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Growth of *Lyropecten* (*Nodipecten) subnodosus* (Sowerby, 1835) Spat Fed with Three Microalgae Mixtures Diets

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Abstract: The culture of the scallop *Lyropecten* (*Nodipecten*) *subnodosus* could be a profitable economic activity, but the knowledges about its nutritional requirements are not enough. This study evaluated the effect of the nutritional quality of three microalgae mixtures diets on the growth of *L.* (*N.*) *subnodosus* during seven weeks (M1: *Isochrysis* sp.-*Pavlova lutheri*; M2: *Pavlova lutheri-Chaetoceros calcitrans* and M3: *Chaetoceros calcitrans* - *Isochrysis* sp.). The spat were fed with three microalgae mixtures diets and they were kept at 21±1°C. The best filtration rates was obtained with an initial algae concentration of 200,000 cell mL^{-1}, at which near total food utilization was achieved; at higher concentrations the spat produced pseudofeces and unconsumed food remained in the vessels after 24 hrs. The major increases in growth (73.4% in length and 57.83 mg of wet weight) were obtained in the spat fed with the diet M2 (*P. lutheri* and *C. calcitrans*), in this diet the high protein content (52.17%) and quantity of the essential fatty acid 20:4n6, 20:5n3 and 22:6n3 were in an appropriate proportion.

Key words: Scallops, growth, microalgal mixtures diets, biochemical composition, filtration rate

INTRODUCTION

Microalgae production is one of most important factors in marine bivalves culture, providing their principal food during several development stages. Microalgae provides the optimum nutritional quality for the organisms, depending on its stage of development[1]. The nutritional quality of the microalgae has been studied by several authors to obtain the best quality nutrition, thereby increasing the growth rate of some organisms[2-4].

Bivalve molluscs have been over-exploited in their natural habitat because some of them, such as the scallops, have high commercial value[5]. Therefore, several laboratories have been trying to raise the growth rates of the organisms by increasing the nutritional quality of food, using diets with two or more microalgae[6]. The conjunction of biochemical components such as proteins, carbohydrates, lipids and fatty acids has improved the nutritional quality of the diets[7-9]. The polyunsaturated fatty acids (PUFA'S) principally the 20:4n6, 20:5n3 and 22:6n3 are components that raise the nutritional quality of the diets. Higher growth rates have been achieved with organisms fed with microalgae rich in these fatty acids compared with others fed diets poor in them[9,10-12].

However, few research has been done on the growth and the nutritional requirement of bivalve such as the scallop *Lyropecten* (*Nodipecten) subnodosus*. In Baja California Mexico this species is considerate of high potential in the aquaculture, because it has a high growth rate (from 9 to 10 cm in shell height per year) and its maximum adductor muscle weight is approximately 43 g [13].

The bigger problem in the culture of this species is in the first development stages, because in these stages the mortality increase and the quantity of spat for culture are little. In the present study we have evaluated the effect of three different diets: M1, *Isochrysis* sp. - *Pavlova lutheri; M2*, *Pavlova lutheri-Chaetoceros calcitrans* and M3, *Chaetoceros calcitrans-Isochrysis* sp. on the growth of *Lyropecten* (*Nodipecten) subnodosus* spat.

MATERIALS AND METHODS

Culture of microalgae: The microalgae *Isochrysis* sp. (IS-XI), *Pavlova lutheri* (PA-LI) and *Chaetoceros calcitrans* (CH-CL) were obtained from the microalgae collection of the Departamento de Acuicultura of the Centro de Investigación Científica y de Educación Superior de Ensenada (CICESE). The microalgae were

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cultured in 15 L containers and were kept in a semi-continuous system and maintained in the late exponential growth phase with a daily dilution rate of 20%. The microalgae were cultured in controlled conditions of temperature (22±1°C); pH between 7.5 and 8 (controled by CO_2 injections); dark/light 12:12 hrs, salinity of 35 ppt and the culture medium "f"[14].

The microalgae were harvested in the late exponential growth phase and were used in three microalgae mixtures diets: M1: *Isochrysis* sp. - *P. lutheri*; M2: *P. lutheri*-*C. calcitrans* and M3: *C. calcitrans* - *Isochrysis* sp. In this study the proportion of each microalgae in the diet was based on cells concentration of each species of microalgae. The microalgae *C. calcitrans* contains up to 50% of ash, therefore in the diets that had this microalgae its proportion was 1: 2 (2 proportion of *C. calcitrans*). In the diet with *P. lutheri* and *Isochrysis* sp. the proportion of the microalgae was of 1:1.

Each third day, triplicate 20 mL of the three microalgae mixtures diets (above mentioned) were concentrated on glass microfibre filters Whatman previously washed with distilled water, ashed and weighed. After filtration, the filters were rinsed with a 3% ammonium formate solution and placed in a convection oven at 60°C for 48 h and weighed to obtain the Total Dry Weight (TDW). The filters were then ashed at 490°C in a muffle furnace to obtain the ash content and calculate by difference the Organic Content (OC).

The biochemical composition of the microalgae mixture diets also was determined each third day using similar triplicate samples. The method used were Lowry *et al.*,[15] for protein; carbohydrates according to Dubois *et al.*,[16], after the treatment and lipids with the colorimetric method of Pande *et al.*,[17] after extraction following Bligh and Dyer[18].

The samples for fatty acids analyses were centrifuged, froze-dried at -70°C, freeze dried and weighed The fatty acids were analysed following Sato and Murata[19], after total lipid extraction[18], using a Hewlett Packard GC/MS gas chromatograph equipped with an Omegawax TM 250 fused silica capillary column (30 m x 0.25 mm i.d., Supelco). Helium was used as carrier gas and the fatty acids were identified by comparison of retention times to those of known commercial standards (SIGMA) and with the NIST library of the GC/MS.

Culture of *Lyropecten* (*Nodipecten) subnodosus*: The scallop spat used in this experiment were obtained from the mollusks laboratory at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR), in La Paz, Baja California Sur, México. These were acclimated at 21°C and were fed with *Isochrysis* sp. during the condition.

The organisms acclimated were distributed at random in nine plastic chambers of 3.4 L (each chamber contained 25 spat). The chambers were made with plastic containers in which the bottom was a mesh of 50 μm. To minimize the sedimentation rate of the microalgae in the vessels a closed system air lift was used into the chambers. Each chamber was put inside vessels containing 20 L of seawater filtered at 1 μm and passed through UV, the water temperature in the vessels was of 21±1°C. The vessels were cleaned daily to minimize the contamination. To avoid the damage in the shell of the spat and a possible stress of them during the change of water in the vessel, the plastic chamber with the spat was change immediately in other vessel with clean water at the same temperature condition.

The cell concentration and filtration rate: Previously at the feeding experiment, we evaluated each microalgae mixture diet in three concentrations corresponding at 200,000; 300,000 and 450,000 cell mL^{-1} to obtain the initial optimum concentration of each diet for *L. (N) subnodosus*. Each concentration of the diet was supplied and evaluated during two days. The spat were starved for 24 hrs between each concentration to clean the gut content and avoid errors in the following test. The filtration rates of spat were calculated according to the method of [37]: $R.F = [(\log_e N_1 - \log_e N_2)V]/TN$, where N1 and N2 are the initial and final concentration of microalgae, V is the water volume of culture, T is the time in hrs or minutes and N is the number of scallops in the vessel (Fig. 1).

The light of the lamps used in previous feedings experiments produced the reproduction of the microalgae in the containers, therefore, the new feeding experiments were realized in semidarkness to avoid their reproduction.

Experimental desing: When the initial optimum concentration of the diets was obtained, spat of similar size (4 mm) were distributed at random in the chambers as described previously. Each microalgae mixture diet (M1, M2 and M3) was tested by triplicate with a total spat number of 75 spat by treatment. The length and the width of the shell of each organism was measured weekly. A number of live spat was obtained each week to know the percentage of survival. Fifty spat were used to obtain the initial Wet Weight (WW), Total Dry Weight (TDW) and Organic Content (OC). Also, 30 spat by diet (10 for each replicate) were measured weekly to obtain their growth increment. At the end of the experiment all organismss of each treatment were measured to obtain the final WW, TDW and final OC. The TDW was determinated in a convectional oven at 60°C for 24 hrs, after which they

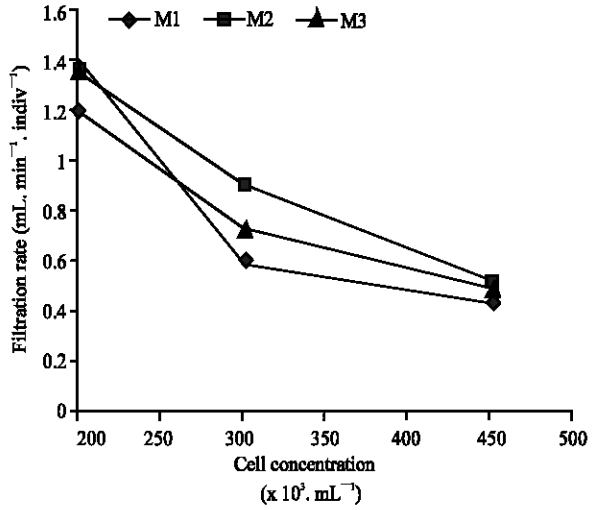

Fig. 1: Mean filtration rate (mL min-1ind-1) to the fourth hour with different concentration of cells (A: 200,000; B: 300,000; C: 450,000 cell mL^{-1}) by *Lyropecten (Nodipecten) subnodosus* fed with three microalgae mixtures diets. M1: *Isochrysis* sp.-*Pavlova lutheri*; M2: *Pavlova lutheri*-*Chaetoceros calcitrans* y M3: *Chaetoceros calcitrans*-*Isochrysis* sp. n = 75 in each diet, ntotal = 225 for concentration

were burned in a muffle furnace at 490°C for 12 hrs and were reweighed to obtain the ashes content. The OC was obtained by differences between TDW and the ashes content. Cultures were kept for seven weeks. The initial food ration was adjusted weekly to compensate the increase in weight of the spat due to growth during the experiment. At the end of the experiment the final concentration was 375, 000 cell mL^{-1}.

Statistical methods: The homogeneity of variances and normality of the data were tested. Comparison among the different parameters of evaluation were carried out by an ANCOVA with a level of signification of $p>0.05$. The shells growth and weights of scallops were evaluated by ANCOVA of one way without interaction. The biochemical composition (protein, carbohydrates, lipid and fatty acid) was evaluated by one way ANCOVA.

RESULTS

Biochemical composition of the diets: The biochemical composition (protein, carbohydrate, lipid and fatty acids) of the microalgae mixtures diets were based on their OC (Table 1). The major percentage of protein was obtained in the spat fed with the diet M2 (52.17%) with respect to M1 and M3 ($p<0.05$). However, no significant difference between M1 and M3 were found ($p>0.05$). The maximum content of carbohydrates was obtained in the spat fed with the diet M1 (26.26%) and M3 (24.43%) with respect to M2 ($p<0.05$); between M1 and M3 no significant differences were found ($p>0.05$). In lipid the greater percentage was found in M2 (28.69%), however no significant difference was found among the three diets ($p>0.05$).

Table 1: Percentage of proteins, carbohydrates and lipids in organic base content, of three microalgae mixtures diets supplied at *Lyropecten (Nodipecten) subnodosus* during seven week. M1: *Isochrysis* sp. - *Pavlova lutheri*; M2: *Pavlova lutheri* - *Chaetoceros calcitrans* y M3: *Chaetoceros calcitrans* - *Isochrysis* sp. (standard error in parentheses). n=24 in each diet for each component, n_{total}=288

	Proteins	Carbohydrates	Lipids
M1	45.14 (3.39)^b	26.26 (1.08)^a	28.58 (2.11)^a
M2	52.17 (7.42)^a	19.13 (2.59)^b	28.69 (0.79)^a
M3	41.92 (3.85)^b	24.43 (2.62)^a	33.64 (2.92)^a

Significant differences: a > b

Table 2: Composition and percentages of fatty acids of three microalgae mixtures diets supplied at *Lyropecten (Nodipecten) subnodosus* during seven week. M1: *Isochrysis* sp. - *Pavlova lutheri*; M2: *Pavlova lutheri* - *Chaetoceros calcitrans* y M3: *Chaetoceros calcitrans* - *Isochrysis* sp. (standard error in parentheses). n=24 in each microalgae, n_{total} = 72

Fatty acid	M1	M2	M3
SFA			
14:0	14.4 ^{b}	14.8^b	17.1^a
15:0	0.41 ^{b}	0.61a	0.60 ^{a}
16:0	17.5 ^{b}	19.2 ^{a}	16.5 ^{b}
18:0	0.41 ^{b}	0.61^a	0.60 ^{a}
Total	32.72	35.27	35.30
MUFA			
16:1	15.8 ^{b}	18.1^a	16.2^b
18:1	6.79 ^{a}	2.49^b	7.30^a
Total	22.64	20.64	23.50
PUFA			
18:2	4.15 ^{a}	0.69^b	4.17^a
18:3	4.03 ^{a}	0.61^b	4.24^a
20:4ω6	1.08 ^{b}	1.82^a	1.24^b
20:5ω3	18.0 ^{b}	28.9^a	15.9^b
22:6ω3	15.9 ^{a}	9.76^b	12.9^b
Total	43.21	41.78	38.56

SFA: Saturated Fatty acids; MUFA: Monounsaturated Fatty Acids; PUFA: Polyunsaturated Fatty Acids. Significant differences: a > b > c

Table 3: Mean shell length and shell width (mm), of *Lyropecten (Nodipecten) subnodosus* fed with three microalgae mixtures diets. M1: *Isochrysis* sp. - *Pavlova lutheri*; M2: *Pavlova lutheri* - *Chaetoceros calcitrans*; M3: *Chaetoceros calcitrans* - *Isochrysis* sp. (Standard error in parentheses). n=75 in each diet, n_{total}=225

	Length			Width		
Diets	Initial (mg)	Increases (mg)	(%)	Initial (mm)	Increases (mm)	(%)
M1	4.03 (0.32)	1.74 (0.08)	43.2	3.72 (0.32)	1.75 (0.08)	47.0
M2	4.03 (0.32)	2.96 (0.05)	73.4	3.72 (0.32)	2.98 (0.04)	80.1
M3	4.03 (0.32)	1.26 (0.06)	31.3	3.72 (0.32)	1.27 (0.06)	34.1

Table 4: Mean Wet Weight (WW), Total Dry Weight (TDW) and Organic Content (OC) of *Lyropecten (Nodipecten) subnodosus* fed with three microalgae mixtures diets. M1: *Isochrysis* sp. - *Pavlova lutheri*; M2: *Pavlova lutheri* - *Chaetoceros calcitrans*; M3: *Chaetoceros calcitrans* - *Isochrysis* sp. (standard error in parentheses). n=75 in each diet, n_{total}=225

	WW			TDW			OC		
Diets	Initial (mg)	Increases (mg)	(%)	Initial (mm)	Increases (mm)	(%)	Initial (mg)	Increases (mg)	(%)
M1	9.83	38.09	387.5	9.51	14.0	247	5.63	3.65	164
	(1.16)	(3.98)		(1.34)	(1.07)		(0.60)	(0.09)	
M2	9.83	57.83	588.3	9.51	18.5	294	5.63	5.69	201
	(1.16)	(0.42)		(1.34)	(2.09)		(0.60)	(0.99)	
M3	9.83	25.16	256.0	9.51	12.1	227	5.63	1.57	128
	(1.16)	(6.87)		(1.34)	(1.07)		(0.60)	(0.00)	

The percentages in the content of the fatty acids in the microalgae mixtures diets were showed in the Table 2. Four saturated fatty acids were identified (14:0, 15:0, 16:0 and 18:0), two of the monounsaturated (16:1 and 18:1) and five polyunsaturated (18:2, 18:3, 20:4ω6, 20:5ω3 and 22:6ω3). Significant difference in the contents of fatty acids among three diets were found ($p<0.05$). The major contents of 16:0, 16:1, 20:4ω6 and 20:5ω3 were found in the diet M2 ($p<0.05$). The maximum percentage of the fatty acid 14:0 was found in the diet M3 ($p<0.05$). The diets M1 and M3 had the major content of 18:1, 18:2 and 18:3 ($p<0.05$). The major contents of 22:6ω3 were found in the diet M1 ($p<0.05$).

Cell concentration and filtration rate: The results of the concentration of food and filtration rates of the scallops fed with the three mixed diets to fourth hour are in the Fig. 1. The highest filtration rates in the three concentrations were obtained during the first hrs after supplying the food; subsequently these diminished until they reached (after 4 or 6 hrs) a stable value. The highest filtration rates were obtained with the spat fed with the concentration of 300,000 cell mL^{-1} in the three diets during the three first hrs, however, were reduced 50% after this time. However, the two highest concentrations of food had more food remaining after 24 hrs than the smallest concentrations. Moreover, the spat fed with the concentration of 450,000 cell mL^{-1} produced pseudofeces, this were identified through of microscope due to their content of complete microalgae cell. For this reason the ration of 200,000 cell mL^{-1} was selected as the initial concentration for the feeding experiments with the three diets of microalgae.

Growth of *Lyropecten (N.) subnodosus*: The best results and maximum increases in length, width, wet weight and dry weight (shell + meat, meat and shell) were obtained with the spat fed with the diet M2 that contained the microalgae *P. lutheri* and *C. calcitrans* (Table 3 and 4). The spat increased 73.4% in length and 80.1% in shell width. The increase in wet weight was 588.3%, the dry weight increased 294% (shell + meat), 432% (meat) and 201% (shell). Significant differences were found among diet M2 with respect to the diet M1 and diet M3 ($p<0.05$), however, no significant differences were found between M1 and M3 ($p>0.05$). The major percentages of survival were obtained in the spat fed with the diet M2 (60%) and M3 (61%); the percentage of survival with the diet M1 was of 39%.

DISCUSSION

The biochemical composition of microalgae in semi-continuous system was kept stable throughout the feeding experiment, it was checked with the biochemical analysis. Previously the optimum diluted rate for each species of microalgae was tested.

The nutritional quality of the microalgae depended principally on its content of proteins, carbohydrates, lipids and fatty acids. The polyunsaturated fatty acids had resulted essential for the growth and survival in the majority of the organismss studied[10,21]. The content of fatty acids, mainly of polyunsaturated, in diets used as food in the pacific oyster *Crassostrea gigas*[3] and the clam *Tapes philippinarum*[22] raise their rate of growth.

In this work, significant differences in the biochemical content among three mixed diets were found. These differences were due to the different biochemical content of each microalgae used in the three diets. The values of the biochemical composition were similar to those reported by Brown *et al.*,[23], Cordero[24] and Renaud *et al.*,[25]. The content of fatty acids obtained in *Isochrysis* sp., *P. lutheri* and *C. calcitrans* is in the range reported by Thompson *et al.*,[26], Cordero *et al.*,[27], Albentosa *et al.*,[28], Reitan *et al.*,[29] and Renaud *et al.*,[25]. The greatest content of polyunsaturated fatty acids 20:4ω6 and 20:5ω3 was obtained in the microalgae *P. lutheri* and *C. calcitrans*; for the fatty acid 22:6ω3, the greatest

content was in the microalgae *Isochrysis* sp; these results are similar to those reported by Hatate *et al.*,[4].

For feeding and growth tests involving nutritional evaluation of diets it is necessary to measure two parameters which influence the amount of food ingested: Filtration rate and optimum concentration of food. Walne found that the growth in juvenile bivalves depended of the concentration of algae cells in the water. The food concentrations used for the scallops are similar those used by several authors in other species[2,3,8]. In this work for *L. (N.) subnodosus* a cell concentration of 200, 000 cell mL^{-1} was selected. This selection was due to the relation present among each concentration of food and its filtration rate through the time decided on. Bricelj and Shumway[25] observed that the majority of bivalve mollusks when exposed to high food concentration, they are able to control the total amount of food ingested by reducing their filtration rates or increasing the amount of material rejected in pseudofeces. However, scallops do not produced large amounts of pseudofeces compared to mussels and oysters. In this work the pseudofeces were produced only in the maximum concentration of microalgae (450,000 cell mL^{-1}). This could support the idea that the scallop controls the material ingested principally by reducing the filtration rate more than by production of pseudofeces[30].

The values obtained for filtration rates showed little variation, mainly in the spat fed with the concentration of 300,000 cell mL^{-1} and 450,000 cell mL^{-1}. Griffiths [31] reported for the black mussel *Choromytilus meridionalis* a similar tendency and concluded that it may occur due to digestive gland activity, saturation and reduction of the filtration rate due to pseudofeces in the water. This caused the valve of the organismss to partly close. The relation among the filtration rate, the optimum concentration of food and growth rate, has been evaluated to measure the energy budgets of the bivalvae. However, the studies on the energy budgets for some species of bivalve mollusks as *Lyropecten* (*Nodipecten) subnodosus* have been insufficient and are necessary more studies on the physiology of these species.

The larvae and juvenile of several bivalvaes had major growth if they were fed with a diet consisting of more than one species of microalgae. The studies of feeding using a monoalgae diet, *C. calcitrans*, *Isochrysis* sp. and *P. lutheri* showed to have good nutritive value for oyster, mussels and crustacean[32,33]. When these microalgae were used in mixed diets for shrimp *Penaeus* spp. and the scallop *Pecten maximus*, they raised the best growth rate mainly in diets with *P. lutheri* and *C. calcitrans*[33,34]. In the present study the best increments in length and weight for *L.* (*N.*) *subnodosus* were obtained with the diet of the *P. lutheri* and *C. calcitrans*. This could be due to the content of proteins and a greater concentration of the fatty acid 20:4ω6 and 20:5ω3 in this microalgae mixture diet. Different results were obtained by Uriarte and Farías[35], they observed that the different protein level affected significantly the growth in postlarval of *Argopecten purpuratus* but no affected the growth in spat of this species. However, the nutritional requeriments in the several stages of development among species are differents and the quality of the protein in the diet influences the growth. Pérez-Camacho *et al.*,[36] observed that the results of growth in *Ruditapes decussatus* could be modify by the quality of the source of the protein, charbohydrate and lipid in the diet. The polyunsaturated fatty acids, principally the acid 22:6ω3 in the diet of the clam *Tapes philippinarum* increased its growth[37]. Volkman *et al.*,[21]also reported that the majority of the bivalvae show the best growth with diets that have the fatty acid 22:6ω3. However, *Isochrysis* sp. had the major content of this fatty acids but the diet with this microalgae was poorly in its nutritional value. Therefore, for this scallop the presence of the fatty acid 22:6ω3 in the diet did not positively affect the growth, in contrast to the fatty acids 20:4ω6 and 20:5ω3.

Exist a variation in the results obtained among different authors about the filtration rate, optimum concentration of food and growth in the different species of mollusks, due to principally at the different conditions used in each experiment[30].

CONCLUSION

- The higher percentage of protein was in the diet M2 (*P. lutheri* and *C. calcitrans*)
- The major contents of 20:4ω6 and 20:5ω3 fatty acids were found in M2 (*P. lutheri* and *C. calcitrans*).
- The best results and maximum increases in length, width, wet weight and dry weight were obtained with the spat fed with the diet M2 (that contained the microalgae *P. lutheri* and *C. calcitrans*).
- The conjunction of protein and the fatty acids 20:4ω6 and 20:5ω3 in the diet improved the growth of *L.* (*N.*) *subnodosus* spat.
- A diet consisting of the microalgae *P. lutheri* and *C. calcitrans* is recommended for *L.* (*N.*) *subnodosus* spat.

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