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# The wedge clam *Donax trunculus* Linnaeus, 1758 (Bivalvia Donacidae) as bioindicator for monitoring of the Annaba gulf (Algeria): measurements of lipid and malondialdehyde rates during the reproduction (Spring 2021)

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**ABSTRACT** The present study aimed to assess the potential impact of pollution on total lipid levels as well as the response of a lipid peroxidation biomarker, malondialdehyde (MDA) in a sentinel and edible species, *Donax trunculus* Linnaeus, 1758 (Bivalvia Donacidae). The samples were collected during the major spawning phase (spring) over three months (March, April, May) in year 2021 from two selected sites in the Gulf of Annaba: El Battah (reference site), and Sidi Salem (polluted site). Biochemical analyses showed a significant increase in total lipid levels determined in the mantle of *D. trunculus* in individuals from El Battah compared to those from Sidi Salem. The highest values are observed during the first two months (March, April), which corresponds to the accumulation of reserves at the beginning of reproduction. The lowest rates are recorded at the end of the breeding season (May) corresponding to the period of emission of gametes which is privileged by the increase in temperature. Sex differences reveal an accumulation of reserves and a greater energy allocation in females compared to males during reproduction. Measurement of MDA indicates an increase in rates recorded in individuals from Sidi Salem compared to those from El Battah. Moreover, sex differences demonstrate a greater response in females compared to males. Overall, the results obtained demonstrated that the Gulf of Annaba is subjected to stress conditions from anthropogenic origin that are more pronounced in the populations of *D. trunculus* from Sidi Salem, linked to the level of pollution of this site. Thus, *D. trunculus* appears to be an appropriate sentinel species for the assessment of ecotoxicological risk in the Gulf of Annaba.

**KEY WORDS** *Donax trunculus*; Gulf of Annaba; Biomarkers; Lipids; Malondialdehyde; peroxidation.

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## INTRODUCTION

The Gulf of Annaba is continuously affected by various contaminants from urban, agricultural, harbor and industrial activities (Belabed et al., 2017), notably by heavy metals (Rabei et al., 2018). In addition, heavy metals were detected in tissues from a sentinel and edible species, *Donax trunculus* Linnaeus, 1758 (Bivalvia Donacidae) (Beldi et al., 2006) and in sediments from the Gulf of Annaba (Amira et al., 2018).

*Donax trunculus* is widely used as a sentinel species in the environmental assessment of marine pollution (Tlili & Mouneyrac, 2019; Amamra et al., 2019; Tlili et al., 2020, Lamine et al., 2021). In Algeria, this clam is abundant in sand beaches from the Gulf of Annaba (Beldi et al., 2006; Hafsaoui et al., 2016) and the most consumed bivalve by the local population for its nutritional value (Merad et al., 2017). Moreover, this edible species was also successfully used as a bioindicator to monitor water quality in the Gulf of Annaba (Soltani et al., 2012; Merad et al., 2016; Amira et al., 2018; Amamra et al., 2019).

The quality of the marine ecosystem was evaluated based on biomarker measures in sentinel species, especially marine bivalves (Signa et al., 2015). Biomarkers include a variety of molecular, cellular, or physiological alterations measurable in organisms in response to pollutants or other environmental stress factors (Birnstiel et al., 2019; Cortez et al., 2019). Thus, *D. trunculus* was used as bioindicator species for the assessment of marine pollution in the Gulf of Annaba through the measurement of several biomarkers like glutathione S-transferase (GST), acetylcholinesterase (AChE), catalase (CAT) or metallothioneins (MTs) rates (Soltani et al., 2012; Amira et al., 2018; Rabei et al., 2018; Sifi & Soltani, 2019).

Energy reserves can be used as good biomarkers of the health of the body and its environment (Yeung et al., 2016; Moreira et al., 2017; Boukari et al., 2021). Among these reserves, lipids have particularly two fundamental roles in metabolism: energy storage in the form of triacylglycerols and distribution as membrane components in the form of phospholipids (Delgado et al., 2004; Nie et al., 2016). Malondialdehyde (MDA) formed during the action of free radicals on polyunsaturated fatty acids is largely used as a biomarker of lipid peroxidation and more generally of oxidative stress (Viarengo et al., 2007; Maurya et al., 2021).

Chemical contaminants exposure was found to affect the reproductive cycle of bivalves (Lintelmann et al., 2003; Hafsaoui et al., 2016; Hamdani et al., 2020). *D. trunculus* has been the subject of several studies on growth, population dynamic and reproductive biology in the Annaba Gulf (Sifi, 2009; Hamdani & Soltani-Mazouni, 2011; Soltani et al. 2012; Belabed, 2013; Hamdani et al., 2014; Benradia et al*.,* 2016; Merad et al., 2017; Amira et al*.,* 2018; Rabei et al*.,* 2018; Sifi & Soltani, 2019; Amamra et al*.,* 2019; Hamdani et al*.,* 2020). Thus, Hamdani et al. (2020) reported that in *D. trunculus* from the Gulf of Annaba the gametogenesis takes place from December to August in both sites, with an autumnal resting phase, while the spawning occurred between March and August with two major spawning phases: April and August in El Battah vs May and August in Sidi Salem.

The present study investigated the potential impact of pollution on total lipid levels as well as the response of a lipid peroxidation biomarker, malondialdehyde (MDA) in a sentinel and edible species, *D. trunculus* (Bivalvia, Donacidae) collected during the major spawning phase (spring) in year 2021, from two selected sites in the Gulf of Annaba, one being exposed to different sources of pollutants (Sidi Salem) and the other being relatively clean (El Battah).

## MATERIAL AND METHODS

### *Presentation of sampling sites*

The samples were collected at two sites from the gulf of Annaba (Fig. 1). El Battah site (36 ° 50 'N, 8 ° 50' E), located to the west of Mafrag Estuary, between Chatt beach and Cape Rosa, is far from any human activities and is expected as a relatively clean site (Rabei et al., 2018). This site, also characterized by higher hydrodynamic conditions that may contribute to the dilution of pollutants, is exploited by fishermen (Hamdani et al., 2020). Sidi Salem (36 ° 50 'N, 7 ° 47' E) located to the east side of the Seybouse Estuary is a contaminated site. It is exposed to various discharges from urban, agricultural and industrial sources (Rabei et al., 2018; Amira et al., 2018).

### *Physicochemical parameters*

The physico-chemical parameters of seawater were recorded using a multiparameter (Multi 340 i, Germany) during the study period at the two sites, El Battah and Sidi Salem. The following parameters were considered: temperature (°C), pH, salinity (PSU) and dissolved oxygen (%) (Table 1).

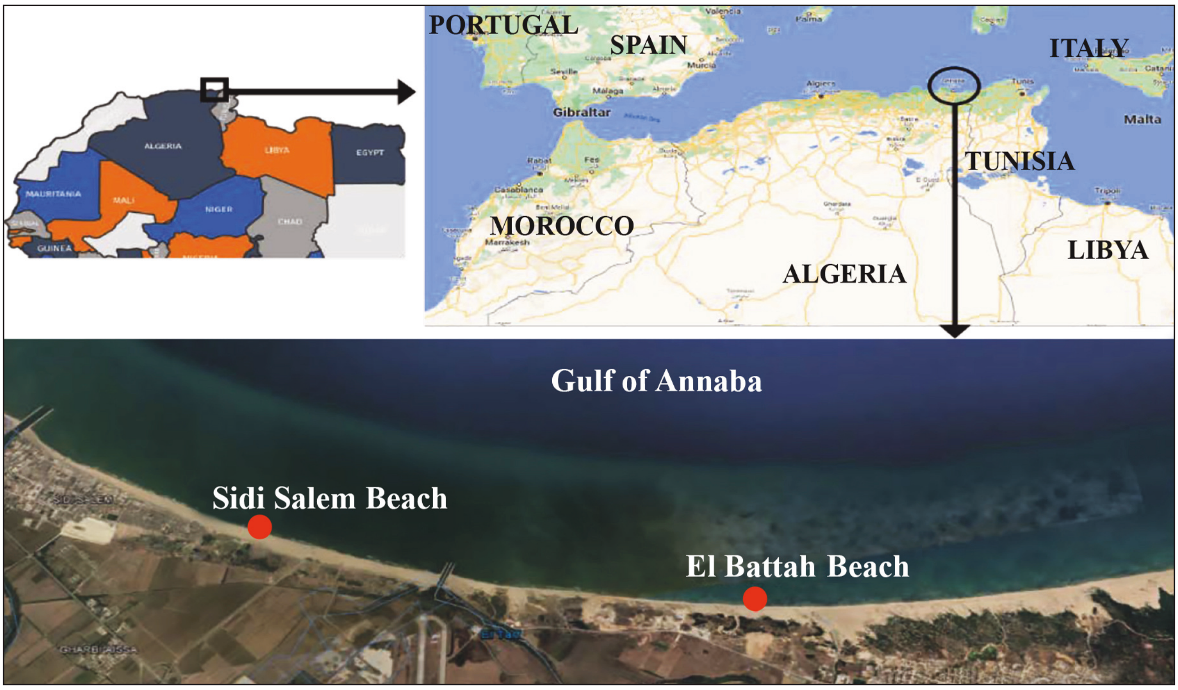


Figure 1. Location of sampling sites of *D. trunculus* in the Gulf of Annaba: the reference site (El Battah) and the polluted site (Sidi Salem).

| Site / Parameters | El Battah | Sidi Salem |
|---|---|---|
| **Temperature (°C)** | 18.24 ± 1.88 **a** | 18.47 ± 1.9 **a** |
| **pH** | 8.01 ± 0.75 **a** | 8.02 ± 0.82 **a** |
| **Salinity (PSU)** | 30.88 ± 1.99 **a** | 31.34 ± 1.14 **a** |
| **Dissolved oxygen (%)** | 4.55 ± 0.45 **a** | 4.69 ± 0.44 **a** |

Table 1. Physicochemical parameters of water at the two sampling sites. For each parameter, mean values followed by the same letter are not significantly different (p> 0.05).

### *Samples collection*

The sampling of *D. trunculus* is carried out by the artisanal fishing process during March, April and May of the year 2021 corresponding to the spring reproduction period (Hamdani et al., 2020). The gender distinction is made only during the period of sexual activity (Merad & Soltani, 2017) and is based on the color of the gonad. When mature, the reproductive system of males appears as a white or yellowish gland and the sex products are white and viscous (dense) while females show an intense blue (deep) blue gonad with blue and granular sex products (Hafsaoui et al., 2016; Merad & Soltani, 2017). The gonadal cycle is synchronous in both males and females. (Mouëza & Frenkiel-Renault, 1973; Hamdani & Soltani-Mazouni, 2011). Living individuals of this species with an average size of 27 ± 1 mm, are dissected with their mantle being removed and cleared of the siphons then separated vertically into two halves where, each half will be used for a different biochemical dosage.

### *Extraction and dosage of lipids*

Specimens of standardized shell size (length 27 ± 1 mm) were dissected and the mantles (weight 40–45 mg) removed for biochemical analysis. Lipids from mantles were extracted according to Shibko et al. (1966) as previously described (Boukari et al., 2021). Each mantle sample was individually homogenized with ultrasound (Sonifier B-30) in 1 ml of trichloroacetic acid (20%) and then centrifuged (5000 g, 10 min). The supernatant II recovered will be used for the determination of the total lipids. The pellet added with a mixture of ether and chloroform (1V/1V) was subjected to a second centrifugation (5000 g for 10 min). The resulted supernatant was used to quantify the lipids based on the vanillin method of Goldsworthy et al. (1972) and absorbance of the samples was measured at 532 nm. wavelength.

### *Malondialdehyde assay*

Lipid peroxidation was estimated by quantifying MDA levels in mantles, according to the method of Draper & Hadley (1990) based on the colorimetric measurement of the reaction between Thiobarbituric Acid (TBA) and malondialdehyde (MDA) giving a reddish-brown product whose intensity is measured at a wavelength of 532 nm. The fragments of mantles are ground with ultrasound in 1 ml of tris-HCL buffer (50 mM, pH 7.5) and centrifuged for 10 min at 10,000 rpm, this being followed by adding 2.5 ml of TCA (TCA 0.1 g/mL) to 0.5 mL homogenate and placed in a boiling water bath for 15 min. After cooling in tap water, centrifugation is carried out again at 10,000 rpm for 10 min. Two ml of the supernatant is then taken, to which is added 1 ml of TBA solution. After a second heating (100 °C., 15 min) and cooling, 1.5 ml of butanol is added. After agitation, a final centrifugation (10,000 rpm, 10 min) is carried out with the supernatant being recovered, as well as an absorbance measurement at 532 nm. The rate of MDA was expressed as µmol.mg−1 protein.

### *Protein quantification*

The proteins were quantified according to the method of Bradford (1976), which consists in measuring protein concentrations in the supernatants by using bovine serum albumin (Sigma) as standard and Coomassie Brilliant Blue (BBC) as a reactant. Absorbances are read at a wavelength of 595 nm.

### *Statistics*

The results obtained are expressed as mean ± standard deviation (m ± SD) and various statistical tests are carried out using Prism version 9 software (GraphPad software, La Jolla California, United States, www. Graphpad.com). Different tests were carried out:

-Student's t test to compare the two-by-two mean values of the physicochemical parameters of seawater;

- Three-way analysis of variance (ANOVA) (month, site and sex) was used.

- Finally, the Tukey's test was applied allowing to rank the different mean values and determining the differences between the studied groups.

## RESULTS

Biochemical analyzes reveal that the lipid levels of individuals from El Battah were higher compared to those from Sidi Salem during the three months of the study (Table 2). Maximum values were recorded in females from the El Battah site (21.84 ± 0.64 µg / mg of tissue) in April against a minimum values in males from the Sidi Salem site (7.35 ± 0.48 µg / mg tissue) in May. The comparison of total lipid levels for each sex by Tukey's test (Table 3) indicates a significant difference ($p < 0.0001$) between the two sites during the three months. Three-way ANOVA shows significant effects ($p < 0.0001$) of both month ($F_{2, 48} = 467$), site ($F_{1, 48} = 3594$) and sex ($F_{1, 48} = 524.2$). In addition, a month / site ($F_{2, 48} = 67.07$) and site / sex ($F_{1, 48} = 109.1$) interaction at $p < 0.0001$ as well as a month / sex interaction ($F_{2, 48} = 9.022$) and month / site / sex ($F_{2.48} = 8.845$) at $p = 0.0005$ were recorded, respectively.

The results obtained concerning the MDA rates are summarized in Table 4; they reveal that the MDA rates of individuals from El Battah were lower compared to those from Sidi Salem during the three studied months. Maximum values were recorded in females from the Sidi Salem site with an average of 33.95 ± 0.47 µM / mg of protein in May against minimum values in males from the El Battah site with an average rate of 15.28 ± 0.17 µM / mg protein in March. Comparison of MDA rates for each sex by Tukey's test, presented in Table 5, reveals significant differences between sites during the three months ($p < 0.0001$). Three-way ANOVA (month, site and sex) of MDA levels (µM /mg protein) at the two study sites during the spring breeding season of *D. trunculus* reveals significant effects ($p < 0.0001$) of both month ($F_{2, 48} = 1436.46$), site ($F_{1.48} = 1269.96$) and sex ($F_{1.48} = 1468.34$). As well as a month / sex (F 2.48 = 46.57), site / sex (F 1, 48 = 23.81) and month / site / sex (F 2.48 = 34.82) significant interactions ($p < 0.0001$) were recorded (Tables 4, 5)

| Site | El Battah | | Sidi Salem | |
|---|---|---|---|---|
| Sex / Month | Female | Male | Female | Male |
| March | 20.24 ± 0.39 **a** A | 16.64 ± 0.40 **b** A | 10.47 ± 0.61 **a** A | 9.11 ± 0.35 **b** A |
| April | 21.84 ± 0.64 **a** B | 18.38 ± 0.35 **b** B | 12.15 ± 0.78 **a** B | 10.34 ± 0.32 **b** B |
| May | 16.60 ± 0.64 **a** C | 10.36 ±0.44 **b** C | 8.87 ± 0.28 **a** C | 7.35 ±0.48 **b** C |

Table 2. Total lipid levels (m ± SD, n = 5; µg/mg tissue) in the mantle of females and males *D. trunculus* from the Gulf of Annaba collected during the spring breeding season of 2021. Mean values followed by the same lowercase letter are not significantly different (p> 0.05) between sexes for the same month at each site, while those followed by the same uppercase letter are not significantly different (p> 0.05) between months for the same gender at each site.

| Sex | Female | | Male | |
|---|---|---|---|---|
| Site / Month | El Battah | Sidi Salem | El Battah | Sidi Salem |
| March | 20.24 ± 0.39 **a** | 10.47 ± 0.61 **b** | 16.64 ± 0.40 **a** | 9.11 ± 0.35 **b** |
| April | 21.84 ± 0.64 **a** | 12.15 ± 0.78 **b** | 18.38 ± 0.35 **a** | 10.34 ± 0.32 **b** |
| May | 16.60 ± 0.64 **a** | 8.87 ± 0.28 **b** | 10.36 ± 0.44 **a** | 7.35 ± 0.48 **b** |

Table 3. Total lipid levels (m ± SD, n = 5; µg / mg tissue) in the mantle of females and males of *D. trunculus* from the Gulf of Annaba collected during the spring breeding season of the year 2021. Mean values followed by the same lowercase letter are not significantly different (p> 0.05) between sites for the same month and the same sex.

## DISCUSSION

Bivalves are among the most consumed and traded molluscs in the world (Tacon & Metian, 2013). They are associated with a high nutritional value, holding high contents of proteins, fats, carbohydrates, essential amino acids and fatty acids, vitamins, minerals and trace elements (Larsen et al., 2011; Tacon & Metian, 2013). Bivalve body constituents represent energy reserves that are used as an indicator of pollutant stress (Telahigue et al., 2019). Among these reserves, lipids are the most efficient source of energy. In Bivalve Molluscs, energy is stored in the form of lipids and glycogen, and is used, among other things, during gametogenesis (Nie et al., 2016).

In the present study, total lipid rates were quantified in the mantle of *D. trunculus* during the spring breeding season of 2021. The results obtained show higher levels in individuals from the El Battah site compared to those from Sidi Salem confirming previous studies (Hamdani & Soltani-Mazouni, 2011; Soltani et al., 2012; Sifi et al., 2013; Hamdani et al., 2014; Bensouda-Talbi & Soltani- Mazouni, 2014; Sifi & Soltani, 2019). Moreover, biochemical analysis performed in the flesh of an edible fish, *Liza aurata* from the gulf of Annaba, revealed a significant decrease in protein, lipid and carbohydrate amounts in Sidi Salem comparatively to El Hnaya (Bouzenda et al., 2021). This difference between the two sites may be due to their pollution level.

The sexual activity of Bivalves is influenced by several factors, such as temperature, availability of nutrients (Boukadida et al., 2019), as well as contaminants (Bonnefille et al., 2018; Ke & Wang, 2018) Most organisms, under normal conditions, use their reserves and sources of energy for general

| Sex | Female | | Male | |
|---|---|---|---|---|
| Site / Month | El Battah | Sidi Salem | El Battah | Sidi Salem |
| March | 20.14 ± 0.68 **a** | 25.52 ± 0.34 **b** | 15.28 ± 0.17 **a** | 20.80 ± 0.99 **b** |
| April | 20.03 ± 0.29 **a** | 25.37 ± 0.39 **b** | 15.96 ± 0.34 **a** | 20.61± 0.63 **b** |
| May | 31.20 ± 0.49 **a** | 33.95 ± 0.47 **b** | 21.18 ±0.54 **a** | 28.78 ±0.70 **b** |

Table 4. Malondialdehyde rates (m ± SD, n = 5; µM / mg protein) in the mantle of females and males of *D. trunculus* from the Gulf of Annaba collected during spring breeding in 2021. For the same sex and month, mean values followed by the same lowercase letter are not significantly different (p> 0.05) between sites.

| Site | El Battah | | Sidi Salem | |
|---|---|---|---|---|
| Sex / Month | Female | Male | Female | Male |
| March | 20.14 ± 0.68 **a** **A** | 15.28 ± 0.17 **b** **A** | 25.52 ± 0.34 **a** **A** | 20.80 ± 0.99 **b** **A** |
| April | 20.03 ± 0.29 **a** **A** | 15.96 ± 0.34 **b** **A** | 25.37 ± 0.39 **a** **A** | 20.61± 0.63 **b** **A** |
| May | 31.20 ± 0.49 **a** **B** | 21.18 ±0.54 **b** **B** | 33.95 ± 0.47 **a** **B** | 28.78 ±0.70 **b** **B** |

Table 5. Malondialdehyde rates (m ± SD, n = 5; µM / mg protein) in the mantle of females and males of *D. trunculus* from the Gulf of Annaba collected during spring breeding in 2021. Means followed by the same lowercase letter are not significantly different (p> 0.05) between sexes for the same month at each site. The means followed by the same capital letter are not significantly different (p> 0.05) between times (months) for the same sex at each site.

metabolism, growth and reproduction (Gonçalves et al., 2020; Silva et al., 2021). The energy allocation of organisms can be altered by an exposure to contaminants. This stress leads to the depletion of reserves because the synthesis, mobilization and use of antitoxic defenses (Jeon et al., 2013; Yeung et al., 2017). Lipid levels decrease in *D. trunculus* exposed to cadmium with more marked effects in females (Merad & Soltani, 2017). Smolders et al. (2004) and Guerlet et al. (2007) also noted a reduction in lipid levels in the mussel *Dreissena polymorpha.* The same results are observed in *D. trunculus* transplanted into an environment contaminated by heavy metals (Rabei et al., 2018; Boukari et al., 2021). The high rates observed in March and April would correspond to gonadal maturity and gametogenesis, while the low rates in May correspond to the period of gamete emission (Borkovic et al., 2005; Hamdani & Soltani-Mazouni, 2011) favored by the increase in temperature. Sex differences were also demonstrated at the two studied sites with relatively high rates in females than in males. This could be due to a difference in energy demand between male and female gametes (Beninger et al., 2003). In fact, males produce small spermatozoa with little energy reserves compared to females which develop vitelline reserves for the development of oocytes (Beninger & Le Pennec, 1997) and have high energy requirements for the oogenesis process.

The environmental stress induced by pollution causes toxicity through oxidative stress which manifests itself in an increase in the production of oxygen-derived species (ROS) (Chiarelli & Roccherri, 2014; Deidda et al., 2021). The fragmentation of membrane lipids and the lipid peroxidation of polyunsaturated fatty acids due to oxygen radicals cause an alteration of the structure, fluidity, transport and antigenic properties of the cell membrane inducing the formation of lesions in tissues and organs (Valavanidis et al., 2006). Malondialdehyde (MDA) is closely related to cell membrane degradation. It is therefore an early indicator of oxidative stress (Lykkesfeldt, 2007). High rates of MDA are recorded in individuals from the site of Sidi Salem compared to those of El Battah. Our results are in agreement with those reported by Tlili et al. (2010) in Tunisia and Soltani et al. (2012) in the Gulf of Annaba (Algeria). Similar observations have been reported by Bergayou et al. (2009) in *Scrobicularia plan* and *Cerastoderma edule* in Morocco. This suggests that *D. trunculus* individuals from Sidi Salem are subjected to more marked stress causing physiological damage (Sifi & Soltani, 2019). This stress is caused by exposure to various chemicals (Yeung et al., 2016) or metal elements. Indeed, the site of Sidi Salem is exposed to various pollutants from domestic, agricultural and industrial sources (Ounissi et al. 2016; Rabei et al., 2018; Kheroufi et al., 2021; Kroini et al., 2021). Significantly elevated levels of MDA in May have been observed. This is due to pollution and seasonal climate changes with low hydrodynamics (Ounissi et al., 2016). On the other hand, the increase in temperature promotes the evaporation of water and the rise in salinity, leading to an increase in the concentration of pollutants (Soucek, 2007). MDA data also indicate a sex effect since MDA levels are relatively high in females compared to males. This is probably due to their sensitivity to pollution during physiological processes such as reproduction as suggested by Drif et al. (2019).

## CONCLUSIONS

The results of this investigation showed a decrease in lipid levels and an increase in MDA rates in specimens from the Sidi Salem site compared to those from El Battah. This suggests that specimens of *D. trunculus* from Sidi Salem are subjected to oxidative stress related to the different sources of pollution of the site. A more pronounced rise in MDA levels is recorded in females, due to their sensitivity to pollution during reproduction. This concludes that this species is worth being used as a bioindicator in ecotoxicological studies.

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