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Julio López-Alvarado
Prometeo Researcher, Instituto Nacional de Pesca, Letamendi 102 y La ría, Guayaquil, Ecuador.

Correspondence
Julio López-Alvarado
Prometeo Researcher, Instituto Nacional de Pesca, Letamendi 102 y La ría, Guayaquil, Ecuador.

Effect of broodstock diets on biochemical composition and larval growth of Manila clam, *Ruditapes philippinarum* (Adams and Reeve, 1850)

Julio López-Alvarado

Abstract
The effect of broodstock diets on the subsequent larval growth of Manila clam, *Ruditapes philippinarum* (Adams and Reeve), was assessed. For the broodstock, two dried diets (a diet of dried *Tetraselmis suecica* (Kylin) Butch, and a mixed diet of 70 % dried *T. suecica* and 30 % dried *Cyclotella cryptica* Reimann *et al*.), and three live diets (*Skeletonema costatum* (Grev.) Cleve, *Dunaliella tertiolecta* (Butcher) and *T. suecica*) were tested. An unfed group was used as a control. The larvae produced were reared for 7 days on the same diet (*Chaetoceros calcitrans*). All broodstock diets produced eggs with a high growth potential. Larval growth was worse for the starved broodstock group. The fatty acid composition of the eggs was affected by the broodstock diet. Fatty acids in the larvae after 7 days of culture was very similar for all groups, indicating that the early effect of broodstock diets was later replaced by the effect of larval feed.

Keywords: Manila clan, fatty acids, dried algae, broodstock, larvae

1. Introduction
Although many algal diets have proved to be ideal for clam larvae [1, 2], the culture and maintenance of these algae gives many problems in a commercial hatchery [3]. The space needed for algal culture and the time and expertise necessary to maintain the cultures and produce the appropriate volumes at the time required increases the production cost of the final product. To try and overcome these problems, many investigations have been carried out to develop artificial diets. Yeast diets were used without great success [4]. Later, microencapsulated diets were developed, but showed bad results for bivalve larvae [5], but were used successfully with fish larvae [6, 7].
Dried unicellular algae were first used as food for clam larvae [8]. Spay-dried algae produced by the Micro Algae Research Institute of Japan, was used to feed larvae of the hard shell clam, *Mercenaria mercenaria*. In these first studies clam larvae were reared successfully to metamorphosis, although the growth rate was lower for animals fed dried algae than for the animals fed live algae.
Live algae have shown to play an important role in the gametogenesis and larval vigor of bivalves. European flat oyster, *Ostrea edulis*, receiving supplementary food of *Tetraselmis suecica*, produced more larvae than oyster receiving only natural phytoplankton in the water supply [9]. The performance of the larvae is related to the reserves accumulated during gametogenesis, especially long chain polyunsaturated fatty acids (PUFAs). The gametogenic cycle in bivalves is closely linked to cycles of glycogen storage and subsequent de novo synthesis of lipids during vitellogenesis [10].
The aim of this research was to study the effect of broodstock diets on the performance and body composition of Manila clam larvae.

2. Materials and Methods
2.1 Broodstock conditioning
Manila clam broodstock were reared under controlled conditions as described previously [11]. Six groups were established, with the following diets:
1) Dried *Tetraselmis suecica*;
2) Dried *T. suecica* (70 %) + dried *Cyclotella cryptica* (30 %);
3) live *T. suecica*;

4) live *Dunaliella tertiolecta*;
5) live *Skeletonema costatum*;
6) Starved control.
Spawning was induced after 7 weeks of conditioning by heat shock at alternate baths of 30 ºC and 20 ºC [12, 13].

2.2 Larval culture
The larval culture technique is similar to that described for other bivalve species [14]. D-larvae from each treatment were grown up to pediveliger stage. A total of 95.000 larvae per replicate, and 3 replicates per treatment were placed in 40-l bins filled with 30 l of filtered sea water to give a concentration of approximately 3 larvae / ml. Water was exchanged every third day collecting all the larvae with a 35 μm mesh. A sample was taken at this time to estimate larval numbers and growth. Feeding rate was aimed at keeping algal concentrations of 200 cells of *Chaetoceros calcitrans* per μl of culture. If grazing was bigger than 50 %, the ration was increased by 50 %, to 300 cells per μl. The algal clearance rate was measured every day by counting the algal numbers with a Coulter Counter. Growth was estimated as increase in larval shell length. Larval shell lengths were estimated three times per week by measuring with a Video Measuring Gauge FOR IV-560 coupled with a Hitachi video camera and a screen to a Nikon microscope. For each sample, around 60 larvae were measured for each treatment.

2.3 Analytical methods
Dry weight and ash were calculated by gravimetric method. Carbohydrates were analyzed following the anthrone method [15]. Lipids were estimated by gravimetric method after extraction on chloroform/methanol. Fatty acids were analyzed by gas chromatography, after extraction of lipids and esterification of the constituent fatty acids using the method of Lepage and Roy [16].

2.4 Statistical analysis
The statistical analysis was carried out using a SAS statistical package (SAS Institute). The Analysis of Variance (ANOVA) was calculated for all parameters measured. For the parameters in which the results of ANOVA were significant, T-tests were done for comparison between all pairs of groups.

3. Results
3.1 Algae used for the broodstock
Gross biochemical composition of the algae used is shown on Table 1. Live algae had higher protein content and lower carbohydrate content than dry algae.

Table 1: Gross biochemical composition of the algae used as broodstock food.

Species	Lipid as % AFDW	Carbohydrate as % AFDW	Protein as % AFDW
T. suecica (live)	4.7	20.0	75.3
S. costatum (live)	9.0	2.7	88.3
D. tertiolecta (live)	19.0	10.9	70.1
T. suecica (dry)	4.7	67.6	27.7
C. cryptica (dry)	11.4	40.2	48.4

AFDW: ash-free dry weight

The qualitative fatty acid composition of the algae used is shown on Table 2. Among the live algae, *S. costatum* had a high percentage of long chain HUFAs, *T. suecica* had low amounts of EPA and no DHA, while *D. tertiolecta* had no HUFAs in its content. Among the dried algae, *C. cryptica* had high percentages of HUFAs, and *T. suecica* had low amounts of HUFAs including DHA, but higher than its live counterpart.

Table 2: Fatty acid composition of the algae used as diets for Manila clam broodstock (% of total fatty acids).

Fatty acid	S. costatum	T. suecica	D. tertiolecta	C. cryptica (dried)	T. suecica (dried)
14:0	22.52	29.43	23.00	7.04	19.06
16:0	17.13	27.48	22.50	20.96	32.28
18:0	2.74	2.93	4.10	0.85	4.10
16:1 n-9	-	2.42	0.60	-	-
16:1 n-7	24.25	0.99	1.25	22.16	2.81
18:1 n-7	-	1.25	2.20	3.44	32.09
18:1 n-9	3.41	11.94	11.42	0.31	-
18:2 n-6	1.71	8.10	9.90	1.07	3.20
18:3 n-3	-	6.97	24.60	0.19	1.95
18:4 n-4	2.17	6.68	0.40	10.03	0.62
18:4 n-6	-	-	-	-	-
20:5 n-3	24.67	1.85	-	30.78	3.26
22:5 n-3	-	-	-	-	-
22:6 n-3	1.94	-	-	3.18	0.65
Total	100.00	100.00	100.00	100.00	100.00

3.2 Fecundity
The fecundity of Manila clam broodstock fed the different algae has been reported before [11]. Fecundity in millions of eggs per female were high for the broodstock fed live algae (1.969 million for *T. suecica*, 2.265 million for *S. costatum*, and 3.261 million for *D. tertiolecta*), and lower for the broodstock fed dry algae (0.726 million for T. suecica and 1.004 million for T. suecica + C. cryptica). For the starved broodstock, fecundity was much lower (0.421 million eggs per female).

3.3 Size and biochemical composition of the eggs.
Dry weights and lipid content of the eggs used in our larval growth trial are shown on Table 3. The eggs from the

broodstock fed dried algae, live *T. suecica*, and the starved group were bigger than the eggs from the broodstock fed *D. tertiolecta* and *S. costatum*.

Table 3: Size and biochemical composition of the eggs.

Broodstock food	Dry weight ng/egg	AFDW ng/egg	Lipid ng/egg
S. costatum	27.0	25.0	8.1
D. tertiolecta	21.5	18.1	7.8
T. suecica (live)	60.1	37.9	11.3
T. suecica (dry)	59.7	47.4	13.5
T. suecica (dry) + *C. cryptica* (dry)	46.1	26.5	5.9
Starved broodstock	43.3	38.7	10.7

3.4 Recovery of D-larvae

Table 4 shows the recovery of D-larvae 24 hours after hatching. The high percentage of recovery registered for the starved broodstock group is remarkable.

Table 4: Recovery of D-larvae 24 hours after hatching.

Broodstock food	Embryos cultured	D-larvae recovered	%
S. costatum	95,000	19,950	21
D. tertiolecta	95,000	38,950	41
T. suecica (live)	95,000	42,750	45
T. suecica (dry)	95,000	57,000	60
T. suecica (dry) + *C. cryptica* (dry)	95,000	43,000	45
Starved broodstock	95,000	62,100	65

3.5 Growth of larvae

Growth of the larvae from the six broodstock groups is shown in Fig. 1. Larvae from broodstock conditioned with dried diets and live *Tetraselmis suecica* grew to significantly greater final length than the larvae from broodstock conditioned with *D. tertiolecta* and S. *costatum*. Larvae from the starved broodstock had final lengths intermediate between larvae from broodstock conditioned with dried diets and live *Tetraselmis suecica* and larvae from broodstock conditioned with *D. tertiolecta* and S. *costatum*.

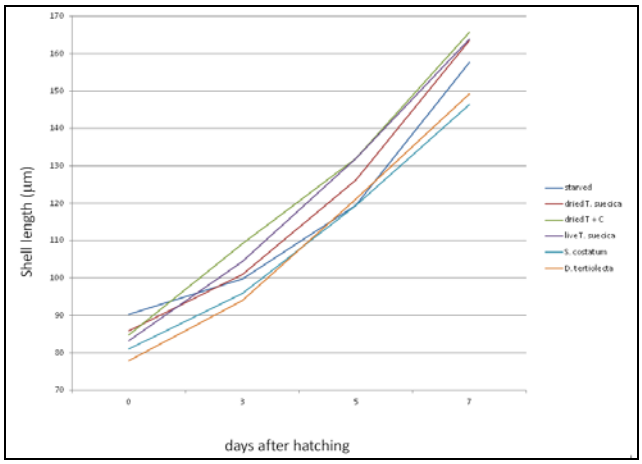

Fig 1: growth of Manila clam larvae produced from broodstock fed different feeds.

The specific growth rate of the larvae are shown on Table 5. It is clear from SGR data that the larvae from the starved broodstock grew at a slower rate than the larvae from any of the fed broodstock groups. The bigger final length (compared to larvae from broodstock conditioned with *D. tertiolecta* and *S. costatum* is due to the bigger size of the eggs, although the SGR was lower.

Table 5: Specific growth rate of manila clam larvae from broodstock conditioned with different diets.

Broodstock diet	Live *Tetraselmis suecica*	Dried *T. suecica* + *C. cryptica*	Live *Dunaliella tertiolecta*	Dried *Tetraselmis suecica*	Live *Skeletonema costatum*	Starved
SGR of the larvae	9.67^{a}	9.57^{a}	9.28^{b}	9.19^{b}	8.45^{bc}	7.96^{c}

Values with the same index are not significantly different

3.6 Fatty acids in eggs and larvae

The fatty acid composition of eggs and larvae are presented on Tables 6 and 7. All groups had a significant amount of HUFAs, compared to the composition of the diets fed to the broodstock.

Table 6: Fatty acid composition of eggs from broodstock fed different algae.

Fatty acid	S. costatum	T. suecica	D. tertiolecta	C. cryptica (dried)	T. suecica (dried)	Starved broodstock
14:0	26.26	22.05	18.82	17.67	28.28	23.08
16:0	26.95	28.03	26.14	26.34	28.08	25.92
18:0	5.04	4.92	5.44	4.36	5.55	5.07
16:1 n-9	-	1.99	-	-	2.26	2.54
16:1 n-7	8.68	2.70	5.73	8.89	3.28	3.28
18:1 n-7	9.88	13.58	12.21	12.34	14.69	11.03
18:1 n-9	4.43	3.37	3.06	2.78	3.61	3.47
18:2 n-6	1.44	3.33	2.66	1.65	1.78	1.54
18:3 n-3	-	3.27	8.91	0.65	0.64	0.69
18:4 n-4	0.78	2.39	1.76	3.78	1.40	1.89
18:4 n-6	-	1.27	4.21	1.36	-	-
20:5 n-3	10.46	7.58	4.36	10.67	3.64	6.85
22:5 n-3	1.06	0.79	1.12	1.87	1.04	1.28
22:6 n-3	5.02	4.72	5.58	7.64	5.77	13.38
Total	100.00	100.00	100.00	100.00	100.00	100.00

Table 7: Fatty acid composition of larvae from broodstock fed different algae.

Fatty acid	S. costatum	T. suecica	D. tertiolecta	C. cryptica (dried)	T. suecica (dried)	Starved broodstock
14:0	28.50	30.66	24.32	23.95	24.37	29.93
16:0	28.38	27.53	27.03	26.41	28.69	27.31
18:0	5.40	5.31	5.68	5.34	5.68	5.81
16:1 n-9	-	-	-	-	1.68	1.98
16:1 n-7	6.97	3.35	4.68	6.95	5.18	2.04
18:1 n-7	11.67	15.58	13.50	13.43	14.46	14.96
18:1 n-9	4.19	2.27	3.06	3.36	3.43	2.52
18:2 n-6	1.66	2.81	2.66	1.88	1.74	1.80
18:3 n-3	-	1.55	6.10	1.06	0.99	-
18:4 n-4	-	1.27	0.87	2.42	2.46	0.99
18:4 n-6	-	0.68	3.05	1.53	-	-
20:5 n-3	8.25	4.03	3.48	7.37	5.21	3.69
22:5 n-3	-	0.84	-	0.85	0.95	0.77
22:6 n-3	4.98	4.12	5.59	5.45	5.16	8.21
Total	100.00	100.00	100.00	100.00	100.00	100.00

4. Discussion

Growth of larvae from broodstock conditioned with a mixture of dried algae was similar to the growth of larvae from broodstock conditioned with live *T. suecica*, considered to be a good food for Manila clam, and it was better than for larvae from broodstock conditioned with *D. tertiolecta* and *S. costatum*. The larvae from broodstock conditioned with only one dried algae (*T. suecica*) had a similar growth as larvae from broodstock conditioned with *D. tertiolecta* and *S. costatum*. The larvae from the starved broodstock group had final lengths greater than the larvae from broodstock conditioned with *D. tertiolecta* and *S. costatum*, but the specific growth rate is in fact much lower than for the rest of the groups. Larvae from starved broodstock had final length larger than larvae from broodstock conditioned with *D. tertiolecta* and *S. costatum* only because the initial egg size was bigger for the broodstock-starved group. If the fecundity and egg weight are compared, it can be noticed that the starved group produced a small amount of eggs, but bigger in size than the eggs from the animals fed *D. tertiolecta* and *S. costatum.* This bigger size results in high recovery rates of D-larvae, and high final length of the larvae, even though the relative growth rate of these larvae was lowest among all groups. In Fig. 1 it can be appreciated that growth of larvae from the starved broodstock was quite slow for the first five days of culture. These larvae, that had the highest initial size, were overtaken by all other groups during these five initial days of culture. However, after day five the growth of these larvae improved, reaching a final length that was intermediate between the best and worst groups. This seems to indicate that there is a very strong effect of broodstock diet on the egg and larval quality, and this effect lasts for a few days, but after a few days receiving a good larval feed, the effect of the larval feed is stronger than the effect of the broodstock diet. The larvae can recover and continue to grow at rates similar to larvae from fed broodstock.

The fatty acid composition of the eggs do not exactly reflect the fatty acid composition of the algae fed to the broodstock. All the groups had a significant quantity of EPA and DHA, essential fatty acids in most marine organisms. These fatty acids are not present in *D. tertiolecta*, and are only present in very small quantities in *T. suecica*. The eggs from the broodstock fed *S. costatum* had high quantities of HUFAs, reflecting the high proportion of these fatty acids en the algae. But a significant amount of HUFAs must have been synthesized *de novo* by the broodstock, elongating short chain fatty acids. Otherwise, the presence of HUFAs in eggs from broodstock fed algae without these fatty acids could not be explained. The high quantity of HUFAs in the eggs of the starved group seen to suggest that the clam is synthesizing these HUFAs from body reserves. The fact that a starved bivalve transfers more materials to the eggs than a well fed one has been demonstrated by other researchers [17].

From the results of the fatty acid analysis in the larvae, it can be noticed that HUFAs (EPA+DHA) are being consumed (its levels in the larvae are lower than in the eggs), and larvae from eggs with higher quality of HUFAs consume them faster. The reduction in HUFAs ranged from 41.16 % for the group coming from starved broodstock, to only 8.8 % for the group coming from broodstock fed *D. tertiolecta*. With this trend, after some days of larval culture, the HUFA composition of all larvae should be very similar, provided that all the larvae are given the same diet, as it was the case in this trial.

Bivalves seem to have a limited ability to convert 18:3 n-3 to HUFAs [18]. The fatty acid composition in a bivalve usually reflects the fatty acid composition in the algae fed. D*unaliella tertiolecta* is supposed to be a poor diet for bivalves [19]. In fact, the fecundity and growth of the larvae from the broodstock fed *D. tertiolecta* were similar to the larvae from the broodstock fed *S. costatum*. Clams (*Mercenaria mercenaria*) were able to utilize mixed algae + yeast diets, as opposed to oysters (*Crassostrea virginica*) [20]. From the results of the present study, it seems that adult Manila clam has the ability to use short chain fatty acids, elongating then to form long chain HUFAs. The larvae produced from eggs in the different treatments had a very similar amount of DHA, probably reflecting the composition of the larval feed used. The effect of the broodstock diet is very strong on the eggs and early larvae, but as the larval culture progresses, the effect of the larval feed takes more importance. This observation is also in agreement with the effect of the broodstock diet on the larval growth, having a strong effect on the early days of larval rearing, and less effect as the culture progressed.

In a previous report, the effect of broodstock diets on fecundity and size of the eggs was reported. This larval growth trial indicates that broodstock diets have an impact on the egg quality and on the early larval growth of Manila clam. This early influence loses importance as the larval culture progresses, and the influence of the larval feed takes a more important role.

5. Conclusion

In this research, the effect of broodstock diets on the larval growth of Manila clam was studied. All diets tested produced

viable eggs, and larvae had good growth. Broodstock diets had an influence in the size and quality of eggs and early larvae of Manila clam. This early influence was later lost to the influence of the larval feed used. The eggs produced by the starved broodstock were fewer in quantity, but bigger. This resulted in a high recovery of D-larvae and good growth of the larvae when given a good larval diet. The use of dried algal diets shows promising results in bivalve culture. Further research is needed to assess the effect of mixed live-dried algal diets, and the level at which dried algae can replace live algae in broostock diets without a significant decrease in fecundity.

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