

# The effects of temperature on two spiny lobster species, *Panulirus homarus* and *Panulirus versicolor* (Decapoda: Palinuridae) juveniles' exoskeleton microstructures

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**Abstract.** Understanding the thermal tolerance of lobster is essential for developing sustainable aquaculture practices and assessing the potential impact of environmental changes. Consequently, continuous research is required to monitor how lobsters adapt to changing temperatures and identify the long-term impacts on their populations and ecosystems. Lobsters have various responses to temperature changes. High temperatures can cause thermal stress in lobsters which also affect their molting cycle. Changes on the structure and characteristics of lobster exoskeleton is a good approach and an appropriate model for studying the physiological effects of temperature on lobster molting cycles. This study aimed to observe the effect of temperature treatment on the exoskeleton microstructure of juvenile lobsters by using a scanning electron microscope (SEM). The exoskeleton structure of untreated and thermally-treated juveniles exoskeleton of *Panulirus homarus* and *Panulirus versicolor* was investigated. Qualitative energy dispersive X-ray (EDX) spectra of samples were recorded using an EDX detector mounted on the scanning electron microscope. According to the SEM inspections, there were slight changes in the exoskeleton microstructures between the treated and untreated groups, in which the treated sample seemed to contain more canal pores. Composition-wise, however, there are only slight differences in terms of atomic percentage of elements in the lobster's exoskeleton structure between the control and treatment groups. There was no alteration in elemental composition between the two groups due to temperature exposure. In this research, temperature treatment had little influence on the overall exoskeleton structure of *P. homarus* and *P. versicolor*.

**Key Words:** cephalothorax, materials, heat, temperature.

**Introduction.** Lobster is one of the important species with high economic trade. According to FAO (2021), Indonesia is one the country which is leading the lobster seed producer. Indonesia has been exporting lobster seed to many countries in Asia, such as Vietnam, Hongkong, Singapore, Thailand and other Asian countries (Apriliani et al 2021). There are 7 commercial lobster species of Palinuridae family exploited in Indonesian waters, among them are scalloped spiny lobster (*Panulirus homarus*), ornate spiny lobster (*Panulirus ornatus*), long-legged spiny lobster (*Panulirus longipes*), stripe-leg spiny lobster (*Panulirus femoristriga*), pronghorn spiny lobster (*Panulirus penicillatus*), painted spiny lobster (*Panulirus versicolor*) and mud spiny lobster (*Panulirus polyphagus*) (Kembaren & Suman 2022). Lombok Island is one the highest lobster seed producers which has reached 80% of total production in Indonesia. Most lobster species found in Lombok are spiny lobster species, *P. homarus* and *P. versicolor*, which are commercially important. Because of its high selling value, the exploitation of lobster was extremely high, threatening the lobster population (Adhiatma 2019), also causing the decrease of seed from natural sources. One

of the factors that determine the success of lobster farming to support growth and survival is feed. Quality feed that is easily digested, has a complete nutritional content and does not contain harmful substances (Yunaidi et al 2019). In addition, water temperature is one of the most important environmental factors determining the growth rate of crustaceans (Chittleborough 1975; Ren et al 2021) also influencing the performance of aquatic ectotherms in culture (Uy et al 2023; Payette & McGaw 2003). Besides that, temperature variations can also affect how crustaceans use energy, which can alter the metabolite levels of their most vital organs which are the muscle and hepatopancreas. (Jimenez & Kinsey 2015). However, very little is known about the responses of the exoskeleton microstructure of juvenile lobsters. According to Nielsen & McGaw (2016), the juvenile life stage is a critical period, has high levels of mortality, combined with specific behavioral responses, which can disconnect larval settlement from patterns of abundance of adults. Beside the behavioral responses such as how thermal preferences can be altered by the presence of shelter and food, thermal preferences also affect the skeletal tissues which are composite materials with associated organic fibrils and mineral particles (Romano et al 2007). These shifts in energy storage may reflect changes not only due to a direct effect of temperature, but also to indirect adjustments, for example, in activity level and feed intake (Jimenez & Kinsey 2015). According to Sacristán et al (2017), a common response to lower temperatures in crustaceans is a drop in feed intake, which, when combined with metabolic rate depression, may have an impact on energy metabolism depending on its severity. Further it has also been demonstrated that, when feed intake is restricted, the energy metabolism can be modulated by dietary composition (Vinagre & Silva 1992). Many studies about the effect of temperature on exoskeleton lobster have been done in other countries. However, very limited study was done in Indonesia, nevertheless the thermal tolerance of lobster is essential for developing sustainable aquaculture practices. The exoskeleton consists of several chemical compositions such as calcium carbonate (Ca), chitin-protein (C), magnesium (Mg) and some other compositions (Romano et al 2007). Those chemicals affect the hardness of the skeleton microstructure through mineralization which may affect the growth and molting period (Raabe et al 2005). Those materials provide a wide range of mechanical properties through local variations in composition and structure (Travis 1963). In addition, understanding the thermal tolerance of lobster is essential for developing sustainable aquaculture practices and assessing the potential impact of environmental changes on these important marine species. The aim of this study was to observe the changes in the exoskeleton microstructure of lobster using scanning electron microscopy both surface and cross-sectional images.

**Material and Method.** *P. homarus* (N=90) and *P. versicolor* (N=90) juveniles with the total length (TL) between 9.57 mm to 12.59 mm (with carapace length (CL) from 3.42 mm to 4.74 mm) were collected from Telong Elong, East Lombok, West Nusa Tenggara (Figure 1), being captured by divers. The wet weight of both species, *P. homarus* and *P. versicolor*, was between 0.20 g to 0.45 g. The lobsters were then transported to the three 1000 L glass aquariums at North Lombok of the Research Center for Marine and Land Bioindustry, National Research and Innovation Agency (BRIN), Pemenang, West Nusa Tenggara, Indonesia. The acclimated glass aquarium was then set at 27°C water temperature level (the natural temperature conditions of the collected lobster) and kept at natural salinity conditions, ambient dark light conditions and 100% dissolved oxygen. All juvenile lobsters were allowed to develop at this temperature for three days and then were subsequently transferred to the experimental tank for further subsequent experiment. Juvenile lobster was subjected to experimental treatments of two different water-temperature levels of (i) ambient temperature, 26-28°C, as control (without heater), (ii) a simulated heatwave temperature of 30°C. Monitoring the water temperature was done daily to adjust the aquarium heater temperatures. For the ambient treatment, we recorded the temperature changes in a three days interval for our records. All juvenile lobsters were fed with circular pellets for shrimp (MS Megami, PT. Matahari Sakti, Indonesia with 48% protein content) daily at 06:00 PM *ad libitum* for 1 hour, and uneaten food as well as lobster's feces were siphoned out from the culture tanks. After 18 days of treatment, lobster juveniles were euthanized in a cold water-bath, weighed and put to the oven for 24 hours in 75°C. The

exoskeleton structure of untreated and thermally-treated juveniles' exoskeleton of *P. homarus* and *P. versicolor* was investigated using scanning electron microscopy (SEM). Qualitative energy dispersive X-ray (EDX) spectra of samples were recorded using an Edax (AMATEK Z1 Analyzer) EDX detector mounted on the scanning electron microscope.



Figure 1. Sampling location for juvenile lobster in Telong Elong, East Lombok, West Nusa Tenggara.

## Results

**SEM images of the surfaces of the exoskeletons.** The microstructures of both untreated and thermally-treated lobster exoskeletons were analyzed using SEM. Figure 2 illustrates the surface microstructures of the exoskeletons of *P. homarus*. Specifically, Figures 1a and 2b present SEM images of the exoskeleton surfaces with and without thermal treatment, respectively. Examination of the microstructures in Figures 2a and 2b reveals that both samples exhibit a relatively rough, scaly surface topology. Additionally, both surfaces display evenly distributed, pimple-like protrusions. Notably, the mound-like structures on the thermally-treated sample in Figure 2b appear more pronounced compared to those on the untreated sample in Figure 2a, suggesting an increased surface roughness resulting from thermal treatment. Figures 2c and 2d showed the SEM images of the exoskeleton surfaces of *P. versicolor*, also with and without thermal treatment. Consistently with the observations for *P. homarus*, the microstructures of *P. versicolor* exhibit scaly textures with pimple-like features. Similarly, the thermally-treated sample in Figure 2d displays more pronounced mound-like particulates compared to the untreated sample. Thus, it can be concluded that the surface microstructures of both species are comparable, with mound-like structures observed on scaly surfaces. The thermal treatment induces a slight modification in the surface topography of the exoskeletons.

**Cross-sectional SEM images of the exoskeletons.** Figure 3 presents the cross-sectional SEM images of the lobster exoskeletons. Specifically, Figures 3a and 3b depict the cross-sections of untreated and thermally-treated *P. homarus* exoskeletons, respectively. Both figures reveal a layered structure characterized by porous, spongy-like features. The porosity appears uneven along the vertical axis of the material in both samples. Comparative analysis of Figures 3a and 3b shows no substantial differences in the microstructures of the thermally-treated and untreated exoskeletons. Similarly, Figures 3c and 3d illustrate the cross-sectional microstructures of untreated and thermally-treated *P. versicolor* exoskeletons, respectively, which exhibit a comparable layered, spongy structure. There are no significant microstructural changes attributable to the thermal treatment observed in Figures 3c and 3d. Therefore, it can be concluded that a thermal treatment at 30°C does not induce notable alterations in the inner microstructures of the exoskeletons of both *P. homarus* and *P. versicolor*, as confirmed by the cross-sectional SEM images.

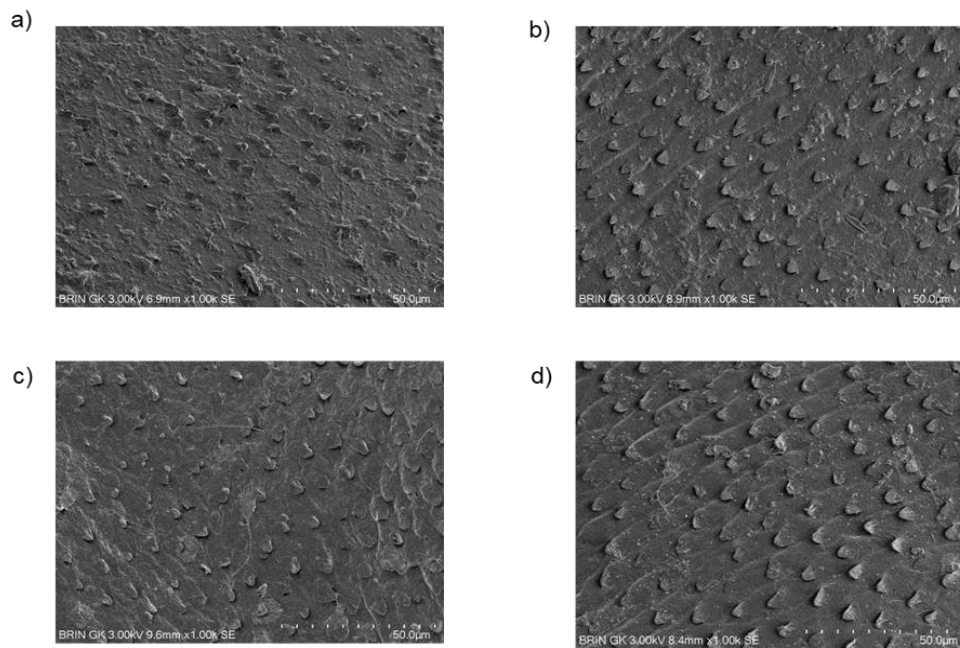


Figure 2. SEM images of the surfaces of the exoskeletons of (a) *Panulirus homarus* as a control (RT); (b) with a treatment at 30°C; (c) *Panulirus versicolor* as a control (RT); (d) with a treatment at 30°C.

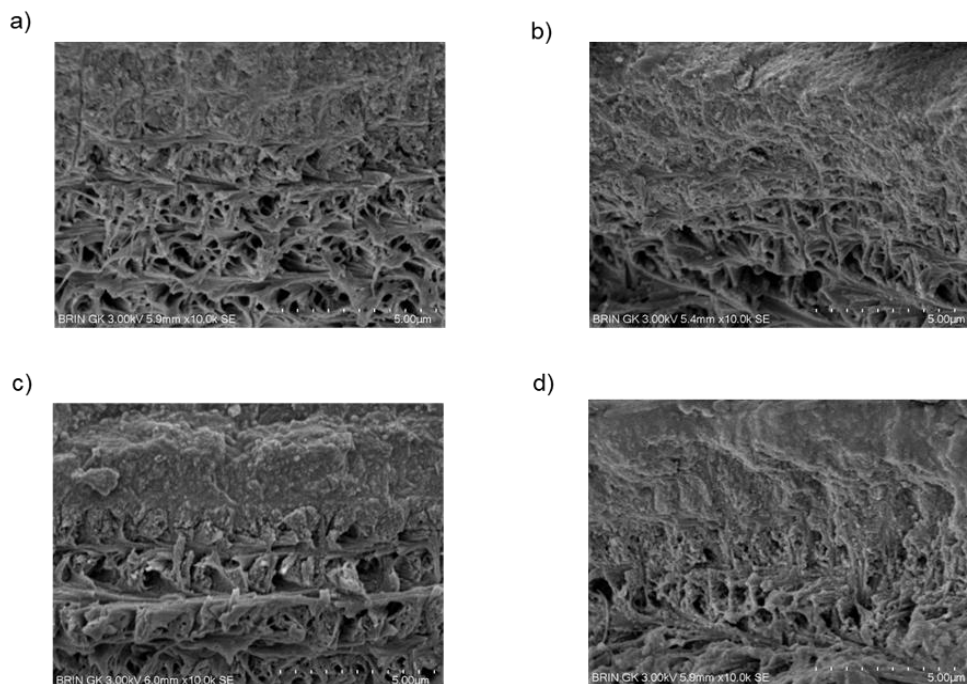


Figure 3. Cross-sectional SEM images of the exoskeletons of (a) *Panulirus homarus* as a control (RT); (b) with a treatment at 30°C; (c) *Panulirus versicolor* as a control (RT); (d) with a treatment at 30°C.

**Cross-sectional chemical compositions.** Figure 4 illustrates the cross-sectional chemical compositions of *P. homarus* exoskeletons, analyzed using EDX. Specifically, Figures 4a and 4b present the EDX analysis curve and the EDX elemental mapping of the untreated control samples, respectively. Figures 4c and 4d show the corresponding data for the exoskeletons subjected to a thermal treatment at 30°C. To provide a comprehensive analysis, the average elemental concentrations are also reported in atomic percent (at %) on the right-hand side of the elemental maps. The comparative examination of the elemental distribution maps in Figures 4b and 4d reveals notable differences between the

treated and untreated samples. For example, the distribution of calcium (Ca) appears more heterogeneous in the thermally-treated sample, with a concentration of Ca clusters observed closer to the surface. Quantitative compositional analysis indicates significant changes between the two conditions: sodium (Na) content increased from 2.9 at.% in the untreated samples to 9.2 at.% in the treated samples, whereas calcium content decreased from 14.1 at.% in the untreated samples to 9.2 at.% in the treated samples. These observations suggest that thermal treatment induces alterations in both the distribution and quantitative composition of specific elements in the *P. homarus* exoskeletons. These results also found that the increase of temperature could affect the distribution of chemical composition in *P. homarus* exoskeleton, especially in C, Na, and Cl. The quantitative elemental concentrations from Figure 4 are also provided in atomic percent (at.%).

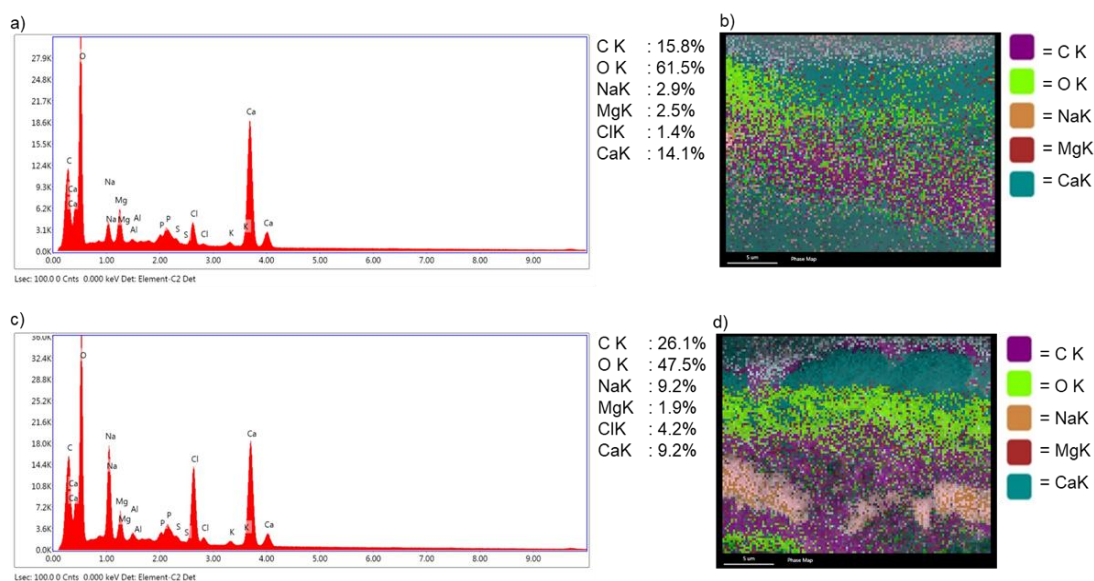


Figure 4. The chemical compositions of the *Panulirus homarus* exoskeletons: (a) EDX analysis curve; (b) EDX elemental mapping of the untreated control samples; (c) EDX analysis curve; (d) EDX elemental mapping of the thermally-treated samples (at 30°C). C =Carbon; O=Oxygen; Na=Sodium; Mg=Magnesium; Cl=Chlorine; Ca=Calcium; K refers to K-electron shell.

Figure 5 presents the chemical compositional data for *P. versicolor* exoskeletons surface. Specifically, Figures 5a and 5b display the EDX analysis curve and EDX mapping of the untreated control samples, respectively, while Figures 5c and 5d show the corresponding data for exoskeletons subjected to thermal treatment at 30°C. Comparative analysis of the quantitative chemical compositions between the untreated and thermally-treated samples of *P. versicolor*, and in relation to the data for *P. homarus* presented in Figure 3, reveals only minor differences. For instance, the sodium (Na) content in the untreated *P. versicolor* sample is 12 at.%, increasing slightly to 13.4 at.% in the thermally-treated sample. These findings indicate that, unlike *P. homarus*, there are no significant changes in the chemical compositions of the untreated and thermally-treated exoskeletons of *P. versicolor*. Overall, there are only slight differences in chemical composition in exoskeleton between thermally treated and untreated in *P. versicolor*. In addition, microstructural analysis using EDX revealed there are no significant differences in the arrangement of the Na, Mg and Cl within the exoskeletons of *P. versicolor*.

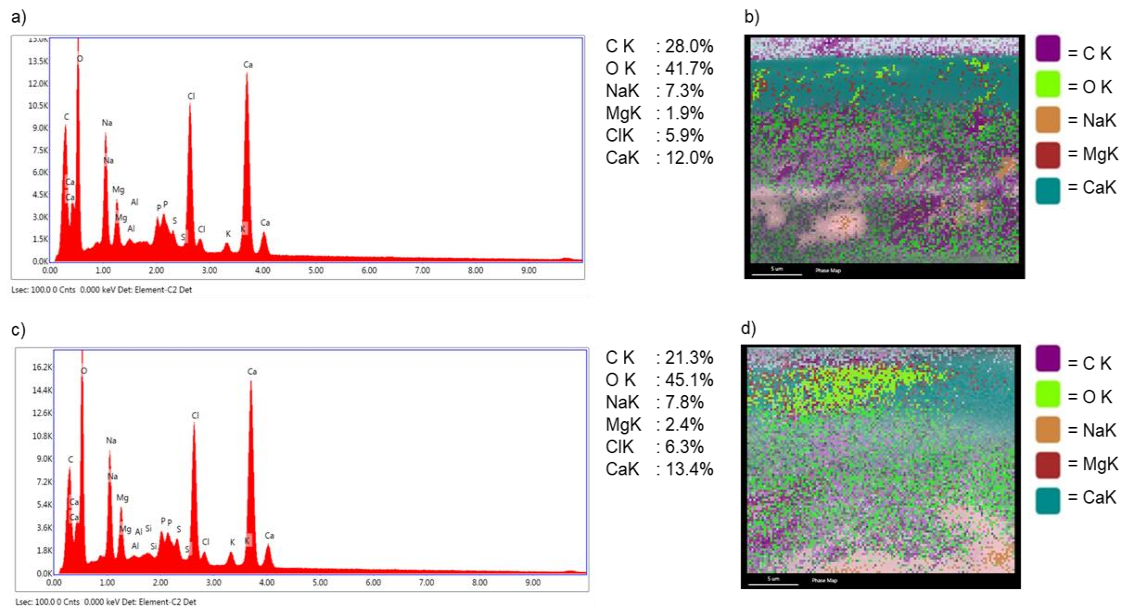


Figure 5. The chemical composition of the *Panulirus versicolor* exoskeletons are depicted in the following figures: (a) EDX analysis curve; (b) EDX elemental mapping of the untreated control samples; (c) EDX analysis curve; (d) EDX elemental mapping of the thermally-treated samples (at 30°C).

**Discussion.** In this study, *P. homarus* exhibited a more layered structure at optimal temperatures, facilitating better mechanical strength. In contrast, *P. versicolor* had a more compact structure at elevated temperatures, suggesting a different adaptive strategy to maintain structural integrity. The temperature affects the size and distribution of microfibrils within the exoskeleton. It causes the overall toughness and elasticity to become more irregular which could lead to increased susceptibility to physical damage (Verma & Tomar 2015). Understanding how temperature affects these microstructural properties has significant ecological implications. It may affect the altered exoskeleton characteristics that also impact the survival rates, reproductive success, and population dynamics of these species (Nielsen & McGaw 2016). According to Glandon et al (2018), the increase of temperature may alter the biomineralization processes involved in their formation. For example, more flexible exoskeletons may lead to increased vulnerability to predation, while rigid structures may impede mobility (Lowder et al 2022). Evolutionarily, these findings suggest that both species may undergo different adaptive changes in response to climate change. *P. homarus*, with its softer exoskeleton under heat stress, may be more prone to selection pressures that favor individuals with enhanced protective traits. Meanwhile, the inherent rigidity of *P. versicolor* may confer advantages in survival in warmer waters, possibly leading to shifts in species distribution (Boavida-Portugal et al 2018). The effects of temperature on the exoskeleton microstructures of *P. homarus* and *P. versicolor* highlight the complexities of crustacean adaptation to environmental changes. In many crustacean groups the hard parts of the exoskeleton and endocuticle are mineralized, essentially by precipitation of crystalline calcium carbonate (Romano et al 2007). In this study, increasing temperature in *P. homarus* could affect the distribution of chemical composition in the exoskeleton, especially in C, Na, and Cl, which also shows that the porosity appears uneven in the exoskeleton surface between thermal treated and untreated. Romano et al (2007) reported that thermal treatment on homarus species affects the decalcification using temperature treatment below 30°C and showed the smoothness of the structure surface. However, there are only slight differences in chemical composition in exoskeleton between thermally treated and untreated in *P. versicolor*. According to Zhang et al (2021), one of the chemical compositions in exoskeleton which affect the crustacean growth and development is chitin which also affects the periodic molting time of crustaceans. Furthermore, the research is essential to understand the long-term implications of these findings and could add a more different range of temperature

treatment, especially as global temperatures continue to rise, which will affect the adaptation of lobster. Monitoring these changes supports the conservation and management of lobster populations in the face of climate change.

**Conclusions.** The current study provides information about the changes in the exoskeleton microstructure of lobster using scanning electron microscopy of both surface and cross-sectional images using two different species, *P. homarus* and *P. versicolor*. This study demonstrates that exposure to elevated water temperature (30°C) causes slight changes in the surface microstructure of lobster exoskeletons in both *P. homarus* and *P. versicolor*, without significantly altering their internal microstructures. SEM analysis revealed mound-like surface structures in both species, while EDX analysis showed that temperature influenced the elemental composition, particularly in *P. homarus*, where increases in carbon, sodium, and chlorine were observed. In contrast, *P. versicolor* showed no significant elemental changes between temperature treatments. These findings highlight species-specific responses to thermal stress and suggest that further studies using a wider range of temperature treatments are needed to better understand microstructural and compositional changes in lobster exoskeletons under climate change scenarios.

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**Conflict of interest.** The authors declare that there is no conflict of interest.

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