Songklanakarin J. Sci. Technol.
36 (1), 45-49, Jan. - Feb. 2014

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Changes in the fatty acid composition of wild harlequin shrimp, Hymenocera picta Dana, 1852 from eggs, newly hatched zoea and juvenile stages: an insight into the fatty acid requirements for aquaculture

Jarunan Pratoomyot* and Nisa Siranonthana

Institute of Marine Science, Burapha University, Mueang, Chon Buri, 20131 Thailand.

Received 29 May 2013; Accepted 8 October 2013

Abstract

The colourful harlequin shrimp, *Hymenocera picta*, is a valuable marine ornamental species but low survival remains a bottleneck to successful commercial culture. Understanding the biochemical composition, notably through the determination of the fatty acid profiles in wild eggs, newly hatched and juvenile shrimp, can provide important information on the nutritional requirements of *H. picta*. Following analysis, the rank order of fatty acid composition was saturates > monoenes > polyunsaturated fatty acids (PUFAs). Within the PUFA content, n-3 highly unsaturated fatty acids (HUFA) was the major representative in all three stages; n-6 PUFA was found in lower amounts, and, arachidonic acid (20:4n-6) was not detectable. Observed increases in EPA and DHA from the eggs through to newly hatched zoea and juvenile shrimp indicate the importance of n-3 HUFA for growth and survival, *i.e.* as components in the formation of cell membranes. These findings should be given due consideration as a first approach to understanding the fatty acid requirements of harlequin shrimp.

Keywords: Harlequin shrimp, *Hymenocera picta*, fatty acid, DHA, EPA, ARA

1. Introduction

Whilst harlequin shrimp, *Hymenocera picta* Dana 1852, make a colourful addition to any marine ornamental collection, most specimens, typically breeding pairs, have been obtained directly from the wild. Harlequin shrimp are difficult to maintain in captivity and in the absence of sustainable aquaculture practices, most wild broodstock represent a dead end in that their nutrition needs are poorly understood, which commonly leads to early mortality or minimal survival of their offspring in captivity. The practice of removing adults from the wild with no compensatory restocking, places this species in danger of overexploitation. To address this, it is imperative that research effort is invested in developing sustainable culture techniques that result in the high survival of both larvae and adults, thereby preventing the current reliance on animals taken from the wild. One of the first steps in the development of a successful aquaculture programme, therefore, is acquiring an understanding of the nutritional requirements of wild shrimp.

Several factors, including appropriate nutrition, have been reported as influencing larval survival (Lin and Zhang, 2001; Rueda and Martinez, 2001; Lin and Shi, 2002; Raabe and Raabe, 2007). Calado (2008), for example, demonstrated that the fatty acid (FA) composition of the diet effected the growth and survival of several crustacean species including marine ornamental shrimp. In the wild, the diversity of live natural prey fulfills the nutritional requirements, which includes the FA, of planktonic larvae (Jones, Yule and Holland, 1997). The FAs accumulating in larval tissues should, therefore, be similar to their live diets (Mourente *et al.*, 1995; Leonardo and Lucas, 2000; Fernandez-Reiriz *et al.*, 2011). The FA content in the tissues of each wild aquatic animal, therefore, can be used as an index to determine the

* Corresponding author.
Email address: jarunan@buu.ac.th

specific FA requirements of that species (D'Abrahamo and Sheen, 1993).

The present study sets out to determine the FA content of the eggs, newly hatched larvae (*i.e.* zoea stage1) and the juvenile stage of wild *H. picta*. The relevant FA composition and differences between each stage can provide important information that can then be used in developing protocols for the culture of harlequin shrimp.

2. Materials and Methods

2.1 Sample collection and preparation

Three juvenile *H. picta* each ~0.2 g in weight and ~2.1 cm in length, and 12 pairs of broodstock shrimp were obtained from a trader operating in Jatujak market, Bangkok. The three juvenile *H. picta* were immediately frozen and stored at -40°C until required. Each breeding pair of adults were maintained in 30 L aquaria in the research hatchery facility within the Institute of Marine Science, Bangsaen. The water quality of each tank was checked regularly and maintained within the following operational ranges: temperature 28-29°C, salinity 32-34 ppt., alkalinity 90-110 mg L^{-1}, ammonia < 0.1 mg L^{-1}, nitrite < 0.03 mg L^{-1}, nitrate<2.5 mg L^{-1}. Adult *H. picta* were maintained by feeding them regularly to satiation on their natural diet consisting basically of the comet sea star, *Linckia multiflora* (Lamarck, 1816). After a period of ~3 months, three sets of mature eggs were collected from the ventral, abdominal surface of separate brooding females and were prepared for analysis. Additional berried females, which were ready to release their eggs, were transferred to separate 30 L aquaria and three sets of newly hatched zoea stage 1 (n = 300-500 per set) were collected within 12 h of their release. In addition, a sample of *L. multiflora* were collected for fatty acid analysis. All samples were stored at below -40°C until they were analysed.

2.2 Lipid extraction and fatty acid analysis

Total lipids from *L. multiflora* and from the eggs, the newly hatched zoea stage 1 and the juvenile shrimp were extracted by homogenizing each sample in 20 volumes of ice-cold chloroform:methanol (2:1, by vol.) and the non-lipid phase was separated by adding 0.88% (w/w) KCl (about 25% of the total volume) and then centrifuged at ×1500 rpm for 5 min (Folch *et al.*,1957). Fatty acid methyl esters (FAME) were prepared from the total lipid by subjecting samples to acid-catalysed transesterification (Christie, 1993). These were quantified by gas-liquid chromatography (Agilent Technologies GC 7820A, USA) and the individual FAMEs identified by comparison to known standards (PUFA No. 3, menhaden oil, Supelco, USA). The FAMEs were split injected through a wall-coated capillary column (HP-Innowax column, 30 m × 0.25 mm id, 0.25µm film thickness, Agilent J&W, USA) and detected with a flame ionisation detector (FID) at 250°C. Helium gas was used as the carrier at a constant flow rate of 1.2 mL min^{-1}. The temperature program used was an initial 150°C for 0.5 min, increasing to 170°C at a rate of 5°C min^{-1}, hold at 170°C for 10 min, then increasing to 190°C at a rate of 3°C min^{-1}, and, then hold at 190°C for 28 min. Temperatures at the injection and detection ports were 230°C and 250°C respectively.

2.3 Statistical analysis

All data are presented as the mean ± S.D. Differences between samples were determined using a one-way analysis of variance (ANOVA) and S-N-K *post-hoc* test using SPSS17 for Windows. Statistical significance was set at $p<0.05$.

3. Results

The lipid content and FA composition of the *L. multiflora* and of the harlequin shrimp are shown in Table 1. The lipid content of the juvenile shrimp was significantly lower than that measured in the eggs and in the newly hatched zoea 1. All the shrimp samples showed a similar trend in their FA composition, with saturated FA (SFA) being higher than both monounsaturated FAs (MUFA) and polyunsaturated FAs (PUFA). The n-3 highly unsaturated FAs (PUFA) component was considerably higher than the n-6 PUFA content (Table 1). A comparison of the FA composition across all three stages revealed a number of significant differences ($p<0.05$) (see Table 1). The eggs and newly hatched zoea stage 1 had significantly higher levels ($p<0.05$) of SFA and MUFA than those found in juveniles. Palmitic acid (C16:0) and stearic acid (C18:0) were the main SFAs found, whilst palmitoleic acid (C16:1n-7) and oleic acid (C18:1n-9) were the main MUFAs that were encountered in all samples (Table 1). The eggs and newly hatched zoea stage 1 also contained higher levels ($p<0.05$) of palmitic and palmitoleic acid than those found in the juveniles, however, the palmitoleic acid content of the newly hatched zoea stage 1 was less than that in the eggs (Table 1).

Over 90% of the PUFA content found in all shrimp samples consisted of n-3 PUFA and n-3 HUFA, while less than 1% n-6 PUFA was present with no arachidonic acid (ARA, C20:4n-6) being found. Across all samples, the levels of PUFA, n-3 PUFA and n-3 HUFA, notably eicosapentaenoic acid (EPA, C20:5n-3) and docosahexaenoic acid (DHA, C22:6 n-3), were higher in the juveniles and the newly hatched zoea than the levels found in the eggs. The juveniles though had the highest levels of EPA and DHA among the developmental stages of *H. picta*. Although the EPA content was higher than that of DHA, both FAs showed a marked increase between the egg and zoea stage 1. The juveniles were found to contain a higher level of EPA indicating that levels continue to rise, but the levels of DHA across the samples remained constant. The DHA/EPA ratio across the samples appeared to remain fairly constant whilst the n3/n6 ratio was found to double between the egg and juveniles stages (*i.e.* 21 to 44 respectively; see Table 1).

Table 1. Total lipid and fatty acid composition (% of total fatty acid) of the eggs, newly hatched zoea stage 1 and juveniles of wild harlequin shrimp, *Hymenocera picta,* and of the comet sea star, *Linckia multiflora*, the sole food item given to juvenile shrimp.

Parameters	Eggs	Zoea stage 1	Juveniles	*L. multiflora*
Total lipid	4.0 ± 0.1[a]	4.8 ± 0.7[a]	1.3 ± 0.0[b]	0.7 ± 0.1
Fatty acids				
C14:0	6.3 ± 0.1[a]	3.7 ± 0.4[b]	3.0 ± 0.1[c]	0.2 ± 0.2
C16:0	22.6 ± 0.5[a]	22.0 ± 1.6[a]	14.2 ± 0.5[b]	3.5 ± 0.1
C18:0	9.4 ± 0.6	11.2 ± 3.4	10.8 ± 0.3	8.1 ± 0.6
C20:0	2.2 ± 0.8[a]	tr	1.1 ± 0.0[b]	n.d.
C22:0	0.6 ± 0.5[b]	0.5 ± 0.5[b]	2.7 ± 0.1[a]	n.d.
SFA	42.2 ± 1.0[a]	38.8 ± 3.8[a]	32.6 ± 1.0[b]	12.5 ± 0.3
C16:1	8.7 ± 0.8[a]	5.3 ± 0.1[b]	3.0 ± 0.0[c]	0.9 ± 0.1
C18:1	24.9 ± 0.6	25.7 ± 0.5	24.6 ± 0.8	5.1 ± 0.7
C20:1	0.4 ± 0.1[b]	2.8 ± 1.4[a]	1.7 ± 0.4[a]	1.6 ± 0.2
MUFA	34.0 ± 1.4[a]	33.9 ± 0.9[a]	29.4 ± 1.1[b]	7.6 ± 0.7
C18:2n-6	0.3 ± 0.0[c]	0.9 ± 0.1[a]	0.6 ± 0.0[b]	1.7 ± 0.2
C20:4n-6	n.d.	n.d.	n.d.	1.6 ± 0.2
n-6PUFA	0.3 ± 0.0[c]	0.9 ± 0.1[a]	0.6 ± 0.0[b]	3.3 ± 0.4
C18:3n-3	0.7 ± 0.2	tr	0.6 ± 0.0	2.7 ± 0.2
C18:4n-3	1.1 ± 0.5[a]	n.d.	0.4 ± 0.1[b]	0.2 ± 0.2
C20:3n-3	n.d.	n.d.	n.d.	n.d.
C20:4n-3	0.6 ± 0.2	0.3 ± 0.5	0.5 ± 0.0	n.d.
C20:5n-3	2.6 ± 0.4[c]	8.1 ± 2.3[b]	18.5 ± 0.4[a]	13.1 ± 0.7
C22:6n-3	1.3 ± 0.5[b]	6.0 ± 2.6[a]	6.2 ± 0.0[a]	3.2 ± 0.2
Total n-3PUFA	6.2 ± 0.7[c]	14.5 ± 2.7[b]	26.1 ± 0.3[a]	19.3 ± 1.2
Totaln-3HUFA	4.5 ± 1.0[c]	14.3 ± 3.1[b]	25.1 ± 0.4[a]	16.3 ± 0.9
TotalPUFA	6.5 ± 0.7[c]	15.5 ± 2.8[b]	26.7 ± 0.3[a]	22.5 ± 1.3
TotalHUFA	4.5 ± 1.0[c]	14.3 ± 3.1[b]	25.1 ± 0.4[a]	18.0 ± 1.1
n-3/n-6	20.7 ± 3.9[b]	15.6 ± 2.6[c]	43.6 ± 1.5[a]	6.0 ± 0.9
DHA/EPA	0.5 ± 0.1	0.8 ± 0.3	0.3 ± 0.0	0.2 ± 0.0

For each value the mean ± S.D. is given. All values are based on triplicate samples.
Abbreviations: HUFA = highly unsaturated FAs; MUFA = monounsaturated FAs;
n.d. = not determined; PUFA = polyunsaturated FAs; SFA = saturated FAs; tr = $\leq$0.2 %.

4. Discussion

Triacylglycerides (TAG) are neutral lipids and the main source of energy storage in shrimp, particularly during embryogenesis (Clarke, 1982). In the present study, high total lipid contents were found in the eggs and newly hatched zoea stage 1 of *H. picta* when compared to the levels found in juveniles. The low levels of lipid found in the juveniles, however, are a reflection of the levels found in their natural diet which consists basically of *L. multiflora.* The SFAs palmitic and stearic acid and the MUFAs palmitoleic and oleic acid were high in all samples, notably the eggs and newly hatched zoea. There was an important reduction in the levels of these FAs, and particularly of the SFA C16:0 in the juveniles, indicating that these are preferentially mobilised as energy sources for growth (Calado *et al.*, 2005b; Calado, 2008).

ARA, EPA and DHA are required for the formation of phospholipids which serve as structural components of healthy tissue (Harrison, 1990; Sargent *et al.*, 2002). The present study found high levels of PUFA, notably EPA and DHA, in newly hatched zoea and juveniles, which are required for tissue growth as the harlequin shrimp develop. Interestingly, very little n-6 PUFA and no ARA were found in the shrimp samples. These results indicate that harlequin shrimp, like many other marine organisms, require lower amounts of n-6 PUFA than n-3 PUFA (Sargent *et al.*, 2002).

In addition to this, the harlequin shrimp had lower levels of linoleic acid (C18:2n-6), linolenic acid (C18:3n-3) and ARA than those provided in the diet indicating that these FAs were selectively catabolised as energy sources.

EPA and DHA have been reported to promote the growth and survival of crustaceans (Hamasaki *et al.*, 2002; Suprayudi *et al.*, 2002a,b), however, it is the ratio of DHA/

EPA that is more critical for growth performance in aquatic animals including shrimp (Palmtag and Holt, 2007; Tziouveli and Smith, 2012). Low DHA/EPA ratios have been documented to result in the high production of larvae (Woods, 2003; Biswas *et al.*, 2006). Newly hatched *Lysmata amboinensis* De Man, 1888 (see Tziouveli and Smith, 2012) and starved phyllosoma spiny lobster, *Jasus edwardsii* (Hutton, 1875) (see Smith *et al.*, 2004) with a DHA/EPA ratio of ~1 were able to molt to the second zoea l stage. The current study found that the wild *H. picta* samples had a DHA/EPA ratio of <1, which is in the same range as that reported for other marine shrimp species, *e.g. Palaemon elegans* (Rathke, 1837) and *Palaemon serratus* (Pennant, 1777) (0.2 and 0.4 respectively; Morais *et al.*, 2002), newly hatched *J. edwardsii* (0.9; Smith *et al.*, 2003); phyllosoma stage of *J. edwardsii* (0.5; Smith *et al.*, 2004); ornamental *Lysmata seticaudata* (Risso, 1816) (0.6; Calado *et al.*, 2005a), and, newly hatched *Panuliru shomarus* (L.) (0.4; Chakraborty *et al.*, 2010). The ratio DHA/EPA found in the newly hatched zoea of another ornamental shrimp species *Lysmata amboinensis,* reared under aquaculture conditions, was found to be close to 1, thereby explaining their survival and development to the next stage (Tziouveli and Smith, 2012). Further work, however, is required to define the appropriate DHA/EPA for optimal growth and survival but it is suggested that it may lie within the range of 0.3-1 of the *H. picta* requirements.

The n-3/n-6 ratio is an index used to determine the n-3 and n-6 PUFA requirements for growth in aquatic animals (Holman, 1986; Sargent, 1995). In this study, a high n3/n6 ratio was found in all samples indicating that harlequin shrimp require high levels of n-3 PUFA, particularly EPA and DHA, for growth, but have limited need for n-6 PUFA, as suggested for other aquatic species (Wickens *et al.*, 1995; Calado et al., 2009).

5. Conclusion

As the FA composition of the tissues reflects levels found in the respective diets of each species (Mourente *et al.*, 1995; Leonardos and Lucas, 2000; Fernandez-Reiriz *et al.*, 2011), the high n-3/n-6 ratio, high levels of SFA, EPA, DHA and a low DHA/EPA ratio provide critical clues as to the nutritional requirements of *H. picta*. This information should be carefully factored into the preparation of enrichment techniques for the sustainable culture of harlequin shrimp.

Acknowledgments

The authors gratefully acknowledge the Office of Higher Education Commission, Thailand, who provided financial support.

References

Biswas, A.K., Nozaki, J., Kurata, M., Takii, K., Kumai, H. and Seoka, M. 2006. Effect of *Artemia* enrichment on the growth and survival of Pacific bluefin tuna *Thunnus orientalis* (Temminck and Schlegel) larvae. Aquaculture Research. 37 (16), 1662–1670.

Calado, R. 2008. Marine Ornamental Shrimp.Biology, Aquaculture and Conservation. Wiley-Blackwell, West Sussex, U.K., p.263.

Calado, R., Rosa, R., Nunes, M.L. and Narciso, L. 2005a. Amino and fatty acid dynamics of *Lysmata seticaudata* (Decapoda: Hippolytidae) embryos during early and late reproductive season. Marine Biology. 147, 341–351.

Calado, R., Rosa, R., Nunes, M.L. and Narciso, L. 2005b. Seasonal variation in the biochemical composition of embryos of the Monaco shrimp *Lysmata seticaudata* (Decapoda: Hippolytidae). Marine Biology. 147, 341-351.

Calado, R., Vitorino, A., Reis, A., Lopesda Silva, T. and Dinis, M.T. 2009. Effect of different diets on larval production, quality and fatty acid profile of the marine ornamental shrimp *Lysmata amboinensis* (de Mann, 1888) using wild larvae as a standard. Aquaculture Nutrition. 15, 484-491.

Chakraborty, K., Chakraborty, R.D. and Radhakrishnan, E.V. 2010. Fatty acid profiles of spiny lobster (*Panulirus homarus*) phyllosoma fed enriched *Artemia*. Aquaculture Research. 41, e393-e403.

Christie, W.W. 1993. Preparation of derivatives of fatty acids for chromatographic analysis. In: Christie, W.W. (Ed.). Advances in Lipid Methodology Two. The Oily Press, U.K., pp. 69-111.

Clarke, A. 1982. Lipid synthesis and reproduction in the polar shrimp *Chorismus antarcticus*. Marine Ecology Progress Series. 9, 81-90.

D'Abramo, L.R. and Sheen, S.-S. 1993. Polyunsaturated fatty acid nutrition in juvenile freshwater prawn *Macrobrachium rosenbergii*. Aquaculture. 115, 63-86.

Fernandez-Reiriz, M.J., Perez-Camacho, A., Peteiro, L.G. and Labarta, U. 2011.Growth and kinetics of lipids and fatty acids of the clam *Venerupis pullastra* during larval development and post larvae. Aquaculture Nutrition. 17, 13-23.

Folch, J., Lees, M. and Sloane-Stanley, G.H. 1957. A simple method for the isolation and purification of total lipids from animal tissues. Journal of Biological Chemistry. 226, 497-509.

Hamasaki, K., Suprayudi, M.A. and Takeuchi, T. 2002. Effect of dietary n-3 HUFA on larval morphogenesis and metamorphosis to megalops in the seed production of mud crab, *Scylla serrata* (Brachyuran: Portunidae). Suisan Zoshoku. 50, 333-340.

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

Holman, R.T. 1986. Nutritional and biochemical evidence of acyl interaction with respect to essential poly-unsaturated fatty acids. Progress in Lipid Research. 25, 29-39.

Jones, D.A., Yule, A.B. and Holland, D.L. 1997. Larval nutrition. In: D'Abramo, L.R., Conklin, D.E., Akiyama, D.M. (Eds.), Crustacean Nutrition-Advances in World Aquaculture. The World Aquaculture Society, Baton Rouge, Los Angelis, U.S.A., pp.353-389.

Leonardos, N. and Lucas, I.A.N. 2000. The use of larval fatty acid as an index of growth in *Mytilus edulis* L. larvae. Aquaculture. 184, 155-166.

Lin, J. and Shi, P. 2002. Effect of broodstock diet on reproductive performance of the golden banded shrimp Stenopus scutellatus. Journal of the World Aquaculture Society. 33, 383–386.

Lin, J., Zhang, D. and Creswell, R. 2001. Aquaculture of marine ornamental shrimps: an overview. Aquaculture Book of Abstracts. The World Aquaculture Society, Orlando, Florida, U.S.A, p. 378.

Morais, S., Narciso, L., Calado, R., Nunes, M.L. and Rosa, R. 2002. Lipid dynamics during the embryonic development of *Plesionika martia* (Decapoda; Pandalidae), *Palaemon serratus* and *P. elegans* (Decapoda; Palaemonidae): relation to metabolic consumption. Marine Ecology Progress Series. 242, 195-204.

Mourente, G., Medina, A., González, S. and Rodríguez, A. 1995. Variation in lipid content and nutritional status during larval development of the marine shrimp *Penaeus kerathurus*. Aquaculture. 130, 187-199.

Palmtag, M.R. and Holt, G.J. 2007. Experimental studies to evaluate larval survival of the fire shrimp, *Lysmata debelius*, to the juvenile stage. Journal of the World Aquaculture Society. 38, 102-113.

Raabe, C. and Raabe, L. 2007. The care and breeding of the harlequin shrimp. Available at: https://www.home2.pacific.net.ph/~sw-eetyummy42/harlequinshrimp.html (January 20, 2013)

Sargent, J.R. 1995. Origin and functions of egg lipids: nutritional implications. Bromage, N.R., Roberts, R.J., editors. Broodstock Management and Egg and Larval Quality, Blackwell Science, London, U.K., pp. 353-372.

Sargent, J.R., Tocher, D.R. and Bell, J.G. 2002. The lipids. Halver, J.E., Hardy, R.W., editors. Fish Nutrition, 3rd Edition. Academic Press, San Diego, California, U.S.A., pp. 181-257.

Smith, G., Ritar, A.J., Johnston, D. and Dunstan, G.A. 2004. Influence of diet on broodstock lipid and fatty acid composition and larval competency in the spiny lobster, *Jasus edwardsii*. Aquaculture. 233, 451-475.

Smith, G.G., Thompson, P.A., Ritar, A.J. and Dunstan, G.A. 2003. Effects of starvation and feeding on the fatty acid profiles of stage I phyllosoma of the spiny lobster, *Jasus edwardsii*. Aquaculture Research. 34, 416-426.

Suprayudi, M.A., Takeuchi, T., Hamasaki, K. and Hirokawa, J. 2002a. The effect of n-3 HUFA content in rotifers on the development and survival of mud crab, *Scylla serrata*, larvae. Suisan Zoshoku. 50, 205-212.

Suprayudi, M.A., Takeuchi, T., Hamasaki, K. and Hirokawa, J. 2002b. Effect of *Artemia* feeding schedule and density on the survival and development of larval mud crab, *Scylla serrata*. Fisheries Science. 68, 1295-1303.

Tveiten, H., Jobling, M. and Andreassen, I. 2004. Influence of egg lipids and fatty acids on egg viability, and their utilization during embryonic development of spotted wolffish, *Anarhichas minor* Olafsen. Aquaculture Research. 35(2), 152–161.

Tziouveli, V., and Smith, G.G. 2012. A comparison of the fatty acid profiles of adult tissues, and newly hatched, fed and starved *Lysmata amboinensis* larvae. Aquaculture Research. 43 (4), 577-587.

Wickens, J.F., Beard, T.W. and Child, A.R. 1995. Maximizing lobster, *Homarus gammarus* (L.), egg and larval viability. Aquaculture Research. 26, 379-392.

Woods, C.M.C. 2003. Effects of varying *Artemia* enrichment on growth and survival of juvenile seahorses, *Hippocampus abdominalis*. Aquaculture. 220, 537-548.