35

USE OF SERUM PROTEIN CONCENTRATION OPTIMIZE PENAEID SPAWNER QUALITY

AQUACOP

CNEXO-COP BP 7004 TARAVAO TAHITI FRENCH POLYNESIA

ABSTRACT

From June 1981 to September 1982, the serum protein concentration has been used as an index of shrimp's health for P.monodon female selection in the maturation units at the COP (Vairao, Tahiti). Three lots were separated according to the food supply during the pregenitor's life and the spawning index (number of spawn per female and per month) was then monitored for each female. With a captive broodstock, a minimum serum protein concentration is needed for spawning ability.

Female reared in earth pond and fed on fresh food once a week + dry pellet have a higher spawning index (Ip = 2.1) then females reared in concrete ponds and fed on dry pellets (Ip = 0.75) and there is a good correlation between the spawning index and the serum protein concentration for each rearing method tested.

A method is proposed for further management of a rearing unit with a spawning index up to 1.65.

INTRODUCTION

The availability of a sufficient number of shrimp spawners remains a limiting factor for the development of Peneid cultures. In many countries where local species are found, the spawners are captured directly in the sea. In countries where there is no local commercial species, it is necessary to be able to control the whole cycle and to produce spawners completely reared in captivity. The complete control of broodstock in captivity will probably become in the future necessary for everybody involved in shrimp culture as recent data show an emergence of virus problems transmitted through the eggs. The only way to prevent heavy losses will be to work with disease-free-broodstocks regularly controled.

The "Centre Océanologique du Pacifique" (COP) has developed since 1974 intensive studies on different species which are maintained in ponds and tanks through successive generations. (10th generation for P.monodon). The rearing technic in the maturation has been already described (Aquacop, 1977 ; Aquacop, 1980 ; and one of the main problem is the quality of the food given to the spawners. It has been prooved Aquacop, 1983 : Chamberlain et al, 1981 ; that the supplementation of fresh food is necessary to obtain good results in maturation and spawning.

This paper deals with the experiments developed to test different alimentary diets during the growth of the Penaeus monodon broodstock which lasts between 6 to 9 months. From June 1981 to June 1982 each female entering the maturation tanks has been checked individualy concerning the rearing conditions (food supply during the growing out and the number of spawnings on a two months production period in the maturation tanks. The serum protein concentration has also been checked for each female entering the maturation unit prior to the eyestalk ablation .

METHOD

1) Constitution of broodstock

a) The ponds

Two kinds of ponds have been used for the rearing of P.monodon :

- Earth ponds : 700 m2 to 2500 m2. These ponds are 1,20 m deep and have compressed clayey earth bottom and dikes.

- Concrete ponds : 400 m2 to 1200 m2. These ponds are 1,80m deep and have earth dikes covered with a layer of concrete and polyester sheet and bottom made of compacted coral. Some of these coral bottoms are partly covered with a layer of gravel and a layer of sand separated by a permeable synthetic cloth. Moderate aeration is provided through a longitudinal perforated PVC pipe. The water is flowed into drain pipes embedded in the gravel, under the sand ("double bottom" ponds).

b) Food supply

- 60% protein compound pellet .

- Fresh food : frozen squid and pork liver.

- Natural phyto and zooplancton present in earth ponds (table 5).

c) rearing methods

- The broodstock received different sorts of food that can be defined as three basic types :

  - Rearing method type 0 : the pregrowing (1 to 10g) is carried out in earth ponds (initial density = 10 to 20 m2) and the final growing is made in concrete tanks (density = 2 to 4 m2). The animals are fed adapted pellets without fresh food.

  - Rearing method type 2 : the pregrowing and the growing is carried out in earth ponds until harvesting and the animals are fed adapted pellets + fresh food once a week.
    - pregrowing density. 10/20 m2
    - final growing density 1 to 2 m2.

  - Rearing method type 1 : this intermediate type mixes animals reared in earth ponds until harvesting and fed adapted pellets without fresh food and animals reared in concrete ponds and fed adapted pellets + fresh food once a week.

2) Maturation

a) Equipment and material : the maturation area is a tanslucide covered 200 m2 surface including 12 circular tanks (Aquacop, 1983) Holding capacity is about 12 m3 for each tank with 60 individuals with a sex ratio of 1/1.

b) The food : previous studies Aquacop, 1980 have shown clearly that a mix diet (fresh food + pellets) is necessary to obtain good results in maturation. The food is distributed twice a day. In the morning, fresh food is used : frozen squid, live mussel (Perna viridis) and troca (Trocus niloticus) ; during the afternoon, 60% protein compound pellets (Japanese pellet) are distributed. The daily feeding rate is 4% of the biomass.

c) Water quality : temperature ranges from 25,5 to 30°C, salinity is 34 ppt and ph is 8,2.

d) Animals : for each female entering the maturation unit, samples of hemolymphe are taken and the serum protein concentration is calculated with a refractometer. Females are then epedonculated by simple pinching of one eyestalk(Aquacop, 1977) and double tagged : a ring of coloured elastic silicone bearing a label is inserted around the remaining eyestalk and an other label with a number is glued on the cephalothorax (Aquacop, 1983).

This technic allows to follow each female individually in a tank for ovarian development and molting.

e) Serum protein concentration calculation : 0,5cc hemolymph is drawned from the cardiac sinus with a syringe and allowed to clott in glass tubes. The serum supernatant is dropped on the prism of a refractometer(Leavitt et al, 1977)which gives the refractometric index. Standardization is made with a standard protein solution commercialy available (proteitrol 99 g/l lab BD Mérieux France).

The molting stage is determined by simple examination of the animals in the maturation tanks. Stage A = the day of molting
B = two days post molting
D = four days pre molting
C = seven to ten days.

This simple technic was controled by microscope examination of the telson setae according to Drach's publication (Drach, 1939).

f) Data processing :

Quantitative data : Serum protein concentration index (P) = a standard serum protein index is used for tabulations. This index is calculated for each female with a corrective coefficient applied to the molting stage.

Spawning index (IP)= the number of spawn for each female and per month is calculated at the end of the maturation trials which lasts two months in the COP.

Qualitative data : 3 types of rearing methods (0,1,2) are tested.

RESULTS

I - Serum protein concentration variation related to the molting stage

a) Healthy animals : data are tabulated in table 1. ANOVA shows highly significant (P 0,001) differences related to the molting stages A B C and D. For healthy animals, the serum protein concentration level ranges from 84 g/l to 106 g/l.

b) Diseased animals : 58 females originated from various ponds were checked for serum protein concentration. These animals showed gross pathological symptoms - Soft shell syndrome
- Brownish carapace coloration
- Septicemia (Vibrio alginolutium).

Differences between healthy animals are highly significant (P 0,001).

II - Proteinemia variation related to the rearing method

A one way analysis of variance indicates that differences are highly significant (P 0,001). Data are shown on table 2.

When rearing conditions are not able to supply the animals with sufficient zooplancton (type 0), the females fed only with pellets have a lower serum protein concentration level compared to animals fed fresh food once a week (type 2) or reared in ponds with sufficient zooplancton supply (type 1).

III - Spawning index variation related to the rearing method

A one way analysis of variance has been performed for each class of protein serum concentration. Data are listed in table 3 and fig.1 and show highly significant differences (P 0,001) : the spawning index of animals reared with method 2 is the best and can reach 2,5.

There is good evidence that the fresh food supply in the rearing ponds induces better spawning for the females which have a sufficient serum protein concentration (90 to 130 g/l) .

- with a lower protein serum concentration, (P 90 g/l) the spawning index is very low especially when animals are reared with method 0. With a proteinemia below 80 g/l (43 animals tested) the animals are not able to spawn and only 4 females reared with method 1 gave spawn during the experiment.

IV - Corelation between the spawning index and the protein serum concentration

The animals were separated into three subgroups corresponding to the rearing methods (0,1,2). The results are shown as a plot of serum protein concentration versus spawning index. The resulting plot was found to fit closely a straight line($P < 0,001$) for each subgroup tested (fig.2) (table 4).

- rearing method 0 : $Ip = 0,219 + 0,0049\ P$ n = 113
- rearing method 1 : $Ip = 0,325 + 0,0083\ P$ n = 111
- rearing method 2 : $Ip = 0,44 + 0,0138\ P$ n = 80

TOTAL N = 304

DISCUSSION

I - Protein serum concentration variation related to the molting stages.

a) Healthy animals

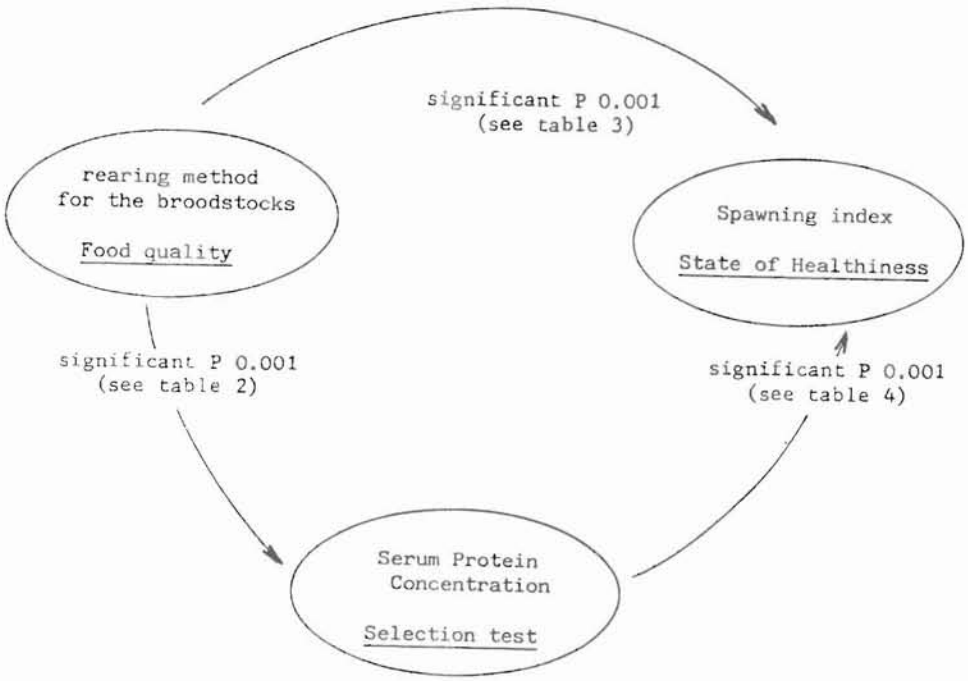
According to Alexander et al 1980, the concentrations obtained with the refractometer are approximately one and a half times those obtained with other methods (automated biuret, Lowry et al phenol reagent, Copper sulfate specific gravity). Data presented here agree with several authors

			stage AB	C	D
Chemical method	Bursey et Lane 1971	P. duorarum	66 g/l	74g/l	81g/l
Chemical method	Bourguet et Exbrayat 77	P. japonicus	25	66	85
Chemical method	Cuzon et al (1980)	P. japonicus	47 to 55	63	81
Chemical mehtod	Balazs et al (1974)	M. rosenbergii		104à144	
Physical method	This study	P. monodon	76à84	94	105

b) Diseased animals : A low serum protein concentration has been corelated to diseases of fishes (op. cit in Alexander et al, 1980) but scarcely to diseases of crustacean : A pallipes (chaisemartin 78), C maenas parazitised by Sacculina or Thelohania (Andrieux, 1976)(Vivares et al, 1981).

II - Proposed model of interaction

Precedent results show interactions between the following parameters.



1) Protein serum concentration related to the rearing method

It is well known that the protein serum C of different species is related to the feeding pattern H.americanus (Stewart et al, 1967) C. maenas (Busselen 70), A fluviatilis (Chaisemartin 78), P.japonicus (Cuzon 71, Deshimaru 76). A pellet with high protein level induces high protein serum concentration for 6 hours (Nakamura 1980.) In this experiment the animals were fed the same pellet from 5g (pregrowing) up to 60g (final growing). The differences shown in table 2 would probably be due to a qualitative effect of the fresh food given in very small quantities which provides essential elements and can prevent carencies and diseases. This hypothesis agree with the occurence of a nutritional carency affecting P.monodon in 1979 (Blue disease) when animals were reared in concrete tanks without fresh food for a long period.

2) Spawning index variation related to the rearing method

Very few informations are available on this subject because P.monodon ♀ are generaly captured in the sea and placed directly in the maturation tanks. Higher spawning index would be probably due to better food supply during the rearing period. The fresh food and natural zooplancton are certainly rich in essential aminoacids and essential fatty acids involved in the maturation process (Middleditch et al., 1980).

3) Spawning index variation related to the protein serum concentration

It is well known in zootechnic that "an healthy animal is an animal able to reproduce and to grow bigger . Stewart and Li, 1969 applied this concept to a study of wild lobster and proposed the measuring of serum protein concentration as a selection test for crustaceans.

Our observations(table1)agree with Stewart's : the serum protein index can be applied for selection of the females entering the maturation unit.Moreover COP'S recent experiments (Aquacop unpublished data) show clearly that the occurence of Vibriosis in the rearing ponds can be a limiting factor for the species reared in captivity.

CONCLUSION

The measurement of the serum protein concentration provides a "good simple method" which can be used as an index of shrimp's health for the selection of females entering the maturation unit. The fresh food supply during the pregenitor's life is also an important factor for spawning ability and the COP has developped intensives studies on this subject. The rearing method 2 described here is by far the most efficient method for the rearing of a P.monodon broodstock at the COP.

1st stage pregrowing	4 months from PL to 10g initial density 10 to 20/m2 expected survival 70% earth pond adapted pellet + fresh food once a week.
2nd stage intermediate growing	4 months from 10g to 25g initial density 1 to 2/m2 expected survival 80% earth pond adapted pellet + fresh food once a week.
3rd stage final growing	4 months from 25g to 50g (♀ 75 ♂ 25g) initial density 1/m2 expected survival 70% earth pond adapted pellet + fresh food once a week.

harvesting and female selection by serum protein concentration.

Besides, the serum protein concentration is an important zootechnic factor which can be used during the growing period so as to prevent diseases ; and attempts are made at the COP to correlate the serum protein concentration with various pathological conditions.

LITERATURE CITED

Alexander JB - Ingram GA, 1980. A comparison of five of the methods commonly used to measure protein concentration in fish sera J.Fish Biol vol 16 n°2 = 115 - 122.

Aquacop, 1977. Reproduction in captivity and growth of Penaeus monodon in French Polynesia. Proc World Mariculture Soc 8 : 927 - 945.

Aquacop, 1980. Reared broodstock of Penaeus monodon. Symposium on coastal Aquaculture Marine Biological Association of India Cochin. January 12 - 18.

Aquacop, 1983. Constitution of broodstock, maturation, spawning and hatching systems for penaeid shrimps in the Centre Oceanologique du Pacifique. CRC - Handbook in press .

Balazs GH - Olbrich SE and Tumbleson ME 1974 :
Serum constituents of the Malaysia Prawn (Macrobrachium rosenbergii) and pink shrimp (Penaeus marginatus) Aquaculture 3 : 147 - 157.

Bourguet J.P. Exbrayat JM, 1977. Quelques aspects de la croissance, du développement sexuel et du métabolisme chez P.japonicus : influence des facteurs externes et internes. 1977.These USTL Montpellier France.

Bursey CR - Lane CE, 1971. Osmoregulation in the pink shrimp Penaeus Duorarum . Comp Biochem Phyriol 39 : 483 - 493

Busselen P., 1970. Effects of moulting cycle and nutritional conditions on hemolymph proteins in C.maenas. Comp Biochem Physiol 37 = 73 - 83.

Chaisemartin C, 1978. Effet du taux des protéines alimentaires sur différents "indices" physiologiques chez l'écrevisse Austropotamobius pallipes. Freshwater Crayfish 4 : 43 - 52.

Chamberlain GW - Lawrence AL, 1981. Maturation, reproduction and growth of P.vannamei and P.stylirostris fed natural diets. J of the World Mariculture Society vol. 12 n°1 : 209 - 224.

Cuzon G. Cahu C. Aldrin JR. Messager JL. Stephan G. and Mevel M., 1980. Starvation effect on metabolism of Penaeus japonicus J. of the World Mariculture Society New Orléans March 5-8 11 : 410-423.

Deshimaru O., 1976. Studies on a purified diet for prawn. VIII changes in free amino acid contents in muscle, hepatopancreas and blood of prawn after feeding. Bull - Jap. Soc. Sci. Fish 42 (6) : 655 - 660.

Drach P., 1939. Mue et cycle d'intermue chez les crustacés décapodes Ann. Inst. Oceanogr. Monaco 19 : 103 - 391.

Leavitt DF - Bayer RC, 1977. A refractometric method of determining serum protein concentration in the american lobster. Aquaculture 12 : 169 - 171.

Middleditch BS - Missler SR - Hines HB, Chang ES, Mc Vey JP, Brown A and AL Lawrence , 1980. Maturation of penaeid shrimp : lipids in the marine food Web. Proc World Mariculture Society 11 : 463-470.

Nakamura K, 1980. Quantitative analysis on the feeding patterns of the Crayfish related to the molting cycle. Mem. Fac Fish Kagoshima University 29 : 225 - 238.

Stewart JE and Li MF, 1969. A study of lobster (Homarus americanus) ecology using serum protein concentration as an index. Canad J Zool 47 : 21-28.

Stewart JE - Cornick JW - Foley DM - Li MF and Bishop CM, 1967. Muscle weight relationship to serum proteins, Hemocytes and hepatopancreas in the lobster, Homarus americanus. J Fish Res. Board Can. 24 : 2339 - 2354.

Vivares CP and Cuq JL, 1981. Physiological and Metabolic variations in Carcinus mediterraneus parasitized by Thelohania maenadi (Microspora). An ecophysiopathological approach. J Invert Pathol 37 : 38 - 46.

Table 1. - SERUM PROTEIN CONCENTRATION VARIATION RELATED TO THE MOLTING STAGE.

HEALTHY ANIMALS

Molting stage	A	B	C	D
Number of animals	20	71	146	85
Serum Protein concentration	84.19	76.87	94.01	105.57
Standart deviation	14.83	19.64	22.43	15.05
Corrector factor for the molting stage	1.1	1.2	1	0.9

Diseased animals

Molting stage	A	B	C	D
Number of animals	9	20	14	15
Serum Protein Concentration	43.86	49.72	47.15	50.61
Standart deviation	9.86	9.09	8.59	20.30

Table 2. - PROTEIN SERUM CONCENTRATION VARIATION RELATED TO THE REARING METHOD

Subgroup rearing method	N number of animals	P Protein serum concentration	
0	114	83.78	1 = 23.26
1	111	112.73	1 = 20.11
2	80	115.27	1 = 23.99

ANALYSIS OF VARIANCE df

Source of variation	SS		Variance	F
Between subgroup	77 417.6	2	38 708.58	84.54
Within subgroup	138 276.7	302	457.87	
Total	215 693.8	304		

with F 0.001 (2.302) = 6.91 differences are highly significant ($P < 0.001$)

Table 3. - SPAWNING INDEX VARIATION RELATED TO THE REARING METHOD FOR EACH CLASS OF SERUM PROTEIN CONCENTRATION.

ANALYSIS OF VARIANCE

Protein serum concentration g/l	Source of Variation		df	SS	F
120 - 130	between subgroup	15.54	2	7.77	14.88*
	within subgroup	17.23	33	0.52	
	total	32.77	35		
110 - 120		27.28	2	13.64	9.47*
		47.67	33	1.44	
		74.85	35		
100 - 110		17.48	2	8.74	7.95*
		50.38	46	1.10	
		67.86	48		
90 - 100		29.72	2	14.86	12.05*
		65.36	53	1.23	
		95.08	55		
80 - 90		8.86	2	4.43	10.29*
		16.79	39	0.43	
		25.65	41		

* highly significant $P < 0.001$

Table 4 : LINEAR CORRELATION BETWEEN THE SPAWNING INDEX (I.P.) AND THE SERUM PROTEIN CONCENTRATION (P).

REARING METHOD	ANALYSIS	LINEAR CORRELATION
0	n = 113 f = 5,76*	I.P. = 0,219 + 0,0049 P
1	n = 111 f = 2,23*	I.P. = 0,325 + 0,0083 P
2	n = 80 R_2 = 0,85*	I.P. = 0,440 + 0,0138 P

* SIGNIFICANT : $P < 0,001$.

Table 5. - ESTIMATION OF NATURAL ZOOPLANCTON OCCURING IN PONDS.

* Qualitative studies

Most common = Copepods Acartia fossac in ponds T_2 T_6
Cyclogoide 675 um in pond C.

Miscel aneous: Rotifers, Mysids, Sergestids, Carid shrimps

* Quantitative studies from August to September 1982

Concrete pond C : 2 200 m3 high density 64/m2

Earth pond T_6 : 3 400 m3 low density 0.8/m2

Earth pond T_2 : 1 000 m3 medium density 14/m2

date	pond C	pond T_6	pond T_2
08/13	5.0*	2.1	2.3
08/19	0.18	4.0	0.5
08/20		11.0	0.2
08/24	0.05	5.0	0.1
08/25	3.0		0.9
08/26	1.7	29.0	
08/27	13.0	19.0	0.4
09/03	0.09	147.0	

* mg/m3 natural zooplancton.

FIG. 2 : LINEAR CORRELATION BETWEEN THE SPAWNING INDEX AND (I.P.) THE SERUM PROTEIN CONCENTRATION (P)

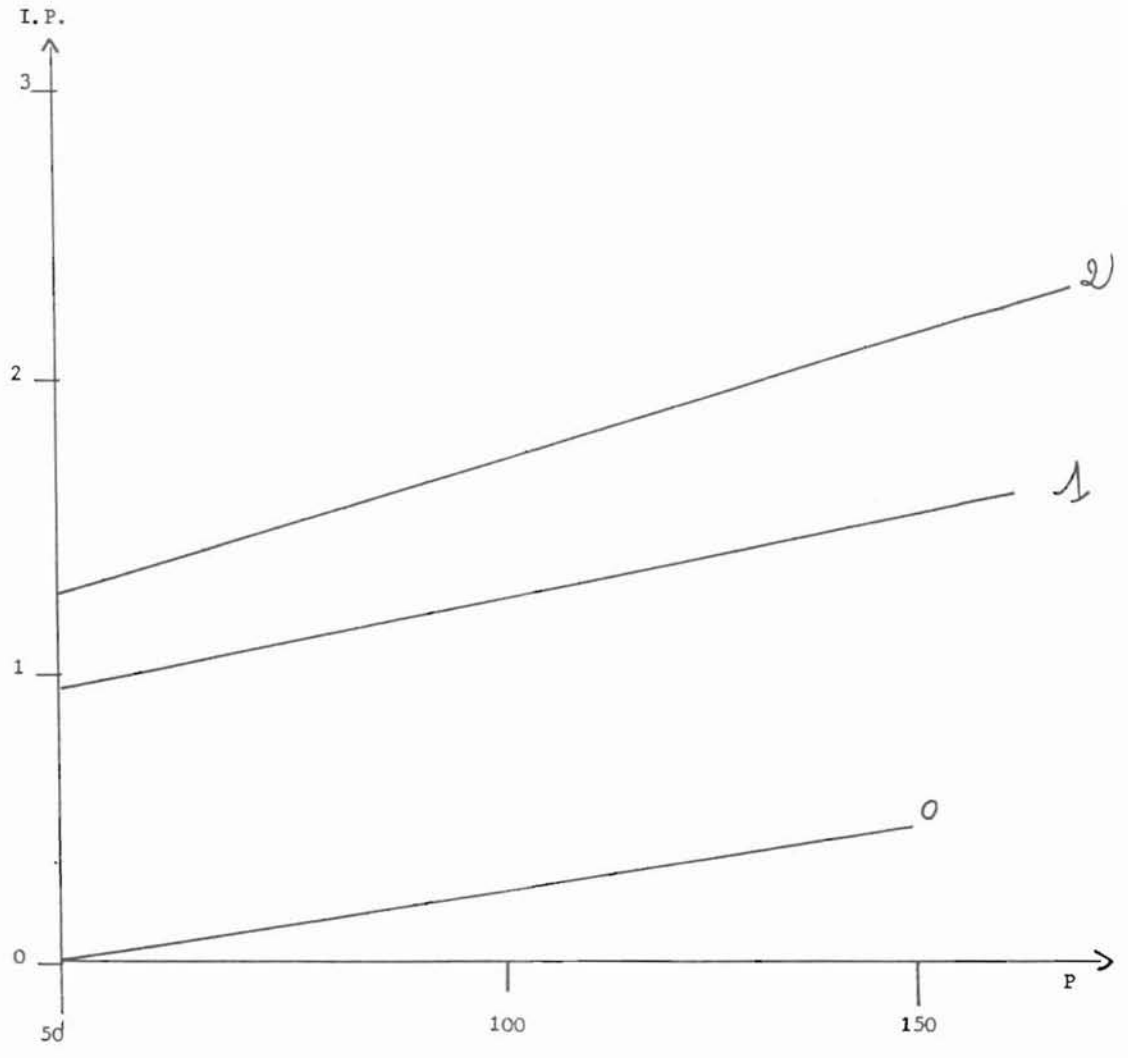
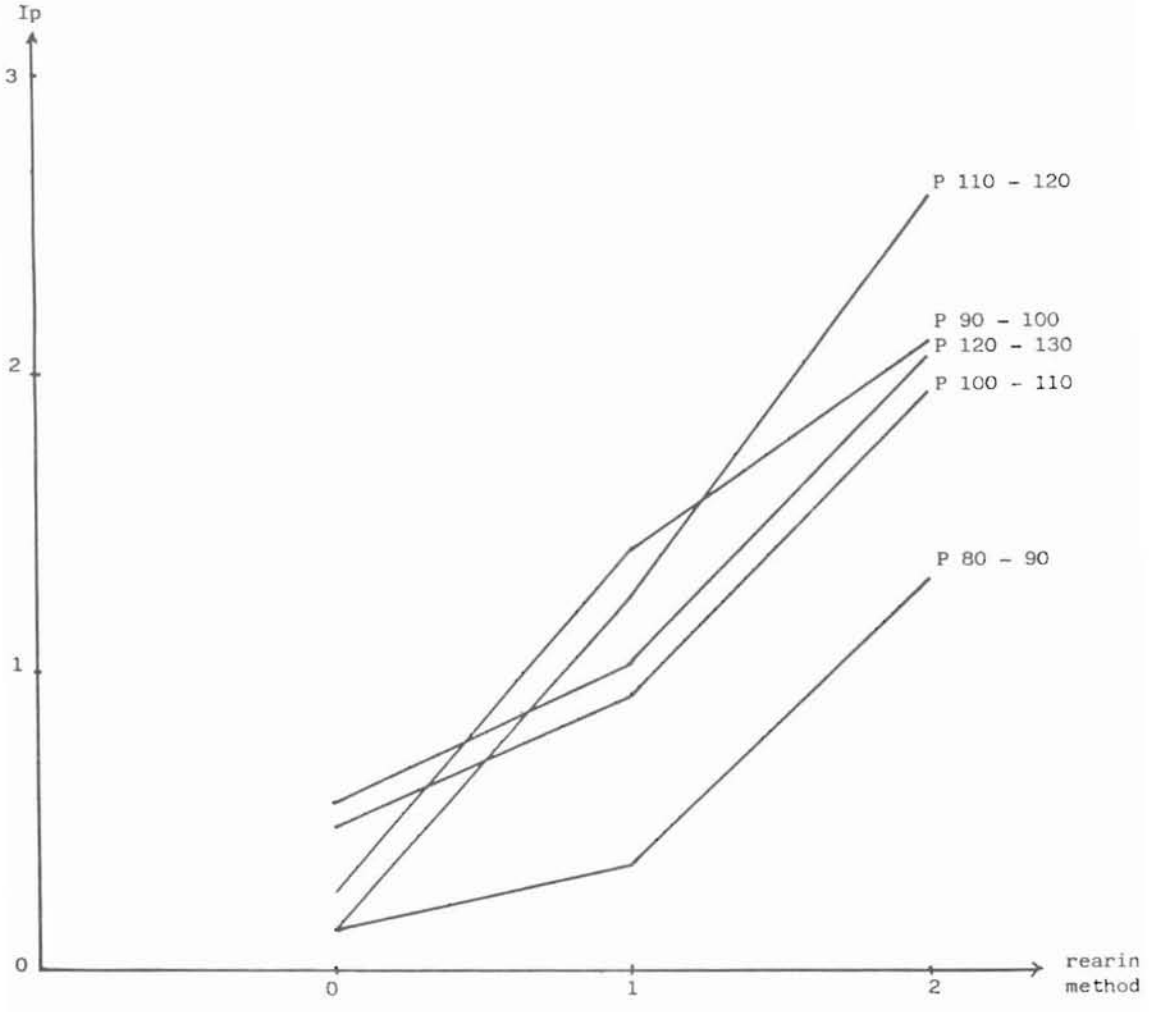


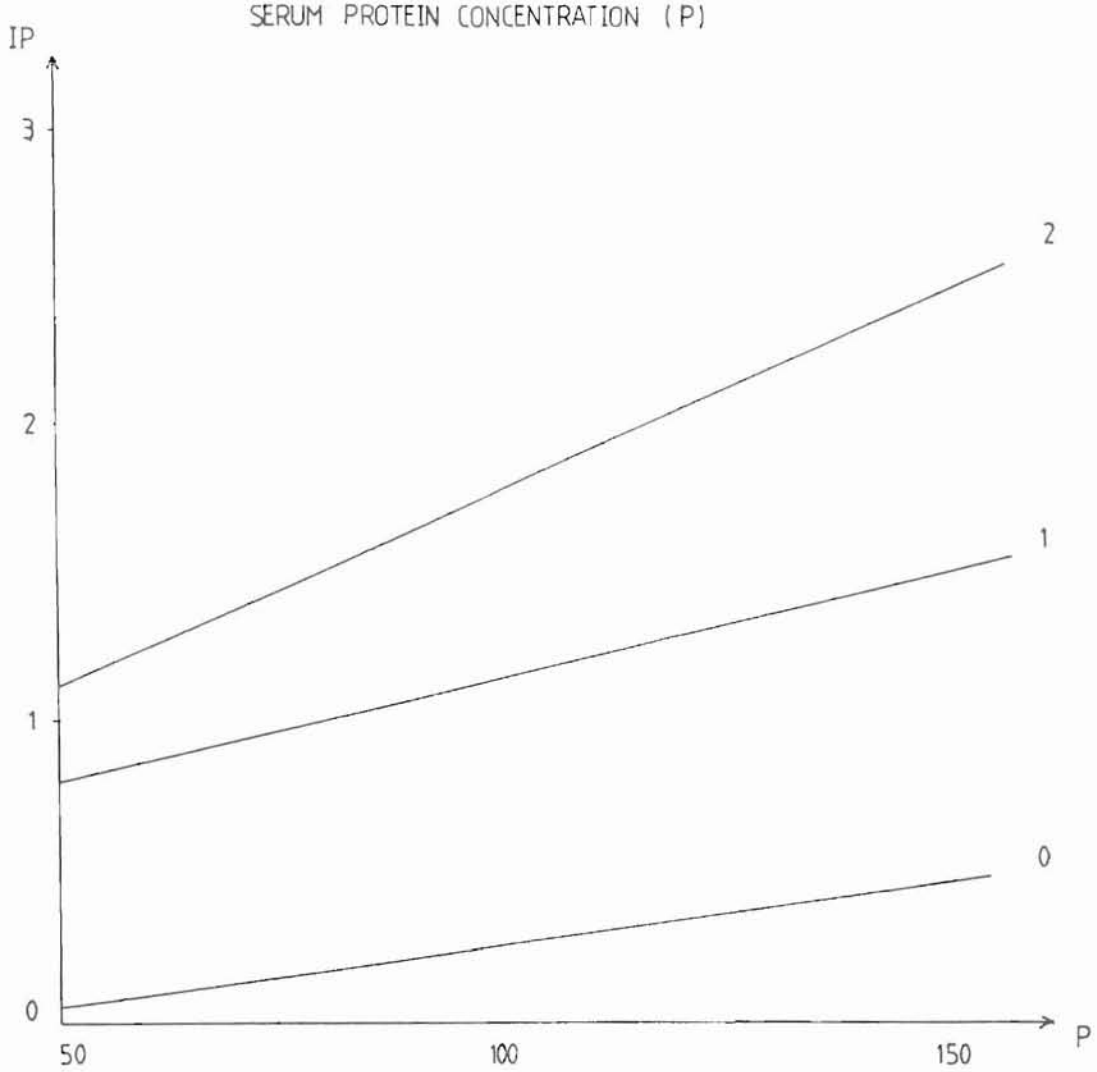
FIGURE 1. - SPAWNING INDEX VARIATION RELATED TO THE REARING METHOD.

Ip = Spawning index

P = Serum protein concentration (g/l)



LINEAR CORRELATION BETWEEN THE SPAWNING INDEX (IP) AND THE SERUM PROTEIN CONCENTRATION (P)



REARING METHOD	ANALYSIS	LINEAR CORRELATION
0	N = 113 F = 5,76 *	IP = 0,219 + 0,0049 P
1	N = 111 F = 2,23 *	IP = 0,325 + 0,0083 P
2	N = 80 R^2 = 0,85 *	IP = 0,440 + 0,0138 P

* significant $P < 0,001$