

**OBSERVATIONS ON THE MATURATION, RE-MATURATION AND SPAWNING IN CAPTIVITY OF *PENAEUS MONODON* (SUGPO) FABRICIUS IN MSU-IFRD**

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Sugpo (*P. monodon* Fabricius) is an Indo-Pacific species particularly abundant in the Philippines, Taiwan and Indonesia where it is raised with *Chanos chanos* (Ling, 1972). Postlarvae of penaeids are caught in the field but hatcheries have been developed in more recent years. Main data on the biology and larval rearing of penaeids in the hatcheries are available from the works of Villaluz (1969), Liao (1973), Forster and Beard (1974), MSU-IFRD team (1975) and SEAFDEC team (1977). However, rearing of commercial penaeids in many countries of the world is entirely dependent on the availability of wild spawners. In MSU-IFRD sugpo hatchery, the problem of continuous supply of spawners is far from being solved. The gradual depletion of this species in our source, Panguil Bay, by indiscriminate fishing is a primary threat. In recent observations, the use of a fine-mesh net (*sanggab*) by fishermen in the area claims even juveniles. In this regard, studies on gonadal maturation and re-maturation of sugpo in captivity is deemed necessary.

Records of reproduction in captivity of penaeids are few and quite recent. It has been said that Fujinaga had obtained several generations of *P. japonicus* in captivity but no details are available. Shokita (1970) obtained spawning of *P. latisulcatus* maintained in aquarium. Idyll (1971) and Caillouet (1973) induced ovarian maturation of *P. duorarum* by a double eyestalk ablation but no spawning occurred. Liao (1973) noticed maturation of *P. penicillatus* and *P. monodon* in captivity, but the eggs were not fecundated. Moore, et al. (1974) obtained viable eggs from *P. californiensis* which have matured in captivity. Aquacop (1975) reports having obtained in captivity numerous

spawnings and multiple generations of *P. merguensis* (F<sub>6</sub>) and *P. aztecus* (F<sub>3</sub>) and some spawnings of *P. japonicus* (F<sub>2</sub>) and *M. ensis*. Arnstein and Beard (1975), after unilateral eyestalk ablation, induced the maturation of *P. orientalis*, *P. monodon*, and *P. occidentalis*, but the eggs were not fecundated. The induction of maturity and spawning in sugpo by unilateral eyestalk ablation was reported by Alikunhi (1975), a SEAFDEC team (1976) and Aquacop (1977).

The present study is geared toward the maturation of non-gravid females and re-maturation of spent spawners that were collected from Panguil Bay and had successfully spawned at MSU-IFRD hatchery, as well as the induced sexual development of hatchery-produced sugpo postlarvae and juveniles in order to have a ready and continuous supply of spawners.

### Materials and Methods

*Obtaining spawner stock.* Sugpo (average weight: 127.29g) utilized in the study were taken from Panguil Bay and were transported to MSU-IFRD laboratory in oxygen inflated plastic bags stored in styrofoam boxes. Spawners were utilized first in the mass production of sugpo fry project and after successful spawning, these same specimens were utilized for maturation study. Postlarvae (0.78 g to 1.18 g) and six-month old sugpo (19g) harvested from the hatchery and fishponds were also in the study.

*Maintaining spawners.* The environment and rearing conditions were similar to those described for the reproduction in captivity of other penaeid species (Moore *et al.*, 1974; Aquacop, 1975): salinity 34 to 35 ppt, water temperature 25 to 29°C, pH 8.15 to 8.35, reduction of natural light by Coralex roofing, artificial food pellets. Clam meat and dried squid were also introduced as feeds. Hollow blocks and PVC pipe shelters were used instead of a sandy-muddy substratum.

*Maturation and spawning in captivity.* The specimens were measured and weighed before extirpation. They were handled with extra care so as not to subject them to undue stress. After measurement, the

In 1976, Stage III gonad development was again observed in one specimens were stocked in specified containers equipped with aeration. Feeding was administered immediately after stocking. Maturation was induced by pinching the eyestalk between fingers to remove one or both eyes. This was done on spent females from 65 to 185 grams. Females were examined every four days. This was done by handling the live animal against the light and observing any changes in the shape of the ovary. Color, shape and texture of the ovary were noted. Females which were about ready to spawn were isolated in a 16-tonner concrete tank paired with males on its second molting period. Everyday feeding with pellets, fresh clam meat and dried squid was done together with observations on molting frequency and feeding consumption of ablated and unablated females as well as males. To minimize stress on specimens as a result of handling, growth measurement by weight determination was not made daily and was optional for spent spawners.

*Developing artificial feed.* Based on the studies on artificial feeds for sugpo conducted by Destajo (1978), the composition of pelletized formula feed was used. The amount given was a percentage of the estimated biomass following the ratio given by Parker *et al.*, (1974). Adjustments were made by direct examination of the remaining food three to twelve hours after the distribution.

## Results

*Ablation experiments.* In 1975, the first batch of bilateral ablation was performed on three age-groups: four P<sub>35</sub>, 10 P<sub>65</sub> and eight 6-month old sugpo taken from the fishpond of MSU-IFRD. P<sub>35</sub> (average weight, 0.79 g) and P<sub>65</sub> (average weight, 1.18 g) were stocked in three-liter jars at one postlarvae per jar. The 6-month old sugpo were stocked in 0.5 ton wooden tank with bamboo screen divider. Each compartment was stocked with 2 specimens, one male and one female. Ablation was done on 50% of the total number of each age-group.

Table 1 and Fig. 1 show the length and daily length increments in P<sub>35</sub> consequent to eyestalk ablation. Trial 1 for ablated specimens resulted in 64.44% increase in length in 48 days giving 1.34% average increase in length per day. Trial 1 for the control gave 42.86% increase in length in 51 days with the rate of average percent increase in daily length equals to 0.84%. In trial 2, for the ablated one, 86% increase in

length was obtained in 42 days with 2.05% average daily increase in length; for the unablated, 62.5% increase was obtained in 51 days which resulted 1.23% increase per day. Daily percent increment in the control was lower than that of the ablated specimens.

Table 2 and Fig. 2 present the weight and percent weight increments in P<sub>35</sub> consequent to eyestalk ablation. Trial 1 specifies 683.33% increase in weight in 48 days for the ablated, which resulted to 14.24% average daily increase; for the control, 138.33% increase in weight in 51 days resulting to 2.71% average increase in daily weight. Trial 2 for the ablated resulted in 541.67% increase in 42 days, which equals to 12.90% average daily increase in weight; for the control, 412% was obtained in 51 days which gave 8.08% average increase in daily weight. The average daily increase in weight was greater in ablated (specimens) than in unablated specimens.

Table 3 shows the growth of P<sub>65</sub> sugpo consequent to eyestalk ablation. The percent increment in weight in ablated specimen was contrary to what was expected. Table 4 summarizes the result of the molting frequency of ablated and unablated six-month old hatchery-produced specimens. Molting frequency decreased with age.

Bilateral ablation on spent spawners was conducted in batches depending on the availability of stock specimens in 1975, 1976 and 1978. In 1975, four completely spawned spawners and one male sugpo taken from Gango, Misamis Occidental were conditioned in a 3-ton capacity wooden tank. After one week of conditioning, they were ablated of both eyestalks and were transferred to a 20 m<sup>3</sup> concrete tank. They were fed with 300 g of fresh/frozen *amahong* and 200 g wheat germ given alternately per feeding to ensure abundance and variation of food. It was observed that molting after eyestalk ablation on spent spawners was not frequent compared to molting of P<sub>35</sub> and P<sub>65</sub> specimens. Every 4 days, each spent spawners was examined for re-matured gonad. One week after ablation, one spawner was observed to have re-developed its ovaries approximately Stage III (based on Rao, 1963). Unfortunately, this specimen died.

Spent spawners were observed to have grown abnormally long and twisted pincers and pereopods so that it could hardly grasp and hold the food given until they die of starvation. Fungal infection of the appendages also set in.

of three ablated spent spawners stocked in compartmentalized 0.5-ton wooden tanks (Table 5). This specimen died after having jumped out of the compartment. No gonadal development was observed in three other unablated ones, just like in previous set-ups.

In 1978, 19 spent spawners were utilized in the gonadal development experimental set-up. These were stocked in nylon screen cages placed in a 20 m<sup>3</sup> concrete pool. Ten were extirpated of both eyestalks and 9 were left unextirpated. Pairing with male sugpo was done after the females had molted twice. Molting frequency is shown in Table 6. The operation was terminated after 37 days (on March 29, 1978) when all the extirpated females died. The mortality of extirpated females was attributed to poor water management in the 20 m<sup>3</sup> compartment of the concrete pool where the nylon cages were situated. Besides, algal bloom could not be minimized since the set-up was under direct sunlight. However, from this set-up, it was determined that feeding 10 grams of clam meat in the morning and 15 grams in the afternoon is sufficient for 2 specimens in one cage. The observation of molting frequency gave an average rate of once for every 16-22 days for females and once every 24-30 days for males.

In the same year, on May 18, 1978, 11 spent females were used as control and 12 were unilaterally ablated. The cages are now placed in the 20 m<sup>3</sup> concrete tank in the coralex-roofed hatchery. After extirpation the specimens were paired with males. One hundred percent survival was attained in both experimental and control specimens. Aside from clam meat and dried squid, pelletized formula feed were given. Penetration of light was reduced by the coralex roof. Free-flowing of fresh seawater was done once or three times daily. No gonadal development was observed. After 2 months the specimens were released from the nylon screen cages to the 20 m<sup>3</sup> concrete tank. A month after their release, 4 ablated spent spawners showed ovarian development: Stage III and Stage IV and 3 unablated spent spawners also displayed ovarian development: Stages II and III. These specimens were isolated separately in two groups in two spawning tanks with 2 males per group. Three of the ablated specimens spawned with an average of 113,000 eggs per spawner. The unablated specimens with developed ovary were found to have a complete regression of their ovaries 4 days after stocking in the

spawning tanks. Details of the larval rearing of the F<sub>1</sub> generation of the spent spawners will be given in a separate report.

### Discussion

Removal of eyestalk in prawns and other crustaceans hastens molting and results in increased absorption of water following each molt (Carlisle and Knowles, 1959; Green, 1967; Waterman, 1960). Farges (1975), on the other hand, stated that the role of the neurosecretory cells of the eyestalk glands of decapod and their specific action on the molt and the maturation cycles are not well known. In the experiment with P<sub>35</sub> and P<sub>65</sub> specimens, the unilateral eyestalk ablation resulted to greater molting frequency and greater increase in percent weight and percent length increments of ablated sugpo than the control animals in the same experimental conditions and the same age. However, sexual maturity was not observed after a series of molting in P<sub>35</sub> and P<sub>65</sub> specimens. It is probable that these age groups are too small for the induced sexual development set-up. Aquacop team (1977) reported that at 40 to 100 g size range, sugpo regularly molted every 3 weeks and immediately impregnated prior to any ovarian development, and maturation signs only show when one eye was pinched and after 7 days, ovarian development occurred.

Ovarian development in unablated specimens was not observed by the maturation team in French Polynesia as well as in SEAFDEC Tigbauan, Iloilo. In the case of unilaterally ablated spent spawners (65 to 127.29 g) in this report, maturation and spawning at an average of 113,000 eggs per spawning was exhibited and in the control of the same size range, ovarian development was also observed. Aquacop (1977) reported that in 6 females between 45 and 130 g in 18 spawnings give an average of 180,000 eggs per spawning. The fecundation process therefore, and the viability of eggs of ablated spawners are not satisfactory. At this point, it is too early to give an explanation. In the control of the size range 65 to 127.29 g, another thing happened. Complete regression of the ovaries was noticed 4 days after isolation. Regression of ovaries was also noticed in bilaterally ablated *P. aztecus* (Aquacop, 1975). The same phenomenon was observed in *P. monodon* by the Aquacop team (1975) on unilaterally ablated females 2 months after

maturation. Recently, Aquacop (1977) reported that regression of ovaries in (*P. monodon*) happens if handling takes place more than 48 hours before spawning. This is highly probable in the unablated specimens in this report.

Bilateral ablation induces complete development of ovaries in *P. aztecus* (Aquacop, 1975). In spent *P. monodon* spawners which were bilaterally ablated, it was observed that the operated females are weak and gradually die as observed in the result of the 100% mortality of the specimens in the experimental set-up.

Light intensity seems to be an important factor. Aquacop (1975) observed more maturations in a tank with a cover. In the experimental setup conducted under coralex roofing, mature and spawner stocks were obtained only when the specimens were released from cages to a wider surface area. Space could be a factor other than light intensity.

Tuma (1967) working on wild stocks of *P. merguensis* writes, "It appears that most adult females undergo insemination after each ecdysis until finally ripe ovaries occur in conjunction with insemination. Aquacop (1975) reported that in captivity, *P. merguensis* insemination took place after each ecdysis but always with undeveloped ovaries except for *P. aztecus* with the eyestalks removed. In *P. monodon*, whether or not fertilization is necessary to induce the maturation process still remains a question. However, Aquacop (1977) reported that on sugpo, although maturation is always obtained, it is observed that ovulation process does not go on until spawning.

At this juncture, we recognize that there remain many questions to be answered by means of other experiments. Hence, this study is still going on.

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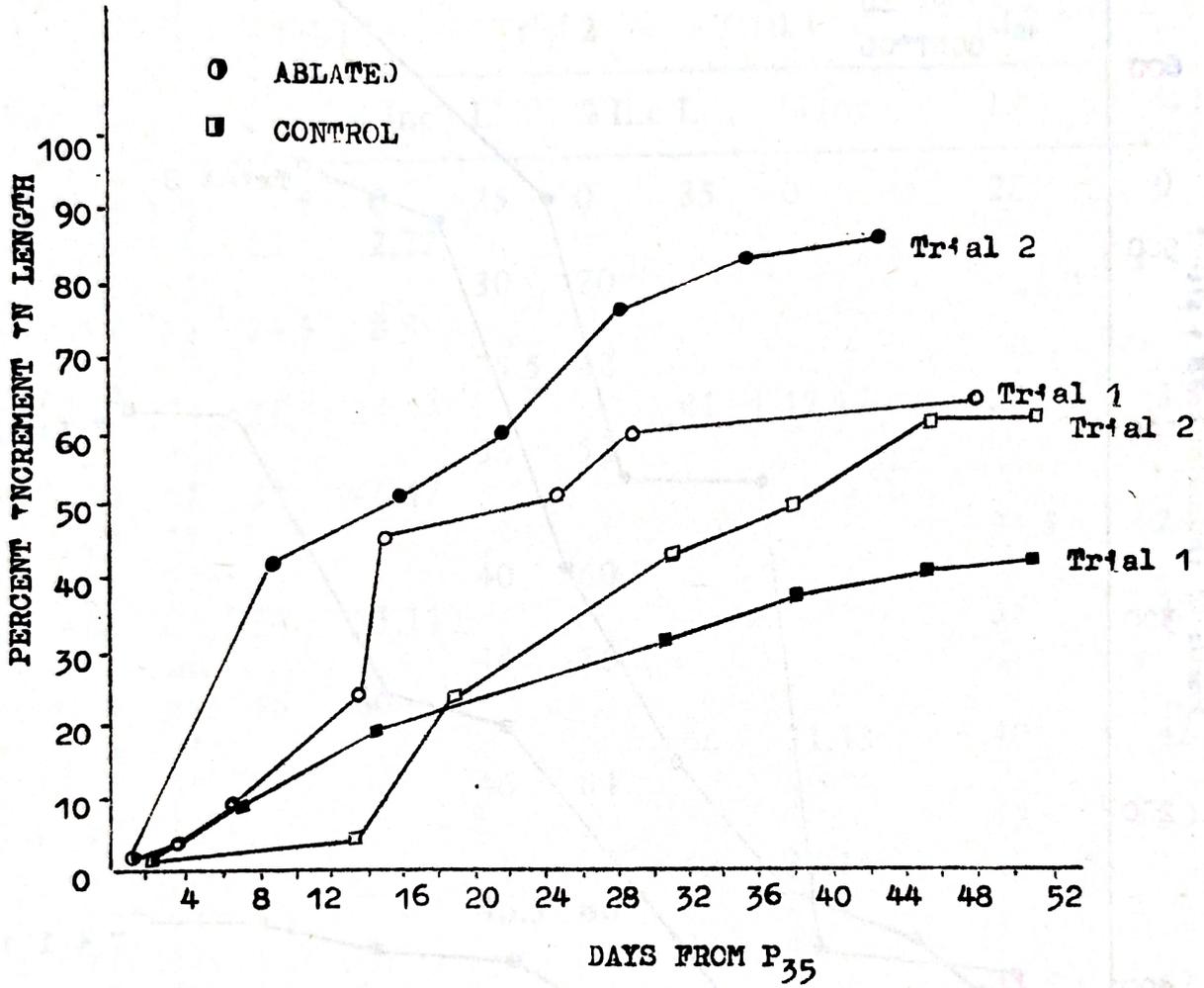


Fig. 1. Percent length increments from P<sub>35</sub> in ablated and unblated specimens

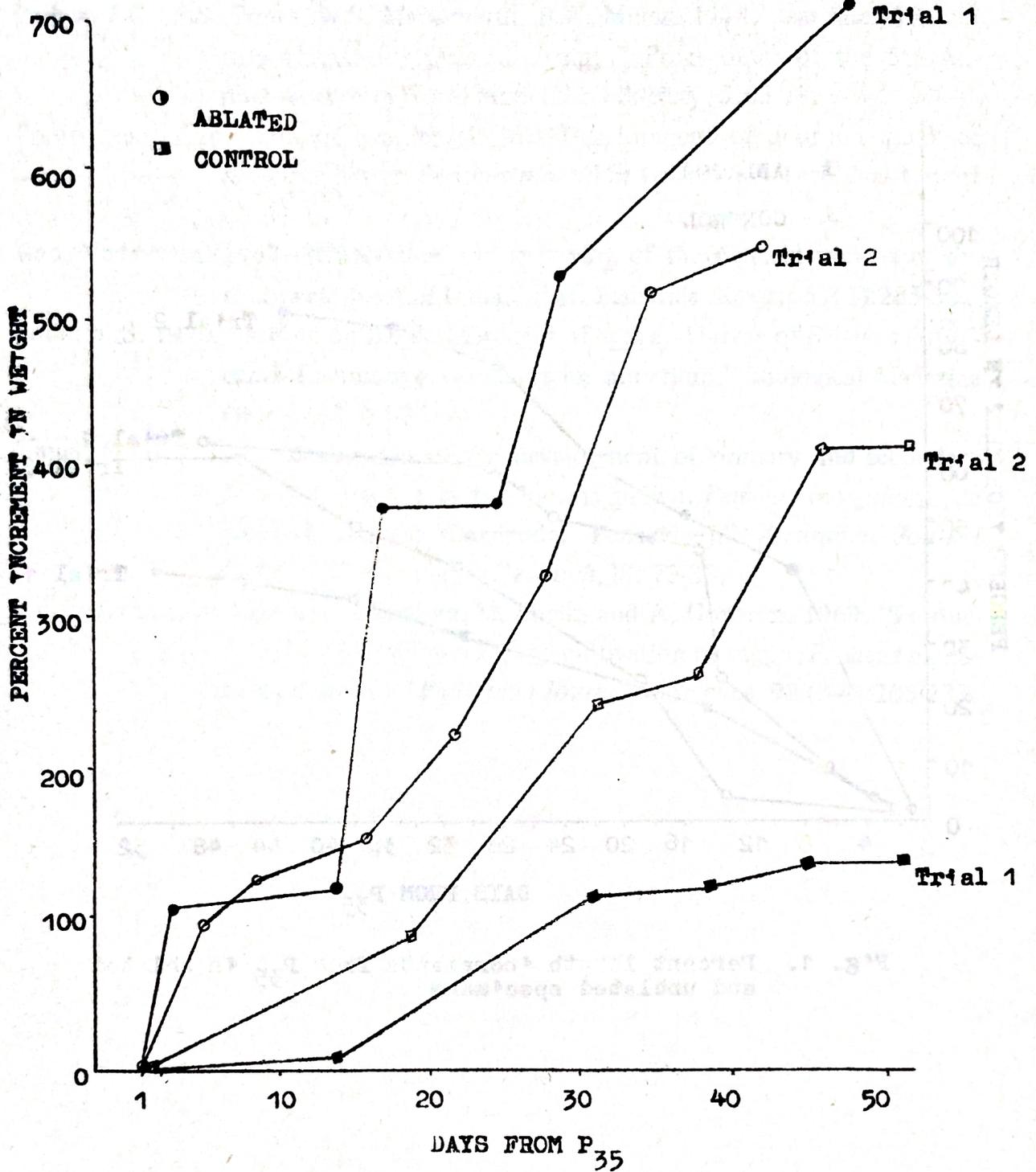


Fig. 2 Percent weight increments from P<sub>35</sub> in ablated and unablated specimens.

Table 1. Length and daily length increments in P<sub>35</sub> consequent to eyestalk ablation.

Date	Day	ABLATED				CONTROL				
		Trial 1		Trial 2		Trial 1		Trial 2		
		L	% Inc							
May	4	1	22.5	0	25	0	35	0	28	0
	6	3	23	2.22						
	8	5			30	20				
	10	7	24.5	8.89						
	12	9			35.5	42				
	17	14	28	24.44			41	17.14	29	3.57
	19	16			38	52				
	20	17	33	46.47						
	23	19							34.5	23.21
	25	22			40	60				
	28	25	34	51.11					35	25
	31	28			44	76				
June	1	29	36	60						
	3	31					46	31.43	40	42.86
	7	35			46	84				
	10	38							42	50
	11	39					48	37.14		
	14	42			46.5	86				
	17	45					49	40		
	18	46							45.5	62.5
	20	48	37	64.44						
	23	51					50	42.86	45.5	62.5

**Table 2. Weight and percent weight increments in P<sub>35</sub> consequent to eyestalk ablation.**

Date	Day	ABLATED				CONTROL			
		Trial 1		Trial 2		Trial 1		Trial 2	
		Wt	% Inc	Wt	% Inc	Wt	% Inc	Wt	% Inc
<b>May</b>									
4	1	.12	0	.24	0	.60	0	.25	0
6	3	.25	108.33						
8	5			.47	95.83				
12	9			.53	120.83				
17	14	.26	116.67			.64	6.67	.40	60
19	16			.61	154.17				
20	17	.57	375.00						
22	19							.47	88
28	25	.57	375.00						
31	28			1.01	320.83				
<b>June</b>									
1	29	.75	525.00						
3	31					1.29	115.00	.85	240
7	35			1.48	16.67				
10	38							.90	260
11	39					1.32	120.00		
14	42			1.54	541.67				
17	45					1.43	138.33		
18	46							1.28	412
20	48	.94	683.33						
23	51					1.43	138.33	1.28	412

Table 4. Molting frequency of 6-month old hatchery produced specimens consequent to eyestalk ablation.

		T A N K N O.			
		A	B	C	D
Date		1	2	1	2
2-19-76	Start of the experiment				
2-20-76	M				
2-23-76	M				M
2-24-76				M	
2-28-76					
3-1-76	D				
3-10-76				M	M
3-14-76					M
3-17-76			M		

Legend: M -- molted    D -- dead

Table 5. Molting frequency of spent spawners placed in compartmentalized wooden tanks after ablation.

Date	TANK NUMBER											
	A	B	C	D	E	F	M	F	M	F	M	F
1976												
Feb. 26		X	X	X	X							
27	X											
March 5			X									
7				X								
9		X										
13			X									
15				X								
17			X									
19	X											
25						G,D						

Legend: A, B, C = Ablated  
D, E, F = Control

F = Female X = Molted

M = Male G = Ovarian development (Stage III) observed but the specimen died



Table 6. (Cont'd.) Molting frequency of ablated and unablated spent spawners.

DATE	1978																			
	ABLATED										UNABLATED									
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	
March																				
13			M																	
14				M	M						M							M		
15							D													
16			M	M																
17		D																		
18													M			M				M
19															M					
20								D	D			M								
21																M				M
22																				
27			D																	
28																				D
29																				D

Legend: M - molted. D - dead.

Table 3. Growth of P<sub>65</sub> sugpo fry consequent to eyestalk ablation.

Day	Length (cm)				Weight (g)			
	Ablated		Control		Ablated		Control	
	Ave L	% Inc	Ave L	% Inc	Ave Wt	% Inc	Ave Wt	% Inc
1st sampling	42.75	0	35.00	0	1.21	0	.69	0
2nd sampling	44.19	3.37			1.34	10.74		
3rd sampling	47.94	12.14	37.25	6.43	1.40	15.70	.87	26.09
4th sampling	50.19	17.40	41.00	17.14	1.70	40.50	1.03	49.28