EFFECT OF DIFFERENT LIVE PREY, *Grapsus grapsus* Linnaeus 1758 AND *Plagusia depressa* Fabricius, 1775 ZOEAS, OVER HISTOLOGY AND BIOCHEMICAL COMPOSITION OF COMMON OCTOPUS, *Octopus vulgaris* Cuvier, 1797 PARALARVAE

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Abstract

Since larval rearing is still the main bottleneck for the development of octopus culture, the aim of the present study was to obtain some information on the feeding strategy and nutritional requirements during this larval stage. For that purpose just hatched out octopus paralarvae were fed with live preys in three different combinations, trying to match their natural food. Enriched *Artemia* metanauplii, *Grapsus grapsus* zoeas supplemented with enriched *Artemia*, and *Plagusia depressa* zoeas supplemented with enriched *Artemia*. Larval treatments were carried out during 28 days in triplicates; fibre glass 120 l tanks in flow through system were used. Growth, in terms of dry body weight, mantle length and width was determined each seven days. A histological study of the paralarval development was carried out. Biochemical composition of preys and paralarvae were determined. Growth was significantly better in paralarvae fed with zoeas and *Artemia* than in those fed only with *Artemia*, from day 8 after hatching. Besides a clear effect on the digestive gland histology morphology was observed.

1. Introduction

On growing of octopus at a commercial scale is a common practice in the northwest of Spain and fast growth is being also achieves in several experimental experiences are been conduced in Canary Islands. Juveniles are feed with frozen crabs and fisheries by-products, reaching growth rates close to 1kg/month (García-García 2001), and compound feeds are being developed for this species. But such production is exclusively based on the capture of young octopus from the wild, since its life cycle has not been yet completed in captivity. Thus, mass production of octopus settled juveniles is still the main bottleneck for commercial octopus culture. Despite pioneer studies showing the complete development of larval stages of *Octopus vulgaris* and obtaining settled individuals were early conducted (Itami et al., 1963), studies on the further development of the rearing techniques have only recently been addressed. Few authors have tried different culture systems, live preys and feeding regimes with various degrees of success (Villanueva, 1994, Iglesias et al., 2000, Moxica et al., 2002, Roo et al., 2003), but unable to produce a large enough amount of settled juveniles. Recently, special attention has been pointed out on octopus paralarvae quantitative requirements for essential fatty acids (EFA) and aminoacids and several trials using artificial diets and live preys have been carried out (Navarro and Villanueva, 2000, 2003). Paralarvae digestive system development and functioning is still poorly understood, several physiological and histological studies during early paralarval stages being conducted in our laboratory at present (Roo et al., 2003). As part of such research, the present study focuses on the effect of two live preys, alternative to *Artemia*, which are commonly found among paralarvae preys in the wild, in order to improve culture success in terms of growth or survival and the available knowledge on the nutritional utilization of live preys.

2. Material and methods

One thousand five hundreds just hatched Octopus paralarvae (density equivalent to 15 paralarvae per L) were stocked in each of 12 cylinder-conical fibber glass of 100 l filled with natural seawater (37‰) filtered through a 50µm mechanical filter in an open flow system.

Three feeding regimes were established for 28 days. The first one consisted in enriched *Artemia* metanauplii (2 indv/ml) (A); the second one *Grapsus grapsus* zoeas (0.05 zoea/ml/day) supplemented with enriched *Artemia* (AG); and *Plagusia depressa* zoeas (0,12 zoeas/ml/day) supplemented with enriched *Artemia* were fed in the last feeding regime (AP). Feed was supplied once a day in each experimental tank.

Paralarval growth was determined by dry weight, obtained after drying 15 individuals per tank in an oven for 48 h at 100 ºC at days 7, 16, 22 and 28. Mantle length and wide were measured in these days. Dead paralarvae were daily collected and survival was determined at day 22 and at the end of the experiment by individual counting of the living octopus. Larvae, were stained with Hematoxiline eosine (H&E), and Peryodic Acid- Reactive schiffs - hematoxiline (PAS-H).

At the beginning of the experiment and at days 22 and 28, samples of paralarvae, as well as samples of preys, were collected for analysis of biochemical composition. Total lipid extraction was carried out using a chloroform/methanol (2:1, v/v) mixture as described by Folch et al. (1956). Fatty acid methyl esters (Fame's) were prepared by transesterification an analysed in a Shimadzu GC-14A gas Chromatograph. Crude protein was determined by Kjedahl method.

3. Results

3.1. Growth and survival

Treatment	Day	ML (mm)	s.d.	n	Day	ML (mm)	s.d.	n
A	22	2.00 [a]	0.14	29	---	---	---	---
AG	22	2.44 [b]	0.18	45	28	2.32 [d]	0.04	28
AP	22	2.21 [c]	0.18	45	28	2.32 [d]	0.09	30

Table I. Statistically significant differences at the 95% confidence level. ML: mantle length.

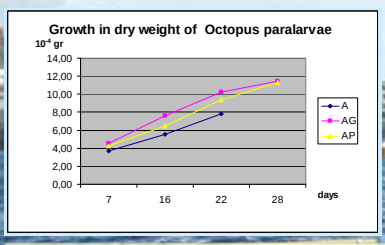


Figure I Growth in dry weight of octopus paralarvae (p<0.05).

Treatment	Equation	r²	SE	n
A	DW= 0.2587 exp (0.0488*d)	0.99	0.04	5
AG	DW= 0.3456 exp (0.0466*d)	0.95	0.09	11
AP	DW= 0.3042 exp (0.0485*d)	0.88	0.15	11

Table II. Dry weight growth equations for *O. vulgaris* paralarvae from different treatments. DW: total dry weight (mg); d: age in days; SE: standard error; n: groups of paralarvae.

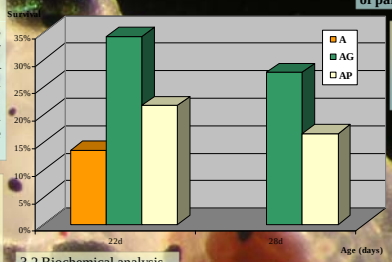


Figure II. Survival of octopus paralarvae at day 22 and at the end of the experimental period. 28d (P<0.05)

3.2 Biochemical analysis

Preys	Water content (%)	Lipids (%)
Artemia nauplii	89.48	17.97
Enriched *Artemia* nauplii	94.84	29.46
Grapsus zoeas	85.19	10.19
Plagusia zoeas	93.01	12.17
Treatment		
Initial Paralarvae	88.07	14.20
A 22d	95.34	55.03
AG 22d	88.04	21.14
AG 28d	92.03	19.95
AP 22d	94.39	37.43
AP 28d	89.67	19.73

Table III. Proximate analysis (% dry basis) of diets and paralarvae.

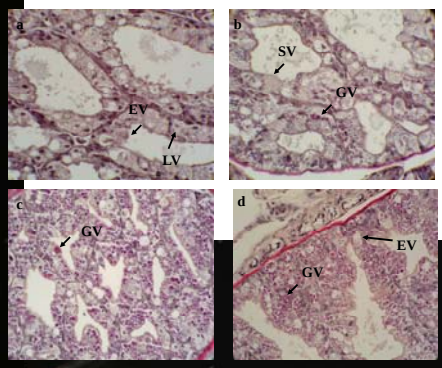
Fatty acids	*Artemia* nauplii	Enriched *Artemia* metanauplii	*Grapsus* zoea	*Plagusia* zoea
16:0	13,952	12,377	15,499	21,972
16:1 n-7	5,315	6,564	2,196	3,634
16:4n1	0,000	0,141	2,853	2,091
18:0	4,053	4,544	8,363	10,944
18:1 n-9	31,492	24,243	16,703	13,986
18:1 n-7	7,001	5,641	4,821	4,338
18:2n-6	4,895	6,693	5,405	3,194
18:3 n-3	18,299	8,060	1,704	1,467
20:4 n-6	0,843	1,269	12,037	9,351
20:5 n-3	2,323	12,068	10,931	10,183
22:6 n-3	0,155	5,390	4,794	4,270
Saturated	20,566	20,917	28,290	36,960
Monounsaturated	45,876	38,720	26,088	24,237
n-3	24,457	28,834	20,192	18,150
n-6	6,959	9,431	20,559	15,967
n-9	32,602	25,896	18,216	15,414
n-3HUFA	2,853	18,865	17,953	15,919
AA/EPA	0,363	0,105	1,101	0,918
EPA/DHA	15,017	2,239	2,280	2,385
Oleic acid/DHA	203,622	4,498	3,484	3,276
Oleic acid/n-3 HUFA	11,039	1,285	0,930	0,879
n-3/n-6	3,514	3,057	0,982	1,137

Table IV. Total lipids fatty acid composition (% of total fatty acids) of the different preys.

Fatty acids	Initial Paralarvae	A 22d	AG 22d	AP 22d	AG 28d	AP 28d
16:0	20,1607	11,6508	12,3363	14,3680	15,7679	16,5663
16:1 n-7	0,4189	4,3764	4,0506	3,7735	3,9791	3,1733
18:0	12,9583	6,6806	7,0932	7,6124	9,8990	9,6457
18:1 n-9	1,8635	20,0805	18,6370	16,8218	20,3401	15,5301
18:1 n-7	2,9867	5,0023	5,0146	4,7948	5,4692	4,8176
18:2 n-6	0,9281	4,6558	4,2335	3,8043	3,9995	3,2238
18:3 n-3	0,2172	7,6172	6,3645	5,3758	5,0052	3,7522
20:4 n-6	6,2845	2,9915	4,4411	4,1354	4,0617	5,2179
20:5 n-3	11,2605	15,3256	14,8504	15,1835	10,7330	12,0126
22:6 n-3	17,2555	9,4000	10,4787	11,1611	7,8655	11,0319
Saturated	35,8348	21,0233	21,7862	24,8218	28,3388	29,2583
Monounsaturated	13,6417	32,0688	30,2766	27,8645	32,9095	26,4738
n-3	32,7999	35,2581	34,5444	34,4123	25,8960	29,3882
n-6	9,7292	9,0845	10,1121	9,3824	9,3843	10,1023
n-9	7,4081	21,9398	20,7362	18,6470	22,3682	17,8981
n-3HUFA	31,6813	26,7015	27,4267	28,3453	20,3318	25,0065
AA/EPA	0,5581	0,1952	0,2991	0,2724	0,3784	0,4344
EPA/DHA	0,6526	1,6304	1,4172	1,3604	1,3645	1,0889
oleic/DHA	0,1080	2,1362	1,7785	1,5072	2,5859	1,4077
oleic/n-3HUFA	0,0588	0,7520	0,6795	0,5935	1,0004	0,6210
n-3/n-6	3,3713	3,8811	3,4161	3,6677	2,7594	2,9091

Table V. Total lipids fatty acid composition (% of Total fatty acids) of the reared paralarvae.

3.3 Histological study



PICTURE 1. a) 22d A b) 22d AG c) 22d AP, d) 28d AP digestive gland morphology in reared paralarvae (SV) Secretion vacuoles, (EV) Excretion vacuoles, (LV) lipids vacuoles and (GV) Glicoproteic vacuoles.

4. Discussion

Paralarvae fed *Artemia* alone showed a progressive mortality along the third week of feeding, leading to the end of the experiment for this feeding treatment on day 22. Best survival rate was obtained when *Artemia* was supplemented with *Grapsus grapsus* zoea, improving that obtained by Itami et al. (1963) and being closed to those reported by Villanueva (1994). Growth was also highest with this feeding treatment and similar to the Iglesias (2000) growth results, probably associated to prey type and feeding regimes reared but lower than the ones obtained by Villanueva et al. (2002), which could be related with the higher prey density in the experiment of the later author, or a difference in the nutritional value of live preys used as discussed bellow.

Better growth and survival obtained with the supplementation with zoeas seem to be related with the marked differences in their biochemical composition in comparison with *Artemia*. In one hand, *Artemia* metanauplii lipid contents were very high in comparison with the newly hatched octopus paralarvae, whereas contents for both zoeas were closer to the paralarvae and adult octopus (lower than 10%). Besides clear differences were found in the fatty acid profiles of the preys, enriched Artemia metanauplii being particularly lower in AA, whereas levels of this fatty acid in both zoea types were slightly higher than in newly hatched paralarvae. AA content of paralarvae fed *Artemia* alone were markedly reduced and in paralarvae fed supplemented zoea were kept similar to those of the newly hatched. Regarding fatty acid composition there is a general lack of information for this species. Newly hatched octopus presented high levels of n-3 HUFA (EPA and DHA) and AA, the essentiality of these fatty acids being clearly demonstrated in fish larvae (Watanabe and Kiron 1994, Izquierdo,1996, Sargent 1999, Izquierdo et al., 2000). Thus, differences in growth and survival in *Artemia* treatment could be related with the level of other essential fatty acid as AA which in this case was ten times lower for *Artemia* diet than in crab zoeas. This fatty acid is specifically involve in stress response which could be directly related with obtained survival (Izquierdo et al., 2000).

The *Artemia* and crab zoeas used in this trial show similar levels of EPA and DHA, with a EPA/DHA relation of 2.2 for all of them, in contrast with the results find for newly individuals reflecting a relation of 0.6. Navarro and Villanueva 2000, found higher levels of n-3 HUFA especially EPA and DHA with EPA/DHA relation of 1:1 in *A. longicornis* and *P. prideaux* zoeas, closer to that of newly hatched octopus. These difference in the fatty acid profiles of preys from both studies may be related with the higher survival found by Navarro and Villanueva (2000), and suggests that prey combination used in this experiment do not support the high n-3 HUFA demand suggested for this species at least in terms of EPA and DHA content.

Big brown vacuoles identified in the digestive gland cells were considered as excretions vacuoles (EV) as it was reported by Boucher-Rodoni, (1976) and Budermann et al. (1997) in adult individuals, who described them as brown or yellow vacuoles that seems to content metabolic residues regularly released into the lumen of the tubules. The second type of vacuoles were conformed for many small eosinophilic granules, this type identified as enzymatic secretion vacuoles (SV). There were not histological evidential differences in form or number of these vacuoles among treatments. The third and fourth type are absorption vacuoles of lipidic (LV) or glicoproteic (GV) nature, respectively. In the present trial paralarvae, glicopreoteic absorption vacuoles increased in paralarvae co-feed with crab zoea, in comparison with those fed *Artemia* alone. This suggest the presence in zoea of a nutritional stimuli improving absorption mechanism and feed utilisation. According with Nixon (1996) young paralarvae are planktonic feeders before settling on the bottom, during this first stage are active predators, consuming a lot of different preys as larval stages of different crustaceans, mollusc and fish, that provide the adequate amount of each essential nutrients. Feeding habits and, particularly feed manipulation and sucking previously to ingestion in this species, difficult to develop an appropriate find a prey to substitute the natural ones. So although *Artemia* does not seem to be the right prey to feed early life stages of octopus, still constitutes one of the life preys which can support a constant demand for octopus industrial rearing, while artificial diets or specific *Artemia* enrichment procedures are being developed for this specie.