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Short Communication

Early Feeding by Cultured Paralarvae of *Octopus sinensis* d'Orbigny 1841: Comparison of Survival, and Fatty Acid and Amino Acid Profiles, Using Two Species of *Artemia*

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Abstract

Experiments were performed to compare the effect on survival patterns of early paralarvae of the East Asian common octopus, *Octopus sinensis* d'Orbigny 1841, fed with one of two species of *Artemia*. Comparison of predator and prey nutritional content (amino acids and fatty acids) revealed that *O. sinensis* paralarvae raised on *Artemia* are relatively deficient in b-alanine, cysteine, carnitine and 20:01 unsaturated fatty acid. Using *Artemia tibetiana* Abatzopoulos, Zhang & Sorgeloos 1998 as sole prey resulted in better survival than with *Artemia franciscana* Kellog 1906 but the latter has better supply and pricing reliability and consistency in hatching. Feeding paralarvae on *A. franciscana* supplemented with a microalgal culture resulted in a survival pattern similar to that when paralarvae were fed with unsupplemented *A. tibetiana*. Therefore, future feed development will be based around amino acid supplementation of *A. franciscana* grown with microalgae.

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Introduction

Aquaculture of the common octopus has so far failed to become commercially viable. A major stumbling block is feeding and maintaining planktonic juveniles during the period from hatching to settlement (Iglesias and Fuentes 2014; Vidal et al. 2014). In the wild, larval crabs dominate the food eaten by paralarvae of the common octopus: *Octopus vulgaris* Cuvier 1797, in the northeastern Atlantic and Mediterranean, as investigated by Roura et al. (2012); and *Octopus sinensis* d'Orbigny 1841, in East Asian waters (Gleadall, in press), as investigated in another study by the present authors (unpublished research). Crab zoeae are regarded as the best aquaculture feed (Iglesias and Fuentes 2014; Vidal et al. 2014) but no suitable, commercially reliable source of zoeae is available at the present time.

The brine shrimp *Artemia* (Crustacea: Branchiopoda) is commonly used to raise aquacultured species but its success with octopus paralarvae has been mediocre (Seixas et al. 2010; Iglesias and Fuentes 2014; Vidal et al. 2014). Recently, two different species of *Artemia* were compared as prey for octopus paralarvae and it was found that survival using *A. tibetiana* is superior to other *Artemia* species (Yamazaki et al. 2015). The objectives of the present study were (i) to investigate the limitations of using *Artemia* as a prey animal and (ii) to obtain information on the nutritional differences among *Artemia* treatments in raising octopus paralarvae successfully.

Materials and Methods

Young octopuses (*O. sinensis* of around 100-300 g) caught by octopus pot fishermen in the Kami-Amakusa area were obtained from Hato-no-kama fish landing, Ōyano Island, Kumamoto Pref., Kyushu, Japan, and transported by road in a specially prepared truck to Tohoku (northeastern Honshu, Japan). They were maintained indoors in a large concrete pool containing ambient seawater (open circulation, temperature 15-20 ℃) within dens constructed from commercial octopus pots or pieces of PVC piping (diameter and length around 10 and 20 cm, respectively). The octopuses were allowed to mate freely and were fed on thawed frozen Pacific sand lance (*Ammodytes personatus* Girard 1856) once daily until the females retired inside a den to lay eggs. The males were then removed and the females were left undisturbed for about 2 weeks.

Brooding females were each transferred (still inside their den) to individual cylindrical tanks of black PVC, diameter 1 m, with a gently sloping conical floor and a capacity of 1,000 L. The tanks received ambient seawater under ambient lighting, with gentle aeration through an airstone behind a retaining net near the central outflow. The females were monitored while they looked after the developing eggs naturally. After hatching commenced and around 5,000 paralarvae had been released, the den with the female and remaining eggs was removed (thereby lowering the oxygen consumption and burden of nitrogenous excretory products).

The experiments were conducted during September-October, 2014. Two species of *Artemia* were prepared from canned dried cysts: *A. franciscana* Kellog 1906 (obtained from Kitamura, Tokyo); and *A. tibetiana* Abatzopoulos, Zhang and Sorgeloos 1998 (Tibetan salt lake Qi Long brand). Feed supplements for *Artemia* were cultures of *Nannochloropsis* or *Chlorella* (algae known for high production and accumulation of fatty acids; *e.g.*, Roncarati et al. 2004). *Artemia* nauplii were introduced to paralarvae once daily from the second day after hatching, at an initial concentration of approximately 1.33 million individuals per tank per day (around 250 *Artemia* per paralarva), progressively reducing the number added in line with the number of surviving paralarvae calculated the previous day based on daily mortality counts. For combinations with algae supplementation, 20 mL algal culture water were added twice daily to the aquaria after the addition of *Artemia*. When aquaria were sampled, those containing algae were sampled 6 h after the last addition of algae, to allow adequate time for uptake, digestion and assimilation by the *Artemia* present.

Samples of both predators (3-4 paralarvae) and prey (5 mg wet-weight *Artemia*) were taken at intervals of 10-14 days. After removing excess liquid, the samples were frozen at -60 °C until analysis for fatty acid and amino acid content. Lipids were extracted as described by Folch et al. (1957) using butylated hydroxytoluene (Wako Pure Chemical Industries, Japan) as an antioxidant. The total lipid extract was stored in dry chloroform before transmethylation. Aliquots of the lipid extracts were dried under nitrogen gas and saponified at 95 °C for 60 min in KOH-methanol. Fatty acids in the saponifiable fraction were methylated at 95 °C for 2 min with 14% BF_3 in methanol. Methyl esters were then dissolved in hexane for injection into a gas chromatograph equipped with a flame-ionization detector (Agilent 6890N, Palo Alto, CA). Separations were performed using a capillary column (0.25 mm x 30 m DB-WAX, Agilent, Palo Alto, CA). Initial column temperature was 150 °C programmed at 2 °C $min.^{-1}$ to 220 °C, then final column temperature was kept at 220 °C for 15 min. Injector and detector temperatures were at 250 °C. Helium was used as the carrier gas with a splitter ratio of 1:50 and a linear gas velocity of 30 $cm{\cdot}s^{-1}$. To determine the total content of free and bound amino acids, samples were hydrolysed in 6N HCl at 110 °C for 24 h (Helrich 1990) and then processed in an automatic amino acid analyser (Hitachi L-8800, Tokyo).

Results

The results of feeding experiments on the survival of *O. sinensis* paralarvae are summarized in Fig. 1, which shows that feeding with *A. tibetiana* produces the best survival performance, clearly much better than with *A. franciscana* as live feed. Supplementing *A. franciscana* by culturing with either *Nannochloropsis* or *Chlorella* brings survival performance close to that of *A. tibetiana*.

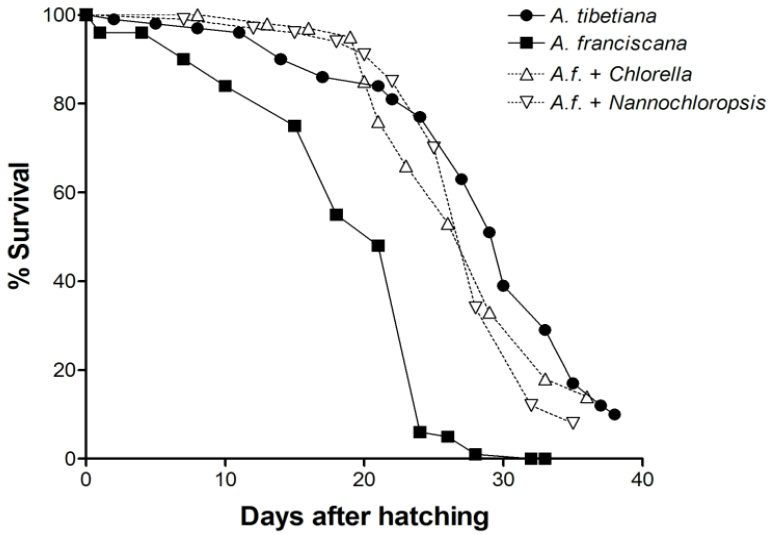

Fig.1. Comparison of the effects of several different kinds of live feed presented daily to cultures of octopus paralarvae (starting with approximately 5,000 paralarvae per tank). Filled symbols and solid lines are survival curves for paralarvae fed on unsupplemented *Artemia* species. Open symbols and dotted lines are for paralarvae fed on *Artemia franciscana* (*A.f.*) supplemented with an algal monoculture of *Chlorella* or *Nannochloropsis.*

Table 1. Fatty acid profiles of *Artemia* fed to octopus paralarvae and of octopus paralarvae before and after being fed different *Artemia* prey items over a period of days after hatching.

Fatty acid	*Artemia* (prey)			Paralarvae	Fed on *Ati*			Fed on NF*Afr*		
	Ati	*Afr*	NF*Afr*	0 d	25 d	34 d	43 d	10 d	20 d	40 d
14:00	2.0	1.5	0.8	1.6	1.0	0.9	0.9	1.1	1.5	0.8
16:00 (PaA)	21.2	21.1	26.0	31.6	10.3	15.3	15.8	22.0	19.4	20.0
16:01	6.5	1.9	2.9	0.4	7.7	4.6	3.7	1.2	2.8	1.2
17:0	---	---	---	2.3	---	---	---	---	---	---
18:00	8.7	8.5	13.9	12.9	7.3	12.1	14.3	15.3	10.8	14.6
18:1 (n-7)	15.3	14.8	15.4	1.3	18.8	14.3	14.3	10.0	19.6	13.0
18:1 (n-9)	6.6	5.8	7.4	3.0	10.1	7.2	6.3	4.8	6.3	3.6
18:02	4.3	4.0	4.3	---	5.0	3.4	3.3	2.4	5.5	3.3
18:03	4.8	17.4	15.3	---	6.1	3.3	2.9	6.8	10.5	4.5
20:01	0.4	2.5	1.6	4.4	1.6	2.4	2.2	1.9	1.6	2.1
20:04	1.3	0.8	1.5	2.2	2.4	3.6	3.0	2.1	1.4	2.1
20:05 (EPA)	9.6	4.6	5.7	9.4	18.0	22.2	16.5	12.0	7.6	10.0
22:05	0.8	8.0	---	0.8	---	---	---	---	---	0.5
22:06 (DHA)	4.9	6.5	1.7	20.4	2.7	5.3	2.9	12.6	6.2	8.8
Other	13.7	2.6	3.5	9.7	9.0	5.4	13.9	7.8	6.8	15.5

All values expressed as a percentage of total fatty acids present. 0d, unfed paralarvae hatchlings; *Afr*, *Artemia franciscana*; *Ati*, *A. tibetiana*; NF*Afr*, *Nannochloropsis*-fed *Afr*. Other abbreviations: DHA, Docosahexaenoic acid; EPA, Eicosapentaenoic acid; PaA, Palmitic acid; ---, not detected.

To investigate the influence of supplementation on survival, paralarval octopus predators and *Artemia* feed prey supplemented with *Nannochloropsis* were investigated further. Comparing the fatty acid profiles (Table 1), paralarvae have a very high content of the saturated fatty acids palmitic acid (16:00) and stearic acid (18:00), which are retained best when their *Artemia* prey have been feeding on *Nannochloropsis*. The paralarvae tended to retain and even gain amounts of docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) but survival declined severely beyond 25 days post-hatching. Levels of 20:01 unsaturated fatty acid were not supplemented by any of the treatments recorded in this study. Comparing amino acid content (Table 2), glutamate and aspartate were consistently the most abundant. The paralarvae hatchlings in the present study contained significant amounts of b-alanine, cysteine and carnitine but these were barely detectable after experimental feeding.

Table 2. Amino acid profiles of *Artemia* fed to octopus paralarvae and of octopus paralarvae before and after being fed different *Artemia* prey items over a period of days after hatching. (Column heading notation and abbreviations as in Table 1).

Amino acid	*Artemia* (prey)			Paralarvae	Fed on *Ati*		Fed on NF*Afr*	
	Ati	*Afr*	NF*Afr*	0 d	25 d	43 d	20 d	40 d
Ala	6.9	6.2	6.3	5.4	5.6	5.5	5.7	5.7
b-Ala	---	---	---	2.2	---	---	0.1	0.2
Arg	6.4	7.4	6.7	8.1	8.7	8.5	8.6	8.6
Asp	9.4	8.3	8.5	11.4	10.7	10.5	10.8	10.6
Cys	---	1.0	1.1	2.2	0.4	0.2	0.4	---
Glu	13.3	12.6	12.9	13.4	14.0	14.0	13.8	14.0
Gly	4.4	4.2	4.6	6.6	4.6	4.4	4.6	4.5
His	1.9	3.1	2.5	2.2	2.8	2.6	2.7	2.6
Ile	3.6	3.3	2.7	4.4	3.6	3.1	3.5	3.0
Leu	7.5	7.8	6.9	8.0	7.7	7.2	7.6	7.3
Lys	7.3	8.0	7.1	7.9	9.7	9.2	9.5	9.2
Met	4.3	2.5	2.5	4.2	3.3	3.4	3.4	3.4
Phe	9.7	13.2	10.9	4.7	10.8	10.0	10.3	9.5
Ser	5.0	3.5	4.1	5.9	---	4.0	4.1	4.8
Thr	4.1	4.0	3.7	4.9	4.4	4.3	4.5	3.1
Tyr	5.3	5.1	4.9	---	4.7	4.5	4.7	4.6
Val	8.6	6.3	5.3	4.6	7.4	6.6	3.5	6.5
Car	0.7	0.1	0.9	1.4	0.0	0.2	0.2	---
Tau	0.4	0.6	0.3	1.0	0.3	1.0	0.3	0.8

Discussion

The experiments reported here confirm that *A. tibetiana* produces better paralarval survival than *A. franciscana* (Yamazaki et al. 2015). Cephalopods are known to require high levels of lipid and protein in their diet for successful development (Vidal et al. 2014). Fatty acids have been highlighted as probable limiting factors in the nutrition of aquacultured animals, and this includes paralarval cephalopods (Navarro et al. 2014).

Paralarvae contain large amounts of palmitic acid, stearic acid, DHA and EPA (Table 1), paralleling the findings of studies on *O. vulgaris* (Navarro et al. 2014). The omega-3 fatty acids DHA and EPA have been implicated as essential for successful culture of octopus paralarvae (Navarro et al. 2014). Using a *Nannochloropsis* microalgal culture as a supplement for *A. franciscana* eliminated the difference in survival between feeding with *A. tibetiana* or *A. franciscana* (Fig. 1). However, the high levels of DHA seen in the paralarvae at hatching were not retained (Table 1), even though supplementing *A. franciscana* with *Nannochloropsis* resulted in higher levels of DHA than with *A. tibetiana*. The similar mortality pattern for *A. tibetiana* and supplemented *A. franciscana* (Fig. 1) therefore suggests that factors other than DHA deficiency are contributing to the collapse in survival between 20 and 30 days post-hatching. These findings are in agreement with those reported for *O. vulgaris* by Seixas et al. (2010). Since merely increasing the DHA content of *Artemia* feed does not improve paralarva survival, *A. franciscana* remains more attractive than *A. tibetiana* for commercial use because of more consistent quality and price, and closely synchronized hatching of cysts.

Glutamate and aspartate were consistently the most abundant non-essential amino acids (Table 2), a feature in line with the results of studies on the composition of juveniles of other cephalopod species (Navarro et al. 2014). The proportions of glutamate and aspartate did not vary much among the different preparations, however, so it seems that neither these nor any of the essential amino acids were limiting survival in the present set of experiments. Inspection of Table 2 also reveals that the hatchlings contained significant amounts of b-alanine, cysteine and carnitine but these were barely detectable after experimental feeding: in particular, b-alanine was barely detected in any of the feeds given to octopus paralarvae. The significance of these three amino acids for octopus paralarvae therefore requires further investigation.

A large protein to lipid ratio has been identified as a critical factor for octopus paralarvae (Seixas et al. 2010; Vidal et al. 2014) and the high growth rate of cephalopods is strongly associated with protein assimilation, and therefore amino acid assimilation (Navarro et al. 2014). Accordingly, trials are in progress involving various natural protein supplements for early feed formulas, in addition to investigation of other types of potential prey with larval stages present as part of the normal planktonic fauna associated with octopus paralarvae.

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References

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

Gleadall, I.G. *Octopus sinensis* d'Orbigny, 1841 (Cephalopoda: Octopodidae): valid species name for the commercially valuable East Asian common octopus. Species Diversity (in press).

Helrich, K. 1990. Official methods of analysis of the Association of Official Analytical Chemists. Vol 1, 15th ed., pp 69-88. Association of Official Analytical Chemists, Washington DC.

Iglesias, J. and L. Fuentes. 2014. *Octopus vulgaris*. Paralarval culture. In: Cephalopod culture (eds. J. Iglesias, L. Fuentes and R. Villanueva) pp 427-450. Springer, New York.

Navarro, J.C., Ó. Monroig and A.V. Sykes. 2014. Nutrition as a key factor for cephalopod aquaculture. In: Cephalopod culture (eds. J. Iglesias, L. Fuentes and R. Villanueva), pp. 77-95. Springer, New York.

Roncarati, A., A. Meluzzi, S. Acciarri, N. Tallarico and P. Meloti. 2004. Fatty acid composition of different microalgae strains (*Nannochloropsis* sp., *Nannochloropsis oculata* (Droop) Hibberd, *Nannochloris atomus* Butcher and *Isochrysis* sp.) according to the culture phase and the carbon dioxide concentration. Journal of the World Aquaculture Society 35:401-411.

Roura, Á., Á. González, K. Redd and Á. Guerra. 2012. Molecular prey identification in wild *Octopus vulgaris* paralarvae. Marine Biology 159:1335-1345.

Seixas, P., M. Rey-Méndez, L.M.P. Valente and A. Otero. 2010. High DHA content in *Artemia* is ineffective to improve *Octopus vulgaris* paralarvae rearing. Aquaculture. 300:156-162.

Vidal, E.A.G., R. Villanueva, J.P. Andrade, I.G. Gleadall, J. Iglesias, N. Koueta, C. Rosas, S. Segawa, B. Grasse, R. M. Franco-Santos, C. B. Albertin, C. Caamal-Monsreal, M.E. Chimal, E. Edsinger-Gonzales, P. Gallardo, C. Le Pabic, C. Pascual, K. Roumbedakis and J. Wood. 2014. Cephalopod culture: current status of main biological models and research priorities. Advances in Marine Biology 67:1-98.

Yamazaki, H., T. Katayama and Y. Yamamoto. 2015. Present state of the technological development for seed production of common octopus (*Octopus vulgaris*) for Japan. Aquabiology (Kaiyo to Seibutsu) 37 (216):25-30 (in Japanese with English abstract).

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