Aquacult Int (2017) 25:1531–1542
DOI 10.1007/s10499-017-0130-5

A comparison of the fatty acid profiles of newly hatched, fed, and starved juveniles of *Amphioctopus fangsiao* (d'Orbigny 1839)

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Received: 19 October 2015 / Accepted: 15 November 2016 / Published online: 16 March 2017

Abstract The paper provides a first look into the fatty acids (FA) of young *Amphioctopus fangsiao*. Laboratory-hatched, 1-day-old juveniles (NH) were analyzed to identify the basal FA profile. To determine dietary effects on FA, individually kept juveniles (FD) were fed mysids once daily. Others were subjected to starvation (ST) to examine which FA may be used and which would be conserved. Treatments run for 25 days post-hatch, at which time ST and FD were analyzed to record FA changes. The dominant FA were 16:0, eicosapentaenoic (EPA), and docosahexaenoic (DHA), overall accounting for 40–60%. Monounsaturated FA (MUFA) were the highest in FD at 24.2% whereas highly unsaturated FA (HUFA) were most prominent in ST at 45.5%, followed by NH at 40.2%. Among n-3 HUFA, DHA was dominant in ST at 22.9 mg g^{-1} dry weight (DW), the role assumed by EPA in FD at 11.5 mg g^{-1} DW. Consequently, the DHA/EPA ratio was the lowest in FD. Arachidonic was the most abundant n-6 HUFA, representing >5% in total FA. However, n-6 FA were not prevalent, resulting in high n-3/n-6 in all juveniles. It could be argued that young *A. fangsiao* require n-3 HUFA, particularly DHA and EPA at a ratio of ideally >1.5 and to a lesser extent n-6 HUFA. Juveniles fed on a low lipid, high n-3 diet increased their MUFA content while maintaining high HUFA. Despite changes in the FA of ST individuals, it appears food-deprived *A. fangsiao* do not depend on FA mobilization for energy production.

Keywords Octopus · Juveniles · Fatty acids · Starvation · Diet effect

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Introduction

Cephalopods are valuable fishery resources, whose exploitation is expected to rise, owing to the decline in many finfish stocks and to their nutritional quality. The only credible alternative to protect existing cephalopod stocks and satisfy demand is aquaculture (Vidal et al. 2014). Compared to the culture of other molluscs and of fish and crustacean, cephalopod culture is still in early stages. This is especially true for octopuses. *Octopus maya* may be the most advanced example of commercially promising octopus aquaculture, although it cannot yet compete with the wild product. Industrial production of *Octopus vulgaris* occurs by ongrowing wild-caught subadults in marine cages in Spanish waters (Vidal et al. 2014).

For fishery and aquaculture purposes, octopuses are distinguished to merobenthic and holobenthic. Merobenthic species (e.g., *O. vulgaris*) lay hundreds of thousands of eggs and the hatchlings, termed paralarvae (Young and Harman 1988), spend variable times in the plankton before assuming a benthic life. The low fecundity (few hundred–few thousand eggs) of holobenthic species (e.g., *O. maya*) is counterbalanced by direct development (i.e., no paralarval form), whereby hatchlings are replicas of the adults and called juveniles (Naef 1928).

Whether merobenthic or holobenthic, all hatchlings have to deal with a critical shift from endogenous (i.e., yolk) to exogenous (i.e., prey) sources to fulfill their energetic requirements and are vulnerable to starvation during this period (Robin et al. 2014). Consequently, nutrition has been suggested as the most influential factor in octopus rearing success (Iglesias et al. 2007; Uriarte et al. 2011b). The diversity of species involved, plus limited information on feeding habits of wild paralarvae and juveniles (Roura et al. 2012), makes implementing a dietary regime challenging.

Studies on the biochemical composition of early stages are useful as a first approach to determine dietary needs in order to improve survival and support large-scale larviculture (Iglesias et al. 2007; Seixas et al. 2010b; Viciano et al. 2011). The profile of paralarvae or juveniles in regards to lipids, fatty acids (FA), amino acids (AA), vitamins, and minerals has been generated and compared with other developmental stages in the life cycle and, where possible, with wild specimens (Navarro and Villanueva 2003; Villanueva et al. 2004, 2009; Villanueva and Bustamante 2006; Noyola et al. 2013).

Although cephalopods have a predominantly AA metabolism, lipids may become energetically important during starvation and other stresses (García-Garrido et al. 2010). Studies on lipid composition of early stage octopuses have shown requirements to be high in phospholipids, cholesterol, polyunsaturated fatty acids (PUFA) and highly unsaturated fatty acids (HUFA) (Navarro and Villanueva 2000; Iglesias et al. 2007). Good results in paralarval rearing have been achieved with shrimp or crab zoeae (Iglesias et al. 2007; Uriarte et al. 2011a), whose biochemical profile is characterized by high phospholipids, low triglycerides, and high PUFAs (Navarro and Villanueva 2000).

García-Garrido et al. (2010) suggested arachidonic acid (ARA) may play a pivotal role in the common octopus, *O. vulgaris*, and Navarro and Villanueva (2000, 2003) stressed the importance of eicosapentaenoic (EPA) and docosahexaenoic (DHA) for the species. These HUFA are necessary for various physiological processes, assisting immune and inflammatory responses and neural processes and ensuring normal cellular structure and function and continuous membrane synthesis in the fast growing young (Miliou et al. 2006; Seixas et al. 2010a; Noyola et al. 2013).

A holobenthic octopus caught on a regular basis in Tokyo Bay and the Seto Inland Sea, Japan, is the ocellated octopus, *Amphioctopus fangsiao* d'Orbigny, 1839, formerly known as *Octopus ocellatus* Gray, 1849. Despite mentions to its aquaculture potential, there is limited information on its biology/ecology, with much of the early literature in Japanese, Chinese, or Korean. Moreover, confusion in its scientific nomenclature (Norman and Hochberg 2005) questions the reliability of available data. Characterized by a pair of golden/brown ringed ocelli near the base of arm III and a rectangular cream-colored patch between the eyes, it reaches ≤300 mm at maturity (Okutani et al. 1987). The lifespan of *A. fangsiao* may be about 1 year around the mainland Japan. The octopuses become sexually mature in winter and spawning occurs in spring, females producing 300–400 large eggs (approx. 7.0–7.5 mm). Time to hatching takes ~50 days in 25 °C. The hatchlings average 12.7 mm, as opposed to *O. vulgaris* paralarvae at 2.8 mm, are benthic from the start and resemble morphologically and behaviorally the adults (Segawa and Nomoto 2002; Fujisawa et al. 2005; Isshiki et al. 2012).

A defining parameter in establishing *A. fangsiao* rearing programs would be to understand the dietary requirements of juveniles so as to develop adequate feeding protocols during early growth. To that scope, we determined the FA composition of newly hatched individuals (NH), as a reference of the ideal/natural profile. Changes to the FA of NH during feeding would point to dietary effects and changes during starvation would indicate the relative importance of certain FA through preferential conservation. This is a first insight into the FA requirements for the species.

Materials and methods

Broodstock maintenance

Mid April 2012, nine gravid *A. fangsiao* females were collected at 10–30 m depth by fishermen in the Akashi Strait, Seto Inland Sea, Japan. They were transferred to the Kobe Municipal Suma Aqua Life Park, Japan, in aerated containers and separated equally in three 182-L (90 cm L × 45 cm W × 45 cm H) holding tanks, referred to as broodstock tanks, filled to 147 L. The semi-closed recirculating system was composed of two parallel lines of four rectangular tanks each and was connected to a sump of 1.9 m^3 capacity, with fresh seawater input at 52 L h^{-1} and a fluid bed filter (silica sand, effective size 0.6 mm). Water conditions in the tanks were constant, with a flow rate at 126 L h^{-1}, temperature 17 ± 0.6 °C, salinity 28 ppt, dissolved oxygen (DO) 6.8 ± 0.4 ppm, and ammonia levels (Tetra test NH_3/NH_4^+) below detectable levels. Twice weekly, full water exchange was performed to remove waste and shed skin. A 12 h light:12 h dark photoperiod was maintained by fluorescent lamps, and tanks were supplied with rectangular ceramic airstones and fitted with tight lids, covered with black mesh to reduce light intensity. Containers (either ceramic pots or dark plastic jars) were provided to the broodstock at introduction to the tanks as shelter and for egg laying. Females (one per container) were not fed, as during the spawning period, they cease to eat and were checked for egg deposition daily. The reason for keeping three females in each tank was to acquire a mixture of hatchlings to ensure genetic variability and more accurate representation of the nutritional profile.

Juvenile collection and experimental treatments

Juvenile collection for trial setup took place between mid June to late July 2012. Active 1-day-old juveniles (NH) were removed from the broodstock tanks early morning by a fine-mesh

hand net. Unless they were allocated to one of two treatments, fed (FD) or starved (ST), they would be processed immediately (sacrificed by immersion in ice cold seawater, rinsed with distilled water, then put on blotting paper to remove excess water), labeled, and stored in a −25 °C freezer. Treatment juveniles were kept individually in perforated plastic containers (12.5 cm H × 6.0 cm W) to ensure proper feeding in the FD and to avoid cannibalism in the ST. Four empty tanks of the aforementioned system (see broodstock maintenance) were used to hold juveniles, two for ST and two for FD. The number of containers per tank varied, as juveniles were added to participate in trials while others were removed to be processed, not exceeding 50 for the FD treatment and approx. double that for the ST at any one time. In the tanks, salinity had risen to 31 ppt and DO levels had dropped to 6.1 ± 0.4 ppm; the temperature was kept at 18 ± 0.3 °C by introducing two 400SP coolers (Zensui Co., Ltd.) to the sump tank. Individual containers were labeled with hatch date and broodstock tank number from where the juveniles originated. Each broodstock tank provided individuals to the NH, FD, and ST in triplicate; there were three broodstock tanks, resulting in nine samples per treatment. Feeding, where applicable, was once daily for 25 days, as in preliminary trials, this was the average survival time of ST individuals. The food was small mysids, *Neomysis awatschensis* Brandt, 1851, also known as *Neomysis intermedia* Czerniavsky, 1882, purchased daily from Izu Chuo Aqua Trading Co., Ltd. Initially, 2–3 mysids were fed per juvenile (based on preliminary results), adjusted as the juveniles grew. When either FD or ST individuals reached 25 days post-hatch (DPH) they would be collected following the NH procedure. By late August, all experimental juveniles had been harvested. Prey samples were collected in triplicate and analyzed for lipid and FA composition as a dietary reference. Thirty-one randomly selected individuals from each the NH, ST, and FD treatments were weighed to 0.01 g (Ohaus SP602 scout scale) at day 1 to record initial weight and, for ST and FD, day 25 to record final weight. Weight changes were calculated by weight loss or gain × initial weight^{-1} × 100. Specific growth rate (SGR, % day^{-1}) was determined as (LnW2-LnW1) × t^{-1} × 100, where W2 and W1 are the final and initial weight, respectively, Ln is the natural logarithm, and t is time of the experimental period.

FA analysis

Lipids were extracted from whole octopus and mysid samples with chloroform/methanol (1:1 *v*/*v*) using the method of Bligh and Dyer (1959). Total lipid (TL) was determined gravimetrically by drying for 30 min at 37 °C under N_2 gas current. The FA components of lipids were derivatized to their fatty acid methyl esters (FAME) using 14% boron trifluoride in methanol (BF3-MeOH; Wako Pure Chemicals, Japan) as a catalyst (Morrison and Smith 1964). Following, FAME were purified using SEP-PAK silica cartridges (Waters, USA) eluted with diethyl ether:petroleum ether (5:95 *v*/*v*). Distilled hexane was added to the purified methyl esters at 20 mg mL^{-1} just before injection into the gas chromatograph (GC) port. FAME were determined using a SHIMADZU GC 17A gas chromatograph fitted with a flame ionization detector (column temperature 220 °C, detector temperature 260 °C, helium as carrier gas at 30 mL min^{-1}) and capillary column (SUPELCOWAX 10, 30 m L × 0.32 mm ID, 0.25 mm D_f) (Supelco Inc., Japan). Peaks were recorded using a SHIMADZU CHROMATOPAC C-R4A data processor. Individual FA were identified by comparison of retention times to those of authentic FAME standards (Wako Pure Chemicals, Japan; Funakoshi Co., Japan; Sigma-Aldrich, USA) and quantified by means of response factor to an internal standard, tricosanoic acid (23:0).

Statistical analysis

The package R 3.1.2 was used for statistical analysis. The level of significance was $p < 0.05$. Parallel coordinate plots (PCP) were used to contrast the profile of NH, ST, and FD juveniles and of FD and their diet by producing a graphical summary of the multivariate data set. PCP was followed by one-way ANOVA for TL, FA, and FA groups of juveniles. Where differences were found, a post-hoc Tukey's honest significant difference (HSD) test was applied. Tukey's HSD was also the preferred test (as it controls for a familywise error rate) for comparisons of TL, FA, and FA groups of FD vs diet. Q-Q plots of the residuals were used to assess assumptions of normality of one-way ANOVA (or Tukey's HSD) while the Fligner-Killeen test, along with boxplots, of homogeneity. Where assumptions were violated, the data were transformed (absolute FA concentrations $\log_e$-transformed, relative FA content arcsine root transformed).

Results

Initial wet weight of *A. fangsiao* hatchlings was 0.09 ± 0.017 g ($n = 93$) while final weight of ST and FD was 0.05 ± 0.011 g ($n = 31$) and 0.34 ± 0.064 g ($n = 31$), respectively. A negative SGR of −3.0 ± 1.18% day^{-1} was recorded for ST, while SGR for FD was 5.0 ± 0.96% day^{-1}. Total lipids ranged from 12.3% of dry weight (DW) in ST to 12.9% in NH; differences were not significant ($p = 0.161$) (Table 1).

Qualitatively, saturated fatty acids (SFA) and HUFA were the most abundant FA groups in all juvenile treatments (Table 1). The ST had the highest HUFA content at 45.5% ($p < 0.05$) and SFA content at 34.0% ($p < 0.05$) and the lowest PUFA content at 3.2% ($p < 0.05$), whereas the highest monounsaturated fatty acid (MUFA) percentage was observed in FD at 24.2 ($p < 0.05$).

The dominant FA across juvenile treatments were 16:0, EPA, and DHA, overall accounting for 40–60% in total FA (Table 1). Apart from 16:0, 18:0 among SFA was high, being the lowest in the NH at 6.6% ($p < 0.05$). The most abundant MUFA were 16:1n-9, the highest in FD at 7.2% and the lowest in ST at 3.1% ($p < 0.05$), and 18:1n-11+18:1n-9, the lowest in NH at 6.8% and the highest in FD at 11.9% ($p < 0.05$). The latter also showed the highest 18:2n-6 (linoleic, LOA) at 3.6%; the equivalent value for ST was 0.7% ($p < 0.05$). The HUFA profile was dominated by EPA, the lowest in NH at 12.5% ($p < 0.05$), and DHA, the lowest in FD at 13.7% ($p < 0.05$). ARA was similarly abundant in the juveniles, with not significantly higher levels in NH (5.5%) ($p = 0.880$).

While DHA accounted for 20.2% of total FA in NH (Table 1), quantitatively 9.4 ± 3.31 mg g^{-1} DW were present, significantly less than the amount in ST at 22.9 ± 0.84 mg g^{-1} DW ($p < 0.05$) (Table 2). Due to the significantly low total FA of NH (45.6 ± 5.29 mg g^{-1} DW) ($p < 0.05$), the pattern was repeated across several important FA, including EPA at 5.7 ± 1.07 mg g^{-1} DW, 18:0 at 3.1 ± 2.16, and 16:0 at 8.0 ± 1.08 mg g^{-1} DW. The equivalent amounts in ST were 14.7 ± 1.19, 10.2 ± 0.93, and 18.8 ± 1.30 mg g^{-1} DW, respectively. Total FA content of FD was 76.9 ± 8.17 mg g^{-1} DW. Both LOA and, especially, 18:3n-3 (linolenic, LNA) were low across all juvenile treatments, with FD exhibiting the highest concentrations at 2.8 ± 0.65 and 0.5 ± 0.21 mg g^{-1} DW, respectively ($p < 0.05$).

The DHA/EPA ratio was similar in NH and ST at 1.6 but significantly lower in FD (0.9) ($p < 0.05$), which had high EPA/ARA ratio at 3.7, being lower in NH at 2.4; however,

Table 1 Qualitative fatty acid composition (% of total FA) of newly hatched, starved, and fed *A. fangsiao* juveniles (mean ± SD, $n = 9$) and of the mysid feed (mean ± SD, $n = 3$)

FA (%)	Juveniles			Feed
	Newly hatched	Starved	Fed	Mysids
14:0	1.8 ± 0.39^{a}	1.6 ± 0.25^{a}	3.8 ± 1.25^{b1}	2.3 ± 0.32
14:1n-5	0.4 ± 0.21^{a}	0.5 ± 0.14^{a}	0.7 ± 0.16^{b1}	0.9 ± 0.20
15:0	0.6 ± 0.25^{b}	0.5 ± 0.15^{ab}	0.3 ± 0.12^{a1}	1.2 ± 0.25
16:0	17.8 ± 3.68^{a}	19.6 ± 1.42^{a}	11.5 ± 1.72^{b1}	18.7 ± 0.54
16:1n-9	5.8 ± 1.78^{a}	3.1 ± 0.39^{b}	7.2 ± 1.16^{a}	5.9 ± 1.20
17:0	0.9 ± 0.35^{b}	1.7 ± 0.22^{a}	1.9 ± 0.63^{a}	1.6 ± 0.52
17:1	0.7 ± 0.47^{ab}	0.9 ± 0.33^{b}	0.4 ± 0.23^{a}	0.8 ± 0.20
18:0	6.6 ± 4.09^{b}	10.6 ± 1.04^{a}	9.8 ± 0.86^{a1}	2.4 ± 0.30
18:1n-11+18:1n-9	6.8 ± 1.78^{a}	8.9 ± 1.59^{b}	11.9 ± 1.01^{c1}	10.2 ± 0.86
18:2n-6	3.2 ± 2.08^{a}	0.7 ± 0.10^{b}	3.6 ± 0.58^{a1}	1.6 ± 0.18
18:3n-6	0.1 ± 0.04^{a}	0.4 ± 0.38^{b}	0.2 ± 0.04^{ab1}	0.1 ± 0.03
18:3n-3	0.1 ± 0.06^{a}	0.2 ± 0.07^{a}	0.7 ± 0.27^{b}	1.0 ± 0.24
18:4n-3	0.0 ± 0.02^{a}	0.0 ± 0.02^{a}	0.4 ± 0.20^{b}	0.3 ± 0.00
19:0	0.1 ± 0.07^{a}	0.1 ± 0.02^{a}	0.2 ± 0.18^{a}	0.1 ± 0.00
20:0	0.3 ± 0.32^{a}	0.0 ± 0.01^{b}	0.4 ± 0.13^{a1}	0.1 ± 0.02
20:1n-11+20:1n-9	3.1 ± 0.86^{a}	0.4 ± 0.17^{b}	2.4 ± 0.65^{a1}	1.0 ± 0.10
20:3n-6	0.5 ± 0.10^{ab}	0.4 ± 0.07^{b}	0.6 ± 0.24^{a1}	0.3 ± 0.04
20:4n-6	5.5 ± 1.08^{a}	5.3 ± 1.38^{a}	5.1 ± 2.51^{a}	3.4 ± 0.38
20:3n-3	1.1 ± 0.43^{a}	1.4 ± 0.19^{a}	0.7 ± 0.25^{b1}	0.3 ± 0.18
20:4n-3	0.1 ± 0.08^{a}	0.1 ± 0.02^{a}	0.2 ± 0.15^{b}	0.2 ± 0.02
20:5n-3	12.5 ± 1.31^{b}	15.2 ± 1.01^{a}	15.1 ± 2.37^{a}	14.4 ± 0.40
22:0	0.1 ± 0.07^{a}	0.1 ± 0.01^{b}	0.2 ± 0.03^{c1}	0.2 ± 0.04
22:1n-11+22:1n-9	1.2 ± 0.64^{a}	0.5 ± 0.19^{b}	0.8 ± 0.52^{ab1}	0.1 ± 0.02
22:3n-6	1.8 ± 1.50^{b}	0.2 ± 0.05^{a}	0.3 ± 0.16^{a}	0.2 ± 0.06
22:5n-6	0.7 ± 0.36^{a}	0.3 ± 0.08^{b}	0.8 ± 0.18^{a1}	1.8 ± 0.37
22:5n-3	1.2 ± 0.59^{ab}	0.6 ± 0.28^{b}	1.6 ± 0.74^{a}	2.1 ± 0.38
22:6n-3	20.2 ± 5.30^{a}	23.9 ± 0.53^{a}	13.7 ± 2.27^{b1}	17.6 ± 1.70
24:1	0.4 ± 0.50^{ab}	0.2 ± 0.07^{b}	0.6 ± 0.29^{a1}	TR
Others	6.3 ± 1.33^{a}	2.9 ± 0.41^{b}	4.9 ± 2.68^{ab1}	10.5 ± 1.36
∑SFA	28.4 ± 1.59^{a}	34.0 ± 1.53^{b}	28.1 ± 2.72^{a}	26.5 ± 1.10
∑MUFA	18.3 ± 2.33^{a}	14.4 ± 1.81^{b}	24.2 ± 1.86^{c1}	18.9 ± 0.69
∑PUFA	6.8 ± 3.64^{a}	3.2 ± 0.30^{b}	6.0 ± 0.79^{a1}	3.4 ± 0.50
∑HUFA	40.2 ± 5.44^{a}	45.5 ± 0.55^{b}	36.8 ± 3.68^{a}	39.7 ± 2.40
n-3	35.3 ± 4.91^{a}	41.4 ± 1.52^{b}	32.3 ± 1.55^{a1}	35.9 ± 2.37
n-6	11.7 ± 3.06^{a}	7.3 ± 1.76^{b}	10.5 ± 2.98^{a1}	7.2 ± 0.66
n-9	16.9 ± 1.92^{a}	12.9 ± 1.97^{b}	22.4 ± 1.99^{c1}	17.2 ± 0.58
TL (% of DW)	12.9 ± 0.58^{a}	12.3 ± 0.62^{a}	12.5 ± 0.69^{a1}	4.3 ± 0.19

Values in the same row with no superscript letters in common are significantly different ($p < 0.05$). The *superscript 1* indicates a significant difference between the fed juveniles and the mysid diet

SFA saturated, *MUFA* monounsaturated, *PUFA* polyunsaturated ($\sum_{FA}$ of 2 and 3 ethylenic bonds), *HUFA* highly unsaturated ($\sum_{FA}$ of >3 ethylenic bonds), *TR* trace

differences were not significant ($p = 0.160$) (Table 2). The n-3/n-6 ratio was significantly higher at 5.9 in ST ($p < 0.05$) while lower but similar for NH and FD. The latter treatment had the highest n-9 content at 17.3 ± 2.97 mg g^{-1} DW ($p < 0.05$).

The mysid diet had low TL content at 4.3% DW, as opposed to 12.5% of the FD ($p < 0.05$) (Table 1). HUFA were the most abundant group in the mysids at 39.7%, while PUFA least abundant at 3.4%. The dominant FA constituents in the mysids were 16:0 at 18.7%, 16:1n-9 at 5.9%, 18:1n-11 + 18:1n-9 at 10.2%, EPA at 14.4%, and DHA at 17.6%. 16:0 and DHA were significantly higher in the mysid diet compared to FD ($p < 0.05$), which showed significantly higher 18:0 at 9.8%, as opposed to 2.4% in their diet ($p < 0.05$). The mysid diet had high n-3/n-6 ratio of 5.0 in comparison to the 3.2 of FD ($p < 0.05$) and not significantly higher EPA/ARA at 4.3 ($p = 0.426$) and DHA/EPA at 1.2 ($p = 0.136$) (Table 2).

Discussion

Average body weight of NH *A. fangsiao* was at the lower end of the range reported for the species by Segawa and Nomoto (2002), whereby hatchlings weighed 0.11 g (range 0.10–0.13 g), and close to values of 1-day-old *O. maya* (0.10 ± 0.001 g; Noyola et al. 2013), another holobenthic species. Total lipids in NH comprised 12.9% of DW; the equivalent amount in *O. vulgaris* paralarvae in the study by Seixas et al. (2010b) was 11.9%. Paralarvae of *Robsonella fontaniana* hatched with 11.5% lipid while protein occupied >50% of their biochemical profile (Uriarte et al. 2011a). Low to moderate lipid reserves (11–13%) have been recorded for other cephalopod hatchlings and regarded typical since the main source of energy for growth is considered to be protein (Navarro and Villanueva 2000; Villanueva et al. 2004). The most prominent FA in NH were 16:0, EPA, and DHA, similar to reports on other cephalopods, for example, *R. fontaniana*, *O. vulgaris*, *Sepia officinalis*, *Loligo vulgaris* (Navarro and Villanueva 2000; Uriarte et al. 2011a; Reis et al. 2016). Percent FA group in NH approximated closely that of *O. vulgaris* hatchlings as reported by Navarro and Villanueva (2000). Specifically, SFA represented 28.4% in total FA in *A. fangsiao*, compared to 27.2% in *O. vulgaris*. Regarding MUFA, the content was higher in *A. fangsiao* at 18.3%, as opposed to 13.5% in *O. vulgaris*, mainly due to a difference in 16:1n-9 levels. In the present study, PUFA and HUFA are reported separately, while in the study by Navarro and Villanueva (2000), these were grouped together. As such, PUFA made up 49.2% of total FA in *O. vulgaris*, the corresponding amount in *A. fangsiao* would be 47.0%, in either case the prevalent FA group. In *O. maya* hatchlings, PUFA and SFA were equally abundant (Tercero et al. 2015), while SFA predominated *R. fontaniana* profile (Uriarte et al. 2011a).

A reduction in body weight of *A. fangsiao* by 51% after 25 days of starvation with no significant reduction in absolute TL content implies juveniles may be relying primarily on muscle protein to satisfy metabolic requirements. Hatchlings of *O. vulgaris* lost 28% of their body weight (Villanueva et al. 2004) and those of *O. maya* 17% after 4 days fasting (George-Zamora et al. 2011). Although it is difficult to make direct comparisons due to prolonged starvation of *A. fangsiao*, life history strategies could be one parameter affecting the rate of weight loss, for example, the metabolically expensive swimming mode of the planktonic *O. vulgaris* as opposed to the necto-benthic and benthic lifestyle of *O. maya* and *A. fangsiao*, respectively. Both aforementioned studies (Villanueva et al. 2004; George-Zamora et al. 2011) reported mobilization of AA during fasting. García-Garrido et al. (2010) also suggested the use of muscle protein for energetic support in *O. vulgaris* juveniles starved for 27 days, despite

Table 2 Quantitative fatty acid composition (mg g^{-1} dry weight) of newly hatched, starved, and fed *A. fangsiao* juveniles (mean ± SD, n = 9) and of the mysid feed (mean ± SD, n = 3)

FA (mg g^{-1} DW)	Juveniles			Feed
	Newly hatched	Starved	Fed	Mysids
14:0	0.8 ± 0.12^{a}	1.5 ± 0.23^{b}	2.8 ± 0.65^{c1}	0.6 ± 0.09
14:1n-5	0.2 ± 0.11^{b}	0.5 ± 0.13^{a}	0.5 ± 0.10^{a1}	0.2 ± 0.05
15:0	0.3 ± 0.10^{a}	0.5 ± 0.15^{b}	0.3 ± 0.07^{a}	0.3 ± 0.06
16:0	8.0 ± 1.08^{a}	18.8 ± 1.30^{b}	8.8 ± 1.64^{a1}	4.7 ± 0.11
16:1n-9	2.7 ± 0.90^{a}	3.0 ± 0.41^{a}	5.6 ± 1.20^{b1}	1.5 ± 0.32
17:0	0.4 ± 0.21^{b}	1.6 ± 0.20^{a}	1.5 ± 0.51^{a1}	0.4 ± 0.13
17:1	0.3 ± 0.19^{a}	0.8 ± 0.32^{b}	0.3 ± 0.15^{a}	0.2 ± 0.05
18:0	3.1 ± 2.16^{a}	10.2 ± 0.93^{b}	7.5 ± 0.82^{c1}	0.6 ± 0.07
18:1n-11+18:1n-9	3.2 ± 1.15^{b}	8.6 ± 1.61^{a}	9.2 ± 1.35^{a1}	2.6 ± 0.19
18:2n-6	1.3 ± 0.83^{a}	0.7 ± 0.10^{a}	2.8 ± 0.65^{b1}	0.4 ± 0.05
18:3n-6	0.0 ± 0.02^{a}	0.4 ± 0.36^{b}	0.1 ± 0.03^{ab1}	0.0 ± 0.01
18:3n-3	0.1 ± 0.03^{a}	0.2 ± 0.07^{a}	0.5 ± 0.21^{b}	0.3 ± 0.06
18:4n-3	0.0 ± 0.01^{a}	0.0 ± 0.02^{a}	0.3 ± 0.12^{b1}	0.1 ± 0.00
19:0	0.1 ± 0.03^{a}	0.1 ± 0.02^{a}	0.1 ± 0.12^{a}	0.0 ± 0.00
20:0	0.1 ± 0.14^{a}	0.0 ± 0.01^{a}	0.3 ± 0.10^{b1}	0.0 ± 0.01
20:1n-11+20:1n-9	1.4 ± 0.32^{a}	0.4 ± 0.17^{b}	1.9 ± 0.55^{c1}	0.2 ± 0.03
20:3n-6	0.2 ± 0.06^{b}	0.4 ± 0.07^{ab}	0.5 ± 0.23^{a1}	0.1 ± 0.01
20:4n-6	2.5 ± 0.72^{a}	5.1 ± 1.27^{b}	4.1 ± 2.38^{ab1}	0.9 ± 0.09
20:3n-3	0.5 ± 0.16^{a}	1.3 ± 0.17^{b}	0.5 ± 0.21^{a1}	0.1 ± 0.04
20:4n-3	0.0 ± 0.03^{a}	0.1 ± 0.01^{ab}	0.2 ± 0.11^{b}	0.1 ± 0.00
20:5n-3	5.7 ± 1.07^{a}	14.7 ± 1.19^{b}	11.5 ± 1.38^{c1}	3.7 ± 0.06
22:0	0.1 ± 0.03^{a}	0.1 ± 0.01^{a}	0.2 ± 0.03^{b1}	0.0 ± 0.01
22:1n-11+22:1n-9	0.5 ± 0.25^{a}	0.5 ± 0.19^{a}	0.7 ± 0.46^{a1}	0.0 ± 0.01
22:3n-6	0.8 ± 0.64^{b}	0.2 ± 0.05^{a}	0.2 ± 0.09^{a1}	0.0 ± 0.02
22:5n-6	0.3 ± 0.13^{a}	0.3 ± 0.08^{a}	0.6 ± 0.14^{b}	0.5 ± 0.10
22:5n-3	0.5 ± 0.24^{a}	0.6 ± 0.28^{a}	1.3 ± 0.63^{b1}	0.5 ± 0.09
22:6n-3	9.4 ± 3.31^{a}	22.9 ± 0.84^{b}	10.7 ± 2.82^{a1}	4.5 ± 0.43
24:1	0.2 ± 0.21^{a}	0.2 ± 0.07^{a}	0.5 ± 0.21^{b1}	TR
Others	2.8 ± 0.46^{a}	2.8 ± 0.38^{a}	3.6 ± 1.60^{a}	2.7 ± 0.35
Total FA	45.6 ± 5.29^{a}	96.1 ± 1.98^{b}	76.9 ± 8.17^{c1}	25.1 ± 0.28
∑SFA	12.9 ± 1.33^{a}	32.6 ± 1.08^{b}	21.5 ± 2.02^{c1}	6.7 ± 0.20
∑MUFA	8.4 ± 1.64^{a}	13.8 ± 1.91^{b}	18.7 ± 2.92^{c1}	4.8 ± 0.20
∑PUFA	2.9 ± 1.43^{a}	3.1 ± 0.27^{a}	4.7 ± 0.93^{b1}	0.9 ± 0.13
∑HUFA	18.6 ± 4.46^{a}	43.7 ± 1.22^{b}	28.5 ± 5.55^{c1}	10.1 ± 0.59
EPA/ARA	2.4 ± 0.71^{a}	3.0 ± 0.81^{a}	3.7 ± 1.82^{a}	4.3 ± 0.43
DHA/EPA	1.6 ± 0.46^{a}	1.6 ± 0.08^{a}	0.9 ± 0.28^{b}	1.2 ± 0.12
DHA/EPA/ARA	0.7 ± 0.22^{b}	0.3 ± 0.06^{a}	0.3 ± 0.09^{a1}	1.4 ± 0.07
n-3	16.3 ± 3.85^{a}	39.8 ± 2.08^{b}	24.9 ± 3.36^{c1}	9.1 ± 0.58
n-6	5.2 ± 0.92^{b}	7.0 ± 1.62^{ab}	8.3 ± 3.11^{a1}	1.8 ± 0.18
n-9	7.8 ± 1.61^{a}	12.4 ± 2.04^{b}	17.3 ± 2.97^{c1}	4.4 ± 0.17
n-3/n-6	3.3 ± 1.33^{a}	5.9 ± 1.33^{b}	3.2 ± 0.74^{a1}	5.0 ± 0.32

Table 2 (continued)

FA (mg g^{-1} DW)	Juveniles			Feed
	Newly hatched	Starved	Fed	Mysids
TL (mg g^{-1} DW)	128.8 ± 5.77[a]	123.0 ± 6.24[a]	125.2 ± 6.88[a1]	42.7 ± 1.93

Values in the same row with no superscript letters in common are significantly different ($p < 0.05$). The *superscript 1* indicates a significant difference between the fed juveniles and the mysid diet

SFA saturated, *MUFA* monounsaturated, *PUFA* polyunsaturated ($\sum_{FA}$ of 2 and 3 ethylenic bonds), *HUFA* highly unsaturated ($\sum_{FA}$ of >3 ethylenic bonds), *TR* trace

noticing a decrease in TL of mantle and TL and FA of digestive gland (DG). Ideally, along with the FA profile, the AA profile should have been generated to allow more accurate conclusions regarding energy substrates in *A. fangsiao* juveniles at times of stress. Preferential retention of FA during nutritional stress may indicate which are physiologically important for an organism. HUFA were the prevalent group in ST, with DHA being proportionally high, followed by EPA. For starved 7-day-old *S. officinalis*, DHA and ARA, a competitor of EPA for the enzymes involved in eicosanoid production, were maintained despite nutritive status (Sykes et al. 2012). On the contrary, in *O. vulgaris* juveniles, 16:0, DHA, and EPA were among the FA mostly mobilized from the DG during the starvation period (García-Garrido et al. 2010). The next most abundant FA group in ST was saturates. However, it has been argued that accretion of short-chain saturates like 16:0 and 18:0 may be due to the ease of synthesis and storage rather than an absolute requirement for these (Olsen 1998). Indeed, 16:0 and 18:0 were the dominant SFA in early eggs of *R. fontaniana* (Uriarte et al. 2011a) and late embryos of *O. vulgaris* (Navarro and Villanueva 2003).

Juveniles in the FD treatment nearly quadrupled their weight in the 25 days of the experiment, which is higher than the two- or threefold increase in 30-day-old *O. vulgaris* paralarvae fed on *Artemia* + pellets or *Artemia* only, respectively (Navarro and Villanueva 2000), but lower than the almost fivefold increase of *O. maya* juveniles fed for 20 days on shrimp-squid paste (Noyola et al. 2013). Segawa and Nomoto (2002) reported an instantaneous growth rate in weight of *O. ocellatus* (current *A. fangsiao*) of 5.6%, higher than the 5.0% recorded in our study; higher rearing temperature and an ad libitum crab diet may be the differentiating factors between their results and ours. Deviations in the lipid and FA profile of reared octopuses from hatchlings are assumed to be of dietary origin. Navarro and Villanueva (2003) observed an increase in TL and MUFA of *O. vulgaris* paralarvae, induced by the diets, after 10 days of rearing. Although MUFA increased, TL content in FD *A. fangsiao* was not affected by the diet, indeed very low in TL. When Viciano et al. (2011) further separated TL of *O. vulgaris* paralarvae into polar and neutral, they noted a dietary effect not apparent in the TL content. Whether the same would apply for *A. fangsiao* is uncertain since lipids were not differentiated into their polar and neutral fractions in the current study. The profile of FD *A. fangsiao* contained higher MUFA than either the NH or ST. A similar level of MUFA (23%) to *A. fangsiao* was reported for fed *O. maya* juveniles raised at 18 °C (Noyola et al. 2013). For cultured *O. vulgaris* paralarvae, increased MUFA, at the expense of SFA and PUFA, were recorded by Seixas et al. (2010b). An increase in monounsaturates, particularly 18:1 isomers, has been associated with maintenance of the unsaturation index under environmental conditions that promote octopus growth (Miliou et al. 2006).

The major FA in the mysid diet were 16:0, 18:1n-11+18:1n-9, EPA, and DHA. Ando and Nozaki (2007) also identified these as the prominent FA in *N. intermedia* from Japanese

waters. Although DHA was more abundant than EPA in the mysids, the opposite was true for the FD juveniles. Consequently, FD had a DHA/EPA ratio of 0.9, which is lower than the remaining treatments but higher than values of 25 DPH *O. vulgaris* paralarvae from different dietary regimes, whereby DHA/EPA ranged from 0.3 to 0.6 (Navarro and Villanueva 2003; Seixas et al. 2010a). In NH and ST *A. fangsiao*, the DHA/EPA ratio was 1.6, similar to *O. vulgaris* (Navarro and Villanueva 2000; Seixas et al. 2010a) and *S. officinalis* (Reis et al. 2016) and triple that of *R. fontaniana* (Uriarte et al. 2011a) hatchlings. García-Garrido et al. (2010) indicated the DHA/EPA ratio of starved *O. vulgaris* remained unchanged and close to previously reported values. In wild *O. vulgaris* juveniles, the ratio averaged 1.4 (Navarro and Villanueva 2003), possibly indicating an ideal relation between the two FA. The DHA/EPA ratio is considered more critical to lipid and FA metabolism than the absolute concentration of either FA, with values ≥1.5 yielding normal embryonic development and good survival and growth in cultured cephalopods (Okumura et al. 2005; Sykes 2007). EPA was more abundant than ARA in the juveniles, evident by the high EPA/ARA ratio in all treatments. Competition between EPA and ARA for site specific enzymes has been documented in marine species (Izquierdo 2005; Miliou et al. 2006; García-Garrido et al. 2010). Despite ARA being the most abundant n-6 HUFA in *A. fangsiao* treatments at >5% in total FA, overall, n-6 FA were not prevalent. In *O. maya* hatchlings, ARA content remained high despite low levels in parental diets (Tercero et al. 2015). *Octopus vulgaris* paralarvae were also rich in ARA at >7% for newly hatched and between approx. 5–7% for cultured ones, depending on diet (Navarro and Villanueva 2000). ARA concentration in mantle and DG of *O. vulgaris* juveniles did not change despite prolonged starvation (García-Garrido et al. 2010).

Based on the high EPA, DHA, and a high n-3/n-6 ratio, it may be proposed that young *A. fangsiao* have an increased requirement for n-3 HUFA and a lesser requirement for n-6 HUFA, in agreement with other octopus species, paralarvae and juveniles, cultured and wild (Navarro and Villanueva 2000, 2003; Iglesias et al. 2007). Findings in this study constitute a first step to identify dietary requirements of *A. fangsiao* juveniles. Next, more in-depth studies of lipid classes and FA and their occurrence in various tissues and organs of the young should be conducted to better understand the role of lipids. Given the widely accepted protein metabolism of cephalopods, analyzing the AA profile of hatchlings and exploring corresponding changes in starved and fed individuals constitute a crucial subject in *A. fangsiao* rearing. Furthermore, the influence of broodstock/parental diets on hatchling biochemical profile and quality and the effect of rearing conditions and diets on cultured juveniles should be considered in future trials.

Acknowledgements Financial support was provided by the Kobe Municipal Suma Aqua Life Park. Special thanks to K. Baba for the advice on aquaculture matters, to Juan Jose Ronceros for the feedback on this MS, and to Marios Aristophanous and, especially, Richard Rowe for the assistance with statistical analysis.

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