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Short Communication

Tracing of aquaculture-escaped meagre *Argyrosomus regius* through otolith microchemistry

P. Arechavala-Lopez[a,b,*], S. Pérez-Mayol[a], M. Palmer[a], J.M. Valero-Rodriguez[c], P. Sanchez-Jerez[c], B. Morales-Nin[a]

[a] Fish Ecology Group, Dept. of Ecology and Marine Resources, Mediterranean Institute of Advanced Studies (IMEDEA – UIB/CSIC), Esporles, Spain
[b] Fish Ethology and Welfare Group, Centro de Ciencias do Mar (CCMAR), Faro, Portugal
[c] Sustainable Aquaculture Research Group, Dept. of Marine Sciences and Applied Biology, University of Alicante, Alicante, Spain

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ABSTRACT

Escape incidents of farmed fish involve economic losses to fish farms, interactions with local fisheries and environmental impacts to coastal ecosystems. In order to ensure a sustainable aquaculture activity it is advisable to gather any kind of information about the escapees for further management strategies. In this study, we aimed to trace the life history of escaped meagre (*Argyrosomus regius*) through otoliths microchemistry, based on the assumption that escaped fish experience different environmental conditions once they are outside the net-pen compared to their farmed conspecifics. Strontium (Sr^{88}) and barium (Ba^{138}) composition was analyzed using laser-ablation ICP-MS along core-to-edge transects of right sagittal otoliths on escaped and farmed meagre from the same coastal area in the W-Mediterranean Sea. Overall, results showed similar patterns of Sr concentrations throughout the otolith transects between farmed and escaped meagre, although some differences can be observed at specific periods of fish life for Ba concentrations. Consequently, temporal variations regarding otolith Sr:Ba ratios differed between farmed and escaped meagre, suggesting that farmed and escaped fish shared the same origin (rearing at coastal farms) but inhabited in different conditions from a certain time of their life. However, core-to-edge Sr:Ba values also differed among escaped individuals, which might indicate that each individual escaped in different periods. Consequently, the otolith Sr:Ba ratio seemed to be a good indicator of differences between fish groups, showing different temporal patterns. Nevertheless, the limitation of the low number of sampled individuals prevented to draw clearer conclusions. Further research is necessary in order to investigate the potential use of otolith microchemistry as a practical tool to trace of escaped fish, and consequently, to help solving potential conflicts among coastal users improving management of potential negative socioeconomic and ecological impacts.

1. Introduction

Fish farming production in floating cages has been increasing in recent decades in the Mediterranean Sea, and particularly for rearing meagre *Argyrosomus regius* (Asso, 1801) where it is considered an emerging species due to its high flesh quality and flavour, fast growth, large size, high feed conversion rate and high adaptation capacities to environmental changes (Monfort, 2010; Duncan et al., 2013). However, the rapid growth of the fish farming industry leads to an increased potential number of farmed fish in the wild. Escape incidents of farmed fish involve economic losses to fish farms, interactions with local fisheries and environmental impacts to coastal ecosystems (Arechavala-Lopez et al., 2018). These potential impacts might be more problematic when the escapees are non-indigenous species (Toledo-Guedes et al., 2014; Atalah and Sanchez-Jerez, 2020). This might be the case of meagre, whose natural populations are currently limited to specific geographic areas (Haffray et al., 2012), but it is being farmed throughout the Mediterranean Basin (Arechavala-Lopez et al., 2015). In addition, meagre escapees from coastal floating facilities have been already reported in different Mediterranean areas where it is considered a local-absent species (Dulčić et al., 2009; Arechavala-Lopez et al., 2015; Valero-Rodriguez et al., 2015; Mavrič and Dragičević, 2018). In order to

* Corresponding author at: Fish Ecology Group, Dept. of Ecology and Marine Resources, Mediterranean Institute of Advanced Studies (IMEDEA – UIB/CSIC), Esporles, Spain.
E-mail address: pablo@fishethogroup.net (P. Arechavala-Lopez).

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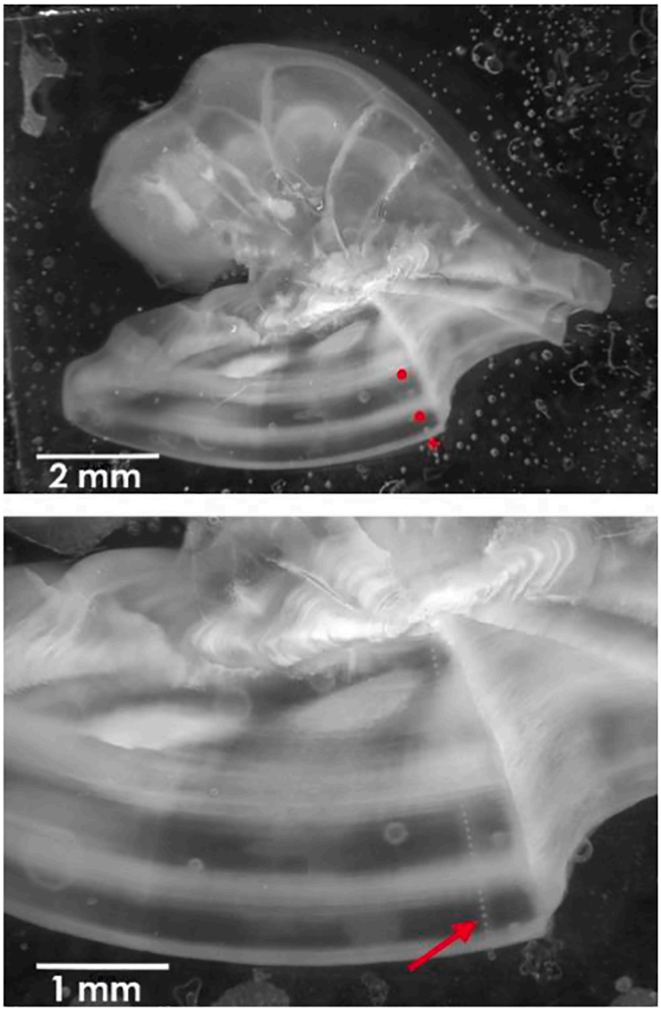

Fig. 1. Example of otolith section of a 2+ years old meagre (up), and transect of laser ablation (LA) spots for ICP-MS analyses (down).

ensure a sustainable aquaculture activity and effective mitigation plans, it is essential to identify escapees and track back their life, so that the origin and possible causes of the incident can be investigated (Dempster et al., 2018).

Diverse methods have been suggested to distinguish escaped fish from wild conspecifics, and otoliths are considered a useful tool to identify the origin of escapees, either by morphometric differences or the presence of specific trace elements, which are likely to reflect the different environmental and feeding characteristics of fish (Arechavala-Lopez et al., 2013). On the other hand, life history and migration patterns of wild meagre were successfully assessed by otolith daily growth (González-Quirós et al., 2011) and variations in otolith elements (i.e. Sr and Ba) along core-to-edge transects (Morales-Nin et al., 2012). Naturally occurring elemental signatures (or composition) in the otoliths of fish are an ideal natural tag due to the metabolic inertness of otoliths (Campana, 1999), continuous growth including daily increments and incorporation of elements that are influenced by environmental variables (Gillanders, 2005). Moreover, differences in concentration of certain elements can be the outcome of individual differences in growth rate (Catalán et al., 2018). Therefore, based on the assumption that escaped fish might experience different environmental conditions once they are outside the net-pen compared to their wild or feral conspecifics (Arechavala-Lopez et al., 2017), the aim of this study was to trace of the life history of aquaculture-escaped meagre through otolith microchemistry transects analysis. The resulting knowledge will help to better-understand its potential use as a tool to investigate the fish origin, improving monitoring of meagre populations and escapes mitigation actions.

2. Material and methods

Escaped meagre individuals ($n = 7$, mean length ± SE = 54.3 ± 2.9 cm; mean weight ± SE = 1.37 ± 0.15 kg) were sampled in winter 2010 from Santa Pola harbor in the SE of Spain, Western Mediterranean Sea. These fish were escapees because the species is not found naturally in the area (Arechavala-Lopez et al., 2015). In parallel, two farmed meagre (mean length ± SE = 67.5 ± 1.5 cm; mean weight ± SE = 4.1 ± 0.11 kg) were obtained one year later (2011) from the nearest fish farm facility (Guardamar del Segura, Spain). Right sagittae otoliths were removed from sampled individuals, rinsed, embedded in epoxy, and transversally cut using a diamond-edged precision saw. The sections were ground on silicon carbide grinding papers of decreasing grain size (from European P-grade 800 to 2400) and polished with 0.3 μm alumina until the nucleus was reached and seasonal marks observable. Individual age was visually estimated through the number of annual increments on transverse sections following the method described in González-Quirós et al. (2011). Otolith sections were randomly mounted on two petrographic glass slides and cleaned, dried and stored following standard methods for chemical purposes. Then, calcium (Ca^{43}, used as internal standard), strontium (Sr^{88}) and barium (Ba^{138}) elements were analyzed using Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS; laser settings: ø = 30 μm, distance = 60 μm, dwell time = 45 s.) along otoliths transect from core (nucleus) to the edge or border (Fig. 1). Three Certified Reference Material (CRMs) were analyzed in a bracketing way with samples and with the same laser settings: NIST614, FEBS-1 (Sturgeon et al., 2005), and NIES-22 (Yoshinaga et al., 2000).

For each one of the laser shots in each transect, the spectrometer provides a temporal profile (intensities over time in counts per second, cps) that is composed by a *blank* interval at which no sample is ablated and a *plateau* interval at which cps values reaches a noisy but steady level after returning to the blank level again. The *blank* and *plateau* intervals of each laser shoot were delimited by an observer using the R package ELEMENTR (Sirot et al., 2017). The distribution of cps for a given laser shot and element was determined by subtracting the distribution of cps at the *plateau* from the distribution of cps at the *blank*. When the plateau-blank difference was not different from zero (prob>0.05), the shoot was excluded from further analyses. Next, the difference plateau-blank for any given element was normalized (ratio) by the difference plateau-blank for Ca^{43}. This normalization (known as internal standard normalization) is a widely applied procedure for correcting cps disparities related only with structural differences between (synthetic and homogeneous) CRMs and biogenic samples. The element: Ca ratio from CRMs (dependent variable) is assumed to be a lineal combination of the certified concentration for each element (ppm), the session (categorical variable accounting for session-specific random effects common to all the shoots of a given working session) and time elapsed since the session's start (i.e., accounting for any lineal temporal drift of cps throughout a given session, which is a common technical issue in LA-ICPMS). The parameters of this statistical model were estimated using a Bayesian approach. The model was then used to estimate the concentration (ppm) of any shoot in an otolith transect from its element:Ca ratio, after accounting for the effects of session and temporal drift. Finally, the otolith core-to-edge Sr:Ba ratio was estimated for all individuals.

3. Results

Visual inspection of otoliths transversal sections revealed that the age of all escaped individuals was 2+, whereas the age of farmed meagre was 3+. As the farmed meagre were sampled one year later, the birthdate of all fish should be similar. Moreover, as the pens and the sampling

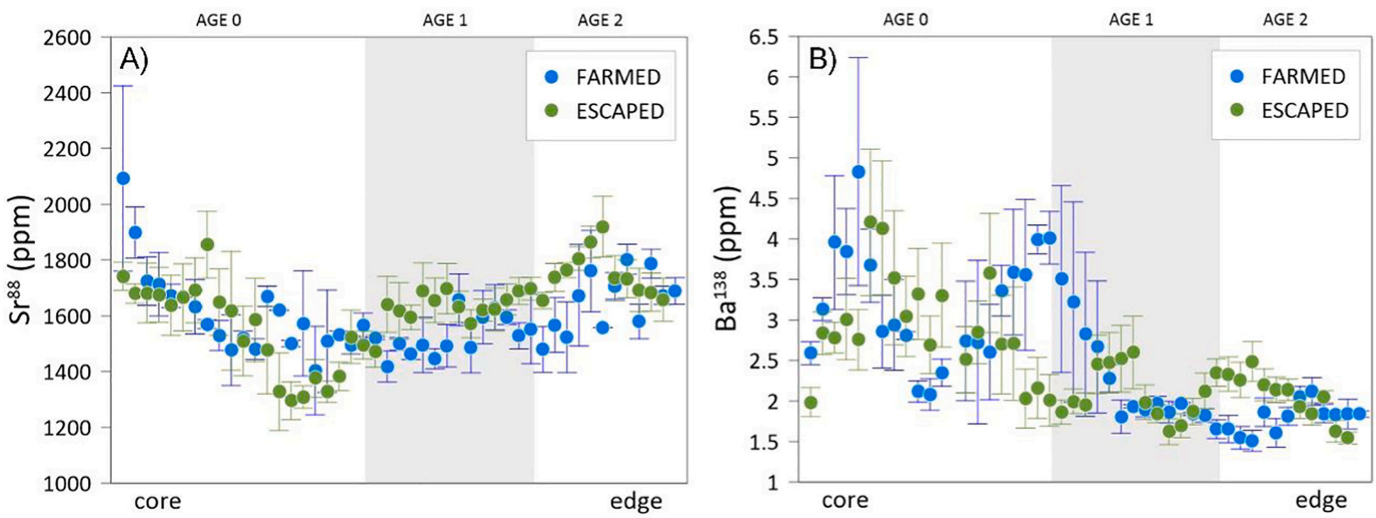

Fig. 2. Scatter plots of mean concentrations of Sr and Ba microelements from meagre otoliths through analyzed core-to-edge transects. Whiskers show standard error (S.E.) values.

harbor were about 6 kms distance, environmental conditions experienced by all the fish should be similar (Gillanders et al., 2001). It must be noted that the values corresponding to the last year of farmed meagre were removed in order to foster data comparison (Figs. 2 and 3). Mean concentrations of otolith Sr along transects were similar between farmed and escaped meagre although showing different temporal trends. Sr concentrations of farmed meagre otoliths remained more or less constant over the transect, whereas Sr concentrations of escaped meagre otoliths decreased during the first year of life and then increase during the rest of the fish life (Fig. 2a). Mean concentrations of Ba along the otolith transects showed different trends between farmed and escaped meagre. Escapees showed lower Ba concentrations than farmed meagre at age 0–1, whereas higher Ba concentrations were detected at age 1–2 (Fig. 2b). Consequently, temporal variations regarding otolith Sr:Ba ratios differed between farmed and escaped meagre, but also among escaped individuals (Fig. 3).

4. Discussion

Elemental composition (i.e. Sr, Ba) along otolith transect revealed temporal variations and differences between fish groups, probably due to escaped fish experience different environments once they are outside the net-pen, compared to their farmed conspecifics. However, core-to-edge Sr:Ba differences among escaped individuals might indicate that each individual escaped in different periods of time. Using LA-ICP-MS along otolith transects, Morales-Nin et al. (2012) evidenced that wild meagre uses different water masses during its life history through microchemical profiles of otoliths. The conceptual life history model for wild meagre can be reflected by low Sr:Ca and high Ba:Ca ratios corresponding to the young-of-the-year period, followed by increased Sr:Ca and decreased Ba:Ca ratios as the juveniles move offshore. The final adult pattern presented seasonal fluctuations in Sr:Ca and Ba:Ca reflecting annual migrations to the estuary for spawning (González-Quirós et al., 2011; Morales-Nin et al., 2012). Comparing with our results, there was a clear decrease of Ba concentration in meagre otolith transect at the age 0–1, and consequently an increase of otolith Sr:Ba ratio, which might indicate the period when farmed meagre break out from the rearing cage and signalling the beginning of its adaptation to the new wild environment. However, core-to-edge Sr:Ba differences among individuals might also indicate that each individual escaped in different periods of time.

Meagre escapees rapidly disperse from the farm facility, mostly moving next to the sea bottom, towards deep waters and probably pressured by the recent unknown habitat and the presence of predators (Arechavala-Lopez et al., 2017). It is known that hatchery-reared meagre experience adverse conditions during the first days after release in the wild and at least some individuals are able to adapt to the natural environment after a few months at liberty, surviving for years (Gil et al., 2014a, 2014b). Differences in growth rates during this adaptation period may cause differences in otolith microchemistry of fish living in the same water mass (Catalán et al., 2018). On the other hand, the higher concentrations of Ba (and lower otolith Sr:Ba ratio) at the age of 1–2, approximately one year of the estimated escape incident, supports the idea of surviving escapees inhabiting coastal waters. Escaped meagre can move towards coastal areas, where it can predate on coastal resources (Valero-Rodriguez et al., 2015) or be captured by local fishermen (Arechavala-Lopez et al., 2017). Moreover, male and female meagre can reach maturity stage at 2 and 3 years old respectively (Cárdenas, 2010), so as the fact that escapees migrate to shallow or estuarine areas for spawning cannot be ruled out, coming back to deeper waters with more stable environmental conditions in winter. It is then that they inhabit similar water masses than those of farmed meagre (i.e. open sea). Although otolith microchemistry can be used to investigate the fish origin and path life, including the precise moment when the fish escapes, further studies must consider in detail specific variations of element concentrations over fish life-time, fluctuations on environmental conditions and individual grow rate (Gil et al., 2014b).

After an escape event, fish farmers should be advised to put in place mitigation or eradication measures to eliminate or minimize any negative effects on the sustainability of uses of the coastal zone (e.g. fisheries, tourism, marine protected areas) or ecosystem services by removing the escaped fish from the wild, if possible in collaboration with local fishermen. However, they should first inform the administration if an escape incident occurs (Arechavala-Lopez et al., 2018). In this case, knowing the period when an escape incident may have occurred through otolith microchemistry will provide "a posteriori" highly relevant information to take into account on mitigation plans. It is of special interest on meagre since in many areas of the Mediterranean this species is clearly identifiable as escapees, which facilitates the reconstruction of the life history and origin of the fish. However, the farm-origin identification could be easily addressed if all farmed fish are marked with tags that enable company-, farm- or even individual-level recognition (Dempster et al., 2018). In this sense, batch marking otoliths by immersion in chemicals such as oxytetracycline hydrochloride (OTC) and alizarine red (ALR) have been successfully tested on juvenile meagre, and leads to good mark retention, minimum mortality, and is less-time consuming in

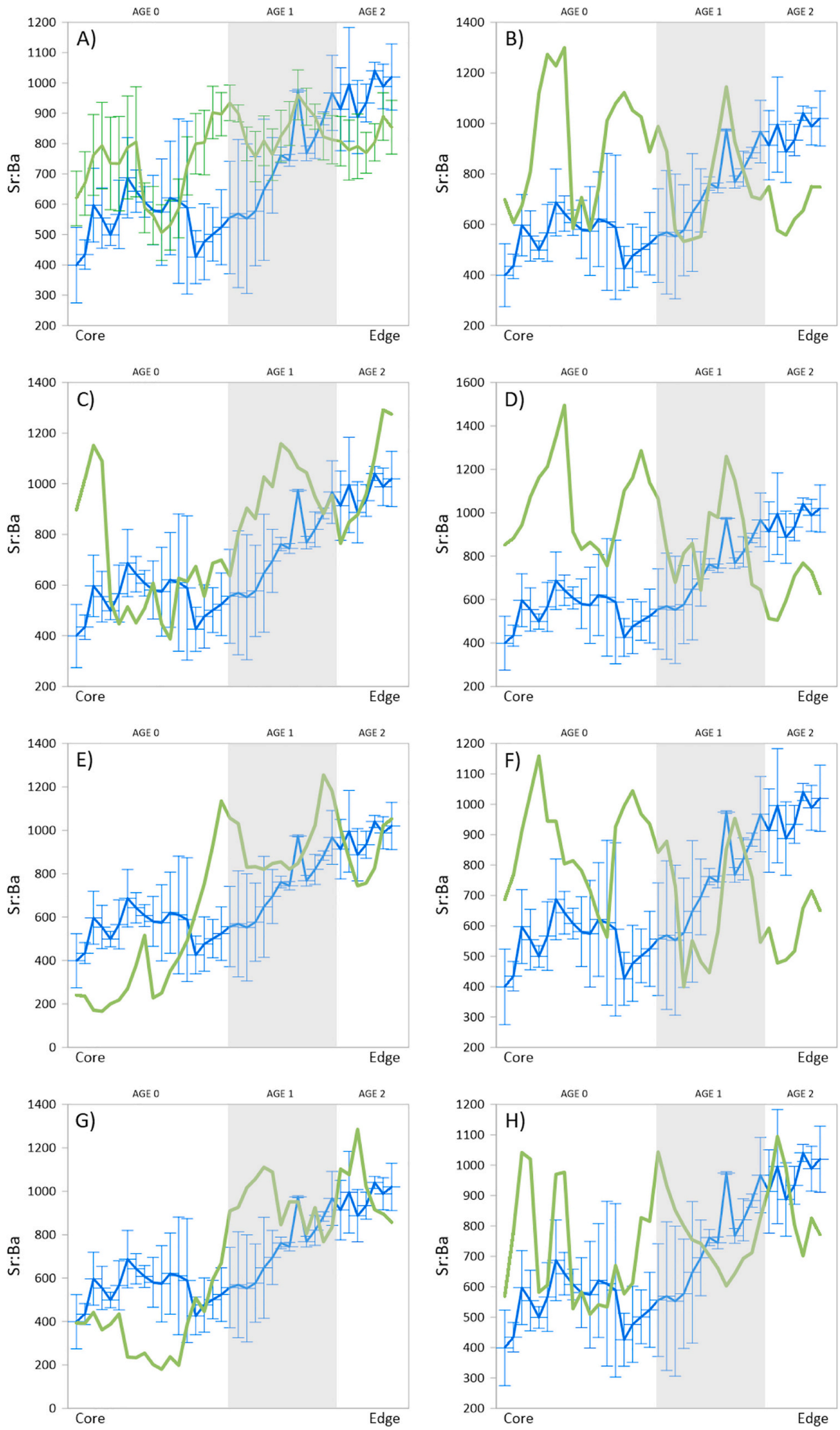

Fig. 3. Core-to-edge Sr:Ba signature of farmed meagre otoliths ($N = 2$; blue line) plotted versus: A) average value of all escaped meagre ($N = 7$; green line), and B—H) otolith Sr:Ba signature for each of the escaped individuals (N = 7; green line). Whiskers represents standard error. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

comparison with other marking methods (Morales-Nin et al., 2010; Gil et al., 2017). Although the use of antibiotics, such as OTC, are widely use aquaculture worldwide (Rigos and Smith, 2015; Leal et al., 2019), their potential effects on the individual and the environment are of serious concern, and further studies are still needed before being implemented as management measures by the industry.

In conclusion, this study has enhanced our understanding of how otolith microchemistry, and in particular the assessment of Sr:Ba ratios along otolith transects, might be a potential tool to trace the life-history of escaped fish, and consequently, to help solving ecological impacts, potential conflicts among coastal users and improving the existing management strategies regarding escapes. Nevertheless, the low number of sampled individuals in the present study prevented to draw clearer conclusions, and further studies are recommended.

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Declaration of Competing Interest

The authors have no competing interests or conflict of interest.

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