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Single Extirpation of Eyestalk in the Freshwater Crab *Barytelphusa cunicularis* (Westwood, 1836)

Anne Rebecca A.[1]*, Keerthi P.[2] and Sowdeswari R.[3]

[1]Department of Zoology, Thiruvalluvar University, Vellore, Tamil Nadu, India
[2]PSG College of Arts and Science, Coimbatore, Tamil Nadu, India
[3]J.K.K. Nataraja College of Arts and Science, Komarapalayam 638 183, Tamil Nadu, India

**Corresponding Author*

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Abstract: India significantly faces a contemporary undernutrition issue. *Barytelphusa cunicularis*, a freshwater crab has potential alternative protein source with therapeutic values. However, the rearing and seed production of these crabs are not well documented. This study investigated the possibilities of seed production in *B. cunicularis* by artificially inducing gonadal maturation by unilateral eyestalk ablation (UEA), finding that growth and reproductive indices were significantly higher in induced crabs after 25 days. Induced male and female crabs showed increased daily growth rates (0.06±0.02 and 0.13±0.06 g), increased gonado-somatic index (0.40±0.02 and 0.59±0.14 %), reduced heart index (0.12±0.009 and 0.13±0.017 %), reduced hepato-somatic index (5.43±0.87 and 5.45±0.53 %), and higher protein, carbohydrates, and lipid content in gonads of induced male and female crabs compared to the control (144.28±6.92 and 238.36±18.96; 3.30±0.17 and 79.51±3.63; and 0.68±0.1 and 0.72±0.10 g, respectively). Muscle biochemical indices indicated the reduction of biochemical components from muscle and aggregation of these components in gonads. The ablated group exhibited higher testicular and ovarian indexes, with the ablated males having a higher testicular index (0.40±0.02 %) and the ablated females having a higher ovarian index (0.59±0.14 %). Induced male crabs showed lower survival rate (75%) and the histological examination of gonads in UEA induced crabs revealed gonadal stimulation exhibited by increased follicle size and increased follicle number in females and the increased spermatids in males as compared to the control.

Keywords: *Barytelphusa,* Eyestalk ablation, Crustacean induced breeding, Gonadal maturation, Testicular index, Protein, Carbohydrates, Lipid

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Introduction

Freshwater crabs are fast growing and belongs to the phylum Arthropoda (Padghane and Chavan, 2018) and are semi-terrestrial crustaceans (Padghane and Kajalka, 2021). The demand for

crab products is growing implying that aquaculture production must rise as well. According to Tran *et al.* (2020), the crab aquaculture sector has grown quickly in nations and regions.

Fisheries and aquaculture are socio-economically important sectors for most nations of the Asia-Pacific region, most of which have high fish consumption rates. India is one of the major contributors for global farmed fish production (FAO 2020). India is one among the 56 countries in the world to have large number of undernourished people (FAO, IFAD, UNICEF, WFP and WHO, 2021). The cost and affordability of healthy foods reasonably affects food security causing poverty and inequality, especially considering the economic downturns during COVID-19 pandemic, its alarming aftermath circumstances and climate extremes. In addition, millions of people around the world suffer from food insecurity and different forms of malnutrition because they cannot afford the cost of healthy diets.

Freshwater crabs are being consumed in many parts of the world. They are reported to have high nutritional and therapeutic values, especially for cardiac heart disease patients and are edible as well as nutritious like other crustaceans such as shrimp, prawns, and marine crab (Das *et al.*, 2015).

Tropical regions have a maximum diversity of crabs in the world (Yeo, 2008; Tsang *et al.*, 2014). Accordingly in India, about 389 different crab species have been reported (Roy and Nandi, 2008). *B. cunicularis* a freshwater crab belonging to Brachyurian sub-order, is reported to be highly suitable for inland capture farming as compared to *Scylla serrate*, a highly commercially exploited freshwater crab reared by coastal capture farming. *B. cunicularis* have lot of rearing potential with high tolerance to climate and diseases (Turner, 2018). Moreover *B. cunicularis* have lot of advantage over fish farming and other similar freshwater crab species like *Barytelphusa guerini*, as it can survive for 3-4 days without food and water in low moisture burrows and therefore possess a series of rearing, marketing and food durability qualities. The species is also being captured by local communities and tribals in India and sold in Indian local fish markets, which is also reported to have high demand (Padghane *et al.*, 2016). Role of freshwater crabs is vital in aquatic and terrestrial food webs and as biological indicators of environmental pollution.

The main challenge in freshwater crab farming is the availability and supply of seeds during demand. Further, raised crabs lack the ability to mature sexually (Amin-Safwan *et al.*, 2019). Eyestalk ablation, which removes the gonad inhibiting hormone (GIH) and vitellogenin inhibiting hormone (VIH) released from the X-organ sinus gland complex within eyestalks, might cause ovarian maturation. Therefore, in crustaceans, the removal of the eyestalk is crucial for both molting as well as reproduction (Revathi *et al.*, 2013; Shen *et al.*, 2013).

Hunger is more prevalent and has grown more in nations experiencing conflict, bad weather, economic downturns, or high levels of inequality (FAO 2021). Mud crab (*Scylla serrata*) farming is extensive along the coast (Turner, 2018). Away from the coast, there is another option provided by the widely dispersed freshwater crab *Barytelphusa cunicularis*, which is harvested for both local trade and subsistence use unproven cultural potential may exist, and it may provide a diet that is both environmentally and socially sustainable and resistant to illness and climate change.

Therefore, *B. cunicularis* is an inexpensive source of protein which possesses a high tolerance to food and water scarcity. It is extremely resistant to illnesses and climatic change, and may be a viable offshore substitute for *S. serrata*. In order to assess and improve the seed production of *B. cunicularis* for farming, gonadal stimulation was carried out in the current study *via* eyestalk ablation.

Materials and Methods

Collection and maintenance of B. cunicularis:

40 crabs were collected from Semmedu lake in the district of Coimbatore at the state of Tamil Nadu, India. Animals were handpicked with gloves and placed in a thermocol box with perforations for aeration. Then the animals were transported to the laboratory and were introduced into the aquarium glass tanks of size 35x25 cm and maintained under room temperature. Water and sand were provided adjacently inside the tank to a level of about 2 cm. Crabs were acclimatized for 7 days. During this period, the crabs were fed with commercially available fish feed daily once in proportion of 10% of their body weight. The initial length and weight of the crabs was measured.

Determination of morphometric indices, experimental setup and eye stalk ablation:

10 crabs were randomly used to determine morphometric indices. After anaesthetizing the animals with chloroform cotton balls, carapace length (CL), carapace width (CW), Total weight (TW), major chelate length (MACL) and minor chelate length (MICL) were measured using a graduated twine (Table 1). Collected animals were divided into four groups as non-ablated males (control), non-ablated females (control), ablated males and ablated females. Ablation was carried out after acclimatization period. For unilateral eye stalk ablation, the right eyestalk was removed from the base using a sterilized surgical scissors. After ablation the wound was cauterized immediately with a hot sterile blunt needle and immediately the crab was immersed in cold water at 18°C in order to reduce stress and loss of haemolymph, which cause mortality. During the experimental period the mortality of the ablated and non-ablated crabs were recorded to determine survival rate.

At the end of 30 days from ablation, crabs were dissected to obtain the tissues such as muscles, hepatopancreas, heart, ovary and testis. The tissues were weighed and stored in deep freezer at -80 ℃ and were used to study growth (Table 2), survival (Table 2), reproductive (Table 3), biochemical (Tables 4, 5) and histological indices (Figs. 8-11).

Following formulas were used for determining survival and growth indices:

Survival Rate (SR) % = Total number of crabs - Total number of dead crabs / Total number of crabs X 100

Daily Growth Rate (DGR) g = Final weight – Initial weight / Total number of days

Hepatosomatic index (HSI) % = Wet weight of the hepatopancreas / Total wet weight of the crab X 100

Heart Index (HI) % = Wet weight of the heart / Total wet weight of the crab X 100

Gonadosomatic index (GSI) % = Wet weight of the gonads / Total wet weight of the crab X 100

Biochemical indices:

Muscle tissues, testis and ovaries of ablated and non-ablated crabs were subjected to biochemical indices. Carbohydrates were estimated by the method of Roe *et al.* (1955). Total proteins were estimated by the method of Lowry *et al.* (1951). Lipids were estimated by the method of Folch *et al.* (1957).

Physical examination of gonads:

The color and appearance of the ovary and testes were recorded in ablated and non-ablated crabs.

Histological indices:

Testis and ovaries of ablated and non-ablated crabs were subjected to histological study with differential stain (H & E). Photomicrographs were obtained under 40X of light microscope.

Statistical analysis:

All data obtained from growth, reproductive and biochemical indices were analyzed using SPSS statistical V-25 software. Data are expressed as mean ± standard deviation of triplicate analysis (Zar, 2009).

Table 1: Morphometric indices of *B. cunicularis* before extirpation

Indices	Male	Female
CL (cm)	5.03±0.40	5.36±0.61
CW (cm)	6.06±0.45	6.5±0.78
Major chela (cm)	10.96±0.68	10.63±1.0
Minor chela (cm)	8.8±0.4	8.6±0.26
Weight (g)	65.16±9.05	65.56±12.06

Data obtained as a result of triplicate analysis expressed as mean± S.D. All values are significant at P≤0.05.

Table 2: Growth and survival indices of *B. cunicularis*

Indices	Male		Female	
	Non-Ablated	Ablated	Non-ablated	Ablated
Survival rate (%)	100±0.00	75±5.00	100±0.00	100±0.00
Daily growth rate (g)	0.04±0.006	0.06±0.02	0.03±0.03	0.13±0.06
Heart index (%)	0.21±0.005	0.12±0.009	0.14±0.02	0.13±0.017
HSI (%)	6.05±0.40	5.43±0.87	7.14±0.26	5.45±0.53

Data obtained as a result of triplicate analysis expressed as mean± S.D. All values are significant at P≤0.05.

Table 3: Reproductive (GSI) indices of *B. cunicularis*

Indices	Non-ablated male	Ablated male
Testicular index (%)	0.32±0.05	0.40±0.02
Ovarian index (%)	Non-ablated female	Ablated female
	0.49±0.04	0.59±0.14

Data obtained as a result of triplicate analysis expressed as mean± S.D. All values aresignificant at P≤0.05.

Results

Survival, Growth and Biochemical indices:

Hepatosomatic index (HSI), heart rate, and muscle-protein, carbohydrates and lipids of ablated crabs decreased. However, daily growth rate, reproductive indices and gonadal-protein, carbohydrates and lipids of ablated crabs increased (Tables 2-5).

The survival rate for ablated males was 75%, compared to 100% for ablated females. The daily growth rates for ablated males (0.06±0.02) and females (0.13±0.06) were higher compared to non-ablated males (0.04±0.006) and females (0.03±0.03). The heart index for ablated males decreased to 0.12±0.009 compared to 0.21±0.005 for non-ablated males. In contrast, there was little difference between ablated females (0.13±0.017) and non-ablated females (0.14±0.02). Ablation led to a reduction in the hepatosomatic index (HSI). For ablated males, the HSI was 5.43±0.87, compared to 6.05±0.40 for non-ablated males. For ablated females, the HSI was 5.45±0.53, while it was 7.14±0.26 for non-ablated females. The

Table 4: Muscle Biochemical indices of *B. cunicularis*

Indices	Male		Female	
	Non-ablated	Ablated	Non-ablated	Ablated
Protein (mg)	140.89±5.12	72.40±1.46	157.30±18.45	142.88±16.70
Carbohydrate (mg)	5.88±1.76	2.07±0.20	5.53±0.66	6.28±1.08
Lipid (mg)	0.35±0.03	0.43±0.007	0.73±0.09	0.45±0.005

Data obtained as a result of triplicate analysis expressed as mean± S.D. All values aresignificant at P≤0.05.

Table 5: Gonadal biochemical indices of *B. cunicularis*

Indices	Male		Female	
	Non-ablated	Ablated	Non-ablated	Ablated
Protein (mg)	88.12±5.25	144.28±6.92	79.51±3.63	238.36±18.96
Carbohydrate (mg)	3.12±0.62	3.30±0.17	2.53±0.41	13.28±0.87
Lipid (mg)	0.28±0.046	0.68±0.1	0.26±0.06	0.72±0.10

Data obtained as a result of triplicate analysis expressed as mean± S.D. All values aresignificant at P≤0.

gonadosomatic index (GSI) was higher in both ablated males (0.40±0.02) and females (0.59±0.14) compared to non-ablated males (0.32±0.05) and females (0.49±0.04).

Ablation has led to a reduction in proteins, carbohydrates and lipids in muscle tissues of both males and females, while increasing their concentrations in the gonads. Muscle protein was higher in the non-ablated group, with males showing values of (140.89±5.12 mg) and females (157.30±18.45 mg). In contrast, the ablated group had lower values, with males at 72.40±1.46 mg and females at 142.88±16.70 mg. Regarding gonads, the ablated group exhibited significantly higher protein content. Specifically, ablated males had 144.28±6.92 and females 238.36±18.96 mg, while non-ablated males had 88.12±5.25 mg and females 79.51±3.63 mg.

In muscle, non-ablated males (5.88±1.76 mg) and females (5.53±0.66 mg) had a higher proportion of carbohydrates. For ablated males, the value was 2.07±0.20, and for ablated females, it was 6.28±1.08. In the gonads, ablated males had 3.30±0.17 mg and females had 13.28±0.87 mg. In contrast, gonads of non-ablated males had 3.12±0.62 mg and females had 2.53±0.41 mg, which were lower than those of the ablated crabs.

Lipid content in muscle tissues of female crabs significantly reduced due to ablation. Non-ablated females had 0.73±0.09 mg and ablated females had 0.45±0.005 mg in their muscles. Consequently, the lipid content in gonads significantly increased due to ablation. Ablated males showed 0.68±0.1 and females reported 0.72±0.10 mg in contrast to non-ablated males (0.28±0.046 mg) and females (0.26±0.06 mg) in their gonads.

Physical examination of gonads:

UEA in *B. cunicularis* females resulted in larger milky-colored testis, compared to non-ablated male crabs (Figs. 4, 5) and larger mature ovaries with dark yellow eggs (Figs. 6, 7).

Histological Indices:

The ablated ovary in females exhibits an increased diameter of follicles, a greater number of follicles, and oocytes that have undergone post-

Fig. 1: Dorsal view of *B. cunicularis.*

Fig. 2: Ventral view of Male *B. cunicularis.*

Fig. 3: Ventral view of Female *B. cunicularis.*

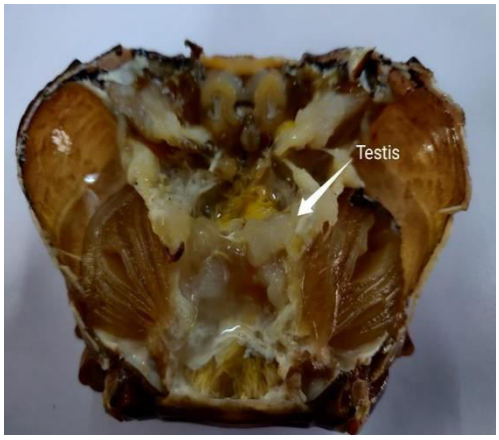

Fig. 4: Testis of non-ablated Male *B. cunicularis.*

Fig. 5: Testis of ablated Male *B. cunicularis.*

Fig. 6: Ovary of non-ablated Female *B. cunicularis.*

Fig. 7: Ovary of ablated Female *B. cunicularis.*

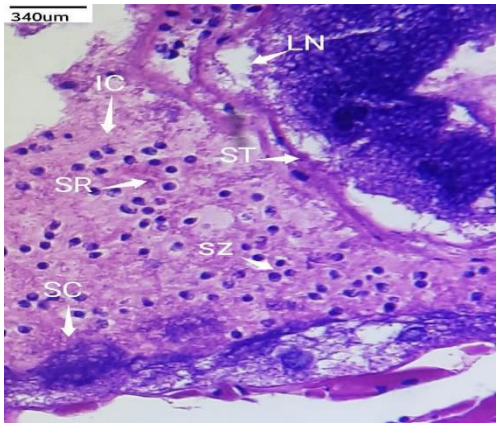

Fig. 8: Cross section of non-ablated Male *B. cunicularis.* **IC-** Interstitial Cell, **SR-** Sertoli Cells, **ST-** SeminiferousTubule, **SC-** Spermatocytes, **SZ-** Spermatozoa, **LN-** Lumen.

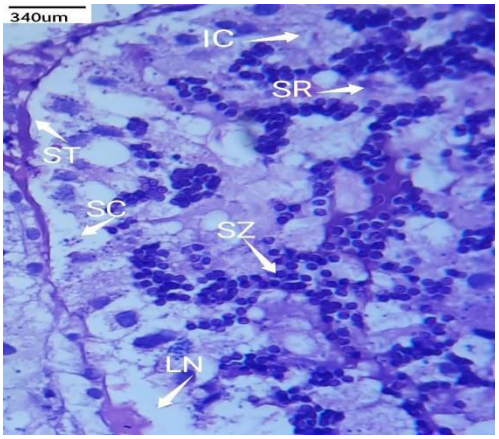

Fig. 9: Cross section of ablated Male *B. cunicularis.* **IC-** Interstitial Cell, **SR-** Sertoli Cells, **ST-** SeminiferousTubule, **SC-** Spermatocytes, **SZ-** Spermatozoa, **LN-** Lumen.

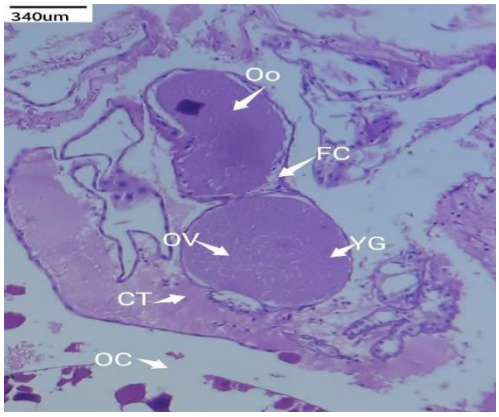

Fig. 10: Cross section of non-ablated Female *B. cunicularis.* **NU-** Nucleus, **Oo-** Oocyte, **YG-** Yolk Granule, **FC-** Follicular Cells, **OV-** Ovum, **CT-** Connective Tissue.

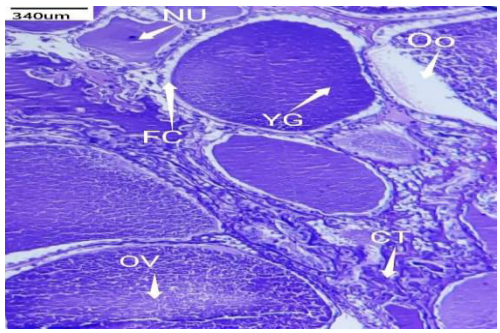

Fig. 11: Cross section of ablated Female *B. cunicularis.* **NU-** Nucleus, **Oo-** Oocyte, **YG-** Yolk Granule, **FC-** Follicular Cells, **OV-** Ovum, **CT-** Connective Tissue.

vitellogenesis. In ablated male crabs, there is an augmentation of spermatozoa within the seminiferous tubules (Figs. 8-11).

Discussion

Research on crustaceans reveals that eye ablation can affect molting, development, reproduction, and metabolic physiology (Ikhwanuddin *et al.*, 2019). The eyestalk complex, composed of the X-organ sinus gland, secretes inhibitory hormones controlling growth and reproduction (Banoo *et al.*, 2018). Eye ablation eliminates these hormones, causing ovarian maturation and moulting patterns (Kale. 2017).

The present study examines the effects of unilateral eye stalk ablation on muscle and gonadal growth, survival, and biochemical changes in male and female *B. cunicularis* crabs compared to non-ablated crabs. Results showed that non-ablated crabs have a 100% survival rate for both sexes and 75% for ablated male. Ablation in crustaceans is reported to cause mortality due to extreme stress experienced (Koshio *et al.*, 1992).

Improvement in gonadal maturation has resulted in an improved growth rate. The growth rate for ablated crabs was higher. This might be due to the quick maturation of the ovary. The ablated group had a significantly lower hepatosomatic index. This might be a result of vitellogenesis, in which reserves and nutrition are transported from the hepatopancreas storage region to the gonads, which require a lot of nutrients and minerals for fast growth. As a result, amylase activity can be high in ablated crabs, indicating higher hepatopancreatic metabolism (Wu *et al.*, 2013). There was only a slight reduction in the heart index for the ablated male and female crabs. In contrast, according to Pervaiz *et al.* (2011), *Scylla serrata* demonstrated an increase in heart rate in relation to eyestalk ablation.

In this study, ablated crabs showed higher reproductive indices compared to non-ablated crabs. The testicular index in ablated males was notably higher (0.40±0.02%) than in non-ablated males (0.32±0.05%). Similarly, the ovarian index in ablated females exceeded (0.59±0.14%) that of non-ablated females (0.49±0.04%). This difference was attributed to a decrease in gonadal inhibiting hormone secretion from the X-organ sinus in the eyestalk complex, leading to accelerated gonad growth in ablated crabs (Kale, 2017). According to a study conducted by Sukardi *et al.* (2019), eye stalk ablation has been used to obtain soft shell crabs in a fast time and it is one of the methods for quick maturity in crabs. Ablated males exhibited larger testes with wider, convoluted milky vas deferens, while non-ablated males had smaller testes with narrow vas deferens. Ablated females had large, dark yellowish-orange ovaries containing visible eggs, contrasting with the smaller, slightly yellow ovaries of non-ablated females.

Muscle biochemical indices indicated the reduction of biochemical components from muscle and aggregation of these components in gonads. Muscle carbohydrates significantly reduced in ablated crabs. Carbohydrate indices also reflects glucose, sucrose, fructose, and other mono and polysaccharides (Okuzumi and Fujii, 2000). Similarly, muscle protein content reduced in ablated male and female crabs (72.40±1.46 and 142.88±16.70) than the non-ablated crabs (140.89±5.12 and 157.30±18.45). Muscle lipid content greatly reduced in ablated female crabs. This can be due to utilization of lipid content during maturation of ovum, as its requirement is high.

The gonadal biochemical indices represent a greater level of growth and metabolism (Murugesan *et al.*, 2008). Gonadal biochemical indices in the present study reveals elevated levels of carbohydrates, proteins and lipids in the gonads of ablated male and female crabs. Explicitly, when comparing the protein content in ablated female (238.36±18.96 mg) with the non-ablated female (79.51±3.63 mg), the protein content in the gonads is significantly improved which denotes the growth and maturity they have attained. Similarly, the lipid is essential for a crustacean in

their metabolism and reproduction (Gangotri, 1978). Eyestalk ablation promotes the growth of gonads (Hussain *et al.*, 2014). The increase in lipid content of gonads was due to mobilization of lipids from hepatopancreas due to vitellogenesis (Alava *et al.*, 2007).

Histological study of male testis revealed increased production of spermatids in ablated male crabs (Fig. 5). The spermatids are characterized by the round shape with prominent nucleus. Later they develop into spermatozoa (Ghorpade, 2016). The tubules are highly coiled and large with densely packed spermatids. The gonadal development is found to be accelerated in unilaterally ablated male. The histological study of female ovary revealed mature spherical ovary with stained nucleus and contains moderate amount of ooplasm which is in accordance with the findings of Kumaran *et al.* (2015). The oocytes were seen surrounded by follicular cells. This is attributed to the occurrence of vitellogenesis owing to reduction of gonadal inhibiting hormone (GIH) thus resulting in growth of ovary in a short span of time.

The amount of primary oocyte is found to be high in case of the unilaterally ablated female than the non-ablated female. Nur *et al.* (2021) stated that, eyestalk ablation is a quick way to mature ovaries even if it is regarded as a disruptive and divisive reproductive technique. Extirpation of eyestalk is also a simple way to obtain soft-shelled, molted crabs without using hormone boosters. Therefore, eye ablation in crustaceans is used to regulate growth and reproduction. However, the stress from ablation can be recovered with continuing growth. Unilateral eyestalk ablation promotes quicker maturation of follicles, spermatids, and spermatozoa. In females it fastens ovarian maturation and quantitative primary oocyte development. These findings were firmly established on histological investigations.

Conclusion

The present study indicates that unilateral eyestalk ablation in *B. cunicularis* markedly affects gonadal growth and maturation, presenting a cost-effective and efficient technique for induced breeding as evident from Daily Growth Rate, Hepato-somatic Index, Gonado-somatic Index, biochemical alterations in muscle, gonads and histological examination of gonads.

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