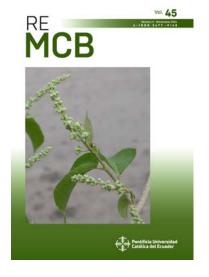
# Fauna close to sea cucumber Isostichopus fuscus (Echinodermata: Holothuroidea) in collectors on Santa Cruz Island, Galapagos

Fauna aledaña del pepino de mar Isostichopus fuscus (Echinodermata: Holothuroidea) en recolectores de la isla Santa Cruz, Galápagos

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Recibido: 29-02-2024 Aceptado: 12-11-2024 Publicado: 26-11-2024 **Abstract.-** *Isostichopus fuscus* is an invertebrate species distributed from Baja California to the Galapagos Islands. *I. fuscus* holds significant economic value in international markets; however, overfishing has led to its classification as an endangered species. Additionally, the surrounding fauna of I. fuscus remains unknown; such information would be valuable for establishing sea cucumber production sites, which rely on the biodiversity surrounding the larvae of this species. This study aimed to determine the accompanying fauna found in Isostichopus fuscus collectors and identify the most representative marine inhabitants to understand their ecological roles. Multiple collectors were installed to simulate the marine habitat where sea cucumbers develop. Associated fauna was collected from two different sites on Santa Cruz Island: La Fe and Las Palmas. The presence or absence of species was compared using diversity index. Las Palmas exhibited a low level of biodiversity (Shannon = 1.792) and low evenness (Simpson = 0.253). La Fe showed higher biodiversity (Shannon = 2.088) and greater evenness (Simpson = 0.459). Dominant species recorded included: *Polyonyx nitidus, Megabalanus vinaceus*, and polychaetes. Both sites have a rich influx of fauna, which is beneficial for the conservation of *I. fuscus*.

**Key words:** sea cucumber, dioecious, collector, diversity index, marine invertebrate, taxonomy

Resumen.- Isostichopus fuscus es un invertebrado distribuido desde Baja California hasta las Islas Galápagos. Los I. fuscus son económicamente importantes debido a su alto valor en mercados internacionales; sin embargo, la sobrepesca ha llevado a su categorización como especie en peligro de extinción. Además, se desconoce la fauna que se encuentra en los alrededores de *I. fuscus*; dicha información sería útil para generar sitios de producción de pepinos de mar, lo cual depende la biodiversidad rodea a las larvas esta especie. En el presente estudio se determinó la fauna acompañante que se encuentra en colectores en los alrededores de Isostichopus fuscus, se identificaron los habitantes marinos más representativos para conocer sus roles ecológicos. Se instalaron varios colectores que emulan el hábitat marino donde se desarrollan los pepinos de mar. Se recolectó la fauna asociada en dos sitios diferentes de la isla Santa Cruz: La Fe y Las Palmas. Se comparó la presencia y ausencia de las especies mediante índices de diversidad. Las Palmas obtuvo un bajo nivel de biodiversidad (Shannon = 1.792), al igual que una baja equidad (Simpson = 0.253). Mientras que en el sitio La Fe mostró una mayor biodiversidad (Shannon = 2.088), al igual que una mayor equidad (Simpson = 0.459). Entre las especies dominantes se registraron: Polyonyx nitidus, Megabalanus vinaceus y poliquetos. Ambos sitios tienen gran afluencia de fauna, lo cual es favorable para la conservación de I. fuscus.

**Palabras clave:** pepinos de mar, dioica, recolectores, índices de diversidad, invertebrados marinos, taxonomía.

## Introduction

Fauna living on the seafloor requires a suitable substrate to develop properly, as the life cycles of different species involve various larval stages that may last for weeks or months, depending on the species (García-Sainz 2010). This substrate is a crucial ecological component, as it attracts benthic organisms in need of nutrients. Artificial collectors have enabled researchers to gain insights into the colonization patterns of marine communities. Various models and sizes of these collectors provide optimal conditions for the settlement of marine species, making them valuable tools for both qualitative and quantitative ecological studies in coastal areas (Mendoza and Cabrera 1998). For several years, the





Galápagos National Park Directorate (GNPD) and several scientists have used artificial collectors to identify settlement areas of commercially important species, such as the spiny lobster and the sea cucumber. The information gathered has been instrumental in developing management strategies aimed at ensuring the long-term sustainability of these resources (Espinoza, Nicolaides, Vásquez, and Nagahama 2006; Espinoza, Masaquiza, Moreno 2015).

Sea cucumbers (holothurians) are long, soft-bodied animals that have a calcareous skeleton comprising microscopic spicules embedded in the body wall and is engared (Brusca and Brusca, 2002). In general, adult holothurians range between 19 and 25 cm in length. They are highly diverse invertebrates, with approximately 1400 described species, including *Isostichopus fuscus* (Fajardo-León, Michel-Guerrero and Singh-Cabanillass 1995; Toral et al. 2003; Vergara et al. 2015). They are dioecious and carry out both sexual and asexual reproduction in hermaphrodite individuals in their population (Herrero-Pérezrul, Reyes-Bonilla, García-Domínguez and Cintra-Buenrostro 1999). *I. fuscus* (Echinodermata: *Holothuria*) is distributed throughout the rocky coasts and coral reefs of the Eastern Tropical Pacific Ocean, ranging from Baja California to the Galapagos Islands of Ecuador (Solís-Marín, Arriaga-Ochoa, Laguarda-Figueras and Durán-González 2009; Purcell, Hair, Mills 2012; Herrero et al. 2005). These organisms are benthic and inhabit soft substrates or rocks (Fagetti 2014); they are found in almost all latitudes, from the intertidal zone to oceanic trenches (Kerr and Kim 2001). They feed on suspended organic matter dispersed throughout the seafloor as sediment mixtures (Quintanal-López et al. 2013; Ruiz et al. 2007).

In the Galápagos Marine Reserve, sea cucumbers are of high commercial interest due to their high demand in Asian markets, where they are valued for their purported aphrodisiac and curative properties (García 2015). When sea cucumber fishing began in the 1990s, no scientific studies had been conducted in Ecuador to support controlled fishing of these organisms, leading to the official closure of the fishery in Galápagos in August 1992. The following year, government authorities and research centers conducted biological and population studies of *I. fuscus* in the Bolívar Channel (0°19'60" S, 91°22'0" W) (De Paco, McFarland, Martínez and Richmond 1993). In 1999, the sea cucumber fishery was reopened for two months, and increased monitoring began in areas around the islands of Isabela, Fernandina, San Cristóbal, Española, and Floreana (Toral et al. 2003). In 2000, significant recruitment was detected, and minimum population density values had been reached (Granda and Marina, 2001). Subsequently, in 2002, a biweekly fishing calendar, a key tool for fishery management in the Galápagos, was developed based on previous research. This calendar has become essential for the Galápagos National Park Directorate (GNPD) and the Ministry of Environment in controlling the overexploitation of the Galápagos Islands' fauna. This biological criterion was formally established for the Islands (Granda and Marina 2001). In early 2005, the Galápagos National Park, the Japan International Cooperation Agency's marine research strengthening program, and the Charles Darwin Foundation launched the project "Monitoring of the Most Frequently Caught Species in the Galápagos Marine Reserve" to control the decline of the archipelago's fauna (Maffare-Cotera and Muñis-Vidarte 2015). As an additional measure for the management and conservation of sea cucumbers, the project "Ecology and Recruitment of Sea Cucumbers (Isostichopus fuscus) in the Larval Stage within the Galápagos Marine Reserve" was proposed in 2019. This project aims to collect sea cucumber larvae using artificial collectors to repopulate areas with deficient populations. It also seeks to further understand the larvae's ecology and physiology (Espinoza et al. 2019).

Studying the fauna in the sea cucumber larvae's immediate environment deepens knowledge of specific biodiversity. This information can be used to establish potential production niches, as well as aid in the analysis of climate change indicators, such as marine environment quality and area overfishing. Further, monitoring locations with a high sea cucumber population is key for reopening artisanal fishing; these data are available in annual reports from the GNPD (information on sea cucumber population is included in censuses done by the GNPD in 2018 and 2019: https://galapagos.gob.ec/monitoreo-poblacional-de-pepino-de-mar-y-langosta-espinosa/).

This study thus aimed to identify the fauna close to *I. fuscus* using artificial collectors on Santa Cruz Island and subsequently analyze the area's biodiversity. This information can help expand the understanding of the great diversity of existing marine species (mollusks, crustaceans, etc.), as each plays an important role on the ocean floor and especially in the highly variable environments of the Galapagos Islands. Many of these species could be used as bio-indicators of changes occurring in the Galapagos marine system (Maffare-Cotera and Muñis-Vidarte 2015). Although information on the biological characteristics, distribution, and environmental role of *I. fuscus* in Ecuador exists, the fauna in *I. fuscus*'



surrounding environment is not known with certainty. Therefore, it is necessary to identify the main organisms in the sea cucumber's vicinity to determine if the ecosystem is diverse and the populations abundant, which would indicate a healthy sea cucumber habitat. This is especially important considering it is one of the most biologically and commercially important species of the Galapagos Islands.

## Materials and methods

**Study area**.- The Galapagos Islands archipelago, renowned for its unique biodiversity, lies in the Pacific Ocean approximately 1000 km off the coast of mainland Ecuador. Within this remarkable ecosystem, our study focused on two distinct sites situated on Santa Cruz Island: Las Palmas and La Fe. Las Palmas, positioned northeast of the island. The study site is characterized by a depth of approximately 15 meters at both collection points, with a moderate temperature averaging around 19°C. Point A, located within Las Palmas, features a rugged, rocky substrate, providing an ideal habitat for a variety of marine species. In contrast, Point B at Las Palmas has a mixed substrate of rock and sand, supporting a different range of organisms (Table 1).

Similarly, La Fe, situated on the southwestern coast of Santa Cruz Island, presents its own distinct features. The depths at La Fe range from 15 to 20 meters, with an average temperature slightly higher at 19.5 °C. Site A at La Fe also has a rocky substrate, mirroring the geological characteristics found throughout many parts of the Galápagos Islands. In contrast, Site B at La Fe combines rocky and sandy substrate, creating a unique environment that fosters a diverse community of marine life (Table 1).

Table 1. Georeferenced and general characteristics of study sites

Sites	Collectors	Concrete dead depth	Latitude	Longitude	Temperature	Visibility	Sustrate
Las Palmas	COL-9M-2	15 m	00.68474°	090.54497°	18 °C	5 m	Rocky
	COL-3M-2	15 m	00.68415°	090.54572°	20 °C	10 m	Rocky sandy
La Fe	COL-6M-2	20 m	00.77017°	090.4175°	21 °C	10 m	Rocky
	COL-6M-1	15 m	00.68186°	090.54605°	18 °C	10 m	Rocky sandy

Field phase.- This phase constituted collector installation and population monitoring, which took place between the months of April to October With previous sea cucumber surveys (using the standardized circular transect method), a collector was installed in each of the four selected areas within the study. Using a 5.74 m rope was used to cordon off the area where sea cucumbers were found during previous monitoring by the PNGD. Artificial collectors are cylindrical structures, composed of plastic threads in the form of algae or vines. In addition, their interior is filled with substrate suitable for benthic fauna, which are stones found at the bottom of the sea. The collectors shelter a great diversity of marine fauna. (García-Sanz, Tuya, Angulo-Peckler and Haroun 2011). The procedure for placing the collectors was as follows: buoys were tied to the collector to keep it suspended so that it could be observed at the surface, and it was also tied to a 50 kg concrete weight on the seafloor to keep it still (Espinoza, Nicolaides, Vásquez and Nagahama 2006). To obtain a large sample of area fauna, the collectors remained on the seafloor for 40 to 45 days at depths of 6 and 9 m. The equipment was then removed from the seafloor and taken to the GNPD laboratory for sample processing. The recovered substrate was sieved and rinsed with potable water to remove excess sediment. The trapped specimens were placed in jars containing a 70 % alcohol solution for immediate preservation. Organisms were observed through a stereoscope for morphological identification and taxonomic classification from order to genus levels using keys and marine invertebrate key manuals (Hickman and Rojas Lizana 1998; Hickman and Finet 1999; Hickman 2008; Hickman et al. 2009).

**Data analysis.**- The data were organized in an Excel spreadsheet, and descriptive analysis was performed using PAST, a free, user-friendly data analysis software. The program allows for the calculation of various diversity indices, such as Shannon, Simpson, richness, and abundance, based on species abundance and distribution within the data (Moreno et al. 2011; Magurran 2004). Additionally, a rarefaction curve (Figure 1) was constructed to assess sampling effort and determine whether the sampling was sufficient to capture the majority of species in the study area. The rarefaction curve was generated by performing repeated random resampling of the data at progressively larger sample sizes, calculating the number of species observed at each sample size. This procedure was repeated several times to estimate the expected species richness as a function of sample size, which allowed for the comparison of diversity between different sampling efforts (Hurlbert 1971; Gotelli and Colwell 2001).



#### Results

The morphological characterization of the accompanying fauna of *I. fuscus* revealed many different species at the two sites. Of the two collectors at Las Palmas, the COL-9M-2-PALMAS collector (Table 1) recorded approximately 517 specimens from various orders and families of marine invertebrates. *Chaetopterus charlesdarwinii* was the most represented, with 380 specimens (73.50 % of the entire collector sample). Some species were represented by just one individual, such as *Telmatactis panamensis*, *Alpheus bellimanus*, *Platypodiella gemmata*, *Cancellaria obesa*, and *Lysmata spp*. In the COL-3M-2-PALMAS collector (Table 2), approximately 137 specimens were identified, including *Megabalanus vinaceus*, with 80 individuals (58.39 % of the entire collector sample). Other species, such as *Cancellaria obesa*, *Teleophry cristulipus*, *Polyonyx nitidus*, *Lophoxanthus lamellipes*, and *Ophiactis savignyi*, consisted of a single individual (0.73 %). In summary, 654 individuals from 32 species were identified at this site.

The collector COL-6M-1-LA FE at the La Fe site (Table 2) collected 364 specimens. *Polyonyx nitidus* was the most represented species, with 159 individuals (43.68 %). *Cronius ruber* was represented by only one individual (0.27 %). The second collector, COL-6M-2-LA FE (Table 2), collected 413 specimens; *Polyonyx nitidus* was the most represented, with 229 individuals (55.45 %). The least represented was *Platypodiella spp* (0.24 %), *Leucozonia tuberculata* (0.24%), and *Lythrypnus rhizophora*, with one individual each (0.24%). The amount of *Polyonyx nitidus* specimens was similar in both collectors. In total, 777 specimens and 14 species were identified at the La Fe site.

Table 2. Accompanying fauna in the sea cucumber (I. fuscus) habitat from four collectors in Santa Cruz, Galapagos Islands

Phylum	Order	Family	Species	Collectors			
				COL-9M-2- Palmas	COL-3M-2- Palmas	COL-6M-1-La Fe	COL-6M-2- La Fe
Arthopoda	Decapoda	Alpheidae	Alpheus bellimanus	1		16	
	Decapoda	Xanthidae	Xanthodius cooksoni	2			
	Decapoda	Xanthidae	Clycloxanthops vittatus	3			
	Decapoda	Xanthidae	Platypodiella gemmata	1		30	21
	Decapoda	Xanthidae	<i>Platypodiella</i> spp				1
	Decapoda	Xanthidae	Microcassiope xantusii			13	
	Decapoda	Palaemonidae	Brachycarpus biunguiculatus	2			
	Decapoda	Lysmatidae	Lysmata spp	1			
	Decapoda	Majidae	Teleophry cristulipus	10	1	17	
	Decapoda	Porcellanidae	Petrolisthes glasselli	2			
	Decapoda	Porcellanidae	Polyonyx nitidus	21	1	159	229
	Decapoda	Panopeidae	Lophoxanthus lamellipes		1		
	Decapoda	Inachidae	Stenorhynchus debilis			6	
	Decapoda	Portunidae	Cronius ruber			1	
	Stomatopoda	Gonodactylidae	Neogonodactylus pumilus				3
	Sessilia	Balanidae	Megabalanus vinaceus(spp)	47	80		
	Amphinomida	Amphinomidae	Eurythoe complanata	3	6		
Annelida	Amphinomida	Amphinomidae			3		
	Phyllodocida	Nereididae	<i>Nereis</i> spp	4	4	10	
	Phyllodocida	Phyllodocidae		10	2	75	123
	Sipunculiforme	S		6	15	37	34
	Spionida	Chaetopteridae	Chaetopterus charlesdarwinii	380			
Chordata	Perciformes	Gobiidae	Lythrypnus rhizophora				1
Cnidaria	Actiniaria	Isophellidae	Telmatactis panamensis	1			
Echinodermata	Ophiurida	Ophioctidae	Ophiactis savignyi	14	1		
	Nudibranchia	Discodorididae	Tayuva licina	2			
Mollusca	Cidaroida	Cidaridae	Eucidaris galapagensis		3		
	Negastropoda	Marginellidae	Volvarina nyssa	6			
	Negastropoda	Cancellaridae	Cancellaria obsesa	1	1		
	Neogastropoda		Leucozonia tuberculata		_		1
	Littorinimorpha		Trivia pacifica		3		
	Gastropoda	Terebridae	Terebra jacquelinae		2		
	Gastropoda	Terebridae Ovulidae	Terebra spp		5 7		
	Littorinimorpha Arcoida	Arcidae	Pseudocypraea adamsonii Barbatia rostae		2		
Total	AI CUIUd	AI LIUde	Dai Valla i USlae	517	<u>∠</u> 137	364	413
IUldl				51/	137	304	413



The Shannon index calculated from Las Palmas site data was 1.792. The Shannon index varies from 0.5 to 5.0; values greater than three indicate high biodiversity, while those less than 2 indicate low biodiversity (Soler et al. 2012). Thus, the Las Palmas site had low biodiversity. Simpson's index for the same site is 0.253. This index represents the probability that two randomly selected individuals belong to the same species. Values range from 0 to 1, with 0 indicating an even population distribution of each species (i.e., more diversity) and 1 no diversity. The study's result is close to 0, which means some species were more dominant relative to the others. For the La Fe site, the Shannon index was 2.088, which indicates higher diversity than for Las Palmas. Simpson's index, 0.459, indicates average dominance.

On the other hand, the results of the rarefaction curve were constructed by sampling two sites on Santa Cruz Island: La Fe and Las Palmas. Where it is shown that at the beginning the curves overlap, that is to say that neither of the two is more diverse than the other. Subsequently, a significant difference is observed (Figure 1).

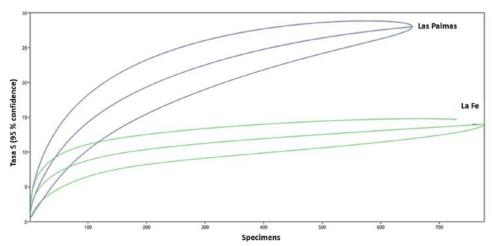


Figure 1. Rarefaction curve in Las Palmas and La Fe

## Discussion

During the study period, collectors at Las Palmas and La Fe on Santa Cruz Island collected 1 431 individuals, of which 54 % corresponded to La Fe and 46% to Las Palmas. For both sites, the phylum Arthropoda was predominant (40%), followed by Annelida (55%) and other phyla (5 %). In the research of Masaquiza (2018), the presence of Arthropoda (32.3%) and Echinodermata (1.6 %) was reported in the Galápagos Islands, similar to the results found at Las Palmas and La Fe, where Arthropoda was also prevalent. The high presence of Arthropoda in both studies may be attributed to the adaptability of this group to the marine substrates of Galápagos, particularly in benthic habitats where they are commonly found. Moreover, in Santa Elena province (a coastal region of Ecuador with seabed characteristics similar to those of Galápagos), Sáa (2015) reported similar findings, with Arthropoda (32.3 %) and Echinodermata (2 %) also being dominant. This further suggests that these taxa are widespread in benthic environments, particularly in habitats that are home to sea cucumbers (holothurians) and other marine benthic fauna. These results are consistent across studies, likely because these groups thrive in marine substrates with suitable feeding modes and ecological roles. It is important to note that Masaguiza (2018) specifically focused on the fauna surrounding sea cucumbers, which likely explains the presence of Echinodermata, including holothurians, in the study. The sampling methods used in these studies, including benthic sampling and the collection of marine fauna associated with sea cucumbers, further support these findings

The current study showed that the most frequently collected species, such as *Polyonyx nitidus* (order Decapoda), were found from the lower intertidal zone to depths of 9 meters, which corresponds to the sampling locations of the collectors. Hiller and Werding (2004) reported that *P. nitidus* can be found at depths of up to 46 meters and prefers sandy substrates, similar to the habitat of sea cucumbers. In the current study, *Megabalanus vinaceus* (order Sessilia) was found attached to rocks within the study sites. The distribution of this species ranges from the Gulf of Fonseca in Costa Rica (13° N) to the Gulf of Guayaquil in Ecuador (3° S) (Gómez 2003). A significant presence of diverse polychaetes



(orders Amphinomida, Phyllocida, and Sipunculiformes) was also found. Méndez (2012) mentions that these groups include herbivores, omnivores, and scavengers, which is why they are abundant in areas with rich marine fauna on the seafloor. The aforementioned taxa were found in high abundance at both sites, suggesting the diversity of the seabed, which consists of both rocky and sandy habitats, and differing from other substrate types (Rivera 2004). These areas are favorable for sea cucumbers, which primarily feed on organic particles suspended in the water column, such as detritus, plankton, and microorganisms. The availability of these particles is linked to the dynamic nature of the sediment in these environments, where rocky and sandy substrates contribute to a high degree of water flow and nutrient cycling, creating favorable conditions for the filtration-feeding behavior of sea cucumbers (Zamorano 2005; Conand 2004). Therefore, the combination of these substrate types with high availability of suspended particles likely explains the abundance of sea cucumbers and their association with these areas.

The Shannon indices in Las Palmas (1.792) and La Fe (2.088) were different and lower than those found by Masaquiza (2018), whose study was conducted on the same island in Galapagos, taking into account that the present study was in areas more remote from the population and in less time. On the other hand, Simpson's index in Las Palmas (0.253) showed that some species (at least three taxa) were dominant, this is due to the easy development of these individuals and adaptability to the seabed. The opposite occurred in La Fe (0.459), where there was medium dominance. These data show that La Fe has greater biodiversity than Las Palmas; these results differ from those found by Masaquiza (2018), since this study had a longer duration and the results were greater in abundance and diversity, because they had more material and availability of boats and diving equipment within reach. This information is corroborated by the construction of the rarefaction curve, which shows that there is a difference between La Fe and Las Palmas, where La Fe predominates in biodiversity.

Rivera (2004) found that being distant from populated areas and human activity promotes the better development of marine species. This is particularly relevant in the case of the current study, where both collection sites, Las Palmas and La Fe, were located far from ports and coastal settlements, providing a relatively undisturbed environment conducive to the development of marine fauna, particularly sea cucumbers. The distance from human activity likely contributes to reduced disturbance and allows for more stable ecological conditions, which can positively influence the population dynamics of marine species.

In Las Palmas, collecting specimens from the artificial collectors proved challenging due to the depth and the influence of high tide, with some specimens being lost when the collectors were removed. Despite these challenges, the artificial collectors used in this study were designed to replicate the natural substrate, ensuring that they were attractive to the fauna associated with sea cucumbers. By using a substrate similar to that found in the natural environment, the study was able to attract and retain a diverse set of species, facilitating the accurate assessment of the benthic community dynamics in these areas. As Espinoza et al. (2015) suggest, the use of artificial collectors with appropriate substrate is a reliable method for studying the species that interact with sea cucumbers, particularly benthic organisms. This approach also supports the hypothesis that undisturbed sites, such as those studied in this research, provide more favorable conditions for the development of diverse and stable marine populations.

Overall, the combination of undisturbed locations, the use of appropriate artificial collectors, and the favorable ecological conditions likely contributed to the higher biodiversity observed in the study, which is consistent with the findings of Rivera (2004), who noted that remote areas tend to foster the development of more resilient and diverse marine communities.

Finally, the results from both La Fe and Las Palmas show that taxa such as *Chaetopterus charlesdarwinii*, *Megabalanus vinaceus*, and polychaetes, all of which are considered biological indicators, were found in large quantities. The presence or absence of these taxa helps clarify the seafloor conditions with respect to sites suitable for the development and growth of species in the Galapagos Islands. In the case of *Isostichopus fuscus*, the presence of these species in areas far from coastal zones is beneficial for its development and conservation, as undisturbed habitats are crucial for the proper growth of sea cucumbers. However, a larger sample size, using additional collectors, is recommended to obtain more precise data that will further facilitate the conservation of sea cucumbers.

Given the longer larval stage and slower growth of *I. fuscus*, it is clear that improving the fishing protocols for this and other commercially valuable species is essential. Studies such as those by Conand (2004) and Lovatelli et al. (2004) emphasize the need for more



sustainable fishing practices, especially in sensitive areas like the Galapagos Islands. The existing artisanal fishing calendar for the Galapagos needs to be refined to ensure that it aligns with the biological cycles of *I. fuscus*, including its reproduction and growth phases. A more flexible fishing protocol, with specific closed seasons during the peak reproductive periods, could contribute to the long-term sustainability of the species and its populations, ensuring both ecological balance and economic benefits for local communities.

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#### Authors contributions

RF: conception and design of the study, data acquisition; RF, HN and JY: data analysis and interpretation, drafting of the initial version of the manuscript and revision of the manuscript.

#### Conflict of interest

The authors declare that they have no conflicts of interest.

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