only maternal diets containing a combination of high ARA and LOA contents contributed to significant larval tolerance to extremely low salinity. Because LOA is a major fatty acid in all tissues (approximately 15-20%), it may have provided additional energy required during energy-intensive developmental stages, such as embryogenesis and larval development (Kanpanich *et al*., 2016). Higher dietary LOA and ARA (as in treatment B2) contents resulted in larvae with high relative contents of ARA and an n-3 lcPUFA, DHA, required for tissue and organ formations in embryos and larvae.

The findings from our study has an important implication for inland hatchery operations where transport of seawater can be costly. A current hatchery practice relies on mixing seawater and freshwater to maintain water salinity at ~14 ppt. Daily water exchange rate can be as high as 50%. Depending on the distance from the coast, transporting seawater to distant farm locations, such as in the North and Northeast Thailand, could be very expensive (16,000–20,000 Baht per 16 m^3). Ability to raise larvae at a salinity level lower than 14 ppt would minimize seawater usage at this stage of larval production.

In conclusions, supplementing ARA at 0.8% in a LOA-rich maternal diet (B2) improved larval ability to tolerate extremely low salinity (2-6 ppt) for at least the first 24 hours after hatching ($p<0.05$). The n-3 to n-6 ratio of this diet was 0.59. The next-step research may include the evaluation of the performance of larvae fed the optimal diet throughout the production.

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(Received: 7 December 2016, accepted: 8 January 2017)