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# Preliminary analysis of the genetic variability of two natural beds of the Scallop *Euvola ziczac* (Linnaeus, 1758) in Brazil

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## ABSTRACT

Euvola ziczac *(formerly* Pecten ziczac*), a simultaneous hermaphroditic scallop was heavily fished in Brazil between 1972 and 1980. The production peaked in 1980 with 8,800 tons and was followed by the total collapse of the resource. In order to investigate the possible loss of genetic variability of the stock associated to overfishing and self-fertilization, the polymorphism of phosphoglucomutase (PGM) and glucose phosphate isomerase (GPI) was analyzed by electrophoresis of the adductor muscle of scallops from São Francisco (26°20.583S; 48°16.507W) and Bom Abrigo (25°28.735S; 47°37.621W) beds; the southern and northern extremes of the scallop fishing ground, respectively. Animals from São Francisco showed a strong deficiency of heterozygosity for GPI and PGM. In addition, PGM showed [*]exclusive alleles for each bed. Such results coupled with other information about the species suggested the following hypothesis: a) the stock was a metapopulation with at least two populations; b) some reproductive isolation might be occurring which might be influenced by conditions of larval transport and by the extremely low densities of scallops; c) presently, the stock seemed to be mostly maintained through self-fertilization; d) São Francisco could constitute a source-area, contributing with larvae and recruits to Bom Abrigo and other areas; e) both beds were suffering a genetic homogenization more evident in São Francisco. Such hypothesis needed to be investigated in order to furnish guidelines for future programs of recovery and management of the resource.*

**Key words:** *Genetic variability, Stock depletion, Scallop,* Euvolaziczac*, Brazil*.

## INTRODUCTION

Among the marine invertebrates, scallops can be considered one of the most important fishing resources, owing to the expressive economic yields obtained by commercial exploitation of their natural beds and aquaculture. In Brazil the family Pectinidae is represented by 18 species (Rios, 1994) but only *Euvola ziczac* (Linnaeus, 1758) (formerly *Pecten ziczac*) and *Nodipecten nodosus* (Linnaeus, 1758) have shown some economic importance. The former species was heavily fished on the southeastern Brazilian shelf during the 1970s and early 1980s. The latter has been cultured but still in a incipient state. Recently, Pezzuto & Borzone (1997a) reviewed the data on the *E. ziczac* fishery in Brazil. The exploitation of the species started between 1972 and 1973, as a response to high international prices of scallop meat and decreasing yields of penaeid shrimps, the target species of the double-rig trawler fleet that operated in the region. Between 1972 and 1980, there were two peaks of scallop production, the highest reaching 8,800 tons in 1980. After this peak the stock was completely collapsed and *E. ziczac* became a minor by-catch item in the shrimp fishery, with current annual landings not exceeding 1-10 t (Pezzuto & Borzone, 1997a; Perez & Pezzuto, 1998).

Comparing data collected during surveys carried out in 1974-1975 and 1995-1996, Pezzuto & Borzone (*unpubl. data*) estimated a reduction of 80% in the area, 89% in the abundance and 99% in the biomass of the scallop beds. The best yields obtained by the authors in the hauls conducted during the 1995-1996 cruises were only 35 individuals per hour, using a heavy 2-m-wide beam-trawl at trawling speeds of 3-4 knots, which corresponded to a trawled area of *ca.* 14,800 $m^2$ (Borzone & Pezzuto, 1997; Pezzuto & Borzone, 1997b). These figures and the continuous drop in the catches observed since 1981, suggested a critical state of the species that probably had its natural restocking capacity transposed by severe overfishing. In addition, it could also be possible that such extreme depletion might have contributed to a significant decline in the genetic diversity of the species, because many pre-existent alleles could have disappeared with the organisms. This situation seems to be even more likely since *E. ziczac* is a functional hermaphroditic (Betancourt *et al.*, 1995). Therefore, while the self-fertilization could be used by the species as a reproductive strategy to overcome or minimize the effects of strong fluctuations in abundance (Betancourt *et al.*, 1995), it could also contribute to the loss of genetic variability of the population, with a consequent increase of homozygosity in the stock that could in turn reduce the chances of success of its recovery.

This article presents some data on genetic variability of *E. ziczac* collected in two natural beds in Brazil, and addresses some hypotheses about the consequences of the overfishing and the spatial structure of the population.

## MATERIAL AND METHODS

Specimens of *E. ziczac* were collected during the "Scallop Project Cruise XII", carried out between 18 and 20 of February 1997 (see Pezzuto *et al.*, 1988, for details of the cruise). Ten individuals were obtained in Bom Abrigo (25° 28.735S; 47° 37.621W) and 20 in São Francisco (26° 20.583S; 48° 16.507W) beds (Fig. 1b), corresponding to animals collected in one and two hauls, respectively. Although the number of individuals was low, it could be considered representative of the beds since their densities were extremely low, as discussed above.

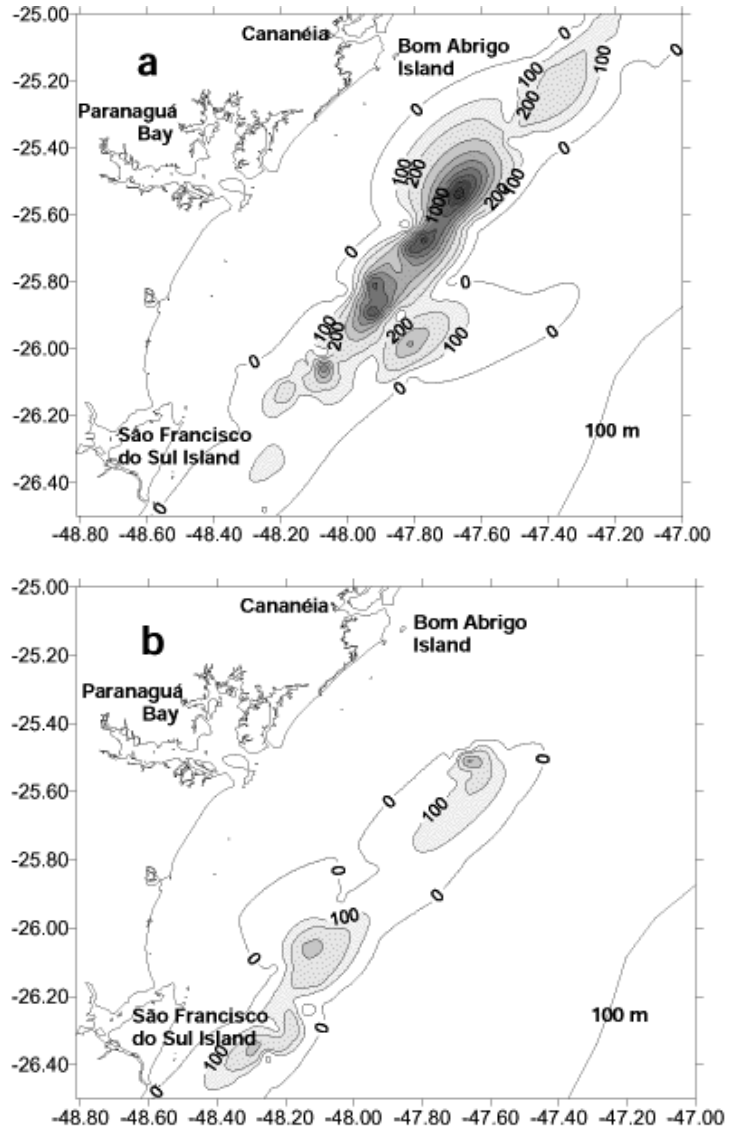


**Figure 1** - Distribution and yields of *Euvola ziczac* in southern Brazil. Values are catches of scallop by hour of trawling using a standard otter trawl. a: catches for 1974-1975; b: catches for 1995-1996.

After sampling, specimens were maintained alive aboard in aerated local seawater. In the laboratory, living animals (five from Bom Abrigo and 13 from São Francisco) were measured (shell height in mm) and dissected for extraction of adductor muscle sections. The muscular tissue was maintained in labeled Eppendorf tubes frozen with liquid nitrogen and stored at -70° C for further analysis. The enzymatic polymorphism of phospho-glucomutase (PGM) and glucose phosphate isomerase (GPI) was analyzed by vertical electrophoresis in acrylamide gels, using SIGMAä acrylamide for electrophoresis at 9 %. Sections of the adductor muscle were manually homogenized with distilled water and centrifuged for 15 min. at 30,000 x g. The electrophoresis was conducted on a 4° C refrigerated chamber connected to a 90-100V continuous electric current font at 3 mA for three hours. The revelation solutions were prepared according to Schaal & Anderson (1974) and Harris & Hopkinson (1976), with minor modifications. In order to prevent overstaining it was used a fixative solution of 10 % acetic acid + 90 % distilled water.

## RESULTS AND DISCUSSION

In both beds, GPI was polymorphic and codified only by two alleles. In the active form this enzyme is a dimere and therefore, individuals can show one or three bands whether they are homozygotes or

heterozygotes, respectively. Animals from São Francisco showed a strong deficiency of heterozygosity. Except by one individual that was homozygote for the allele 115, all specimens were homozygotes for the allele 100. On the other hand, three of the five specimens from Bom Abrigo were heterozygotes for the GPI (Table 1).

**Table 1** - *Euvola ziczac*. Individual phenotypes observed for phosphogluco-mutase (PGM) and glucose phosphate isomerase (GPI) extracted from adult specimens from São Francisco and Bom Abrigo beds. Shell height in millimeters.

| | Shell height | GPI | PGM |
|---|---|---|---|
| São Francisco | 75 | 100\100 | 100\100 |
| | 76 | 100\100 | 85\85 |
| | 63 | 100\100 | 100\100 |
| | 64 | 100\100 | 100\100 |
| | 64 | 100\100 | 100\100 |
| | 73 | 115\115 | 100\85 |
| | 71 | 100\100 | 100\100 |
| | 79 | 100\100 | 100\100 |
| | 66 | 100\100 | 100\100 |
| | 69 | 100\100 | 100\100 |
| | 55 | 100\100 | 100\100 |
| | 60 | 100\100 | 100\100 |
| | 75 | 100\100 | 100\100 |
| Bom Abrigo | 66 | 100\100 | 100\100 |
| | 70 | 100\115 | 100\100 |
| | 72 | 100\100 | 100\115 |
| | 72 | 100\115 | 100\115 |
| | 74 | 100\115 | 100\100 |

Unlike GPI, PGM was a monomeric enzyme showing one or two bands. The same pattern of excess of homozygosity found for GPI in São Francisco was observed for PGM in this bed with the exception that one individual was heterozygote. The enzyme showed three alleles: 115; 100 and 85. Allele 115 was exclusive of individuals from Bom Abrigo and occurred only in heterozygosity, while allele 85 was found only in São Francisco, either in homozygosity or heterozygosity (Tables 1 and 2).

**Table 2** - *Euvola ziczac*. Allelic frequencies and heterozygosity of phosphoglucomutase (PGM) and glucose phosphate isomerase (GPI) extracted from adult specimens from São Francisco (SF) and Bom Abrigo (BA) beds. N: number of individuals; $H_o$: observed heterozigosity; $H_e$: expected heterozygosity; D: deficiency of heterozygosity.

| Bed | Enzyme | N | Allelic frequencies | | | Heterozigosity | | D |
|---|---|---|---|---|---|---|---|---|
| | | | 100 | 115 | 85 | $H_O$ | $H_E$ | |
| BA | GPI | 5 | 0.70 | 0.30 | - | 0.6 | 0.42 | 0.43 |
| SF | GPI | 13 | 0.92 | 0.08 | - | 0 | 0.14 | - |
| BA | PGM | 5 | 0.80 | 0.20 | - | 0.4 | 0.32 | 0.25 |
| SF | PGM | 13 | 0.88 | - | 0.12 | 0.08 | 0.20 | -0.60 |

Despite the scarcity of material used in this study, the electrophoretic analysis of GPI and PGM enzymatic systems showed a clear excess of homozygosity in the São Francisco bed, as found in other natural populations of bivalves (Wilkins & Mathers, 1979; Zouros & Foltz, 1984a, 1984b; Gaffney *et al.*, 1990). Specially for PGM, it was noteworthy the presence of exclusive alleles in both beds, which could indicate the development of characteristic alleles resulting from the partial reproductive isolation of the areas. On the other hand, Table 3 shows that the genetic identity and genetic distance between the beds are very similar for both enzymes. The high genetic identity confirmed the inexistence of specific differences between organisms from Bom Abrigo and São Francisco, and suggested the occurrence of an important genetic flow in the past resulting from the high abundances and the continuous distribution of the species in this ground in 1974-1975, as showed in Figure 1a. This pattern of distribution and abundance of the species was severely changed after the collapse of the fishery in 1981, when the population was gradually segregated in the actual two low density beds placed in the

opposite extremes of the area (Fig. 1b).

**Table 3** - *Euvola ziczac*. Genetic identity and genetic distance calculated for specimens from São Francisco (SF) and Bom Abrigo (BA) beds based on electrophoretical analysis of phosphoglucomutase (PGM) and glucose phosphate isomerase (GPI).

| Bed | Enzyme | Genetic Identity | Genetic distance |
|---|---|---|---|
| BA-SF | GPI | 0.9482 | 0.0531 |
| BA-SF | PGM | 0.9620 | 0.0387 |

Whether the distance between these beds constrained their genetic flow needed to be investigated, but preliminary data suggest that some isolation could exist. The center of the two beds were nearly 65 nautical miles apart (*ca.* 121 km) (Borzone & Pezzuto, 1997). Rojas & Seijo (1990) and Betancourt *et al.* (1995) found that larvae of *E. ziczac* needed 10 to 12 days to develop in laboratory conditions at 26° C and 36 ppm. In the study area, similar temperatures and salinities were observed only in surface waters and during short periods on the summer (Borzone *et al.*, 1999). The predominance of lower temperatures during most of the year in the field suggests that the larval development could be longered than observed in laboratory. Thus, assuming a conservative (possibly short) period of 12 days for the larval development on the shelf, the interchange of larvae between São Francisco and Bom Abrigo would be possible with a latitudinal net transport of only 0.2 knots (*ca.* 0.1 $m.s^{-1}$), considering the hypothetical inexistence of physical and behavioral mechanisms of larval retention in the vicinity of the beds.

However, the pattern of currents suggested by Castro-Filho *et al.* (1987) and Castro-Filho (1990) for the southeastern Brazilian shelf suggested that such mechanisms might occur. According to these authors, during winter the inner continental shelf from the coast to the 50 m isobath remains dominated by Subantarctic waters flowing to the northeast and showing a homogeneous vertical profile of temperature and salinity. This pattern changes during summer when a strong vertical stratification occurs, with high temperature water flowing to the southwest in the surface (Coastal waters) and low temperature water (South Atlantic Central water) flowing to the northeast below the thermocline. Observations on the temporal variation of the gonadosomatic index and the recruitment patterns of *E. ziczac* (Pezzuto & Borzone, *unpubl. data*) suggested that the species spawns twice a year in the region, with the first and strongest spawning peak occurring on the summer and the second and weakest on the winter. Hence, the consequences of the circulation and spawning patterns would be the exportation of larvae from both beds to the northeast during the winter and the retention of the larvae over their respective beds on the summer (main spawning period), supposing that they transposed the thermocline by vertical migration and were transported in opposite directions by the two currents in different phases of their development. Therefore, the partial reproductive isolation suggested by the exclusive alleles found in São Francisco and Bom Abrigo scallops could be controlled by the conditions of larval transport between the beds in each reproductive season, which might be also hardly influenced by the extremely low densities of scallops and, consequently, of larvae produced in both beds.

On the other hand, scallops from São Francisco showed the highest homozygosity values as compared to organisms from Bom Abrigo, suggesting the latter might receive more genetic input from other areas, *i. e.* from low concentration of scallops found north of Bom Abrigo and specially from São Francisco bed which represented the southern limit of the species distribution. Therefore, it was possible that São Francisco might represent a source-area, contributing at least sporadically with larvae and recruits to both beds, while receiving few inputs from the north. In addition, the high degree of homozygosity might also be caused by the prevalence of a self-fertilization strategy in the population, since *E. ziczac* is a simultaneous hermaphroditic (Betancourt *et al.*, 1995). Beaumont & Budd (1983) and Orensanz *et al.*, (1991) pointed out that the development of simultaneous hermaphroditism in some pectinids might have a significant role in the stock maintenance during negative fluctuations in their abundance, though such strategy still have not been confirmed with field data. In the case of *E. ziczac*, at least two evidences supported the hypothesis that the self-fertilization could be the main responsible for the maintenance of the existent beds: a) laboratory experiments have showed that contrary to other species (*e.g. Pecten maximus*; Beaumont & Budd, 1983), the occurrence of self-fertilization in *E. ziczac* specimens did not interfere either in the survival or in the growth of their larvae and juveniles, at least in two self-produced generations (Betancourt *et al*., 1995); b) densities of

the actual beds were so extremely low that the success of cross-fertilization in the water column was expected to be very unlikely, even supposing that the spatial pattern of the individuals in micro-scale was strongly clumped. Like other pectinids (*e. g.* Caddy, 1989; Orensanz *et al.*, 1991; Ciocco, 1992), the stock of *E. ziczac* in southern Brazil seems to be another example of self-sustainable metapopulation consisting of a minimum of two populations represented by the São Francisco and Bom Abrigo beds, possibly with different but still not confirmed roles in stock maintenance. As pointed out by Caddy (1975), the dynamic pool axiom, commonly used for finfish management did not apply to sedentary species such as scallops and therefore, in order to implement correct management actions it would be necessary to distinguish their self-sustainable and permanent populations from the edge-populations that could be more dependent and/or that occured in a more transitory form.

The hypotheses raised in this work needs to be investigated in order to furnish guidelines for future programs of recovery and management of the resource in southern Brazil.

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## RESUMO

A vieira hermafrodita simultânea *Euvola ziczac* (anteriormente *Pecten ziczac*), foi intensamente explotada no Brasil entre os anos de 1972 e 1980. O máximo de produção foi atingido em 1980, com 8800 toneladas desembarcadas, após o qual ocorreu o total colapso do recurso. Com o objetivo de reconhecer uma possível perda de variabilidade genética do estoque associada à sobrepesca e à autofecundação, analisou-se por técnicas eletroforéticas o polimorfismo da fosfoglicomutase (PGM) e da glicose fosfato isomerase (GPI) do músculo adutor de vieiras do banco de São Francisco (26° 20,583S; 48° 16,507W) e Bom Abrigo (25° 28,735S; 47° 37,621W), nos extremos sul e norte da área de pesca, respectivamente. Os animais de São Francisco mostraram uma forte deficiência de heterozygocidade para a GPI e a PGM. A PGM mostrou também alelos exclusivos deste banco. Estes resultados, junto com outras informações da espécie, levantam às seguintes hipóteses: a) o estoque é uma metapopulação com pelo menos duas populações; b) algum isolamento reprodutivo pode ocorrer influenciado pelas condições de tranporte larval e pelas densidades extremamente baixas dos adultos; c) atualmente o estoque parece manter-se principalmente por autofecundação; d) o banco de São Francisco pode constituir uma área fonte, contribuindo com larvas e recrutas para o banco de Bom Abrigo e outras áreas; e) ambos bancos estariam sofrendo uma homogenização genética, mais evidente em São Francisco. Estas hipóteses necessitam ser investigadas com o objetivo de prover orientação a futuros programas de recuperação e manejo do recurso.

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