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# The Hulong hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂) has invaded the coastal waters of Hainan Island, China

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Hybrid groupers, including the Hulong hybrid grouper (*Epinephelus fuscoguttatus*♀ × *Epinephelus lanceolatus*♂), have become increasingly popular in mariculture across the southwest Pacific, raising concerns about their potential to cause biological invasions. First developed in Malaysia in 2007 and introduced to mainland China in 2009, the Hulong hybrid grouper has been sighted in the coastal waters of Hainan Island, China, for over a decade. This study examines the species' distribution, survival, and ecological impacts in these waters. From 2019 to 2023, social surveys, specimen collections, and field observations confirmed the widespread presence of the Hulong hybrid grouper, particularly in inshore reefs and artificial structures. Age determination and gonadal analysis demonstrated their long-term survival and reproductive maturity in the wild. The introduction of this species resulted from both unintentional escapes from aquaculture facilities and intentional releases. This research represents the first documented case of an artificial hybrid marine fish invading and establishing a population in the wild, with potential negative ecological impacts such as interspecies competition, disease transmission, and genetic pollution.

## KEYWORDS

biological invasion, artificial hybrid fish, Hulong hybrid grouper, introduced population, Hainan Island

## 1 Introduction

The acceleration of biological invasion events in aquatic ecosystems and the associated negative ecological and economic impacts around the world have garnered significant attention for decades (Diagne et al., 2021; Bellard et al., 2017; Liu et al., 2020). Among these cases, fish have become an important group, contributing a large portion of biological

invasion events in aquatic systems in recent years. Fewer marine fish introductions have been reported compared to freshwater systems. However, marine fish invasions are considered highly consequential and capable of altering biodiversity or community structure (Bellard et al., 2017). The two mechanisms for marine fish invasions are natural population extensions and anthropogenic dispersal. For instance, the expansion of the mariculture industry around the world has considerably increased the distribution of alien fish. Invasions caused by alien fish species used in mariculture have caused concern due to a lack of knowledge about their impacts on local ecology (Chen et al., 2017). One example is the red drum, *Sciaenops ocellatus*, which invaded the northeast Pacific area circa 1998 and has become the most widely spread alien marine fish species in China (Liao et al., 2010; Lin et al., 2020). Because the effects of marine fish invasions are difficult to measure, greater awareness of early prevention is needed.

Not only alien fish species but also distant hybridization fish are increasingly causing potential biological invasion events in aquatic ecosystems (Wringe et al., 2018; Rimmer and Glamuzina, 2019). Distant hybridization technology involves cross-breeding between two different species. This process generates offspring with outstanding traits for aquaculture, such as disease resistance, good growth performance, and environmental tolerance. Dozens of new hybrid fish have been created using this technology for aquaculture purposes in the past decade, mainly within the Family Cyprinidae (Liu et al., 2016). Despite the relatively rare use of distant hybridization technology in marine fish, more than 20 hybrid groupers (Perciformes, Epinephelidae) have been produced for mariculture (Glamuzina and Rimmer, 2022). Until now, the consequences of the dispersal of hybrid fish in nature were still largely unknown.

At the beginning of the hybrid grouper industry, the Hulong hybrid grouper (also known as Sabah giant grouper, Figure 1C) became a key species, with maximum production exceeding 100,000 tons per year in China. This hybrid grouper was produced by

fertilizing the eggs of the tiger grouper (*Epinephelus fuscoguttatus*, Figure 1B) with the sperm of the giant grouper (*Epinephelus lanceolatus*, Figure 1A) through *in-vitro* fertilization (IVF) technology. The Hulong hybrid grouper was first produced in Malaysia in 2007 (Ch'ng and Senoo, 2008). Subsequently, in 2009, IVF technology was further advanced in China, leading to the production of Hulong hybrid grouper juvenile fish in Wen Chang, a major city for aquatic organism breeding on northeastern Hainan Island. With the development of the breeding and mariculture industry of Hulong hybrid grouper on Hainan Island, escapees have been sighted in the coastal waters of Hainan Island (Figure 1D) by anglers, divers, and fishermen in the past few years at multiple locations. This has raised concerns about a potential biological invasion event by this hybrid fish.

This study documents the Hulong hybrid grouper as the first hybrid fish to invade the tropical coastal waters of Hainan Island. The distribution pattern, mechanisms of introduction, and possible ecological consequences of the invading Hulong hybrid grouper were further discussed.

## 2 Materials and methods

### 2.1 Social survey

Sightings of the Hulong hybrid grouper have been reported frequently in the last 5 years across multiple locations on Hainan Island, particularly in regions far from mariculture areas. From March 2019 to September 2023, a series of social surveys and wild observations were conducted around Hainan Island to investigate the current and historical distribution patterns in the wild, development status in the mariculture industry, biological characteristics, resource dynamics, and possible dispersal pathways of the Hulong hybrid grouper. Initial requests for

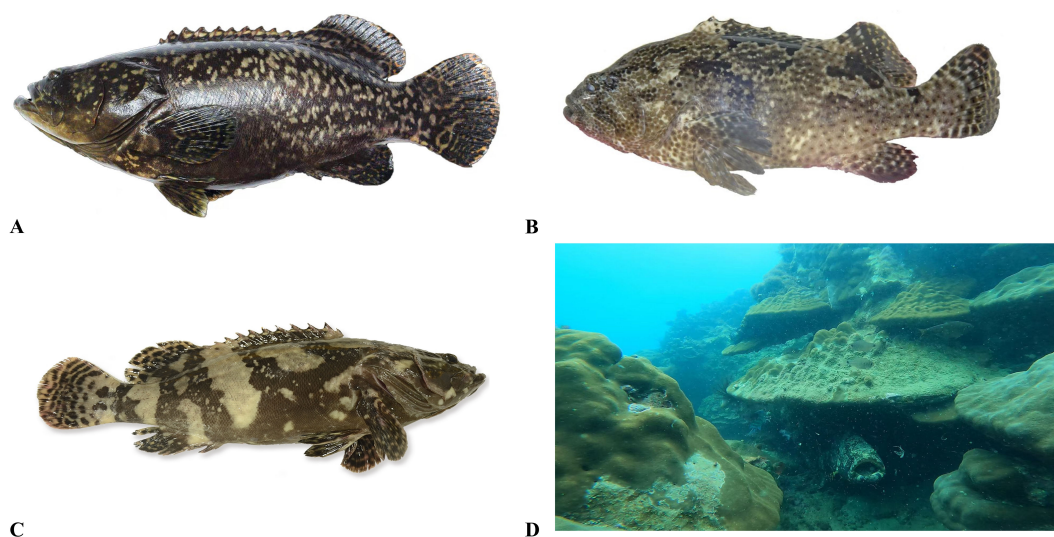


FIGURE 1

Images of (A) *Epinephelus lanceolatus*, male parent species of the Hulong hybrid grouper, (B) *Epinephelus fuscoguttatus*, female parent species of the Hulong hybrid grouper, (C) Hulong hybrid grouper, and (D) Hulong hybrid grouper under local reefs in San Ya, Hainan Island.

observation data of this hybrid grouper were made via social media (Weibo, WeChat groups, and other public websites), local angler/fisherman networks, and online questionnaires to those who witnessed a wild Hulong hybrid grouper (Supplementary File S1), including details such as observation sites, time points, and specimen profiles. Further face-to-face investigations were conducted in the 12 cities around Hainan Island, targeting local fishery enforcement employees, fishing port superintendency agency employees, and fishermen, to accurately profile the historical and current distribution patterns, population dynamics, and potential risks of this hybrid grouper (Figure 2A; Supplementary File S2). Finally, we interviewed major hybrid grouper mariculture companies to summarize the development status of this industry and the biological characteristics and possible dispersal pathways of the Hulong hybrid grouper (Supplementary File S3). For each location, the estimated yields of the Hulong hybrid grouper were calculated by averaging all interviewee feedback about their own predicted number of local Hulong hybrid grouper yields.

## 2.2 Field observation and specimen collection

Scuba diving and angling were used to observe the introduced Hulong hybrid grouper in the wild, including the coastal regions of Wen Chang, Qiong Hai, and San Ya, confirming the data collected in the social surveys. The population status and diet composition of this hybrid grouper were also recorded by filming video and taking pictures onsite using a DJI OSMO Action 3 (DJI, Shenzhen, China).

In total, 28 specimens were collected with different body weights at the local fishing ports of Wen Chang, Qiong Hai, and San Ya, and then photographed while still fresh. Total length, standard length, and total weight for each individual were measured. The specimens were then stored at  $-20^{\circ}\text{C}$  and transferred to the laboratory for further analysis.

## 2.3 Age determination and gonadal histology

Sagittal otoliths extracted from Hulong hybrid grouper individuals were rinsed in water and air-dried before processing. Up to four transverse sections, approximately 0.3 mm thick, were cut to ensure the primordium of each otolith was included in one of the sections. These sections were then cleaned and mounted in polyester resin on microscope slides. Age was estimated by counting the narrower opaque zones closest to the primordium.

Gonads were cut into pieces approximately 2 cm in size after being removed from Hulong hybrid grouper individuals, and fixed in Dietrich's fixative (900 ml distilled water, 450 ml 95% ethanol, 150 ml 40% formaldehyde, and 30 ml acetic acid) for at least 1 week at room temperature. They were then dehydrated, embedded, and sectioned at a thickness of 5–6  $\mu\text{m}$ . The sections were stained with hematoxylin and eosin, mounted on slides, and examined under a light microscope, with micrographs taken for analysis.

## 2.4 Environmental data collection of potential Hulong hybrid grouper habitats

Based on the social survey and field observation data, all the locations of Hulong hybrid grouper wild sightings were marked in ArcGIS. Water temperature, dissolved oxygen, and  $\text{NH}_4^+\text{-N}$  concentration of these potential Hulong hybrid grouper habitats were collected from local environmental monitoring stations. Parameters such as water temperature, dissolved oxygen, and  $\text{NH}_4^+\text{-N}$  concentration were tested using a YSI ProQuatro (Xylem, OH, USA).

## 2.5 Biological characteristics of parental species of Hulong hybrid grouper

To compare the changes in ecological niches between Hulong hybrid grouper and its parental species, the biological traits of the

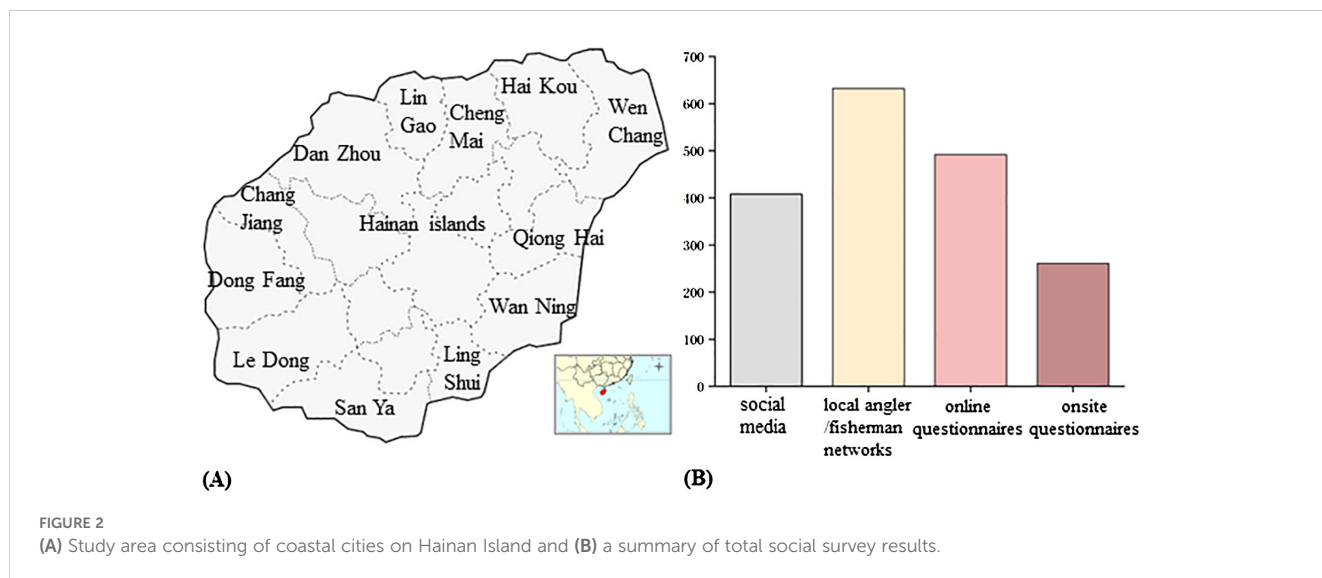


FIGURE 2  
(A) Study area consisting of coastal cities on Hainan Island and (B) a summary of total social survey results.

parental species of Hulong hybrid grouper (*E. fuscoguttatus*♀×*E. lanceolatus*♂) such as environmental tolerance, gonad development, and diet composition were collected from FishBase and other published papers (Froese and Pauly, 2024).

## 3 Results

### 3.1 Social survey profiles

A total of 1,532 wild sighting records of Hulong hybrid grouper were collected, including 408 records from pictures and vlogs from social media, 632 records from local angler/fisherman networks, and 492 records from online questionnaires (Figure 2B). These records covered the period from 2014 to 2023. More specifically, 920 (60.1%) records were from Hainan Island, 564 (36.8%) records were from other provinces in southeast China, and 48 (3.1%) records were from southeast Asian nations. The body weight of wild sightings of the Hulong hybrid grouper ranged from approximately 20 g to 35,000 g, with the majority falling between 2,500 g to 5,000 g (44.8%).

Additionally, 261 valid questionnaires from face-to-face interviews were analyzed. All interviewees around Hainan Island confirmed the existence of the Hulong hybrid grouper in their local coastal waters. Furthermore, we calculated the total witness rate (total witness number/261\*100%) and regional witness rate (regional witness number/regional interviewees number\*100%) for each year to understand the origin and spread pattern of this hybrid grouper around Hainan Island. This hybrid grouper was first recorded in Wen Chang and Qiong Hai in the wild, located in the northeastern region of Hainan Island, in 2012. Its range extended southward to San Ya and northward to Hai Kou and finally encompassed the rest of the coastal areas of Hainan Island circa 2015 (Figure 3). The maximum and minimum body weights of captured Hulong hybrid groupers were 39,800 g (in Wen Chang, northeast city of Hainan Island) and approximately 10 g (in Dong Fang and Chang Jiang, southwest cities of Hainan Island), respectively. The weights of captured Hulong hybrid groupers

were also mainly distributed between 2,500 g to 5,000 g. The estimated yields among these cities ranged from 10 to 150 tons per year in the inshore area, with the highest expected yield on the east coast of Hainan Island.

The results from a detailed investigation of the major mariculture companies producing Hulong hybrid groupers on Hainan Island indicated that the Hulong hybrid grouper mariculture industry began in Wenchang in 2009. Subsequently, rapid expansion to the south (2010 to 2012) and west (2013 to 2014) coasts of Hainan Island was observed (Figure 4). The information obtained from the mariculture companies revealed that Hulong hybrid groupers are typically reared and sold at body weights ranging from 500 g to 1000 g and ages of 1 to 1.5 years. Domestic Hulong hybrid groupers typically reach maturity as females at the age of 4 to 5 years (with threshold weights approximately 5,000 to 6,000 g) and as males at the age of 6 to 7 years (with threshold weights approximately 20,000 g) (Figure 5B).

### 3.2 Biological traits of the Hulong hybrid grouper in the wild

The Hulong hybrid grouper has been found in water temperatures ranging from 15°C to 34°C and salinity from 5 to 36 in the wild. They prefer living in reef areas at depths between 2 to 100 m and in inshore artificial structures and adjacent areas at depths of 1.5 to 5 m. More importantly, this fish can thrive in eutrophic environments with minimum dissolved oxygen ( $DO_{min}$ ) levels of about 1.5 mg/L and a maximum  $NH_4^+-N$  concentration of approximately 4 mg/L, conditions in which its parental species cannot survive.

The feeding habits of the Hulong hybrid grouper were observed through scuba diving and angling. A total of 6 classes and 12 orders were identified as prey of the Hulong hybrid grouper (Table 1). Animals from the Actinopterygii, Bivalvia, and Malacostraca classes accounted for more than 70% of sightings. These prey species were primarily benthic invertebrates and reef fish.

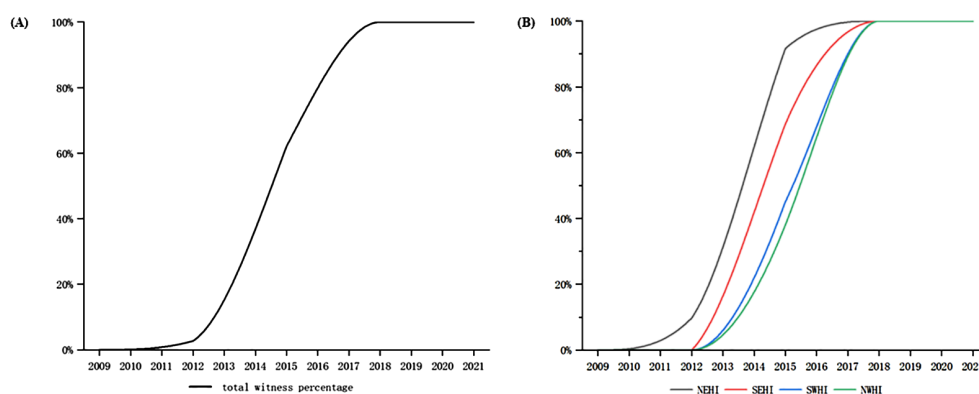
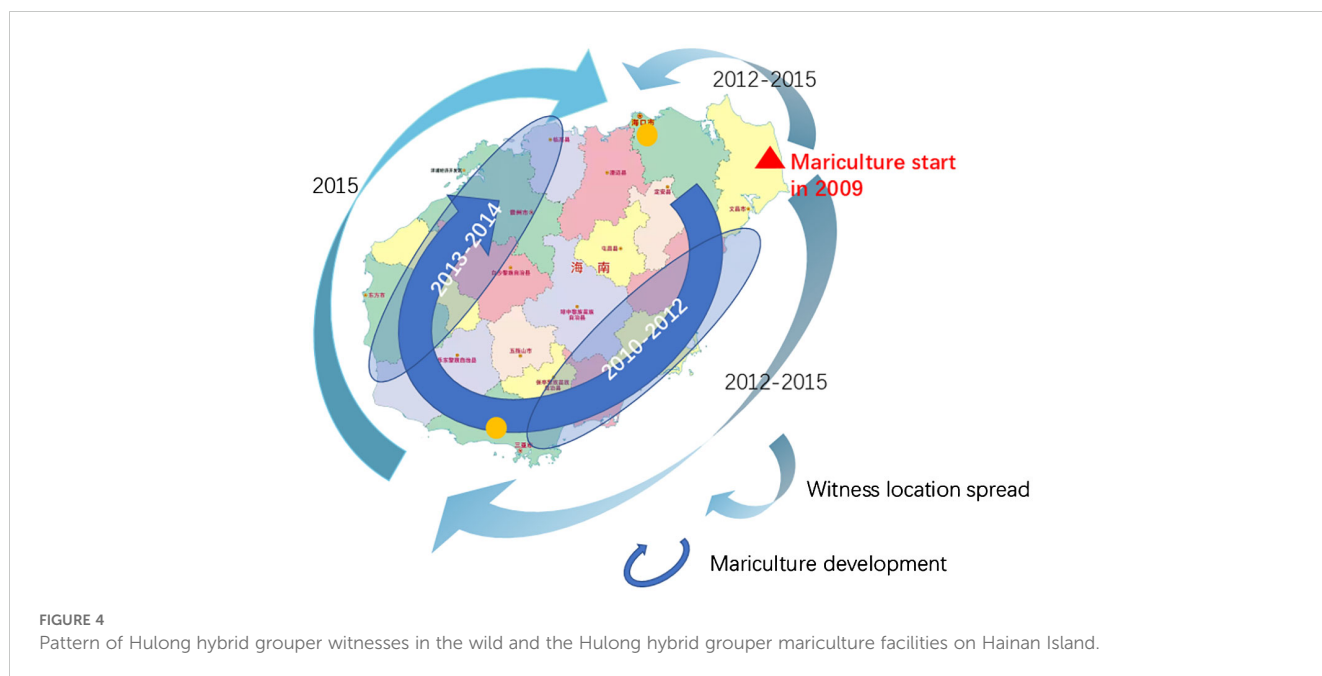


FIGURE 3

The curves of (A) total and (B) regional witness percentages from the face-to-face interview data. NEHI means cities including Hai Kou, Wen Chang and Qiong Hai. SEHI means cities including Wan Ning, Ling Shui and San Ya. SWHI means cities including Le Dong, Dong Fang and Chang Jiang. NWHI means cities including Dan Zhou, Lin Gao and Cheng Mai.



### 3.3 Age determination and gonadal classification of introduced Hulong hybrid grouper

The ages of the Hulong hybrid grouper samples from the wild ranged from less than 1 year to 5 years old ( $n=28$ ). These wild hybrid groupers were undifferentiated in sex at ages 0 to 1 year old ( $n=5$ ), were immature females at ages 1 to 2 years old ( $n=19$ ), and were mature females with ripe eggs at ages 3 years and older ( $n=4$ ). No mature male individual was found in this study (Figure 5).

## 4 Discussions

### 4.1 The distribution pattern of Hulong hybrid grouper in the coastal waters of Hainan Island

A total of 1,532 observation records of Hulong hybrid grouper during the period from 2014 to 2023 indicated the continuous and widespread distribution of the hybrid grouper over a long period around Hainan Island. It is worth mentioning that over 500 records came from the southeast provinces of China (i.e., Guangxi, Guangdong, and Fujian) and even from the southeast Asian nations, highlighting the wide distribution of this hybrid grouper in the wild.

Moreover, 261 valid questionnaires from face-to-face interviews were analyzed (Figure 3). All the interviewees around Hainan Island confirmed the existence of the Hulong hybrid grouper in their local coastal waters. This hybrid grouper was first recorded in the wild at Wenchang and Qionghai in the northeastern region of Hainan Island, in 2012. Its range extended southward to Sanya and northward to Haikou, and then was finally found along the rest of the coast of Hainan Island circa 2015. The maximum and minimum body weight of the captured Hulong hybrid groupers were 39,800 g (in Wen Chang, northeast of Hainan Island) and approximately 10 g

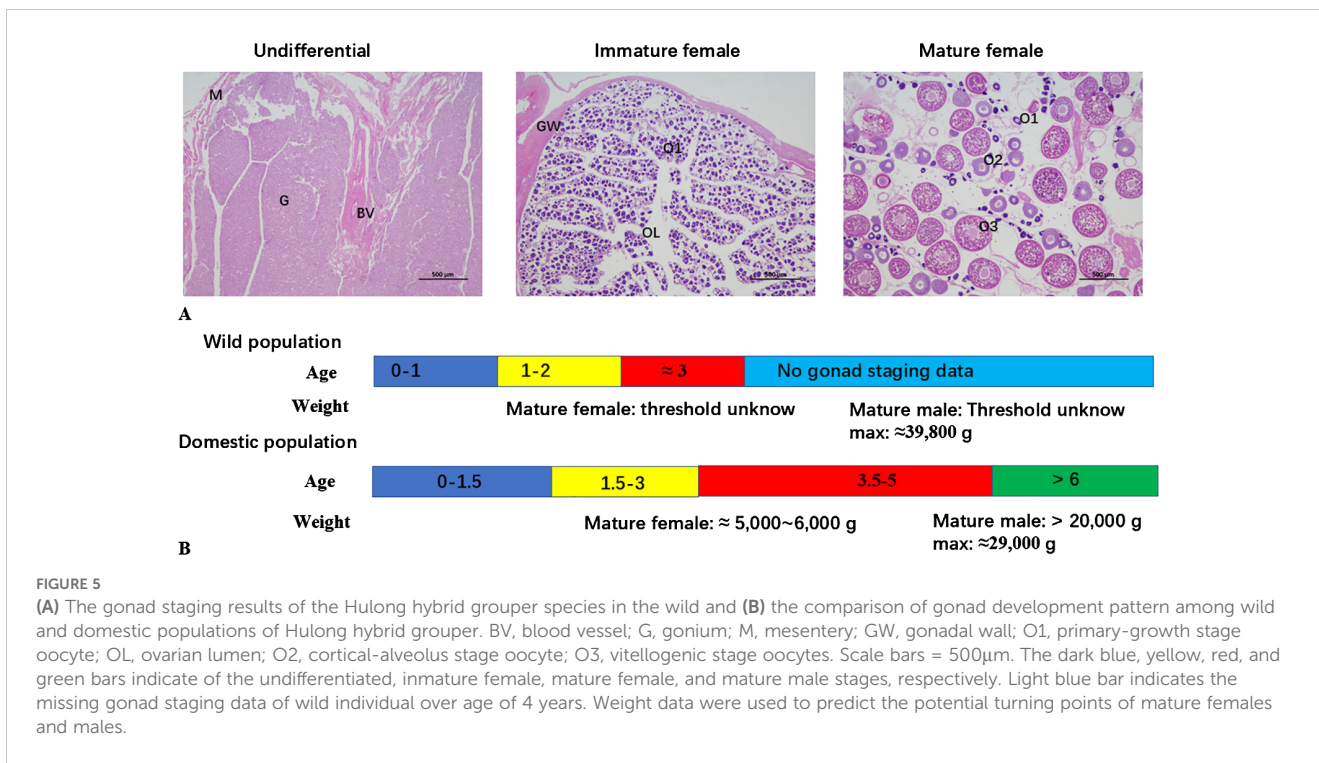
(in Dong Fang and Chang Jiang, southwest of Hainan Island), respectively. The weight of captured Hulong hybrid groupers ranged from 2,500 to 7,500 g. The estimated yields among these cities were 10 to 150 tons/year, with the highest expected yield on the east coast of Hainan Island. Despite the different introduction time points of the Hulong hybrid grouper among the regions, these results indicated the Hulong hybrid grouper acted like settled fish and that a sizable community of this fish has formed in these wild areas.

To determine the reason for the unique distribution pattern of Hulong hybrid groupers, a detailed investigation into the major spawning and mariculture companies of this fish on Hainan Island was performed. The results indicated that the spawning and mariculture industry of Hulong hybrid grouper started in Wen Chang in 2009. It then quickly expanded to the south (2010 to 2012) and west (2013 to 2014) coasts of Hainan Island, which shows a similar expansion pattern with the extension footprint of the Hulong hybrid grouper in the wild (Figure 4).

Based on our current social survey data, currently, the Hulong hybrid grouper has a wide distribution in the coastal waters of Hainan Island and has been introduced to this region for almost 12 years. Meanwhile, the spotting frequency, distribution areas, and stock biomass also increased during this period, which indicates this hybrid grouper survived well and formed an enormous population in the wild. The results also highlighted that the vigorous development of the Hulong hybrid grouper mariculture industry played an important role in the introduction and invasion of this species in the coastal waters of Hainan Island.

### 4.2 Survival of the Hulong hybrid grouper on the coastal waters of Hainan Island

The survival requirements of an invasive species in a new area include a suitable habitat, food sources, environment tolerance, and



**FIGURE 5** (A) The gonad staging results of the Hulong hybrid grouper species in the wild and (B) the comparison of gonad development pattern among wild and domestic populations of Hulong hybrid grouper. BV, blood vessel; G, gonium; M, mesentery; GW, gonadal wall; O1, primary-growth stage oocyte; OL, ovarian lumen; O2, cortical-alveolus stage oocyte; O3, vitellogenic stage oocytes. Scale bars = 500μm. The dark blue, yellow, red, and green bars indicate of the undifferentiated, immature female, mature female, and mature male stages, respectively. Light blue bar indicates the missing gonad staging data of wild individual over age of 4 years. Weight data were used to predict the potential turning points of mature females and males.

interactions with local species (Stauffer, 1984). In regular invasive alien species (IAS) research, the biological traits of an IAS and the environmental factors in the place of IAS's origin are taken into consideration to measure the survival of an IAS in a new area (Liu et al., 2021). As a newly created and potential invasion species, we lack such data on the Hulong hybrid grouper, which greatly hinders the estimation of survival status of this hybrid grouper in the wild. In this study, we used biological trait data and environmental factor of distribution area data of the parental species (*E. fuscoguttatus* and *E. lanceolatus*) and biological traits of the Hulong hybrid grouper in mariculture conditions to measure the survival of this hybrid grouper in the introduced area.

The Hulong hybrid groupers were found in areas with a water temperature between 15°C and 34°C, in reef areas with water depths between 3 to 100 meters, and inshore structures and adjacent eutrophic waters with depths of 2-5 meters. Considering that the natural habitat of the parental species of the Hulong hybrid grouper was rocky areas with water depths between 10 to 100 meters and a water temperature of 18°C-30°C, this result reflected a connection in habitat preferences between of the Hulong hybrid grouper and its parental species, on the one hand. On the other hand, this hybrid grouper utilized areas with human disturbance and eutrophication, which indicated the domestication event shaped the habitat preference and stress resistance of the newly produced hybrid fish and further increased the possibility of successful invasion.

Current diving reports strongly suggest that the Hulong hybrid grouper has a wider diet list compared with its parental species (Table 1). Feeding habit changes of invasion fish have been reported. In this case, the hybrid and further domestication processes may contribute to the feeding habit changes in Hulong hybrid grouper, thus contributing to the survival of this hybrid

grouper in the wild. The prey species of Hulong hybrid grouper includes crustaceans, mollusks, echinoderms, polychaetas, and fish, which are abundant in the coastal waters of Hainan Island. In recent years, there has been a serious problem with overfishing in Hainan Province. As a result, the fish community has changed with a decrease in the abundance of local high-trophic predatory fish. The

**TABLE 1** Diet composition of the Hulong hybrid grouper and its parental species *E. fuscoguttatus* and *E. lanceolatus*.

Class	Order	Hulong hybrid grouper	<i>E. fuscoguttatus</i> and <i>E. lanceolatus</i>
Actinopterygii	Perciformes	+	+
	Scorpaeniformes	+	-
	Pleuronectiformes	+	-
	Tetraodontiformes	+	-
	Clupeiformes	+	+
Cephalopoda	Sepiida	+	-
	Teuthida	+	-
Echinoidea	Echinoimies	+	-
Bivalvia	Veneroida	+	-
Malacostraca	Decapoda	+	+
	Stomatopoda	+	-
Polychaeta	Phyllodocimorpha	+	-

Note: Diet composition of *Epinephelus fuscoguttatus* & *E. lanceolatus* were retrieved from FishBase (<https://fishbase.mnhn.fr/search.php>).

Hulong hybrid grouper is a typically high trophic fish and may have occupied the vacant niche to accelerate the process of invasion.

Overall, the Hulong hybrid grouper shows good environmental adaptation ability and stress resistance in the wild. The coastal waters of Hainan Island have suitable environmental conditions, enough food supply, and vacant niches that are suitable for the invasion of this hybrid grouper.

### 4.3 Successful population establishment of Hulong hybrid grouper

For an invasive species to be considered to have successfully established a population, the species needs to be detected for 10 years and its occurrence is necessary in at least two localities or in 2 consecutive years (Ruiz et al., 2000). In recent years, there has been a serious problem with overfishing on Hainan Island. As a result, the fish community has changed with a decrease in the abundance of local predatory fish species. The vacant niches may be occupied by the more competitive predatory Hulong hybrid grouper. Additionally, repeated releases of the fish increase the probability of the successful establishment of a population.

The distribution and observation data included multiple records from more than 12 cities over 10 consecutive years (2014–2023). In addition, most captured individuals from the wild ( $\approx 78\%$ ) had body weights larger than 2,500g. Since the Hulong hybrid grouper is usually reared and sold at body weights of 500–1,000g, the larger size of individuals in the introduced population shows that they have survived and grown well in the wild.

The gonad histology results indicated a successful Hulong hybrid grouper reproduction population has been established in the wild (Figure 5). These wild hybrid groupers were undifferentiated at the age of 0–1, were developing females at the age of 1–2, and mature females at approximately 3 years old. No sex reversal or mature male individuals were found in our samples due to the difficulty of catching a large hybrid grouper specimen in the wild. Our social survey data from the mariculture industry showed that a domestic Hulong hybrid groupers become mature females at the age of 4 to 5 (weight of 5,000 to 6,000 g) and mature males at the age of 6 to 7 (weight > 20,000 g). Currently, the largest recorded domestic mature male of this hybrid grouper was 12 years old at approximately 32,000 g. The growth rate of Hulong hybrid groupers in the wild was faster than their counterparts in the mariculture system, which is highly correlated with the development of gonad maturity (Mawa et al., 2021). Despite the absence of mature male specimens, a mature male population must exist in the wild based on the following reasons: 1) the age threshold of mature females and males seems earlier in the wild population; 2) this hybrid grouper population had a considerable number of individuals with weights over 20,000 g, which is usually used as a key criterion for the selection of individual mature males; and 3) two self-breeding F1 Hulong hybrid grouper individuals from the wild were found and identified using multiple nuclear genes (Figure 6C, unpublished data).

In summary, we believe the Hulong hybrid grouper has survived well and has successfully established populations in the coastal waters of Hainan Island.

## 4.4 Mechanisms of introduction

The mechanisms of fish introductions by aquaculture activities can be unintentional releases or deliberate introductions to improve fisheries (Baltz, 1991). Our results show that the mechanisms for the Hulong hybrid grouper invasion in the coastal waters of Hainan Island included unintentional escapees from the aquaculture industry as well as intentional release.

It is unlikely that the Hulong hybrid grouper was created directly in the coastal waters of Hainan Island. Judging by the fact that there was no report of the Hulong hybrid grouper on Hainan Island before 2011, the fish observed locally were most likely human introductions. At the early stage of invasion (2011–2015), the main introduction pathway of the Hulong hybrid grouper was escapees from aquaculture ponds or marine net cages. Hainan Island is a major typhoon landfalling location in the west Pacific region with an average of 1.3 per year. The heavy rainfall and high tides accompanying a typhoon landfall often cause severe damage to mariculture infrastructure. They can cause floods and consequently help the fish escape from high-density aquaculture ponds and marine net cages, both of which are used in major grouper mariculture facilities on Hainan Island.

In the period from 2016 until now, intentional release activities for economic profit, improving fishery resources, and religious purposes became an extra pathway for this hybrid grouper to be introduced to the local area. With the development of a Hulong hybrid grouper breeding industry, problems such as germ degeneration or disease occurrence at the juvenile stage trouble companies and individual farmers. To reduce economic losses, companies and individual farmers usually drain the juvenile hybrid groupers or even fertilized eggs directly into adjacent waters without appropriate biosafety treatment, according to our social survey. This process has unquestionably led to a batch of juvenile fish being introduced to the wild and caused the blooming of smaller hybrid groupers in the coastal waters of Hainan Island. Furthermore, invasions can be caused by releasing economic fish larvae into coastal waters for the purpose of improving fishery resources or religious praying activities. Our unpublished data indicated that the body weight of released Hulong hybrid groupers ranged from 20g to 2,500g. However, the precise numbers and frequency of releases were difficult to trace and document. We believe that these repeated releases contributed to the hybrid grouper invasion process in the regions far from the mariculture areas on Hainan Island.

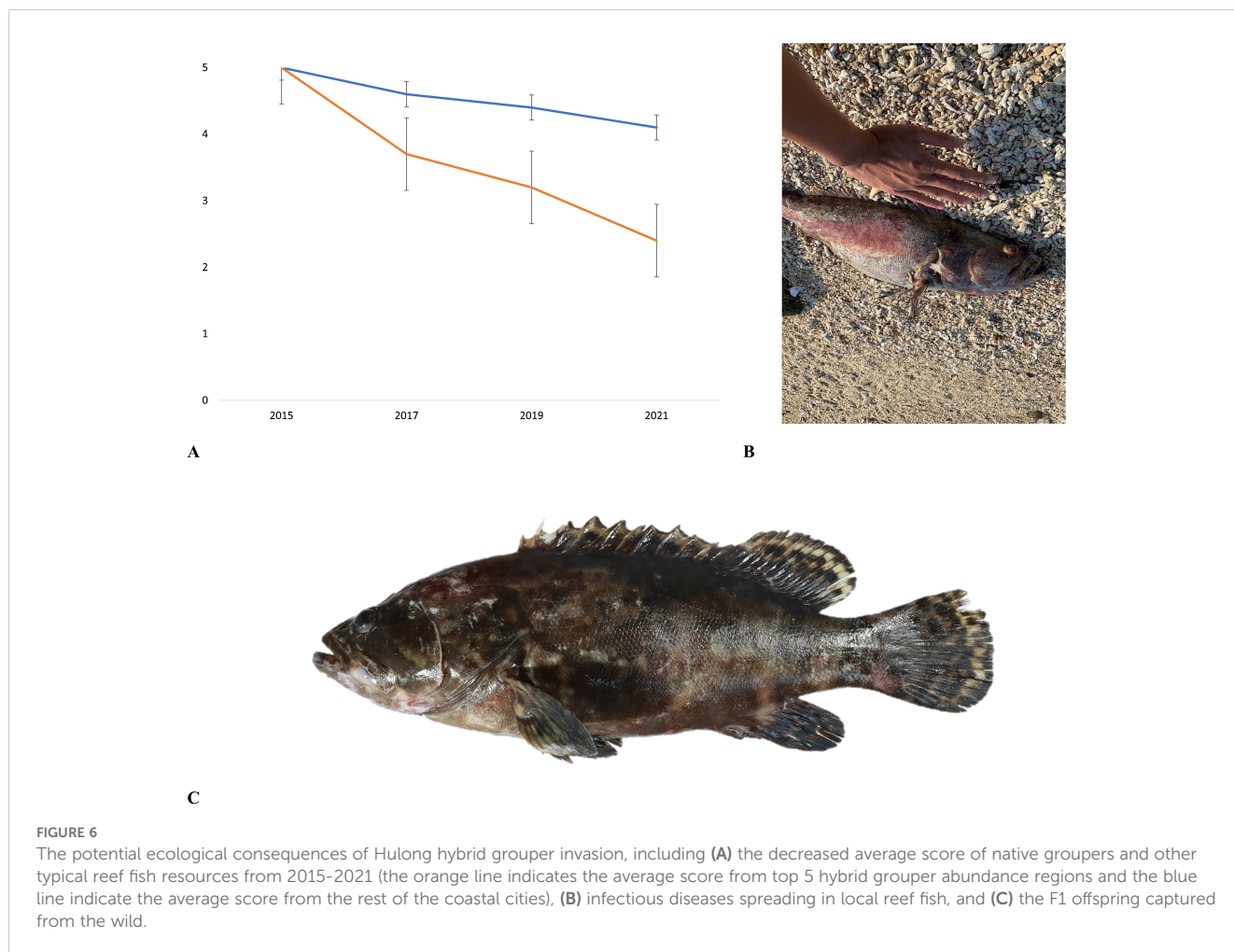
## 4.5 Putative ecological consequences

Invasion species can fundamentally alter native communities and ecosystem processes (Ruiz et al., 1997; Taylor et al., 1984). In this case, the Hulong hybrid grouper showed obvious negative impacts on native communities, similar to other reported invasive fish. This hybrid grouper successfully occupied the vacant niches of Hainan Island coastal waters and acted as a high trophic predatory fish. It caused severe interspecies competition and a top-down effect on food organisms, as well as declines or displacement of native

marine species. Our preliminary results indicated a negative impact of this hybrid grouper on the native groupers and other typical reef fish resources (Figure 6A). Based on our survey data, the average resource score of native groupers and other typical reef fish resource dropped dramatically in the regions (Wen Chang, Qiong Hai, Wan Ning, Ling Shui and San Ya) with larger hybrid grouper populations when compared to the rest of the coastal cities. Furthermore, several reports demonstrated a potential link between the invasion event and an increase in infectious diseases in local reef fish communities (personal communication to Xiang Zhang, Figure 6B). However, we still lack direct etiological evidence to verify this.

Notably, concerns have arisen that the Hulong hybrid grouper could greatly affect the genetic resources of local groupers through direct hybridization and reproductive competition. Groupers are typically reef fish and are ecologically isolated and Hainan Island is a hotspot for grouper resources. Our study demonstrated that the Hulong hybrid grouper has established a reproductive population in the wild. We do not know specific characteristic details of the reproductive biology of this hybrid grouper after its invasion, but we could piece together evidence to gain a better understanding of the consequences of the hybrid grouper for the local grouper community on the genetic level. The Hulong hybrid grouper has the ability to self-

breed and backcross-breed offspring (unpublished data), and has unquestionably demonstrated that it can reproduce with other grouper species in mariculture conditions. Considering the continuous expansion of the Hulong hybrid grouper population and the fish often being mixed with local groupers in the wild, the possibility of direct hybridization among the hybrid grouper and other local grouper species increased. This process would lead to the introgression of a number of heterogeneous chromosomes and further threaten the integrity of local adaptation gene complexes in the natural grouper communities (Eizaguirre and Lenz, 2010; Ding et al., 2018). As the local adaptation gene complex is essential for groupers to survive in a particular environment, direct hybridization could finally lead to a decrease or even extinction of wild grouper resources. Even if direct hybridization does not happen, this hybrid grouper may exert additional reproductive pressure on local groupers by limiting the opportunities of successful reproduction for these species. Interestingly, we captured two self-breeding F1 Hulong hybrid grouper individuals from the wild and their origin is still a mystery (Figure 6C, unpublished data). Thus, the invasion of hybrid grouper could have significant negative impacts on biodiversity and the ecosystem such as interspecies competition, disease transmission, and genetic pollution in the local community.





## 5 Conclusions

In conclusion, the Hulong hybrid grouper has invaded the coastal waters of Hainan Island. This fish has established a population in this area and shown gradual negative impacts on local community, particularly the local grouper species. Compared to previously reported invaded alien fish, the Hulong hybrid grouper is not only the first reported invaded hybrid fish but also reminds us of the potential risks of this fauna. The public and government must be more cautious to prevent such threats from occurring. The prey composition, trophic spectrum, and aggressive behavior of this hybrid grouper should be further evaluated.

## Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding authors.

## Ethics statement

The animal study was approved by the ethics committee of Hainan University. The study was conducted in accordance with the local legislation and institutional requirements.

## Author contributions

XZ: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. JL: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Writing – original draft, Writing – review & editing. SC: Conceptualization, Data curation, Formal analysis, Investigation, Writing – review & editing. QY: Investigation, Writing – review & editing, Formal analysis, Visualization. FJ: Investigation, Writing – review & editing. HL: Data curation, Formal analysis, Investigation, Writing – review & editing. SD: Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Writing – review & editing.

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## Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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## Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fmars.2024.1438895/full#supplementary-material>

### SUPPLEMENTARY FILE S1

Online questionnaire for anyone who witness the wild Hulong hybrid grouper.

### SUPPLEMENTARY FILE S2

Onsite questionnaire for local fishery enforcement employees, fishing port superintendency agency employees, and fishermen.

### SUPPLEMENTARY FILE S3

Onsite questionnaire for major hybrid grouper mariculture companies' managers.

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