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A POPULATION STUDY OF BLUE PILCHARDS (*Sardinops neopilchardus*) IN SOUTH AUSTRALIA WATER USING ALLOZYME ELECTROPHORESIS

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ABSTRACT

The biology and population analysis of Australian pilchards (Sardinops eopilchardus) was reviewed and studied. The Australian pilchards stock was depleted in South Australia whereas in other states are under-exploited. The population study was done using protein analysis. Six enzymes (PEPB, AH, PGM, EST, MPI and AAT), which showed polymorphism, were scored and used in the population study. For future studies, frozen procedure techniques need to be improved and number of samples collected need to be extended.

Keywords: *Sardinops neopilchardus*, allozyme electrophoresis, population study.

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INTRODUCTION

The Australian pilchards, *Sardinops neopilchardus* belongs to the family of Clupeidea. Pilchards are usually found in bays, inlets and water from inshore to the edge of continental shelf. They are distributed from the surface to depths of up to 200m.

The pilchard fishery in Australia has been developed since 1940`s, but its annual production has fluctuated (Syahailatua, 1992). It is recently known that most Australian pilchards stock is heavily exploited in Western Australia whereas in other states are under-exploited.

This paper is a review on the biology and a population study of South Australian pilchards using allozyme electrophoresis. Some biological aspects of pilchards such as, morphology and taxonomy, food and feeding, predators, reproduction and life history and distribution are included. A brief discussion on the population study is also given.

MATERIALS AND METHODS

Specimen Collection

Specimens were collected on April 23, 1997 from two regions of South Australia; SA1 and Port Lincoln 1 and 2 (Fig.2). Fish were frozen and transported to the laboratory on ice. Upon arrival, all specimens were stored whole at –20°C, until required.

Preparation of Starch Gel

Horizontal starch gels were made from 10% (w/v) sigma starch with 3 buffers following Richardson, *et.al* (1986). Starch (30 g) was suspended in 1/3 of 300 ml of gel buffer, put on the stirrer while the remaining 2/3 of buffer was heated for about 150 seconds in a microwave oven. The stirring solution as then transferred to a pre-heated hot plate, and the remaining solution was quickly added. It was kept stirring until the volume reduced and the solution clear with bubbles rising from the bottom of the flask. This solution was the degassed using a Gelman `Litlle Giant` vacuum pump and poured into a square Perspex mould (dimension: 300ml 19.5x 19.5 x 5 cm).

Once poured, the gel was left to cool at room temperature for at least 1 hour, covered with plastic wrap to prevent desiccation and stored overnight in a refrigerator.

Table 1. Electrophoresis Buffer Recipes (Electrostarch)

Buffer		Recipe
TM pH 7.8		
Electrode Buffer		24.2 g Tris
		9.2 g Maleic acid
Gel Buffer	to	2.0 l Milli Q water
		100.0 ml Electrode buffer
	to	1.0 l Milli Q water
Run conditions		50 mA, 200 V, 3.5 hrs
CAM pH 6.1		
Electrode Buffer		16.8 g Citric acid
		19.5 ml N-(3-aminopropyl-morpholine)
Gel Buffer	to	2.0 l Milli Q water
		15.0 ml Electrode buffer
	to	300.0 ml Milli Q water
Run conditions		50 mA, 190 V. 3 hrs.
TC pH 6.8		
Electrode Buffer		131.2 g Tris
		84.1 g Citric acid
Gel Buffer	to	4.0 l Milli Q water
		70.0 ml
	to	2.0 l Milli Q water
Run conditions		50 mA, 200 V, 3 hrs.

* Revised from Richardson, et. al (1986)

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Tissue Preparation

A total of 232 fish samples were partially thawed and liver tissues were removed for electrophoresis. All samples were stored in 1.5 ml eppendorf tubes at –70°C until required for electrophoresis. All fish were then stored at –20°C until they were measured and sexed.

Samples were partially thawed and homogenized with 60 Mm volume of cold homogeny zig buffer. Tissue samples were kept in small ice filled insulated containers during homogenization. The slurry was then centrifuged using a Heraeus Sepatech 17 RS Biofuge at 4°C, 5000 rpm for 10 minutes. Cooling is needed when the centrifuge temperature above 4°C. This process separates cellular debris from the supernatant containing proteins in solution.

Electrophoresis

Three different buffer systems viz: CAM 6.1,TM 7.8 and TC 5.8 were investigated in this study. The recipes of the stains stain buffers and stock solutions used in this study are following Richardson, *et.al* (1986).

After homogenization and centrifugation, the supernatant was observed onto a filter paper wick (Whatman #3 filter paper, 5x2 mm) and using jeweler's forceps, placed onto the edge of a cut starch gel 5cm from the cathodal end of the gel. A wick blotted in bromophenol blue solution was placed in the first lane on the left-hand side of the gel and after the last one. It could also be placed in the middle to make easier for scoring.

Loaded gels were placed in electrophoresis tanks with the appropriate electrode buffer. A direct current was applied across the gel using harmacia power tank according to the conditions prescribed for the buffer as given in the table 1.

Gels were run until the tracking dye had migrated 5-7 cm from the origin (3.5 h for TM 7.8 and 3 h for CAM 6.1 and TC 5.8). Gels were then sliced twice. Each slice was removed using a pre-cut plastic sheet and placed in a plastic tray. The gels were stained using methods modified from Shaw and Prasand (1970) and Harris and Hopkinson (1976) in Dixon et.al (1996). Gels with activity were scored.

Population Study

Three sites of South Australia samples; 157 of SA1, 33 of Port Lincoln 1 and 46 of Port Lincoln 2 were electrophoreses and scored. Gels in the population study were usually run with 24 unscreened individuals with the first individual repeated after the twenty fourth individual. If a specific enzyme with the same individuals repeats is needed then double band run is approved well. This method less time consumed.

Alleles were assigned arbitrary values with the most common allele nominated as 100; faster alleles were assigned numbers in increasing units of 10 in preference to using specific migratory distance. That is, the next anodal allele was 110 and so on. The slower alleles were typed in decreasing values of 10. This allowed for the inclusion of any previously undetected alleles. Such alleles were assigned values according to the mobility in relation to other alleles.

For consistency in typing alleles between gels, each gel was run in two standard of known genotype. The distance between the origin and each allele was measured using dividers.

RESULTS AND DISCUSSION

The Australian and New Zealand pilchards *Sardinops neopilchardus* (**Fig.1**) belongs to the genus *Sardinops*; which consists of four other closely related species, *Sardinops ocellatus* (South Africa), *S. melanostictus* (Japan), *S. sagas* (South America and California) and *S. caeruleus* (Fletcher, 1990). One of the most recent descriptions of this species is given by Baker (1972) in Fletcher (1990:) `Body elongate, oval in cross section. Ventral profile gently curved; dorsal profile less curved. Depth of body is less than the length of head. To elongate scales at the base of caudal fin; body scales very deciduous; scutes weakly keeled. Mouth terminal, with minute teeth only on posterior ventral/surface of maxillary. …Caudal fin deeply forked. Vertebrae 49-52. Standard length to 213mm. Colour: dark blue above…a row of eight to fourteen spots dorso-laterally along the body just behind dorsal fin; below this row, the colour of the flanks changes abruptly to silvery bronze. The tips of jaws are finely spotted with black; dorsal and caudal fins with fine black spots at margins. The anal fin consists of 16-20 soft rays and no obvious lateral line. The dorsal fin is located in the front half of its body with 17-20 soft rays.

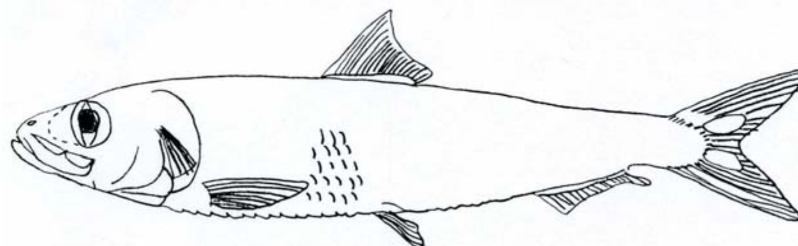

Fig. 1. The Australian Pilchard (*Sardinops neopilchardus*)

Few aspects of the feeding biology of Australasian pilchards (*S. neopilchardus*) have been determined thoughtfully. Roughly (1916) in Fletcher (1990) described the food of the pilchard as consisting of `animalculae` of the surface water. This is including the spawn of fishes, crustaceans and mollluscs.

Blackburn, 1960 then stated that pilchard mostly feed on crustacean (copepod, ostracod, euphausiid, mysid), but larvaceans and chaetognaths are also consumed.

A large variety of fish, bird and mammals are pilchard's predators (Blackburn, 1960; Fletcher, 1990). Pilchards were found in the stomachs of southern bluefin tuna, tunny, John Dory and bonito. They are also a major for item for the little penguins (*Eudyptula minor*) of Victoria and Western Australia (Fletcher, 1990).

The reproductive cycle of pilchard has been studied thoroughly and the sufficient data has been collected show that the spawning season of *S. neopilchardus* varies with the geographical location (**Fig.2**).

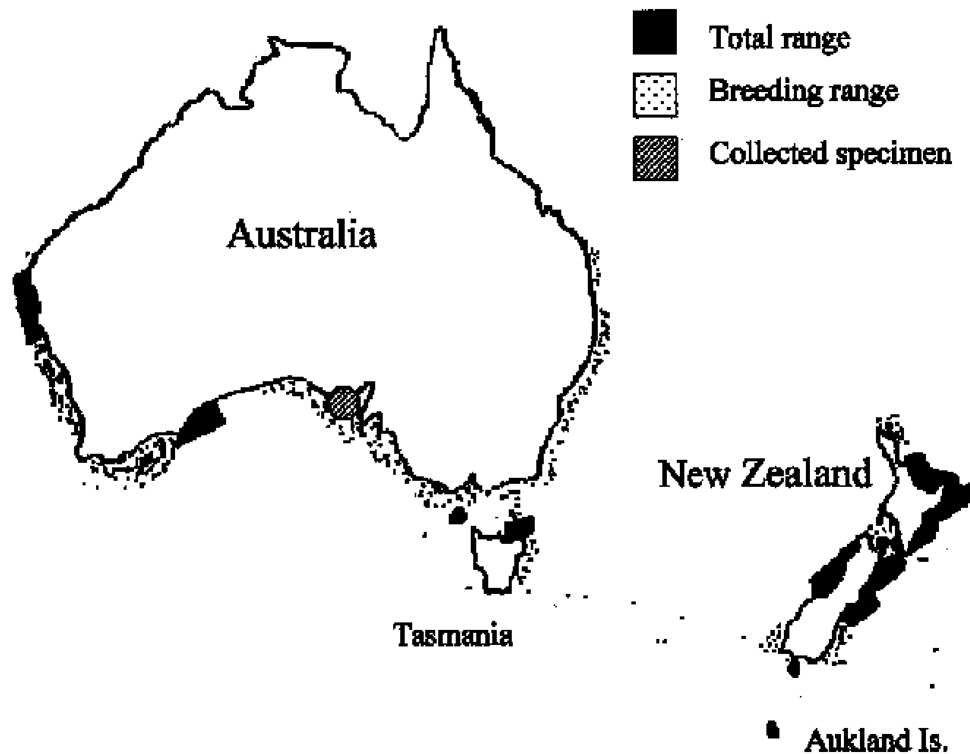

Fig. 2. The total breeding/spawning range in Australia and New Zealand.

Pilchards are bisexual; they are hermaphroditism, heterosexuality and inter sexuality (Blackburn, 1960). The spawning season maybe determined by the period of the pilchards' stage of maturity or shown by the period of abundance of its eggs and larvae (Syahailatua, 1992).

Blackburn (1960) worked with plankton in South Australia found that eggs and larvae were present from December to early March and suggested as a summer/autumn season. Spawning has been recorded only where the surface temperature are between 14 and 21 °C (Kailola et.al, 1993).

Pilchards are schooling behaviour fish, which inhabit the temperate waters of most continents (Kailola et.al, 1993). In Australia pilchards are widespread, have been found from Hervey Bay in Queensland down to around Tasmania, across Victoria, the GAB and up to the west coast of Western Australia to Red Bluff (Fletcher, 1990) (Fig.3).

The distribution of *S. neopilchardus* is relation to temperature was studied by Regan (1916) and Blackburn (1960). Regan found the worldwide distribution of *Sardinops* spp. would in coastal waters with the mean annual temperatures between 12-20 °C.

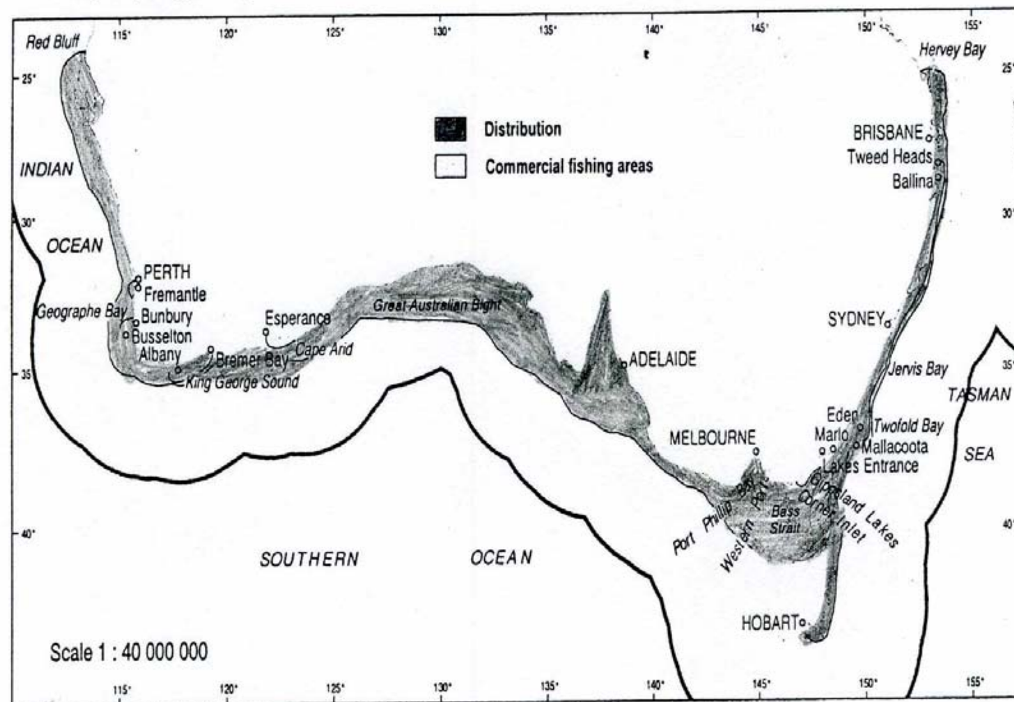

Fig. 3. Geographic distribution and commercial fishing areas for pilchards in Australia water.

Blackburn suggested differently for *S. neopilchardus* that they only occurred in those waters of the continental shelf which had mean winter surface temperature between 9-21°C and surface salinity between 33.5-37 ‰. There is a little functional difference between these two definitions.

Six enzymes were screened in liver tissue on six different buffer systems. PEPB and AH were run with CAM 6.1; PGM and EST were run with TM 7.8 and MPI and AAT (alternative) were run with TC 5.8. PEPB, EST and AAT gave the strongest activity for most of the samples then followed by PGM, MPI and AH. However, samples from Port Lincoln 1 have the worst results. It was assumed due to the lack of frozen procedure.

The last fifteen samples of SA1 also have no activity at all and this was believed due to the time consumed during tissue preparation. The six enzymes (PEPB, AH, PGM, EST, MPI and AAT) showed polymorphism with sufficient resolution for scoring. The lowest locus is found in AH (80) and the highest in AAT and AH (120). The observed banding appears and designed genotypes for the six enzymes screened in the population study showed in Figure 4.

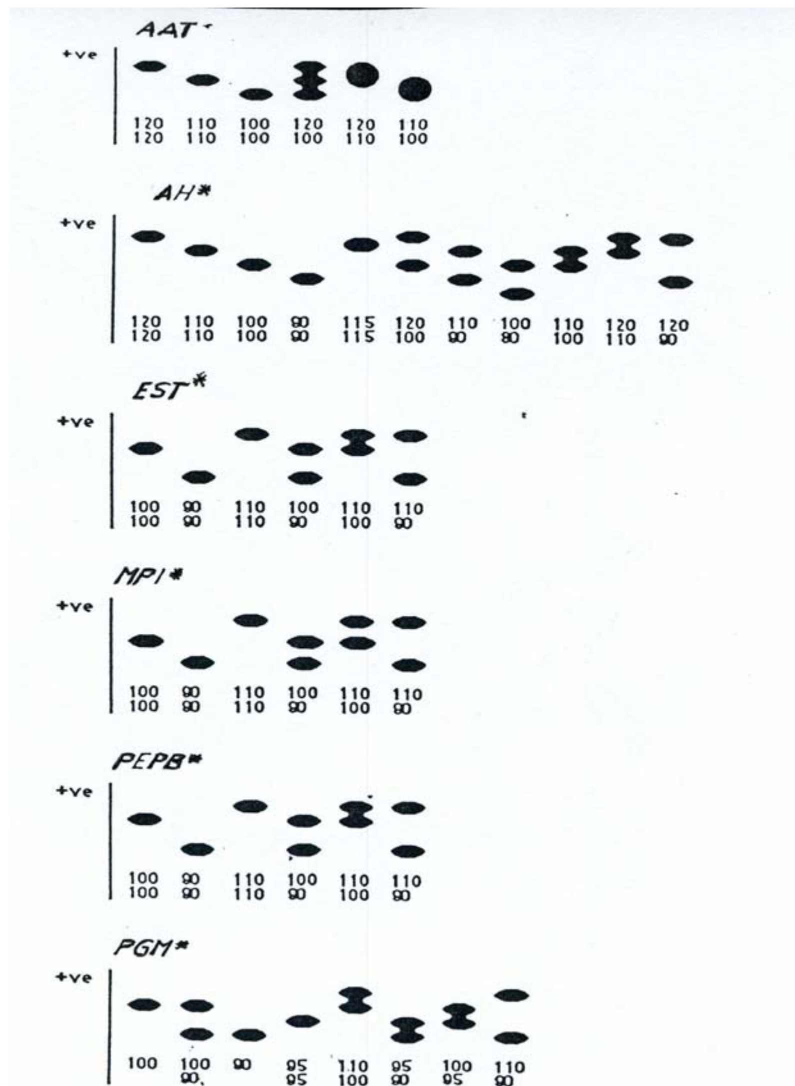

Fig. 4. Observed banding appears and designed genotypes for the six enzymes screened in the population study.

CONCLUSION

The results have shown the ability of allozyme electrophoresis in separating individuals fish based on their enzymatic activities. In this study six enzymes have shown polymorphism (PEPB, AH, PGM, EST, MPI and AAT), but there were samples, which did not show any activities. These problems may be arisen due to frozen procedures and tissue preparation. Therefore, for future research we need to increase the quality of frozen procedure, to work more precisely on tissue preparation and to extend the number of samples used for each site to an equilibrium number.

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