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Ecology and Genetic structure of Giant Clams around Savu Sea, East Nusa Tenggara Province, Indonesia

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Abstract - Giant clams are harvested by coastal communities around Savu Sea for food. As one of the important inhabitants of the reef, their status in terms of abundance of adult population and recruits was assessed. The genetic structure of *Tridacna maxima* was also determined for use in the establishment of network of MPAs around Savu Sea. There were four species identified during the survey: *Tridacna crocea*, *T. maxima*, *T. squamosa* and *Hippopus hippopus* with *T.*

maxima as the most common in all sites. Clam density ranged from 0.33 ind./ m^2 to 19 ind./ m^2.

Population subdivision was found to be highly significant among the five *T. maxima* populations as assessed using the Analysis of Molecular Variance (AMOVA). The percentage of total molecular variation within samples was 73.20%, and that among samples was 26.80%, amounting to FST = 0.26798 which is significant (p-value = 0.014). However, pairwise comparison revealed genetic relatedness between some populations.

Keywords - MPAs, genetic structure, genetic relatedness, giant clams, Savu Sea

INTRODUCTION

Giant clams are important inhabitants of the reef. They belong to the family Tridacnidae and are the largest bivalves. They are commercially important for the food market (Murakoshi, 1986; Shang et al. 1994), shellcraft industry and in the live marine aquarium trade (Knop 2004). Overexploitation of this valuable and multi-facetted resource has led to the decline of natural stocks throughout its natural Indo-Pacific range and the ecological extinction (Pandolfi et al. 2003) in some areas of the larger species (*Tridacna gigas* Linne 1758; *Tridacna derasa* Roding, 1798) (Brown and Muskanofola 1985; Lucas 1988; Juinio et al. 1989). All eight described species are endangered and are included in the Convention of International Trade of Endangered Species (CITES) list (Wells et al., 1983; Hilton-Taylor 2000). Research efforts on giant clams proliferated in the 1980s and culture techniques in the tropics for food and restocking of depleted reefs were established (Lucas, 1988; Braley, 1992; Calumpong 1992). Giant clams are widely distributed in the Indo-Pacific. From the southeast Pacific westwards to East Africa, its distribution extends up north to the Red Sea (Rosewater 1965; Lucas 1988; Braley 1992).

Populations of giant clams in East Nusa Tenggara Region are under heavy threatened by coastal communities. They use the shell of giant clams as a container to evaporate water and leave salt on it, some of them kill giant clams to consume its muscle, the other collect the shell as decoration or ornamental purposes. This paper presents the current

status of giant clam populations, their connectivity around Savu Sea and spawning successes.

MATERIALS AND METHODS

The field survey and broodstock collection

Field surveys were conducted in different reef sites southern and northeast part of Nusa Tenggara. Three 50 m x 5m belt transects were laid in each sampling site through SCUBA diving. Presence of giant clams was determined, identified and measured using a caliper. Clam mantle tissues were also snipped using forceps and scissors and preserved in 99% ethanol for genetic analysis. Broodstocks were also collected during the survey. Members of the community were also asked to help the team collect broodstocks for spawning. Collected clams were placed in holding tanks in the hatchery.

Spawning and Larval Rearing

Two methods of spawning induction namely, thermal shock and serotonin were applied following the protocol of Heslinga, et al., (1990) and Braley (1992). Clams were brushed to remove dirt, epifauna and epiphytes from shell, washed and placed under direct sunlight (or inside the hatchery house where temperature was 35 ºC) for 30 minutes to one hour. Clams were then placed inside basin with filtered seawater. Once valves were opened wide, each clam was injected with 2mM serotonin intragonadally, at least 1'' from the exhalant siphon. Fresh sperm water was collected whenever clam released sperm and set them aside until clam started releasing eggs. When this happened, clam was washed with filtered seawater and transferred to a new basin with filtered seawater and clam was allowed to release more eggs. Fertilization was done after 15 minutes by adding enough amount of sperm water. Fertilized eggs were counted and were transferred to hatching tank with aeration.

On Day 2, swimming veligers were retrieved by siphoning the water in hatching tank and passed through a sieve (50 um mesh) and transferred unto a basin containing filtered seawater and with aeration. This was done until all water in hatching tank were off. Retrieved

veligers were counted and stocked in larval rearing tank at 1-3 veliger per mL stocking density. The tank was provided with moderate aeration and remain static for ten days or until larvae metamorphosed and settled at the bottom of tank. However, fresh filtered seawater was added everyday until it reached to the fullest height/overflew. A very moderate flow through was then applied until one month and was increased till two months. Two liters of phytoplankton (*Nanochloropsis* sp) was given every other right after larvae were placed in larval tank. On Day 8 zooxanthellae were obtained from clam mantle and were given to the larvae.

Genetic Connectivity

*Sampling, Preservation and Total DNA Extraction.*Giant clam populations from the reefs of Semau, Batu Bau, Rote, Sabu, Pura and Batang Islands (Fig 4). A small piece of tissue from the mantle margin was snipped using a pair of surgical scissors while the shell was kept open with a wedge. The tissue was placed in small vials with 95% ethanol and stored at -20°C.

Genomic DNA was extracted from approximately 1 mm^2 *Tridacna maxima* mantle tissue. This was done using TNES-urea digestion buffer (6 M urea, 1M Tris-HCl pH 7.5, 5 M NaCl, 0.5 M EDTA, and 1% SDS or sodium dodecyl sulfate) as described in Wasko et al., (2003) and Proteinase K treatment followed by standard phenol-chloroform extraction method.

Amplification and Sequencing. Partial sequences (500 bp) of the mitochondrial *cytochrome c oxidase* 1 (COI) gene were amplified with a specific primer for *Tridacna crocea* (Tridacna 1F 5'- ACC CTT TAY TTT TTA TTA GCA Y- 3'; Tridacna 3R 5'- CAA TGC TGT AAT CGC CAA TGA C–3') designed by Barber (2006). PCR reactions were performed into a 50 μL reaction volume consisting of 1 x PCR buffer, 2 mM MgCl2, 10mM dNTP, 0.2 μM of forward and reverse primers, 0.5 U of Promega *Taq* polymerase products and 20-25 ng extracted genomic DNA. Cycling times were 1 min at 94 °C (soaking) followed by 6 cycles of 30 s at 94 °C (denaturation), 1 min 30 s at 45 °C (annealing) and 1 min at 72 °C (elongation), 36 cycles of 30 sec at 94 ºC, 1 min 30 sec at 51 ºC, 1 min at 72 ºC plus a final 5 min extension (soaking) at 72 °C. The PCR products were visualized using 1% (w/v) agarose gel electrophoresis.

Clones (forward and reverse stands) were sequenced on an ABI 377 or an ABI 3730 automated sequencer using Big Dye (Applied Biosystems, Foster City, CA) terminator chemistry. Nucleic acid sequences were subjected to BLAST/N (Altschul et al., 1990) searches at the National Center for Biotechnology Information (NCBI).

Genetic diversity and structure.

DNA sequences were aligned and edited using the Geneious v.3 (Drummond et al. 2008). Variation among and within collection sites was determined using the Analysis of Molecular variance (AMOVA) using ARLEQUIN version 3.1 (Excoffier et al. 2006; http::://cmpg.unibe.ch//software// arlequin3). Significance of the fixation indices was determined using a non-parametric approach described in Excoffier et al., (1992). FST was calculated among all populations and between all pairs of populations using DNA sequence data, and the significance of departures from zero of F statistics and genetic variance components was tested using 1,000 permutations (Schneider et al. 2000). Cluster analysis was conducted by constructing a phylogram on Kimura 2-P genetic distance using the neighbor joining (NJ) method. Pairwise comparisons of F-statistics was made to look at significant genetic differences within and among populations.

Finally, isolation-by-distance effects on population genetic structure was investigated using Reduced Major Axis (RMA) regression analysis and significance of the correlation was tested by Mantel Test utilizing the Isolation by Distance Web Service (IBDWS Version 3.15). The statistical significance of correlations between distance matrices was obtained from 10,000 random permutations of matrix elements.

RESULTS AND DISCUSSION

Field Survey and Broodstock Collection. A total of nine reef sites surveyed by the team since August 2010 (Fig. 1): five in southern part of Nusa Tenggara (Semau, Batu Bau, Tablolong, Rote Island and Sabu Island) while four in in the northeastern side which are all parts of Alor islands (Pura Island, Ternate, Batang and Alor). There were four species identified: *Tridacna crocea*, *T. maxima*, *T. squamosa* and *Hippopus hippopus* with *T. crocea* and *T. maxima* as the most common in all sites (Table 1 and Fig. 2). Clam density ranged from 0.33 ind./ 250m^2 to 19

ind./250m^2. *Tridacna crocea* was found to be abundant in Batang Island, Alor with density at 19 individuals/250m^2 and 63% of which were juveniles (size range: 2.1-4.6 cm). Among the nine reef sites surveyed, only Batu Bau and Semau have all the four species. As noticed, *Hippopus hippopus* was only found around the area of Tablolong and Bato Bau while the two closely related species, *Tridacna crocea* and *T. maxima* were the only species seen in Batang, Pura and Alor.

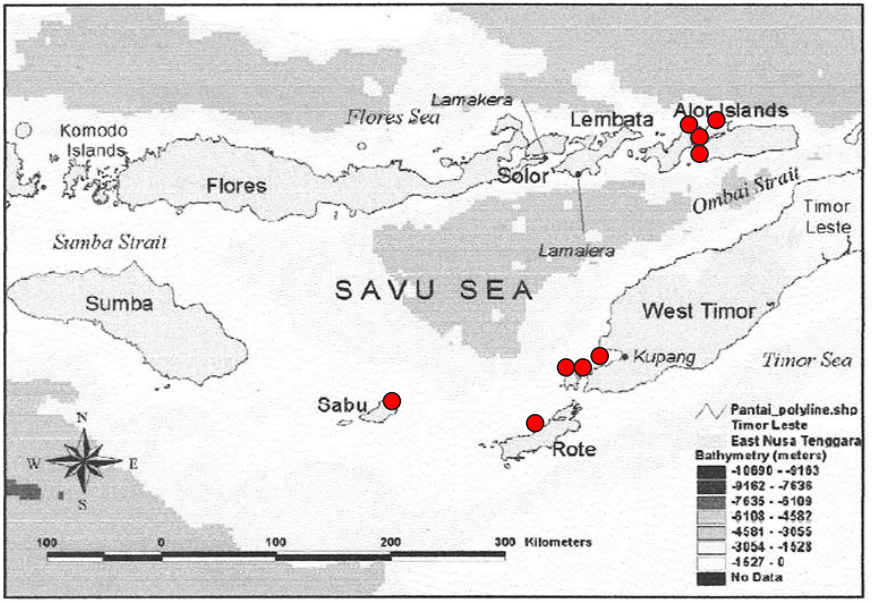

Fig. 1. Map of East Nusa Tenggara showing the surveyed sites.

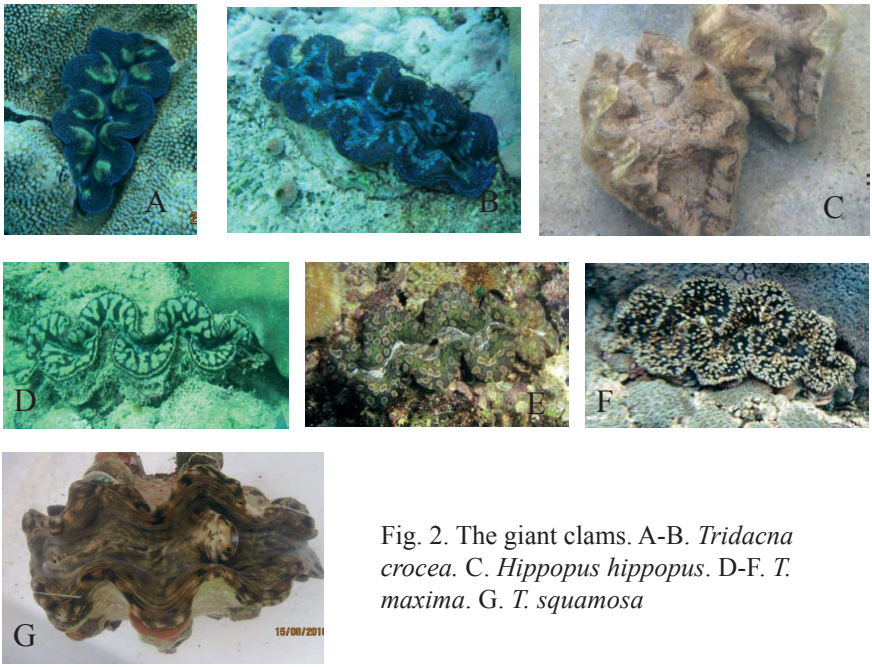

Fig. 2. The giant clams. A-B. *Tridacna crocea*. C. *Hippopus hippopus*. D-F. *T. maxima*. G. *T. squamosa*

Table 1. Number of clams per 250 m^2 and mean lengths in centimeters. (values below are standard deviations).

	Species							
Site	***Tridacna crocea***		***T.squamosa***		***T. maxima***		***Hippopus hippopus***	
	Density	**length**	**Density**	**length**	**Density**	**length**	**Density**	**length**
Bato Bau	2	6.7 ± 1.46	1	10.0 ±1.09	3	13.7 ±4.47	1	35
Semau	1	5.8 ±0.58	0.75	10.8	1	10.1 ±3.01	0.33	18.2
Tablolong			3	28.0 ±3.82	1		2.33	16.8±4.14
Rote	0.33	3.6						
Pura	1	5.7 ±3.50			1.33	9.3 ±4.58		
Ternate	1	2.3 ±1.35	0.33	7.5	0.33	29.5		
Batang	19	5.8 ± 3.12			3.33	9.4 ±3.80		
Alor	6	4.4 ±1.41			2.33	11.0±2.26		
Sabu	0		0.67	12.2 ±6.78	1.67	16.2 ±2.38		

A total of thirty-five (35) broodstocks were collected: nineteen (19) *Hippopus hippopus*, eight (8) *T. squamosa* and eight *T. maxima*. All clams were placed in holding or broodstock tank with aeration and flow-through system and fed with *Nanochloropsis* sp. at a rate of one liter of green water per week. Six *T. maxima* and one *Hippopus hippopus* died after one month.

Spawning and larval rearing. Seven *T. maxima*, 8 *H. hippopus*, four *T. squamosa* were induced to spawned from August to October 2010 using thermal shock and serotonin injection. As shown in Table 2, there were two spontaneous spawnings occurred in August and October 2010 by a single species, *Hippopus hippopus*. None of the *T. maxima* broodstock responded to the induction using serotonin. For *T. squamosa*, only one had successfully spawned using serotonin injection, however, metamorphosed larvae did not survive until the juvenile stage. On the other hand, two spontaneous spawnings and one induced occurred with *H. hippopus* species.. Fig. 3 presents the different larval and juvenile stages of giant clams the team was able to monitor.

Genetic Diversity. A total of 25 *Tridacna maxima* were screened for variation from a 490-bp partial mitochondrial COI sequence resulting in the identification of nine unique haplotypes. Of the 490 bases, only 255 sites were considered (since alignment gaps and missing data were not included) with 39 (15.29%) were polymorphic sites (undergo several substitutions) and 216 monomorphic sites (conservative or do not undergo substitutions) (Table 3). Among the variable sites, only one (1) was singleton (a site which contains at least two types of nucleotides with, at most, one occurring multiple times) while 38 were parsimony informative (a site which contains at least two types of nucleotides, and at least two of them occur with a minimum frequency of two). The average nucleotide composition are 27.6 (A), 35.5 (T), 16.7 (C), and 20.2(G).

Four haplotypes were unique to single individuals: two found in Pura Island (haplotypes 7 and 8), one found in Semau (haplotype 5) and one in Sabu island (haplotype 9).With regard to the rest of the haplotypes, they were either shared by 2, 3 or 12 individuals. Haplotype 3 was the most common which was found in 12 individuals across all sampled populations, except in Batang Island. Haplotype 6 was only made up of samples from Batang Island.

Table 2. Records of spawning and larval rearing.

SPECIES	Method of spawning	Date of spawning	Number of fertilized eggs	No. of Retrieved veligers	No. of juveniles	Remarks
T. squamosa						
Ts1	serotonin	08/15/10	500,000	75000		Not survived
Ts2	serotonin	10/23/10	only released sperm			
Ts3	serotonin	10/23/10	only released sperm			
Ts4	thermal	10/23/10	only released sperm			
T.maxima	serotonin	08/17/10	all not responded			
H. hippopus						
Hh1	spontaneous	08/14/10	5760000	175000	5,000	
Hh2	spontaneous	10/16/10	2000000	570000	10603	
Hh3	serotonin	10/17/10	3560000	3239600	45239	
Hh4	serotonin	10/23/10	only released sperm			
Hh5	serotonin	10/23/10	only released sperm			
Hh6	serotonin	10/23/10	only released sperm			
Hh7	spontaneous	10/23/10	only released sperm			
Hh8	thermal	10/23/10	not responded			

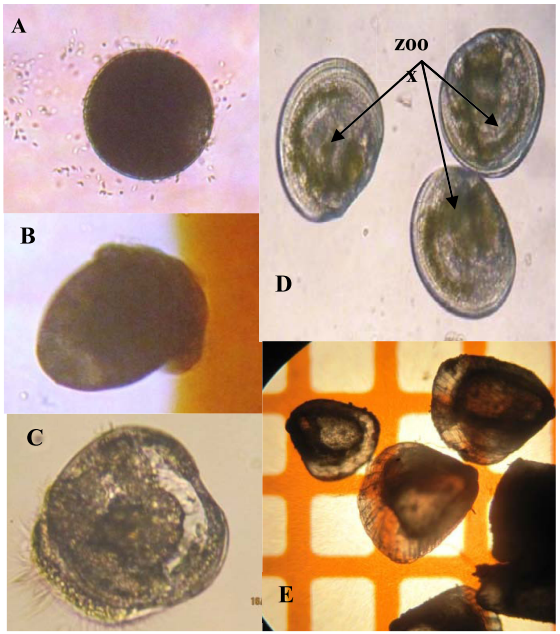

Fig. 3. The giant clam development. A. fertilized egg with sperms around it. B. trochophore stage. C. veliger stage. D. metamorphosed larvae 10X to show the zooxanthellae. E. two-month old juvenile at 4X magnification.

As shown in Table 4, haplotype diversity (also called gene diversity or heterozygosity) was high in all populations (1.0). The further in the past the defining event occurred, and the more that subsequent population growth occurred early, the greater the haplotype diversity for a particular number of descendants will be. On the other hand, if the haplotype diversity is smaller for a particular number of descendants, this may indicate a more recent common ancestor, or that a population expansion has occurred more recently.

Nucleotide diversity is a measure of the degree of DNA polymorphism within a population. It is defined as the average number of nucleotide differences per site between any two DNA sequences chosen randomly from the sample population. In the study, nucleotide diversity was generally small in all populations. Batang population exhibited the smallest nucleotide diversity, which was actually zero, however, this is not conclusive due to the small number of sequences generated. Batu-bau had the highest value (0.289 ±0.190). The overall

haplotype diversity (Hd) was 0.76000 and nucleotide diversity (PiT, π) was 0.02562. The general pattern of relatively high haplotypic diversity and low nucleotide diversity could be due to population expansion following bottleneck (Grant and Bowen 1998). Bottleneck is a large-scale but short term reduction in population size followed by an increase in population size. Moreover, the low nucleotide variability could be attributed to a recent coalescence, while the higher haplotype diversity could be explained by retention of mutations in a rapidly expanding population.

Historical Demography. To test the theory of neutral evolution of the CO1 marker, Tajima's D was calculated. Under the neutral model, the number of pairwise differences and number of segregating sites should equal and the mean Tajima's D statistic should approximate 0. Because rare alleles contribute more to the number of segregating sites than to the number of pairwise differences, negative values reflect an excess of low-frequency polymorphisms. Tajima's D was negative in Semau, Pura and Sabu Islands while zero in Batang and Batubau, however, all were not statistically significant at the 0.05 level (Table 6). This suggests selection, population expansion, or population bottlenecks.

Genetic Relationships and Connectivity Among Populations in Savu Sea. Population subdivision was found to be highly significant as assessed using the Analysis of Molecular Variance (AMOVA) (Table 6). The percentage of total molecular variation within samples was 73.20%, and that among samples was 26.80%, amounting to FST = 0.26798 which is significant (p-value = 0.014).

A neighbor-joining tree constructed from the matrix of corrected pairwise genetic distance between populations is presented in Fig. 4. The Sabu Island population clustered with Batu Bau, Pura Island with Batang while Semau formed a separate group.

Pairwise comparison of F_{ST}s (Fig. 4) detected a significant genetic differentiation of Sabu Island population from Semau (F_{ST} = 0.480, $p \leq 0.05$, 186.34 Km distance), Batang (F_{ST}= 0.994, $p \leq 0.05$, 377.93 Km) and Batu Bau (F_{ST}= 0.482, $p < 0.05$; 185.70 Km). On the other hand, genetic relatedness were seen between Semau and Batu Bao populations

(FST=0.048, p>0.05; 8.68 Km), Semau and Batang(FST=0.111, p>0.05; 262.17 Km), Semau and Pura Island (FST= -0.143, p>0.05; 244.72 Km), Batang and Batu Bao (FST= 0.274, p>0.05; 251.39 Km), Batang and Pura Island (FST=0.092, p>0.05; 16.58 Km), Batu Bao and Pura island (FST= -0.092, p>0.05; 243.66 Km), Pura island and Sabu Island (FST=0.536, p>0.05; 374.16 Km).

Table 3. Variable nucleotides from the *cytochrome c oxidase I* sequences of *Tridacna maxima*. Numbers on top refer to positions in the original alignment; s=singleton; p=parsimony informative. Nucleotides identical to the consensus are shown as dots.

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                  ----------------------------------------------------------------------------------------------
                  pppppppppppppppppppppppppppppppppppppsppppp
                           1111111111122222222222
POSITIONS                  1123334566888999012234557890012233445 6
                  2511241468369258795810253810601703684 0
                           ----------------------------------------------------------------------------------
                        *      *      *
                           'TM06_Sem'  GGTACGGTGCTTATTCGAAACTCGAACTCCAGGATCCGG
                            'TM01_Sem'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTGAAT.TAAA
                            'TM13_Sem'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TMS6_Sem'  A.CGTA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TMS7_Sem'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM16_Sem'  A.CGTA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM33_Sem'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM03_Sem'  AAC.TA.CAAC.TACAAGGGGCTAGGTATTG.AT.TAAA
                            'TM05_Sem'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                                        'TM26_Sem'  .....................................
                            'TM11_Bat'  AAC.TAACAACCTA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM10_Bat'  AAC.TAACAACCTA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM18_Bat'  AAC.TAACAACCTA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM12_Bau'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM20_Bau'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM23_Bau'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM22_Bau'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM14_Pur'  A.C.TA.CAAC.TACAAGGGGCTAGGTATTG.AT.TAAA
                            'TM15_Pur'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                             'TM24_pur'  A.C.TA.C.AC.TACAAGGGGCTAGGTA.T..AT.TAAA
                            'TM21_Pur'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTGAAT.TAAA
                            'TM17_Sab'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.ATCTAAA
                            'TM19_Sab'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM29_Sab'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                            'TM09_Sab'  AAC.TA.CAAC.TA.AAGGGGCTAGGTATTG.AT.TAAA
                              ----------------------------------------------------------------------------
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Table 4. Intrapopulational molecular diversity of *Tridacna maxima* mitochondrial *cytochrome oxidase 1*(C01).

Population	No.of Gene Copies	No. of Polymorphic Sites	No. of Haplotypes	Haplotype Diversity (Hd)	Nucleotide Diversity (Pi, π)	Theta s (θ_s)
Sabu Island	4	3	2	1.00 ±0.176	0.003 ±0.002	1.636 ±1.186
Semau	10	235	5	0.89 ±0.075	0.192 ±0.102	18.028 ±7.601
Batu Bau	4	200	1	0.67 ±0.200	0.289 ±0.190	0.00
Pura Island	4	201	4	1.00 ±0.176	0.221 ±0.145	5.454 ±3.270
Batang	3	0	1	1.00 ±0.272	0.00	0.00

Table 5. Neutrality (Tajima's D and Fu's Fs). s=significant ($p \leq 0.05$); ns= not significant.

	Neutrality Tests	
Population	**Tajima's D**	**Fu's Fs**
Semau	-0.112 ^{ns}	10.641 ^{ns}
Batang	0.000 ^{ns}	**
BatuBau	0.000 ^{ns}	12.931 ^{ns}
Pura Island	-0.033 ^{ns}	2.878 ^{ns}
Sabu Island	-0.754 ^{ns}	1.716 ^{ns}

**cannot be computed since only single haplotype

The deep genetic structure between Sabu island and Batang maybe attributed to isolation by distance, with Batang as being located on the northern side of Alor Archipelago (Fig. 1). Based on the Sewell Wright's island model and stepping-stone model, Sabu Island and Semau, as well as Sabu Island and BatuBau could have been genetically similar. A similar study conducted by DeBoer et al. (2008) using *T. crocea* CO1 in the Coral Triangle, indicated significant fine-scale structuring across the 2800 km of central Indonesia which they have attributed as a result from isolation during periods of low sea level. During glacial

maxima, sea levels dropped up to 130 m below present, constricting the waterways of the Indonesian Archipelago (Voris 2000). However, it should be noted that DeBoer et al. (2008) only had one site within Savu Sea.

Table 6. AMOVA results (1023 permutations).

Source of variation	d.f.	Sum of squares	Variance components	Percentage of variation
Among populations	4	427.280	14.41239 Va	26.80
Within populations	20	787.400	39.37000 Vb	73.20
Total	24	1214.680	53.78239	

Fixation Index FST : 0.26798 P-value = 0.01369+-0.00367

The genetic relatedness of some populations within Savu Sea can be possibly influenced by its predominating water currents. Although secondary and actual data on local oceanography have not been obtained up to this writing, a westward water flow maybe existing in the area brought about by the strong Indonesian Throughflow (moves up to 19 million m^3 of water per second) that may partly enter via the Ombai Strait or by an eastward flow of the South Java Current (Fig. 5). Data from mtDNA COI revealed genetic affinity between Philippine and Indonesian populations, made up of haplotypes from a single clade (DeBoer et al., *in press*). High genetic similarity between Philippine and Indonesian populations has been reported in marine gastropods (Crandall et al. 2008), vetigastropod (Imron et al. 2007), marine snails (Reid et al. 2006), scleractinian coral (Knittweis et al. 2009), seahorse (Lourie et al. 2005), and stomatopod species (Barber& Boyce 2006) and has been inferred in part attributable to the intense Indonesian Throughflow current, which moves from northeast to southwest between these two areas, providing a mechanism for larval

dispersal within the region (Knittweis et al., 2009; Gordon, 2005; Gordon & Fine, 1996).

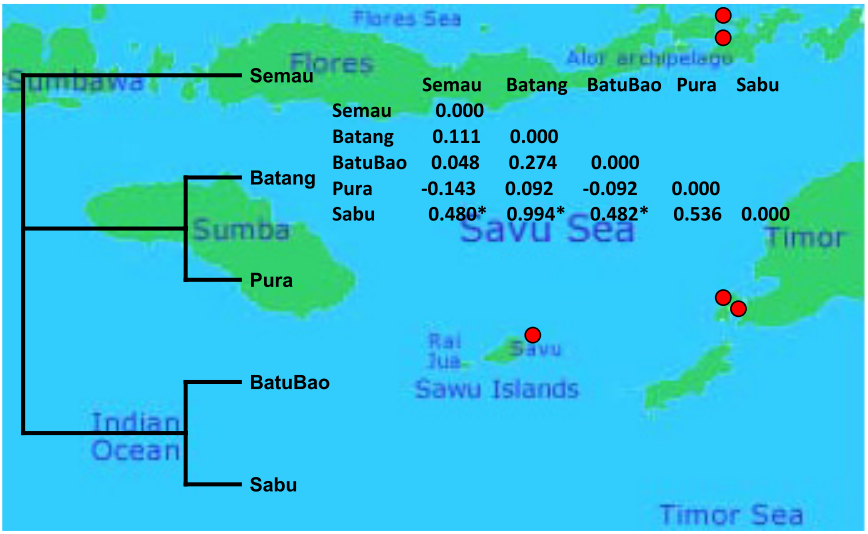

Fig. 4. Phylogram showing the genetic relationship of the five *Tridacna maxima* populations from Savu Sea, East Nusa Tenggara, Indonesia. Pairwise differences of F_{ST} (1023 permutations) were clustered using Neighbor-joining implemented in PHYLIP v. 3.69 and TreeView. * significant, $p \leq 0.05$. Super-imposed is a map of Savu Sea. White dots indicate study sites.

Conservation Implications. At the moment, there are only four species of giant clams in the southern and northeastern parts of East Nusa Tenggara. Only *Tridacna squamosa* and *Hippopus hippopus* represented the third larger species which have been exploited by coastal villagers for food (meat) and salt making (shells) as revealed by hundreds of dead shells seen scattered in Tablolong and Semau (Fig. 1). Even the smallest species were rarely seen, except in Batang Island, Alor. There is really a need to conserve and restock the reefs through partnership with the fishing communities. Hence, production of more giant clams is required particularly the larger species.

Genetic connectivity patterns are very important in the formulation of marine conservation plans (Sale et al. 2005). Identification of sources

and sinks of marine taxa is critical requirement in designing network of marine protected areas (MPAs) around Savu Sea. According to Sale and Kreitzer (2003), the objectives of establishing MPAs are not realized when the spillover of biomass and subsidies of recruits from no-take reserves to non-MPA areas is negated with the spilling in of adults and recruits to the MPA. In this case the MPA has no net benefit to the fishery. The same thing will happen when the MPA and the non-reserve area are a single unit and the surrounding resources are devastated, providing a very slim opportunity for the population within the MPA to survive.

Results of this study have indicated some connectivities of *T. maxima*, although not substantial due to limited sampling sites (only five) and samples (only few with successful sequencing results). It should be noted that this is the only fine scale study ever conducted in Savu Sea using *T. maxima*. Hence, it is highly recommended that further investigation with increased sampling sites and samples should be made coupled with actual water current studies to verify the influence of the Indonesian Throughflow and South Java Current in Savu Sea and to determine the presence of local gyres and eddies that may affect larval settlement and recruitment.

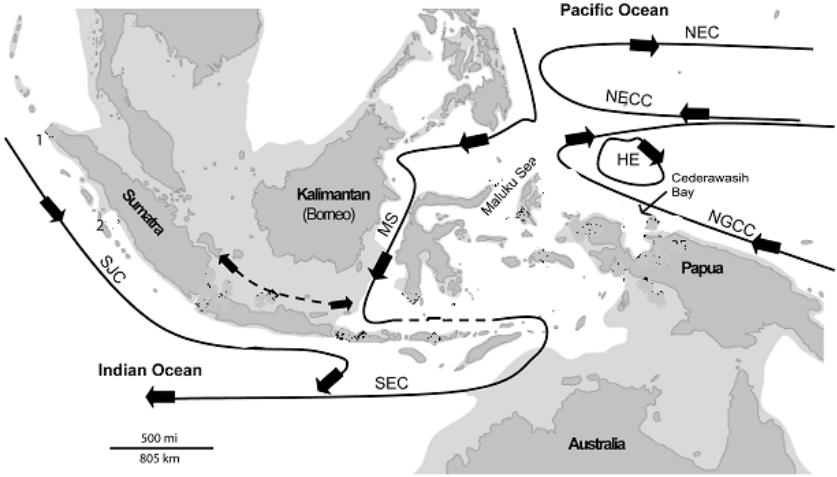

Fig. 5. The dominant (solid lines) and seasonally reversing currents (dashed lines) in the Indo-West Pacific Region (source: Barber et al.,

2002; DeBoer et al., 2008). NEC (North Equatorial current); NECC (North Equatorial Counter Current); NGCC (New Guinea Coastal Current); HE (Halmahera Eddy); MS (Makassar Strait, the main passageway of the Indonesia Throughflow); SEC (South Equatorial Current); SJC (South Java Current). Red box indicate the coverage of the study in Savu Sea.Light gray shading indicates coastal margins during Pleistocene low sea level stands, after Voris (2000).

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