Discovery of *Holothuria leucospilota* juveniles on Pai Island, Biak-Papua, and an overview of sea cucumber nursery grounds in Indonesia

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Abstract

To date, little research has been conducted on the ecology of sea cucumber juveniles. This project investigated a nursery ground of *Holothuria leucospilota* on Pai Island, Biak-Papua. Sea cucumber abundance, sea grass cover and density, and juvenile abundance within algal clumps were measured to characterise the nursery. The abundance of juveniles, seagrass cover, density and height, and algal biomass all decline seaward. This suggests that vegetation is an important factor for nursery sites, as well as land contour, substrate type, and organic material. An overview of sea cucumber nursery grounds throughout Indonesian waters, based on a literature survey and direct interviews with divers, showed that only limited data are available, and mostly in central and eastern parts of Indonesia. Documentation of juvenile nursery sites of *H. leucospilota* in particular is just a small snapshot of Indonesian discovery, with broad opportunities and challenges for further research remaining.

Introduction

The number of sea cucumber species fished around the world is increasing. In Indonesia, 54 species are commercially fished (Setyastuti and Purwati 2015). The number of species fished is increasing compared with the previous data by Choo (2008), who noted 35 species of sea cucumber were fished for trade, and Purwati (2005) who stated that as many as 26 species were fished. However, the condition of sea cucumber stocks in Indonesian waters is still uncertain. The only information about natural stocks is from community communications in particular areas in Indonesia that reported either stable conditions or overexploitation and declining populations. These limited published data about natural stocks are in contrast with the vast sea cucumber habitat that Indonesia possesses.

Successful management cannot be achieved without key ecological and socioeconomic information (Conand et al. 2006). From an ecological viewpoint, comprehensive information on life history of the small juvenile stages is still inadequate (Wiedemayer 1994). However, a clearer understanding of habitat and ecological requirements of juvenile sea cucumbers will enhance the success of conservation management programmes (Shiell 2004; Purcell 2004; Wiedenmayer 1994). In Indonesia, studies on juvenile life history and ecology of sea cucumbers are limited. Most studies are about restocking techniques using seed from nature (Hana 2011; Tangko and Mustafa 2008; Marizal et al. 2013), and these techniques were highly dependent on the availability of a natural seed stock that is invariably irregular (Pangkey et al. 2012). To address this shortcoming, a programme on sea cucumber cultivation is maintained to produce hatchery-product juveniles in several research institutions in Indonesia, but follow-up studies of released seed products in the wild are lacking (UPT LPBIL Mataram 2015). Recently, at Candi Manik Village (Lombok Island), more than 500 individuals of hatchery-reared juveniles from BBIL-LIPI Mataram failed because of the decline in salinity (Setyastuti, pers. obs.). It was not ascertained whether the population failed because of heavy rainfall or freshwater intrusion from the river. This finding indicates that our understanding of the microhabitat area for nursery ground and successful restocking are still in their infancy.

Most of the existing literature on juvenile ecology or nursery habitat is from fortuitous field observations (Wiedemayer 1994; Shiell 2004; Taquet et al. 2011; Palazzo et al. 2016). Most sea cucumber juveniles are enigmatic until they are large enough to avoid predation (Wiedemayer 1994; Purcell 2004; Shiell 2004).

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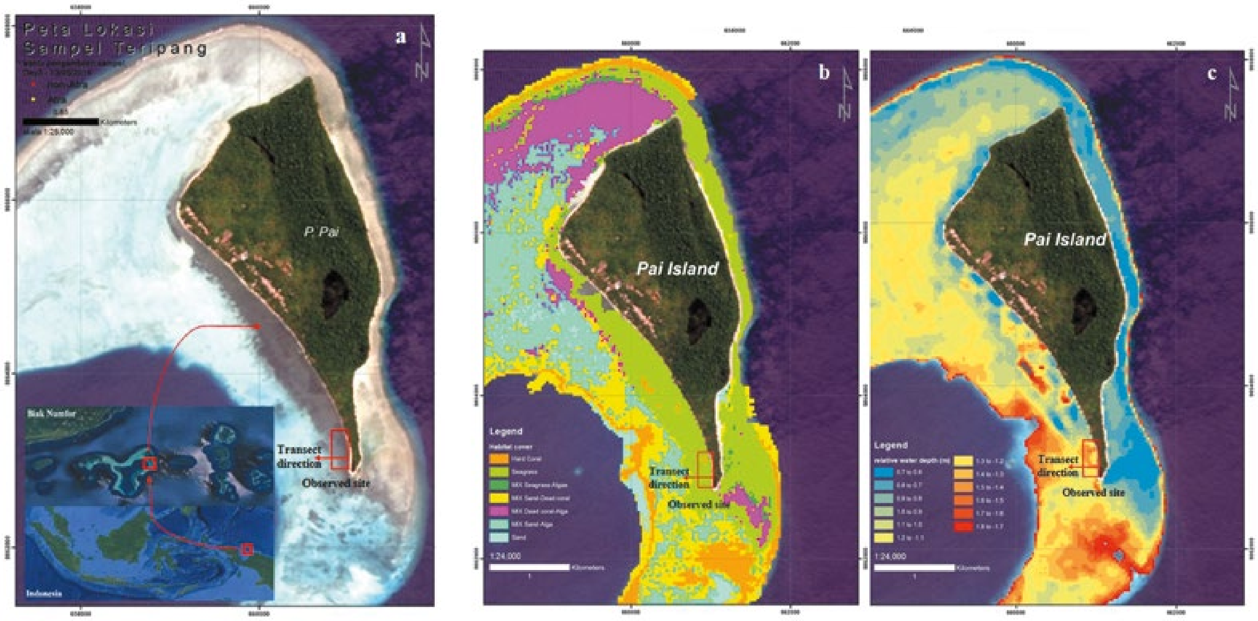

Figure 1. a: Survey location at Pai Island, Biak-Papua, Indonesia;
b: Habitat cover on observed site using satellite;
c: Relative depth of water based on satellite altimetry.

P2O-LIPI, a high number of *H. leucospilota* juveniles were fortuitously observed in Pai Island, Biak-Papua, Indonesia. These small individuals were only in a specific area on Pai Island. To take advantage of this observation, we set out to assess the density of juveniles in relation to the density of seagrass, algae and depth, to better understand these relationships. We also assembled available data on the occurrence of other nursery areas in Indonesian waters to provide context.

Methods

Pai Island is part of Padaido archipelago located in Biak Numfor Regency, Papua Province, Indonesia (Figure 1). Padaido archipelago has been established as a TWP (*Taman Wisata Perairan*/Marine Park for tourism) area by the decision of the Indonesia Ministry of Marine Affairs and Fisheries (No. 68/MEN/2009). Pai Island is surrounded by a vast (about 467.7 hectares) intertidal to shallow sub tidal characteristic reef and sand flat habitats (KKP 2014).

Surveys were conducted along the south side of Pai Island on 13 May 2016 at around 2–4 pm East Indonesia Time (WIT). Before deciding the specific location for sampling, rapid assessment of habitat using satellite imagery was conducted to determine the best location with high variability in vegetation cover and depth (Figures 1a and 1b).

Sampling methods were as follows:

1. Sampling was along a 1 x 100 m long transects. This single transect was purposefully conducted in order to get the precision density of juveniles *only*; not for an ecological approach.

2. Population density of sea cucumber juveniles, and cover and density of sea grass were measured in five 0.5 x 0.5 m quadrats along the transect that was orthogonal to the coast.

3. Algal clumps hold particularly high density of sea cucumbers. Five algal clumps were sampled in addition to the above by collecting entire clumps, counting all sea cucumber juveniles in the clump, then measuring the dried biomass of the algae.

4. Five sediment samples were taken at each sea grass quadrat transect and the total organic matter was determined by burning the sediment at 550°C in a furnace, as recommended by Fourqurean et al. (2012).

Statistical data were analysed using R Studio 1.0.136. The mean variances of juvenile abundance, seagrass density, seagrass coverage, and total organic matter were analysed using ANOVA and Tukey tests, respectively. The relationship among parameters was examined by Spearman rank analysis.

Results

Large numbers of juvenile black sea cucumbers were observed on the south side of Pai Island, Biak-Papua, Indonesia. Field identification of juvenile sea cucumbers as *Holothuria leucospilota* was based on morphological appearance (long black body, uniformly brown body colour when preserved in alcohol, smooth tegument), behaviour of expelling the cuvierian tubules when disturbed (hand contact), and confirmed by laboratory observations on ossicles in sampled specimens (Figure 2).

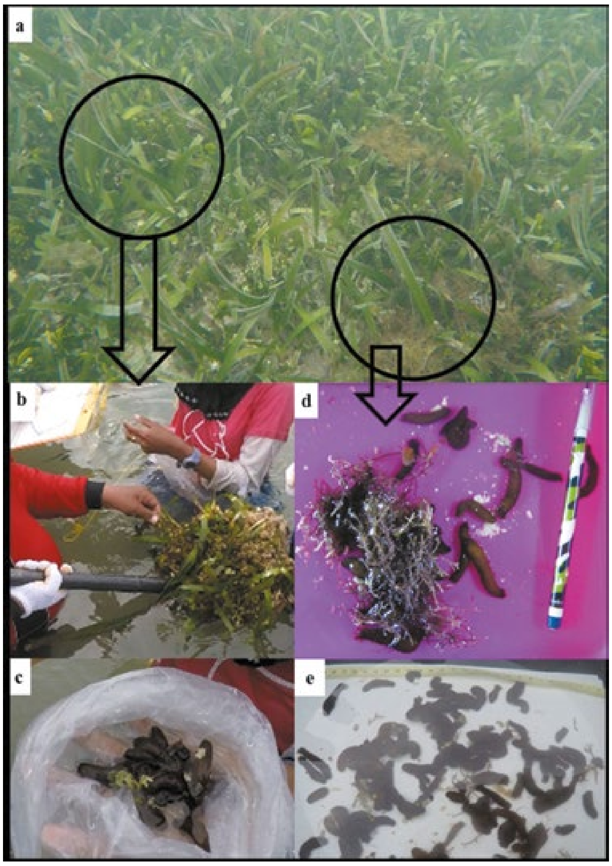

Figure 2. a: Vegetation at observed area; b-c: digging the bottom of the seagrass stand, where juveniles were collected, counted and measured; d-e: algae clump of *Hypnea* sp. and juveniles from the clump.

The juvenile microhabitats preference was observed on two areas, firstly on the sandy, mixed coral rubble or shell fragments bottom within the seagrass bed (Figure 2a). The seagrass stands on the spot were only *Thalasia hemprichii* and *Enhalus acoroides*. Secondly, the juveniles' habitat preference was adhered to clumps of the specific alga *Hypnea* sp. (Figure 2d). During the observation it was noted that species composition and cover of seagrass decreased seaward (Figure 3). Thus algal clumps were only collected along the first 40 m from the shoreline (Figure 4).

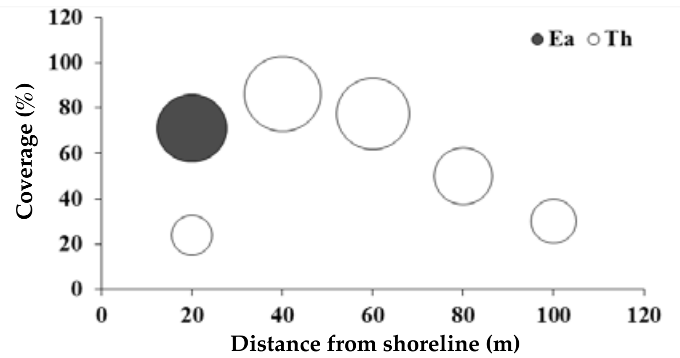

Figure 3. Seagrass cover along transect at Pai Island, Biak-Papua (Ea = *Enhalus acoroides*; Th = *Thallasia hemprichii*).

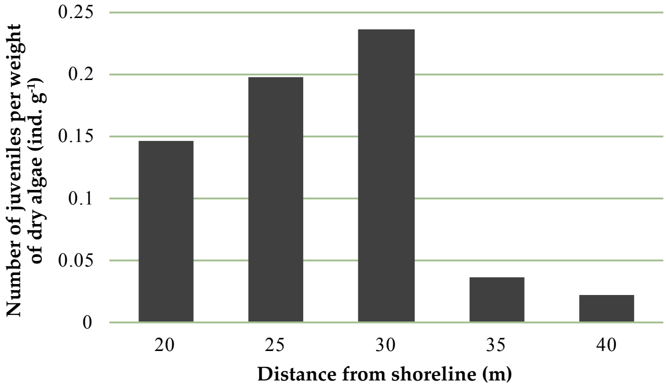

Figure 4. Abundance of juveniles per dry algal biomass.

Seagrass cover and density decreased constantly from landward to seaward (91.25 ± 5.00% to 30.00 ± 7.56%; Figure 5). The 20 m plot was dominated by *E. acoroides* with a leaf height of 0.5–1 m, which was the highest among all plots. The 40–100 m sites were dominated by *Thallasia hemprichii* with a leaf height that gradually dropping seaward. The abundance of *H. leucospilota* juveniles decreased constantly seaward, and they were reaching zero at 100 m (Figure 5).

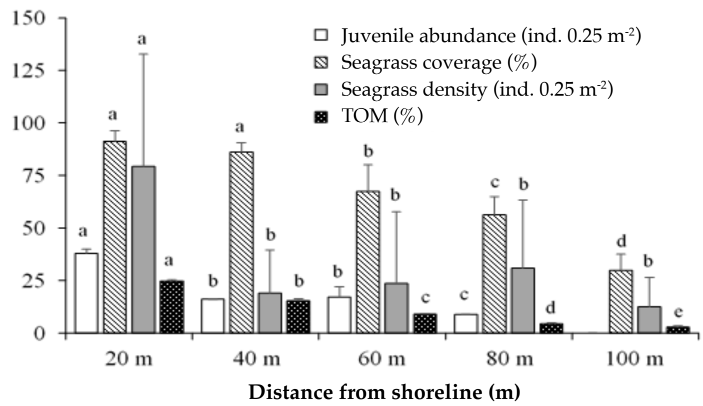

Figure 5. Juvenile abundance; seagrass cover and density; and total organic matters along the transect. (abcde : Tukey test result among five sites. Same letter on each parameter represented no significant difference among sites).

Seagrass cover and juvenile abundance were positively correlated (Spearman correlation coefficient of (0.831, P<0.01) (Table 1). Total organic matters (TOM) was also positively correlated with both seagrass cover and density (0.884, P<0.01) and *H. leucospilota* juvenile abundance (0.931, P<0.01) (Table 1). Dense algal clumps also had a high density of sea cucumbers (Figure 4), and in these the holothurians were up in the algae, while in seagrass they remained on the sediment surface.

In the review of literature and the internet, most records on juvenile sea cucumbers in Indonesia related to observations of only single specimens (Table 2). Only three other observations pertained to large abundance of juveniles, two of *H. leucospilota* and one of *H. edulis* (Table 2). All four observations of the abundance of *H. leucospilota* encountered animals in a similar microhabitat.

Table 1. Spearman correlation coefficient among juvenile abundance, seagrass profile and substrate total organic matter (TOM).

Parameters	Juvenile abundance	Seagrass		
		Density	Coverage	Structure
Juvenile abundance				
Seagrass density	0.385			
Seagrass coverage	0.831**	0.175		
Seagrass structure	0.240	0.539*	-0.047	
Total organic matter	0.931**	0.362	0.884**	0.173

* significance level at alpha = 0.05

** significance level at alpha = 0.01

Discussion

Recruitment of larvae is more likely to occur in certain habitats (Purcell 2004). Juveniles and adults frequently occupy different habitats in terms of substrate type, food availability and type, and depth (Slater and Jeffs 2010). Several microhabitat characteristics of the south side of Pai Island make it a nursery ground for *H. leucospilota* juveniles. Firstly, suitable sandy mixed coral rubble/shell fragment substrate was available there. Based on Slater et al. (2010) this kind of substrate may not only be suited for larval settlement but also for predator avoidance. Secondly, the contour of the site acts like a basin. At low tide the area is still underwater. When currents come from the eastern side, they are hindered by the south edge of the island. When currents come from the western side, they are constrained by the coral reef (see map on Figure 1). These conditions shelter the observed site from high currents. Thirdly, the observed site has dense vegetation stands of seagrass or algae clumps. In littoral areas, vegetation has an ecological role as a nutrient trapper as well as a shelter for juveniles, not only from predators but also from waves and currents (Fonseca and Fisher 1986; Mercier et al. 2000b; Komatsu et al. 2004; Duffy 2006; Dissanayake and Steffansson 2011). Finally, Table 1 and Figure 5 indicate that there is a close relationship between the number of juveniles and the levels of organic matter, as well as with the density of seagrass cover. The high aggregation of juveniles in this study area suggests a preference towards high levels of organic material, as expected for deposit feeders. The importance of organic material to habitat choice by sea cucumbers has also been observed by several researchers (Conand 1990; Conand and Mangion 2002; Purcell 2004; Purcell et al. 2009; Slater and Jeffs 2010; Dissanayake and Steffansson 2011).

The hypotheses above fit with the observations of high densities of *H. leucospilota* juveniles found on the south side of Pai Island. Meanwhile, no adults were observed at the site. The best habitats for larvae settlement and juvenile growth may not be the best habitats for adults (Purcell 2004). Ontogenetic habitat shifts in some echinoderms, including sea cucumber, have been reported (Bos et al. 2011; Eriksson et al. 2013). Migration to deeper water or more exposed sandy areas were reported in several publications (Conand 1981; Conand 1993; Hamel and Mercier 1996; Mercier et al. 2000a; Hamel et al. 2001; Purcell 2004; Bos et al. 2011; Eriksson et al. 2013). Our working hypothesis about adult origins is that currents bring planktonic larvae to the observation site and the larvae are then trapped and settle in the restricted nursery ground. Most juvenile sea cucumbers are believed to have enigmatic behaviour during the earliest stage of their life cycle (following settlement) that may be related to avoiding predation (Shiell 2004; Slater et al. 2010). We found no predators of juveniles of this size (big sea stars, molluscs, crustaceans or fish) on the observed site during the survey.

Juvenile abundance at bottom of seagrass or adhering on *Hypnea* sp. algae generally decreases seaward (Figures 3–5). This pattern may be due to the cover and density of seagrass and biomass of algae declining seaward, although there is probably a more complex relationship between algal biomass and sea cucumber density (Figure 4). Decreasing vegetation is also correlated with a decline in nutrient concentration in the substrate (Table 1 and Figure 5) and the absence of settlement surface and sheltering area. Thus, the concentration of both seagrass and algal vegetation is likely to have a considerable effect on distribution of juvenile sea cucumbers. Slater and Jeffs' (2010) experimental

Table 2. *In situ* observations of juvenile sea cucumbers in Indonesia waters.

Species observed	Approx. size and numbers	Location	Habitat	Time	Date	Adults present	Observers' name(s) and affiliation / Source of further information
H. leucospilota	3–5 cm, 151 specimens observed on the transect	South side of Pai Island, Biak-Papua, East Indonesia	Bottom of seagrass bed and adhere on the *Hypnea* seaweed colony, 1–2 m water depth	2–4 pm WIT*	May 13, 2016	No	This study
H. leucospilota	3–5 cm, specimen not counted (a lot)	Coastal water of Bindusi to Orwer Village, East Biak-Papua, East Indonesia	Bottom of seagrass bed (*T. hempricii, C. rotundata, H. pinnifolia* and *E. acoroides*), 1–2 m depth	Day time	2009	Yes	Ludi Parwadani Aji; Conservation Unit for Biak Marine Life - LIPI
H. leucospilota	1.5–3.5 cm, 35 specimens	Pantai Kalinaun, Eastern side of Manado Peninsula, Nort of Bitung, Sulawesi, Center Indonesia	Seagrass bed, 50 cm water depth	3–7 pm WITA**	May 15, 2010	No	Taquet et al., 2011
H. edulis	2–3 cm, specimen not counted (a lot)	Bilangan Gili, Sekotong Lombok Barat	Sand, coral	Day time	June, 2006	Yes	Taufan; gala-aksi.blogspot.co.id
B. argus	1 specimen	Selat Lembeh, Madidir, North Sulawesi	Corall	Day time	2010	-	Rakus Groeneveld and Sanne Reijs; www.diverosa.com
P. graeffei	1 specimen	Kubu, Batu Kembar, Bali	Corall	Day time	2011	-	Rakus Groeneveld and Sanne Reijs; www.diverosa.com
S. horrens	1 specimen	Seraya Slope, Bali	Corall	Day time	2016	-	Rakus Groeneveld and Sanne Reijs; www.diverosa.com
Actinopyga sp.	5 cm, 1 specimen	Hative Besar, ambon Bay, Maluku, East Indonesia	Adhered under the dead coral, 4 m water depth	3 pm WIT*	October 15, 2009	No	Ana Setyastuti; RC Oceanography LIPI. Data published in field report of SBL Dikti 2009-UPT BKBL LIPI-Ambon
Actinopyga sp.	5 cm, 1 specimen	Tanjung Tiram, Ambon Bay, Maluku, East Indonesia	Seagrass bed of *Enhalus acoroides*, 0.5 m water depth	3 pm WIT*	April 30, 2014	No	Ana Setyastuti, RC Oceanography LIPI.
S. horrens	10 cm, 1 specimen	Wainuru, Ambon, Maluku, East Indonesia	Adhered on the stone, 1 m water depth	Day time	March 19, 2014	Yes	Ana Setyastuti, RC Oceanography LIPI.

* WIT: East Indonesia local time

** WITA: Centre Indonesia local time

work specifically demonstrated the importance of shell fragments as initial settlement surfaces. They noted that shell fragments were one of the important factors of *Australostichopus mollis* juvenile survival. Another possible explanation of juvenile distribution in this study could be that there were sufficient seagrass beds and algae clumps landward, which provide suitable shelter even under the influence of tidal change, while the reef slope is constantly impacted by waves and current, making it an unsuitable habitat. Setyastuti (2014) also noted the importance of seagrass (*Enhalus acoroides*) for small individuals of *Holothuria atra* in Baluran National Park, Indonesia.

The abundance of the alga *Hypnea* sp. was conspicuous during the survey. *Hypnea* sp. clumps always had abundant *H. leucospilota* juveniles. The algae may facilitate the initial settlement of larvae, serve as a source of detritus and a place for predation avoidance (review of Slater and Jeffs 2010; Mercier et al. 2000b). Juvenile abundance fluctuates with algal biomass, rising steadily to a peak of 30 m from shore, then dropping sharply seaward (Figure 4). This suggests that algal biomass is not the primary driver of juvenile abundance. Instead, juvenile abundance may be tracking the general abundance of algal clumps, which decrease after 30 m and disappear after 40 m from shoreline.

To date, studies of sea cucumber nursery grounds in Indonesia are few. Based on the *in situ* observation list data presented in Table 2, similar observations to this study were made by Taquet et al. (2011) and Ludi Purwadani Aji (pers. obs.). They both observed high abundance of juveniles *H. leucospilota*, at Pantai Kalinaun, Manado Peninsula and coastal water of Bindusi to Orwer Village, East Biak-Papua, respectively. Juveniles were found on the bottom among seagrass beds with sandy mixed coral rubble substrate in both studies. These three studies suggest that *H. leucospilota* utilises nearshore, sand/coral rubble with seagrass habitats as nursery grounds.-

Findings herein support the studies of Shiell (2004), Purcell (2004), Slater and Jeffs (2010) and Palazzo et al. (2016) that among sea cucumbers, juveniles have a wide range of habitat preferences. At present, our understanding of the habitats occupied by juvenile sea cucumbers is based mostly on anecdotal observations of a few species in a limited area (Table 2). Given the diversity of species found in the commercial market of Indonesia and the few studies available on early life history and ecology of only a handful of species, more studies are needed to inform sustainable practices of this important fishery. Research challenges and opportunities for both taxonomically and regionally are vast.

Conclusions

Comprehensive studies on factors that support an area as a nursery ground for sea cucumbers are needed to inform recommendations for designation of marine protected areas (MPA). Conservation programmes will find these studies useful as they will not only indicate likely regions for natural recruitment, but also identify regions that are likely to be successful for restocking hatchery-product juveniles. Herein, we found that vegetation coverage, local geomorphology, substrate type and organic material levels may be important factors for making an area successful as a nursery ground for juvenile sea cucumbers. The denser the vegetation and the taller the seagrass stands, the more juveniles there were. The more complex the substrate, the more successful larvae settlement will be. In the future, studies on nursery grounds throughout Indonesian waters should be encouraged, in order to get a bigger picture of potential areas for both re-stocking, conservation, and management of waters that are used for fishing.

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