CHAPTER IV

DISCUSSIONS

Osmotic Study of *Penaeus monodon*

1. Osmoregulation Type

Penaeus monodon can regulate their haemolymph concentration in the studied range (20 $^{o}/oo$ to 45 $^{o}/oo$). They behave as a hypo-hyperosmoregulators. At salinities 30 $^{o}/oo$ and 40 $^{o}/oo$, a hypersaline condition take place in *P. monodon*. Present results indicate that *P. monodon* can regulated body fluid concentration either in high or low salinities water. Thus, prawn offshore migration should be caused by other biological demands such as maturation rather than their ability of osmotic regulatory. Indirect effects of salinity are essentially of two types; osmotic shock and salinity stress which may also become a limiting factor when acting together with one or several other stressful conditions (Gilles and Pe'queux, 1983; Vernberg, 1983b). Furthermore, Vernberg (1983a) noted that while osmotic regulation is important, the selective regulation of specific ions or ionic regulation is essential, since protoplasmic integrity can not be maintained in absence or excess of certain ions. For example, calcium added to low salinity water which is normally lethal to organism may enable that organism to survive.

There are strong possibilities of interactions between salinity and molting effect on osmoregulation (Chu *et al.*, 1988).

Thus, studies made salinity effects generally avoided this possible interactions by using only intermolt prawn. For *Penaeus monodon*, haemolymph concentration would already be closed to intermolt value within 1 day after molting, (Ferraris *et al.*, 1986). *P. monodon* during molting or immediate postmolt is less able to regulate haemolymph concentration compared to intermolt prawn (Ferraris *et al.*, 1986, 1987)

Osmoregulation of prawn can be affected in two successive steps. The first is to keep the osmoregulation of haemolymph constant regardless of the salinity of the external medium. The second is to maintain the intracellular fluids isosmotic to the extracellular fluid (haemolymph). Both steps tend to avoid water movement at the cellular level (Gilles and Pe'queux, 1983). From the present study the results show that *Penaeus monodon* could be characterized as a highly efficient osmoregulator because they can regulate either in high or low salinities. They can reach the steady state rapidly in hypersaline.

Gilles and Pe'queux (1983) stated that good osmoregulator will rely on very efficient mechanisms of osmoregulation. However, osmoregulation and ionic regulation usually work together (Gilles, 1979; Mantel and Farmer, 1983). Thus, maintenance of osmotic and ionic equilibrium in low and high salinity must involve active uptake process by gill, antennal gland or green gland and permeability of body surface (Lockwood, 1969; Potts and Parry, 1964; Robertson, 1960; Muramoto, 1988; Ferraris *et al.*, 1987).

The subadult *Penaeus monodon* hyperregulate their haemolymph concentration in diluted seawater, salinity below 28 o/oo and hyporegulate in seawater at salinity higher than 28 ^{o}/oo. Thus, the isosmotic point of subadult *P. monodon* during intermolt stage is approximately 28 ^{o}/oo or 789 $\pm$ 40 mmol/kg (Figure 5). Similar result was also observed by Ferraris *et. al.*(1987).

At isosmotic point the prawns has less physiological stress than other conditions. Thus, the isosmotic point may be the best condition for aquculturing. However, the isosmotic point of organism also depends on the stages of the life history (Ferraris *et al.*, 1986, 1987; Gilles and P'equeux, 1983).

1.1 Effect of Eyestalk Ablation on Osmoregulation

Not only salinity affected on osmoregulation of *Penaeus monodon* but also eyestalk ablation as well. Moreover, the results of the present study indicated that salinity and eyestalk ablation have an interaction effect on osmoregulation. There is significant difference between haemolymph concentration of ablated prawn and un-ablated one at hypersalinity (45 ^{o}/oo). It suggested that hypersalinity and eyestalk removal may superimpose onto osmoregulation control mechanisms, neurosecretory system and/or energetic.

Lockwood (1969) suggested that while eyestalk ablation induced maturation, its side effect of hormonal manipulation also reduces production of gonad inhibiting hormone (GIH). Consequently, it may affect number of other functions other functions such as molting, metabolic rate and osmoregulation.

Tombes (1977) and Muramoto (1988) stated that when eyestalk was removed, ability to adjust water balance and blood sugar level were changed, since the concentration of the molt inhibiting hormone (MIH) decreased after experimental extirpation to eyestalk.

There is, however, stronger evident that osmoregulation is linked to specific hormone such as Na^{+}-K^{+} ATP-ase, MIH, GIH (Lerey, 1984). The eyestalk neurosecretion hormone, MIH in haemolymph is reduced, followed by an influx of water and dilution of the haemolymph concentration. These conditions has been duplicated by the removal of the eyestalk or sinus gland. This result of the fact that the X-organ-sinus-gland (SG) complex in the eyestalk is the major neuroendocrine control center in crustaceans. The release of secretory material from SG is under neural control (Beltz, 1988; Fingerman, 1976; Muramoto, 1988; Tombes, 1977; Waterman, 1960)

2. Relationship between Osmoregulation and Hypersaline Acclimation Time on Osmoregulation

In subadult prawn during intermolt stage, haemolymph concentration is unaltered by time. However, this can suggest that the longer the prawn which held in osmotic stress condition the more energy loss occurs in order to regulate it osmotic concentration. Even in the absence of mortality, the osmotic stress becomes some chronic effects (Prusch, 1983; Lasserre, 1976).

Ferraris, *et al.* (1987) showed that haemolymph osmolality displayed a narrow range during intermolt and almost constant when it held in constant salinity, suggesting high ability in regulating of haemolymph concentration. Moreover, haemolymph osmolality change as function of salinity, when molt stage is fixed (Gilles, 1979; Gilles and P'equeux, 1983; Sastry, 1983). Ferraris *et al.* (1986) found that the haemolymph osmolality of juvenile *Penaeus monodon* would already be close to intermolt value within a day after molting. Thus, after 45 days acclimation in the present work should be adequate for osmotic adaptation of these prawns.

In laboratory condition, the prawns must not be affected by other stress factors such as temperature, crowding, and handling. Seawater is one of a source of variation. Salinity and ionic composition may affect osmoregulation though this salinity variation cannot be detected by salinometer.

Calcium concentration in rearing water which is altered by recirculated time may be another factors influencing ionic regulation and variation in osmotic concentration of medium and haemolymph (Wilson, 1975). The influence of calcium at salinity 30 $^{o}/oo$ should be less than at 40 $^{o}/oo$ because the Ca:Salinity ratio of 40 $^{o}/oo$ is more different from natural water than that of 30 $^{o}/oo$. The diluted brine water should be recirculated through filter bed before using in order to adjust of ionic compositions such as calcium free ion.

Ovarian Maturation Experiments of *Penaeus monodon*

Most of the studies on reproduction of penaeids are focused on female maturation (AQUACOP, 1985; Beard and Wickins, 1980; Halder, 1978; Lumare, 1979; Primavera, 1978, 1985; Crocos and Kerr, 1986). Subadult prawns which begin the onset of sexual maturation and adult stage characterized by the completion of sexual maturity under optimum condition (Motoh, 1981, 1985) are usually used in maturation researches.

1. Adult Prawn Maturation in Hypersalinity

Management of environmental conditions has been used successfully to induce maturation of several species of penaeids (Primavera, 1985; Chanberlain and Lawrence, 1981, Crocos and Kerr, 1986; Khoo, 1988). Salinity, one of the environmental factors that affect on maturation and spawning of *Penaeus monodon*, was studied in the present work. The results show that hypersalinity (30 ^{o}/oo and 40 ^{o}/oo) is sufficient to induce ovarian development for unablated female adult female prawn.

Life history of *Penaeus* spp. consists of a estuarine phase and a marine phase with the offshore migration of subadult and adult (Motoh, 1981, 1985; Primavera, 1985). In fact, there is no difference between subadult and adult habitat. Adult *Penaeus monodon* spawning is presumed to take place mainly offshore (outer littoral area) within high saline water (33-36 ^{o}/oo) (Motoh, 1981; Penn, Hall and Capati, 1985). Adiyodi (1985) suggested the offshore existence of adult penaeid prawns during reproduction. The most successful cases of captive reproduction may rely on some environmental factors such as salinity like this present work. By using hypersalinity aimed to induce ovarian maturation in unablated *P. monodon*. The results imply that there is a relationship between hypersalinity acclimation time and ovarian index at salinity 30 ^{o}/oo and 40 ^{o}/oo under recirculating water system. That is, ovarian maturation increases with acclimation duration.

Under the hypersaline condition adult prawn can develop its reproductive organ as in open sea. The hypersalinity may affect on neural system that control reproductive mechanism and may initiate the oogenesis. Primavera (1985) suggested that ovarian development of *Penaeus* spp. may start in estuaries but the complete maturation and spawning take place after returning to open sea. The physico-chemical factors at offshore area may provide the stimuli for lowing of gonad inhibiting hormone (GIH) levels, and the ovarian maturation is induced by gonad stimulating hormone (GSH). One of the important factors is salinity, which increase from 20 ^{o}/oo in estuaries to more than 30 ^{o}/oo in spawning ground (Kunvankij, 1985; Primavera, 1978, 1985; Beard and Wickins, 1980).

The present result indicates that at salinities 30 ^{o}/oo and 40 ^{o}/oo there is no significant difference in maturation rate of unablated female *Penaeus monodon*. Similarly, Primavera (1985) also found that *P. indicus* kept in tank at salinity 32 ^{o}/oo and 42 ^{o}/oo has no difference in maturation rate. These results may be caused by high captivity adaptation and salinity tolerance of *Penaeus* spp. (Vernberg and Vernberg, 1972; Motoh, 1981, 1985; Penn, Hall and Capati, 1985).

Breeding migration or offshore migration is essential to reproduction (Dall, 1985; Penn, Hall and Caputi, 1985). Racek (1959) found that *Penaeus plebeius* which prevented from migrating never mature for two years are still sexually immature to the end of this period, although they have attained normal adult body size (Racek, 1959 cited by Vernberg and Vernberg, 1972; Penn, Hall and Capati, 1985; Vernberg and Vernberg, 1972).

Although *Penaeus monodon* female are more difficult to mature in captivity than those of other penaeid species (Kunvankij, 1985). Hypersalinity acclimation like the present study showed the possible induction on their maturation processes without ablation. We have now considerable evidences that ablation of captive reared prawns result in reducing reproductive performance in term of lower fecundity, egg viability, and hatch rate compared to unablated prawn (Lumare, 1979; Emmerson, 1983; Crocos and Kerr, 1986).

Eventhough the biological requirement for maturation remains poorly understood, utilization of environmental stimulation of maturation may eliminate the need for the more traumatic ablation techniques.

The comparison between the oocyte dedelopment of the prawns in hypersaline condition and other *Penaeus* spp. in natural water found no different pattern.

The condition index or fatness is also independent on hypersaline acclimation. This may be a result of physiological and morphological adaptation in adult prawn under hypersalinity in laboratory condition. However, in the aspect of energy budget of aquatic animals it might be predicted that ovarian development of prawns would be better at salinity 30 ^{o}/oo than 40 ^{o}/oo due to a lower energy allocation for osmoregulation at salinity 30 ^{o}/oo for living (Brafield and Llewellyn, 1982; Vernberg, 1987)

Fatness and ovarian index have no relationship. This fact confirms that ovarian index can be used as indicator for ovarian development of this prawn by assumption of Grant and Tyler (1983a,1983b).

The influence of hypersalinity on ovarian development and condition index of *Penaeus monodon* can be a benefit of using subadult prawn having first maturity as the broodstock. If salinity can accelerate its maturation rate, hypersaline condition would be used effectively to induce adult maturation.

2. Prawn Size, Hypersaline Acclimation and Eyestalk Ablation Effect on Maturation of Subadult *Penaeus monodon*

This part is focused on ovarian development of two size groups of subadult prawn (small size group rearing in laboratory and large size subadult collected from intensive farms) in hypersalinity and the response to eyestalk ablation. The present result confirms a positive effect of hypersalinity on maturation. Motoh (1981) reported that the first maturity of *Penaeus monodon* occurs during subadult stage (4-6 months age). Moreover, five-month-old female prawn can be successfully matured and spawned in captivity by ablation (Primavera, 1978; Emmerson, 1983).

The present study shows that hypersaline acclimation, 30 ^{o}/oo and 40 ^{o}/oo initiates precocious ovarian development of subadult prawn either in small or large size group. Though the increase in ovarian index is well developed in the adult stage, the subadult prawns group are positively response to hypersaline acclimation. However, the ovarian index of the subadult is lower in magnitude than those of the adult prawn. This may be the result of the complete maturity in adult prawns (Motoh, 1985).

The average ovarian index indicates that prawns acclimated at salinity 30 ^{o}/oo is better for ovarian development than that of acclimated at 40 ^{o}/oo. Moreover, 40 ^{o}/oo may be supraoptimal salinity for small size prawn which had been previously held at 20 ^{o}/oo under laboratory condition. The long-term exposure to dilute seawater in captivity can cause physiological adaptation (Lockwood, 1976; Vernberg and Vernberg, 1972). Osmoregulatory at high salinity may require high energy resource. Therefore, these prawns cannot allocate energy to reproduction as much as the large subadult prawn. In addition, small size subadult prawns have low condition index at the beginning (control group) but the condition index is improved after acclimation (Table 10). Thus, the low condition index may cause the delay of maturation of *Penaeus monodon* broodstock.

Although subadult stage can be induced by using hypersalinity, e.g. 30 ^{o}/oo, adult prawns have better response under this condition. In addition, large size subadult prawn which has high condition index is preferred for using as broodstock when compared to small size prawn. This result may be affected by a good physiological condition of the large size prawn condition.

Adiyodi (1985) have demonstrated that eyestalk removal produced precocious gonad development in decapod crustaceans depending upon the interaction of ambient environment and age of animal. Beard and Wickins (1980) and Santiago (1977) also suggested that inhibiting of ovarian maturation in captive female *Penaeus monodon* has been overcome by unilateral eyestalk removal in large subadult prawn resulted in ovarian index increment within one month of acclimation.

Biological condition of prawn is influenced on vitellogenesis after ablation. On the other hand, eyestalk ablation shows inversely effects on mortality. These may be caused by direct result of worsening condition of prawn, not from a direct result of eyestalk ablation (Browdy and Samocha, 1985).

The present study, mortality of ablated prawn at salinity 30 ^{o}/oo and 40 ^{o}/oo in both size groups is higher than the normal ones. It is possible that handling techniques and injuring raise the physiological stress and cause high mortality. Waterman (1960) and Yano (1984) stated that mortality in the ablated prawn is caused by damaging the optic nerve as well as ablation technique used (Kelemec and Smith, 1980). The whole eyestalk removal in this study can produce an open wound and cause trauma with greater haemolymph loss than other technique (Makinouchi and Primavera, 1987). Mortality of ablated prawn at salinity 40 ^{o}/oo seems to be higher than at 30 ^{o}/oo indicating that osmoregulatory stress also as well as handling technique is another cause of stress.

Although eyestalk ablation accelerates maturation rate, ovarian development of *Penaeus monodon* can be induced by hypersaline acclimation without ablation since subadult stage. However, Hypersaline acclimation in conjunction with ablation seems to give the highest ovarian index.

3. Manipulation Techniques to Induce Ovarian Maturation of Subadult *Penaeus monodon*

3.1 Hypersaline Effect

The results as mentioned earlier indicate that maturation has been induced in penaeids as young as 5-month-old or subadult stage. Motoh (1981) also reported that subadult stage of *P. monodon* can be used for maturation purpose. The size of subadult broodstock corresponds to about 40 to 80 grams.

Bray and Lawrence (1984) stated that the best exact age for broodstock is not defined , but as a general rule the animals should be at least the size of wild animals at their first maturity, the onset of maturation. Eventhough the ovarian index of subadult prawns are not significantly increased (Table 11, P>0.05) after hypersaline acclimation (compared with the control group (TRM-12)), oocytes in yolk vesicle and cortical vesicle are found. The mature ova are found in the ovarian index of 1.59% . The most oocytes were still in primary vitellogenesis; early perinucleolar, late perinucleolar; and early yolk deposit stage. That is, hypersalinity may be able to stimulate the oocyte maturation by short-term acclimation. Tombes (1977) suggested that the primary vitellogenesis can occur continuously in reproductive season but development rate can be accelerated by external factors and neurohormone.

Salinity, one of the external cues which may induced CNS to promote the first phase of vitellogenesis by inhibiting the production of gonad-inhibiting-hormone (GIH) and favoring the release of gonad-stimulating hormone (GSH) (Highnam and Hill,1977; Khoo, 1988). However, the effects of GIH on the primary vitellogenesis is usually less on the secondary vitellogenesis. Without eyestalk ablation primary phase of oogenesis can be occurred by environmental manipulation, but GIH is highly efficient in inhibiting the yolk deposition in secondary vitellogenesis. Thus, the eyestalk ablation is still necessary to induce the secondary phase of ovarian development. Primavera (1978) and Santiago (1977) also indicated that eyestalk ablation influences on subadult maturation by accelerating ovarian growth resulting in increasing of ovarian index.

From the present experiments, hypersaline condition, 30 ^{o}/oo and 40 ^{o}/oo can accelerate the oogenesis during 30 days of acclimation. In contrast, long-term acclimation (longer than 30 days) may produce some severe effects on physiology of prawn, the ovarian index decreases and the prawns die especially in high salinity (40 ^{o}/oo). Kinne (1967) and Vernberg and Vernberg (1972) suggested that a short duration of salinity exposure produces an adaptative response, whereas a prolonged exposure to the same intensity results in death. Hence, both time and quantity of the effector must be studied to assess its environmental role.

After acclimation the prawn could reach the new steady state compensatory response to the alteration of salinity. The new equilibrium of functions and metabolic adaptation for stress may not support the reproduction process (Vernberg and Vernberg, 1972).

The results indicate that two-salinity acclimation (30 ^{o}/oo and 40 ^{o}/oo) and as TRM-6 and TRM-7 (TRM definition are showed in Table 10) were not optimal to induce maturation. They show similar trend as single salinity acclimation. It confirmed that high salinity is not a major factor on maturation (Table 10). Bray and Lawrence (1984) suggested that salinity used for experiments on penaeid reproduction should be maintained from 28 ^{o}/oo to 35 ^{o}/oo. Moreover, salinities approaching 40 ^{o}/oo are detrimental to egg development when doing long-term acclimation. Among the TRM trial, hypersaline acclimation by using 30 or 40 ^{o}/oo in short duration (30 days) can induce the ovarian maturation. However, the results of this experiment is still not clear to conclude the effect of salinity on maturation because of under laboratory condition the prawns are stressed by several factors such as culture techniques, tagging and size determination disturbance.

3.2 Combination Effects between Hypersalinity Manipulation and Eyestalk Ablation

One of the major advances in captive reproduction of *Penaeus monodon* occurs with the application of eyestalk ablation of females in the mid-1970's (Tombes, 1977). Although the process induces rapid ovarian maturation, the success of the operation is dependent upon the physiological condition and captive culture condition. The combination between hypersaline condition and eyestalk ablation can induce maturation process in subadult *P. monodon*

The ovarian development of subadult prawns by TRM-9 TRM-10 and TRM-11 is influenced by combinated effects of hypersaline manipulation and unilateral eyestalk ablation. TRM-11 (eyestalk ablation and sudden acclimated at salinity 40 $^{o}/oo$ for 30 days, then transferred to salinity 30 $^{o}/oo$ for 15 days acclimation) is a well organized way for ovarian development. That is to say, the short-term acclimation at salinity 40 $^{o}/oo$ together with eyestalk ablation is highly efficient method.

Adiyodi (1985) and Charniaux-Cotton and Payen (1988) suggested that under optimal condition decapod crustaceans such as prawn, crab and crayfish can reach the end of primary vitellogenesis without eyestalk ablation. Unilateral eyestalk ablation accelerates yolk deposition in oocytes in which secondary vitellogenesis due to a removal X-organ and sinus gland complex which produce gonad inhibiting hormone (GIH). However, eyestalk ablation has the long-term disadvantage, other functions such as growth, metabolic rate, water balance and heart rate (Beltz, 1988; Giese and Pearse, 1974; Highnam and Hill, 1977; Lockwood, 1968). Thus eyestalk ablation should be used to induce for short-term period under optimal condition. The results indicate that the optimal acclimation time for eyestalk ablation in hypersaline condition are about 15 days.

The TRM-11 is the most efficient method which found among these experiment. Eyestalk ablation and short-term acclimation at 40 ^{o}/oo can stimulate the first phase of oocyte development. Sudden transferring the prawn to salinity 30 ^{o}/oo may decrease osmotic stress and improve physiological condition. These prawns continuously develop their oocyte to mature stage under 30 ^{o}/oo during 15 days.

By paraffin histological data, they indicate that oocyte development (in case of ova size and morphology) does not dependent on age of prawn, salinity, and eyestalk ablation. It can suggest that the oogenesis of *Penaeus monodon* is not disturbed by the hypersaline (30 ^{o}/oo and 40 ^{o}/oo) acclimation and eyestalk ablation.

In conclusion, environmental manipulation and hormonal manipulation act together on maturation process. It can be predicted from this results that if adult prawn was treated by TRM-11, it would be well matured.

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Molting of *Penaeus monodon* Broodstock

The molting process and reproductive cycle in crustacean including *Penaeus monodon* are closely related. Highnam and Hill (1977) suggested that reproductive activity and molting are antagonistic. Both processes require high energy supply and are controlled by neurohormonal system (Brafield and Llewellyn, 1982; Cooke and sullivan, 1982; Vernberg, 1987). Ovarian development of closed-thelycum penaeid prawns occurs in the intermolt stage of molt cycle (Choy, 1987; Muramuto, 1988; Primavera, 1978, 1985; Tseng, 1987). Furthermore, the ovarian developing rate of *Penaeus* spp. relate to molting interval and hormonal change during molt cycle (Highnam and Hill, 1977).

Although the eyestalk ablation may accerelate molting and/or ovarian development of prawn, external and internal condition at time of ablation are important for alternating the response of these prawns between molting and reproduction (Charniaux-Cotton and Payen, 1988; Webster, 1985). If molting interval is not altered after eyestalk ablation, the hormonal effects should influence only reproductive cycle.

1. Molting of Non-ablation Prawn

Apparently, in this present study there is no sexually dimorphism in molting interval of adult *Penaeus monodon*. Thus, the growth rate of this stage may not differ between female and male. All adult prawns which are used in the experiment are about 8-month-old.

Adult and subadult prawns in these experiments have a similar response of hypersaline acclimation. That is, salinity (30 $^{o}/oo$ and 40 $^{o}/oo$) has no significant ($P>0.05$) influence on molting interval. The adult prawn has longer molting interval than subadult because intermolt period lengthen with increase size and age (Hartnoll, 1982). This similar trend was found in *Palaemonetes pugio* (Cheng and Chen, 1990)

2. Molting of Eyestalk Ablated Prawn

Molting is stimulated in crustacea by one or more closely related to steroid hormones, the ecdysteroids (Skinner, 1985; Chan, Rankin and Keeley, 1985; Lockwood, 1969). Generally, eyestalk ablation is a common method used to induce molting process in decapods. In present experiment, the eyestalk ablated subadult *Penaeus monodon* is not accelerated the in onset of molting in both salinities, 30 $^{o}/oo$ and 40 $^{o}/oo$.

The ablation experiments assume that any observed effects are due to the removal of the x-organ-SG complex. This assumption is probably valid. However, it is important to remember that when these neurosecretory centers once removed, it also produces severe trauma, destroying a major portion of central nervous system (CNS) and render the animals blinds. Thus, eyestalk removal can raise physiological stress (Tombes, 1970; Beltz, 1988; Skinner, 1985). Consequently, the molting interval of ablated prawn is delayed. Furthermore, Highnam and Hill (1977) and Beltz (1988) suggested that MIH may reside not only in the sinus gland (SG) but also in other neurosecretory cells in brain and other parts of the CNS. Thus, MIH from other sources may inhibit some mechanisms of molting of ablated prawn under these hypersaline condition.

As mentioned above, reproduction and molting is antagonistic. There is both a molting-inhibiting hormone (MIH) and ovary-inhibiting (inhibitory gonadrotropin) hormones produced by the eyestalk, both being somewhat antagonistic to another (Adiyodi, 1985). After ablation some prawns lead to accelerated gonad development coincident with inhibiting somatic growth and retarding molting. Thus, the mean molting interval of overall are not different between ablated prawn and control group.

Low light intensity and constancy in rearing tank may inhibit some physiological process and role on Y-organ releasing molt inhibiting hormone. Thus, under these conditions molting interval of ablated prawn did not differ from non-ablation prawn. Moreover, the failure in acceleration of molting by eyestalk ablation in subadult prawns may be caused by other unknown factors either intrinsic or extrinsic factors in rearing unit (Webster, 1985). Morphological and physiological stress caused by damaged of appendages from transportation, handling, tagging, and eyestalk removal possibly result in delay of molting in these specimens either ablated prawn or normal eyes prawn (Primavera, 1985; Choy, 1987).

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