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Effect of Density and Nutritional Quality of Diet on Survival and Growth of Lion's-Paw *Lyropecten* (*Nodipecten*) *Subnodosus* Spat

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Abstract: The effect of the density and nutritional quality of the diet on the growth and survival of the organism had been evaluated in different species of Mollusk. In bivalve low growth and survival had been registered in high densities of culture and poorer nutritional quality of the diet. The scallop *Lyropecten* (*Nodipecten*) *subnodosus* is an organism with a high commercial value. However, in this species few researches had been performed to evaluate the effect of the density and nutritional quality of the diet on the growth and survival, principally in stages of larvae and spat. The objective in this study was to evaluate the effect of the survival and nutritional quality of the diet on the growth and survival of *L. (N.) subnodosus* spat. In this study, the results of growth and survival obtained to show significant differences between densities and nutritional quality of the diet. The major results of length, total dry weight, organic content and survival were obtained in the organism cultured in the lowest density and fed with the mixture diet M1 (*Pavlova lutheri* and *Chaetoceros calcitrans*). The mixture diet M1 registered the higher content of protein, carbohydrates and polyunsaturated fatty acids. These components had been essential to the growth and survival of the spat stage, due to its high requirements of protein and fatty acids used for the somatic growth. For this reason the high growth and survival were registered in the organism cultured in the lowest density and fed with high protein content and adequate percentages of the polyunsaturated fatty acids (M1).

Key words: Density, growth, mixtures diet, nutritional quality, biochemical composition

INTRODUCTION

The culture of some marine mollusks with a profitable economic activity had been increased in the last year due to its high value market[1]. The interest in improvement the techniques of culture are due to obtain enough products to supply the market without affected the natural environment of the organism. The scallop *Lyropecten (Nodipecten) subnodosus* is a bivalve mollusk with a high potential in the aquaculture due to its growth characteristic and elevated commercial value principally in Europe and United States[2]. However, actually little researches on the growth and the nutritional requirement has been performed in this species.

The effect of some factors like the nutritional quality of the diet, the temperature and density on the growth and survival of the organism had been studied in researches different[3-5]. The effect of the temperature and nutritional quality of the diet on the growth and survival of the organism depend of different factors like the species, its development stage and the optimal ranges of temperature[6]. Friedman *et al*.[7] and Flores-Vergara *et al*.[5] had observed that temperatures above of 30°C had a negative effect on the growth of *Crassostrea gigas* independently of the nutritional quality of the diet. However, the variation of density between the cultures must be affected negatively the final results of growth and survival independently of supply an optimal temperature and appropriated nutritional quality of the diet for the organism.

The optimal density of the organism is an essential factor for the income-yield capacity of the culture[8]. High densities of organism had showed a negative effect on the growth and survival in the majority of the cultures[9,10]. The lowest growth and survival were registered in *Sepia officinalis*[11] and *Litoria aurea*[3] cultured in high densities. In mollusk had been registered a similar tendency, where the major growth and survival were obtained in the cultures with lowest densities[12,9,10]. The effect of the density on the growth and survival in cultures of the scallop *Lyropecten. (N.) subnodosus* had been little studied. The majority of the researches had

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been performed principally in larvae and reproductive stage, likewise in other species of the same genus, *Lyropecten* (*Nodipecten) nodosus*.

In previously researches were registered high growth in spat of *Lyropecten.* (*N.*) *subnodosus* cultured at 23°C and fed with mixtures of the microalgae *Pavlova lutheri*, *Chaetoceros calcitrans* and *Isochrysis* sp. Moreover, the density were a possibly factor to affect the growth of this species[13]. Therefore, the objective of this study was to evaluate the effect of the density on the growth and survival of *L. (N.) subnodosus* spat cultured at 23°C and fed with two mixtures of microalgae.

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 cultured in controlled conditions of temperature (22±1°C); a pH of 7.5-8 (controlled by CO_2 injections); salinity of 35 ppt and the culture medium f (Guillard and Ryther 1962). The microalgae were cultured in 15 L containers and were kept in the late exponential growth phase through of a semi-continuous system with a daily dilution rate of 20%.

The microalgae were used in two mixtures diets: M1: *P. lutheri-C. calcitrans* and M2: *C. calcitrans-Isochrysis* sp. The proportion of each microalgae in the diet was based on cells concentration of each species. The proportion of the microalgae *C. calcitrans* in both diet was 1:2 (2 proportions of *C. calcitrans*), due to this microalgae contains up to 50% of ash.

Each third day, triplicate of 20 mL of the three microalgae mixtures (above mentioned) were concentrated on glass microfibre filters Whatman GF/C filters (Ø 47 mm) previously washed with distilled water, ashed and weighed. After filtration, the filters were rinsed with a 3% ammonium formate solution (for salt removal) and placed in a convection oven at 60°C for 48 hrs 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 was evaluated each third day. The mixtures diets were filtered by triplicate through Whatman filters GF/C (Ø 27 mm). The method used were Lowry *et al.*[15] for protein; carbohydrates according to Dubois *et al.*[16], after the treatment as Whyte[17] and lipids with the colorimetric method of Pande *et al.*[18] after extraction following Bligh and Dyer[19].

The samples for fatty acids analyses were centrifuged, froze-dried at -70°C, freeze dried and weighed. The fatty acids were analyzed following Sato and Murata[20], after total lipid extraction[19], 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 *Lyropecten (Nodipecte) subnodosus* spat (≈4 mm of length) were acclimated at 23°C and fed with *P. lutheri* and *C. calcitrans* during this condition. The temperature of water was maintained by a heating of 50 watts to connect at temperature regulator.

Experimental design: Three densities of 5, 10 and 15 spat by liter were evaluated. Likewise, two mixtures of microalgae (M1: *P. lutheri-C. calcitrans* and M2: *C. calcitrans-Isochrysis* sp.) were proportioned by triplicate in each density. The initial concentration of microalgae in the mixtures diets was 200,000 cells mL^{-1}. The feeding trials were kept by four weeks and the concentration of food was adjusted weekly (25, 000 cells mL^{-1}) to compensate the growth during the experiment. The final concentration of microalgae in the diets were 300, 000 cells mL^{-1}.

The spat acclimated at 23°C were distributed at random in 18 plastic chambers with 8 L of seawater. An air lift system inside of the chamber was used to minimize the sedimentation rate of microalgae. The vessels were cleaned daily to minimize the contamination by pseudofeces and to avoid some damage in the shell of organism.

Measurements of growth: An initial sample of 30 spat was measurement to obtain the length, Total Dry Weight (TDW) and Organic Content (OC). Weekly a sample of 30 spat by treatment was measured to obtain their increment of length. The length of shell was measurement in a stereomicroscope with an adapted ruler (Wild Heerbrygg) Max Erb at 25x. A sample of 9 spat by treatment in the lowest density and 12 spat by treatment in the other two densities were sacrificed weekly to obtain the increase in TDW and OC. The TDW was determinate in a convectional oven at 60°C for 48 hrs, after which they 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.

The instantaneous daily growth rates in TDW (k) were calculated with the equation: $k= \ln (M_t/M_o)/ t$, where M_t= measurement at day t, M_o= measurement at day zero, t= day. The survival rate was calculated weekly throughout all the experiment.

Statistical analysis: The obtained results were analyzed to obtain the homogeneity of variances and normality test. The effect of the density and nutritional quality of the mixed diets on the growth and survival was evaluated using a two-way ANOVA with the time like covariance. The level of significance was 0.05. The biochemical composition (protein, carbohydrates, lipid and fatty acid) was evaluated using one-way ANOVA with an accepted level of significance of 0.05. When a significant difference was found, the post hoc comparisons were made by Tukey's honest significant difference test HSD.

RESULTS

Biochemical composition of the diets: The biochemical composition (protein, carbohydrate, lipid and fatty acids) of the mixtures diets was based on their OC (Table 1). The results of biochemical composition showed significant differences between diets ($p<0.05$). The major percentages of proteins and carbohydrates were registered in the mixture M1 to respect at M2 ($p<0.05$). The high percentages of lipids were obtained in the mixture M2 to respect at M1 ($p<0.05$).

Significant differences were found in the content of the fatty acids between the mixtures ($p<0.05$). The higher percentages of Saturated Fatty Acids (SFA) and polyunsaturated fatty acids were registered in the mixture M1 (Table 2). The higher content of monounsaturated fatty acids were obtained in the mixture M2. Significant differences were not found in the content of the fatty acids 14:0 and 16:1 between the diets ($p>0.05$). The major content of the fatty acids18:0, 18:1ω7, 18:1ω9, 18:2, 18:3 and 22:6ω3 were registered in the mixture diet M2 to respect at M1 ($p<0.05$). The major percentages of the fatty acids 16:0, 18:4, 20:4ω6 and 20:5ω3 were obtained in the mixtures diets M1 to respect at M2 ($p<0.05$).

Growth of *Lyropecten (N.) subnodosus*: The major results in increases in length were obtained in the organism fed with the mixture M1 and cultured in the lowest density (5 spat by liter) to respect at the others treatments. The values registered were in a range of 60.47 to 88.06% (Table 3).

The growth rate (k) on base of TDW registered similar values between densities and mixtures diets with values of 0.086 to 0.089. The survival of the spat registered percentages above of 50% in all treatment. Significant differences in the percentages of survival were found between densities and diets ($p<0.05$). The lowest survivals were registered in the organism fed with the diet M2 in all densities (Table 3). The higher percentage of survival was obtained in the organism fed with the mixture M1 and cultured in the lowest density to respect at the others treatments ($p<0.05$).

Table 1: Mean percentages of proteins (PROT), carbohydrates (CHOS) and lipids (LIP) on base of organic content of *Lyropecten (Nodipecten) subnodosus* spat fed with two mixtures diets (MI: *Pavlova lutheri* - *Chaetoceros calcitrans*; M2: *Isochrysis* sp. - *Chaetoceros calcitrans*) and kept at 23°C. Means with different subscripts in the same column are significantly different ($p<0.05$), a>b. The n in each component by treatment was 39

Diets	Prot	Chos	Lip
M1	47.64_a	24.98_a	27.38_b
	(0.45)	(0.59)	(0.80)
M2	46.31_b	23.32_b	30.37_a
	(0.48)	(1.01)	(1.04)

Table 2: Mean percentages of total fatty acids of *Lyropecten (Nodipecten) subnodosus* spat fed with two mixtures diets (MI: *Pavlova lutheri*-*Chaetoceros calcitrans*; M2: *Isochrysis* sp. -*Chaetoceros calcitrans*) and kept at 23°C. SFA: saturated fatty acids; MUFA: monounsaturated fatty acids and PUFA: polyunsaturated fatty acids. Means with different subscripts in the same row are significantly different ($p<0.05$), a>b. The n in each treatment was 39

Fatty acids	M1	M2
SFA		
14:0	23.53_a	23.47_a
16:0	25.58_a	22.40_b
18:0	0.71_b	2.29_a
Total	49.52	48.16
MUFA		
16:1	16.89_a	16.48_a
18:1ω7	2.80_b	6.27_a
18:1ω9	2.76_b	6.27_a
Total	22.45	29.02
PUFA		
18:2	1.32_b	2.33_a
18:3	1.41_b	3.11_a
18:4	0.64_a	0.44_b
20:4ω6	2.36_a	1.23_b
20:5ω3	17.19_a	8.44_b
22:6ω3	5.11_b	7.27_a
Total	28.03	22.82

Table 3: Increases in length (mm) (%), growth daily rate (k) (on base of TDW) and survival (S) of *Lyropecten* (*Nodipecten*) *subnodosus* cultured at three densities (D_1=5 org.L^{-1}; D_2=10 org.L^{-1}; D_3=15 org.L^{-1}) and fed with two mixtures diets (M1: *Pavlova lutheri* - *Chaetoceros calcitrans*; M2: *Isochrysis* sp. - *Chaetoceros calcitrans*) and kept at 23°C. Initial length was 3.77 mm (±0.42). Means with different subscripts in the same column are significantly different ($p<0.05$), a>b>c

		I (%)	S (%)	k
D_1	M1	88.06	89.16_a	0.089
	M2	72.94	82.16_b	0.088
D_2	M1	72.41	77.08_c	0.087
	M2	66.31	75.83_c	0.087
D_3	M1	68.16	71.94_d	0.086
	M2	60.47	67.77_e	0.086

Table 4: Mean final (F) and Increases (I) of total dry weight (TDW) and organic content (OC) in mg and % of *Lyropecten* (*Nodipecten*) *subnodosus* spat cultured at three densities (D_1=5 org.L^{-1}; D_2=10 org.L^{-1}; D_3=15 org.L^{-1}) and fed with two mixtures diets (M1: *Pavlova lutheri* - *Chaetoceros calcitrans*; M2: *Isochrysis* sp. - *Chaetoceros calcitrans*) and kept at 23°C. The n_{total} in D_1= 32, D_2= 96, D_3=96. Initial: TDW (4.11 ± 0.00 mg) and OC (0.51 ± 0.00 mg). Means with different subscripts in the same column are significantly different ($p<0.05$), a>b>c

Density	Diet	TDW I (%)	OC I (%)
D_1	M1	393.6_a	1115.6_a
	M2	367.8_b	1058.8_b
D_2	M1	364.9_b	1056.8_{bc}
	M2	341.6_{cd}	1027.4_{cd}
D_3	M1	346.9_{bc}	1017.6_{de}
	M2	320.4_d	990.1_e

Significant differences in TDW and OC were found between densities and mixtures diets ($p<0.05$) (Table 4). The lowest result of TDW and OC were registered in the organism fed with the mixture M2 in all densities. The higher values in increases in TDW and OC were registered in the organism fed with the mixture M1 and cultures in the lowest density to respect at the others treatments ($p<0.05$). The values of TDW and OC were found in intervals of 320.4 to 393.6 mg and of 990.1 to 1115.6%, respectively.

DISCUSSION

The temperature, the nutritional quality of the diet and the density of the culture had been important factors in the culture of the different aquacultures species. The interaction of these factors affected the growth and survival of the organism in the culture[1]. Therefore, the time for reached the optimal commercial size of growth to change.

In general, the organisms cultured out side of the optimal interval of temperature have a negative effect on the growth and can to produce their death. Some species of the organism to survive at this negative condition because are able to regulate their metabolic processes[21]. The temperature of 23°C used in this study were selected on base of the result obtained in own previous researches and the interval of temperatures registered by Bernard[22] for *L.* (*N.*) *subnodosus*. The last condition to avoid that the temperature had an effect on the growth and survival of the *L.* (*N.*) *subnodosus* spat and the results were not reliable.

The adequate availability and optimal nutritional quality of the food have an important effect on the increment of the growth and survival of the organism[23,24]. However, the density of the organism in the culture had played an important roll although the diet satisfies the requirement nutritional of them. An inadequate density provoked a stress stage in the organism due to the territoriality or the competition by the food[8,25]. The last condition affected the metabolic rate of the organism and the energy destined for growth had been used in other physiological process like the pseudofeces production and the motion to diminish the growth[26,27].

Some time the optimal initial density of the organism can be affected significantly due to suddenly higher mortalities. The last condition resulted in significant differences of density between cultures and the forecast production were reduced due to the size of the organism is significantly different between cultures. For this reason is very important to know the effect of the density of the culture on the growth in each species to establish the income-yield capacity of the culture[28,8].

In general, high densities in the cultures reduced the growth and survival in the majority of the species, this to depend of its biology like the characteristics of behavior and feeding. In cultures of crustaceae and amphibious[30] had been registered a negative effect in the survival and growth in high densities. In cultures of mollusk had registered the same tendency on the growth of the organism in high densities, due to the competition for food and bad water quality[31,23,10]. In pectinidae due to characteristic of motion, the density plays an important roll in its culture. The pectinidae *yropecten (Nodipecten) subnodosus* is a species with few investigations on the effect of the interaction of the density and nutritional quality of the diet on its growth and survival. The researches in this species had been made in other organism of the same genus like the scallop *Lyropecten (Nodipecten) nodosus*[32,8].

The result obtained in this study to indicate that the high density had a negative effect on the growth and survival of *L. (N.) subnodosus* spat, these results to confirm the obtained by other authors in species of the same genus[32,8]. In this study the interaction of the density and the nutritional quality on the growth registered a negative tendency to increase the density of culture. The last result were obtained due to the energetic requirement derivate by the motion stress in the organism cultured in high densities were not satisfied by any diets. Likewise, the *L. (N.) subnodosus* spat kept in the lowest densities and fed with high content of protein and polyunsaturated fatty acids (M1) to register the major growth. Similar results were obtained by Cerón-Ortiz *et al.*[13] in *L. (N.) subnodosus* spat cultured in low densities and fed with the microalgae *P. lutheri* and *C. calcitrans*.

The microalgae used in this work (*Isochrysis* sp., *P. lutheri* and *Chaetoceros* sp.) have been some microalgae species more recommended like food, due to

high protein content and essential fatty acids[33-35]. Their nutritional quality of the microalgae will be to improve used diet with more of one species[36,5]. The last is due to the biochemical component of each microalgae must be integrated in the mixture to increase its nutritional value[37,35]. In cultures of the *Placopecten magellanicus* and *Aequipecten tehuelchus* fed with microalgaes of *Pavlova* genus had registered high growth[38,35].

High protein content in the diet of spat and juveniles had been very important due to its requirement for the somatic growth[39,40]. In post larvae and spat of *Argopecten purpuratus* a high protein content in the diet to increase the growth of this species[25]. Farías and Uriarte[40] had showed that the content of essential fatty acids in the diet to increase the growth rate of the organism. In bivalve the content of fatty acids 20:4ω6, 20:5ω3 and 22:6ω3 in the diet to increase the growth and survival. These fatty acids have very important functions like precursors of active metabolites and a structural roll, moreover these to play a roll in the fluidity of the cell membrane for the interchange of substances[41,5]. The fatty acids 20:4ω6, 20:5ω3 and 22:6ω3 had been essential component in the diet of bivalves[25], however, the requirements of each fatty acid are different between the species[42]. In juveniles of *Tapes philippinarum* the fatty acid 22:6ω3 had a major effect on their development than the fatty acids 20:5ω3[43]. The major result of growth and survival were obtained in the *L. (N.) subnodosus* spat fed with high protein content and the fatty acids 20:4ω6 and 20:5ω3[13]. Similar results in the growth and survival of *L. (N.) subnodosus* spat were registered in this study.

The growth rate of the organism had been affected by the species, the nutritional quality, the development stage and the culture time[31,44]. The growth rate on base of total dry weight of *L. (N.) subnodosus* registered in other researches was major to obtain in this study[2,6]. The differences in the results of growth rate registered between this study and others can be to explain principally at the different conditions used in each experiment.

The effect to the density in the culture on the survival of the organism is different between species. In juveniles of the genus *Lyropecten (Nodipecten)* were not registered a negative effect on the survival of the organism cultured in high densities[8]. This results were contraries to obtain with *L. (N.) subnodosus* spat in own study. The differences in the results between researches were due to the differences in the densities proved, the initial size, the species and the condition of culture. In this study, the optimal quantity of food was proportioned to depend of each treatment. Therefore, the results of survival in *L. (N.) subnodosus* spat were explained by the effect of the density and nutritional quality of food. The survival of *L. (N.) subnodosus* spat in this work showed a tendency to diminish in high densities. The major survivals were obtained in the organism fed with M1 (*P. lutheri* and *C. calcitrans*). The same tendency was registered in the growth. Therefore, the effect of the density and nutritional quality of diet on the survival of *L. (N.) subnodosus* spat must be explanted by the argument above mencionated.

CONCLUSION

- The density of the culture and the nutritional quality of the diet had an effect on the growth and survival of *L. (N.) subnodosus* spat.
- The spat of *L. (N.) subnodosus* must be culture in lowest density.
- A diet with high content of protein and polyunsaturated fatty acids (20:4n6 and 20:5n3) to increase the growth and survival of *L. (N.) subnodosus* spat.
- The conjunction of the microalgae *P. lutheri* and *C. calcitrans* had a positive effect on the growth and survival of *L. (N.) subnodosus* spat.

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