Int Aquat Res (2016) 8:169–178
DOI 10.1007/s40071-016-0132-3

SHORT COMMUNICATION

Stable isotope analysis suggests the existence of multiple populations of streaked spinefoot (*Siganus javus* L.) in Bandon Bay, Southern Thailand

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Received: 16 September 2015 / Accepted: 20 April 2016 / Published online: 3 May 2016

Abstract The stock unit used in fisheries resource assessment and management is generally based on the morphological and genetic characteristics of a particular population or species to avert problems caused by the treatment of multiple populations as one stock, which can lead to the overestimation of population sizes and genetic pollution. Furthermore, since the linkage of microhabitats is an important factor affecting the reproduction of marine organisms in coastal areas, an understanding of the food web in each microhabitat is essential to establish sustainable fisheries management practices. We investigated spatial variations in the food sources and feeding habits of immature stage of *Siganus javus* using genetic population analyses and stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$). These species are commonly harvested by small-scale fisheries, and it inhabits Bandon Bay in the Surat Thani Province of Southern Thailand. Genetic variation within sampling sites was greater than that between sites. The $\delta^{13}C$ values of *S. javus* differed between sites, which suggest that the different ecological habitats exhibit different rates and patterns of carbon flow even among sites located in the same bay. Our results suggest that studies combining genetic population analyses and stable isotope analyses are required to confirm the delineation of fine-scale management units intended for the development of coastal fishery resources.

Keywords *Siganus javus* · Feeding habit · Food web · Population genetics · Stable isotope · Thailand

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Introduction

The EU and other countries, such as Japan, that are located at middle and high latitudes typically set output controls on economically valuable species to manage fishery resources. These controls are calculated using the maximum sustainable yield theory for each species (European Commission 2014; Fisheries Agency 2015). Species have generally been treated as the minimum unit of management, based on the premise that genes associated with certain ecological characteristics, such as feeding and reproductive habits, are shared within species. However, this approach of targeting species for fisheries resource assessment is sometimes difficult to conduct, and is insufficient for establishing sustainable resource management procedures (Caddy 2009).

In tropical regions, such as Southeast Asia, small-scale fisheries are important economic resources for local residents, especially in terms of food security (Pauly 2006; Béné 2006). However, small-scale fisheries are a potential impediment to effective resource management, because they can affect the accuracy of resource assessments. These fisheries are an unregulated and unmonitored form of resource use (Varkey et al. 2010) in which by-catch species go unreported and are discarded (Peckham et al. 2007) and which have low compliance rates of fisheries activities (Boonstra and Nguyen 2010). The stock assessment of fisheries resources is usually conducted by governmental bodies and based on statistical data; however, the statistical data collected from small-scale fisheries are generally incomplete because of limited budgets and the large number of target species. In terms of biodiversity, Randall and Lim (2000) reported that over 3000 species of fishes are found in the biodiversity hotspot of the South China Sea. In such a rich environment, numerous fishes are being caught by small-scale fishery activities. However, demersal fishes caught using a large fishing gear, such as mackerel gill nets and trammel nets, are caught in greater quantities in the Andaman sea, Thailand (Seilert and Sangchan 2001). Rabbitfishes (*Siganus* spp.) are valuable fish species captured by small-scale fisheries, and are commonly sold at local markets for consumption by local residents (Kohno 2001). The establishment of efficient and cost-effective assessment of fish stocks is necessary for the proper management of small-scale fisheries in tropical regions.

Genetic population analysis is one of the most powerful methods for determining genetic divergence, population structure, and evolutionary history (Begg et al. 1999; Avise 2000; Pullin 2000; Bernatchez 2001) and provides information critical for conservation and management (Takagi et al. 2010, 2011). However, metapopulation theory suggests that the genetic analysis is insufficient when attempting to identify the minimum management units of marine resources (Rosenberg et al. 2000; Thorrold et al. 2001), because the results of genetic analyses do not elucidate habitat linkages based on spatial and temporal food web structures. These ecological features have the potential to divide populations into regional or local ecological units that are smaller than the area's genetic population units are. Therefore, tropical fishery resource management should be reconsidered using both population genetics and food web structure analysis to identify concrete and rational management units for coastal fisheries resources.

We analyzed the genetic population and food web structure suggested by stable isotope measurements of streaked spinefoot, *Siganus javus* in Bandon Bay of Surat Thani Province in Thailand to identify the minimum management unit of the species in this bay. An additional aim of this study was to evaluate the efficacy of combining both genetic and stable isotope analyses in the stock assessment of fishery resources in the tropics. Siganid species inhabit the benthos in shallow water and school in proximity to rocks (Woodland 1990). *Siganus javus* is an omnivore, its diet including zooplankton, such as copepods (Yuniar et al. 2007), and seaweed (brown microalgae) (Noda et al. 2011). In addition, the analyses of otolith microelements in a related species, *Siganus fuscescens*, have revealed juvenile seasonal population changes (Soliman and Yamaoka 2010); and migration to, and habitation in, river estuaries in its early life stages (Yamada and Baba 2009). A study in Mikawa bay, Japan, indicated that the adult stage of *S. fuscescens* tends to be inhabiting around the mouth of the bay where close to the open water; however, there was no significant spatial difference in immature stage between two different sites in shoreline (Kamohara et al. 2007). *Siganus javus* primarily feeds on brown macroalgae and organic materials (Hoey et al. 2013), with the juveniles found in neritic-demersal habitats near sandstone and coral reefs where they feed on encrusted algae (Mansor et al. 1998). To identify a model for stock in narrow bodies of shallow water in the tropic, this study selected young stage of *S. javus* to examine the unit in sedentary species.

Materials and methods

Study site

Bandon Bay is located in the Surat Thani Province in Southern Thailand and has two main rivers flowing into the central part of the bay: the Tapi River basin, covering 5460 km^2, and the Phumduang River basin, covering 6125 km^2 (Paw 1988). Mean water depth in the bay is 2.9 m, and the tidal amplitude ranges from 0.7 m at neap tides to 1.9 m at spring tides (Wattayakorn et al. 2001). The shoreline of the bay and the Tapi River delta are lined with mangrove vegetation. Surat Thani Province has a tropical monsoon climate according to the Köppen Geiger system of climate classification (Peel et al. 2007), with a rainy season lasting from September to December and a dry season from January to June. Two sampling sites were selected at the mouth of Bandon Bay. The first site was located at the eastern part of the bay near an extensive oyster aquaculture zone and consisting of muddy sediment covered by microphytobenthos and lacking seagrass and seaweed habitats (site A). At this site, the water was approximately 2 m deep, with a salinity of 13.3–27.1 and temperature ranging from 28.8 to 29.7 °C. Transparency was 0.5 m in muddy color water. The other site was in the inner bay at the mouth of the Tapi River located between a mangrove tidal flat and a blood cockle aquaculture zone also consisting of muddy sediment covered by microphytobenthos and lacking seagrass or seaweed habitats (Site B). At this site, the water was approximately 1.3 m deep, with a salinity of 4.2–9.3, temperature ranging from

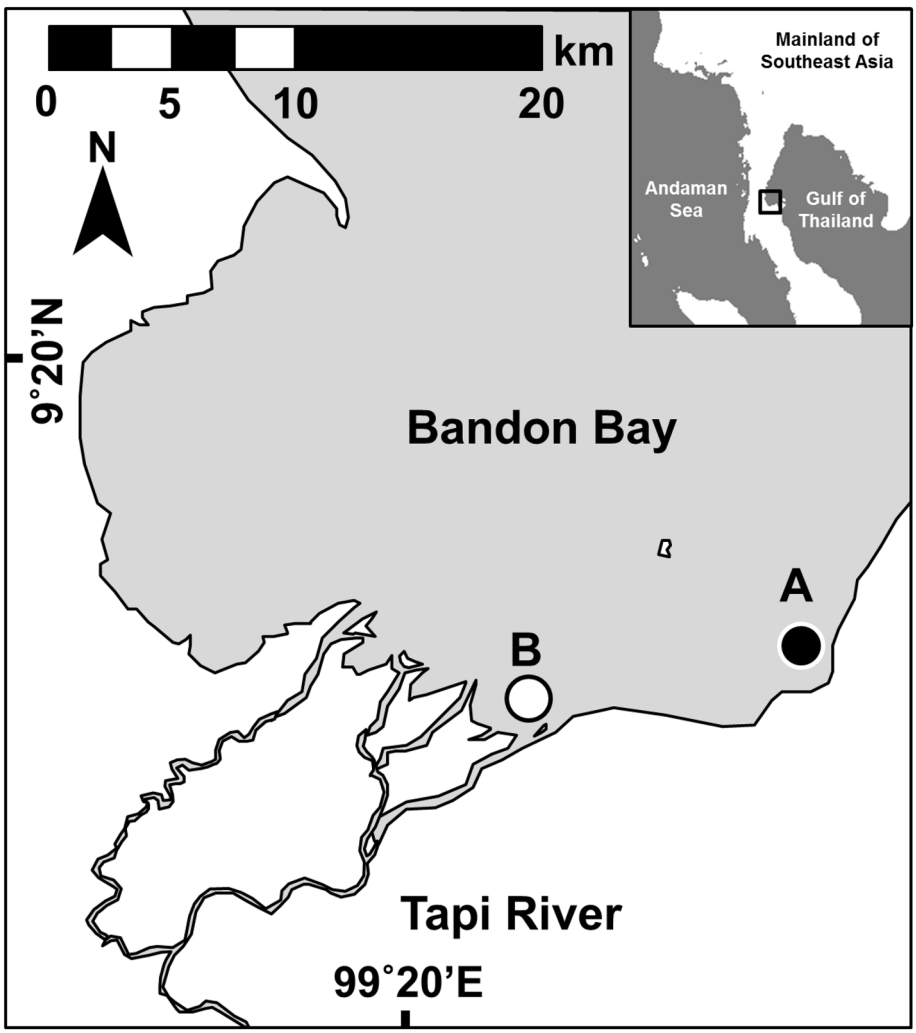

Fig. 1 Sampling sites in Bandon Bay, Surat Thani Province, Thailand

Table 1 Number (*n*), body length (mm), and values of $\delta^{13}C$ and $\delta^{15}N$ of *Siganus javus* specimens collected from Bandon bay, Surat Thani province, Thailand

	Site A				Site B			
	n	Mean body length (range ± SD)	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	*n*	Mean body length (range ± SD)	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$
March 2013	5	156.6 (143.0–168.0, ± 9.4)	−18.64 ± 0.6	11.52 ± 0.8	5	56.1 (46.0–77.1, ± 13.2)	−20.84 ± 1.2	11.43 ± 0.6
September 2013	11	102.9 (57.0–184.0, ± 44.6)	−18.61 ± 0.3	12.35 ± 0.5	7	64.4 (49.0–84.0, ± 13.6)	−17.66 ± 0.4	11.76 ± 0.6

29.3 to 32.1 °C, and transparency was 0.4 m in muddy color water. Temperature and salinity for both sites were measured using a digital thermo-salinity meter (YSI Pro2030; YSI, Yellow Springs, OH, USA).

Field sample collection

A total of 28 *S. javus* specimens were collected from two sites (A and B) in Bandon Bay in March and September 2013 (Fig. 1; Table 1). All specimens were caught by local fishermen using fish/crab traps, cast nets, or gill nets in similar/common sizes of mesh of the nets among local fishers. The body lengths (BL) of the specimens were measured on site, and muscle tissue samples were collected and dried prior to preservation. The muscle tissue of 22 specimens was used for subsequent genetic analysis, and the 28 specimens were used to conduct stable isotope analyses. In addition, zooplankton fraction particulate organic matter (ZPOM) was collected by filtering the water through 200-μm-mesh plankton net in September 2013.

Genetic analysis

We employed population genetic analysis methods to account for the possibility that specimens from sites A and B belong to different reproductive units. Twenty-two *S. javus* specimens for which tissue samples suitable for DNA extraction were available, collected from 10 and 12 specimens from sites A and B, respectively, were subjected to mitochondrial DNA (mtDNA) sequencing. The sequences were then analyzed using a method commonly employed to delineate reproductive units in a wide range of organisms (Avise 2004). Total genomic DNA was extracted from the muscle tissue samples using the Wizard® Genomic DNA Purification Kit (Promega) according to the manufacturer's protocol. Approximately 650 base pairs (bp) from the 5′ end of the cytochrome oxidase subunit I (*COI*) gene sequences of the mtDNA were amplified using a primer cocktail ["COI-3" in Ivanova et al. (2007)]. The COI-3 primer cocktail included four M13-tailed primers: VF2_t1, 5′-TGT AAA ACG ACG GCC AGT CAA CCA ACC ACA AAG ACA TTG GCA C-3′; FishF2_t1, 5′-TGT AAA ACG ACG GCC AGT CGA CTA ATC ATA AAG ATA TCG GCA C-3′; Fish R2_t1, 5′-CAG GAA ACA GCT ATG ACA CTT CAG GGT GAC CGA AGA ATC AGA A-3′; and FR1d_t1, 5′-CAG GAA ACA GCT ATG ACA CCT CAG GGT GTC CGA ARA AYC ARA A-3′ (Ivanova et al. 2007). PCR was conducted in a 8.0-μL reaction volume containing 4.0 μL of 2× EmeraldAmp PCR Master Mix (TaKaRa), 0.125 μM of each primer, and 1.0 μL of template DNA, using the following PCR program: initial denaturation at 94 °C for 2 min, followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 °C for 40 s, extension at 72 °C for 1 min, and a final extension at 72 °C for 10 min. The PCR products were purified using ExoSAP-IT (Affymetrix). DNA sequencing was performed using the Big-Dye® Terminator Cycle Sequencing Kit v.3.1 (Life Technologies) on an Applied Biosystems 3500 Genetic Analyzer (Life Technologies). The mtDNA sequences were edited using the sequence alignment editor BioEdit 7.0.5.3 (Hall 1999) and aligned using the program Clustal X (Larkin et al. 2007). Estimation of genetic structure among sampling sites, based on haplotype frequency and uncorrected genetic distances between haplotypes (Φ_{ST}), was performed using Arlequin 3.5 (Excoffier and Lischer 2010). The significance of the Φ_{ST} value was tested using 10,000 random permutations. The minimum spanning network (MSN) (Bandelt et al. 1999) of the haplotypes was constructed and implemented in PopART 1.7 (Leigh and Bryant 2015). The sequences generated in this study have been deposited in GenBank (accession numbers: LC071531-LC071552).

Stable isotope analysis

The muscles of the Streaked Spinefoots were separated at the nape using a scalpel and tweezers, then dried in an oven at 50–60 °C for 48 h, after which they were transferred to glass vials for cooling. Next, the tissues were ground into powder and transferred to tin capsules. The collected samples of ZPOM were filtered using glass microfiber filters (Whatman GF/C; GE Healthcare, Buckingamshire, UK), air-dried for 48 h, and transferred into tin capsules. The samples were analyzed using an isotope ratio mass spectrophotometer (Delta V™ Advantage; Thermo Scientific, MA, USA) coupled with an elemental analyzer (Flash EA 1112; Thermo Scientific, MA, USA) via a CONFLO III interface (ConFlo III, Thermo Scientific, Bremen, Germany) to facilitate continuous-flow analysis at the Research Institute for Humanity and Nature. The isotope ratios in the samples were calculated from a linear calibration derived from a standard (DL-α-Alanine; Wako Pure

Chemical Industries, Osaka, Japan) and a blank correction. The natural abundances of ^{13}C and ^{15}N in the samples were expressed as per mil (‰) deviations using the following equation:

$$\delta_{\text{sample}-\text{standard}} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 1000,$$

where R_{sample} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the sample, R_{standard} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio in the calibration material, and $\delta_{\text{sample}-\text{standard}}$ is the isotopic composition of the sample relative to the standard reference materials. These materials include Vienna Peedee Belemnite relative isotope-amount ratio scales for $\delta^{13}C$, which are based on NBS 19, limestone (NIST, National Institute of Standards and Technology, MD, USA) and LSVEC lithium carbonate (IAEA, International Atomic Energy Agency, Vienna, Austria), and atmospheric nitrogen measurements for $\delta^{15}N$, which are relative to IAEA-N1 (IAEA) and IAEA-N2 (IAEA). All $\delta^{13}C$ and $\delta^{15}N$, body length data were examined with a Mann–Whitney's *U* test using the Excel software to evaluate differences between sites and seasons.

Results

Body length

The average body length of *S. javus* collected at site A was 156.6 mm (SD = 9.4) in March and 102.9 mm (SD = 44.6) in September. Average BL of the specimens collected at site B was 56.1 mm (SD = 13.2) in March and 64.4 mm (SD = 13.6) in September. In both seasons, the body length of the specimens collected from site A was greater than that of specimens from site B, and there was a significant difference in body length between the two sites in March ($P < 0.01$). Seasonal differences in body length were only observed at site A ($P < 0.05$).

Genetic diversity of *S. javus* in Bandon Bay

Within the amplified region of mtDNA, 579 bp of the *COI* gene sequences from 22 specimens were successfully aligned, containing nine variable sites and defining a total of six haplotypes. The Φ_{ST} value between sampling sites (sites A and B) was negative and not statistically significant ($\Phi_{ST} = -0.073$, $P = 0.87$), indicating that genetic variation within sampling sites was greater than between sampling sites. In addition, the MSN of the haplotypes showed no separation between sampling sites, with three haplotypes being shared between them (Fig. 2). These results suggest that these specimens belong to a single gene pool detectable using its mtDNA variation.

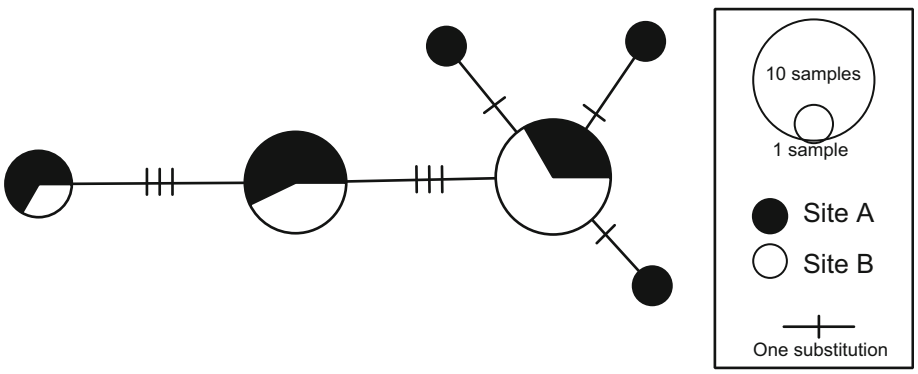

Fig. 2 Minimum spanning network of the mtDNA *COI* gene sequence haplotypes (579 bp) of *Siganus javus*. Sample size is shown by size of the circle. *Black* indicates a haplotype from the population at *site A*. *White* indicates a haplotype from the population at *site B*. *Hatch marks* show the number of substitutions between haplotypes

Seasonality and spatial differences of $\delta^{13}C$ and $\delta^{15}N$

The values of $\delta^{13}C$ ranged from −22.93 ‰ (site B) to −17.09 ‰ (site B) and varied seasonally at site B ($P < 0.01$); however, there was no seasonality in site A (Table 1). The value of $\delta^{15}N$ ranged from 10.26 ‰ (site A) to 12.95 ‰ (site A), and the value of $\delta^{15}N$ at site A exhibited slight seasonality ($P < 0.05$), while there was no seasonality in site B. In addition, the $\delta^{13}C$ values of *S. javus* collected from each site in March and September 2013 were compared, and the spatial variation among the $\delta^{13}C$ values of *S. javus* muscles was confirmed in each season ($P < 0.01$); however, there were no spatial differences in the value of $\delta^{15}N$ between sites A and B in either season. In addition, there was a spatial difference of $\delta^{13}C$ in ZPOM in September ($P < 0.05$) (Table 2).

Discussion

In general, genetic populations are recommended as the standard units of fishery resource management policies (Pullin 2000), and the importance of using genetic analyses to establish units for resource management has previously been discussed (Begg et al. 1999). The results of the genetic analysis in this study suggest that *S. javus* specimens collected from sites A and B were homogenous in both seasons in 2013, although the body length differed between sites (Fig. 2). According to ordinal management methods, the Bandon bay population of this species should be treated as a single unit based on these results. However, the results of the stable isotope analysis suggest that the specimens collected at sites A and B belong to two different food webs (Table 1). When there is a mismatch between the population assessments made by the two analyses (i.e., genetic and stable isotope), the smaller unit should be adopted as the precise assessment and management unit. In the case of Gadidae, a mismatch between the use of a genetic population as the unit for fishery resource management and the current practice of fishery resource management has been reported (Reiss et al. 2009). Such studies emphasize the importance of taking into account inter-population dynamics for the examination of genetic populations.

The results of the stable isotope and genetic analyses of this study indicate that fine-scale ecological (feeding habit) divergence could take place even within a single stock (as defined by mtDNA variation, which is a widely used genetic marker in the fisheries context) (Hauser and Carvalho 2008). The tropics are usually recognized as areas of high diversity and low population density, as compared to the middle and high latitudes. However, small-scale fisheries target mainly small fishes and demersal fishes, such as *S. javus*, which are found in specific locations or microhabitats. Such sedentary habits might lead to differentiation in characteristics, such as feeding habit, even within genetic populations. Seasonal changes in diet (Noda et al. 2002) and the diversification of diets in different populations of Siganid species have been reported (Lundberg and Golani 1995). In addition, Fox and Bellwood (2011) described differences in diel feeding patterns of a tropical reef fish, *Siganus lineatus*, observed in two habitats in two coral reefs. Therefore, the variation in $\delta^{13}C$ observed in *S. javus* is a logical reflection of regional and habitat-based feeding habits.

The value of $\delta^{13}C$ has been applied to describe how regional differences in the environment include potential foods (Marchais et al. 2013; Antonio et al. 2010). In the case of *Siganus fuscescens,* sexual maturation occurs at a body size greater than 170 mm (Katayama et al. 2009), and whereas body size differed between the two sites, the majority of our samples was at a younger stage with a body length less than 170 mm. There was no research on sexual maturation, specifically on *S. javus*, therefore, it is difficult to omit the effect of body size; however, these growth stages in Siganid species might be referable to our samples that being in similar growth stages. Therefore, our results indicate regionality in the different habitats: site A is

Table 2 Number (*n*) and values of $\delta^{13}C$ and $\delta^{15}N$ of zooplankton fraction particulate organic matter collected from Bandon bay, Surat Thani province, Thailand in September 2013

	Site A			Site B		
	n	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$	*n*	$\delta^{13}C \pm SD$	$\delta^{15}N \pm SD$
September 2013	5	−21.30 ± 0.2	8.00 ± 1.7	4	−24.76 ± 0.3	6.53 ± 2.0

characterized by seasonality, and site B is characterized by the effect of constant inflow from the rivers that limits drastic seasonal change, included the difference of body size in immature/young stage.

Regarding food sources at each site, we had complemental samples and data of $\delta^{13}C$ and $\delta^{15}N$ on zooplankton fraction particulate organic matter (ZPOM), and the value of $\delta^{13}C$ suggested difference between two sites (Table 2). These data were limited in one particular season; however, the differences of $\delta^{13}C$ in ZPOM possibly indicated the differences of the contents/composition of zooplankton. The importance of animal components in the diet of *S. fuscescens* has been reported (Shibata et al. 2010), and these differences might be triggered the differences in $\delta^{13}C$. In addition, spatial differences of Chlorophyll *a* in Bandon Bay in 2014 had been reported (Plongon and Salaenoi 2015). Thus, these variations of potential food sources may be represented the differences in $\delta^{13}C$ between the two sites.

The present study examined a limited sample of only one species of Siganidae in Bandon Bay; further studies on additional species and other sites are required for a comprehensive understanding of the population genetics and food web relationships of these species. However, our findings suggest the importance of conducting in-depth studies using a combination of genetic and carbon and nitrogen isotope analyses. Together, such analyses can be used to confirm delineations of the conventional genetic units and to reveal fine-scale regional ecological units for use in the appropriate fishery resource management of small-scale fisheries, including demersal and sedentary fishes at local sites in the tropics.

Acknowledgments The authors declare that there is no conflict of interest associated with this manuscript. We are grateful for the support of many people, especially the students of Kasetsart University, Yamagata University, and Kitasato University; the staff of the research project "Coastal Area Capability Enhancement in Southeast Asia" at the Research Institute for Humanity and Nature; the Southeast Asian Fisheries Development Center; and the local residents and fishermen in Surat Thani Province, Southern Thailand. In addition, this study was supported by the research project "Coastal Area Capability Enhancement in Southeast Asia (Research Institute for Humanity and Nature)" and a Grant-in-Aid for Scientific Research (no. 24241083) and Young Scientist (B: 26840131) from the Japan Society for the Promotion of Science (JSPS), Tokyo, Japan. In addition, we would like to thank Editage for their assistance with English language editing.

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