www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 10: 403-410 (2010) DOI: 10.4194/trjfas.2010.0314

Effects of Eyestalk Ablations on Growth and Ovarian Maturation of the Freshwater Prawn *Macrobrachium lanchesteri* (de Man)

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Received 11 Novomber 2008
Accepted 05 Agust 2010

Abstract

The effects of unilateral and bilateral eyestalk ablations on growth, ovarian maturation, carbohydrate, protein and energy contents were investigated in *Macrobrachium lanchesteri* (de Man). Daily growth as well as ovarian maturation were accelerated by eyestalk ablation in these prawns. The mortality in ablated prawns was more than that of intact ones. The biochemical components like carbohydrate and protein were altered by eyestalk ablations. The energy content of the different tissues were also showing some variations due to the absence of eyestalk hormones. Eyestalk ablation, especially unilateral eyestalk ablation could be used for inducing rapid growth and spawning in this particular species of freshwater prawns.

Keywords: Eyestalk ablation, ovarian maturation, ovarian index, growth, energy content.

Göz Sapı Kesim Yönteminin Tatlı Su Karidesinin *Macrobrachium lanchesteri* (de Man) Büyümesi ve Yumurtalığının (Ovarium) Olgunlaşması Üzerindeki Etkileri

Özet

Macrobrachium lanchesteri'de tek yanlı ve iki yanlı göz sapı kesim (ablasyon) yönteminin; büyüme, yumurtalığın olgunlaşması, karbonhidrat, protein ve enerji içeriğinin üzerindeki etkileri araştırılmıştır. Hem günlük büyüme hem de yumurtalık olgunluğu; bu karideslerde göz sapı kesimiyle birlikte hızlanmıştır. Kesilmiş karideslerde ölüm, intakt (kesilmemiş) olanlardan daha fazla olmuştur. Karbonhidrat ve protein gibi biyokimyasal bileşenler göz sapı kesimiyle değişmiştir. Göz sapı hormonları olmadığından, farklı dokulara ait enerji içeriği de bazı değişiklikler göstermiştir. Tatlı su karidesinin bilhassa bu türünde hızlı büyümeyi ve yumurtlamayı tetiklemek için göz sapı kesimi, özellikle de tek yanlı göz sapı kesimi kullanılabilir.

Anahtar Kelimeler: Gözsapı kesimi, yumurta olgunlaşma, ovaryum indeksi, büyüme, enerji içeriği.

Introduction

Crustacea is remarkably a successful group, both in number of living species and in the colonization of varied habitats. Among decapod crustaceans, the suborder Natantia (Prawns and shrimps), form a major aquatic resource for human exploitation. The increasing demand for prawns has created a global awareness and interest in the development of prawn culture fisheries (Bardach *et al*., 1972; Pillay, 1979). Freshwater prawn fisheries in India is mainly based on capture fisheries rather than on aquaculture. Freshwater prawn culture has undergone a phenomenal growth in the past two decades. (Mariappan *et al.*, 2003).

Reproduction is an energy demanding process. In reptantians like crabs various aspects of reproduction like egg maturation, ovulation, spawning and incubation of eggs are completed within the intermoult stage. Spawning follows ecdysis in natantians (Charniaux-Cotton, 1985) i.e., somatic and reproductive growth occur simultaneously in natantian decapods (Sarojini *et al*., 1983). In Caridian prawns like *Palaemon serratus* (Panouse, 1947), *Palaemon paucidens* (Kamiguchi, 1971) and *Macrobrachium nobilii* (Pandian and Balasundaram, 1982) spawning is obligatorily preceded by a moult. Synchronous occurance of the moulting and reproduction demands apportioning of the available energy for both processes. Since eyestalk ablation removes the regulatory effects of gonad inhibiting hormone (GIH) and moult inhibiting hormones (MIH)

on reproduction and moulting, it enhances growth and reproduction (Manjunath, 1989). Hence it would be interesting to know the flow and fluxes of energy to the ovary from their storage organs like the hepatopancreas and the muscle when eyestalk ablation is performed in female prawns of different ovarian maturity stages.

Of the 100 species of freshwater prawns belonging to the genus *Macrobrachium* recorded world wide, 40 species are found in India (Mariappan *et al.*, 2003). Among the *Macrobrachium* species, the effects of different diets (Manik, 1976; Millikin *et al.*, 1980), salinity (Smith *et al.*, 1982) and water quality (Cripps and Nakamura, 1979; Menasveta, 1982) have been studied using intact *M. rosenbergii*. The effects of unilateral and bilateral eyestalk ablations on the food conversion efficiency of *M. lanchesteri* (de Man) have been reported (Ponnuchamy *et al.*, 1981). Effects of unilateral eyestalk ablation on moulting, growth, reproduction and energy budget of *M. nobilii* have been documented (Sindhukumari and Pandian, 1987a). Koshio *et al.* (1992), studied effects of unilateral eyestalk ablation and feeding frequencies on the growth, survival and body composition of juvenile freshwater prawn, *M. rosenbergii*. Till now, very few investigations regarding the energy content per unit weight of the intact and eyestalk ablated prawns have been undertaken (Sindhu Kumari and Pandian, 1987a). Most of the studies depend on indirect energy measurements to estimate the energy budget of fresh water and marine prawns. Hence an attempt has been made in this present study to analyse the effects of unilateral and bilateral eyestalk ablations on growth, ovarian maturation, carbohydrate, protein and energy contents of female *Macrobrachium lanchesteri* (de Man) of different ovarian maturity stages.

Materials and Methods

The freshwater habitats in and around Bangalore city (77°33' E- 12°55' N) harbour the Palaemonid prawn *Macrobrachium lanchesteri* (de Man). These prawns were collected from a freshwater habitat (Ramohalli tank) of Bangalore. Healthy female prawns of *M. lanchesteri* were collected and acclimatized to laboratory conditions for 2-3 days in aquaria containing 15 L freshwater. Throughout the experimental period, a daily photoperiod cycle of 10 L: 14D (L=$08.^{00}$-$18.^{00}$ hr, D= $18.^{00}$-$08.^{00}$ hr) was maintained and the water temperature was 23.2±1.6°C. The pH of water was maintained close to 7. Live *Tubifex tubifex* (Muller) worms which are known to constitute a rich source of nutrition for freshwater prawns (Ponnuchamy *et al.*, 1981) were used as food in the present experiment. During the experimental period, water was aerated continuously. Feeding and collection of undigested food was undertaken daily between $10.^{00}$ hr and $12.^{00}$ hr. To determine the state of maturity of the ovary in the live prawn, the ovarian index was established by the formula:

Ovarian index (OI)= (Ovarian length (mm)/ Carapace length (mm))x 100

Based on the OI, female prawns were segregated into 3 groups:

1) OI < 30
2) OI 30-45
3) OI 50-70

Three series of feeding experiments were carried out. In the first series normal prawns (control) were held in groups of 16 in each aquarium containing 15L freshwater. In the second and third series, unilateral eyestalk ablated (UEA) and bilateral eyestalk ablated (BEA) prawns were held in similar densities respectively. Each group of experiments were carried out in triplicates.

The eyestalk of each prawn was immersed in ice-cold water (4-10°C) for 6-10 seconds. Then the operation was performed by cutting off the eyestalk at its base with sterilized scissors. Immediately after eyestalk ablation the prawns were introduced into the aquarium and the water was aerated continuously. The experimental prawns in each series were fed on *ad libitum Tubefix tubefix* worms till the individuals attained their next respective maturity stages i.e., prawns of OI< 30 till they reached an OI of 30, OI 30-45 till they attained an OI of 60 and OI 50-70 till they became ovigerous (spawning). During this period, their moulting frequency, daily food intake and growth were determined.

After completing the feeding period, all the individuals in the three series of experiments were weighed, ovarian indices were recorded, ovaries & hepatopancreas were dissected out. The remaining animal including the exoskeleton was grouped as carcass. In each of these tissues, carbohydrate, protein and energy contents were estimated.

Protein: Protein was determined by the Folin-Ciocalteau method (Lowry *et al.*, 1951).

Carbohydrate: Carbohydrate was determined by estimating the glycogen content following Phenol-sulphuric acid method (Dubois *et al.*, 1956).

Energy: Direct energy measurements were made by making use of the Microbomb Calorimeter (Parr Illinois, Co. USA). Energy measurements were made following the protocol given in the instruction manual provided by the company. The bomb calorimeter was standardized by burning a pellet of Benzoic acid.

Statistical Analysis

All the series of experiments were done in

triplicates and the standard error was calculated. The significance was calculated using one-way analysis of variance (ANOVA) and Student's t-test of SPSS statistical software. A value of $P<0.05$ was taken as statistically significant. And the results were calculated as mean with standard error (±SE) values.

Results and Discussion

Ovarian Maturation

In group I (OI<30), the control prawns attained an ovarian index of 30 by the end of 30 days feeding period. Unilateral (UEA) and bilateral eyestalk ablation (BEA) in individuals of OI<30 failed to induce accelerated ovarian maturation (Figure 1). Similar observations were made by Rao (1983). Eyestalk hormones (GIH and MIH) are essential for initial growth and maturation of the ovary. This is in contrary to the reports of Sindhukumari and Pandian (1987) that in *Macrobrachium nobilii* UEA brings about accelerated moulting frequency and onset of sexual maturity. According to reports by Okumara (2007), in immature female prawns, unilateral ablation does not induce ovarian maturation and vitellogenin(VTG) gene expression, whereas bilateral ablation induces rapid ovarian maturation and VTG gene expression. But in our experiment we could not get accelerated ovarian maturation by both unilateral and bilateral eyestalk ablations in immature (i.e, OI <30) prawns.

The control prawns of OI 30-45 group (Figure 2), took 25 days to attain an ovarian index of 60. On the other hand, unilateral and bilateral eyestalk ablated prawns showed an accelerated ovarian growth and took only 12 days to attain the ovarian index of 60. Similarly, the control individuals of OI 50-70 group took 20 days to become ovigerous (spawn) (Figure 3), while the unilateral and bilateral eyestalk ablated prawns attained ovigerous state in 12 days. Similar reports that unilateral and bilateral eyestalk ablated *Macrobrachium nipponense* had accelerated ovarian maturation were made by Han and Kim (1993). Sindhukumari and Pandian (1987) reported that in *M. nobilii* also application of unilateral eyestalk ablation increases moulting and spawning frequency.

Food Intake and Growth

The daily food intake of control and unilateral and bilateral eyestalk ablated individuals in the three

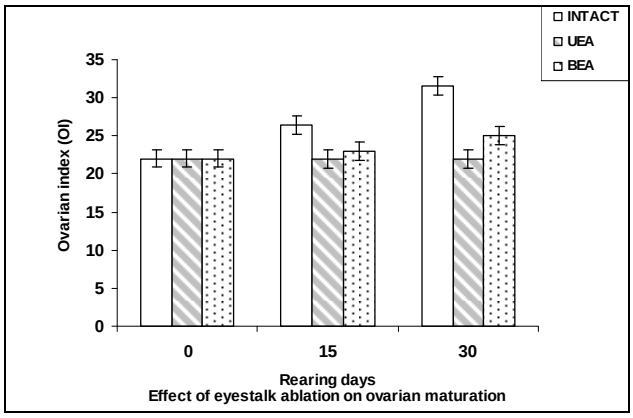

Figure 1. Effect of unilateral (UEA) and bilateral eyestalk ablation (BEA) on ovarian maturity of female *Macrobrachium lanchesteri* (de Man) of ovarian index (OI) <30.

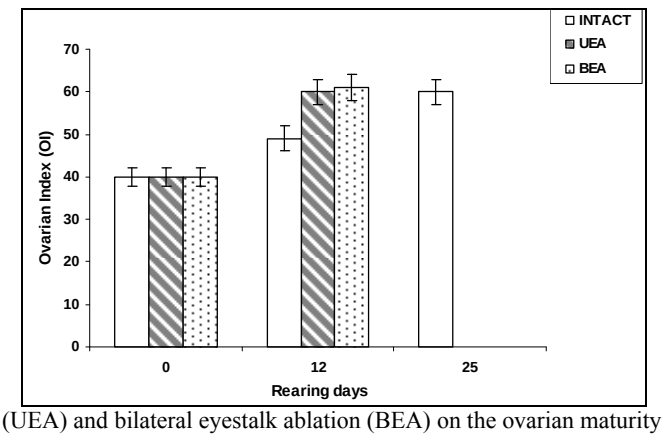

Figure 2. Effect of unilateral (UEA) and bilateral eyestalk ablation (BEA) on the ovarian maturity of female *Macrobrachium lanchesteri* (de Man) of ovarian index (OI) 30-45.

ovarian maturity stages of female *M. lanchesteri* have been given in Figure 4. Daily food intake was increased in all the three classes of prawns by eyestalk ablation. Similar observations were made by other workers (Pandian and Sindhukumari, 1985; Aktas and Kumlu, 1999). Sindhukumari and Pandian (1987) reported an increase of 1.1 times in food consumption by the ablated female *M. nobilii*. Ponnuchamy *et al.* (1981), reported higher food conversion efficiency for unilaterally and bilaterally ablated *M. lanchesteri*. Venkitraman *et al*. (2004), reported that bilateral eyestalk ablation decreased the food consumption by 21.5% than the controls and unilateral eyestalk ablation did not alter food consumption rate significantly when compared to the control prawns.

Daily growth was also found to increase in unilateral and bilateral eyestalk ablated prawns (Figure 5). In the control prawns, the daily growth decreased with increase in maturity stage of the ovary. Similar results that unilateral eyestalk ablation accelerates growth and conversion efficiency of *Metapenaeus dobsoni* (de Man) were reported by Venkitraman *et al.* (2004). In our results we observed that growth is enhanced by both unilateral and bilateral eyestalk ablations but according to the results of Venkitraman *et al.* (2004), dry weight decreased 28.42% by BEA and only UEA resulted in an increase in the dry weight by 4.55% when compared to the control intact prawns. We got similar results only in the mature prawns of OI 50-70. In these prawns of OI 50-70, growth was maximum in unilateral eyestalk ablated prawns, while in bilateral eyestalk ablated prawns it was reduced significantly (0.77 mg. dry / prawn day^{-1}). This reduction could be due to the loss of eggs after spawning in these prawns. Bilateral eyestalk ablated prawns failed to retain eggs in their brood pouch. Eyestalk hormones are apparently necessary for the display of normal parental care behavior in these prawns. Similar reports were made by Manjunath (1989).

Survival

The percentage survival of the three groups of female *M. lanchesteri* of OI<30, OI 30-45 and OI 50-70 are plotted in the Figure 6. Control prawns of OI <30 showed 92% survival in 30 days feeding period, the percentage survival decreased to 80 in unilateral eyestalk ablated prawns and in bilateral eyestalk ablated individuals it was further reduced to 50%. In the other two groups of prawns also the percentage survival was lower in unilateral eyestalk ablated

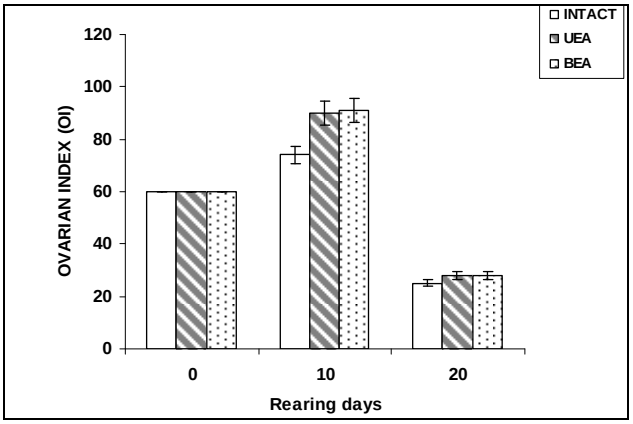

Figure 3. Effect of unilateral (UEA) and bilateral eyestalk ablation (BEA) on the ovarian maturity of female *Macrobrachium lanchesteri* (de Man) of ovarian index (OI) 60.

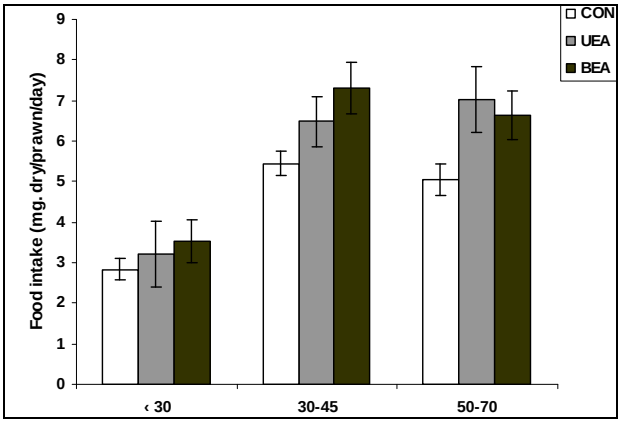

Figure 4. Effect of eyestalk ablation on the food intake of female *M. lanchesteri* (de Man) of diff. ovarian indices.

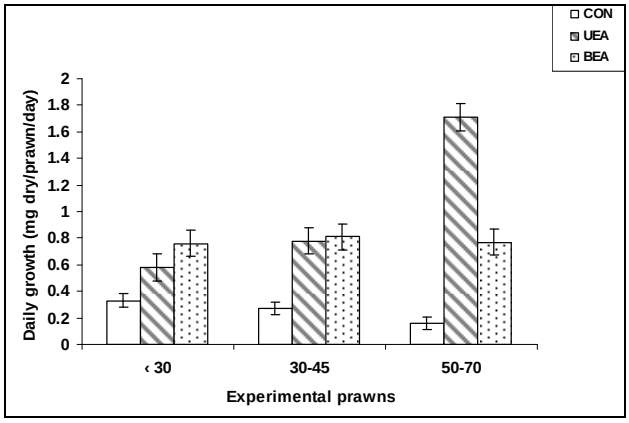

Figure 5. Effect of eyestalk ablation on the growth of female *M. lanchester* (de Man) of diff. ovarian indices.

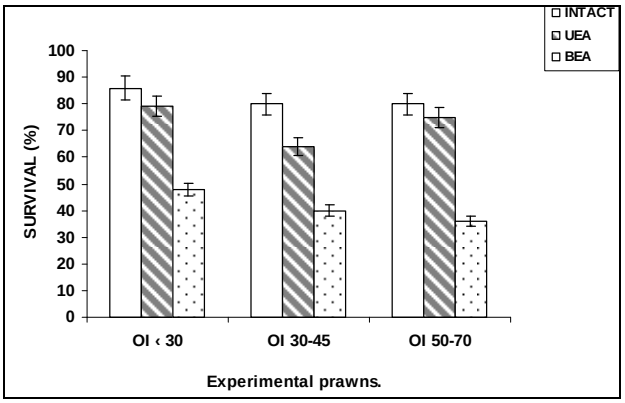

Figure 6. Effect of eyestalk ablation on the survival of female *M. lanchesteri* (de Man) of diff. ovarian indices.

prawns and very much lower in bilateral eyestalk ablated ones. This can be improved by controlling environments such as stocking density, tank size and rearing methods. In shrimps and other crustaceans eyestalk ablation is known to result in heavy mortality (Primavera *et al.*, 1978; Radhakrishnan and Vijayakumaran, 1984; Koshio *et al*., 1992). According to the results of Juinio-Meñez and Jesselita Ruinata (1996), at the end of two months, only 6% of the bilaterally ablated *Panulirus ornatus* survived compared with 73% and 75% for unilaterally ablated and unablated lobsters respectively.

Protein Content

Variations in the protein content of the ovary, hepatopancreas and carcass of the female *M. lanchesteri* (de Man) due to unilateral and bilateral eyestalk ablations are shown in the Figure 7. In females of OI<30, protein content was increased by both UEA and BEA in all the tissues. Whereas in the case of females of OI 30-45, protein content was decreased due to eyestalk ablations in both the hepatopancreas and carcass and increased in the ovary indicating their mobilization from hepatopancreas and carcass towards faster ovarian maturation. In the case of females of OI 50-70, protein content decreased in the hepatopancreas by UEA and BEA and showed an increasing trend in both the ovary and the carcass. In the case of the eggmass, the protein content decreased by both UEA and BEA. These results are in contrary to the reports of Koshio *et al.* (1992), who said that protein contents were not altered by UEA in *M. rosenbergii.* According to Okumura (2007), bilateral eyestalk ablation resulted in an increase in the vitellogenin level of the ovary and hemolymph of *Marsupenaeus* (*Penaeus*) *japonicus* and there was no significant increase in the case of unilaterally ablated prawns than the controls.

Carbohydrate Content

The eyestalk hormones are known to regulate the carbohydrate, nitrogen and lipid metabolism in crustaceans (Highnam and Hill, 1979). The changes in the carbohydrate content of hepatopancreas, ovary and carcass are shown in the Figure 8. In the control prawns OI<30, the carbohydrate of hepatopancreas decreased by unilateral and bilateral eyestalk ablations. In the ovary carbohydrate content decreased in unilaterally ablated ones but showed an increase in the bilaterally ablated prawns. In the carcass, it

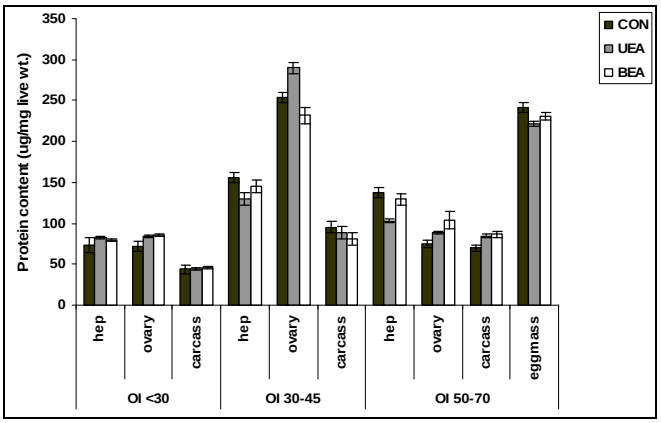

Figure 7. Effect of unilateral and bilateral eyestalk ablations on the protein content of the ovary, hepatopancreas and carcass of the three ovarian index classes of female *M. lanchesteri* (de Man).

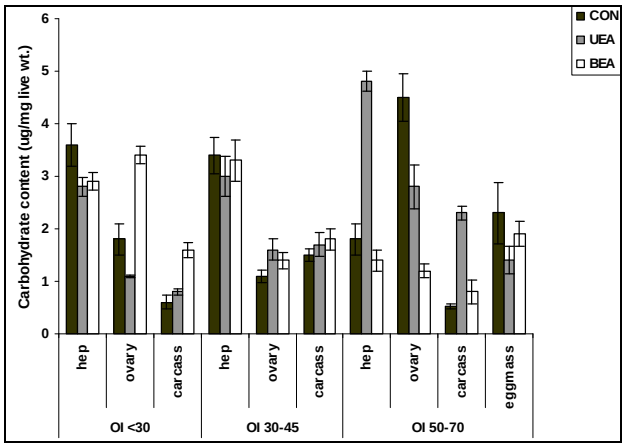

Figure 8. Effect of unilateral and bilateral eyestalk ablations on the carbohydrate content of the ovary, hepatopancreas and carcass of the three classes of female *M. lanchesteri* (de Man).

showed an increasing trend by both UEA and BEA. These results indicated the loss of metabolic regulatory mechanisms situated in the eyestalk. In the case of females of OI 30-45, eyestalk ablations resulted in a reduction in the carbohydrate content of hepatopancreas and increase in the case of the ovary as well as the carcass. But in the case of females of OI 50-70, carbohydrate content increased in the hepatopancreas by UEA and not by BEA. Ovary carbohydrate content showed a declining trend by both UEA and BEA. In the eggmass, carbohydrate content decreased by eyestalk ablations. These results are contrary to that of Soundarpandian and Ananthan (2008), who reported that carbohydrate content did not vary significantly by eyestalk ablation in M. malcomsonii when fed with different feeds.

Energy Content

Estimating the calorific value of the edible animals is very important from the nutritional point of view. When the energy content of the ovary, hepatopancreas and carcass of the prawns of OI 30 were compared (Figure 9), it was found that the energy content of the ovary and hepatopancreas were decreased by both unilateral and bilateral eyestalk ablations. Whereas, in the carcass there was not much difference in the energy content per unit dry weight. In the case of females of OI 30-45, when they were reared till they attained an OI of 60, it was found that, the energy content of all the tissues were increased by unilateral and bilateral eyestalk ablations. In the case of females of OI 50-70, the energy content per unit dry weight of all the tissues were decreased by both UEA and BEA. An exception was the energy content of the eggmass, which was increased by eyestalk ablations when the prawns were reared in the laboratory till they spawned. The energy content of eggmass was comparable to that of the ovaries of females of OI 30-45 when they matured to an OI of 60 showing the magnitude of energy allocation for reproduction. Sindhukumari and Pandian (1987) also reported an increase in energy investment for egg production by the ablated *M. nobilii.* According to the results of Venkitraman *et al.* (2004), production i.e, energy investment was increased by UEA and decreased drastically by BEA. This is contrary to our results where we observed that in maturing prawns

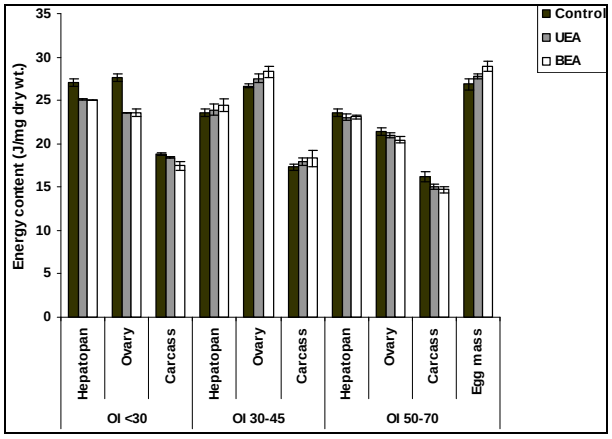

Figure 9. Effect of unilateral and bilateral eyestalk ablations on the energy content of the different tissues of the three classes of female *M. lanchesteri* (de Man).

(OI 30-45) both UEA and BEA resulted in an increase in the energy contents of all the tissues and in mature females (OI 50-70), energy contents of all the tissues except eggmass were decreased by both UEA and BEA.

In the present study, it is seen that in *M. lanchesteri*, eyestalk ablation brings about an increase in food intake in females of all the three ovarian index classes. The eyestalk ablation had induced rapid ovarian maturation and enhanced growth of these prawns. The present study documents for the first time the direct calorific content of the different tissues of female *M. lanchesteri* of varying ovarian maturation stages. The carbohydrate and protein contents were altered by eyestalk ablations. The energy content of the different tissues were also showing variations by both UEA and BEA. This report is in contrary to that of Koshio *et al.* (1992), who observed that water, protein and lipid profiles were not affected by unilateral eyestalk ablation in *M. rosenbergii*.

Conclusion

Although a high potential of unilateral eyestalk ablation for promoting growth and ovarian maturation of *Macrobrachium lanchesteri* (de Man) was demonstrated in the present study, further studies on the nutritional requirements of ablated prawns should be conducted. This freshwater prawn, being nutritious and tasty and consumed by the local people, can be commercially exploited. If the rural fish-folk around Bangalore can adopt unilateral eyestalk ablation and improved culture practices, they should be able to get a marketable size of the prawns earlier than naturally expected. This would help to eliminate rural poverty and unemployment.

Acknowledgement

Financial assistance from the University Grants Commission, New Delhi, is gratefully acknowledged.

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