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Fatty Acid Requirement of Moon Jelly *Aurelia coerulea* at the Ephyra Stage

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Abstract

Moon jelly *Aurelia coerulea* has been gaining attention as a new production target species in Japan because of its increasing demand as a feed candidate for larval finfishes and shrimps and as an exhibit animal in an aquarium. In this study, rearing experiments of *A. coerulea* ephyrae using *Artemia franciscana* nauplii enriched with several oils containing various fatty acids (FA) as feed were conducted to evaluate the FA requirement of this species. As a result of two rearing experiments, feeds not containing DHA gave unfavorable growth rates compared to feeds with DHA, and the highest growth rate was obtained in feeds enriched with EPA:DHA=62:38 oil (DHA/EPA ratio of 0.6). These results indicate the importance of dietary FAs, especially EPA and DHA, on the somatic growth of *A. coerulea* ephyra and EPA may play a more crucial role than DHA to maximize the growth of the ephyrae. This study is the first to provide a criterion of suitable FAs composition in a feed for the efficient production of this species under artificial breeding.

Key Words: Aurelia coerulea, Jellyfish, Ephyra stage, Nutritional requirement, Fatty acid

Introduction

Nutritional requirement is one of the most important study topics for the successful production of marine animal species. Especially at the early developmental stages, essential fatty acids (EFA) are indispensable factors that affect not only growth and survival but also the metamorphosis of marine animals[1-2]. A lot of research performed so far has demonstrated that a lack of dietary EFA induced a low growth rate, high mortality, and malfunction in metabolism for marine animals[1]. Unlike terrestrial animals and freshwater finfishes, marine animals usually require n-3 highly unsaturated fatty acids (HUFA) in diets as EFA due to their low capacity to synthesize $C_{20\text{-}22}$ n-3 HUFA from C_{18} fatty acid (FA) precursors. For instance, Sargent et al.[2] suggested in their work that marine fishes and invertebrates required three kinds of HUFA, such as docosahexaenoic acid (DHA, C_{22}:6n-3), eicosapentaenoic acid (EPA, C_{20}:5n-3), and arachidonic acid (ARA, C_{20}:4n-6), for their normal growth and reproduction. On the other hand, Xu et al.[3] reported that penaeid shrimps required linoleic acid (LA, C_{18}:2n-6) and linolenic acid (LNA, C_{18}:3n-3) as EFA for their growth. González-Félix et al.[4] indicated that the EFA requirement of marine animals is species-dependent and also varies depending on the supplementation level of dietary lipid. Therefore, in order to obtain an efficient production process for target marine animals, it is paramount to establish an optimum feed strategy in accordance with distinctive FA requirements.

Nowadays, the moon jelly *Aurelia coerulea*, which is the most common jellyfish in Japanese coastal sea

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areas, has been gaining attention as a new production target species in Japan because of its increasing demand as a feed candidate for some marine animals[5-8] and as an exhibit animal in aquariums. In some Japanese hatcheries and aquariums, a long-term rearing of some jellyfish species including *A. coerulea* has been conducted by feeding with enriched *Artemia* spp. and minced shellfish. However, this feeding process has been conventionally and/or practically conducted and there are very few empirical investigations on the nutrient requirement of *A. coerulea*. In rearing experiments for other marine animals, it has been reported that the growth inhibition occurred when fed with no-enriched *Artemia* spp., considered to be due to a lack of dietary EFA[1]. Ueno et al.[9] preliminarily demonstrated that *Artemia* sp. with HUFA enrichments proved to be potentially effective in the growth of *Sanderia malayensis*. On the other hand, EFAs and their importance in *A. coerulea* remain unclear.

The aim of this study is to elucidate the FA requirement of *A. coerulea*, contributing to estimating the efficient production process of this species in a hatchery. The rearing experiments of *A. coerulea* ephyrae were preliminarily carried out using *Artemia franciscana* nauplii (described as *Artemia* below) enriched with several FA oils.

Materials and methods

Two rearing experiments using enriched *Artemia* were carried out to investigate the effects of dietary LA, LNA, and DHA (Experiment 1) and different ratios between EPA and DHA (Experiment 2) on the growth of *A. coerulea* ephyrae.

Protocols of HUFA enrichment to Artemia

Artemia used in this study was obtained from Pacific Trade Co. Ltd. (Fukuoka, Japan). The FA enrichment of *Artemia* was carried out according to Yoshimatsu and Kitajima.[10] The oils used for the enrichment were soy oil for LA enrichment (Kanto Kagaku, Tokyo), linseed oil for LNA enrichment (Kanto Kagaku, Tokyo), EPA oil (Bizen Chemical CO. LTD., Okayama), and DHA ethyl ester (described just as DHA oil below). DHA oil was provided by the Laboratory of Marine Bioscience, Miyazaki University. The oils were individually emulsified by mixing 0.2 g of each oil with 1 drop of raw chicken egg yolk and 100 ml of fresh water. The FA enrichment of *Artemia* was conducted in glass beakers, containing 180 ml of artificial seawater and 20 ml of the emulsified oils, at room temperature of 24℃ for 4 hours with vigorous aeration.

Experiment 1: The effects of dietary LA, LNA, and DHA on A. coerulea growth

A. coerulea polyps were sampled from Ago bay, Mie prefecture in Japan. The polyps were reared with non-enriched *Artemia* and maintained at a temperature of 13℃ for 20 days to obtain ephyrae. Hatched 12 ephyrae were transferred into a 200 ml conical flask containing artificial seawater of 34 psu, prepared for each experimental group. The rearing water was kept at 24℃ and statically aerated. The whole water and the flasks were exchanged once a week, and 50% of the rearing water was exchanged every day after cleaning the flasks. The rearing experiment was carried out for a period of 36 days. The *A. coerulea* ephyrae were fed once a day with a saturated amount of *Artemia* enriched either with (1) soy oil, (2) linseed oil, or (3) DHA oil, and the treatment with (4) no enrichment was also set as a control. The bell diameters of the ephyrae were measured every six days under a stereomicroscope.

Experiment 2: The effects of DHA/EPA ratio on A. coerulea growth

The protocol to obtain *A. coerulea* ephyrae was the same as in Experiment 1. Newly hatched ephyrae were reared by feeding non-enriched *Artemia* for 15 days and then used for this experiment. Ten ephyrae were transferred into a 500 ml round bottom flask containing sterilized sea water of 34 psu, prepared

for each experimental group. The rearing water was kept at 22.5℃ and statically aerated. 50% of the rearing water was exchanged every day after cleaning the flasks, and the whole water and the flasks were exchanged once a week. The rearing experiment was carried out for a period of 15 days, and *A. coerulea* ephyrae were fed once a day with *Artemia* enriched either with (1) soy oil, (2) EPA and DHA oils (v/v, 30 : 70), (3) EPA and DHA oils (62 : 38), or (4) EPA and DHA oils (90 : 10). The bell diameters of the ephyrae were measured every five days under a stereomicroscope.

FA analyses

Analysis of FA composition was performed on the enriched *Artemia* on a dry basis and the oils used for the *Artemia* enrichment. The enriched *Artemia* was collected and rinsed with distilled water, and then kept at -30℃ until analyses. The samples were freeze-dried with a centrifugal evaporator (CVE-1200, EYELA, Tokyo) and cold trap (UT-1000, EYELA, Tokyo). Lipids were extracted from approximately 10 mg of the freeze-dried samples using Fatty Acid Methylation Kit (bacarai tesque, Kyoto) following the manufacturer's instructions, and then analyzed with gas chromatography (GC-2025, Shimazu, Kyoto). Samples of 1 μl were injected into a split (split ratio 1:10) using an autosampler (AOC-20i, SHIMADZU Co., Kyoto, Japan) into a ULBON HR-SS-10 column (Shinwa Chemical Industries Ltd., Kyoto, Japan, 30 m, 0.32 mm inner diameter). The carrier gas was helium at a flow rate of 4.5 ml min^{-1}. Both the injection port and FID detector were set at 220℃. The initial oven temperature was maintained at 150℃ for 1 min, and raised to 220℃ at a rate of 3℃ min^{-1}, and held for 3 min. The results were expressed as the percentage of total FA.

Statistical analysis

All statistical analyses were performed using Statcel ver. 3 software (OMS Publishing Inc.). The mortality rates of ephyrae fed with *Artemia* enriched with different oils were analyzed by Chi-square test. One-way analysis of variance (ANOVA) was used to evaluate the growth of *A. coerulea* ephyrae, followed by a post hoc Tukey-Kramer test. The statistical differences were accepted when $P < 0.05$.

Results

FA profiles of Artemia enriched with each oil

The FA profiles of oils used for enrichments and enriched *Artemia* are shown in Table 1. The highest FA component in each oil was LA (C_{18}:2n-6, 53.6%) in soy oil, LNA (C_{18}:3n-3, 56.9%) in linseed oil, EPA (C_{20}:5n-3, 98.5%) in EPA oil, and DHA (C_{22}:6n-3, 76.4%) in DHA oil, respectively. As shown in Table 1, the FA profile in *Artemia* enriched with each oil exactly reflected these compositions both in Experiments 1 and 2.

Survival and growth of A. coerulea ephyrae

In Experiment 1, the mortality rates during the 36 days of culture were 16.7% (2 of 12 individuals) in soy oil, 16.7% (2 of 12 individuals) in linseed oil, and 8.3% (1 of 12 individuals) in no enrichment, and no mortality was observed in the ephyrae fed with *Artemia* enriched with DHA oil. The significant difference in the mortality rate was not detected between groups ($P > 0.05$).

Changes in bell diameters of *A. coerulea* ephyrae in Experiment 1 were shown in Fig. 1. In all treatments the mean bell diameter gradually increased during the experiment. The mean bell diameters of the ephyrae fed with *Artemia* enriched with DHA oil increased from 2.2 ± 0.1 to 19.1 ± 0.8 mm (mean ± SE) through the experiment, providing significantly higher growth rates than in other treatments after 18 days of culture.

In Experiment 2, mortality of the ephyrae was not observed in all treatments. Changes in bell

Table 1 The FA compositions of oils used for enrichments and enriched *Artemia* as feeds (% total fatty acid)

		C16:0	C16:1	C18:0	C18:1	C18:2n-6	C18:3n-3	C20:5n-3	C22:6n-3	Others
Oils	Soy	10.8	nd*	3.2	24.8	53.6	6.9	nd	nd	0.7
	Linseed	5.1	nd	2.4	19.9	15.5	56.9	nd	nd	0.2
	EPA	nd	nd	nd	nd	nd	nd	98.5	nd	1.5
	DHA	0.2	nd	0.4	1.8	0.1	0.1	6.1	76.4	14.9
Artemia (*Experiment 1*)	Soy	13.2	3.4	6.1	31.1	17.2	23.3	1.2	nd	4.5
	Linseed	12.1	2.9	5.2	29.1	8.0	31.8	1.4	nd	9.5
	DHA	7.7	3.4	3.3	25.9	5.2	27.8	3.6	8.2	14.9
	no enrichment	11.4	3.8	4.9	26.8	5.7	30.3	2.2	nd	14.9
Artemia (*Experiment 2*)	Soy	11.1	2.6	5.6	22.6	9.6	27.6	1.6	nd	19.3
	EPA&DHA (30:70)	10.3	2.7	5.2	20.4	5.1	27.6	3.8	5.0	19.9
	EPA&DHA (62:38)	10.3	2.9	5.2	20.5	5.0	26.9	5.4	3.5	20.3
	EPA&DHA (90:10)	10.6	3.1	5.4	20.8	5.2	27.6	5.9	1.2	20.2

* not detected.

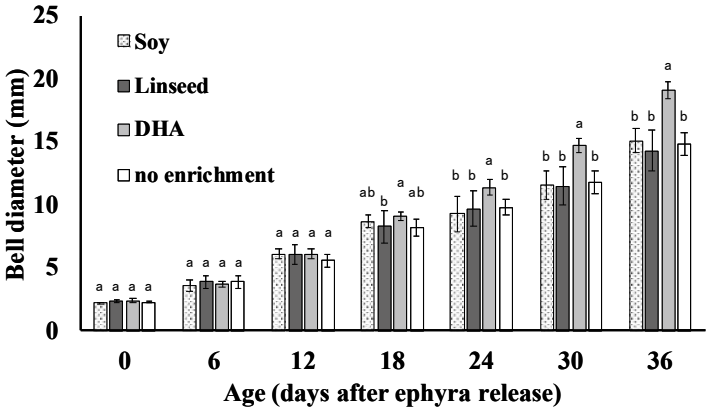

Fig. 1 Bell diameters of *Aurelia coerulea* ephyrae fed with *Artemia* enriched with different oils. Data are expressed as the mean ± SE (n = 12 per treatment, and n = 10 and 11 after ephyrae died). Different letters indicate significant differences between treatments ($P < 0.05$).

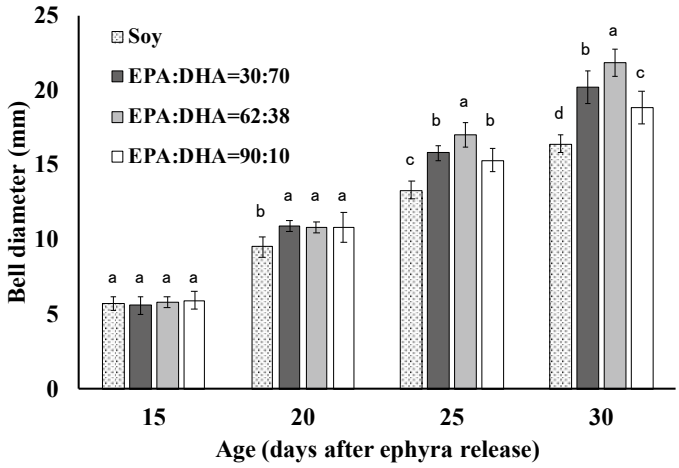

Fig. 2 Bell diameters of *Aurelia coerulea* ephyrae fed with *Artemia* enriched with different DHA/EPA ratios. Data are expressed as the mean ± SE (n = 10). Different letters indicate significant differences between treatments ($P < 0.05$).

diameter of *A. coerulea* ephyrae were shown in Fig. 2. The mean bell diameter of the ephyrae fed with *Artemia* enriched with EPA and DHA oils (62 : 38) increased from 5.8 ± 0.4 to 21.8 ± 0.9 mm, which was significantly higher than in other treatments, while the mean bell diameter of the ephyrae fed with *Artemia* enriched with soy oil increased from 5.7 ± 0.5 to 16.4 ± 0.2 mm, provided significantly lower growth than in other treatments after 20 days of culture.

Discussion

It has been globally recognized that a large outbreak of jellyfish is a nuisance greatly damaging the health of marine ecosystems and fisheries in coastal waters. Up till now, research on jellyfish mainly focused on the causes of blooming and appropriate countermeasures[11-14]. Most of these works have been carried out during the benthic polyp stage because the scale of jellyfish bloom is largely determined by the number of polyps[15-16]. Thus, exhaustive studies on polyps can be easily found on the influence of prey HUFA[17-18]. Moreover, the feeding ecology of jellyfish medusa has been studied, contributing to the monitoring of the incidence of jellyfish blooming[19]. Our rearing experiment was conducted from a different perspective from the prior studies, which was to establish an efficient production process of *A. coerulea* for industrial purposes, newly providing a nutritional criterion for the breeding of this species in a hatchery.

In this study, the FA profiles in prey *Artemia* reflected those of oils, and which definitely affected the breeding performance of *A. coerulea* ephyrae. As mentioned, several researchers have so far investigated the role of prey FAs on the growth of the jellyfish polyp. However, some of them are not in agreement with our results with the ephyrae. For example, Chi et al.[18] suggested that in their study, feed containing high HUFA didn't improve somatic growth of *A. aurita* polyps and the HUFA content of food was not the direct factor determining polyps somatic growth. In contrast, in our results, feed enriched with DHA clearly resulted in the favorable somatic growth of *A. coerulea* ephyrae compared to other feeds. This inconsistency between our results and theirs may be attributed to the different developmental stages of jellyfish or the experimental designs. The experiment design by Chi et al.[18] did not set up a treatment that did not contain DHA and the single efficiency of each HUFA on the growth of a jellyfish was not speculated. On the other hand, our experiments were carried out using the preys in which the levels of internal FAs, such as EPA and DHA, were precisely manipulated. Unlike the role of dietary DHA in other aquatic animals, supporting normal development, reproduction, and adaptability to differential experimental conditions[20-21], *in vivo* function of DHA in jellyfish ephyra is still less documented. Our results clearly indicate that dietary DHA plays a vital role in the healthy growth of this species, contributing to providing evidence for the importance of DHA on the healthy growth of *A. coerulea* ephyra. However, in general, the importance of n-3 HUFA in aquatic animals is greater during the early developmental stages[1, 23]. In this case, it should be greater in the polyp stage than in the ephyra stage. This is thought to be due to the unrevealed role of n-3 HUFA in the individual developmental stage of a jellyfish. Further detailed studies on *in vivo* functions of n-3 HUFA in a jellyfish are needed.

In Experiment 2, a favorable DHA/EPA ratio on the growth of *A. coerulea* ephyra was investigated. Besides the respective effects of EPA and DHA, the importance of the DHA/EPA ratio in a feed has been extensively well known in the aquaculture scenario and has been used as an indicator to estimate the nutritional value of a feed[2, 21]. A lot of studies have demonstrated that a suitable DHA/EPA ratio in a feed improved the performance of reproduction and somatic growth in some aquacultural fishes such as Siberian sturgeon[22], red sea bream[23], golden pompano[24] and yellowtail flounder[25], and in invertebrates

such as Chinese mitten crab[26] and European oyster[27]. According to these studies, a higher level of DHA relative to EPA (DHA/EPA ratio of 1.1 or higher) provides favorable growth, survival, and reproduction performance of marine animals. In our study, however, the most favorable growth of *A. coerulea* ephyra was obtained at the treatment of EPA:DHA=62:38 (DHA/EPA ratio of 0.6). Interestingly, Stenvers et al.[13] reported that a wild *A. aurita* similarly contained more EPA than DHA (DHA/EPA ratio of 0.4 to 0.5 in the ephyrae stage and 0.3 to 0.4 in mature medusae) in their study, where seasonal FA variability in wild *A. aurita* was investigated, suggesting that EPA in a jellyfish plays a more crucial role than DHA. In fact, EPA in the feed of Experiment 2 showed better growth than all treatments of Experiment 1, indicating the effectiveness of EPA enrichment. On the one hand, the extremely high level of EPA (DHA/EPA ratio of 0.2) did not result in the favorable growth of the ephyrae in our results. We cannot fully explain the internal function of n-3 HUFA in a jellyfish here but dietary EPA and DHA can likely have interplay in the jellyfish's normal growth. Although not included in the present data, morphological abnormalities in oral arms were observed in the ephyrae fed with *Artemia* not containing DHA. This suggests that DHA may play an important role in the ephyrae stage of the jellyfish.

The aim of this study was to elucidate the FA requirement of *A. coerulea* for the efficient production of this species. The study highlights the importance of dietary FAs, especially EPA and DHA, on the somatic growth of *A. coerulea* ephyra. Unlike previous reports on other marine animals, EPA may play a more crucial role than DHA to maximize the growth rate of the ephyrae, and a DHA/EPA ratio of 0.6 in a feed seems sufficient for optimal growth. The biosynthesis of FAs in the ephyrae was not considered in this study. On the other hand, Chi et al.[17] anticipated low activity of biosynthesis pathway around n-3/n-6 HUFA in jellyfishes, implying validity of our results and the importance of dietary n-3 HUFA provision when considering feed selection. Although this study was preliminarily conducted as the experimental replicates were not adequately set, it is the first to report a criterion of suitable FAs composition in a feed for the efficient production of this species under artificial breeding and could provide considerable data for future study. This study used the same clonal ephyrae as a preliminary test; therefore, more exhaustive studies examining the fatty acid requirement of *A. coerulea* using several strains of ephyrae are needed.

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ミズクラゲ *Aurelia coerulea* エフィラ期における必須脂肪酸要求

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要　旨

ミズクラゲ *Aurelia coerulea* のエフィラ幼生について，高度不飽和脂肪酸を含む油脂を用いて脂肪酸強化されたアルテミアを給餌する飼育試験を行い，脂肪酸要求特性について調査した。飼育試験の結果，DHA を含まない試験区ではエフィラ幼生に対する成長阻害が確認された。さらにこの試験区では，対照区の個体と比較して口腕部が真直するといった発達異常が観察された。餌料中の異なる DHA/EPA 比がエフィラ幼生の成長に与える影響を調査した試験では，DHA/EPA 比が 0.6 の試験区において最も高い成長率が得られた。これらの結果から，餌料に含まれる DHA および EPA 含量が，ミズクラゲのエフィラ幼生期の成長，生残に対して正の影響を及ぼすことが明らかとなった。