

## **Depth and Seasonal Effects on the Settlement Density of Two Mussel Species (*Perna Perna* and *Mytilus Galloprovincialis*) in Offshore, Agadir (Morocco)**

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### **Abstract**

A study of spat settlement of two mussel species was carried out in Agadir area on the North Atlantic Coast, between Jan 2002 and Jan 2003. The preferred depth of settlement and settlement period of both species were monitored on collectors suspended in offshore at three different depths (1, 5, 10 m). For *Perna perna*, the effect of season on settlement was consistent, with relatively higher settlement both in spring (286-462 spats.m<sup>-1</sup>) and in summer (406-594 spats.m<sup>-1</sup>). Similar abundances of settlers were found at 1 m and 5 m depth whatever the season, which suggests a homogeneous distribution of settlers of *P. perna* in the first 5 m of the sea water. For *Mytilus galloprovincialis*, the settlement was less patchy in time in regard to *P. perna* ( $p < 0.05$ ). Thus, the settlement was continuous from spring to autumn until 10 m depth. The filamentous structures (laces in polypropylene) used in this study are often designed to enhance the amount of settlers, however, the settlement densities of both species were very low. Consequently, the results suggest that larval supply has been the limiting factor in the settlement success, but not the lack of suitable substrates. Moreover, the study area has poor spat falls and seems to be not suitable for collection of mussel spat.

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**Keywords:** Mussels spat, offshore, depth, season

### **Introduction**

The mussel culture still depends on the use of natural spat because of

the generally abundant supply. The seasonal nature of spat collection also allows recovery periods of up to a year, which should be ample time for the recovery of the ecosystem. However, the supply of mussel seed is often critical for the development of industrial mussel cultivation (Fuentes & Molares, 1994). Thus, supplies of seed mussels from natural sources have been scarce over the last few years severely affecting the profitability of mussel fisheries. While wild beds are still in use for juvenile supply in several countries, reliability was obtained through the development of spat collecting techniques (ropes, shell). Spat recruitment using rope collectors has been used about 65 years ago in Galician estuaries as an alternative to scraping (Fuentes & Molares, 1994; Cáceres-Martínez & Figueras, 1998). Interestingly, the use of mussel juveniles from collector ropes has been recommended because of their significantly larger size and weight at harvest than juveniles from intertidal beds (Fuentes et al., 1998).

In Morocco, the development and scale-up of the mussel aquaculture sector are limited. The mussel seed used in cultivation is still obtained by scraping directly from intertidal exposed rocky shores where mussel seed is attached. The gathering of mussel seed from artificial collector ropes was carried out only on an experimental basis (Aghzar et al., 2012). Due to overfishing, the mussel beds tend to disappear on the exposed rocky shores thus making the manual collection of spat less efficient. The feasibility of using techniques such as rope cultures has been investigated in offshore, in Agadir area (Id Halla et al., 2017). The results indicated that the submerged longline system tested in the study area could be commercially convenient. Thus, about 90% of mussels in both species reached the commercial size ( $\geq 60$  mm) after 10 months of culture. However, it is necessary to move towards a culture technique which combines seed collection and growth in suspended culture to allow shorter production cycle.

The offshore mussel culture system has been often suggested as a solution to meet the growing demand (Langan & Horton, 2003; Karayücel et al., 2015). Well-protected and bays are preferred than open unprotected areas for mussel culture. However, these ecosystems are often subject to environmental constraints associated with pollution from industrial and domestic sources. Major difficulties in establishing the rope cultures in offshore are the high tidal currents, which require special mooring systems and the reliance on natural larval supply. A mussel cultivation business is dependent on a regular annual supply of seeds for growing on to market size. Such supplies may not be sufficiently regular to sustain restocking on an annual basis. The capture of wild stocks of mussel spat for use in aquaculture was more reliable through accurate forecasting of peak settlement period and knowledge of the preferred depth of settlement of different species. Thus, the present study was designed to test the influence of season and depth on spat

settlement of two coexisting mussel species (*Mytilus galloprovincialis* and *Perna perna*) in offshore, in Agadir area. This is the first investigation of mussel settlement using an offshore submerged system in Morocco. The study also aims at investigating the seed availability of both species for commercial purpose.

## Material and Methods

### Experimental design

The study was conducted 25 km north of Agadir at 20-22 m depth of the offshore site in the North Atlantic Ocean (Fig. 1). This marine area, commonly called “PK25” (30°34’N, 9°46’W), was chosen due to its harsh weather conditions (currents and high waves), far from any pollution sources (domestic and industrial). The designated area located at 1 mile from the coast was exposed to relatively energetic waves from the North Atlantic. More than 70% of waves in winter have significant heights ranging 1.5-3.5 m (Aouiche et al., 2016). In summer, the directional range also covers the North. More than 90% of incoming waves during this season have significant heights ranging 1-2 m. This clear seasonal wave pattern highlights the predominance of a swell-dominated regime in winter and more fair-weather mixed swell and shorter-fetch wave conditions in summer. Tides are semi-diurnal and mesotidal with a mean spring range of 2.9 m and a mean neap range of 1.3 m.



Fig 1. Map showing the study area

A submerged longline system was deployed at 1 m below the water surface. This system was designed according to site conditions. It was anchored at both sides with heavy cement blocks (350 kg), and buoys (30 L) attached along the longline were used to keep it afloat. Four marker buoys were used on the edges of the experimental area to mark and protect the site for navigation purposes. Experimental collector ropes were suspended on the longline system from February 2002 to January 2003. During this spat collecting trial, one type of collector rope was installed. Four 15 m (0.8 cm in diameter) nylon ropes with filamentous lacing sections (40 cm of length) were

used. These polypropylene sections were attached to the ropes at three different depths (1, 5, 10 m). Five kg concrete weights were tied to the ends of the ropes to guard against the wave action and the tangling of ropes. The collectors were hung on the mainline.

### **Sampling procedure**

Collectors deployed in February 2002 and data collection started in March 2002. Monthly sampling was conducted to determine the density of spat on ropes at three different depths (1, 5, 10 m) throughout the study period. Four replicates were sampled by divers at each depth. Individual samples were grazed from a 40 cm length of the filamentous lacing sections. The spat was transferred into a 20-L tank filled with sea water and brought to the laboratory. Monthly mussel density on the ropes was determined by counting spat on the 40 cm sections of each filamentous lace. The settlers of each species were identified by shell morphology, counted and preserved in 70% alcohol.

### **Environmental variables**

Temperature and salinity were measured monthly during the experimental periods using a probe (6600 V2 YSI). Seawater samples were collected at the experimental site from a 5 m depth using a Niskin bottle of 3 L. Three replicas were considered. The water samples were transferred to a laboratory and filtered onto Whatman GF/C filters after acetone extraction to determine chlorophyll a ( $\mu\text{g L}^{-1}$ ).

### **Statistical analysis**

Spatial and temporal trends in spat abundance were analysed using ANOVA. A series of ANOVAs were therefore used on balanced subsets of the data to assess settlement variability. Two-way ANOVA was performed to test the influence of season and depth on the settlement of both species. Where significant differences ( $p \leq 0.05$ ) were recorded, post-hoc comparisons were done using Tukey's test. We used a three-way balanced factorial ANOVA ( $n=4$ ) to examine the effects of season, depth, and species on the settlement.

## **Results**

### **Environmental variables**

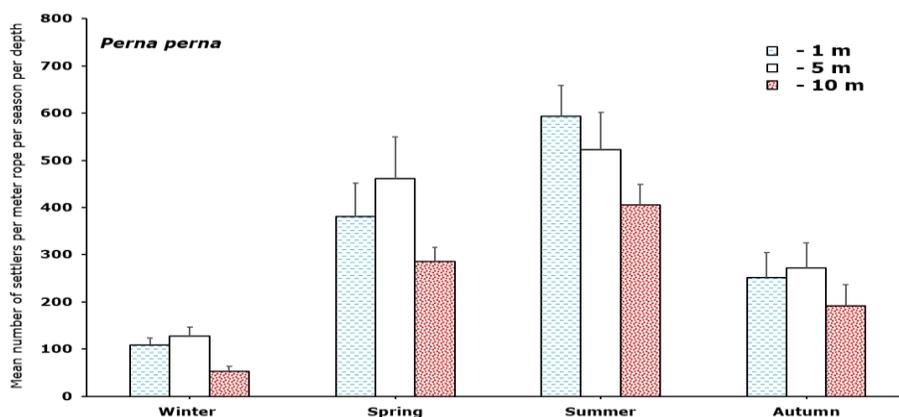
The temperature ranged between minimum values of  $15.7^{\circ}\text{C}$  in winter and  $21.1^{\circ}\text{C}$  in summer (Table 1). Salinity varied within a narrow range (35.3-35.9). Chlorophyll-a concentration displayed significantly marked seasonal changes. Chlorophyll-a concentration presented minimum value ( $0.77 \pm 0.32 \mu\text{g L}^{-1}$ ) in winter and maximum value ( $1.95 \pm 0.23 \mu\text{g L}^{-1}$ ) in summer. There was a significant correlation between chlorophyll-a and temperature ( $r=0.87$ ,  $\rho < 0.001$ ).

**Table 1.** Seasonal changes in mean temperature, salinity and chlorophyll a during the experimental period.

| Seasons | Temperature<br>(°C) | Salinity<br>(‰) | Chlorophyll-a<br>( $\mu\text{g L}^{-1}$ ) |
|---------|---------------------|-----------------|---|
| Winter  | 15.7±0.42           | 35.3±0.33       | 0.77±0.32                                 |
| Spring  | 18.8±1.34           | 35.7±0.01       | 1.51±0.10                                 |
| Summer  | 21.1±0.42           | 35.9±0.09       | 1.95±0.23                                 |
| Autumn  | 18.8±1.33           | 35.4±0.23       | 1.23±0.22                                 |

### Settlement of *Perna perna*

In winter, settler abundance of *P. perna* on collectors ranged between 52±21 (10 m) and 108±35 (1 m) per meter rope (Fig. 2). The settlers were more abundant in spring and summer whatever the depth. The number of spats settled in spring, ranged between 286±50 (10 m) and 462±88 (5 m) per meter rope. In summer, the abundance of settlers fluctuated between 406±43 (10 m) and 594±65 (1 m) per meter rope. The settlers were more or less abundant in autumn, with a mean maximum value of 272±53 settlers.m<sup>-1</sup>.

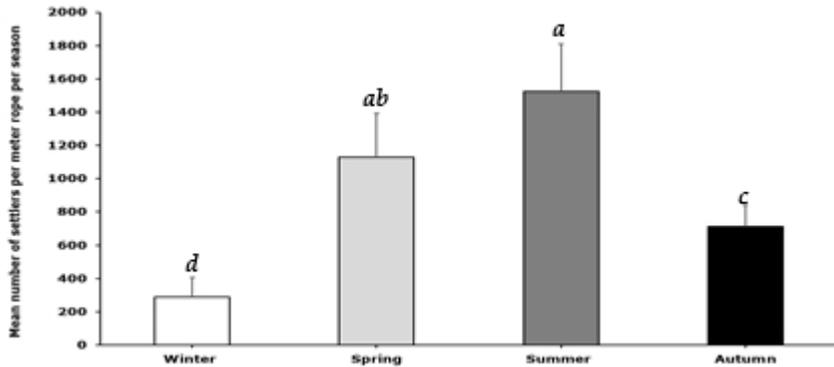


**Fig. 2** Seasonal settlement of *Perna perna* on collectors suspended at different depths (1, 5, 10 m). Means are reported with standard deviations.

Two-way ANOVA was performed to test the influence of season and depth on the settlement of *P. perna*. The results revealed that their effects were highly meaningful ( $\rho < 0.0001$ , Table 2), but not with a significant interaction among them ( $\rho = 0.202$ ). Therefore, the effects of season and depth were evaluated separately without considering their interaction effect. In winter, the settlement of *P. perna* was significantly lower than those recorded in the other seasons (Fig. 3, *post-hoc* comparison  $\rho < 0.01$ ). The settlement occurred mainly over two seasons: spring and summer, with no significant difference among them (*post-hoc* comparison  $\rho = 0.185$ ). Furthermore, the number of settlers decreased significantly from summer to autumn (*post-hoc* comparison  $\rho < 0.0001$ ).

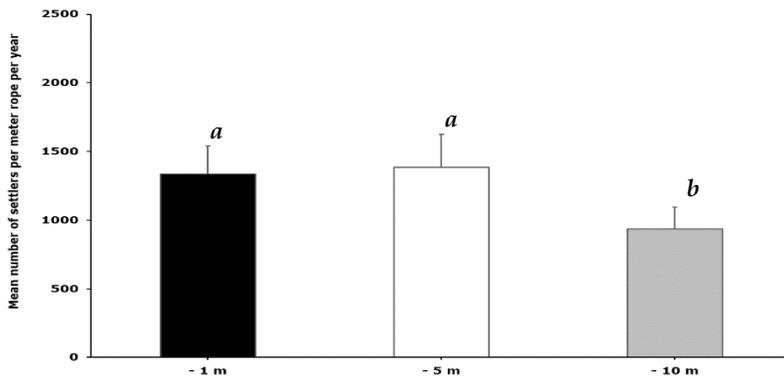
**Table 2.** Two-way ANOVA on the effect of season (fixed, 4 levels) and depth (fixed, 3 levels) on settlement abundance of *Perna perna*.

|                       | SS     | Df | MS     | F      | $\rho$   |
|-----------------------|--------|----|--------|--------|----------|
| Season                | 848981 | 3  | 282994 | 89.839 | < 0.0001 |
| Depth                 | 91706  | 2  | 45538  | 14.456 | < 0.0001 |
| Season $\times$ depth | 29475  | 6  | 4912   | 1.559  | 0.202    |
| Error                 | 75600  | 24 | 3150   |        |          |



**Fig. 3** Settlement of *Perna perna* on suspended collectors: *Post-hoc* comparisons of the effect of season. Letters indicate homogeneous groups (Tukey test,  $\alpha < 0.05$ ).

The depth affected highly the variability of the settler abundance ( $\rho < 0.0001$ , Table 1). However, similar abundances were found at 1 m and 5 m depth whatever the season (Fig. 4, *post-hoc* comparison  $\rho = 0.982$  in winter,  $\rho = 0.819$  in spring,  $\rho = 0.911$  in summer and  $\rho = 0.991$  in autumn). The settlement of *P. perna* decreased significantly from 5 m to 10 m depth both in winter and spring (*post-hoc* comparison  $\rho < 0.05$ ).

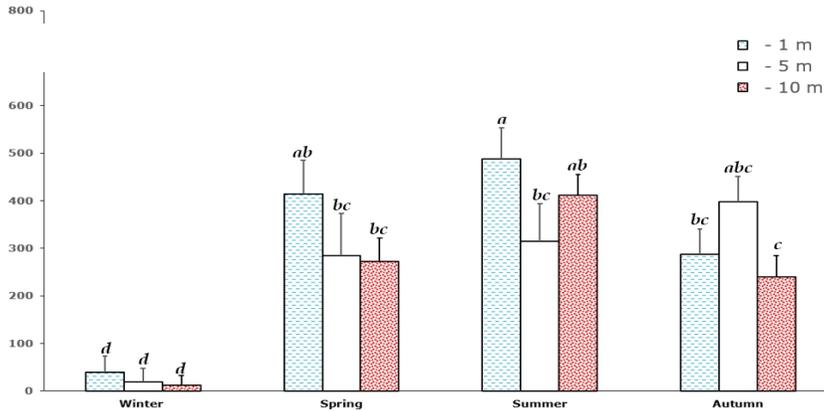


**Fig. 4** Settlement of *Perna perna* on suspended collectors: *Post-hoc* comparisons of the effects of depth. Letters indicate homogeneous groups (Tukey test,  $\alpha < 0.05$ ).

### Settlement of *Mytilus galloprovincialis*

The settlement of *M. galloprovincialis* was extremely low in winter,

ranging between  $12 \pm 7$  (10m) and  $39 \pm 11$  (1 m) per meter rope (Fig. 5). By cons, the settlers were more abundant during the other seasons. In spring, settler abundance ranged between  $272 \pm 34$  (10 m) and  $414 \pm 51$  (1 m) per meter rope. In summer, this abundance fluctuated between  $315 \pm 52$  and  $488 \pm 60$ , recorded at 5 m and 1 m depth respectively. The settlement in autumn reached at 5 m depth, a maximum value of  $398 \pm 49$  settlers.m<sup>-1</sup>.



**Fig. 5** Settlement of *Mytilus galloprovincialis* on suspended collectors: *Post-hoc* comparisons of the season  $\times$  depth interaction. Letters indicate homogeneous groups (Tukey test,  $\alpha < 0.05$ ).

Two-way ANOVA was also performed to test the influence of season and depth on the settlement of *M. galloprovincialis*. The results demonstrated that these two main effects were significant, with a meaningful interaction among them ( $\rho < 0.0001$  for season;  $\rho < 0.01$  for depth and season  $\times$  depth effects; Table 3).

**Table 3.** Two-way ANOVA on the effect of season (fixed, 4 levels) and depth (fixed, 3 levels) on settlement abundance of *Mytilus galloprovincialis*.

|                       | SS     | df | MS     | F      | P        |
|-----------------------|--------|----|--------|--------|----------|
| Season                | 749901 | 3  | 249967 | 90.221 | < 0.0001 |
| Depth                 | 34087  | 2  | 17043  | 6.151  | 0.0069   |
| Season $\times$ depth | 88674  | 6  | 14779  | 5.334  | 0.0012   |
| Error                 | 66494  | 24 | 2177   |        |          |

The settlement of *M. galloprovincialis* was drastically lower in winter whatever the depth, in comparison with the other seasons (*post-hoc* comparison  $\rho < 0.0001$ ). There was not a significant difference in abundances between spring and summer whatever the depth (*post-hoc* comparison  $\rho = 0.841$  (1 m),  $\rho = 0.981$  (5 m), and  $\rho = 0.103$  (10 m)). In autumn, the numbers of settlers decreased significantly at 1 m and 10 m depth ( $\rho < 0.01$  and  $\rho < 0.05$  respectively). However, similar abundances were found at 5 m depth whatever the season, except in winter (*post-hoc* comparison  $\rho = 0.919$  for spring-summer,  $\rho = 0.317$  for spring-autumn, and  $\rho = 0.730$  for summer-autumn).

### Interspecies comparison

The comparison of settlement of both species between seasons and in all depths was performed by using three-way ANOVA (Table 4). This analysis showed a significant interaction between season, depth and species ( $\rho < 0.05$ ). In winter, the settlement of *P. perna* was higher than that of *M. galloprovincialis*, with significant differences at all depths (*post-hoc* comparison  $\rho < 0.05$ ,  $\rho < 0.01$  and  $\rho < 0.05$  at 1, 5 and 10 m depth respectively). At 5 m depth, numbers of settlers of *P. perna* were higher than those of *M. galloprovincialis* both in spring and summer (*post-hoc* comparison  $\rho < 0.05$  and  $P < 0.01$  respectively). By cons, similar settler abundances were recorded for both species at 1 m and 10 m depth (*post-hoc* comparison  $P = 0.746$  and  $\rho = 0.983$  respectively). In autumn, both species displayed similar settlement abundances across all depths (*post-hoc* comparison  $\rho = 0.991$ ,  $\rho = 0.437$ , and  $\rho = 0.975$  at 1, 5 and 10 m depth respectively).

**Table 4.** Three-way ANOVA of the settlement of *P. perna* and *M. galloprovincialis* with season (4 levels), depth (3 levels) and species (2 species) as fixed factors.

|                          | SS      | df | MS     | F       | P        |
|--------------------------|---------|----|--------|---------|----------|
| Season                   | 1520911 | 3  | 506970 | 171.257 | < 0.0001 |
| Depth                    | 98130   | 2  | 49065  | 16.574  | < 0.0001 |
| Species                  | 27966   | 1  | 27966  | 9.447   | 0.003    |
| Season × depth           | 63669   | 6  | 10611  | 3.585   | 0.005    |
| Season × species         | 77970   | 3  | 25990  | 8.780   | < 0.0001 |
| Depth × species          | 27032   | 2  | 13516  | 4.566   | 0.015    |
| Season × depth × species | 54479   | 6  | 9080   | 3.067   | 0.013    |
| Error                    | 142094  | 48 | 2960   |         |          |

### Discussion

Numerous studies on the spat settlement of mussels indicate spatial and temporal variability over a wide range of scales. Our results reported that settlement of *P. perna* was highly patchy in time. Indeed, the effect of season on settlement of *P. perna* was consistent, with significantly higher settlement both in spring and summer. This strongly implies meaningful differences in delivery of spat among seasons. However, the presence of settlers throughout the year reflects the occurrence of different spawning periods for *P. perna*. Peak settlement of spat occurred in April and August whatever the depth. The temporal differences could be assigned to several biotic and abiotic factors involved in larval dispersion and settlement. The timing and magnitude of spat larval supplies (Porri, McQuaid & Radloff 2006), algal coverage (O'Connor, Crowe & McGrath 2006) and microbial coverage (Hunt & Scheibling 1996) are the most evident biotic factors. Abiotic factors combined the local hydrographic regimes implicated in nutrient and spat dispersion (Eyster & Pechenik 1987; Dobretsov & Miron 2001), physico-chemical substratum

characteristics (Pulfrich 1996; Alfaro et al., 2006), temperature (Pineda 1991; Garland et al., 2002), orientation and daylight (Bayne 1964). In this study, the spawning of *P. perna* was stimulated by increased temperature in spring, which is the initial spawning period in the region. The peak settlement of *P. perna* occurred both at 1 and 5 m depth, which suggests that it is quite possible that we have a homogeneous distribution of settlers between 5 m and 1 m depth. Thus, no depth preference can be inferred until 5 m from the data. Seed densities were relatively lower at 10 m throughout the year. Mussel seeds settled on the ropes near the surface because of the high temperature, abundance of light and available food (Karayücel et al., 2002).

The settlement of *M. galloprovincialis* was less patchy in time in regard to *P. perna*. Indeed, the effect of season on settlement of *M. galloprovincialis* was inconsistent from spring to autumn. Overall, this implies no meaningful differences in delivery of spat among these seasons. However, the settlement of *M. galloprovincialis* was extremely low in winter whatever the depth. The vertical variation of *M. galloprovincialis* did not follow the same pattern as that of *P. perna*. Indeed, the settlement of *M. galloprovincialis* was continuous from spring to autumn until 10 m depth. Peak settlement of spat occurred in May and September at 1 m depth.

It has been proved that especially artificial collectors with filamentous structure are effective in the settlement of mussel seeds (Petersen 1984; Lekang et al., 2003). Despite the preferable settlement of spats on filamentous structures using the same laces, reported in many studies (Lutz & Kenish, 1992; Ramón et al., 2007), our results showed lower settlement densities. This suggests that larval supply has been the limiting factor in settlement success, but not the lack of suitable substrates. Cáceres-Martínez et al. (1994) obtained highest settlement in the open sea with almost 60.000 mussels  $m^{-2}$  after five months. Okumuş (1993) reported that density of spat on the collector rope had to be at least 1200 ind.  $m^{-1}$  with commercial mussel farming. Thus, the settlement densities obtained in this study seem to be not suitable for commercial mussel farming. This inappropriate spat settlement revealed that the study area had not suitable hydrological conditions and as a result, it not facilitated mussel larvae settlement on the collectors. Thus, the local oceanographic conditions were reported to play an important role in larval distribution (Blanchette & Gaines, 2007; Blanchette et al., 2007). The basic requirement of all mussel culture practices is secure supply of spats and that's why big culture operations around the world have been located in areas traditionally as where natural seed mussels are readily available every year. Id halla et al. (2017) obtained better productions in this area and suggested offshore cultivation as an opportunity for commercial enterprises without worrying about the shortage of seed. In this case, it is necessary to collect or purchase seed from distant areas which may involve expensive transport costs.

However, transferring seed from one site to another, however, results in extra seed loss (Paul, 1987) and could be very labour intensive. Therefore, there is no doubt that a site suitable for both good settlement and growth is always preferable.

## Conclusion

The mussels exhibited rapid growth under cultivation in the offshore area, hence marketable size ( $\geq 60$  mm) was reached after 10 months of culture. However, considerable effort is required to obtain sufficient seed to develop the mussel farming in offshore. The spat collectors must be installed close to the rocky shores, as the ropes tested did not produce enough seed in offshore for new ropes (or thinning out ropes) where mussels remain until they reach marketable size. If there is not enough spat on the spat collectors, seeds will be collected from natural beds and transferred to the longlines after tubing in bio-degradable socks. However, it must be taken into account that seed density might change year to year depending on environmental factors at the spat collection sites.

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