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ARGULUS OF INDIAN MAJOR CARPS IN SELECTED FISH FARMS OF MYMENSINGH

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ABSTRACT

Studies were conducted to investigate the infestations of *Argulus* of juvenile carps of Mymensingh region. A total of 8 farms, four Government (Govt.) and four Private (Pvt.) fish farms were investigated Indian major carps - Catla (*Catla catla*), Rui (*Labeo rohita*) and Mrigal (*Cirrhinus cirrhosus*) during June, 2010 to May, 2012. Monthly samplings were carried out with 5 fish from each species and each farm. Altogether 2880 fish host were examined of which 940 hosts were found to be infested with *Argulus* during the study period. The *Argulus* were very common in body surface, base of the pelvic, pectoral, anal, caudal fin, muscle and mouth cavity of all fishes. Water quality and management practices were recorded in different farms. The risk of infestation by *Argulus* in carps significantly ($p<0.001$) increased when the water quality parameters were not maintained the optimum level. Prevalence (%), mean intensity and abundance were found to be species specific and also varied with seasons and management systems. Prevalence (%) of *Argulus* in carps was significantly ($p< 0.001$) higher in Pvt. farm than that of Govt. farms. The prevalence (%) of *Argulus* were significantly ($p< 0.01$) higher in rainy season. Rui was more susceptible than Catla and Mrigal by *Argulus*.

Key words: *Argulus*, Infestation, Water quality, Seasons and Indian major carps

INTRODUCTION

One of the major problems of fish culture is the parasitic infestation and disease. Fish parasites can cause mortalities of fishes in culture operations. They attack fishes and destroy them or make wounds or disease on their flesh, thus making them unedible (Woo, 1999). Hence, in order to control fish diseases caused by the parasites, it is essential to study their taxonomy to identify them, their infestations, as well as their effect on hosts. Argulosis is one of the problems in fish culture program in Bangladesh. Fish farmers raise several complains to protect their crop from argulosis both in nurseries and culture ponds. The disease is caused by an ectoparasitic crustacean of the genus *Argulus*, the fish louse, is a small miniature with dorsoventrally flattened body provided with suckers and proboscis. *Argulus*, the crustacean feeds by first inserting a pre-oral sting which injects digestive enzymes into the body. They then suck out the liquidated body fluids with their proboscis-like mouth. The various spines, suckers and hooks that lice use for attachment may also cause additional tissue damage (Shimura and Inoue, 1984).

The parasite community of fishes shows considerable variation with the environmental conditions in where they live. Various physic-chemical factors have strong influence on fish health and their resistance against the disease causing agents (Plumb *et al.*, 1988 and Shresta, 1994). The physiological and biological features of the host affect the composition of parasites (Shafir and Van, 1986). Poor of physic-chemical properties of water are O_2 depletion, excess ammonia, carbon dioxide, excess CO_2 in water and temperature change.

Several works have been done in other countries of the world on argulosis. Thomas and Devaraj (1975) studied the taxonomy of *Argulus* and erected two new species *A. cauveriensis* and *A. fluviatilis*. He also provided a key for identification of the Indian species reported from fish. Gresty *et al*. (1993) stated that *A. japonicus*, an ectoparasite attaching to, and feeding on the skin of its host, is a potentially serious pathogen of native freshwater fishes. Padmavathi and Prasad (1998) studied on the control measures of argulosis using Nuvan and Ekalux.

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Argulus from freshwater wild fish was reported by Ahmed and Sanaullah (1978). Chandra *et al*. (2004) studied on affected fishes and found flesh of both sides of vertebral column of the infested fish was shrunk. When *Argulus foliaceus* and its related species lay eggs attach to fish can be seen with the naked eye. The attached area is often red and irritated the fish show hemorrhagic reaction to the infection. Bakshi *et al*. (2006) stated that June, July and August were the most potential months for *Argulus* infestation. Rui and Mrigal were higher susceptible species for argulosis. Preferable site of infestation on the host body is pectoral region. Brood fishes were more susceptible to *Argulus* infestation, due to weaker physiological condition at the breeding season. Ahmed (2004) worked to the development of environment friendly measures for the treatment of argulosis in carp brood ponds. Though a number of information are available from other countries, no work except BAU yet been done in Bangladesh. Only preliminary information are available from the works of Bakshi *et al*. (2006), Chandra *et al.* (2004) and Rahman (1968). However, for a more detailed information regarding the infestation of Argulus the present work was undertaken on the Seasonal infestations of *Argulus* in different carp species; general pattern of infestation in different seasons and its infestations in different farming systems of juvenile indigenous Indian major carps of Mymensingh district.

MATERIALS AND METHODS

For the study eight fish farms, 4 Government Farm and 4 Private farms were investigated. The study was conducting during June 2010 to May 2012. To observe the seasonal infestation, only main seasons were used as rainy season (June-September); winter season (October-January) and summer (February-May). Catla, Rui and Mrigal were selected as the experimental fish and sampled regularly by visiting the experimental ponds. Samplings were carried out from the farms at monthly intervals. During each sampling 5 fishes from each species were collected every pond with the help of seine net. Fish samples were transported to Disease Laboratory with plastic containers and bags. The young carps or juvenile were examined quickly to observe for *Argulus* infestation, injury, infection and other abnormal conditions of fish body. Water quality and pond management procedures of various parameters were noted.

Collection, fixation, preservation and study of *Argulus*

Most of the *Argulus* were recorded from the base of the pelvic, pectoral, anal, caudal fin, body surface, muscle and mouth cavity. Body surface of the host fishes were carefully observed. Infesting *Argulus* were collected and preserved for further study. Skin scrapping was done from different parts of the body and smears were taken for examined. *Argulus* and their number were recorded as much as possible. Larger crustaceans were removed from the skin by a pair of forceps and thumbs and then placed into a water containing jar or clean water on petridish for allowing them relaxed, swimming and washing. After washing, the live specimens were dropped into a vial containing 70% alcohol for further processing. The slides with the parasites were impregnated with Klein's dry silver impregnation technique (Klein, 1958). Preserved *Argulus* were studied under microscope and their size shape and chitinoid structure was noted identification. Photographs was taken, total length, width and other appendages were drawn with the help of a camera lucida. The measurements were taken with the help of oculormicrometer adjusted with stage micrometer and the microscope. The length of body, carapace length and width, the length and breath of tail, the diameter of the sucking disc, the length of second maxillae and numerous spines were measured. All the measurements are given in millimeters (mm), ranges in parentheses by the arithmetic mean and standard deviation unless other wise stated. For statistical analysis, morphometric measurements of 10 specimens for the species were considered. Measurements were done following the recommendations of Shimura and Inoue (1984) and Chandra (2008).

Identification

Infested fishes were collected from the selected water bodies and various symptoms of diseases were noted according to Amlacher, 1997. Identification and classification of the parasites were done following Thomas and Devraj (1975) and Chandra (2008). The ecological terms for prevalence (%) and intensity of infestation were used after Margolis *et al*. (1982) as

$$\text{Prevalence} = \frac{\text{No. of hosts infected}}{\text{No. of hosts examined}} \times 100$$

$$\text{Mean intensity} = \frac{\text{No. of parasites recovered}}{\text{No. of infected fish}}$$

$$\text{Abundance} = \frac{\text{No. of parasites recovered}}{\text{No. of host examined}}$$

Statistical analysis

Prevalences of *Argulus* were computed by the following formula (Thrusfield, 1995): Chi-square and normal tests had been performed for testing the significance of the variation in prevalences of argulus of different species, seasons and farms (Gupta, 2005). Comparison of two prevalences were made by normal test and chi-square test was used for examining the existence of overall significant differences among more than two prevalences, but their mutiple comparisons were done by Tukey-type test (Zar, 2003). In Tukey-type test, prevalences were transformed by the following arcsin transformation formula (Zar, 2003) due to its preference by many researchers:

$$P' = \frac{1}{2}\left[\arcsin\sqrt{\frac{X}{n+1}} + \arcsin\sqrt{\frac{X+1}{n+1}}\right]$$

$P' =$ Transformed proportion

$X =$ No. of infested fishes

$n =$ No. of examined fishes

Mean intensity and abundance of argulus in different types of juvenile carp fishes corresponding to the different seasons as well as farms were compared by Tukey tests (Zar, 2003). All the statistical analyses were done by SPSS (Statistical Package for Social Science) and MS Excel.

RESULTS AND DISCUSSION

Water quality parameters in carp culture ponds in Mymensingh region

Temperature, dissolved oxygen (DO), pH, ammonia, alkalinity, hardness, transparency and depth of water were recorded from 8 (4 Govt. and 4 Pvt.) ponds in different months and seasons. In the present investigation, these parameters were exhibited significant variations during summer, rainy and winter season. Significantly increased value of ammonia, alkalinity, hardness and decreased value of temperature and dissolved oxygen (DO) were observed during these seasons which were within the unfavorable range of fish culture over the study period as indicated in Table 1.

Table 1. Seasonal fluctuations of water quality parameters of culture fish farms in Mymensingh

Seasons	Water quality parameters (Mean ± SD)							
	Water Temp (^{0}C)	DO (mg/l)	pH	Ammonia (ppm)	Alkalinity (ppm)	Hardness (ppm)	Transparency (cm)	Depth of water (cm)
Rainy	32.97 ±1.27	5.87 ±0.40	7.30 ±0.35	1.70±0.1 0	105.33 ±13.01	93.67 ±11.72	35.00±2.00	173.00 ±2.65
Winter	19.43 ±4.68	4.57 ±0.70	7.07 ±0.15	1.87±0.4 7	154.33 ±12.06	125.00 ±26.96	36.67±4.04	141.00 ±13.23
Summer	27.78 ±3.46	5.53 ±0.60	7.40 ±0.26	1.73±0.4 0	158.00 ±8.89	142.00 ±9.87	34.67±4.04	157.33 ±9.81
Mean ±SD	26.3 ±2.02	5.3 ±0.22	7. 2 ±0.08	1.7±0.09	138.0 ±8.61	118.8 ±8.75	36.3 ±1.15	155.5 ±4.80

Seasonal infestations of argulus in different juvenile carp species

Infestations of argulus in juvenile carp species fluctuated over the study period. In government farm, the highest prevalence of this parasitic disease was recorded as 53.75% for Rui in rainy season followed by 40% in Mrigal in the same season. In case of Catla, the highest prevalence (27.5%) in Govt. farm. In Pvt. farm, the highest prevalence of argulus was 63.75% in Rui in rainy season. Infestations was 50% in Mrigal in the same season. In context of Catla, the prevalence of this parasite is also found as the highest (38.75%) in rainy season in Pvt. farm. The higher mean intensity and abundance were obtained for Rui in rainy season in Pvt. farm, but the lower were observed for Catla in winter in Govt. farm (Table 2). It was evident from the Table 2 that the prevalence of argulus in both the Govt. and Pvt. farm differs significantly ($p<0.05$ or $p<0.01$) among the different species in each of the seasons.

Table 2. Seasonal infestations of argulus in different juvenile carp species during June, 2010 to May, 2012

Seasons	Species	No. of host fishes		Total of argulus	Prevalence (%) (Transformed prevalence, %)[1]	χ^2 (p-value)	Mean intensity	Abundance
		Examined	Infested					
				Govt				
Rainy	Catla	160	44	322	27.50 (31.80^{bc})	11.454 **	7.32^{c}	2.01^{c}
	Rui	160	86	2382	53.75 (47.12^{a})	(0.003)	27.70^{a}	14.89^{a}
	Mrigal	160	64	1082	40.00 (39.30^{ab})		16.91^{b}	6.76^{b}
Winter	Catla	160	12	28	7.50 (16.44^{bc})	9.916**	2.33^{c}	0.18^{c}
	Rui	160	42	232	26.25 (31.01^{a})	(0.007)	5.52^{b}	1.45^{a}
	Mrigal	160	32	230	20.00 (26.83^{ab})		7.19^{a}	1.44^{ab}
Summer	Catla	160	22	148	13.75 (22.13^{bc})	7.731*	6.73^{ac}	0.93^{b}
	Rui	160	50	944	31.25 (34.13^{a})	(0.021)	18.88^{a}	5.90^{a}
	Mrigal	160	46	546	28.75 (32.59^{ab})		11.87^{ab}	3.41^{ab}
Total		1440	398	5914	27.64		14.85	4.11
				Pvt				
Rainy	Catla	160	62	564	38.75 (38.58^{bc})	10.036**	9.10^{c}	3.53^{c}
	Rui	160	102	2980	63.75 (52.88^{a})	(0.007)	29.22^{a}	18.63^{a}
	Mrigal	160	80	1620	50.00 (45.00^{ab})		20.25^{b}	10.13^{b}
Winter	Catla	160	26	102	16.25 (24.09^{bc})	8.401*	3.92^{b}	0.64^{c}
	Rui	160	58	378	36.25 (37.12^{a})	(0.015)	6.52^{a}	2.36^{a}
	Mrigal	160	48	268	30.00 (33.36^{ab})		5.58^{ab}	1.68^{ab}
Summer	Catla	160	36	294	22.50 (28.55^{bc})	7.993*	8.17^{ac}	1.84^{b}
	Rui	160	68	1304	42.50 (40.74^{a})	(0.018)	19.18^{a}	8.15^{a}
	Mrigal	160	62	820	38.75 (38.58^{ab})		13.23^{ab}	5.13^{ab}
Total		1440	542	8330	37.64		15.37	5.78

Level of Significance : * $p<0.05$ and ** $p<0.01$. [1]Transformed prevalences are computed and compared (pairwise) only for significant chi-square values and any two of these prevalences in each season having no common letter are significantly different at 5% or 1% level of probability and the level selection depends upon the p-value of the corresponding chi-square. In each season, any two values of mean intensity and abundance having no common letter are significantly different at 5% level of probability.

Seasonal variation of infestations of argulus in carp fishes

Seasonal variation of infestations of argulus in carp fishes found to be fluctuated. In Govt. farm, the highest mean intensity (19.52) and abundance (7.89) in rainy season followed by 13.88 & 3.41 in summer and 5.70 & 1.02 in winter were recorded. Similar nature of infestation was observed though higher infestation was observed in case of Pvt. farm (Table 3). The seasonal prevalence of argulus in carp fishes varies significantly ($p<0.001$) both in Govt. and Pvt. farms. Even the combined set of data exerts significant ($p<0.001$) difference in prevalences of this parasite (Table 3).

Table 3. Seasonal variation of infestations of argulus in carp fishes during June, 2010 to May, 2012

Farm category	Seasons	No. of host fishes		Total load of argulus	Prevalence (%) (Transformed prevalence, %)[1]	χ^2 (p-value)	Mean intensity	Abundance
		Examined	Infested					
Govt	Rainy	480	194	3786	40.42 (39.50^{a})	29.267 ***	19.52^{a}	7.89^{a}
	Winter	480	86	490	17.92 (25.14^{bc})	(0.000)	5.70^{c}	1.02^{c}
	Summer	480	118	1638	24.58 (29.80^{b})		13.88^{b}	3.41^{b}
Total		1440	398	5914	27.64		14.86	4.11
Pvt	Rainy	480	244	5164	50.83 (45.48^{a})	32.057 ***	21.16^{a}	10.76^{a}
	Winter	480	132	748	27.50 (31.69^{b})	(0.000)	5.67^{c}	1.56^{c}
	Summer	480	166	2418	34.58 (36.06^{bc})		14.57^{b}	5.04^{b}
Total		1440	542	8330	37.64		15.37	5.78
Combined	Rainy	960	438	8950	45.63 (42.50^{a})	60.386***	20.43^{a}	9.32^{a}
	winter	960	218	1238	22.71 (28.50^{c})	(0.000)	5.58^{c}	1.29^{c}
	Summer	960	284	4056	29.58 (32.98^{b})		14.28^{b}	4.23^{b}
Total		2880	940	14244	32.64		15.15	4.95

Level of Significance: *** $p<0.001$. [1]Transformed prevalences are computed and compared (pairwise) only for significant chi-square values and any two of these prevalences in each farm category having no common letter are significantly different at 1% level of probability. In each farm any two values of mean intensity and abundance having no common letter are significantly different at 5% level of probability.

Infestations of argulus in carp fishes in different farms

Among the different Govt. and Pvt. farms the highest prevalence (40.56%) were found in Deshbandhu Pvt. farm and mean intensity (17.59) were found in Pankouri Pvt. farm and abundance (6.27) were found in Deshbandhu Pvt. farm. Minimum prevalence, mean intensity and abundance were found in BFRI Govt. farm. In combined prevalence was (27.64%), mean intensity was (14.86) and abundance was (4.11) were found in Govt farm and similarly cumulative prevalence was (37.64%), mean intensity was (15.37) and abundance was (5.78) were observed in Pvt. farm during the study period. The highest prevalence in Sombhugonj (33.89) was recorded, lowest prevalence (21.11) in BFRI farm (Table. 4). It could be concluded that farm based prevalences of argulus in carp fishes exhibit significant ($p<0.001$) difference in Govt. farm also differ insignificantly ($p>0.05$) in case of Pvt. farm as well. The mean intensity and abundance of argulus do not differ significantly among the farms of Govt. as well as the Pvt. ones.

Table 4. Infestations of argulus in carp fishes for combining 2 years in different farms during June, 2010 to May, 2012

Type	Name of the farm	No. of the host Fishies		Total load of argulus	Prevalence (%)	χ^2 (p-value)	Z-statistic (p-value)	Mean intensity	Abundance
		Examined	Infested						
GOVT	BFRI	360	76	986	21.11	7.576	4.045***	12.97^{ad}	2.74^{bc}
	Maskanda	360	104	1514	28.89	(0.056)	(0.000)	14.56^{ac}	4.21^{ab}
	Sombhugonj	360	122	1954	33.89			16.02^{a}	5.43^{a}
	Gouripur	360	96	1460	26.67			15.21^{ab}	4.06^{ac}
	Total	1440	398	5914	27.64			14.86	4.11
PVT	Brahmaputra	360	138	1788	38.33	1.769		12.96^{ad}	4.97^{ad}
	Deshbandhu	360	146	2258	40.56	(0.622)		15.47^{ac}	6.27^{a}
	Pankouri	360	122	2146	33.89			17.59^{a}	5.96^{ab}
	Sornalota	360	136	2138	37.78			15.72^{ab}	5.94^{ac}
	Total	1440	542	8330	37.64			15.37^{a}	5.78

Level of significance : *** $p<0.001$. Any two values of mean intensity and abundance in each farm category having common letter do not differ significantly at 5% level of probability.

Fish release CO_2 through its skin and gills. This activity is hampered if the amount of this gas is more in the water. As a result fish suffers from suffocation leading to death (Jhingran, 1988). According to Swingle (1967), free CO_2 at a concentration of more than 15 ppm was detrimental for pond fishes. Prolonged exposure to low concentrations of DO can be harmful to fish life because they would die at a level of 1 mg/l. Growth and feeding decreases at 1-5 mg/l of DO and growth and production is optimum at more than 5mg/l (Hossain *et al*., 2007).

Argulids have been recognized as pests of cultured trout in Europe and carp in China since 17th century (Wilson, 1926; Kabata, 1985). They cause mortalities of fish in aquaria, in lakes and estuaries and occasionally cause problems in sea-caged salmonids (Menezes *et al*., 1990; Stuart, 1990). Secondary infections by fungi and bacteria reduce the commercial value of parasitized carp and goldfish (Shimura, 1983). In severe climates they overwinter as eggs (Razmashin and Shirshov, 1981. In more moderate climates, it survives the winter as adults and may breed all the year round (Kimura, 1970). *Argulus* is an estuarine pests, is found recently on culture ponds. Fish mortality associate *Argulus* may cause severe damage to the body surface of the host fish. Host specificity of the species is related to the unusual conditions

From Indian subcontinent eight species of Argulus have been reported. Das and Das (1997) reported *Argulus foliaceus*, *Argulus bengalensis* and *Argulus siamensis* infesting Indian major carps *L. rohita, C. mrigla* and *C. catla*. From Bangladesh only *A. foliaceus* and *A. bengalensis* have been reported from experimental pond (Chandra *et al.* 2004). Hora (1943) reported the fish louse *Argulus foliaceus* L., causing heavy mortality among carp fisheries of West Bengal. *Argulus bengalensis* was also reported by Ramakrishna (1951) from West Bengal and Bombay, India. In Bangladesh Chandra (2006) also reported *A. bengalensis* from *L. rohita, C. mrigla* and *C. catla*. *Argulus bengalensis* infestation is higher in polyculture ponds.

Prevalence of argulus in carp fishes was significantly ($p< 0.001$) higher in Pvt. farm than that of Govt. farm. The prevalence of this parasite was significantly ($p< 0.01$) higher in rainy season than the other seasons. Rui was more infected than Catla and Mrigal by this parasite. But its prevalence differed significantly ($p< 0.05$) only with Catla. Almost similar result was reported by Bakshi *et al*., 2006 who studied Pvt. farms were found to be more affected by parasitic diseases compared to Govt. farm. The causes of higher/lower infestation in prevalence mean intensity or abundance were different due to different nature of management practices like pond preparation, depth of water, transparency, stocking density, feeding of fry/fingerlings etc.

It has also been observed that infestation of *Argulus* in different months varied at various levels. Prevalence, abundance and mean densities of these *Argulus* were also observed and found to vary from season to season in the present work. Whitish to yellowish cysts in the skin and gill, loss of mucus, slight haemorrhage at the base of dorsal, pectoral and caudal fin were observed in case of heavily infected fish. Subasinghe (1992) studied hatchery diseases of freshwater fish in Sri Lanka and reported heavy mortality in major carp fry and fingerlings due to argulosis during nursery operation.

Rainy months were the most susceptible period of the year when *Argulus* are abundant. This could be due to stocking density, water depth, temperature along with other physico-chemical parameters and management practices maintained. Ahmed *et al*., 2009; Banu and Khan (2004) reported that *Argulus* are some of the very important pathogens that have had significant impact on the yield in carp hatcheries and seed production centres. Mean intensity and abundance is highest in *L. rohita* collected from private Fish Farm and lowest in *C. catla* collected from Govt. Fish Farm. It might be due to higher stocking density in Pvt. Fish Farm. Several authors have noted the correlation between outbreak of disease and stocking density (Chandra and Jannat, 2002; Bakshi *et al*., 2006; Johnsen and Jensen, l986). Almost similar result was reported by Barai *et al*. (2005).

During the study period *Argulus* infestations in fish farms of Mymensingh area were observed to occur throughout the year. The infection was the highest in rainy and lowest in winter season. This result agrees with (Akter *et al*., 2007; Banerjee and Bandyopadhyay, 2010). Carps are susceptible host for *Argulus* where Rui and Mrigal were the most susceptible hosts. Carps were more suitable host for *Argulus*. Mainly Rui infestation rate was l00 %. This result was almost similar to Bakshi *et al.* (2006). The mortality rate of fish due to argulosis was 0-5% in the study area. A typical age group was not mentionable in case of mortality. Bakshi *et al*. (2006) reported that motality was higher (10 %) in larger farms than (5%) smaller farms. Argulosis was a major and frequently occurring fish disease in carp and other fish species too. But in present study it was understood that a major portion (45%) of farm owners were not aware about the disease caused by *Argulus*. They were new in fish farming and had little knowledge in fish culture. Occasionally they used banned chemicals before stocking of fishes.

In the present study, *Argulus* sp. were collected from carps though severe infection was not found because of immature and recent attachment on fish. Abnormal swimming, rubbing themselves against the wall of tank were observed in diseased fish. The skin and fins had brownish grey points and hemorrhagic areas. *Argulus* infestations lead to secondary parasitic infestation of the skin (Soulsby, 1982). These for more measure may be undertaken by the farmers to enhance their production.

CONCLUSION

It may be concluded from the present investigation that *Argulus* was found all over the body surface of the host fish. But the most preferable sites of *Argulus* infestation were at the base of pectoral, pelvic and anal fin. Private farm owners used haphazardous types of management practices and were found more infestations. The present study highlighted the infestation and disease problems, including their identity. In addition, culture and economic loss, seasonality of pathogenic infection was also investigated including management related problems. It was reported that there was a direct relationship between disease outbreaks among fishes and water quality parameters. Occurrence of diseases were more severe when most of the water quality parameters were not found within suitable level. Infestation started at the early stage of fish life and *Argulus* infestations were more severe in Rui. Among different seasons, maximum prevalence of *Argulus* were higher in rainy season than in winter season. In culture system, more attention and necessary steps should be given in the rainy season when probability of parasitic infestations and disease outbreak increased in farm fishes of Mymensingh.

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