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Effects of Unilateral Eyestalk Ablation on Growth in Juvenile Blue Crabs *Portunus pelagicus* (L.) (Crustacea: Decapoda: Portunidae)*

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Abstract

The effects of unilateral eyestalk ablation on growth in *Portunus pelagicus* were investigated using the physiological energy budget method and checked by means of actual growth performance and body condition indices.

Unilateral eyestalk ablation significantly increased absorption efficiency and dry meat yield (% of dry weight) by 12.3% and 33.2%, respectively. It also accelerated molting by 3.4 days. The physiological mechanisms of growth enhancement are increased absorption efficiency and possi-bly also increased feeding rate and decreased energetic loss to carapace. The increased absorp- tion efficiency, standard ammonia excretion rate, and low O:N ratio in ablated crabs indicate the need to determine optimal lipid and protein levels in the diet.

Dry meat yield is suggested as a better gauge than whole wet weight for assessing growth response to eyestalk ablation since the reducing effect of ablation on carapace size tends to mask its weight gain enhancement effect.

Introduction

The blue swimming crab, *Portunus pelagicus* ("alimasag"), is the most important species in Philippine crab fishery, followed by *Scylla serrata* (mud crab). Despite its greater contribution (16,687 t in 1986 against 637 t for *S. serrata*) to the Philippine crab fishery and its overall economic importance in the Indo-Pacific region, the biology and culture of the species is less known than those of *S. serrata.*

Growth enhancement is one of the primary aims of aquaculture research efforts. Investigations on a number of crustaceans have shown the efficacy of

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* Best Paper Award, Silver Anniversary Convention, Federation of Institutions for Marine and Freshwater Sciences (FIMFS). Dedicated to Carlo and Kristi for their inspiration.

eyestalk ablation in accelerating molting and enhancing growth and gonadal development (Passano 1960; Bliss 1966; Skinner 1984). This has yet to be investigated in *P. pelagicus*. The present study was made to assess the effects of unilateral eyestalk ablation on the energy budget and growth performance of juvenile female *P. pelagicus*.

Materials and Methods

P. pelagicus female juveniles (15-20 mm carapace length) were collected from the wild. The collected specimens (intermolt stage only) were acclimated to laboratory conditions (34-35 ppt, 25-28°C) for about 1.5 months in 18 x 40 cm compartments (3-4 crablets per compartment) in tanks with a flow-through seawater system. The crablets were fed *ad libitum* with boiled chicken intestine four times a day at 6-hour intervals. Only freshly molted crabs that had completely mineralized were selected for the experiments.

The experiments were arranged in randomized complete block design (RCBD) with four crabs per treatment in each of three blocks, blocks being molting batches.

Ablation was done by severing the left eyestalk as described by Caillouet (1973). The wound was cauterized by placing a hot, blunt rod over it.

The effects of unilateral eyestalk ablation on growth were assessed using the physiological energy budget method and checked by means of actual growth performance and body condition indices. Physiological measurements were done in round chambers 24 hours after ablation to permit adjustment to the treatment and chamber conditions and to allow stabilization of metabolism. Physiological measurements were done for 18 hours to obtain a more representative average rate and to determine feeding rates at different times of the day. Holding water was changed (100%) before each feeding.

Feeding rate was estimated by allowing the crabs to feed on a preweighed amount of boiled chicken intestine at each feeding schedule (0800, 1400 and 2000 hours) and weighing the amount of food remaining after 6 hours. Chicken intestine was used as food to test its usefulness as crab food and also because its supply is fairly constant and its proximate composition appears fairly uniform throughout its length. To account for possible weight changes of the food, blanks (chambers without crabs) were provided in which weight changes in the food were monitored. Feeding rate (F.R.) was then computed using the expression (based on Bakke 1988):

$$\text{F.R. } (\text{mg}\cdot\text{g}^{-1}\cdot\text{hour}^{-1} = [W_0(1+k) - W_t]/T/WA$$

where W_0 and W_t are the wet weights (mg) of the food given and that remaining after 6 hours, k is the mean fraction weight change in blanks, T is feeding time of 6 hours, and WA is the whole animal wet weight (g).

Absorption efficiency was measured using the ratio method of Conover (1966, in Widdows and Johnson 1988) as follows (E in Conover's formula was changed to Fe to avoid confusion with E in the scope for growth equation):

Absorption efficiency = (Fo - Fe)/[Fo(1-Fe)]

where Fo = ash-free dry weight (AFDW):dry weight (DW) ratio of food; Fe = AFDW:DW ratio of feces.

After each feeding, the respiration rates of individual crabs were monitored by confining the crabs in water with known initial oxygen concentration and measuring the amount remaining after a time interval. For each interval, initial oxygen concentration was at full air saturation. A YSI DO meter calibrated by the Winkler method was used. Each chamber was provided with a magnetic stirring rod to ensure an even distribution of oxygen in the medium prior to measurement. After measurement of the initial oxygen content, the chambers were covered with acetate, including an orifice that was provided for the oxygen probe. Blanks (chambers without crabs) were also included to correct for possible oxygen consumption/production by microorganisms in the water. The rate of oxygen consumption (VO_2) was calculated as follows (from Bakke 1988):

$$VO_2\ (\text{mg } O_2 \cdot g^{-1} \cdot \text{hour}^{-1}) = \frac{[(C_0 - C_1) \pm (Cb_0 - Cb_1)]60V}{WA.t}$$

where C_0 and C_1 are oxygen concentrations at start and finish after time interval t (min); Cb_0 and Cb_1 are start and end oxygen concentrations of blanks (deducted from C_0 - C_1 if +; added if -); V is the volume of the medium (1 l); and WA is the crab wet weight.

The rate of ammonia excretion was determined by sampling (with replacement) the holding water 3 hours after each feeding schedule. Water samples (in triplicate) from all treatment chambers including four blanks were analyzed for ammonia using the phenol-hypochlorite method (Solorzano 1969). Ammonia excretion rate (E.R.) was calculated as described by Bakke (1988):

$$E.R.\ (\text{mg } NH_3 \cdot g^{-1} \cdot \text{hour}^{-1}) = \frac{(Ne_t - Ne_0)V}{WA.t}$$

where Ne_t and Ne_0 are concentrations of NH_3 (mg/l) in treatment and blank chambers, after time t of 3 hours; V is the volume of the incubating medium (1 l); and WA is the crab wet weight.

After the 24-hour measurement of physiological parameters, the crabs were allowed to evacuate their guts for about 24 hours for the determination of the standard respiration and excretion rates.

The energy content ($J \cdot mg^{-1}$ AFDW) of the food was determined by using an isothermal jacket bomb calorimeter. The oxienergetic coefficient used was 14.22 $J \cdot mg^{-1}$ O_2 (calculated based on different diets in *M. rosenbergii,* Clifford and Brick 1979). For ammonia, an energy equivalent of 19.7 $J \cdot mg^{-1}$ NH_3 (Clifford and Brick 1979) was used. In converting calories to joules, the joule equivalent of the thermochemical calorie (4.184, Brafield and Llewellyn 1982) was applied.

The scope for growth was computed on a per-hour basis using the formula (Warren and Davies 1967):

$$SFG = A - (R + E)$$

where: A (absorbed ration) = C x absorption efficiency

$$\begin{aligned} \text{C (total consumption)} &= \text{feeding rate } (\text{mg}\cdot\text{g}^{-1}\cdot\text{hour}^{-1}) \times \text{DW} \\ &\quad \times \text{AFDW} \times \text{energy content } (\text{J}\cdot\text{mg}^{-1}) \\ \text{Absorption efficiency} &= (Fo\text{-}Fe)/[(1\text{-}Fe)Fo] \end{aligned}$$

where Fo = AFDW:DW ratio of food
Fe = AFDW:DW ratio of feces

R (respiration) = mg $O_2\cdot g^{-1}\cdot hour^{-1} \times 14.22\ J\cdot mg^{-1}\ O_2$
E (excretion) = mg $NH_3\cdot g^{-1}\cdot hour^{-1} \times 19.7\ J\cdot mg^{-1}\ NH_3$

The maintenance ration was obtained by regressing feeding rates of all individuals per treatment in one block against their corresponding SFG. The feeding rate at which SFG = 0 (or the intercept of the regression line) is taken as the maintenance ration.

Physiological measurements were done within only 1 molt period but rearing was continued for a month to allow detection of molt acceleration and actual growth enhancement. Actual growth and body condition indices were subsequently determined.

Actual growth parameters determined include weight gain after one month, carapace length increment after molting and after one month, and molt interval.

The body condition indices determined were:

Moisture (%) = (WAWW-WADW) x 100/WAWW
Dry weight (%) = (WADW/WAWW) x 100
Dry meat of dry weight (%) = (MDW/WADW) x 100
Dry meat of wet weight (%) = (MDW/WAWW) x 100
Length-specific meat dry weight = MDW/CL
Meat organic content = (MAFDW/MDW) x 100

where WAWW is whole-animal wet weight, WADW is whole-animal dry weight, MDW is meat dry weight, CL is carapace length, and MAFDW is meat ash-free dry weight.

The O:N ratios were also determined as the ratio (in atomic equivalents) of the oxygen consumed to the nitrogen excreted by the animal.

Initial statistical analysis was done using analysis of covariance (ANCOVA) for a RCBD. However, the effects of blocking and that of the covariate were found to be insignificant in most of the parameters analyzed. Hence, the data were re-analyzed using analysis of variance (ANOVA) for a completely randomized design (CRD) to increase the degrees of freedom. Means were compared using LSD test.

Results

Energy Budget and Related Data

The energy budget and related data of ablated and control crabs are shown in Table 1. Although ablated crabs apparently consumed and absorbed more food and spent more energy for respiration, the differences between the two treatments were not significant. As a result, the scopes for growth between the two treatments were also not significantly different. However, analysis of the effect of ablation on food absorption efficiency using one-way ANOVA for RCBD instead of a CRD yielded a significant effect of ablation on this parameter. Absorption efficiency was analyzed using one-way ANOVA for RCBD since this parameter was significantly influenced by blocking (molting batches). The effect of time on the feeding rate of both ablated and control crabs was also significant, with evening rates being significantly higher than morning rates (Table 2).

Table. 1. Energy budget and related data of ablated and control (unablated) *Portunus pelagicus* during an 18-hour measurement period. All values are given in $J \cdot g^{-1} \cdot hour^{-1}$ (mean rates were converted by multiplying with their corresponding energy equivalent) and efficiencies in %.

	Ablated		Control	
Parameter[1]	N	Mean ± SD	N	Mean ± SD
Consumption	11	21.601 ± 12.926a	11	16.160 ± 9.156a
Absorption efficiency[2]	3	76.980 ± 7.845a	3	68.570 ± 7.11b
Respiration	11	2.005 ± 0.498a	11	1.735 ± 0.483a
Excretion	11	0.001 ± 0.0004a	11	0.001 ± 0.0004a
Scope for growth[3]	8	16.871 ± 9.541a	8	10.434 ± 8.229a
Maintenance ration	2	5.554 ± 2.299	2	3.622 ± 0.882
Standard respiration	10	0.768 ± 0.441a	11	0.768 ± 0.370a
Standard excretion[4]	10	0.0003 ± 0.0002a	11	0.0002 ± 0.0001b

[1] Means in each row with a common letter are not significantly different at 5% level, LSD.

[2] ANOVA for RCBD, significant at 5% level.

[3] Computed for individual crabs, not computed based on treatment means of the different parameters.

[4] ANOVA, significant at 5% level.

Table 2. Effects of time on the feeding rates[1] (mg·g^{-1}·$hour^{-1}$) of ablated and unablated crabs.

Treatment	Time (hours)	N	Mean ± SD
Ablated	0800	11	2.159 ± 2.996b
	1400	11	3.735 ± 1.981ab
	2000	11	3.802 ± 2.304a
Unablated	0800	11	1.054 ± 2.581b
	1400	11	2.137 ± 1.316ab
	2000	11	3.515 ± 2.657a

[1] ANOVA, significant at 5% level. Means in a column with a common letter are not significantly different at 5% level, LSD.

The differences in maintenance ration could not be statistically tested due to few observations available. However, ablated crabs seemed to have higher (0.488-1.175 mg·g^{-1}·hour^{-1}) maintenance ration requirement than unablated ones (0.410-0.675 mg·g^{-1}·hour^{-1}). The difference in standard respiration rates between the two treatments was not significant, while the difference in standard ammonia excretion rate (higher in ablated crabs) was significant.

Actual Growth and Body Condition

Weight gain appeared to be higher, and carapace length increments and molt interval were shorter in ablated than in normal crabs (Table 3). However, the differences in actual growth performance between the two treatments were not statistically significant.

Table 3. Actual growth performance and body condition indices of ablated and control crabs after a 1-month rearing period.

	Ablated		Control	
Parameter[1]	N	Mean ± SD	N	Mean ± SD
Actual growth				
Weight gain (g)	6	8.12 ± 7.22a	7	7.04 ± 3.67a
Length increment after molting (mm)	5	4.24 ± 0.56a	7	4.37 ± 0.44a
Length increment after 1 month (mm)	8	3.54 ± 3.46a	8	3.92 ± 1.56a
Molt interval (days)	6	21.00 ± 3.83a	7	25.43 ± 5.34a
Body condition indices				
Percent moisture	6	71.26 ± 7.04a	7	72.09 ± 5.19a
Percent dry weight (DW)	6	28.74 ± 7.04a	7	27.91 ± 5.19a
Percent dry meat of DW[2]	6	26.59 ± 5.41a	7	19.97 ± 4.11b
Percent dry meat of WW	6	7.40 ± 1.63a	7	5.77 ± 2.13a
Length-specific meat DW (mg dry meat/mm length)[2]	6	48.17 ± 0.01a	7	32.71 ± 0.01b
Meat organic content (%)	6	86.62 ± 3.93a	7	84.24 ± 4.40a
O:N ratio	11	1,926.6 ± 532.6a	11	2,348.16 ± 857.9a

[1] Means in each row with a common letter are not significantly different at 5% level, LSD.
[2] ANOVA, significant at 5% level.

Of the body condition indices, dry weight, dry meat of dry weight, dry meat of wet weight, length-specific meat dry weight and meat organic content, were seemingly higher in ablated crabs, while per cent moisture and O:N ratio were lower. However, only the differences in per cent dry meat of dry weight and length-specific meat dry weight between the two treatments were significant.

Discussion

The insignificant enhancement of SFG in *P. pelagicus* by eyestalk ablation may be due to its insignificant effects on energy intake and on energy expenditures via respiration and ammonia excretion. The insignificance of the effects is more likely stage-related and/or related to the non-measurement of the amount of energy lost as exuvia. In the lobster *Panulirus homarus* (Vijayakumaran and Radhakrishnan 1984), the weight increase due to eyestalk ablation was less pronounced in juveniles than in adults for the following reasons: 1) the difference in food consumption between ablated and control juveniles is less than that in adults, 2) the difference in conversion efficiency between ablated and control juveniles was negligible while it was more than 30% of the control in ablated adults, and 3) the difference in per cent loss of converted food as exuvia between ablated and control juveniles was only 7% whereas it was about 30% in adults. These findings may also be true for *P. pelagicus*. A juvenile will probably have a less pronounced response to eyestalk ablation because of its generally lower membrane microviscosity (higher fluidity, Shinitzky 1984). Since eyestalk ablation apparently confers a crab with increased membrane fluidity, the effect on an individual with already high fluidity should be less pronounced. Moreover, the non-measurement of the amount of energy lost as exuvia which was conceivably higher in unablated crabs as shown by their higher carapace length increments, may have contributed to the insignificance of the effect on SFG. Inclusion of this energy budget component might have increased the magnitude of difference in SFG between ablated and unablated crabs.

The significant effect of blocking/batches on food absorption efficiency could be due to possible differences in the proportions of dietary components in the different food batches. Chemical composition could vary with age, and qualitative and quantitative differences in dietary components could affect absorption efficiency (MacDonald et al. 1973). The mechanism by which eyestalk ablation enhances absorption efficiency may involve qualitative and quantitative alterations in lipid metabolism and in membrane fluidity. Crustacean eyestalks contain neurosecretory factors which have been shown to influence lipid metabolism (Kleinholz 1976). Mantel (1985) reported that removal of eyestalks in crustaceans leads to an increase in lipid synthesis in general, and that lipid content and composition are important in the regulation of permeability.

The significantly higher feeding rates of both ablated and unablated crabs in the evening show that *P. pelagicus* is a nocturnal feeder. This agrees with the reported increased feeding activity of the species at dusk (Wassenberg and Hill 1987) and indicates the importance of feeding at dusk at rates higher than daytime rates.

The maintenance ration appeared to be higher as a result of eyestalk ablation. This could be ascribed to the stress of ablation which necessitates more energy for maintenance (supported by the significantly higher standard ammonia excretion rates in ablated crabs) without resorting to utilization of tissue reserves. Under conditions as those of the present study, ablated and unablated crabs should be given rations at rates higher than 0.488-1.175 and 0.410-0.675 mg food$\cdot$g$^{-1}\cdot$hour^{-1}, respectively, to ensure growth.

The minimum oxygen and minimum equivalent protein requirements for body maintenance can be derived from the standard respiration and ammonia excretion rates. Standard metabolism is often used to describe the lowest metabolic rate seen under experimental conditions, that is, while the animal is maintained under relatively constant and well-defined conditions of minimal activity (Vernberg 1987). To obtain the equivalent protein from standard ammonia excretion rate, the latter was first converted to NH_4-N then to equivalent protein assuming 16% nitrogen in protein (Winberg 1971). The use of standard ammonia excretion rate to represent the minimum equivalent protein requirement for body maintenance disregards the source of the nitrogen excreted (more than 90% of which anyway comes from protein catabolism, Brafield and Llewellyn 1982) and assumes that it could be replaced by protein nitrogen. Under conditions as those of the present study, the minimum oxygen requirements of ablated and unablated crabs are about the same (0.054 mg $O_2 \cdot g^{-1} \cdot hour^{-1}$), while the minimum equivalent protein requirements are 0.100 and 0.062 µg equivalent protein$\cdot g^{-1} \cdot hour^{1}$, respectively. Marian et al. (1986) also reported that ablated prawns (*Macrobrachium lamarrei*) required higher dietary protein (40%) than non-ablated prawns (36%).

The insignificant effect of eyestalk ablation on weight gain could partly be attributed to the apparent decrease in carapace length increments in ablated crabs. With decreased carapace length increments, carapace weights of ablated crabs would be lesser too, thus reducing the magnitude of difference in weight gain between the two treatments and masking any meat weight gain enhancement effect of ablation. In *Panulirus homarus*, Vijayakumaran and Radhakrishnan (1984) reported that the percentage of converted food lost as exuvia was an average of 43.2% in ablated and 64% in control lobsters.

Of the body condition indices, the significantly higher per cent dry meat of whole-animal dry weight and length-specific meat dry weight are economically desirable characteristics. The higher per cent dry meat and length-specific meat dry weight of ablated crabs might be the combined result of the apparently higher weight gain, less per cent moisture and less energetic loss to carapace formation. The meat organic content approximates the range (85-95%) reported by Siddiqui et al. (1987) for three portunids including *P. pelagicus*. The relatively lower O:N ratio in ablated crabs is indicative of its stressed condition and suggests that more protein was metabolized per mole of oxygen respired by ablated crabs. Optimal diet protein and lipid levels of both ablated and unablated crabs should thus be determined.

Eyestalk ablation resulted in a significant increase in absorption efficiency as well as in dry meat yield. The significant increase in dry meat yield indicates that the improvement in absorption efficiency was more than enough to compensate for any increase in energy expenditures via respiration and ammonia excretion that might have resulted from ablation. The indications of enhancement effects of eyestalk ablation on feeding, respiration and ammonia excretion rates may still be considered useful clues that should be taken into account in the culture of ablated crabs in view of documented enhancement effects on the same parameters in other species.

Furthermore, dry meat yield may be a better indicator of production enhancement compared to weight gain since the latter includes the carapace and water which can be considered as waste products in the commercial sense. Moreover, weight gain may have higher measurement errors due to errors in standardizing removal of excess water. Significant enhancement of weight gain may be masked by the reducing effect of ablation on carapace size. Thus, considering the enhancement effects on absorption efficiency and dry meat yield, the apparently lesser energetic loss to carapace, and the molt acceleration, unilateral eyestalk ablation may be recommended as a technique to enhance meat yield of cultured juvenile blue crabs. However, since the experiment was not designed for immediate practical application, verification of the results on a commercial scale in marine enclosures/cages or in a pond culture system is necessary.

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