

Ecological implications of purple sea urchin (*Heliocidaris crassispina*, Agassiz, 1864) enhancement on the coastal benthic food web: evidence from stable isotope analysis

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ARTICLE INFO

Keywords:

Benthic food web
Ecosystem
MixSIAR
Sea urchin
SIBER
Stable isotope
Stock enhancement

ABSTRACT

A responsible approach to marine stock enhancement is an effective approach to restore fishery resources. While the release strategy of target species has been well investigated, the impacts on local ecological equilibrium and habitat qualities have only been poorly considered. In the present study, we evaluated how the macro-benthic food web in Daya Bay was affected by purple sea urchin (*Heliocidaris crassispina* (Agassiz, 1864) stock enhancement using stable isotope analyses ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Our results indicated that the distribution of local species and trophic diversity were influenced to a certain degree by release of purple sea urchins and changes in the feeding habit of the urchins were observed in line with food abundance, which seasonally varied. When food is abundant, the main food source of sea urchins was microphytobenthos and no significant differences were observed among sites; significant differences in the diet of purple sea urchins were detected when food is less abundant. These results suggested that optimization of the release strategy should include information on seasonal productivity of local recipient sites, food web structure and feeding habits of released species. Such information is essential for building a responsible release approach to maximize production enhancement.

1. Introduction

Wild catches of most commercial species are already beyond maximum sustainable yields and the potential for increases in production is very small (Branch et al., 2011). Thus, as the human population grows and *per capita* consumption increases, aquaculture plays a significant role. This means that existing and future fish stocks must be addressed in a more productive and efficient way, to sustainably maximize the yields in order to meet the increasing demand (Sarà et al., 2018). To date, cage aquaculture in marine, brackish and freshwater occurs within specific waters and is predicted to have great development potential (Halwart et al., 2007). Extensive literatures dealing with the impacts of aquaculture shows that cage aquaculture generates major drawbacks such as potential nutrient alteration at the local scale, increased water mass turbidity and hypoxia events with significant effects on the local biodiversity (Sarà et al., 2004; Sarà, 2007a, b; Sarà,

2007c), potential disease transfer and increasing likelihood of escapes (Halwart et al., 2007; Jensen et al., 2010). Due to these detrimental consequences and the current lack of efficient innovative breakthroughs, cage culture has been heavily regulated in many areas to cope with the steady stable increase of proteins demand from the sea. In the last few decades, new methods and soft technologies have been thought to increase animal production through coastal stock enhancement, which consists in stocking cultured organisms to replenish or increase the abundance of wild stocks (Kitada, 2018). Nowadays, while worldwide stock enhancement of invertebrates and fish include dozens of case-species (Kitada, 2018), the success has often been limited by significant spatial and temporal fluctuations in predation, annual natural recruitment and carrying capacity of recipient sites (Kitada, 2018). Indeed, stock enhancement has many potential ecological effects, given that the release of a large number of individuals belonging to the same species can alter ecological equilibriums by affecting density-dependent

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<https://doi.org/10.1016/j.marenvres.2020.104957>

Received 1 February 2020; Received in revised form 10 March 2020; Accepted 10 March 2020

Available online 14 March 2020

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processes, with potential repercussions on both functional traits (e.g. feeding behaviour, mating system) of wild co-specifics and the structure of recipient food webs. Nevertheless, some global concerns have been raised such as: i) the genetic risks of artificial propagation of hatchery releases on wild populations. Indeed, if hatchery individuals compete with wild organisms in a limited carrying capacity habitat, this may lead to reduce wild fitness and genetic diversity; ii) potential implications of the replacement of wild organisms and, lastly iii) change in population composition and structure (Laikre et al., 2010) with many examples observed in the last years, such as those involving salmon, gilthead sea bream, European sea bass, cod and barramundi escapees (Bolstad et al., 2017; Glover et al., 2012, 2017; Karlsson et al., 2008; Segvić et al., 2011; Somarakis et al., 2013). Following the conceptual framework of Blankenship and Leber (1995) and Lorenzen et al. (2010), stock enhancement - through a physical-biological pathway - directly affects resource, fishing, aquaculture production, habitat quality and environment, stakeholders, markets and governance arrangements. Surprisingly, while the release strategies have been well-investigated (Kitada, 2018), the ecological effects due to the massive release of individuals on local community structure and dynamics has been poorly considered; these include the potential implications for the local food web structure. Although stock enhancement implies releases of large numbers of individuals that can affect local ecological dynamics, the potential effects are still ignored. Such a lack of research may be justified by difficulties in evaluating the efficiency of stock enhancement and influence of released species on a given habitat. Here, based on a large-scale study in a Daya Bay, Guangdong, China, we estimated the effect of stock enhancement of purple sea urchin (*Heliocidaris crassispina* Agassiz, 1864) on both the feeding habit of the wild sea urchin and the recipient food web structure. Carbon and nitrogen Stable Isotope Analysis (SIA) was used to re-build the effect on the trophic structure (e.g. primary food sources, trophic linkages and trophic position) due to stock enhancement and the putative effect on isotopic signatures of wild individuals (Brind'Amour and Dubois, 2013; Layman et al., 2012). In the past, only few studies involved SIA in stock enhancement case studies (Cucherousset et al., 2007) and most of them dealt with otolith chemistry to discriminate the signatures of hatchery-reared and wild individuals. Surprisingly, SIA

was never used to study the potential effects of stock enhancement on food web structure at recipient sites. Nevertheless, the current literature shows that SIA has a large potential to discriminate how biological traits and ecological preferences may shape the food web structure and how feeding behaviour can change in response to natural habitat changes and anthropogenic pressure (De Smet et al., 2015; Naman et al., 2016; Pasotti et al., 2015; Reuter et al., 2016). Accordingly, first of all, this study i) estimated the success of purple sea urchin stock enhancement, and secondly ii) used SIA to investigate whether purple sea urchin enhancement (56 000 juvenile purple sea urchins released in two Chinese bays [Lajia and Yuanzhou Islands] in August 2014) caused changes of the purple sea urchin's feeding habit, and lastly iii) whether stock enhancement affected the macrobenthic food web structure of the recipient sites.

2. Material and methods

2.1. Study area

The study area, including three small islands, is located in the coastal waters of Daya Bay, Guangdong Province, China (Fig. 1). Daya Bay, a semiclosed bay characterized by a subtropical climate, is surrounded by mountains on three sides and contains many islands, which provides a variety of natural habitats, rich of biological resources. The average annual temperature is 22.0 °C. Yuanzhou and Lajia are two small islands belonging to the Zhongyang Key islands, in the centre of Daya Bay. In August 2014, 56 000 juveniles of purple sea urchin, with 6.5 mm in diameter, were released *per site* (Yuanzhou and Lajia), to enhance stocking density. Sanmen Island is at the opening of Daya Bay, at a distance of 10–12 km from the other two sites. No purple sea urchin stock enhancement was implemented at Sanmen Island.

2.2. Sampling

The efficiency and the success in terms of the density levels before and after the stock enhancement of the purple sea urchin transplanting experiments at Lajia and Yuanzhou islands was reported by Chen et al.

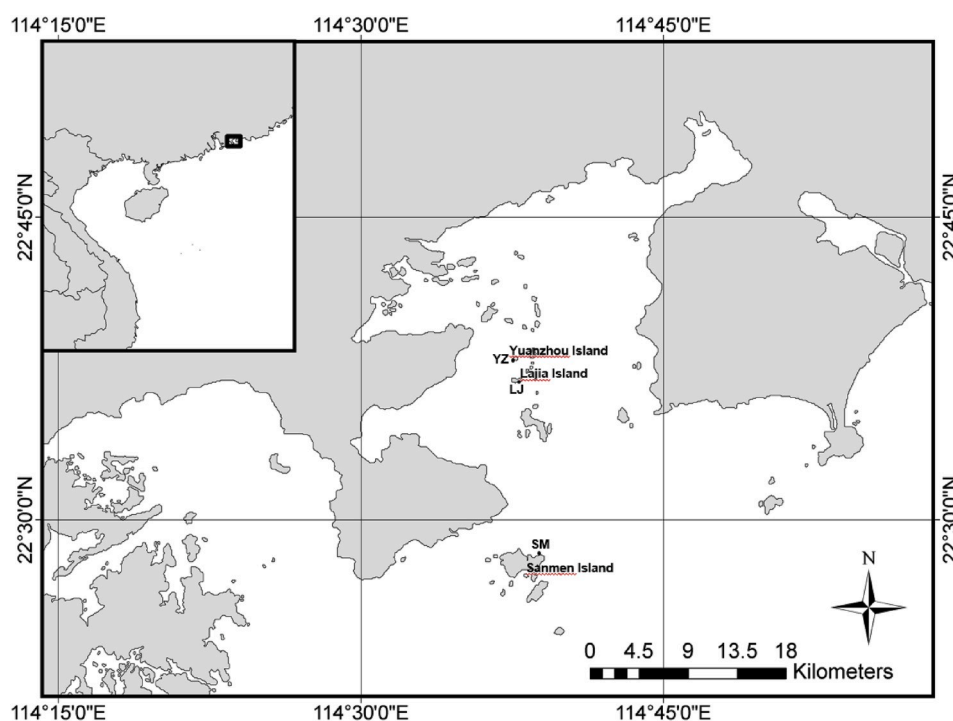


Fig. 1. Sampling sites in the Daya Bay (Guangdong Province, China)

(2016). Briefly, the 600 parent purple sea urchin were collected from Lajia and Yuanzhou Islands in April, then transferred to Shenzhen Experimental Station for breeding. The juvenile purple sea urchin were released to Lajia and Yuanzhou in August, when the juvenile purple sea urchin were over 5–6 mm in diameter. In order to evaluate purple sea urchin enhancement, purple sea urchins were collected during surveys conducted by the South China Sea Fisheries Research Institute and the Chinese Academy of Fishery Sciences in November of 2014. Surveys were conducted at Zhongyang Islands using stratified random sampling, designed to sample at each island. Yuanzhou and Lajia islands were surveyed as being part of the Zhongyang Islands. At every island, 3 to 5 stations were sampled manually over quadrats (1 m²) by scuba diving. Targeted macrobenthic specimens were frozen on board and transported to the laboratory for further analysis. Body weight and size per individual were measured at every station. One year after the purple sea urchin released at study islands, samplings were carried out at the three sites in four seasonal [August (summer), November (autumn), February (winter) and May (spring)] from 2015 to 2016 to assess the stable isotope (SI) composition of main macro benthic taxa belonging to the same trophic web of released sea urchins. Thus, macro-benthos was sampled using scuba diving at 3 sites every season. The purple sea urchins were graded into three groups: large-sized group >50 mm, middle-sized group 40–50 mm and small-sized group <40 mm. After identification, specimens of all benthic taxa sampled for the study were allowed to defecate overnight, measured and killed by freezing. Tissue was separated from the gonads and Aristotle's lanterns of the purple sea urchins and from the foot of the gastropods, while the whole somatic fraction was extracted from the shellfish and used in the analysis.

Macroalgae were collected manually from the bottom at each survey site. Microphytobenthos (benthic microalgae) were collected using nylon bolting nets (5 m long, 1 m wide, 100 µm mesh), which were kept for about ten days under 1 m water depth at each site. Particulate organic matter (POM) was obtained from water samples collected using Niskin bottles from each site; samples were filtered through Whatman GF/F glass fibre filters (47 mm diameter, 0.7 µm pore size). Plankton was collected using a nylon plankton nets (both for zoo- and phytoplankton), pulled for 10–15 min at 2.2 nm. Sediment organic matter samples were obtained via scuba diving, using plastic tubes (6 cm in core diameter). The first 2-cm slices were cut from the core surface. All the samples were diluted with fresh water and stored frozen (−20 °C) until analysis.

2.3. Stable isotope analysis (SIA)

Frozen tissue samples were decalcified using 0.5 N HCl. POM and sediment organic matter (SOM) were acidified with 0.5 N HCl. Then, all the samples were freeze-dried in a vacuum freeze drier (Vacuum freeze drier, China) at −55 °C for 24 h or until completely dry. Dried samples were sent to Dalian Ocean University, Dalian, China and were analyzed using a CNSH-O elemental analyser (Vario PYRO Cube, Elementar, Germany) coupled with a mass spectrometer (IsoPrime 100, Elementar, Germany).

The isotope values (δ13C and δ15N) were computed according to the following equation:

$$\delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 10^3,$$

Where X is ¹³C or ¹⁵N, and R represent the ratios ¹³C/¹²C or ¹⁵N/¹⁴N.

2.4. Data analysis

2.4.1. Food web modelling

For the food web modelling analysis, site-level data were pooled temporarily into 3 sites: Lajia island, Yuanzhou island and Sanmen island, which corresponded to three research islands and four seasons:

summer, autumn, winter and spring. The relative trophic level (TL) of each species was estimated based on its δ¹⁵N, using the formula from Hussey et al. (2014) and Sarà (2007d).

$$TL = \left(\frac{\log(\delta^{15}N_{lim} - \delta^{15}N_{base}) - \log(\delta^{15}N_{lim} - \delta^{15}N_{TP})}{k} \right) + TL_{base}$$

Where δ¹⁵N_{lim} is the saturating isotope limit as trophic position (TP) increases, δ¹⁵N_{TP} is the consumer isotope value at a given TP, k is a rate constant. Calculating k and δ¹⁵N_{lim} are given from the meta-analysis as,

$$k = -\log \left(\frac{\beta_0 - \delta^{15}N_{lim}}{-\delta^{15}N_{lim}} \right)$$

$$\delta^{15}N_{lim} = \frac{-\beta_0}{\beta_1}$$

The values of β₀ and β₁ from full meta-analytical analysis was 5.92 and −0.27 respectively, were taken from Hussey et al. (2014). Baseline consumers used to estimate TL_{base} in the experiment were zooplankton in each site (Hussey et al., 2014).

Initial analyses utilized two-way analysis of variance (ANOVA) to test the effects of seasons, trophic group and sites on TL (Perkins, 2013). To determine where significant differences lay among purple sea urchin sizes within different sites and seasons, subsequent one-way ANOVA were tested with Tukey post-hoc tests, for TL, δ¹⁵N and δ¹³C. All analyses were conducted in R version 3.4.1 (R Development Core Team, 2008).

2.4.2. SIBER modelling

The differences of benthic ecosystem structure at different sites were compared using the SIBER (Stable Isotope Bayesian Ellipses in R) package (version 2.1.3) in R 3.4.1 (R Development Core Team, 2008) developed by Jackson et al. (2011). The δ¹⁵N range (NR) and δ¹³C range (CR) are indicative of trophic length and niche diversifications, respectively. The mean distance from centroid (CD) provides a measure of trophic diversity. The mean nearest neighbour distance (MNND) is a measurement of density and clustering of species within the community. The standard deviation of the nearest neighbour distance (SDNND) gives a measure of evenness of species packing. The total area (TA) is estimated by the convex hull enclosing all the species. The standard ellipse area (SEA) and the corresponding metric corrected for small sample size (SEA_c) are used to assess the niche width.

For the analysis, organisms were classified into 4 trophic groups, namely, primary producer, herbivore (herb), omnivore - filter feeder (omnif) and omnivore - deposit feeder (omnid), which based on published background information on species life traits (Table S1).

2.4.3. MixSIAR modelling

MixSIAR mixing model framework (version 3.1.2) (Parnell et al., 2013) were used to analyze the feeding habit of the purple sea urchin at different seasons and sites. The MixSIAR mixing model is a Bayesian mixing model developed to analyze uncertainty associated with variation in isotope values of both sources and consumers, fractionation values and concentration dependence. In this study, the potential food source of the purple sea urchin was considered to include sediment organic matter (SOM), Microphytobenthos (MPB) and macroalgae, which differed at different seasons and sites. To reduce the number of sources in the model, macroalgae were divided into three groups: red algae (*Porphyra crispata*, *Amphiroa dilatata*, *Ceramium kondoi*), brown algae (*Dilophus okamurai*, *Sargassum hemiphyllum*, *S. parvivesiculosum*, *S. euryphyllum*, *S. siliquastrum*) and green algae (*Ulva lactuca*, *Enteromorpha clathrata*), which depend on their taxon. As POM was considered an important food source for the purple sea urchin in summer (Mo et al., 2017; Ogle, 2008), we also considered the purple sea urchin as a food source. In our case, the purple sea urchin usually feeds on macroalgae as it is an herbivore. In this situation, average trophic enrichment factors (TEFs) of 2.52 ± 2.5‰ for δ¹⁵N and −0.41 ± 1.04‰ for δ¹³C

(Ogle, 2008; Zanden and Rasmussen, 2001) were used to model the diet. Otherwise, based on previous analyses, the survey dataset was divided into two seasons: macroalgae bloom season (MBS, winter and spring) and less macroalgae season (LMS, summer and autumn).

3. Results

3.1. Assessing the success of stock enhancement

In November 2014, the stocking density of the purple sea urchin at three sites was surveyed using scuba diving. Three to five quadrats (1 m²) were used to collect purple sea urchins in order to estimate the stocking density of the purple sea urchin at every site. The stocking density of the purple sea urchin increased from 0.75 to 2.41 individuals/m² to 13.41 to 13.99 individuals/m² at Yuanzhou and Lajia island. In contrast, the stocking density of the purple sea urchin at Sanmen island - not released site - remained at only 3.5 individuals/m².

3.2. Stable isotope profile and food web structure

Forty-nine species were sampled from the three sites in Daya Bay (Lajia island, LJ; Yuanzhou island, YZ; Sanmen island, SM, including 21 gastropods, 12 bivalves, 1 polyplacophora, 1 holothuroidea, 1 echinoida, 3 rhodophyta, 5 phaeophyta and 2 chlorophyta (Table S1, Total Ind. n = 641). The mean $\delta^{13}\text{C}$ values ranged between -17.9 (*Barbatia virescens*) and -9.84 (*Heliocidaris crassispina*) at site LJ, -16.44 (*Pinna atropurpurea*) and -7.6 (*Nerita yoldi recluz*) at site YZ, and -17.07 (*Pinna vexillum*) and -8.81 (*Clypeomorus bifasciatus*) at site SM. The mean $\delta^{15}\text{N}$ values were highest in autumn than in other seasons and showed a broad range at site YZ (Fig. 2). The highest and lowest mean $\delta^{13}\text{C}$ values were observed in summer (-13.76) and spring (-16.52) at site YZ. The isotopic signature of food sources ranged from the more depleted $\delta^{13}\text{C}$ values of phytoplankton (-18.47 ± 2.48) and POM (-18.44 ± 2.54) to the more enriched values of macroalgae (-14.41 ± 2.33) in the three sites (Supplementary Fig. 1). POM showed the widest range of $\delta^{13}\text{C}$ values. In terms of $\delta^{15}\text{N}$ values, macroalgae showed the highest values (9.46 ± 0.08), followed by SOM (8.47 ± 1.83), phytoplankton (7.91 ± 1.70), MPB (5.81 ± 2.76) and POM (4.26 ± 3.05). The mean $\delta^{15}\text{N}$ values of deposit feeder feeding groups were higher than the values of the other

feeding groups. However, filter feeders showed the most depleted $\delta^{13}\text{C}$ values. Trophic groups are defined according to feeding modes (Table S1). The producer group was composed of POM, SOM, MPB, phytoplankton and macroalgae, the food source of other secondary zooplankton species. TL was calculated from the zooplankton $\delta^{15}\text{N}$ values of sampling seasons and sites, individually as a baseline. The longest and shortest TL were both revealed at site YZ ($\text{TL}_{\text{max}} = 3.65$, *Mitra scutulata* and $\text{TL}_{\text{min}} = 2.32$, *Littorina Férussac*), in spring and autumn respectively. Significant differences were detected among seasons, trophic groups, sites and seasons (seasons: $F_{3,209} = 5.5675$, $p = 0.0011$; trophic group: $F_{3,174} = 81.2110$, $p < 0.001$; sites and seasons: $F_{6,209} = 2.8811$, $p = 0.0102$). However, the relationship between sites (sites: $F_{2,209} = 0.6084$, $p = 0.5420$), sites and trophic group ($F_{6,174} = 0.5499$, $p = 0.7696$), seasons & trophic group ($F_{9,174} = 1.0939$, $p = 0.3696$) and sites and seasons and trophic group ($F_{17,174} = 0.6144$, $p = 0.8778$) was not statistically significant.

The distribution of stable isotopes of different sized purple sea urchins, for different sites and seasons, is represented on a dual-plot graph (Supplementary Fig. 2). No differences were detected among sites ($F = 0.0068$, $P = 0.9932$) for $\delta^{13}\text{C}$, and sizes ($F = 19.802$, $P = 0.6020$) for $\delta^{15}\text{N}$. The values of $\delta^{13}\text{C}$ ($F = 28.74$, $P < 0.001$) and TL ($F = 26.342$, $P < 0.001$) during different seasons reveal significant differences. The mean $\delta^{13}\text{C}$ value in spring was lower than in the other seasons, while the shortest TL occurred in autumn.

3.3. Feeding habit and season difference in the trophic structure

The Layman's metrics by season (Group) and site (Community) are summarized in Table 1. The $\delta^{15}\text{N}$ ranges (NRs) of LJ (0.256) was higher than YZ (0.664) and SM (2.043). The $\delta^{13}\text{C}$ ranges (CRs) of LJ (2.276) and YZ (1.999) were similar, whereas SM showed larger NRs than LJ and YZ. The mean distance to centroid (CD) and mean nearest neighbour distance (MNND) showed similar trends with NRs at three sites. Standard ellipse areas, corrected for small sample size (SEA_c), were different per shape and size among sites and seasons (Table 2), while the largest SEA_c was found among seasons at YZ. Summer at SM produced the largest SEA ($34.2\%^2$). The narrowest ellipses ($12.5\%^2$) were observed in spring at YZ. All seasons at LJ, SM and YZ showed almost similar SEA_c trends, decreasing from summer, autumn, spring and winter, but for winter it

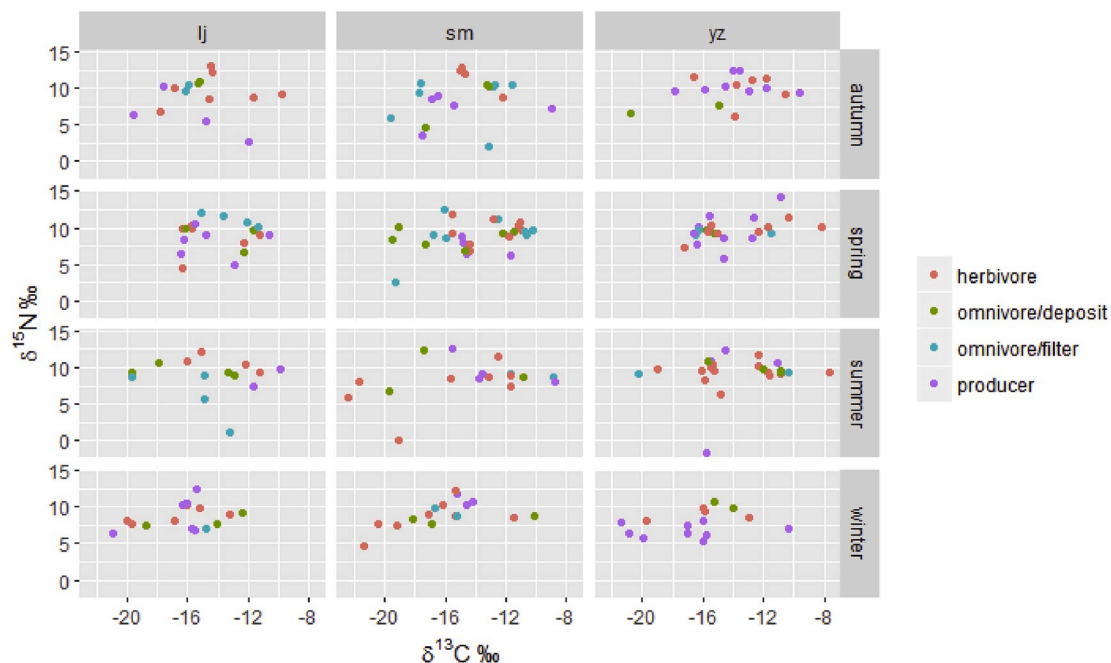


Fig. 2. Dual isotope plot of all samples collected per season and site. Sampling site abbreviations: LJ = Lajia Island; SM = Sanmen Island; YZ = Yuanzhou Island.

Table 1

The Layman's community-wide metrics for three sites per season group.

Site	NR	CR	TA	CD	MNND	SDNND
LJ	0.266	2.276	0.365	0.738	0.740	0.344
SM	2.043	2.755	1.026	1.196	0.993	1.352
YZ	0.664	1.999	0.514	0.655	0.709	0.437

Sampling site abbreviations: LJ = Lajia Island; SM = Sanmen Island; YZ = Yuanzhou Island. NR = $\delta^{15}\text{N}$ range, CR = $\delta^{13}\text{C}$ range, TA = Total area of convex hull, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance.

was higher than for spring at YZ. The Layman's metrics are summarized in Fig. 3. The probability that the standard ellipse area (SEA_b) in summer was larger than autumn SEA_b at LJ, YZ and SM was 0.681, 0.898 and 0.849, respectively. The overlap between the two seasons was 82.66%, 66.89% and 64.81%, respectively. The probability of the spring SEA_b was higher in winter at three sites was 0.669, 0.741 and 0.325, while the overlap was 54.00%, 49.21% and 65.05, respectively.

3.4. Environmental determinants of the trophic structure

Three studied sites showed similar NR (Table 3). The CR at LJ (2.41‰) was smaller than at SM and YZ. TA, CD and MNND at three sites did not show differences. The SEA_c among feeding habits at three sites showed the same trend in that producers covered the largest SEA_c , followed by herbivores, omnivores/deposit and omnivores/filter feeders. The SEA_b showed that the probability of the producers being larger than other groups was higher than 0.985 at all sites (Table 4). The smallest SEA_c was observed for omnivore/filter feeders at three sites. Overlap among sites was very large (Supplementary Fig. 3). The overlap of producer between LJ and SM, LJ and YZ, SM and YZ is 0.57, 0.72 and 0.79, respectively. The overlap of herbivore between LJ and SM, LJ and YZ, SM and YZ is 0.80, 0.76 and 0.83, respectively. The overlap of omnivore/deposit feeders between LJ and SM, LJ and YZ, SM and YZ is 0.54, 0.50 and 0.43, respectively. However, there was no overlap of omnivore/filter feeders between LJ and SM, lower overlap between SM and YZ (0.11). TA calculations (Supplementary Fig. 4) suggested that there was no significant difference among three sites.

3.5. Purple sea urchin feed diet changes in an enhanced area and an un-enhanced area

An initial MixSIAR model was run with the five potential sources in less macroalgae season (LMS, summer and autumn). LMS season and the six potential sources in macroalgae bloom season (MBS, winter and spring) (Fig. 4) showed that the main food resources in LMS season were microphytobenthos ($\geq 34\%$) and SOM ($\geq 24\%$). No significant differences were found among sizes at three sites [Standard deviation = 0.23 (0.01, 1.02)]. However, the contribution of SOM decreased with size, and the contribution of microphytobenthos increased in LMS season (Table 5, Supplementary Fig. 5). Microphytobenthos at SM comprised 56–58% of the diet and SOM 24–26%.

These results for MBS seasons showed that microphytobenthos was the primary nutritional subsidy (Table 5, Supplementary Fig. 6), with a

lower concentration compared to SOM and equal proportions of brown algae, green algae and POM. Small differences in the contributions of the different potential food sources were observed among purple sea urchin sizes: middle sized purple sea urchins fed more on green and brown algae, and less on red algae and microphytobenthos. A larger proportion of large purple sea urchins was derived from microphytobenthos than that of small- and middle-size purple sea urchins. No significant differences were observed among sites.

4. Discussion

4.1. Influence of purple sea urchin enhancing on benthic ecosystem structure

Stock enhancement is a common restoration practice adopted across coastlines worldwide (Kitada, 2018) even though the ecological effects of massive transplantation on a recipient community are still not well-studied and understood. Often, this practice leads to more negative impacts on wild populations than benefits (Kitada, 2018). Understanding the impact of stock enhancement on the ecosystem, particularly on ecosystem structure and function, helps to develop a responsible approach to marine stock enhancement and to design management

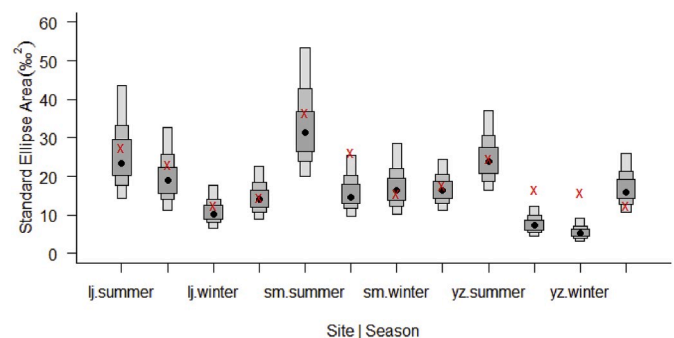


Fig. 3. Standard ellipse area Bayesian estimates (SEA_b). Communities are presented per site (LJ = Lajia Island; SM = Sanmen Island; YZ = Yuanzhou Island) and groups are presented per season (Summer, Autumn, Winter, Spring). Black dots are the mode of the $\text{SEA} (\%^2)$ while the shaded boxes represent the 50% (dark grey), 75% (lighter grey) and 95% (lightest grey) credible intervals. For comparison purposes, small sample size-corrected SEA_c are plotted as crosses.

Table 3

The Layman's community-wide metrics for three sites per feeding group.

	NR	CR	TA	CD	MNND	SDNND
LJ	4.61	2.41	6.60	1.96	2.47	0.79
SM	4.72	3.94	7.66	2.29	2.31	0.04
YZ	4.56	4.28	7.64	2.29	2.52	0.86

Sampling site abbreviations: LJ = Lajia Island; SM = Sanmen Island; YZ = Yuanzhou Island. NR = $\delta^{15}\text{N}$ range, CR = $\delta^{13}\text{C}$ range, TA = Total area of convex hull, CD = mean distance to centroid, MNND = mean nearest neighbour distance, SDNND = standard deviation of the nearest neighbour distance.

Table 2Standard ellipse areas corrected for small sample size (SEA_c) among seasons for three sites.

	LJ				SM				YZ			
	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring	Summer	Autumn	Winter	Spring
TA	57.69	57.48	26.46	31.24	92.64	69.22	36.72	58.92	88.60	41.03	34.44	41.94
SEA	25.38	21.39	11.81	13.67	34.20	24.65	14.56	16.99	23.70	15.46	14.63	11.91
SEA_c	27.50	23.03	12.60	14.47	36.48	26.10	15.60	17.67	24.78	16.57	15.76	12.50

Sampling site abbreviations: LJ = Lajia Island; SM = Sanmen Island; YZ = Yuanzhou Island. TA = Total area of convex hull, SEA = standard ellipse area, SEA_c = standard ellipse areas corrected for small sample size.

Table 4Standard ellipse areas corrected for small sample size (SEA_c , %²) among feed habitats for three sites.

	LJ				SM				YZ			
	producer	herbivore	omnivore/ filter	omnivore/ deposit	producer	herbivore	omnivore/ filter	omnivore/ deposit	producer	herbivore	omnivore/ filter	omnivore/ deposit
TA	49.19	19.32	1.69	8.06	76.91	33.17	0.29	8.38	51.66	39.83	2.05	15.10
SEA	15.31	7.50	0.67	5.43	23.03	9.59	0.28	3.64	18.48	9.55	0.70	6.50
SEAc	16.04	7.90	0.73	6.33	24.08	9.88	0.37	3.92	19.57	9.93	0.75	7.04

Sampling site abbreviations: LJ = Lajia Island; SM = Sanmen Island; YZ = Yuanzhou Island. TA = Total area of convex hull, SEA = standard ellipse area, SEAc = standard ellipse areas corrected for small sample size.

measures. As a main consequence, to provide data to increase our understanding on if this practice is successful and how a recipient community trophically respond in receiving many newly individuals from the external is crucial to appropriately design management strategy (DuFour et al., 2015; Kitada, 2018). Here first, we showed that transplanted individuals were not subject to mass mortality due to ecological resistance after the restocking, showing increasing local density of sea urchins at the two transplanting islands. The information obtained from SI food web analyses carried out at successive temporal steps after the restocking are of particular value as new individuals appeared to be well-accepted by the recipient food webs (at Yuanzhou and Lajia Islands). This result, in light of the present SIA outcome which showed an overall non-significant effect of stock enhancement on the recipient communities, is essential to ascertain the benefits of a successful targeted stock enhancement. While most research on the stock enhancement effects have focused on spatial patchiness (Rossi et al., 2015; Thompson and Townsend, 2005), habitat influence (Giraldo et al., 2017; Whitney, 2016) and feeding habit (deVries, 2017; O'Neill et al., 2017), studies on the consequences of stock enhancement contribution to food web complexity through SIA are uncommon, although SIA is a powerful tool adopted in all ecological fields to assess changes in trophic level and food web structure (Saporiti et al., 2014; Syväranta et al., 2013). In addition, traditional stock enhancement evaluation methods (Garlock and Lorenzen, 2017; Leber et al., 2008, 2016; Lorenzen, 2014; Taylor et al., 2017; Yamashita et al., 2017) were constrained by many factors and show difficulties in disentangling any influence from the ecosystem.

Here, trophic dynamic theory, as revealed by SIA metrics, supported us in disentangling the role of stock enhancement on food web structure and species interactions (Hussey et al., 2014; Trebilco et al., 2013). Our SIA results suggest that TLs at the purple sea urchin enhancing sites did not differ from the control site and to our knowledge this is a new finding that could be of interest for managers. Indeed, we showed for the first time that stock enhancement actions may be absorbed by the recipient communities with no significant shocks of recipient trophic dynamics although there were some SI signals, which revealed that new introductions were not entirely negligible for recipient communities. This is suggested by some trophic features such as trophic diversity and niche width as revealed by isotopic variables (e.g. SEAc, MNND), which slightly (but not significantly) changed in the comparison between the stock enhancement and control (i.e. no-stock enhancement) sites. For example, trophic diversity at control was slightly larger than at stock enhancement sites (i.e. Lajia Island and Yuzhou Island, Table 2); this could be due to the influence of the purple sea urchin release. Also, the SEAc ellipse seasonally varied, suggesting that isotopic niche width in summer and autumn changed, being larger than winter and spring, although it is not easy to disentangle the influence of seasonal shifts in diet preferences on seasonal variations of SEAc (De Smet et al., 2015). Instead, seasonal homogeneity between summer and autumn and between winter and spring as shown by the SEAc summer and autumn (>0.6481) and winter and spring (>0.4921) overlaps have already been evidenced in previous studies, such as those of Brind'Amour and Dubois (2013).

Otherwise, species distribution at Sanmen Island was more even. However, De Smet et al. (2015) found that *Janicea conchilega* aggregation

did not affect trophic redundancy. The SEAc ellipses showed specific patterns for the different trophic traits. Fewer samples of omnivore/filter feeder species led to small SEAc ellipses. Larger overlap among sites for the same trophic group (Table 4, producers, herbivores and omnivores/deposit) implied that the samples at three sites had similar niche space (Brind'Amour and Dubois, 2013). The SEAc for omnivore/deposit at Sanmen Island was smaller than at the other islands, suggesting that species number, size or feeding habit variations (Rossi et al., 2015) at Sanmen Island was lower than other islands.

4.2. Feeding habit changes of the purple sea urchin

Microphytobenthos plays an important role in benthic community (Christianen et al., 2017; Moncreiff and Sullivan, 2001; Wainright et al., 2000; Zheng et al., 2015). The highest contribution of microphytobenthos to the diet of the purple sea urchin was found at three sites during seasons (about 0.57 on average). However, most research implies that sea urchins, such as *Diadema antillarum* (Lewis, 1964), *Lytechinus variegatus* (McConnell et al., 1982), *Triplaneustes gratilla* and *Salmacis sphaeroides* (Klumpp et al., 1993), *Strongylocentrotus purpuratus* (Sonnenholzner et al., 2011), *Heliocidaris crassispina* (Agassiz, 1864) (Lau et al., 2014) usually rely on macroalgae. Zheng et al. (2015) reported that the micro-benthic community utilized more epiphytes than macroalgae; the possible reason is that previous studies did not consider the surface of macroalgae-attached epiphytes. Meanwhile, Mo et al. (2017) found high POM contribution to *H. crassispina* in August. Otherwise, a faster ingestion rate and higher consumption/digestion efficiency were recorded in gastropods feeding on microphytobenthos under experimental conditions (Raw et al., 2017).

Seasonal changes of composition and abundance of macroalgae could influence the purple sea urchin's diet (Chiu, 1985, 1990). In LMS, with fewer of macroalgae species and abundance, sediment organic matter (SOM) was the main food source for the purple sea urchin; in particular, the purple sea urchin fed more on SOM at the two release sites than at control island (Sanmen Island). However, the contribution of SOM in MBS was low (0.02) at all the sites. At the food shortage areas, animals fed on alternative food sources or moved to another area with abundant food. When food was scarce, changes in feeding habits were observed as well as morphological changes in sea urchins (Ebert, 1996; Lau et al., 2009). When food was not abundant, sea urchins relied on more mobile food items, broadening their diet. This was especially significant in the purple sea urchin enhancement areas (i.e. Lajia and Yuanzhou Islands). The purple sea urchin had to feed more on SOM to substitute the microphytobenthos. No significant difference in MBS food contribution among three sites indicated that the purple sea urchin had similar feeding habits.

4.3. Implication for fishery enhancement and conservation

With ocean-ranching programs in Asia and North America, salmon stocks have increased to historical or near historical levels; however, increased competition for food results in smaller body size and diminished forage production (Cooney and Brodeur, 1998). Meanwhile, the biological attributes of enhancement species should be important in

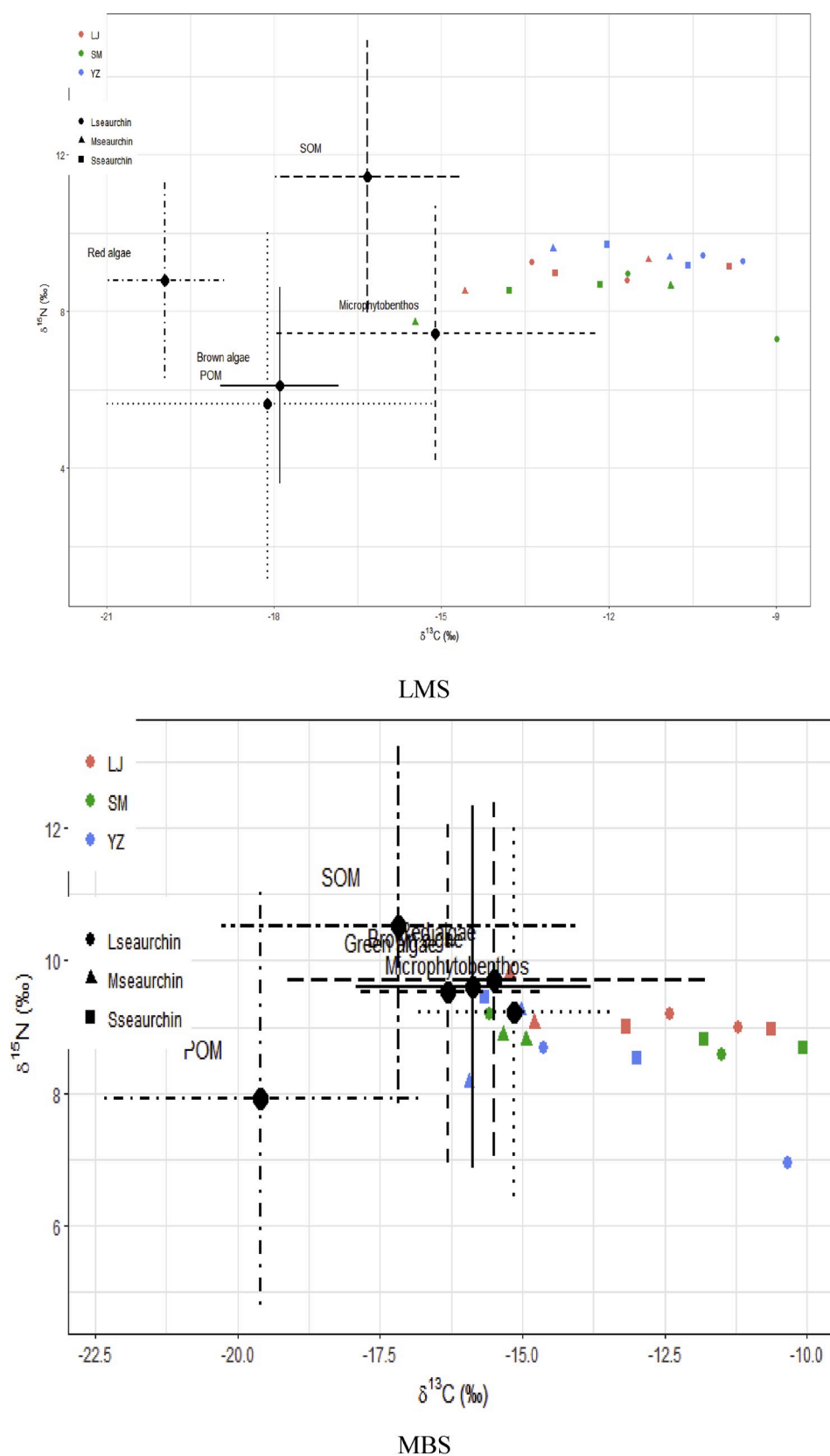


Fig. 4. Stable isotope bi-plots of the purple sea urchin and potential food sources. LMS = less macroalgae season, MBS = macroalgae bloom season. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 5

Estimated contribution of diet to the diet of consumers based on MixSIAR modelling (Burn = 200 000). Values are expressed as mean proportions with median estimates (95% CI).

Site	Size	Brown algae	Green algae	MPB	POM	Red algae	SOM
LMS							
LJ	S-Sea urchin	0.07(0,0.23)	–	0.37(0.11,0.59)	0.07(0,0.24)	0.08(0,0.27)	0.42(0.27,0.56)
	M-Sea urchin	0.07(0,0.23)	–	0.38(0.11,0.61)	0.07(0,0.26)	0.07(0,0.26)	0.4(0.27,0.54)
	L-sea urchin	0.07(0,0.22)	–	0.4(0.12,0.63)	0.07(0,0.26)	0.07(0,0.27)	0.39(0.25,0.53)
SM	S-Sea urchin	0.06(0,0.26)	–	0.55(0.07,0.82)	0.08(0,0.41)	0.06(0,0.31)	0.26(0.1,0.45)
	M-Sea urchin	0.06(0,0.28)	–	0.56(0.07,0.83)	0.08(0,0.43)	0.06(0,0.28)	0.25(0.1,0.43)
	L-sea urchin	0.06(0,0.26)	–	0.58(0.07,0.84)	0.08(0,0.44)	0.06(0,0.26)	0.24(0.08,0.42)
YZ	S-Sea urchin	0.05(0,0.2)	–	0.34(0.06,0.55)	0.05(0,0.21)	0.05(0,0.21)	0.52(0.37,0.66)
	M-Sea urchin	0.05(0,0.21)	–	0.35(0.07,0.55)	0.05(0,0.22)	0.05(0,0.21)	0.51(0.36,0.65)
	L-sea urchin	0.05(0,0.19)	–	0.37(0.07,0.58)	0.05(0,0.22)	0.05(0,0.2)	0.49(0.35,0.64)
MBS							
LJ	S-Sea urchin	0.08(0,0.97)	0.05(0,0.55)	0.72(0,1)	0.04(0,0.31)	0.1(0,0.99)	0.01(0,0.12)
	M-Sea urchin	0.1(0,0.77)	0.2(0,1)	0.6(0,1)	0.05(0,0.32)	0.04(0,0.31)	0.02(0,0.17)
	L-sea urchin	0.05(0,0.58)	0.03(0,0.3)	0.79(0,1)	0.06(0,0.5)	0.06(0,0.64)	0.01(0,0.11)
SM	S-Sea urchin	0.07(0,0.97)	0.05(0,0.56)	0.74(0,1)	0.04(0,0.37)	0.1(0,0.98)	0.01(0,0.09)
	M-Sea urchin	0.09(0,0.82)	0.19(0,1)	0.61(0,1)	0.06(0,0.42)	0.04(0,0.29)	0.02(0,0.15)
	L-sea urchin	0.05(0,0.63)	0.03(0,0.36)	0.8(0,1)	0.06(0,0.5)	0.05(0,0.65)	0.01(0,0.1)
YZ	S-Sea urchin	0.07(0,0.96)	0.05(0,0.53)	0.74(0,1)	0.05(0,0.52)	0.08(0,0.99)	0.01(0,0.09)
	M-Sea urchin	0.08(0,0.8)	0.18(0,1)	0.63(0,1)	0.08(0,0.54)	0.03(0,0.24)	0.01(0,0.12)
	L-sea urchin	0.04(0,0.37)	0.02(0,0.16)	0.81(0,1)	0.09(0,0.82)	0.04(0,0.44)	0.01(0,0.06)

Sampling site abbreviations: LJ = Lajia Island; SM = Sanmen Island; YZ = Yuanzhou Island.

influencing fisheries (Camp et al., 2013). In our study, species distribution at the release sites was more uneven than at the control site, although no significant difference was found in the food web structure among the three sites. Moreover, food limitation leads to behavioural changes and dietary diversity in many species (Berg, 1979; Mangano et al. 2017; Paulsen et al., 2017; Tinker et al., 2008; Trebilco et al., 2013). Therefore, the purple sea urchin at the release sites, as PBS abundance limited, fed more on SOM in summer and autumn. A responsible approach to stock enhancement should consider size at release, release habitat, release season, release magnitude and interactive effect of release species (Blankenship and Leber, 1995; Leber, 2013; Leber et al., 2016; Lorenzen, 2005; Lorenzen et al., 2010). In our study, we have provided evidence about density-dependent feeding habit changes/shifts. Thus, food abundance when it is scarce should be considered as a limiting factor in choosing the release density. Our results further support existing knowledge as we highlighted the potential of stable isotope analysis to evaluate and measure the success of release. SIA can overcome the difficulty of tagging and recapturing release species, thereby further showing the importance of analysing food web structure in stocking enhancement studies.

5. Conclusion

Following a purple sea urchin enhancement program implemented in Daya Bay, stocking density of the purple sea urchin increased. We observed a food habit change in summer and autumn, and that the purple sea urchin fed more on SOM at the release sites. Otherwise, species distribution at the release sites was more uneven. Our analysis provided a basis for comparing the degree of influence of fishery enhancement on coastal benthic food web. These results suggested that release density should be considered in the food-poor seasons. Our evidence underlined the need to optimize the design of release strategy in order to build a responsible release approach and maximize enhancement production. Specifically, we propose that further studies should integrate individual feeding habit and trophic position with food web structure.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

CRedit authorship contribution statement

Chuanxin Qin: Conceptualization, Formal analysis, Methodology, Writing - original draft, Writing - review & editing. **Pimao Chen:** Conceptualization. **Gianluca Sarà:** Writing - review & editing. **Baolin Mo:** Conceptualization, Data curation. **Ankai Zhang:** Conceptualization. **Xiaoguo Li:** Data curation.

Acknowledgement

The authors would like to thank Mr. Jack Botelho and Dr. Ryan Schloesser (Mote Marine Laboratory, Florida, United States) for improving the language of the manuscript. Funding for this project was provided by the National Key R&D Program of China (2018YFD0901605), the National Natural Science Foundation of China (Grand No. 41206119), the Central Public-interest Scientific Institution Basal Research Fund, the South China Sea Fisheries Research Institute, CAFS (Grand No. 2017YD04) and the China Scholarship Council. In addition, we wish to thank the anonymous reviewers who have helped to improve the paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2020.104957>.

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