REPRODUCTIVE BIOLOGY OF YELLOWFIN TUNA (*Thunnus albacares* Bonnaterre, 1788) FROM SOUTHERN PART OF INDONESIAN WATERS AND ITS APPLICATION AS LIMIT REFERENCE POINT (Lm_{50})

Bram Setyadji[1)] and Hety Hartaty[1)]
[1)]Research Institute for Tuna Fisheries, Denpasar, Bali

INTRODUCTION

Microscopic or histological analysis is necessary to determine the classification of the development of gonads precisely to estimate the stage of maturity and reproductive activity for female individuals. In female yellowfin tuna, the diameter of the gonads can be the same size between the vitellogenin and post-spawning atretic fish (regenerating stage after spawning season) (Schaefer, 2001). So there is often a misclassification between adult female fish at the regenerating stage into immature fish. The use of histological analysis to assess the maturation stage of gonads is the most appropriate method (Schaefer, 1998).

Fish are classified as immature individuals when the female has oocytes without early stages yolks or yolks and matures when oocytes are at the atresia stage. In males, fish are classified as adults if there is histological evidence of sperm presence (Schaefer, 2001). Female fish are at the active stage when ovaries contain advanced yolked oocytes, and there is no atresia or only slight alpha atresia. Active females are then classified into spawning classes and non-spawning. Females at the spawning stage have ovaries that show evidence of spawning in the past (there is a postovulatory follicle) or will colonize with a significant presence of hydrated oocytes or migratory nucleus. In contrast, females whose ovaries do not show such evidence are classified as non-spawning.

Yellowfin tuna has an asynchronous type of development and multiple spawners and indeterminate fecundity type (Schaefer, 2001; Zudaire et al., 2013a). In Indonesia, several studies on the reproduction of yellowfin tuna have also been conducted (Andamari et al., 2012; Wagiyo et al., 2015; Mardlijah & Patria, 2016; Arnenda et al., 2019).

Knowledge of the histological classification of gonads accurately is essential in the extended analysis process of the first mature gonads (Lm_{50}) (Griffiths, 2010). This analysis is often used as a parameter of fishery management as a threshold value (reference point). Other parameters usually used include MSY (Walters et al., 2005), biomass, and mortality ratio in

Kobe Plot (Nishida et al., 2011). The minimum allowed size limit approach (Lm_{50}) is already applied to lobster fisheries. Under the Regulation of the Minister of Marine Affairs and Fisheries of the Republic of Indonesia Number 56/PERMEN-KP/2016, lobsters that can be caught are not in egg-laying conditions, carapace length above 8 cm, or weight above 200 grams per head. While for tuna is applied only to pacific bluefin tuna fisheries (NOAA, 2019) where the minimum size limit that can be caught is 73 inches (182.45 cm).

Control over the yellowfin tuna catch allowance is challenging to do in addition to a large area, diverse fishing gear, different landing models, and data collection problems. Therefore, research was conducted to explore the biological aspects of yellowfin tuna reproduction, including the development of oocytes, egg diameter, histological classification, and its application as one of the models of fishery management in the form of a minimum size limit allowed to be caught. This approach is expected to be an alternative to the management of yellowfin tuna fisheries in Indonesia, especially those derived from fishing gear with high selectivities, such as handline and longline tuna.

OOCYTES DEVELOPMENT AND EGG DIAMETER

Samples of yellowfin tuna gonads were obtained from two locations: Benoa Harbour, Denpasar, Bali and Kedonganan Fish Landing Site (TPI), Badung, Bali (Figure 2.1). Both locations are representations of industrial tuna fisheries and small-scale tuna fisheries. A total of 79 samples, of which 36 came from longline tuna, while 43 came from hand lines and troll lines.

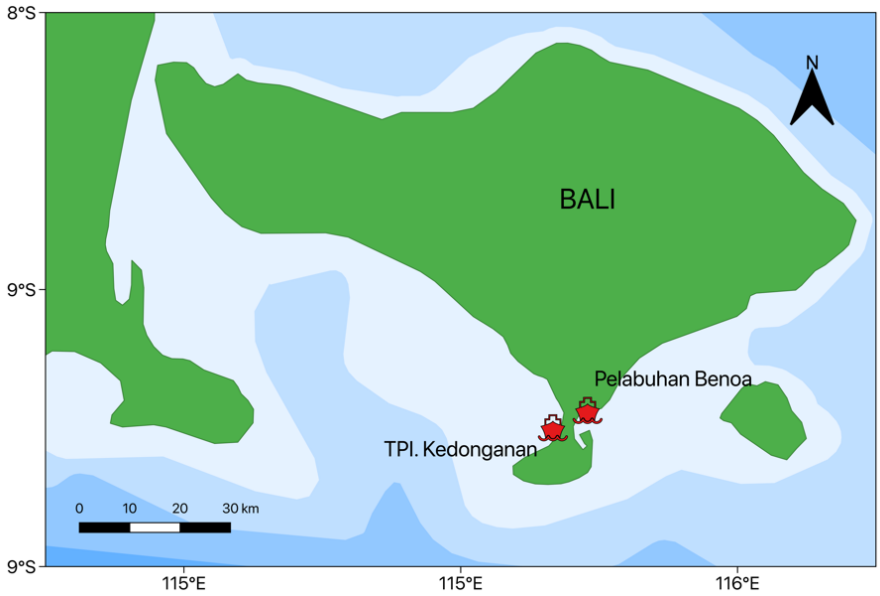


Figure 2.1. Yellowfin tuna ovary sampling locations.

Oocytes develop in the ovary through various stages. Although several differences appear among species, the sequence of oocyte development can be generalized among vertebrate fish into four main stages: primary growth, cortical alveoli or yolk vesicle formation, vitellogenesis and maturation (Wallace & Selman, 1981; West, 1990; Tyler & Sumpter, 1996). Ovary samples (n=35) were collected during March-May 2018. It was collected from the catch of longline tuna based in Benoa Harbour. Data on the length were measured using callipers with a precision of 1 cm. Fish weight and gonads used scales with 1 kilogram and 1 gram precision. Gonads are taken from the site to be frozen or handed over directly to the laboratory. The sub-sample was cut from each gonad then fixated in a 10% neutral buffered formalin solution.

Ovaries from fish lengths between 99-157 cm FL were analyzed histologically in the Research Institute for Tuna Fisheries Histology Laboratory using the Paraffin method and the Harris-Haemotoxilin and Eosin staining method. Gonad maturity levels were classified using criteria used by Farley and Davis (1999) and Farley et al. (2013) based on:

1. The existence of the most advanced group of oocytes (MAGO): unyolked, early yolked, advanced yolked, migratory nucleus and hydrated;
2. Presence and approximate age of postovulatory follicles (POFs);
3. Alpha atresia levels of advanced yolked oocytes: absent, <50%, ≥50%, and 100%;

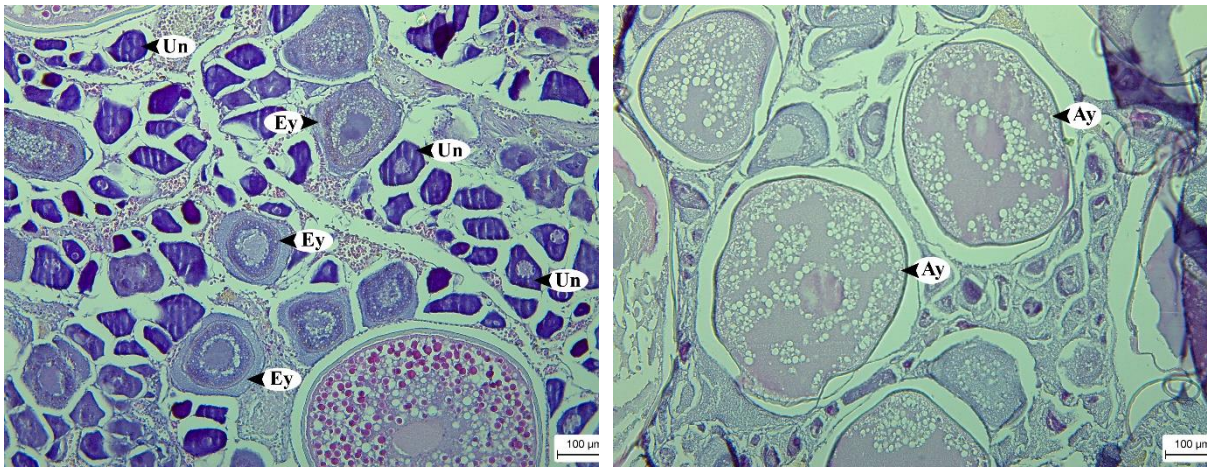
4. The absence of beta atresia levels of advanced yolked oocytes;
5. The absence of markers maturity indicates the previous development of the ovary. Maturity markers used include residual hydrated oocytes that may be encased by connective tissue and the oldest atresia levels (gamma/delta) that are yellow-orange-brown and often referred to as melano-macrophage centres or brown bodies.

Based on the dynamics of the ovary arrangement, Marza (1938) in Murua & Saborido-Rey (2003) and Wallace & Selman (1981) set three types of ovarian developmental arrangements, namely:

1. Synchronous. The whole oocyte develops and ovulates at the same time.
2. Group-synchronous. At least two oocyte populations are developing at the same time. Where one oocyte population is colonized during the spawning season and the other population is colonized afterwards.
3. Asynchronous. Oocytes of all stages of development appear in the absence of population dominance. Oocytes of different stages of development appear randomly. This kind of ovary can be found in fish species that have a continuous spawning season.

The oocyte diameter was observed using sample histology preparations, where each MAGO level is measured as many as five oocytes each and calculated on average. Observation using Zeiss Primostar trinocular microscope with 4x magnification and AxioVision Rel 4.8 software.

By making observations on 35 gonad samples, it is known that yellowfin tuna have asynchronous oocyte development, which can be seen from the maturation of oocytes in an ovary that appears more than one stage seen in Figure 2. 2. The characteristics and sizes determined each oocyte development. Oocyte development found in this research was unyolked stage (undeveloped); early yolked stage; advanced yolked stage; migratory nucleus stage (almost mature); and hydrated stage (mature/hydrated). The Egg diameter of each MAGO level ranges between 54.3 ± 10.8 to 502.2 ± 65.9 (Table 2.1).



(a) (b)

Gambar 2.2. (a) Un=unyolked oocyte, Ey=early yolked oocyte; (b) Ay=advanced yolked

Table 2. 1 Yellowfin tuna oocyte diameter at each level of development

Most advanced group of oocytes (MAGO)	MAGO Development	Egg Diameter (µm)
Un yolked	Undeveloped	30-83
Early yolked	Developing	103-221
Advanced	Early Mature	208-466
Migratory nucleus	Almost mature	232-522
Hydrated	Mature	431-635

Like other tuna, yellowfin is multiple spawners, indicated by the development of asynchronous oocytes in one ovary (Schaefer, 2001; Andamari et al., 2012; Zudaire et al., 2013b; Diaha et al., 2016). The diameter of the observed oocyte size ranges from 38-635 µm, and this size is similar to that found by Zudaire et al. (2013b), which is between 45-780 µm. The diameter of oocyte size at the development of gonads that differ from immature to mature has a sustainable size from the smallest to the largest oocyte size (Table 1). Schaefer (1998) and Zudaire et al. (2013a) reported the same results in previous studies in the Pacific Ocean and the Indian Ocean. According to West (1990), the frequency of continuous oocyte size without any gaps in diameter between undeveloped oocytes to mature in different maturity levels and time has been considered a sign of indeterminate, as it may indicate the continuity of primary oocyte recruitment during spawning season.

HISTOLOGY CLASSIFICATION

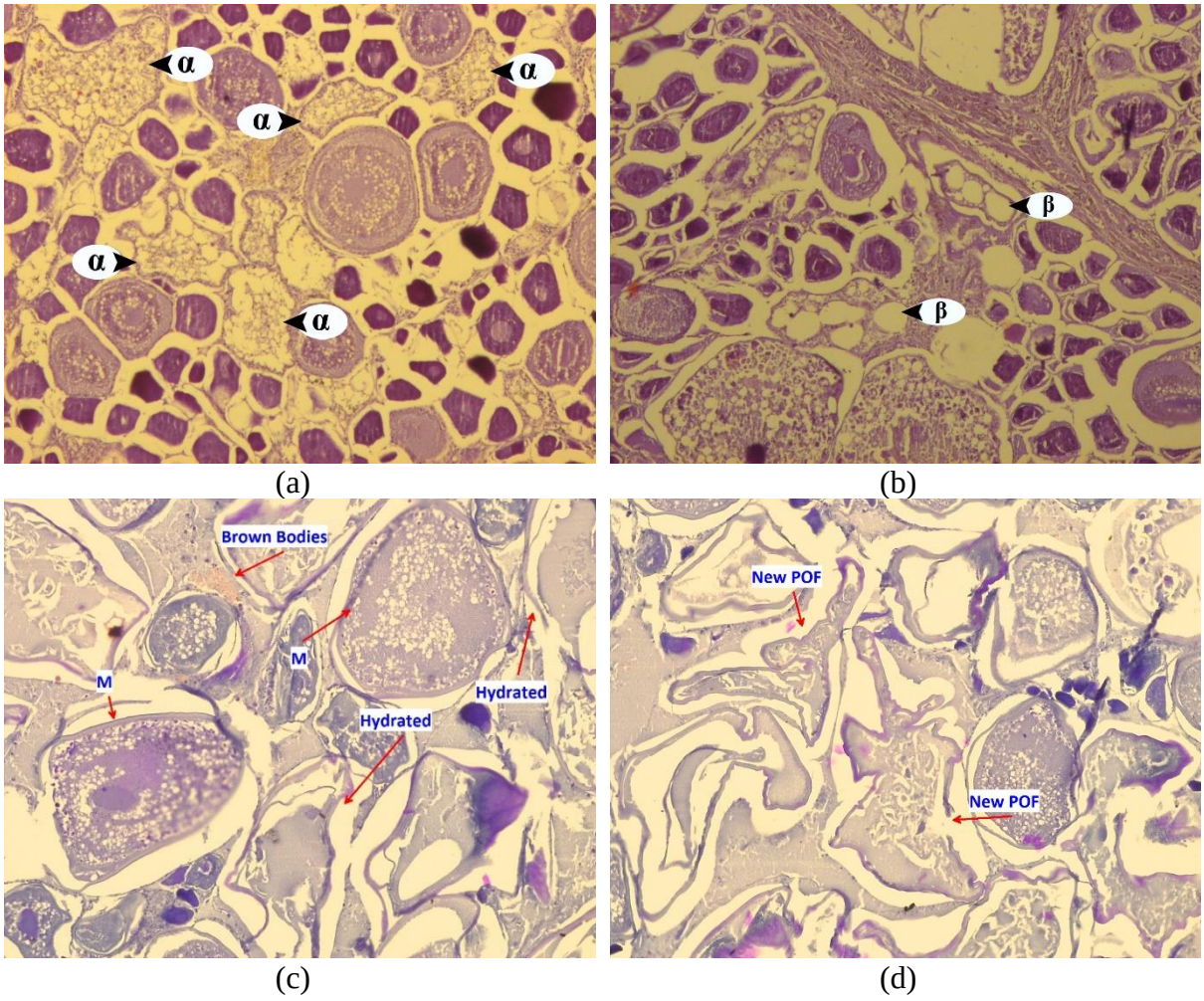
The histological classification of yellowfin tuna ovary is determined based on criteria developed by Farley et al. (2013). Female yellowfin tuna is classified as mature if yolked oocytes (advanced, migratory nucleus or hydrated), atresia (alpha or beta) and/or maturity markers were found. While immature individuals only unyolked or early yolked oocytes were found (Schaefer, 1998; Farley & Davis, 1998; Itano, 2000; Farley et al., 2013) and the absence of atresia and maturity markers. Histological classification is required to determine the development of fish gonad. Without histological analysis, mature females at the regenerating stage are usually misclassified into immature. The histological classification criteria by Farley et al. (2013) on south pacific albacore presented in Table 2.2.

Table 2. 2 Histological classification criteria (Farley *et al.*, 2013)

Kelas	Maturity status	Aktivitas	Kelas Perkembangan	MAGO dan tingkat POF	Atresia α dan β	Maturity markers
1	Immature	Inactive	Immature	Unyolked, no POFs	Absent	Not present
2	Immature	Inactive	Developing	Early yolked, no POFs	Absent	Not present
3	Mature	Active	Spawning capable	Advanced yolked, no POFs	<50% atresia α, atresia β may be present	May be present
4	Mature	Active	Spawning	Migratory nucleus or hydrated and/or POFs	<50% atresia α, atresia β may be present	May be present
5	Mature	Inactive	Regressing (Potentially reproductive)	Advanced yolked, no POFs	≥50% atresia α, atresia β present	May be present
6a	Mature	Inactive	Regressed 1	Unyolked or early yolked, no POFs	100% atresia α, atresia β may be present	May be present
6b	Mature	Inactive	Regressed 2	Unyolked or early yolked, no POFs	No atresia α, atresia β present	May be present
7	Mature	Inactive	Regenerating	Unyolked or early yolked, no POFs	Absent	Present

All samples observed were mature fish because maturity markers were found (Figure 2.3). A total of 86% of individuals were active, while those inactive as much as 14%. The gonad

development classes found include spawning capable 2.86%, spawning 82.86%, regressing 2.86%, regressed1 5.71%, and regenerating 5.71%. Maturity stages based on the histological classification criteria of yellowfin tuna fish analyzed are presented in Table 2.3.



Gambar 2.3. (a) α=atresia alfa; (b) β=atresia beta; (c) M=migratory, hydrated, brown bodies; (d) New POF.

Maturity stages were determined by the maturity markers found in the ovary (Figure 3). Four analyses determine maturity markers, including postovulatory follicle (POF), alpha atresia, beta atresia, and brown bodies (Davis et al., 1999; Farley et al., 2013b, 2016). Maturity markers can distinguish from immature fish and mature fish entering the rest period in reproducing (inactive). Overall, yellowfin tuna observed were mature. The percentage was dominated by females actively spawning and spawning capable by 86% and 14% inactive (post-spawning:

regressing, regressed 1 and regenerating) (Table 2.3). Immature fish were not found because the samples observed came from tuna fishing gear with high selectivity, so only large fish were obtained (99-157 cm FL).

Table 2. 3 Maturity stages of yellowfin tuna based on histological classification criteria

Maturity status	Activity	Development Class	n	FL cm	Body Weight (kg)	Gonad Weight (gr)
Mature	Active	Spawning capable	1	137	44	272
Mature	Active	Spawning	29	100-157	17-63	108-1080
Mature	Inactive	Regressing (potentially reproductive)	1	121	32	311
Mature	Inactive	Regressed 1	2	99-102	16-19	71-81
Mature	Inactive	Regenerating	2	100-105	19	92-185

Females with the development of post-spawning gonads (regressed 1 and regenerating) were smaller than females that spawned, which size was 99-105 cm FL. According to Murua & Saborido-Rey (2003), the early appearance of ovarian regenerating rates occurs during the peak of reproductive activity of adult individuals, and it is not related to the end of the spawning season of young fish. So there is an allegation that the misalignment in reproductive time between sizes is that young females missed the spawning period (Diaha et al., 2016).

In females who are firstly mature, maturation involves significant physiological and behavioral transitions so that if energy is insufficient, then reproduction does not occur. By skipping spawning, the female increases the growth and chances of survival, resulting in increased life span and reproductive outcomes (Rideout & Tomkiewicz, 2011). Therefore, further research on the emergence of regenerating-level female gonads in the young fish group suggests that the female fish has missed the spawning season is indispensable.

RECOMMENDED MINIMUM SIZE LIMITS

Length at 50% sexual maturity (Lm_{50}) is estimated as length where randomly selected specimens have a 50% chance of becoming sexually mature (Roa Lm_{50}et al., 1999; Somerton, 1980). The average length at which 50% of mature individuals were calculated using Bayesian

model-based logistics analysis is contained in the sizeMat module (Torrejon-Magallanes, 2018) in R software version 3.5.2 (R Core Team, 2018). In regression analysis, X is considered as a descriptor and classification of sexual maturity of YFT (immature: 0; adult: 1) as a random changer (binomial). The changes were installed in the logit (logistics) function following the Bakhayokho model (1983). This study used a Bayesian logistics model approach (not a frequency like GLM) where examples of posterior distribution were obtained through the Random Walk Metropolis algorithm.

A total of 79 samples was female. Male specimens were not analyzed due to difficulties in obtaining samples in the complete form. The sample length size ranges from 30-157 cm FL with an average of 80.73 cm FL. Sample weight ranges from 0.8-63.0 kg, with an average of 16.5 kg. The Bayesian logistics model shows a value $Lm_{50}$of 92.40 cm FL, at coefficient R^2= 0.94 (Fig 2. 4).

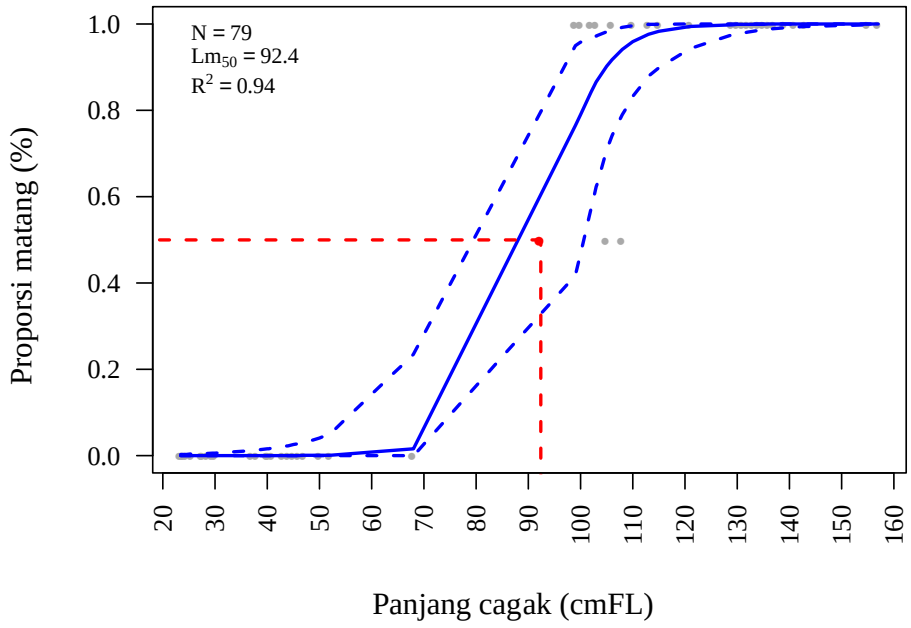


Figure 2. 4. Average length at 50% yellowfin tuna that has matured gonads (Lm_{50})

The model showed that the lack of a sample of medium-sized yellowfin tuna (80-100 cm FL) showed inconsistent values in that range of values. Yellowfin tuna in this size are usually found in longline tuna under 30 GT. In contrast, small sizes usually dominated the catch of troll and hand lines (<50 cm FL) and large (>100 cm) (Muhammad & Barata, 2016; Nurdin, 2017).

Lm_{50} is an essential parameter in studying fish stocks, especially for highly migratory fish such as yellowfin tuna. The value produced by the model is still in the range of similar studies in the western part of the central Indian Ocean (Zhu et al., 2008b) and the western Indian Ocean (Zudaire et al.,2013), which is between 75.0-113.7 cm FL. Research related to the reproductive biology of yellowfin tuna in the eastern part of the Indian Ocean is still constrained by the high cost of samples, especially for fish sizes above 100 cmFL. The cooperation method with fish processing (fillet) can be used as a solution, as is done in this study, so as to save the cost of samples issued.

CONCLUSION

Asynchronous oocytes indicate that yellowfin tuna is a type of fish that repeatedly spawns (multiple spawners). The sustainable size of oocytes (without gaps) indicates the continuity of oocyte recruitment that has not developed during the spawning season. Yellowfin tuna observed were mature fish with active status (spawning and spawning capable) 86%, and 14% inactive (post-spawning: regressing, regressed 1 and regenerating). Classification information of gonad development is beneficial to know the geographical variations in life history and population dynamics of yellowfin tuna. The characteristics found can be used for its management on a regional scale.

Based on this study, the management recommendation that can be formulated is applying the minimum size limit of yellowfin tuna that can be caught above 92.40 cm FL. This strategy may not be applicable to all fishery models, given that yellowfin tuna interact with a wide variety of fishing gear. The solution is education for fishers/fisheries to be more selective in conducting catch efforts. Widening the mesh size, enlarging the hooks, fishing deeper in fisheries associated with FADs, and conducting the opening and closing of the catching season is a combination of management models that can be done to maintain the resources of yellowfin tuna to continue to be utilized sustainably.

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