The Early Development of the Short-Necked Clam, *Paphia undulata* (Born 1778) (Mollusca, Pelecypoda: Veneridae) in the Laboratory

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ABSTRACT

The population of *Paphia undulata* in Negros Occidental waters continues to decline due to unregulated harvesting because of the increasing demand for this resource. One potential effort to mitigate this problem is reseeding or stock enhancement of the natural population. For these efforts to be effective however, successful laboratory rearing and breeding of this species is a prerequisite. Thus, this study was conducted to describe the embryonic and larval development of *P. undulata* to provide information on laboratory larval rearing. Broodstock were collected during the natural spawning peak from Hinigaran, Negros Occidental and were maintained in a static system with a mean salinity and temperature of 36 ppt. and 26.7 ^{O}C, respectively and were fed with *Isochrysis galbana*, *Chaetoceros calcitrans*, and *Tetraselmis tetrahele*. Fertilized eggs started to divide 30 minutes after fertilization. Ciliated swimming blastulas were observed after 1.5 hours with an average shell length (SL) of 40 µm. These further developed into trocophore larvae (average SL 60µm) after 7 hours and into D-veliger (average SL 80 µm) after 12 hours. Fully developed D-larvae (average SL 90 µm) were attained after 17 hours. Larvae with completely developed umbo were observed on day 9 with an average SL of 140 µm. On day 13, larvae (average SL 220 µm) started to settle and metamorphose. Spontaneous spawning was observed during the peak of reproductive activities of *P. undulata* showing that collection of broodstock must be conducted during the peak of reproductive season to ensure successful spawning and production of viable gametes under laboratory conditions. Further experiments should be conducted to determine optimum conditions to improve survival rates of the larvae during settlement. The results corroborate other studies and help explain the derived information on the species' population and reproductive biology.

Keywords: *Paphia undulata*, embryonic and larval development, reproductive biology, laboratory breeding, Negros Occidental

INTRODUCTION

The short-necked clam *Paphia undulata* (Born, 1778) is one of the most economically important bivalves in the Philippines as it is a cheap source of high quality protein. In west central Visayas, Philippines, it is widely distributed in the coastal muddy bottoms of Hinigaran, Binalbagan and Himamaylan, Negros Occidental. Clams collected by compressor diving in these areas are sold to local markets as well as exported (live or processed) to other countries like Taiwan (Villarta & del Norte-Campos, 2010). The population of this commercially important species has declined substantially because of unregulated harvesting caused by increasing demand. Agasen et al. (1998) reported that the population in Negros Occidental was overexploited with an exploitation rate (E) of 0.5. After a period of thirteen years of unregulated fishing, overexploitation has worsened (E = 0.75) due most likely to the continued absence of management policies (del Norte-Campos & Villarta, 2010). This overexploitation with the familiar symptoms of decrease in the sizes of the catch and high proportions of immature in the catch has clearly led to growth overfishing of the natural stock in the area (del Norte-Campos & Villarta, in prep.). One way to mitigate this problem is by reseeding the overfished area or stock enhancement. Reseeding/restocking has the potential to increase fishery yields and rebuild overexploited stocks and dampen fluctuations in catch due to variable recruitment (Rothlisberg, 1999). On the other hand, stock enhancement is the introduction of artificially produced organisms which are released after rearing to a desired stage of development to support or augment the wild population of the species and to increase the eventual yield, or occasionally to create new fisheries (Howarth 1991). Knowledge on the larval biology of *P. undulata* is quite limited with the few existing publications either being sketchy and incomplete (Salleh et al. 1987), or published in more obscure literature (Pongthana, 1990). This knowledge is however a prerequisite to eventually promote successful reseeding or stock enhancement procedures. Knowledge gained from its early growth will also further complement the information on its population biology and help understand the dynamics of the species' recruitment in its natural habitats. This study was therefore conducted simply to describe the early development of *P. undulata* reared under laboratory conditions. Due to logistical constraints, no replications were made. Using the derived information, including the duration of larval stages, inferences on the timing of settlement of the species in its natural habitat were made.

MATERIALS AND METHODS

Clam collection and hatchery maintenance

Broodstock were collected through compressor diving from Hinigaran, Negros Occidental, West Central Philippines (Fig. 1) during the peak spawning season (August-November) (Nabuab et al. PAMS 10) of the species in the area. They were transported without water, chilled (2-4 °C) in an ice chest to the Aquaculture facility of the University of the Philippines Visayas where they were stocked in a tank without substrate with a mean salinity of 36 ppt, mean temperature of 26.7 ^{O}C and mean dissolved oxygen of 5.68 mg L^{-1} for acclimatization prior to spawning. Ten liters each of *Isochrysis galbana*, *Tetraselmis tetrahele* and *Chaetoceros calcitrans* were fed to the broodstock daily. Microalgal starters were obtained from Phycology Laboratory of the Southeast Asian Fisheries Development Center (SEAFDEC/AQD) and were cultured using F-media. Feces and algal debris were siphoned daily to ensure good water quality during the acclimatization period.

Monitoring of Embryonic and Larval Development

When natural spawning took place in the broodstock tank, fertilized eggs were collected using a 25 micron sieve and were incubated in plastic bins at a stocking density of 5 eggs ml^{-1}. Embryonic development was monitored every three hours until D-larvae were observed. Thereafter, larval development monitoring and water change were done every other day. D-larvae were stocked in a flat bottom container with mild aeration at a density of 5 inds·ml^{-1} and fed with 5 x 10^3 cells ml^{-1} of *Chaetoceros calcitrans*. When the D-larvae reached the umbone stage, a mixed diet of *Chaetoceros calcitrans* and *Isochrysis galbana* was given at a cell density of 5 x 10^4 cells ml^{-1}. Settled larvae were transferred in an upwelling system (Pongthana 1990) and were fed with *C. calcitrans*, *I.*

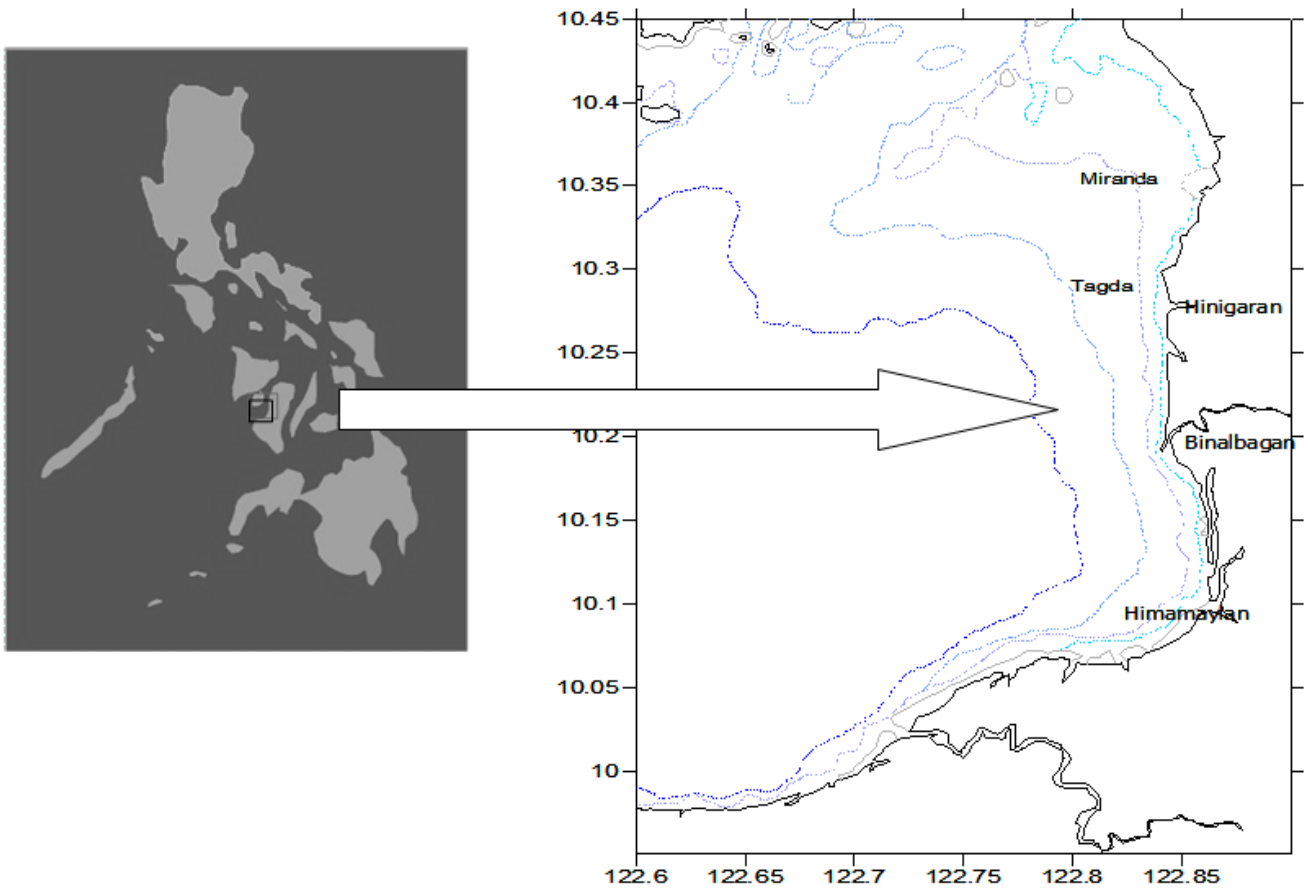


Figure 1. Site of collection of *Paphia undulata* broodstock in Negros Occidental waters.

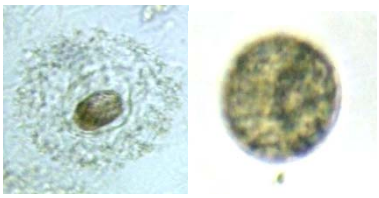
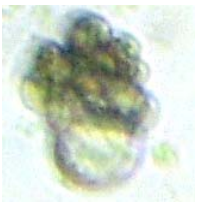
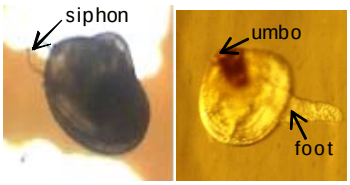
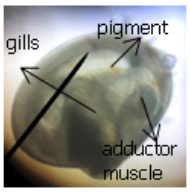
galbana, and *T. tetrahele* at a cell density of 8.3 x 10^4, 3.3 x 10^4, and 3 x 10^3 cells ml^{-1}, respectively (Utting and Spencer 1991). Shell lengths of larvae were measured to the nearest 0.01 µm every other day. Survival (%) and growth rates (µm day^{-1}) were monitored. Three months after spawning, the spats/seed clams were transferred to 40 L plastic basins for further rearing. Seed clams were fed with *C. calcitrans, I. galbana,* and *T. tetrahele* each at a concentration of 1.5 x 10^5 cells ml^{-1}.

RESULTS AND DISCUSSION

Spontaneous spawning was observed among clams kept in the broodstock tank. Such was also further observed in August, the peak of spawning activities in *P. undulata* (Tuaycharoen 1984; Pongthana 1990; and Nabuab et al., in prep.). These show that successful spawning of the short-necked clams in the laboratory is achieved during the peak of their reproductive activities in the wild.

Table 1 summarizes and shows the embryonic and larval development of *P. undulata*. During fertilization, a mass of spermatozoa surrounds the egg and formation of jelly coat was observed. Cleavage was attained after 30 minutes and developed into swimming blastula (SL 40 µm) 1 hr and 34 minutes after fertilization. Trocophore larvae with a SL of 60 µm were actively swimming after 7 hours and 13 minutes which further developed into D-veliger after 11 hours and 55 minutes of fertilization. Fully developed D-veliger (straight-hinged) which is also the feeding stage was attained after 17 hours and 12 minutes. Straight-hinged veligers developed into the umbo stage with visible gut 9 days after fertilization having a mean length of 140 µm. Thirteen days after fertilization, larvae (220 µm) with fully developed foot started to metamorphose and settle at the bottom.

Table 1. Summary of the embryonic and larval development of the short-necked clam *Paphia undulata* reared in the laboratory

Age	Stage	Ave.Length (µm)	Description	Pictures
0	Fertilization	40	swarms of spermatozoa surround the egg	
30 mins	Cleavage	40	cells actively dividing & forming daughter cells	
11 hrs 55 mins	D-veliger	80	straight-hinged larvae; ciliated velum and gut; actively swimming	
9 days	Umbone	140	two identical umbones appeared at the hinge	
13 days	Metamorphosed Larvae	220	disappearance of velum and cilia of the gut; foot fully developed and other adult organs (gills and siphon) start to develop	
1 month	Fully metamorphosed larvae	1000	fully developed organs; development of shell pigmentation	
3 months	Seed clam/ spat	6000	fully developed organs and shell	

Average increase in shell length (µm) of the two batches of larvae is plotted against time (weeks) in Figure 2. Over the first two weeks, the mean growth rates were 29.6 and 20.5 µm day^{-1} for batches 1 & 2 respectively. These values are within the range of that (25.1 µmday^{-1}) estimated by Pongthana (1990). Early growth appears to continue with the same rate until about the 4th week, after which daily growth rates more than double (Fig. 2). Mean survival rate (%) from fertilized eggs to metamorphosis (day 13) was higher in batch 2 (2.8%) than batch 1 (1.7%) (Figs.3A-B). Although low, this higher survival rate for batch 2 would most likely be a reflection of slightly improved lab rearing techniques. In terms of days, the trend is the same for both batches whereby the survival drastically decreased in day 3, coinciding with the D-veliger stage, the stage when the larva starts to feed on its own (exogenous feeding), a time referred to in classic fisheries literature as the "critical period" (Hjort 1914). Further experimentation is obviously necessary to refine methods and thus achieve optimum survival rates.

From a simultaneous study, the growth of the species was studied resulting in a growth curve shown in Figure 4 (del Norte-Campos & Villarta, 2010). Using the von Bertalanffy growth parameters, asymptotic shell length (SL_{∞}= 79 mm) & growth function (K=1.0 yr^{-1}) derived from the study, the modal length of the first curve at approximately 30 mm in mid-April has an equivalent age of 6 months. Projecting this backwards will therefore mean that these individuals were spawned in October the previous year, confirming the findings on the peak spawning reported by Nabuab et al. (2010). Based on the present results, these individuals would also have settled by the middle of October. These results therefore corroborate earlier studies specifically on its growth and settlement in the natural habitat.

ACKNOWLEDGMENTS

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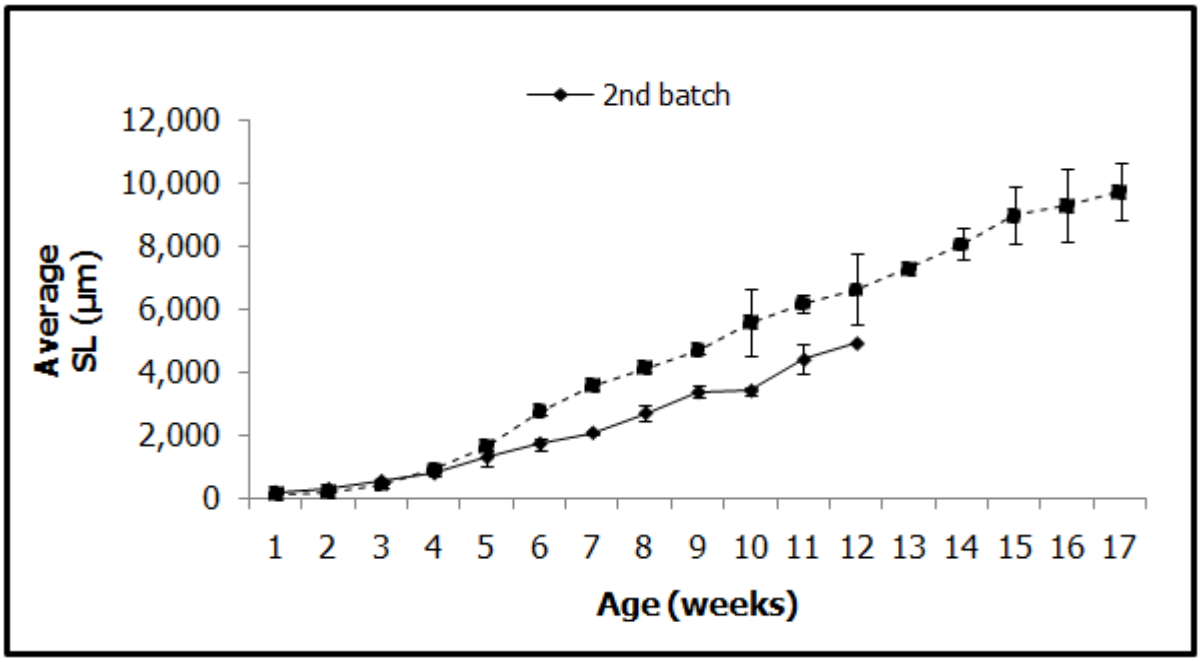

Figure. 2. Increase in average shell length (øm) against age in weeks of 2 batches of *Paphia undulata* spawned at Aug. 20, 2009, and Nov. 4, 2009 and reared in the laboratory.

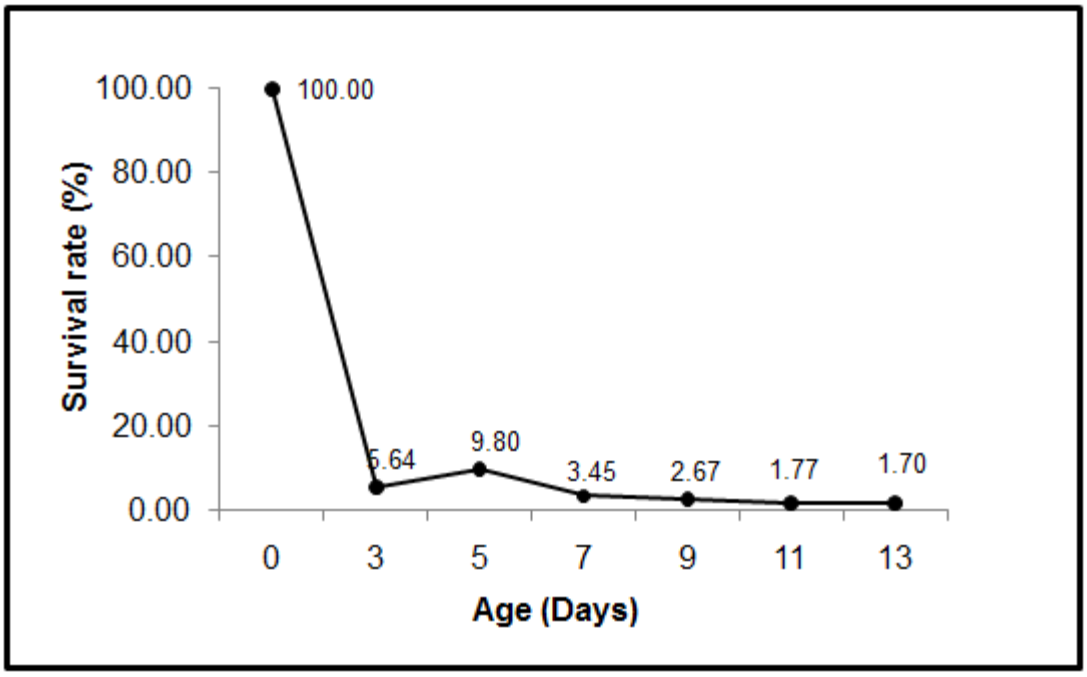

Figure 3A. Survival rate (%) of the first batch of *P. undulata* larvae from fertilized eggs to metamorphosis.

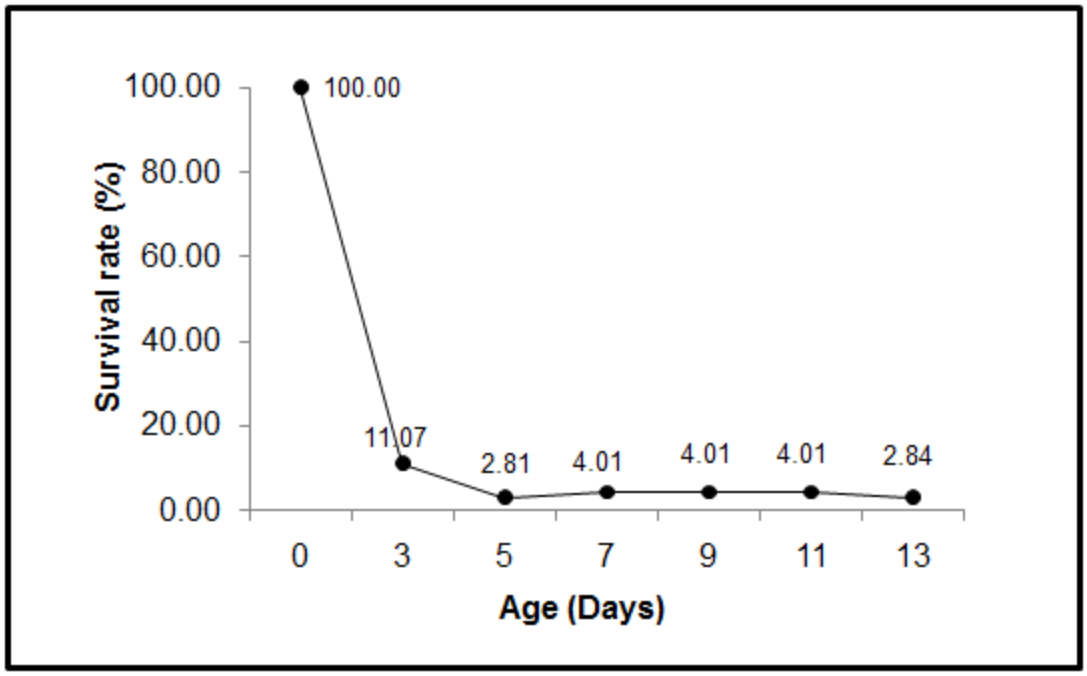

Figure 3B. Survival rate (%) of the second batch of *P. undulata* larvae from fertilized eggs to metamorphosis.

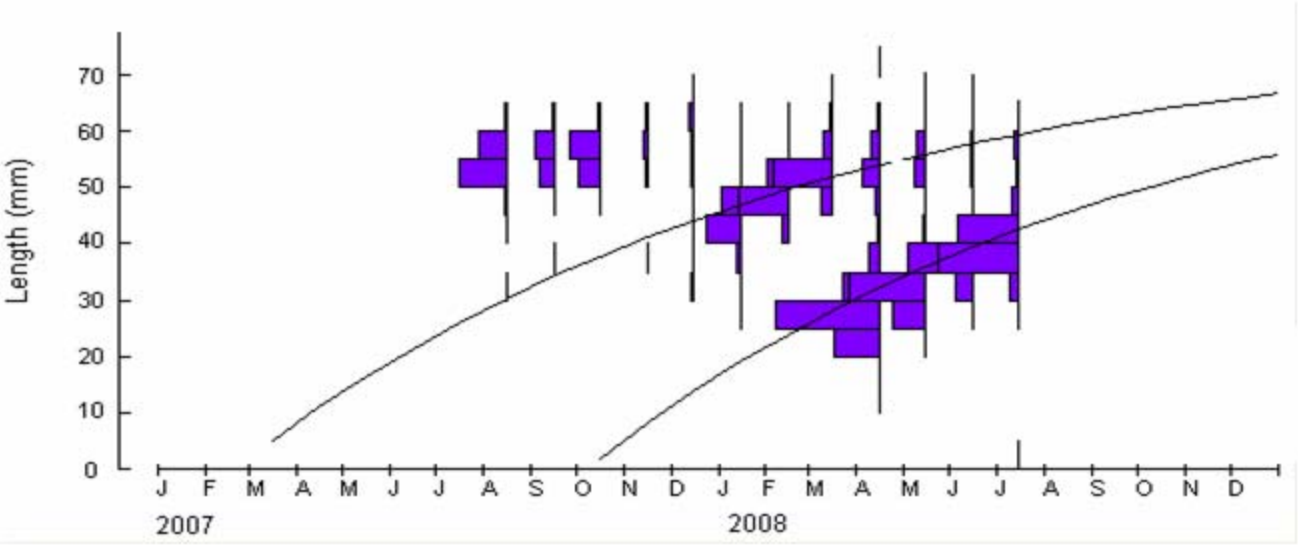

Figure 4. Growth curve of the short-necked clam *Paphia undulata* derived using FiSAT. Von Bertalanffy growth parameters are SL_{∞} = 79 mm and K = 1.0 yr^{-1} (from del Norte-Campos and Villarta, in prep).

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