

# Microsatellite Markers Suggested Moderate Genetic Variation in Indian Mackerel (*Rastrelliger kanagurta*) Populations from the Andaman Sea, Thailand

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## ABSTRACT

The Indian mackerel (*Rastrelliger kanagurta*) fishery in Thailand has experienced declining catches during the past several years and was identified as over-exploited. The study obtained population genetic information to support the ongoing management of the fishery. Six microsatellite loci from short mackerel (*Rastrelliger brachysoma*) were screened to assess the genetic diversity in Indian mackerel stocks from four localities on the Andaman Sea coast. All population samples exhibited similar and moderate genetic variation with an average of 6.5 to 7.8 alleles per locus and average expected heterozygosity at all loci of 0.60 to 0.69. There was no evidence to support population substructure (overall genetic differentiation =  $F_{ST} = 0.012$ ; non-significant pairwise  $F_{ST}$  values). Gene flow was detected through the presence of rare alleles at three loci across samples. The contemporary estimated effective population size was approximately 330 if a single stock unit was considered. This moderate size of the breeding population is crucial for the short-term conservation of genetic variability in Indian mackerel stocks. Genetic data from the present study provides baseline genetic information and supports the management of Indian mackerel as a single unit.

**Keywords:** microsatellite, genetic diversity, gene flow, effective population size, population structure

## INTRODUCTION

Short mackerel (*Rastrelliger brachysoma* Bleeker, 1851) and Indian mackerel (*Rastrelliger kanagurta* Cuvier, 1817) are pelagic species of the family Scombridae, widely distributed in the tropical waters of the West Pacific and Indian Oceans (Collette and Nauen 1983). These two closely related species can be found in both the

Gulf of Thailand and the Andaman Sea. They are important food and export items for Thailand and the Southeast Asian region. Indian mackerel is the major target species for both commercial and small-scale fisheries along the Andaman Sea coast (Krajangdara *et al.*, 2007). The average annual catch of Indian mackerel was 20,000 t during 1997–2007, but decreased to 17,000 t in 2009 (Department of Fisheries, 2010). Recently, stock

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assessment based on catch-per-unit-effort data identified the status of Indian mackerel in Thailand as fully exploited with a total catch of over 30% of the maximum sustainable yield (Sumontha *et al.*, 2010). Moreover, Indian mackerel caught from the Andaman Sea were 67% smaller than the average size at first maturity. As a result, it is likely that the size of spawning stock and recruitment will be drastically reduced in the near future (Sumontha *et al.*, 2010). Further, it is not known whether Indian mackerel populations along the Andaman Sea coasts of Myanmar, Thailand, Malaysia and Indonesia are composed of one or several stocks. However, the Thai Department of Fisheries has managed the stock in Thai waters as a single unit, with no area or sector-specific allocations. The management measures include spatial and temporal closures, the reduction of fishing capacity and licensing of fishing gear.

The genetic diversity of a population is an important issue in the fisheries management of exploited species. Genetic data can provide information about the stock structure and status of the population (Bagley *et al.*, 1999; Hauser *et al.*, 2002). Genetic diversity is necessary for a species to respond to short-term and long-term environmental changes. However, despite its commercial importance, there is no information about the genetic diversity of the Indian mackerel, and the population structure of this species is mostly unknown, in particular for the Andaman Sea.

Marine species are generally believed to possess high levels of genetic diversity within populations, but to display low between-population differentiation. This is due to their large population sizes, high fecundity and extensive gene flow (Waples, 1998). However, this perception has been challenged, as an increasing number of studies have compiled evidence of the genetic structure in marine fish species over both small and large geographical scales. Therefore, the ability to detect a weak population structure is critical for fishery management plans (Zardoya *et al.*, 2004).

In the past, allozyme electrophoresis has been the standard tool in genetic studies of fish populations. However, most studies that employed allozyme markers have reported low levels of genetic variation (Menezes *et al.*, 1993). The use of DNA markers has revolutionized how the structure of populations of marine species is viewed (Waples, 1998). Although other types of genetic markers such as ISSRs (inter-simple sequence repeats) have been developed for short mackerel (Srinuangray and Piyapattanakorn, 2009), these dominant markers are not applicable for estimating the effective population size or migration between populations. Conversely, microsatellites have proven to be useful genetic markers in measuring the genetic diversity and evaluating the dynamics of populations (Bagley *et al.*, 1999; Broughton *et al.*, 2002; Chiang *et al.*, 2006, 2008).

The objectives of this study were to assess the genetic diversity and population structure among four population samples of Indian mackerel. Because microsatellite markers have not been developed for Indian mackerel, microsatellite loci from short mackerel were used in this study. Cross-species amplification of microsatellites was determined in Indian mackerel. The use of short mackerel microsatellites for population genetic analysis, was validated by testing for heterozygosity, Hardy-Weinberg expectations and linkage disequilibrium in a sample of 30 individuals of Indian mackerel from the Gulf of Thailand. The null hypothesis was tested that Indian mackerel comprised a single homogeneous population along the Andaman Sea coast of Thailand. This is the first report on genetic investigations of Indian mackerel from the region.

## MATERIALS AND METHODS

### Sample collection

A sample of 30 individuals of Indian mackerel from the Gulf of Thailand was used for the cross-species amplification and validation of short mackerel microsatellites. During 2009 and

2010, 176 individual Indian mackerel samples were collected from purse seine nets from four fishing sites along the Andaman Sea coast: Ranong (RN), PhangNga (PN), Phuket (PK) and Satul (ST) (Figure 1). To maximize the chance of detecting any genetic differentiation among four locations (if present), similarly-sized samples from these locations were collected at approximately the same time. Fin-clips were placed in 95% ethanol until DNA extraction. The DNA samples were used for assessment of genetic variation.

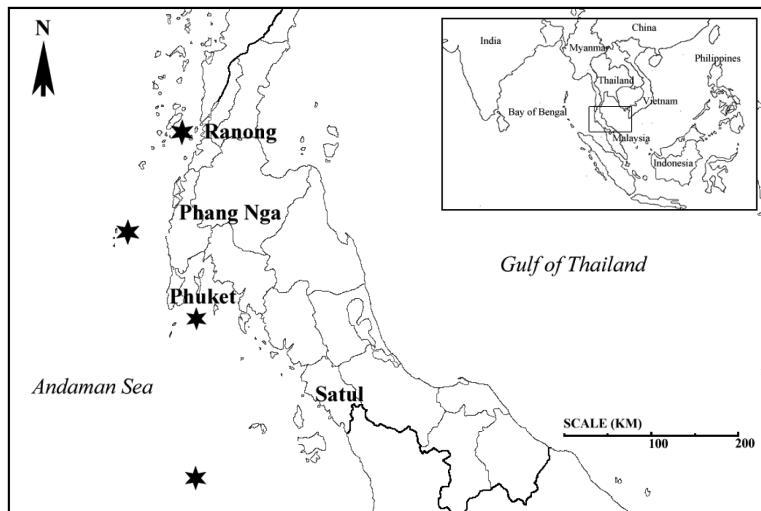
### Microsatellite genotyping

Six microsatellite loci (*Rbr-7*, *Rba-8*, *Rbr-9*, *Rbr-12*, *Rbr-13*, and *Rbr-14*) isolated from short mackerel were tested for cross amplification and validation in Indian mackerel. DNA extraction was carried out from the fin clips of the Indian mackerel samples using a standard phenol-chloroform extraction procedure (Taggart *et al.*, 1992). The polymerase chain reaction (PCR) was performed in a 10 µL reaction volume which contained 20 ng of genomic DNA, 5× PCR buffer (10× *Taq* buffer with (NH4)2SO4), 1.5 mM MgCl2, 200 µM of dNTPs, 0.5 µM of each primer and 0.1 U of *Taq* DNA polymerase (Fermentas; Vilnius, Lithuania). The PCR profile was: initial denaturation at 95 °C for 5 min; 35

cycles at 95 °C for 30 s, 60 °C for 45 s and 72 °C for 1 min; followed by 1 cycle at 72 °C for 7 min in a PTC-100 Programmable Thermal Controller (MJ Research; Waltham, Massachusetts, USA). The annealing temperature was set at 60 °C for all six loci. Following amplification, the reaction products were mixed with sequencing dye (0.1% bromphenol blue, 0.1% xylene cyanol, 2% of 0.5 M EDTA pH 8.0 and 98% formamide). The reaction mixtures were heated for 5 min at 95 °C and subjected to electrophoresis on a 4.5% denaturing polyacrylamide gel in 1× TBE running buffer at 60 W for 2–3 h in a sequencing gel apparatus (Bio-Rad; Hercules, California, USA). The gels were denatured at 100 °C for 30 min before electrophoresis. The DNA bands in the gels were visualized by silver staining. Allele sizes were estimated by comparison to an M13 sequence ladder.

### Data analysis

To validate the use of short mackerel microsatellite loci, estimates of genetic variation, including observed/expected heterozygosities and genotypic disequilibrium were calculated based on the allele frequencies of 30 individuals of Indian mackerel using the computer program GENEPOP version 4.0 (Rousset, 2008).



**Figure 1** Sample collection locations (★) for Indian mackerel along the Andaman Sea coast.

The program MICRO-CHECKER version 2.2.0 (Van Oosterhout *et al.*, 2004) was used to test for the presence of null alleles in four population samples of Indian mackerel. Genetic variation within a population including the mean number of alleles per locus (A), allelic richness ( $A_r$ ) which is allele diversity independent of sample size, and observed ( $H_o$ ) and expected ( $H_e$ ) heterozygosities were calculated using GENEPOL version 4.0. Hardy-Weinberg expectations were tested by the exact  $P$  values and calculated by a Markov chain randomization method (Guo and Thompson, 1992) using ARLEQUIN version 3.0 (Excoffier *et al.*, 2005), which was also used to calculate the pair-wise  $F_{ST}$  (genetic differentiation) values and  $P$ -values between all pairs of samples. The number of migrants between population pairs was estimated using POPGENE V. 1.31 (Yeh *et al.*, 1997).

The single-sample effective population size based on the linkage disequilibrium method was estimated using NeEstimator version 1.3 (Ovenden *et al.*, 2007). The gene flow between pairs of populations based on the frequencies of private alleles was calculated using GENEPOL. The program STRUCTURE version 2.3.4 (Pritchard *et al.*, 2000) was used to assign individuals into  $K$  clusters, without any prior knowledge of the population membership. The likelihood values suggested by STRUCTURE for each  $K$  were relied on to determine whether the  $K$  value was likely to be more than one. If population structure exists, the likelihood values should show some marked changes between successive  $K$  values. Twenty independent runs for  $K = 1$  to 6 were performed using 100,000 iterations after a burn-in period of 25,000 runs. The distribution of  $\Delta K$  (an ad hoc statistic based on the rate of change in the log probability of data between successive  $K$  values) was examined and the  $K$  value most likely to explain the population structure is the modal value of this  $\Delta K$  distribution (Evanno *et al.*, 2005).

## RESULTS

All six microsatellite loci of short mackerel were cross amplified with Indian mackerel samples (Table 1). The number of alleles per locus ranged from three to 14. The observed heterozygosities ranged from 0.17 to 0.96 and the expected heterozygosities ranged from 0.16 to 0.91. All loci conformed to Hardy-Weinberg expectations. There was no significant linkage disequilibrium for all pairs of loci ( $P > 0.003$  after Bonferroni correction).

A total of 176 Indian mackerel individuals were genotyped at six microsatellite loci (Table 2). All four population samples exhibited similar and moderate genetic variation with the average number of alleles per locus varying from 6.50 (ST) to 7.83 (PK) and average allele richness from 4.97 (RN) to 5.14 (ST). The average observed heterozygosities across loci ranged from 0.54 (PN and ST) to 0.56 (RN and PK) and the average expected heterozygosities ranged from 0.60 (PK) to 0.70 (ST). The average observed heterozygosities were lower than expected in all populations. However, neither allele richness nor the expected heterozygosities differed significantly ( $P > 0.05$ ) across all populations. None of the samples showed indications of null alleles at any loci. Significant departures from Hardy-Weinberg expectations were observed at locus *Rbr-7* in the PN population, locus *Rbr-8* in the PK samples and locus *Rbr-14* in the RN population, using the criterion  $P < 0.0083$  after Bonferroni correction for multiple comparisons. Significant ( $P = 0.0083$ ) deviations at the three loci displayed heterozygote deficiencies.

The overall estimate of  $F_{ST}$  (0.012) indicated that genetic differentiation among stocks was very low, that is, only 1% of the detected variation arose from between-population differences and 99% was within population differentiation. All population pairwise comparisons exhibited non-significant  $F_{ST}$

**Table 1** Cross-species amplification of short mackerel *Rastrelliger brachysoma* microsatellite loci in Indian mackerel *R. kanagurta* (N = 30).  
Annealing temperature was set at 60 °C for all six loci.

Locus	GenBank Accession No.	Primer sequences 5'-3'	Repeat motif	Size range (bp)	Number of alleles	$H_O$	$H_E$	P value
<i>Rbr-7</i>	JX488046	F: CCACCCACTACCTGGCAGTC	[TC] <sub>14</sub>	154–160	4	0.60	0.60	0.84
		R: ATGTGCTGGAGCCGCTAAC						
<i>Rbr-8</i>	JX488047	F: GCAACGTTCAAGACACTCAC	[GA] <sub>7</sub>	158–172	7	0.65	0.78	0.11
		R: GAAGGCATCACAACTCCTATCAC						
<i>Rbr-9</i>	JX488048	F: CACTCCCTGAATCTAGAAAGTG	[TG] <sub>9</sub>	206–212	3	0.17	0.16	0.67
		R: CAGCTGCTCCCAGAGATG						
<i>Rbr-12</i>	JX488049	F: ACAAGCCCCGGGGTGTGAC	[TG] <sub>8</sub>	158–164	3	0.17	0.21	0.33
		R: TGCTCCCCAGAGATGAGGTG						
<i>Rbr-13</i>	JX488049	F: ACCTCATCTCTGGGAGCAG	[CA] <sub>5</sub> CCCG[CA] <sub>10</sub>	172–220	14	0.96	0.91	0.69
		R: GTCTGTGAGTCGTAGCATG						
<i>Rbr-14</i>	JX488054	F: ACGTAGAAAGAGCACGCACAG	[CA] <sub>7</sub>	198–214	6	0.50	0.79	0.08
		R: TTTCAGCCCTGGTCACTTGC						

$H_O$  = Observed heterozygosity;  $H_E$  = Expected heterozygosity.

P values for test of Hardy-Weinberg expectations were adjusted for multiple comparisons using the Bonferroni correction.  $P < 0.0083$  (0.05/6)

values ( $P > 0.05$ , Table 3). Estimates of migrants per generation ranged from 4 between PK and ST and between PN and ST to 10 between PN and PK. The effective population size estimated over all four stocks was about 335 individuals (95% confidence intervals (CI): 165–3,233).

Clustering analysis in STRUCTURE revealed that all individuals were grouped together in one cluster (Figure 2). Log likelihood was highest at  $K = 1$ . In addition, the values of  $\Delta K$  changed only slightly between successive  $K$  values.

**Table 2** Genetic variability at six microsatellite loci in four populations of Indian mackerel, including sample size ( $N$ ), total number of alleles ( $A$ ), allelic richness ( $A_r$ ), observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), fixation index ( $F_{is}$ ), and  $P$  value for test of Hardy-Weinberg expectations (HWE).

Population (N)	Locus						Average across loci
	<i>Rbr-7</i>	<i>Rbr-8</i>	<i>Rbr-9</i>	<i>Rbr-12</i>	<i>Rbr-13</i>	<i>Rbr-14</i>	
RN (48)							
A	5	7	5	4	16	8	$7.50 \pm 4.42$
$A_r$	3.80	5.06	2.74	2.58	9.93	5.71	$4.97 \pm 3.23$
$H_o$	0.66	0.72	0.26	0.26	0.87	0.61	$0.56 \pm 0.25$
$H_e$	0.64	0.76	0.26	0.29	0.92	0.78	$0.61 \pm 0.27$
$F_{is}$	-0.03	0.05	-0.02	0.09	0.04	0.23	0.07
HWE	0.9156	0.3311	0.4855	0.3750	0.2748	0.0001	
PN (41)							
A	3	8	6	4	12	7	$6.66 \pm 3.20$
$A_r$	2.99	6.60	3.22	2.89	8.67	5.53	$4.98 \pm 2.36$
$H_o$	0.36	0.74	0.22	0.28	1.00	0.63	$0.54 \pm 0.30$
$H_e$	0.64	0.83	0.29	0.30	0.88	0.78	$0.62 \pm 0.26$
$F_{is}$	0.45	0.12	0.24	0.08	-0.14	0.20	0.14
HWE	0.0003	0.5537	0.0785	0.0657	0.2299	0.0688	
PK (35)							
A	4	6	4	4	21	8	$7.83 \pm 6.66$
$A_r$	3.56	5.28	2.50	2.67	10.45	6.27	$5.12 \pm 2.74$
$H_o$	0.71	0.76	0.22	0.22	0.78	0.64	$0.55 \pm 0.26$
$H_e$	0.61	0.78	0.24	0.27	0.92	0.81	$0.60 \pm 0.28$
$F_{is}$	-0.16	0.03	0.07	0.18	0.16	0.22	0.08
HWE	0.5003	0.2816	0.0792	0.1001	0.0051	0.1000	
ST (52)							
A	7	8	4	4	9	7	$6.50 \pm 2.07$
$A_r$	6.00	6.59	2.81	2.88	7.41	4.97	$5.14 \pm 2.05$
$H_o$	0.78	0.57	0.32	0.38	0.64	0.59	$0.54 \pm 0.16$
$H_e$	0.86	0.81	0.46	0.46	0.84	0.74	$0.69 \pm 0.18$
$F_{is}$	0.10	0.31	0.30	0.17	0.25	0.21	0.22
HWE	0.4029	0.0001	0.1525	0.4748	0.1005	0.1999	

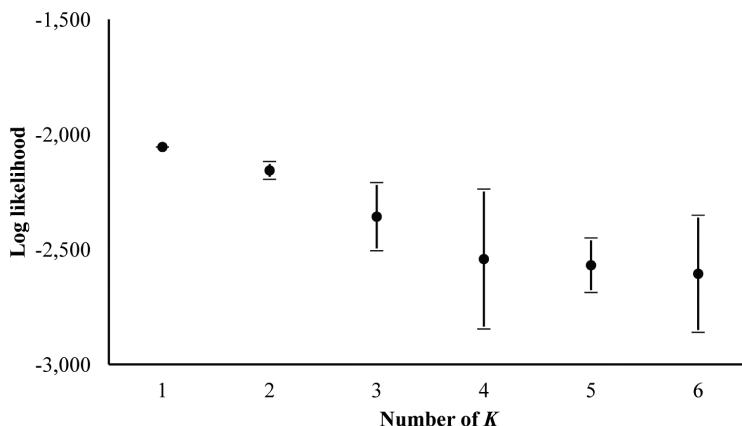
RN = Ranong; PN = PhangNga; PK = Phuket; ST = Satul.

$P$  values were adjusted for multiple comparisons using the Bonferroni correction:  $P < 0.0083$  (0.05/6).

**Table 3** Pairwise genetic differentiation (above diagonal) from program Arlequin V.3.11 and estimated number of migrants between four Indian mackerel populations (below diagonal) from the program POPGENE V.1.31.

	RN	PN	PK	ST
RN	***	0.0049	0.0052	0.0192
PN	5	***	0.0034	0.0193
PK	7	10	***	0.0036
ST	6	4	4	***

RN = Ranong; PN = PhangNga; PK = Phuket; ST = Satul. \*\*\* = Not applicable.



**Figure 2** Mean (closed circles) and standard deviation (vertical lines) of log likelihood values for  $K$ , the number of putative clusters of Indian mackerel calculated using the STRUCTURE program.

## DISCUSSION

The Andaman Sea stocks of Indian mackerel exhibited moderate levels of genetic variability within populations and no population structure ( $F_{ST} = 0.012$ ). The four stocks were very similar in terms of allelic distribution and the amount of heterozygosity. The results suggested that a lack of genetic differentiation was likely due to the impact of migration. For marine species, migration can have a substantial impact on the extent of population substructure (Hellberg, 2009). It is believed that adult Indian mackerel migrate from coastal waters to spawn in the open sea where sufficient food is available and environmental conditions are optimal (Collette and Nauen, 1983). Individual fish may spawn five

times or more per year and release multiple batches of 25,000 eggs per spawn (Sumontha *et al.*, 2010). Indian mackerel are found to spawn year round, peaking in two phases, from December to March and from August to September (Krajangdara, 2007), and they are believed to spawn in the open sea from Ranong to Satul provinces. The migratory behavior of Indian mackerel may lead to a congregation of fish from different geographical origins. To avoid sampling of individuals from the population mixture, Indian mackerel samples from the four locations were collected at about the same time. The samples of Indian mackerel in this study appeared to represent fish of a similar age, based on length-frequency distributions (data not shown). This lack of population structure also was supported by non-significant ( $P > 0.05$ ) pairwise

$F_{ST}$  values and population assignment analysis. All four samples were assigned to the same cluster with the highest probability. For the few studies that have assessed the genetic stock structure of Indian mackerel in Peninsular India and the Andaman Sea (Menezes *et al.*, 1993; Jayasankar *et al.*, 2004), there were no genetic differences reported among populations.

The genetic connection between the four sampled sites is further demonstrated by the number of migrants estimated between populations. Gene flow can be expressed as the product of the effective population size and the fraction of migrants per generation (Hedrick, 2005). Alternatively, gene flow can be determined from the number of migrants using rare allele frequencies from samples of different populations (Barton and Slatkin 1986). Alleles that are relatively rare overall but common in a few populations suggest these populations are connected by gene flow (Hellberg, 2009). The current results indicated that rare alleles were present at loci *Rbr-7*, *Rbr-8*, and *Rbr-13* with frequencies ranging from 0.003 to 0.009 in all samples. The estimated exchange of individuals varied from 4 to 10 across neighboring populations. Migrating Indian mackerel are believed to cover over 100 km (Krajandara *et al.*, 2007). Studies in marine species have indicated that the relationship between the gene flow and distance varies at different geographic scales. At small spatial scales, movement among populations is equally likely and there will be no relationship between the gene flow and distance (Hellberg, 2009). In the present study, it was likely that a lack of association between the gene flow and distance was due to the relatively small area that was studied, where the distance between sampling sites ranged from 140 to 500 km. This observation also was reported for stocks of spotted mackerel (*Scomber australasicus*) off Taiwan (Tzeng, 2007). In other studies of pelagic fishes, there was no evidence of a migration pattern even over large geographical scales for king mackerel (*Scomberomorus cavalla*) in the

western Atlantic Ocean and the Gulf of Mexico (Broughton *et al.*, 2002), for chub mackerel (*Scomber japonicus*) across the Mediterranean Sea and Atlantic Ocean (Zardoya *et al.*, 2004) and for bigeye tuna (*Thunnus obesus*) in the Pacific and the Indian Oceans (Chiang *et al.*, 2006, 2008).

Reductions in the effective population size ( $N_e$ ), which are crucial for maintaining variation, adaptation and survival of populations have been documented in commercial species of marine fish. For example, overfishing was reported to be a major factor causing a significant reduction in the effective population size and reduced genetic diversity in an exploited stock of New Zealand snapper *Pagrus auratus* (Hauser *et al.*, 2002). It is understood that the number of fish in the population is much larger than  $N_e$  because of overlapping generations, skewed sex ratios and the variation in the reproductive success of breeders (Bagley *et al.*, 1999). The effective size of a population can be estimated for the contemporary (local) or long-term time frame. While the long-term  $N_e$  is important for the maintenance of genetic variation and adaptability, the local  $N_e$  is crucial for determining the impact of short-term threats, such as genetic drift and inbreeding (Luikart *et al.*, 2010). Because the contemporary  $N_e$  is more relevant to the current status of Indian mackerel stocks, the present study estimated local  $N_e$  using the linkage disequilibrium (one-sample) method. If a single demographic unit of Indian mackerel were assumed, the estimated effective population size would be moderate ( $N_e = 336$ ; 95% CI: 165-3233) based on the minimum size of 50 individuals for the short-term conservation of genetic diversity (Nelson and Soulé, 1987). The current findings imply that the Indian mackerel stocks seem in little danger of inbreeding and genetic drift. To obtain reasonably precise estimates of  $N_e$  for exploited migratory fish species, researchers may need to sample repeatedly over the course of the spawning period (Hallerman, 2003). However, it would be useful to obtain fisheries data on the spawning stock biomass to estimate the census population

size ( $N$ ) and to obtain the ratio of  $N_e:N$ . Low  $N_e:N$  ratios appear to be characteristic of highly fecund species with high juvenile mortality and the information provides powerful insight into spawning behavior and recruitment of marine species (Hellberg, 2009; Waples and Do, 2010).

## CONCLUSION

This study demonstrated that microsatellite markers developed from short mackerel were useful for estimating the genetic diversity and population structure of its congener, Indian mackerel. Moreover, these estimated genetic parameters were used to reveal the stock structure, migration pattern and effective breeding size of the population. The results suggest that the Andaman Sea stocks of Indian mackerel constitute a single population with moderate levels of genetic variation. These findings also support a management strategy by the Thai Department of Fisheries for Indian mackerel to be considered as a single stock. The effective population size estimate of these Andaman Sea samples was about 330 based on a single stock unit, although a more precise estimate would be needed. The present study provides baseline information on the genetic component of the Indian mackerel. This genetic data can be integrated with population dynamic data to establish the stock extent and composition for the effective management of the fisheries. In addition, sampling across temporal scales would be useful for monitoring genetic changes in the long-term. Sampling over the full extent of the species' range from Africa and the Red Sea in the west to the Pacific islands and Japan in the east would yield a complete picture of any geographic genetic stock structure.

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