Vol. 336: 161–168, 2007 | MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser | Published April 27

# Influence of the North Equatorial Current on the population genetic structure of *Tridacna crocea* (Mollusca: Tridacnidae) along the eastern Philippine seaboard

**Rachel G. Ravago-Gotanco*, Richard M. Magsino, Marie Antonette Juinio-Meñez**

**The Marine Science Institute, Velasquez Street, University of the Philippines, Diliman, Quezon City 1101, Philippines**

ABSTRACT: *Tridacna crocea* populations were sampled from 15 locations throughout the eastern Philippine seaboard and screened for allozyme variation at 7 polymorphic loci in order to examine the influence of the North Equatorial Current (NEC) bifurcation on population genetic structure. Significant genetic differentiation among all populations was detected ($F_{ST}$ = 0.065). Ordination methods and cluster analysis revealed 2 regional groups and a north–south spatial genetic structure broadly concordant with the bifurcation of the NEC into the Kuroshio and Mindanao current branches. Analysis of molecular variance (AMOVA) indicated greater partitioning of genetic variance among groups ($F_{CT}$ = 0.049) than among populations within groups ($F_{SC}$ = 0.029). An isolation-by-distance signal across the entire Philippine seaboard and marked geographical variation of allele frequencies between Kuroshio and Mindanao Current regions suggested that genetic differentiation is likely due to limited larval exchange and genetic drift. The Mindanao current populations are characterized by greater genetic diversity and differentiation (observed heterozygosity, $H_O$ = 0.298; $F_{ST}$ = 0.056) than the Kuroshio populations ($H_O$ = 0.152; $F_{ST}$ = 0.025), attributable to variable atmospheric and hydrographic regional conditions. Weaker connectivity among Mindanao current populations are attributed to complex patterns of hydrographic circulation south of the NEC bifurcation, which may translate to greater entrainment potential, in turn influencing dispersal and recruitment of planktonic propagules at varying spatial and temporal scales. Fine-scale genetic differentiation was also detected within Kuroshio and Mindanao current populations, indicating the influence of small-scale temporal and spatial physical processes that affect larval dispersal and recruitment along the eastern Philippine seaboard.

KEY WORDS: *Tridacna* · Genetic structure · Gene flow · North Equatorial Current · Oceanography · Giant clam



## INTRODUCTION

The eastern Philippine reefs situated along the western boundary of the Pacific Ocean consist of various reef habitat types exposed to wind-driven surface currents. Upon encountering the western boundary along the eastern Philippine seaboard, the westward flowing North Equatorial Current (NEC) in the Pacific Ocean bifurcates into narrow, fast-flowing western boundary currents: the northward flowing Kuroshio and the southward flowing Mindanao Current (Nitani 1972, see Fig. 1). The bifurcation latitude is estimated to lie between 11 and 14.5° N (Nitani 1972), and exhibits seasonal meridional shifting, from 14.8 up to 17° N in summer and winter, respectively (Qiu & Lukas 1996). The divergent current pattern generated by the NEC bifurcation has been suggested as one of the primary determinants in the north-south geographical differentiation of reef fish species composition along the eastern Philippines (Aliño & Gomez 1993). Population genetic studies provide an opportunity to test the hypothesis that the NEC bifurcation significantly affects larval dispersal and gene flow of reef organisms along the Pacific coast of the Philippines.

*Email: rachelr@upmsi.ph

*Tridacna crocea* is the smallest giant clam species. It is widely distributed in the Indo-Pacific region and is the most abundant giant clam among shallow water reefs on the eastern Philippine seaboard. Like other tridacnid species, it is a broadcast spawner and has an estimated planktonic larval period of 7 to 10 d (Lucas 1988), after which the larvae metamorphose into shelled pediveligers, acquiring a foot for attachment and subsequently burrowing into suitable substrate. Despite the dispersal potential mediated by a planktonic larval phase, previous studies reported broad-scale genetic differentiation among populations from the South China Sea, Sulu Sea, northern Palawan, and southern Palawan (Yu et al. 2000, Juinio-Meñez et al. 2003), with genetic connectivity between regions mediated by larval dispersal via predominant surface currents. Fine-scale differentiation among reefs within each region was reported and attributed to stochastic patterns of larval dispersal and recruitment influenced by small-scale (local) physical processes and diverse oceanographic conditions (Juinio-Meñez et al. 2003).

Large-scale oceanic currents like the NEC may play a significant role in shaping the genetic structure of marine populations along the eastern Philippines. As well as the physical, geological and biological oceanographic characteristics that give rise to perceived biogeographic boundaries, the concordance of genetic divergence of broad-ranging reef species with patterns of ocean currents could provide insights into the processes governing species' patterns of larval dispersal and gene flow in the areas influenced by the the Kuroshio and Mindanao Current. Previous studies on other tridacnid species revealed patterns of genetic connectivity that were not consistent with present-day major surface currents (Macaranas et al. 1992, Benzie & Williams 1995, 1997). In contrast, fine-scale genetic structure in the order of tens to hundreds of kilometers was reported for Philippine populations of *Tridacna crocea* in the South China Sea and Sulu Sea (Juinio-Meñez et al. 2003), and along the southeastern Philippine reefs (Magsino et al. 2002).

The present study tests the hypothesis that the NEC bifurcation influences gene flow among *Tridacna crocea* populations along the entire length of the eastern Philippine seaboard. Estimates of genetic diversity and variability were based on allozyme markers, which allowed some degree of comparison with earlier studies that also employed allozymes. Specifically, spatial patterns of genetic differentiation were examined to test whether the NEC bifurcation is a dispersal barrier, restricting larval dispersal and gene flow among *T. crocea* reef populations along the eastern Philippine seaboard.

## MATERIALS AND METHODS

**Sample collection.** Mantle biopsies allowed non-destructive sampling of 630 *Tridacna crocea* clams from 15 reef sites (26 to 75 clam per population) along the eastern Philippine seaboard. Sampling sites were selected to include representative reefs north and south of the NEC bifurcation (Fig. 1). Reef sites sampled included Cagayan (CAG), Isabela (ISA), Aurora (AUR), 3 sites at Polillo (POL-1, POL-2, POL-3), Catanduanes (CAT), Masbate (MAS), Balicuatro (BAL), Div-

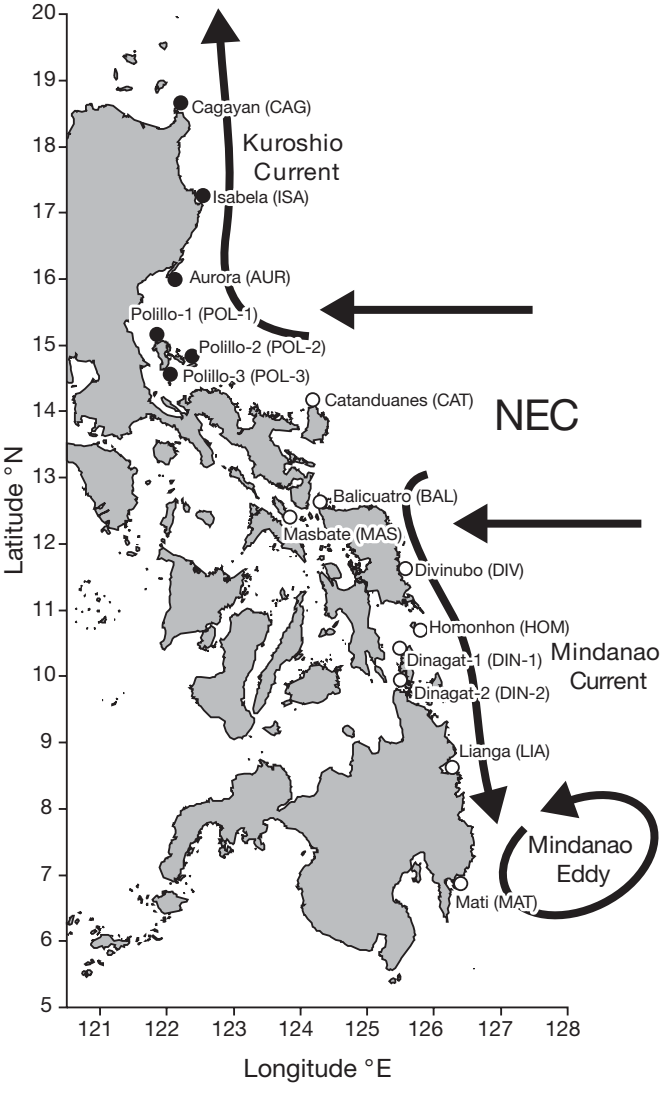


Fig. 1. *Tridacna crocea.* Sampling sites on the eastern Philippine seaboard. Significant oceanographic features are shown. Average current speed and width are 1.5 m $s^{-1}$ and 100 to 150 km for the Kuroshio (Nitani 1972), and ~1 m $s^{-1}$ (max.) and 200 km for the Mindanao Currents (Wijffels et al. 1995). Classification of sites into either Kurushio (●) or Mindanao (○) groups reflects spatial structure based on our results

inubo (DIV), Homonhon (HOM), 2 sites at Dinagat (DIN-1, DIN-2), Lianga (LIA) and Mati (MAT). Collections from most sites were conducted during April and May in 2000 and 2001, with the exception of Cagayan, which was sampled in October 2001. At each reef site, 2 SCUBA divers searched reef areas of approximately 200 to 400 $m^2$, collecting mantle tissue from clams with shell lengths of ~4 to 8 cm as they were encountered. Tissue samples collected from individual clams were frozen in liquid nitrogen and stored at −70°C until electrophoretic analysis.

**Allozyme electrophoresis.** Processing of mantle tissues and horizontal starch-gel electrophoresis of tissue homogenates followed the procedures of Magsino et al. (2002). Histochemical methods for detecting specific enzyme systems followed protocols of Shaw & Prasad (1970). Pilot screening of 20 enzyme systems revealed 7 polymorphic loci resolved using Tris-Citric acid pH 7 buffer: adenylate kinase (*AK**, E.C. 2.7.4.3), glucose-6-phosphate isomerase (*GPI**, E.C. 5.3.1.9), isocitrate dehydrogenase (*IDH**, E.C. 1.1.1.42), lactate dehydrogenase (*LDH**, E.C. 1.1.1.27), malate dehydrogenase (*MDH-1** and *MDH-2**, E.C. 1.1.1.37), and mannose phosphate isomerase (*MPI**, E.C. 5.3.1.8). Allozymes were numbered in order of decreasing anodal mobility. Electromorphs were equated with alleles and assigned numerical designations relative to the mobility of the most common allele (Allele 100). Enzyme names and numbers followed the recommendations of the Commission on Biochemical Nomenclature (Shaklee et al. 1990).

**Statistical analysis.** Allele frequencies and measures of genetic variability (observed and expected heterozygosity, mean number of alleles, percentage of polymorphic loci) were calculated from genotype data using the GENETIX software (Belkhir et al. 2004, available at www.genetix.univ-montp2.fr/genetix/genetix.htm). The inbreeding coefficient of an individual relative to its subpopulation ($F_{IS}$) was used to represent the Mendelian equilibrium, and single and multi-locus deviation from Hardy Weinberg equilibrium expectations was tested using the Markov chain method implemented in GENEPOP version 3.1d (Raymond & Rousset 1995). GENEPOP was likewise used to test non-random association of loci (linkage disequilibrium) within populations. Significance levels for statistical tests were adjusted according to the sequential Bonferroni method (Rice 1989).

Unbiased genetic distances (Nei 1978) were computed for all pairwise population comparisons using GENETIX. To explore population genetic affinities, a neighbor-joining dendrogram was generated from the distance matrix using the NEIGHBOR program in the PHYLIP package (version 3.5, Felsenstein 1985). Topological confidence was evaluated with 1000 bootstrap replicates using the SEQBOOT and CONSENSE programs in PHYLIP. In addition, non-metric multidimensional scaling (MDS) of pairwise $F_{ST}$ values was conducted to visualize the spatial pattern of genetic relationships among samples, using the PERMAP software package (available at www.ucs.louisiana.edu/~rbh8900/permap.html). Significant differences in allelic frequencies among samples was tested using an exact test implemented in GENEPOP.

The extent of genetic differentiation among samples was estimated using Wright's standardized variance in allelic frequencies ($F_{ST}$; Wright 1951), which is the proportion of allelic variation resulting from differences among samples. Single and multi-locus $F_{ST}$ values were calculated in GENEPOP, in order to provide measures of differentiation among samples, among various sample groupings, and between each pair of samples. The significance of $F_{ST}$ values was evaluated using an approximation to a chi-square test, and significance levels were adjusted for multiple simultaneous table-wide tests using the sequential Bonferroni method (Rice 1989).

Partitioning of genetic variance components among geographic regions and within samples was performed using a hierarchical analysis of molecular variance (AMOVA, Excoffier et al. 1992) with the package ARLEQUIN (version 2.0, Schneider et al. 2000). Sample sites were nested into regional groupings in order to evaluate hypothesized patterns of spatial genetic structure. The correlation between geographical distance and genetic differentiation (pairwise $F_{ST}$) was evaluated using a Mantel test (Mantel 1967) implemented in ARLEQUIN.

## RESULTS

Seven polymorphic loci representing a total of 26 alleles were scored and used in this study (Table 1). Significant departures from Hardy-Weinberg equilibrium were detected at 7 loci (7.9%; total of 88 comparisons, Table 1). Of these, 6 cases resulted from heterozygote deficits. Averaging across sites, heterozygote deficits were significant (after Bonferroni correction) at *AK-1** ($F_{IS}$ = 0.357), *GPI-1** ($F_{IS}$ = 0.331), and *IDH-1** ($F_{IS}$ = 0.353). Single-locus disequilibrium was mostly observed at *GPI-1** (5 of 15 comparisons significant), and contributed to multilocus disequilibrium at 5 populations (POL-1, MAS, DIV, DIN-2, MAT). However, *GPI-1** was still included in the analysis because no differences in dendrogram topologies were observed for datasets with or without the locus (results not shown).

Genetic differentiation over all 15 populations was highly significant ($F_{ST}$ = 0.069, $p < 0.001$), with heterogeneity detected at 6 of the 7 loci (only *MDH-2** was

Table 1. *Tridacna crocea*. Allele frequencies and $F_{IS}$ values for eastern Philippine seaboard populations. Significant $F_{IS}$ values (following Bonferroni correction) in **bold**. Values in parentheses indicate no. of individuals analyzed per locus; np: allele was not present in the population. $H_O$ = observed heterozygosity; $P_{99}$, $P_{95}$ = proportion of loci polymorphic at 99 and 95% level, respectively; site abbreviations given in 'Materials and methods'

| Locus/ allele | CAG | ISA | AUR | POL-1 | POL-2 | POL-3 | CAT | MAS | BAL | DIV | HOM | DIN-1 | DIN-2 | LIA | MAT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *AK-1** | (37) | (37) | (23) | (45) | (75) | (59) | (39) | (54) | (38) | (43) | (19) | (37) | (38) | (30) | (38) |
| 98 | np | np | np | 0.067 | np | np | 0.038 | 0.028 | np | 0.023 | np | 0.041 | np | 0.033 | 0.158 |
| 100 | 1.000 | 1.000 | 1.000 | 0.933 | 1.000 | 0.966 | 0.859 | 0.833 | 1.000 | 0.826 | 0.921 | 0.838 | 1.000 | 0.933 | 0.789 |
| 109 | np | np | np | np | np | 0.034 | 0.103 | 0.139 | np | 0.151 | 0.079 | 0.122 | np | 0.033 | 0.053 |
| $F_{IS}$ | np | np | np | 0.060 | np | 1.000 | 0.497 | 0.488 | np | 0.458 | –0.059 | 0.435 | np | 0.036 | 0.108 |
| *GPI-1** | (37) | (35) | (23) | (44) | (62) | (59) | (38) | (54) | (43) | (43) | (22) | (37) | (33) | (34) | (33) |
| 68 | np | np | np | 0.011 | np | 0.051 | 0.026 | np | 0.023 | np | np | np | 0.030 | np | 0.091 |
| 80 | 0.027 | np | np | np | 0.008 | 0.076 | 0.090 | 0.037 | 0.186 | 0.047 | np | 0.162 | 0.258 | 0.250 | 0.106 |
| 100 | 0.946 | 0.929 | 0.804 | 0.818 | 0.927 | 0.822 | 0.692 | 0.694 | 0.581 | 0.744 | 0.455 | 0.757 | 0.591 | 0.647 | 0.576 |
| 105 | 0.027 | 0.043 | 0.196 | 0.159 | 0.048 | 0.017 | 0.128 | 0.102 | 0.163 | 0.116 | 0.386 | 0.041 | 0.121 | 0.059 | 0.091 |
| 112 | np | 0.029 | np | 0.011 | 0.016 | 0.034 | 0.064 | 0.167 | 0.047 | 0.093 | 0.159 | 0.041 | 0.000 | 0.044 | 0.136 |
| $F_{IS}$ | –0.029 | 0.168 | 0.055 | **0.854** | –0.050 | –0.016 | 0.333 | **0.504** | 0.272 | **0.403** | 0.359 | –0.006 | **0.793** | 0.269 | **0.284** |
| *IDH-1** | (37) | (16) | (19) | (37) | (75) | (59) | (41) | (54) | (45) | (43) | (24) | (37) | (23) | (34) | (33) |
| A | np | np | np | np | 0.007 | 0.008 | np | np | np | np | np | 0.135 | np | np | 0.121 |
| B | 0.959 | 0.906 | 0.868 | 0.959 | 0.920 | 0.898 | 0.951 | 1.000 | 0.978 | 0.802 | 0.479 | 0.770 | 0.913 | 1.000 | 0.833 |
| C | 0.014 | 0.094 | 0.105 | 0.041 | 0.053 | 0.093 | 0.049 | np | 0.022 | 0.174 | 0.333 | 0.054 | 0.087 | np | 0.030 |
| D | 0.027 | np | 0.026 | np | 0.020 | np | np | np | np | 0.023 | 0.188 | 0.041 | np | np | 0.015 |
| $F_{IS}$ | –0.019 | 0.651 | 0.127 | –0.029 | 0.120 | 0.089 | –0.039 | np | –0.011 | **0.649** | 0.153 | 0.028 | 0.470 | np | 0.282 |
| *MDH-1** | (37) | (37) | (23) | (45) | (75) | (59) | (41) | (44) | (45) | (43) | (24) | (37) | (38) | (34) | (38) |
| 90 | np | np | 0.022 | np | np | np | 0.012 | np | np | np | np | np | np | np | np |
| 100 | 1.000 | 1.000 | 0.913 | 1.000 | 1.000 | 1.000 | 0.841 | 0.909 | 0.956 | 0.709 | 0.563 | 0.919 | 0.987 | 0.838 | 1.000 |
| 130 | np | np | 0.065 | np | np | np | 0.024 | 0.034 | 0.011 | 0.081 | 0.104 | 0.014 | np | 0.088 | np |
| 148 | np | np | np | np | np | np | 0.122 | 0.057 | 0.033 | 0.209 | 0.333 | 0.068 | 0.013 | 0.074 | np |
| $F_{IS}$ | np | np | 0.214 | np | np | np | 0.041 | –0.063 | –0.026 | –0.082 | –0.092 | –0.061 | 0.000 | –0.124 | np |
| *MDH-2** | (37) | (36) | (23) | (42) | (75) | (59) | (41) | (51) | (45) | (43) | (24) | (37) | (78) | (34) | (38) |
| 100 | 0.743 | 0.861 | 0.783 | 0.774 | 0.847 | 0.780 | 0.793 | 0.824 | 0.800 | 0.826 | 0.792 | 0.784 | 0.974 | 0.824 | 0.789 |
| 120 | 0.108 | 0.097 | 0.043 | 0.155 | 0.087 | 0.093 | 0.122 | 0.069 | 0.122 | 0.116 | 0.042 | 0.135 | 0.013 | 0.132 | 0.053 |
| 160 | 0.149 | 0.042 | 0.174 | 0.071 | 0.067 | 0.127 | 0.085 | 0.108 | 0.078 | 0.058 | 0.167 | 0.081 | 0.013 | 0.044 | 0.158 |
| $F_{IS}$ | –0.031 | 0.115 | –0.202 | –0.012 | 0.073 | 0.086 | –0.034 | –0.146 | –0.169 | –0.145 | 0.052 | –0.185 | –0.007 | –0.153 | –0.194 |
| *LDH-1** | (37) | (37) | (23) | (45) | (75) | (59) | (35) | (54) | (45) | (43) | (24) | (37) | (38) | (33) | (38) |
| 82 | np | np | np | np | np | 0.025 | np | np | np | np | np | np | np | np | np |
| 100 | 1.000 | 1.000 | 0.891 | 0.956 | 0.987 | 0.847 | 0.829 | 0.667 | 0.756 | 0.837 | 0.833 | 0.824 | 0.974 | 0.727 | 0.961 |
| 118 | np | np | 0.109 | 0.044 | 0.013 | 0.127 | 0.171 | 0.333 | 0.244 | 0.163 | 0.167 | 0.176 | 0.026 | 0.273 | 0.039 |
| $F_{IS}$ | np | np | –0.100 | –0.035 | –0.007 | 0.113 | –0.193 | **–0.493** | –0.313 | –0.183 | 0.418 | –0.200 | –0.014 | –0.362 | –0.028 |
| *MPI-1** | (37) | (37) | (23) | (45) | (62) | (59) | (41) | (54) | (45) | (43) | (23) | (37) | (38) | (34) | (38) |
| 96 | 0.041 | np | np | np | 0.016 | 0.068 | np | np | np | np | np | np | np | np | np |
| 100 | 0.932 | 0.986 | 0.783 | 0.800 | 0.935 | 0.898 | 0.756 | 0.769 | 0.900 | 0.826 | 0.522 | 0.757 | 0.974 | 0.853 | 0.763 |
| 115 | 0.027 | np | 0.022 | 0.056 | 0.024 | 0.008 | 0.134 | 0.074 | 0.067 | 0.070 | 0.130 | 0.135 | np | 0.074 | 0.105 |
| 135 | np | 0.014 | 0.196 | 0.144 | 0.024 | 0.025 | 0.110 | 0.157 | 0.033 | 0.105 | 0.348 | 0.108 | 0.026 | 0.074 | 0.132 |
| $F_{IS}$ | –0.040 | 0.000 | –0.226 | 0.151 | 0.093 | –0.075 | 0.033 | 0.081 | –0.073 | 0.471 | –0.084 | –0.211 | –0.014 | –0.109 | 0.067 |
| Overall | | | | | | | | | | | | | | | |
| $H_O$ | 0.108 | 0.061 | 0.247 | 0.146 | 0.096 | 0.182 | 0.275 | 0.277 | 0.238 | 0.266 | 0.412 | 0.340 | 0.056 | 0.290 | 0.264 |
| N alleles | 2.14 | 1.86 | 2.43 | 2.43 | 2.71 | 3.00 | 3.14 | 2.71 | 2.71 | 3.00 | 2.71 | 3.14 | 2.29 | 2.71 | 3.00 |
| $P_{99}$ | 42.86 | 57.14 | 85.71 | 57.14 | 57.14 | 71.43 | 85.71 | 85.71 | 57.14 | 100.00 | 100.00 | 100.00 | 28.57 | 85.71 | 71.43 |
| $P_{95}$ | 57.14 | 57.14 | 85.71 | 85.71 | 71.43 | 85.71 | 100.00 | 85.71 | 85.71 | 100.00 | 100.00 | 100.00 | 85.71 | 85.71 | 85.71 |

not significant; Table 2). MDS analysis revealed a north-south geographical grouping of populations (Fig. 2), concordant with the clustering of populations based on the neighbor-joining dendrogram (tree not shown). Spatial patterns of population genetic affinities indicated that 6 northern populations form one group (Polillo and all sites northward; hereafter referred to as the 'Kuroshio' group), whereas populations from Catanduanes southward form a second major group (the 'Mindanao' group). The first dimension (explaining 32% of variance) distinguished the Kuroshio and Mindanao groups, whereas the second dimension (12% of variance) mainly differentiated the Homonhon population from the rest of the samples.

Table 2. *Tridacna crocea*. $F_{ST}$ values per locus for various sample groupings. Probabilities given in parentheses. Significant values (following Bonferroni correction) in **bold**

| Locus | All populations (n = 15) | Kuroshio (n = 6) | Mindanao (n = 9) |
|---|---|---|---|
| *AK-1** | **0.067** (0.000) | **0.034** (0.000) | **0.039** (0.000) |
| *GPI-1** | **0.072** (0.000) | **0.033** (0.000) | **0.032** (0.000) |
| *IDH-1** | **0.102** (0.000) | 0.001 (1.000) | **0.153** (0.000) |
| *MDH-1** | **0.140** (0.000) | **0.067** (0.000) | **0.106** (0.000) |
| *MDH-2** | 0.008 (0.816) | 0.004 (0.976) | **0.013** (0.000) |
| *LDH-1** | **0.101** (0.000) | **0.065** (0.000) | **0.064** (0.000) |
| *MPI-1** | **0.059** (0.000) | **0.050** (0.000) | **0.048** (0.000) |
| Multilocus | **0.069** (0.000) | **0.025** (0.000) | **0.056** (0.000) |

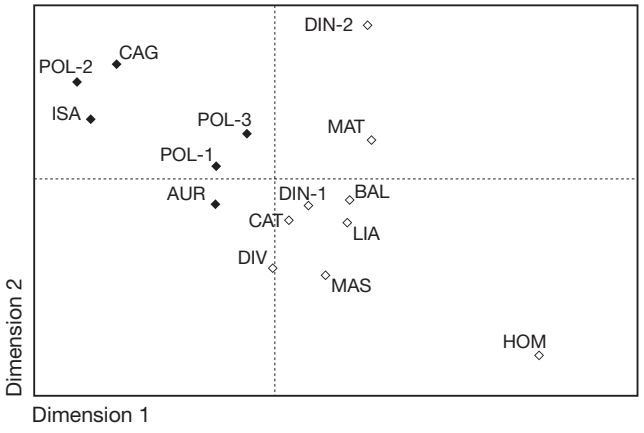


Fig. 2. *Tridacna crocea*. Ordination plot of MDS based on $F_{ST}$ values. ◆ = Kuroshio group populations; ◇ = Mindanao group populations

Hierarchical AMOVA revealed significant differentiation at all levels of organization: among groups ($F_{CT}$), among samples within groups ($F_{SC}$), and within samples ($F_{ST}$) (Table 3), with 4.9% of total genetic variation partitioned among the Kuroshio and Mindanao groups. Single-locus AMOVA revealed that 5 loci accounted for significant between-group differentiation: *AK-1**, *GPI-1**, *LDH-1**, *MDH-1**, and *MPI-1**. Closer inspection of allele frequencies at these loci revealed marked geographical variation in allele frequency distributions. In particular, *AK-1*$^{109}$, *GPI-1*$^{80}$, *GPI-1*$^{105}$, and *LDH-1*$^{118}$ occur at higher frequencies in Mindanao group populations, i.e. from CAT southward. *MDH-1*$^{148}$ is found only in the Mindanao group, whereas *MPI-I*$^{96}$ is only found in the Kuroshio group (at CAG, POL-2, and POL-3) (Table 1). Moreover, the Kuroshio group exhibited lower genetic diversity (mean $H_O$ = 0.152, $P_{95}$ = 61.9, $P_{99}$ = 73.8) than did the Mindanao group (mean $H_O$ = 0.298, $P_{95}$ = 79.3, $P_{99}$ = 92.1). Observed heterozygosity values differed significantly between the Kuroshio and Mindanao samples (Student's *t*-test; p = 0.014).

Table 3. *Tridacna crocea*. AMOVA for partitioning of allele frequency variation. Samples were tested according to 2 hypothesized structures: (A) no geographic subdivision; (B) 2 groups: Kuroshio and Mindanao, based on MDS and dendogram clustering results; p-values, calculated from random permutation tests (10 000 replications) represent the probability of obtaining a more extreme variance component and *F*-statistic than the observed values by chance alone (Excoffier et al. 1992)

| | % variation | *F*-statistic | p |
|---|---|---|---|
| (A) Single group of 15 samples | | | |
| Among populations | 5.59 | $F_{ST}$ = 0.05595 | 0.00000 |
| Within populations | 94.41 | | |
| (B) Two groups (Kuroshio and Mindanao) | | | |
| Among groups | 4.95 | $F_{CT}$ = 0.04948 | 0.00098 |
| Among populations within groups | 2.84 | $F_{SC}$ = 0.02985 | 0.00000 |
| Within populations | 92.21 | $F_{ST}$ = 0.07786 | 0.00000 |

Significant genetic differentiation was detected among populations within groups, with the Kuroshio group characterized by lower levels of differentiation ($F_{ST}$ = 0.025, p < 0.0001) and consequently greater genetic relatedness and gene flow (mean *D* = 0.006) than the Mindanao group ($F_{ST}$ = 0.056, p < 0.0001; mean *D* = 0.029) (Table 2). Geographically proximal populations were significantly different, i.e. Polillo samples POL-1 and POL-3 ($F_{ST}$ = 0.026, p < 0.0001; 60 km separation), BAL and MAS ($F_{ST}$ = 0.0301; p < 0.001; 35 km separation), and Dinagat populations DIN-1 and DIN-2 ($F_{ST}$ = 0.075, p < 0.0001; 20 km separation). Within either Kuroshio or Mindanao groups, genetic differentiation ($F_{ST}$) was not correlated with geographical distance (Mantel r = −0.1255 and −0.2322, respectively; p > 0.05). Across the entire eastern Philippine seaboard, a weak but significant signal of isolation-by-distance was indicated by the positive correlation between $F_{ST}$ and geographic distance (Mantel r = 0.278, p = 0.036) (Fig. 3).

## DISCUSSION

The north-south spatial genetic structure (Kuroshio and Mindanao groups) indicates the influence of the NEC bifurcation as an oceanographic barrier to larval dispersal and gene flow of *Tridacna crocea* along the eastern Philippine seaboard. The marked geographical variation in allele frequencies north and south of the bifurcation may reflect restricted larval exchange and gene flow owing to vicariance. The genetic differentiation of continuously distributed eastern seaboard populations analyzed in this study ($F_{ST}$ = 0.069; ~1400 km) is comparable with differentiation between

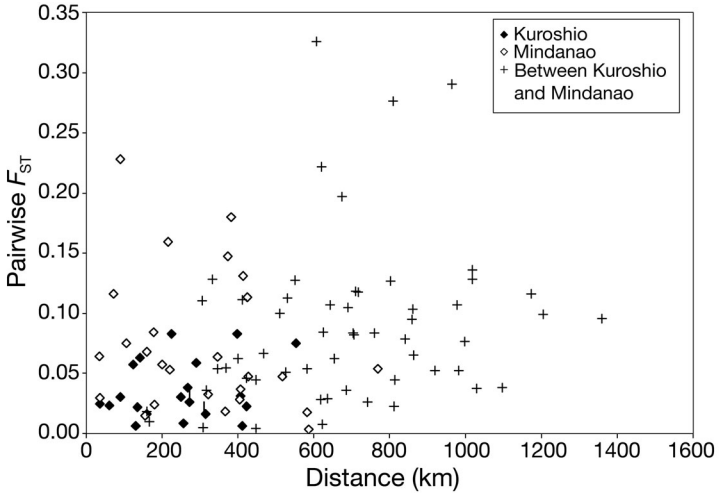


Fig. 3. *Tridacna crocea*. Relationship between $F_{ST}$ values and geographic distance for pairwise comparisons among 15 populations

disjunct South China Sea and Sulu Sea populations ($F_{ST}$ = 0.066; ~750 km straight-line distance; ~1000 km following current flow) (Juinio-Meñez et al. 2003) separated by the presence of a land barrier (Palawan Island). There was also a signal of isolation-by-distance over the entire length of the eastern Philippine seaboard (p = 0.036). Although isolation-by-distance patterns may be confounded by vicariance (Bossart & Prowell 1998), the pattern observed for *T. crocea* does not appear to be driven by the north-south spatial genetic structure (Fig. 3).

The genetic structure of *Tridacna crocea* along the Philippine Pacific seaboard is consistent with other studies correlating genetic divergence with hydrographic barriers to dispersal (Rocha-Olivares et al. 1999, Waters & Roy 2004) and biogeographic boundaries (reviewed by Dawson 2001). Genetic differentiation concordant with the NEC bifurcation was likewise detected among the reef fishes *Siganus argenteus* and *S. fuscescens* (Magsino 2004). However, previous studies of other tridacnid species reported patterns of gene flow inconsistent with present-day surface currents in the Indo-West Pacific (Macaranas et al. 1992, Benzie & Williams 1995, 1997), which were attributed to historical patterns of dispersal. Although consistent with present-day ocean current conditions, genetic differentiation between *T. crocea* populations north and south of the NEC bifurcation may also reflect ancient origins, i.e. restrictions to larval dispersal over geological timescales. Reconstruction of the tectonic history of southeast Asia (Hall 1998) suggest that the eastern Philippine seaboard was configured, positioned, and interacted with the westward-flowing NEC as early as 4 to 5 million years ago (Mya). The oldest occurrence of *T. crocea* in the fossil record is Late Pleistocene (Rosewater 1965); however, molecular data infer an early Pliocene origin for the *T. crocea* lineage (5 Mya; Schneider & Foighil 1999).

The differing levels of genetic diversity and connectivity between Kuroshio ($H_O$ = 0.152, $F_{ST}$ = 0.025) and Mindanao groups ($H_O$ = 0.298, $F_{ST}$ = 0.056) likely reflect population genetic responses to contrasting environmental conditions, i.e. the coastal topography, geology, and oceanography of the 2 regions. The lower genetic diversity of Kuroshio populations may be due to the effect of local-scale adverse environmental conditions, e.g. typhoons, on larval mortality and recruitment success (Juinio-Meñez et al. 2003), with typhoon tracks generally occurring with greater frequency on the northeastern coast relative to the southeastern coast. Greater genetic differentiation characteristic of the Mindanao populations could be attributed to more complex circulation patterns south of the NEC bifurcation, which could affect larval dispersal, mortality, and recruitment at varying spatial and temporal scales. Eddies (e.g. the Mindanao Eddy) resulting from large-scale oceanographic phenomena such as the NEC can alter connectivity of populations both spatially and temporally. Moreover, coastlines along the Mindanao Current are characterized by greater complexity (higher rugosity) relative to coastlines in the northern (Kuroshio) regions (Magno 2005). Greater coastal complexity may translate to higher potential for wake and eddy formation, which may serve as retention-favorable areas for planktonic propagules (reviewed by Sponaugle et al. 2002).

The strong flow of tidal currents through straits may act as additional barriers to longshore dispersal along the southeastern Philippine coast. Turbulent mixing through narrow, shallow straits may also influence dispersal and recruitment of propagules. In particular, differentiation between proximal reef populations of *Tridacna crocea* from Balicuatro and Masbate may arise from variable dispersal and recruitment success as a result of the inflow of water from the Philippine Sea into the Visayan Sea. Similar population genetic differentiation between these 2 reefs was previously reported for the sea urchin *Tripneustes gratilla* (Malay et al. 2002). Likewise, strong tidal currents flowing through the Surigao Strait, coupled with the high rugosity of coastlines in the area, may affect larval dispersal, entrainment potential, and recruitment at Homonhon and Dinagat reefs. Despite the short distances separating these 3 populations (~70 to 90 km between HOM and DIN-1 and DIN-2, and 20 km between DIN-1 and DIN-2), overall $F_{ST}$ values exceeded values of all 15 populations spanning the ~1400 km range of the eastern Philippine seaboard ($F_{ST}$ = 0.135 and 0.069, respectively).

Weaker genetic connectivity among Mindanao current populations could not be attributed to geographical

sampling effects, i.e. larger spatial scales covered by the Mindanao populations (~800 km). The Kuroshio samples covered a spatial range of ~400 km ($F_{ST}$ range = 0.006 to 0.083, mean = 0.036). Greater pairwise $F_{ST}$ values for Mindanao populations separated by 400 km were observed ($F_{ST}$ range = 0.015 to 0.2284, mean = 0.079) (Fig. 3). However, the strong genetic differentiation of the Homonhon population from the rest of the samples largely contributed to this result (Fig. 2). The resulting pattern of HOM as an outlier population may be attributed to accentuated genetic drift in smaller populations. The small sample size (n = 24) reflects the notably lower population density of *Tridacna crocea* at the Homonhon site relative to other reef sites sampled, which may be due to limited available suitable substrate, i.e. limited calcareous substrates at Homonhon (M. Juinio-Meñez pers. obs.). Alternatively, small population size may be due to variable reproductive and recruitment success owing to strong variability in hydrographic conditions, which results from turbulent mixing and complex hydrographic circulation in the area.

Despite the expected homogenizing effect of planktonic larval dispersal by strong, persistent current flows downstream of the NEC bifurcation along the eastern Philippine seaboard, fine-scale genetic differentiation (chaotic patchiness) was observed for *Tridacna crocea* populations within Kuroshio and Mindanao Current regions ($F_{ST}$ within regions = 0.025 and 0.056, respectively). This result is consistent with previous reports of conspecific populations from the South China Sea and Sulu Sea reefs (Juinio-Meñez et al. 2003), and contrasts with earlier population genetic studies of other tridacnid species, which revealed little genetic differentiation over broader geographic scales (reviewed by Juinio-Meñez et al. 2003). Locus-specific heterozygote deficiencies (in this case *GPI-1**) is a pattern frequently observed in bivalves (Luttikhuizen et al. 2003 and references therein), and may be attributed to genotype scoring errors, as well as to biological/reproductive factors e.g. genetic differences between cohorts, differential selection, and subpopulation structure (Wahlund effect). Exclusion of the *GPI-1** locus did not appreciably change the results of the present study, and thus this locus was included in the analysis.

In summary, the north–south geographic structure of *Tridacna crocea* populations along the eastern Philippine seaboard is broadly concordant with the flow of predominant oceanographic currents, reflecting the influence of the NEC bifurcation on the restriction of larval dispersal, which shapes population divergence as a result of random genetic drift. However, the influence of local selection at allozyme loci on the shaping of the observed spatial structure cannot be discounted, i.e. variations at allozyme loci may not be neutral. Correlation between allele frequencies and clinal variations in physical factors, notably temperature and salinity (reviewed by Eanes 1999), points to the role of selection in maintaining polymorphism at allozyme loci. Moreover, the influence of selection at allozyme loci in marine invertebrates has been demonstrated via comparison of genetic differentiation patterns with markers that are presumably neutrally evolving, e.g. mitochondrial DNA or microsatellites (e.g. Dufresne et al. 2004, Veliz et al. 2004). Nonetheless, concordance of genetic patterns observed for *T. crocea* with those of other marine taxa (Magsino 2004), as well as with biogeographic patterns in reef fish community structure (Aliño & Gomez 1993), suggests that these patterns were in part shaped by biogeographic barriers to gene flow. However, the location of the biogeographic break may span a wide latitudinal range for various marine taxa, owing to the temporal and spatial variability in flow strength and bifurcation latitude of the NEC (Qiu & Lukas 1996). The historical origin of this biogeographic pattern, i.e. long-term restrictions to larval dispersal over geological time-frames, is suggested by tectonic reconstructions (Hall 1998) but cannot be confirmed by allozyme data. Direct analyses of intraspecific phylogenies based on DNA sequence data (phylogeographic analyses) may provide better estimates of relative timescales of genetic divergence between the Kuroshio and Mindanao groups. In contrast, fine-scale genetic differentiation suggests that small-scale (temporal and spatial) physical processes affect larval dispersal and recruitment along the eastern Philippine seaboard.

*Acknowledgements.* This research was a component of the Pacific Seaboard Research and Development Program funded by the Department of Science and Technology-Philippine Council for Aquatic and Marine Research and Development (DOST-PCAMRD). We thank the Pacific Seaboard Project-1 team, C. Sajot (SSPSC) and L. Jimenez (DOSTSC) for providing logistical support for sample collection. C. Villanoy and F. Siringan provided valuable oceanographic and geological information and insights. R.M.M. acknowledges a travel grant from S. Planes (EPHE-CNRS 8046), and P. Lenfant (Université de Perpignan) for training on allozyme electrophoretic techniques and population genetic data analysis. The manuscript was improved by comments from a number of anonymous reviewers. This is UP Marine Science Institute Contribution No. 351.

## LITERATURE CITED

Aliño PM, Gomez ED (1993) Philippine coral reef conservation: its significance to the South China Sea. In: Yamazoto K, Ishiyima S, Sakihara S, Taira H, Shimabukuro Z, Teruya F, Nishihir F (eds) Development and conservation of the Asia-Pacific region. Proc Reg Conf East-West Center Assoc. The East-West Center Association, Okinawa, p 222–229

Belkhir K, Borsa P, Chikhi L, Raufaste N, Bonhomme F (2004) 1996–2004 GENETIX 4.05, logiciel sous Windows TM pour la genetique des populations. Laboratoire Genome, Populations and Interactions. Université de Montpellier II, Montpellier

Benzie JAH, Williams ST (1995) Gene flow among giant clam (*Tridacna gigas*) populations in Pacific does not parallel ocean circulation. Mar Biol 123:781–787

Benzie JAH, Williams ST (1997) Genetic structure of giant clam (*Tridacna maxima*) populations in the West Pacific is not consistent with dispersal by present-day ocean currents. Evolution 51:768–783

Bossart JL, Prowell DP (1998) Genetic estimates of population structure and gene flow: limitations, lessons and new directions. Trends Ecol Evol 13:202–206

Dawson MN (2001) Phylogeography in coastal marine animals: a solution from California? J Biogeogr 28:723–736

Dufresne F, Bourget E, Bernatchez L (2004) Differential patterns of spatial divergence in microsatellite and allozyme alleles: further evidence for locus-specific selection in the acorn barnacle, *Semibalanus balanoides*? Mol Ecol 11: 113–123

Eanes WF (1999) Analysis of selection on enzyme polymorphism. Annu Rev Ecol Syst 30:301–326

Excoffier L, Smouse PE, Quattro JM (1992) Analysis of molecular variance inferred from metric distances among DNA haplotypes: application to human mitochondrial DNA restriction data. Genetics 131:479–491

Felsenstein J (1995) PHYLIP — phylogeny inference package, version 3.57c. University of Washington, Seattle, WA

Hall R (1998) The plate tectonics of Cenozoic SE Asia and the distribution of land and sea. In: Hall R, Holloway JD (eds) Biogeography and geological evolution of SE Asia. Backhuys Publishers, Leiden, p 99–131

Juinio-Meñez MA, Magsino RM, Ravago RG, Yu ET (2003) Genetic structure of *Linckia laevigata* and *Tridacna crocea* among the Palawan shelf and shoal reefs. Mar Biol 142: 717–726

Lucas JS (1988) Giant clams: description, distribution and life history. In: Copland JW, Lucas JS (eds) Giant clams in Asia and the Pacific. Aust Cent Int Agric Res Monogr 9: 21–32

Luttikhuizen PC, Drent J, Van Delden W, Piersma T (2003) Spatially structured genetic variation in a broadcast spawning bivalve: quantitative vs. molecular traits. J Evol Biol 16: 260–272

Macaranas JM, Ablan CA, Pante MJR, Benzie JAH, Williams ST (1992) Genetic structure of giant clam (*Tridacna derasa*) populations from reefs in the Indo-Pacific. Mar Biol 113: 231–238

Magno MM (2005) Estimation of entrainment potential in Philippine coastal waters: the physical consequence of island wakes and eddies. MS thesis, University of the Philippines, Diliman

Magsino RM (2004) Patterns of population genetic structuring of rabbitfishes *Siganus argenteus* (Quoy & Guimard, 1825) and *Siganus fuscescens* (Houttuyn, 1782) along the eastern Philippine coasts. MS thesis, University of the Philippines, Diliman

Magsino RM, Juinio-Meñez MA, Planes S (2002) Preliminary analysis of the genetic variation of *Tridacna crocea* among seven reefs. UPV J Nat Sci 7:81–89

Malay MD, Juinio-Meñez MA, Villanoy CL (2002) Population genetic structure of the sea urchin *Tripneustes gratilla* from selected sites in western Luzon and eastern Philippines. Proc 9th Int Coral Reef Symp Bali

Mantel N (1967) The detection of disease clustering and a generalized regression approach. Cancer Res 27:209–220

Nei M (1978) Estimation of average heterozygosity and genetic distance from a small number of individuals. Genetics 89: 583–590

Nitani H (1972) Beginning of the Kuroshio. In: Stommel H, Yoshida K (eds) Kuroshio: its physical aspects. University of Tokyo Press, Tokyo

Qiu B, Lukas R (1996) Seasonal and interannual variability of the North Equatorial Current, the Mindanao Current, and the Kuroshio along the Pacific western boundary. J Geophys Res C 101:12315–12330

Raymond M, Rousset F (1995) GENEPOP (version 1.2). Population genetics software for exact tests and ecumenism. J Hered 86:248–249

Rice WR (1989) Analyzing tables of statistical tests. Evolution 43:223–225

Rocha-Olivares AA, Vetter RD (1999) Effects of oceanographic circulation on the gene flow, genetic structure, and phylogeography of the rosethorn rockfish (*Sebastes helvomaculatus*). Can J Fish Aquat Sci 56:803–813

Rosewater J (1965) The family Tridacnidae in the Indo-Pacific. Indo-Pacific Mollusca 1:347–396

Schneider JA, Foighil DO (1999) Phylogeny of giant clams (Cardiidae: Tridacninae) based on partial mitochondrial 16s rDNA gene sequences. Mol Phylogenet Evol 13:59–66

Schneider S, Roessli D, Excoffier L (2000) ARLEQUIN ver 2.000: a software for population genetics data analysis. Genetics and Biometry Laboratory, University of Geneva, Geneva

Shaklee JB, Allendorf FW, Morizot DC, Whitt GS (1990) Gene nomenclature for protein-coding loci in fish. Trans Am Fish Soc 119:2–15

Shaw CR, Prasad R (1970) Starch gel electrophoresis of enzymes — a compilation of recipes. Biochem Genet 4: 297–320

Sponaugle S, Cowen RK, Shanks A, Morgan SG and 7 others (2002) Predicting self-recruitment in marine populations: biophysical correlates and mechanisms. Bull Mar Sci 70: 314–375

Veliz D, Bourget E, Bernatchez L (2004) Regional variation in the spatial scale of selection at *MPI*⋆ and *GPI*⋆ in the acorn barnacle *Semibalanus balanoides* (Crustacea). J Evol Biol 17:953–966

Waters JM, Roy MS (2004) Phylogeography of a high-dispersal New Zealand sea-star: does upwelling block gene flow? Mol Ecol 13:2797–2806

Wijffels SE, Firing E, Toole J (1995) The mean structure and variability of the Mindanao Current at 8° N. J Geophys Res C 100:18421–18435

Wright S (1951) The genetical structure of populations. Ann Eugenet 15:323–353

Yu ET, Juinio-Meñez MA, Monje VD (2000) Sequence variation in the ribosomal DNA internal transcribed spacer of *Tridacna crocea*. Mar Biotechnol 2:511–516

*Editorial responsibility: Otto Kinne (Editor-in-Chief), Oldendorf/Luhe, Germany*

*Submitted: April 24, 2006; Accepted: August 21, 2006*
*Proofs received from author(s): April 9, 2007*