

## GENETIC VARIATION AND POPULATION STRUCTURE IN THE HUMPBACK GROUPER, *Cromileptes altivelis*, TROUGHOUT ITS RANGE IN INDONESIAN WATERS<sup>\*)</sup>

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### ABSTRACT

Starch gel electrophoresis was used to assess the level and distribution of genetic variation in humpback grouper, *Cromileptes altivelis* sampled from 4 different areas of coral reefs in Raas and Kangean located in Madura as well as Sangeang and Bungin located in Sumbawa regions, between December 1997 and March 1998. Fifteen loci detected from eleven enzyme systems examined. Three loci: *ADH\**, *GPI\** and *IDH-1\** were polymorphic at 95% level. These three loci were in Hardy-Weinberg equilibrium at all samples and heterozygosities ranging from 0.058 to 0.089 with an average of 0.071. No significant difference in allele frequencies between samples within region was detected, but significant differences were revealed between regions. The UPGMA cluster analysis of genetic distance showed that, the population of Madura region was clearly distinct from Sumbawa region and might not belong to one panmictic population.

**KEYWORDS:** *Cromileptes altivelis*, genetic variation, population structure.

### INTRODUCTION

Groupers are popular marine food fish of high market value in many parts of the world including South East Asia, China, Taiwan, Japan, Australia, Mexico and Kuwait. Among these groupers, the humpback grouper, *Cromileptes altivelis* is the most expensive species (Pawiro, 1999)

In juvenile stage, (total length of 5-10 cm), humpback grouper has a high demand for aquarium fish, and in adult size (body weight of 600-1300 g) has high price and potential market in Hong Kong, Southern China and Taiwan. In Indonesia at present the price of juvenile grouper is US\$ 1.0/fish and in Singapore US\$ 3-7/fish, while bigger size and live fish is sold for US\$ 62-87/Kg.

Most of the humpback grouper for both aquarium and consumption are caught from the wild. In some areas this species is rare as a result of over exploitation. Recently, Gondol Research Institute for Coastal Fisheries has successfully mass-produced the juveniles of humpback grouper. The seed production techniques of this species have been applied to backyard hatcheries. There is a growing interest in grouper culture using net cages and restocking in over-exploited area to develop an export industry in Indonesia (Sugama *et al.*, 1999). Because of its economic importance, genetic variation and population structure of this fish need to be studied in more depth throughout its natural

range. Several papers have emphasized on the important of maintaining the genetic variation in cultured stock of fish (Allendorf & Phelps, 1980; Cross & King, 1983; Kincaid, 1983; Taniguchi *et al.*, 1983; Vuorinen, 1984; Sbordonni *et al.*, 1986; Sugama *et al.*, 1992). Loss of genetic variation in hatchery stocks of salmonid and penaeid shrimp have produced harmful effects on a variety of important traits such as fecundity, survival, growth rate, food conversion efficiency and body shape (Leary *et al.*, 1985; Sbordonni *et al.*, 1986). Furthermore, the loss of genetic variation may reduce the ability of fish to adapt to different environmental conditions. These problems are especially important for hatchery bred juveniles, that are used in restocking programs (Ryman & Sthal, 1980; Benzie, 1998).

Knowledge of the genetic structure of wild fish stocks will be vital for sound long-term management. If local strains exist, they are likely to be adapted to local conditions and will have better performance than imported stock. Significant genetic differentiation among population of marine fish has been noted in many studies (Taniguchi & Sugama, 1990; Benzie *et al.*, 1992; Ohtani *et al.*, 1997; Foss *et al.*, 1998; Castilho & McAndrew, 1998) but no published data on genetic variability and population genetic structure of humpback grouper is available so far.

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This study aimed to use allozyme electrophoresis to describe the genetic variability and genetic structure of humpback grouper population throughout its natural range. Data on genetic variation of this species may identify new sources of spawners for seed production in hatcheries.

### MATERIALS AND METHODS

Wild population of humpback grouper, *C. altivelis* were collected from 4 areas (Sangeang and Bungin coral reefs in Sumbawa Island and Kangean and Raas coral reefs in Madura Island (East Java) (Figure 1 and Table 1). The fish were transported alive to the Gondol Research Institute for Coastal Fisheries Laboratory. After dissection,

tissue samples (muscle, liver, heart and eye) were stored at  $-30^{\circ}\text{C}$  until used.

Enzyme variation was assayed by horizontal starch gel electrophoresis (Taniguchi *et al.*, 1983; Taniguchi & Sugama, 1990). The list of enzymes examined, loci detected, buffer and tissue specificity are given in Table 2. Gels were prepared as 12% suspensions of hydrolyzed starch (Sigma S-4501). Gel electrode buffer used were Tris citric acid pH 8.0 (TC-8) and Citric acid aminopropyl morpholine pH 7 (CAMP-7) (Taniguchi & Sugama, 1990).

Loci and alleles were scored by a nomenclature system as outlined by Shaklee *et al.* (1990). Loci were designated consecutive numbers with 1 denoting the fastest anodal migratory system.



Figure 1. Sampling locations of humpback grouper, *Cromileptes altivelis*.

Table 1. Source and number of humpback grouper, *Cromileptes altivelis*, analyzed genetically (see Fig. 1 for geographical location of sampling sites).

Location	Sampling date	Sample size (ind.)	Range of BW (g)
- Sangeang	December '97	50	128.4 - 248.0
- Bungin	January '98	60	173.5 - 312.2
- Kangean	January '98	80	154.6 - 264.4
- Raas	March '98	40	213.3 - 330.8

Allelic variations were designated according to their relative mobility. The most common allele is designated 100 and other alleles were given numbers that indicated their mobility relative to that of the common allele. Cathodal systems were designated in a similar way but were given negative signs.

Allele frequencies at each polymorphic locus were tested for homogeneity or heterogeneity between locations with  $\chi^2$  test or G-test (Sokal & Rohlf, 1981). Genetic relatedness among localities were estimated by Nei's (1972) genetic distance values was constructed using the UPGMA method

of Sneath & Sokal (1973). BIOSYS-1 (Swofford & Selander, 1981) was used to confirm genotypic frequencies to Hardy-Weinberg equilibrium as well as average heterozygosities.

## RESULTS

A survey of 11 enzyme systems has produced 15 consistently scorable loci (Table 2) of these *ADH\**, *GPI\** and *IDH\** were polymorphic ( $P_{0.95}$ ) (Table 3). The remaining 12 putative loci showed no detectable variation. The electrophoretic banding pattern of 3 polymorphic loci was presented in Figure 2. The percentage of polymorphic loci using

Table 2. List of enzymes examined, locus detected, tissue and buffer specificity in humpback grouper, *Cromileptes altivelis*.

Enzymes	Locus	Tissue <sup>1</sup>	Buffer <sup>2</sup>
Aspartate dehydrogenase	<i>AAT*</i>	L	TC-8
Alcohol dehydrogenase	<i>ADH*</i>	L	TC-8
Esterase	<i>EST*</i>	L	CAMP-7
Glucosephosphate isomerase	<i>GPI*</i>	M,L	CAMP-7
$\alpha$ -Glycerophosphate dehydrogenase	<i><math>\alpha</math>-GPD*</i>	M	CAMP-7
Isocitrate dehydrogenase	<i>IDH-1*</i>	M,L	CAMP-7
	<i>IDH-2*</i>	M,L	CAMP-7
Lactate dehydrogenase	<i>LDH-1*</i>	M,E	CAMP-7
	<i>LDH-2*</i>	M,E	CAMP-7
Malate dehydrogenase	<i>MDH-1*</i>	M,L,H	CAMP-7
	<i>MDH-2*</i>	M,L,H	
	<i>MDH-3*</i>	M,L,H	
Malic enzyme	<i>ME*</i>	M	CAMP-7
6-Phosphogluconate dehydrogenase	<i>6-PGD*</i>	L,H	CAMP-7
Phosphoglucomutase	<i>PGM*</i>	M,L	CAMP-7

1. M= Muscle; L= Liver; H= Heart; E= Eye

2. TC-8= Tris-Citric Acid Buffer pH 8; CAMP-7= Citric Acid-aminopropyl Morpholine Buffer pH 7.

Table 3. Allele frequencies, heterozygosities ( $H$ .) and number of allele per locus ( $Na$ .) at polymorphic loci in humpback grouper, *Cromileptes altivelis*,  $N$ = number of individual examined. See Figure 1 for locations.

Locus	Alleles	Location			
		Sangeang (N=50)	Bungin (N=60)	Kangean (N=80)	Raas (N=40)
<i>ADH</i> *	- 75	0.300	0.150	0.150	0.150
	-100	0.660	0.767	0.850	0.850
	-125	0.040	0.083	0.000	0.000
<i>GPI</i> *	100	0.530	0.550	0.575	0.525
	70	0.470	0.450	0.425	0.475
<i>IDH-1</i> *	100	0.780	0.800	0.919	0.937
	80	0.220	0.200	0.081	0.063
$H^1$		0.089	0.077	0.059	0.058
$Na^2$		1.267	1.267	1.200	1.200

<sup>1</sup>  $H$ . = Average observed heterozygosity over 15 loci

<sup>2</sup>  $Na$ . = Average number of alleles per locus over 15 loci

$P_{0.95}$  criteria was 20.0%, average heterozygosities among samples ranged from 0.058 (Raas, Madura) to 0.089 (Sangeang, Sumbawa) and number of allele per locus ranged from 1.200 (Kangean, Madura) to 1.267 (Bungin, Sumbawa). No significant deviation from Hardy-Weinberg expectations was observed in any sample.

Testings for heterogeneity ( $c^2$ ) of all possible pairwise comparisons of localities for main alleles at each locus were conducted. Of the 18 comparisons (3 loci x 6 pair wise comparisons), six were statistically significant (Table 4). The significantly different loci were *ADH*-100\*, between Sangeang-

Kangean ( $c^2_{df=1} = 12.82$ ,  $p < 0.01$ ) and Sangeang-Madura ( $c^2_{df=1} = 8.43$ ,  $p < 0.01$ ), and *IDH-1* 100\* between Sangeang-Kangean ( $c^2_{df=1} = 10.21$ ,  $p < 0.01$ ), Sangeang-Raas ( $c^2_{df=1} = 8.59$ ,  $p < 0.01$ ), Bungin-Kangean ( $c^2_{df=1} = 8.47$ ,  $p < 0.01$ ) and Bungin-Raas ( $c^2_{df=1} = 7.26$ ,  $p < 0.01$ ). There were no significant differences between Sangeang and Bungin and between Kangean and Raas samples at any locus.

Genetic distance values (Nei, 1972) ranged from 0.0002 between Kangean-Raas to 0.0044 between Sangeang-Raas (Table 5).

Table 4. Chi-Square ( $c^2$ ) values of heterogeneity test of each polymorphic locus between locations of humpback grouper, *Cromileptes altivelis*. (See Figure 1 for locations).

Location	d.f.	Locus, Allele		
		<i>ADH</i> -100*	<i>GPI</i> -100*	<i>IDH-1</i> -100*
Sangeang-Bungin	1	3.09	0.09	0.13
Sangeang-Kangean	1	12.82**	0.51	10.21**
Sangeang-Raas	1	8.43**	0.00	8.59**
Bungin-Kangean	1	3.12	0.17	8.47**
Bungin-Raas	1	2.07	0.12	7.26**
Kangean-Raas	1	0.00	0.54	0.25

\*\*  $p < 0.01$

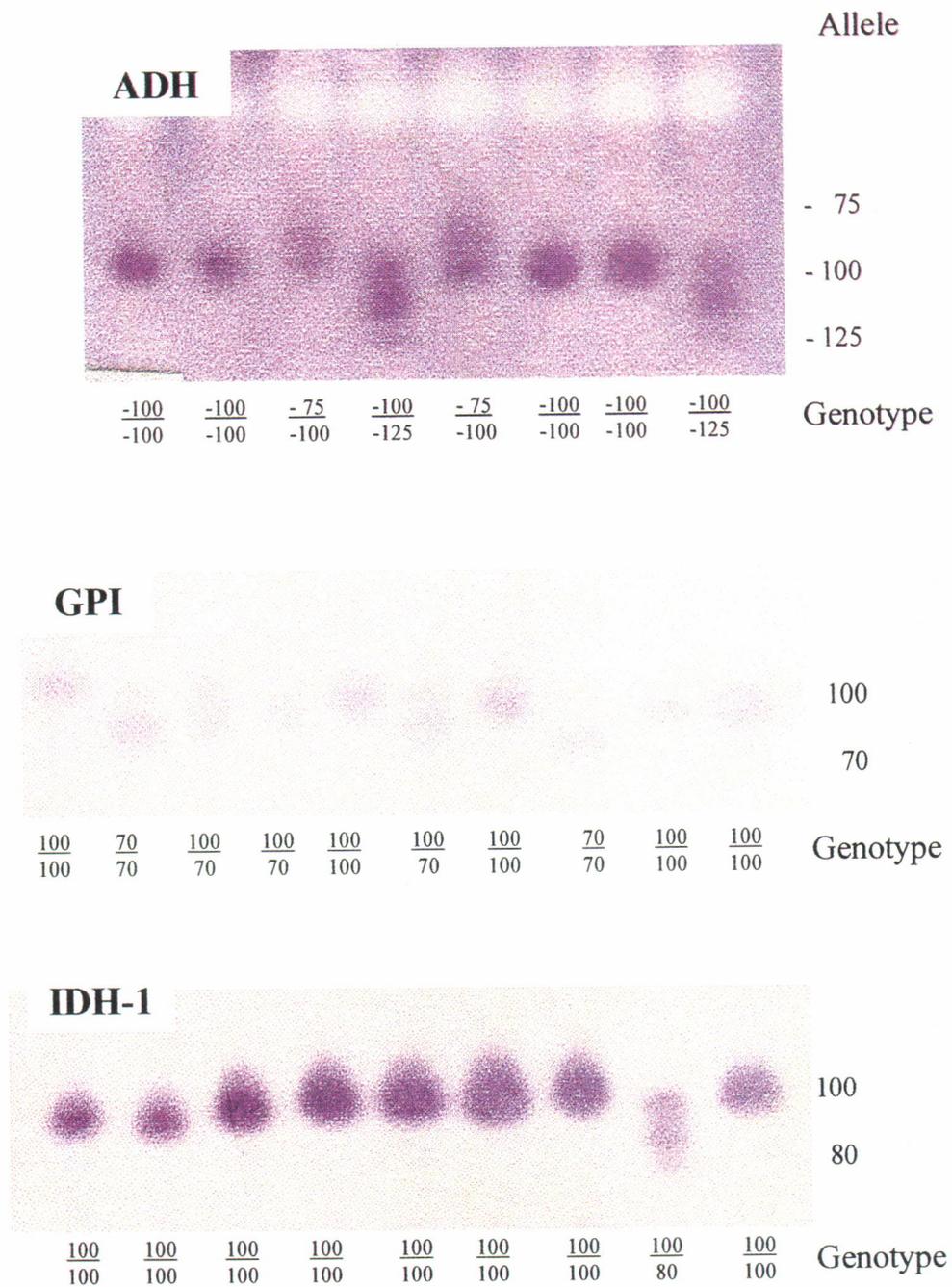


Figure 2. Electrophoretic banding pattern of polymorphic loci (*ADH\**, *GPI\** and *IDH-1\**) in humpback grouper, *Cromileptes altivelis*.

Table 5. Nei's (1972) genetic distances between humpback grouper, *Cromileptes altivelis* populations, based on 15 loci examined. (See Figure 1 for locations).

Location	Sangeang	Bungin	Kangean	Madura
Sangeang	-			
Bungin	0.0016	-		
Kangean	0.0041	0.0017	-	
Raas	0.0044	0.0021	0.0002	-

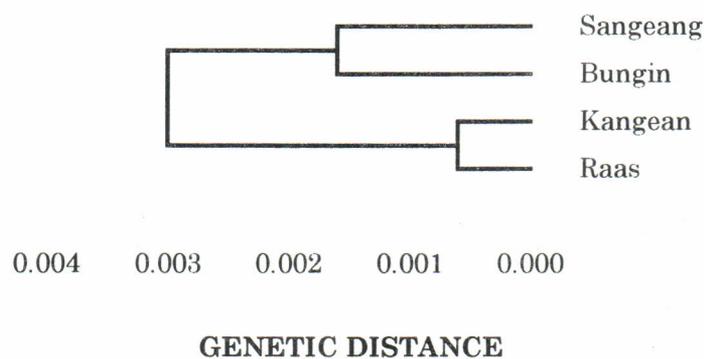


Figure 3. Cluster dendrogram (UPGMA) summarising the genetic relationship among four populations of the humpback grouper, *Cromileptes altivelis* based on their genetic distance as shown in Table 5.

The dendrogram constructed from the matrix of pairwise genetic distance values (UPGMA) revealed two groups: (I) Sangeang and Bungin (Sumbawa group) and (II) Kangean and Raas (Madura group) (Figure 3).

## DISCUSSION

The levels of genetic variation observed in humpback grouper were comparable to those reported for 82 fish by Winans (1980). The genetic distance summation over 15 loci, revealed two genetically distinct populations of humpback grouper throughout its range. One was the coral reefs of Madura Island and the other one was along the coral reef of Sumbawa Island (Figure 1 and 3). Samples collected from coral reef areas of Sangeang and Bungin as well as Kangean and Raas showed similar allele frequencies over three polymorphic loci (*ADH\**, *GPI\** and *IDH-1\**, Table 3). Heterogeneity test of pooled allele frequencies representing the two major coral reef group (Sumbawa and Madura areas) revealed evidence of allele frequencies differentiation at (*ADH 100\** ( $c^2_{df=1} = 9.31$ ,  $p < 0.01$ ) and *IDH-1 100\** ( $c^2_{df=1} = 8.17$ ,  $p < 0.01$ ). Because of the sharply discontinuous

distribution of suitable humpback grouper habitat in the present study separated by distance of 250 km, and the limited mobility of the territorial adults and the relatively brief pelagic larval stage, it was assumed that the lifecycle of humpback grouper inhibited significant levels of sub-population differentiation throughout the 250 km length of sampling area. This expectation is supported by the report of population sub-division for many other marine fish including Damselfish (Shaklee, 1984), Australian bass (Jerry, 1997) and Japanese sea bass (Ohtani *et al.*, 1997).

There are two possible explanations for the genetic differences found between the coral reefs of Madura and Sumbawa areas. One possibility is that this coral reef represents the separate breeding stock maintained through active homing of larvae to the site of their spawning. This explanation seems highly improbable because of the high degree of local current variability and the lack of evidence that fish larvae can actively maintain their position rather than being passively transported by currents. The second explanation for the observed genetic differences is that the habitat into which an individual humpback

grouper settled is genetically determined. From the management point of view, the Madura and Sumbawa humpback groupers should clearly be treated as separate stocks.

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