

Genetic variations in marine natural populations—Measurement and utility in resource management and conservation: A review

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A number of molecular and biochemical tools which can be applied to the identification of species and the detection of genetic variation within species have been developed in recent years. All these methods rely on the ability to distinguish between variants of specific genes (DNA) or their gene products (such as RNA or proteins). The technique of gel electrophoresis coupled with histochemical staining of specific proteins (isozymes) has been widely applied in population biology. This paper reviews the laboratory methods and genetic interpretation of gel phenotypes along with statistical methods for data analyses. The applications and perspectives for identifying and protecting genetic variation within and among marine populations are discussed in the light of studies undertaken on fishes collected from different areas of the marine environment of India.

The need for conservation of fish genetic resources has been recognized by fishery scientists and aquaculturists for some time, especially in relation to overfishing of natural stock, the effects of large-scale alterations to river systems, and domestication of species through aquaculture. In 1980 two symposia were held to address specifically the issue of genetic conservation in fishes, the Fish Gene Pools symposium held in Stockholm¹ and the FAO/UNEP symposium on Conservation of the Genetic Resources of Fish, in Rome². Both symposia recognized and recommended an urgent need to identify and protect genetic diversity within and among populations of fish species and further came out with an action plan. This came as an impetus to research on conservation of biological diversity, which has steadily grown over the years³.

Nevertheless, global activities towards putting genetic conservation into practice in most cases have been minimal. A major cause of this delay in implementation of appropriate conservation measures appears to be ignorance or indifference towards the implications of continued losses of genetic resources⁴. For any oceanic species, one of the first requirements for the formulation of a preservation policy must be a distribution map and the identification of any distinct sub-populations

which may exist. Distribution data will probably be available from relevant fisheries, but the detection of subpopulations may have to be carried out by the use of one or more of the 'labelling' techniques available.

Molecular techniques can be particularly useful in the identification and description of taxonomic units, including species, subspecies, and populations⁵. These methods rely on the ability to discriminate between variants of specific genes (DNA) or their gene products (such as RNA and proteins). Almost all our knowledge about genetics of natural populations has been gained from electrophoretic data, mainly because of the versatile nature of isozyme methodology⁶. Isozymes have been extensively used for describing the genetic architecture and genetic differentiation of fishes collected from different areas of the marine environment (Table 1).

Techniques

Gel electrophoresis is a powerful yet relatively simple technique that separates proteins according to their net charge and size in an electrical field. Proteins are complex molecules built up from a chain of amino acids that are the structural products of genes. The cornerstone of the electrophoretic method is the fact that each

Table 1—Species names, sampling locality and number of specimens examined (N)

Family/Species	N	Locality	Source/ref. no.
Scombridae			
<i>Rastrelliger kanagurta</i>	100	Panaji, Goa	Menezes <i>et al.</i> ^{23,37}
	40	Colva	
	60	Ennur, Madras	
	35	San Thome	
	40	Andaman	
Clupeidae			
<i>Sardinella longiceps</i>	94	Panaji, Goa	Menezes ²⁴
	20	Malpe, Karnataka	
	20	Mangalore	
<i>S. albella</i>	47	Panaji, Goa	Menezes ⁵¹
<i>S. fimbriata</i>	56	Panaji, Goa	
Mugilidae			
<i>Mugil cephalus</i>	103	Caranzalem, Goa	Menezes <i>et al.</i> ¹³
<i>Liza subviridis</i>	46	Caranzalem	
<i>Valamugil cunnesius</i>	12	Caranzalem	
Sciaenidae			
<i>Johnieops dussumieri</i>	100	Calangute, Goa	Menezes <i>et al.</i> ¹⁹
<i>Kathala axillaris</i>	70	Calangute	
<i>Pennahia macrophthalmus</i>	26	Calangute	
<i>Otolithes ruber</i>	149	Calangute	
Stromatidae			
<i>Pampus argenteus</i>	51	Dona Paula, Goa	Menezes ⁴²
<i>Pampus chinensis</i>	4	Dona Paula	
Apolectidae			
<i>Parastromateus niger</i>	46	Dona Paula	

specific protein is encoded by a single gene. Use of this technique has uncovered a wealth of genetic variation in animal and plant species and has led to new theoretical and practical insights in population genetics.

Most proteins studied in electrophoretic surveys are enzymes (Table 2). Samples need to be frozen as soon as possible and to be stored at -20°C or lower. In the laboratory, a range of buffer systems are tested to resolve the specific enzymes^{7,8}. The cell lysate obtained by freezing and thawing is directly subjected to electrophoresis for phenotypic analysis. After a suitable time interval about 4 hours for starch gel, the power is switched off and the gel is removed from the electrophoresis chamber and sliced. Each slice is immersed in a stain that contains the specific enzyme substrate and its coenzyme. It is the coupling of this precise histochemical staining technique with electrophoretic separation that enables the researcher to examine many independent genes for enzymes with comparative ease and speed. Procedures for starch gel electrophoresis are those of Menezes & Taniguchi⁸. Detection of isozymes and nomen-

clature of locus designation is as per Shaklee *et al.*⁹.

Electrophoretic variations of enzymes are reflected as either multilocus isozymes or allelic isozymes. Multilocus isozymes indicate one or more ancient gene duplications followed by evolutionary divergence of the duplicated locus⁵. These multilocus isozymes are usually expressed within all members of a given species, but the level of expression may vary among tissues of the same individual (e.g. heart, liver, skeletal muscle and eye). Genetic variation within a species at a specific locus is referred to as an allelic isozyme (often referred to as an allozyme).

This picture of a single gene with all alleles equally expressed and readily identified contrasts sharply with other characters used in stock discrimination studies. Morphometric and meristic characters and physiological properties, such as spawning time are polygenic (i.e. are under the control of several genes) and their expression is compounded by environmental modifications³.

Electrophoretic data are collected as genotypes (the genetic composition of each locus in an

Table 2—List of enzymes examined, locus names and tissue specificity observed

Enzyme	Enzyme number	Locus	Tissue specificity
Alcohol dehydrogenase	1.1.1.1	<i>ADH*</i>	Liver
Aspartate aminotransferase	2.6.1.1	<i>AAT*</i>	Skeletal muscle
Creatine kinase	2.7.3.2	<i>CK*</i>	Skeletal muscle
Glycerol-3-phosphate dehydrogenase	1.1.1.8	<i>G3PDH-1*</i> <i>G3PDH-2*</i>	Skeletal muscle Liver
Haemoglobin		<i>HB*</i>	Heart
Isocitrate dehydrogenase	1.1.1.42	<i>IDHP-1*</i>	Heart
Lactate dehydrogenase	1.1.1.2	<i>LDH-A*</i> <i>LDH-B*</i>	Skeletal muscle Heart, Skeletal muscle
		<i>LDH-C*</i>	Eye
Malate dehydrogenase	1.1.1.37	<i>MDH-1*</i> <i>MDH-2*</i>	Skeletal muscle Skeletal muscle
Malic enzyme	1.1.1.40	<i>MEP*</i>	Skeletal muscle
Phosphogluconate dehydrogenase	1.1.1.44	<i>PGDH*</i>	Liver
Phosphoglucomutase	5.4.2.2	<i>PGM*</i>	Skeletal muscle
General protein (PROT)		<i>PROT-1*</i> <i>PROT-2*</i>	Skeletal muscle Skeletal muscle

individual) and converted to allele frequencies (the proportion of alleles in a population sample for each locus)¹⁰. One of the first tests is to compare the observed distribution of genotypes with the expected distribution calculated from the allele frequencies. This is the Hardy-Weinberg equilibrium test. Genetic imbalance occurs if there is a significant difference between the proportions of observed and expected genotypes. Imbalances usually indicate the presence of stock mixing and they signify a need for further study.

A variety of statistics are used to denote the amount of genetic variation within a population. The most extensively used measures are the polymorphism (P) and the heterozygosity (H). Polymorphism is simply the proportion of loci found to be polymorphic and H is determined by totalling the number of observed heterozygotes for each locus, dividing this by the total number of individuals in the samples and then averaging over all loci. P is equal to about 45% in plants, 40-50% in invertebrate animals and 15-30% in vertebrates, including man. H is equal to about 17% in plants, 12-15% in invertebrates and 3% to 8% in vertebrate animals and man^{11,12}.

When there is no genetic variation at a particular locus within a species but substantial variation between species, isozymes can be used to rapidly detect species differences between larvae or other

life stages that may be ambiguous from a morphological perspective^{13,14}.

Several measures have been developed to quantify the genetic differences or similarities between samples averaged over many loci¹⁵. The common measure is Nei's genetic distance¹⁶, which identifies the average proportion of nucleotide substitutions that have occurred since the groups diverged. Genetic distance (D) is the natural log of I, or genetic similarity, which is calculated from the allele frequencies x^j and y^j in population x and y respectively. It is summed over all loci, both polymorphic and monomorphic. Genetic distance analyses produce a matrix of values between pairs of samples. These can be visualized by using the unweighted pair-group method of clustering to produce a genetic distance dendrogram¹⁷ showing phylogenetic relationships¹⁰.

Relationships between standard genetic distances observed for various taxonomic categories are about as expected. Higher categories are on the average more different than the lower ones. Nei¹⁶ estimated that for local races of a species, D ranges from nearly 0 to 0.05, for subspecies D = 0.02 to 0.2, and for full species D=0.1 to 2.0. Sibling species, which are morphologically nearly identical, generally have D values two or three times smaller than the D's between morphologically distinct species. For genera, D is usually greater than 1.

Applicability and Perspectives

Isozymes can be used to rapidly detect species differences between larvae or other life stages that may be ambiguous from a morphological perspective. For example, the grey mullets (fish belonging to the family Mugilidae) are highly esteemed for brackish water fish farming. However, mullet farming is dependent on the seasonal supply of mullet fry which enter the estuaries. The identification of fry to be used for stocking purposes, especially *Mugil cephalus* and others, is of practical interest and often causes problems for farmers who do not want to stock the ponds with other species. Two isozyme (*LDH-A**, *MDH-1**) and two general protein (*PROT-2**, *PROT-3**) loci are found to be diagnostic for identification of the four mullet species, *Mugil cephalus*, *Liza subviridis*, *L. macrolepis* and *Valamugil cunnesius*¹³. These loci could help to identify a particular species within a larval assemblage. In the case of sciaenids, conventional taxonomy based on external morphology and anatomical characteristics was found to be confusing¹⁸. Three isozyme loci (*LDH-B**, *MDH-2** and *G3PDH-1**) are found to be the most reliable species specific markers for identification of four species, namely - *Johnieops dussumieri*, *Kathala axillaris*, *Pennahia macrophthalmus* and *Otolithes ruber*¹⁹.

Isozymes have also revealed cryptic species that were not morphologically distinguishable at any life-history stage. Shaklee & Tamaru²⁰, identified two sympatric sibling species in Hawaii bonefish (*Albula*), that were isozymically distinct at 58 out of 84 loci. Other examples of isozymically detected cryptic species and populations have been described in oysters, commercially exploited squids, clupeids, lizardfish, mackerels and other fish species¹⁴.

Genetic diversity, expressed as average heterozygosity (H) is usually higher in species with wider geographic ranges, higher fecundity, greater longevity and larger population sizes²¹. Soule²² identified population size as the most important variable in population genetics theory and long-term maintenance of a large population size as a necessary condition for greater variability. Amongst the fishes studied from the Arabian Sea, H varied from 0.077 to 0.006 (Table 3). The highest value was found in the sciaenid *Johnieops*

*dussumieri*¹⁹ and the lowest in the oil sardine *Sardinella longiceps*²⁴. High levels of polymorphism and average heterozygosity have also been reported in other members of the Sciaenidae family^{25,26}. According to Nevo¹², high heterozygosity values are found in tropical vertebrates that are ecologically generalists (widespread, common, vagile, broad-niched species).

In the marine species of Clupeiformes high genetic variation (H= 0.056 to 0.106) has been found²⁷. Pacific sardines, however, showed little genetic variation both within and between populations²⁸, similar to the oil sardine²⁴. The low genetic variation in Pacific sardines was attributed to a small number of founders. The abundance of oil sardines in the fishery off India, as revealed by landings, was subject to wide fluctuations that were first noticed by Day²⁹. A greatly reduced population size for a single generation can have a drastic effect on genetic variation³⁰. A slow population increase after a bottleneck, or a repeated number of bottlenecks, may result in heavy loss of genetic variation³¹. With the loss of genetic variability, the population may no longer be able to exploit its former ecological niche¹. The great applicability and the strong need for quantitative analyses of the present type in genetic conservation programmes have frequently been emphasized^{3,32}.

Fishery biologists use the "stock" concept as a basis to manage commercially important marine organisms. Most evolutionary biologists consider the stock as the fundamental genetic unit of evolution. Therefore, the identification of stocks and the impact of harvest-induced mortality³³ are critical elements in any management regime. Although morphological, life-history characteristics and other variables have been useful in such studies, the analyses of isozymes and their allelic variants have widely been employed in the study of fish population structure and stock identification. An extension of this approach also allows estimating the geographical origin of fish caught in mixed - stock fisheries.

Shaklee *et al.*³⁴ recently reviewed how isozyme techniques could be used to establish the existence of multiple stocks of Spanish mackerel. Shaklee and his colleagues^{34,35} have also shown that there were several stocks of Australian barramundi (*Lates calcarifer*), and they have used isozyme

Table 3 - Genetic variation in each species

Species	Pop studied (no.)	Loci studied (no.)	Mean no of alleles per locus	Proportion of P		Average heterozygosity		Source/reference
				P99	P95	Observed	Expected	
<i>Rastrelliger kanagurta</i>	2	17	1.71	0.529	0.176	0.063±0.104	0.068±0.126	Menezes <i>et al.</i> ²³
<i>Sardinella longiceps</i>	3	19	1.37	0.368	0.000	0.006±0.010	0.006±0.010	Menezes ²⁴
<i>Sardinella lbella</i>	1	17	1.24	0.235	0.235	0.048±0.093	0.045±0.086	Menezes ⁵¹
<i>Sardinella fimbriata</i>	1	17	1.29	0.353	0.176	0.044±0.092	0.043±0.083	Menezes ⁵¹
<i>Mugil ephalus</i>	1	19	1.53	0.421	0.158	0.039±0.087	0.044±0.111	Menezes ¹³
<i>Liza ubviridis</i>	1	19	1.42	0.316	0.000	0.014±0.024	0.014±0.024	Menezes <i>et al.</i> ¹³
<i>Valamugil cunnesius</i>	1	19	1.11	0.158	0.105	0.035±0.093	0.030±0.080	Menezes <i>et al.</i> ¹³
<i>Johnieops dussumieri</i>	1	18	1.89	0.667	0.278	0.077±0.122	0.119±0.199	Menezes <i>et al.</i> ¹⁰
<i>Kathala axillaris</i>	1	18	1.67	0.556	0.167	0.061±0.104	0.054±0.086	Menezes <i>et al.</i> ¹⁹
<i>Pennahia macrophthalmus</i>	1	16	1.19	0.188	0.063	0.033±0.100	0.027±0.081	Menezes <i>et al.</i> ¹⁹
<i>Otolithes ruber</i>	1	16	1.94	0.750	0.250	0.050±0.077	0.060±0.093	Menezes <i>et al.</i> ¹⁰
<i>Parastromateus niger</i>	1	16	1.25	0.250	0.188	0.042±0.100	0.039±0.088	Menezes ⁴²
<i>Pampus argenteus</i>	1	16	1.50	0.440	0.000	0.013±0.018	0.013±0.018	Menezes ⁴²

P. polymorphic loci

techniques to define their inter-stock boundaries. Utter *et al.*³⁶ have used isozyme techniques to demonstrate the presence of several chinook salmon fisheries in the northwest Pacific. Shaklee *et al.*³⁴ have reviewed the extensive isozyme literature and shown that several distinct chum salmon stocks also contribute to mixed fisheries in the Pacific Northwest.

In the Indian mackerel, *Rastrelliger kanagurta*, out of the 11 loci studied, one locus (*PROT-2**) was found to be significantly different between the west coast/ east coast and the Andaman Sea samples³⁷. The genetic distance between them was 0.059 which is indicative of a local race¹⁶. The Andaman Sea population of *R. kanagurta* also showed differences in many biological and fisheries characteristics from the populations in the coastal waters of the Indian peninsula^{38,39}. The potential for genetic differentiation between populations of a species depends upon a number of variables, including migration rate, the number of individuals within a population, and natural selection at different loci.

Genetic differences between populations within a defined body of water may conceivably decrease with time, due to gene flow between subpopulations. Alternatively, genetic differentiation could become accentuated through time (perhaps because subpopulations become increasingly associated with discrete breeding areas)⁴⁰. In the sciaenids of Japan^{8,26} the average

genetic distance (D) varied between 0.0006 to 0.0018 in *Pennahia argentata* from 6 locations, 0.001 to 0.0094 in *Nibea albiflora* from 3 locations and was 0.0067 in *Nibea mitsukurii* from 2 locations. The low D values in *P. argentata* suggests the more frequent migration /gene flow between these subpopulations. This is supported by the fact that this species has its habitat and spawning ground more offshore than the other two and that it has a wide distribution⁴¹. In *N. mitsukurii* population, the spatial genetic discontinuity between Tosa Bay and Atsumi Bay suggests a barrier preventing gene flow between these two localities. The Ariake Sea population of *N. albiflora* seemed to be well isolated from the other subpopulations such as those of the East China Sea and the Inland Sea. The D is much larger in the Ariake Sea subpopulation compared to the other subpopulations. The mean genetic distance (D = 0.0094) is almost equal to the value of 0.01 that is indicative of a local race¹⁶. The differentiation of Ariake Sea subpopulations may have been accelerated by the closed nature of the sea for a long geological time²⁶.

The average D value among congeneric species (*N. mitsukurii* and *N. albiflora*) was 0.092, which is quite low⁸. Although these two species are considered to be sibling species principally having allopatric distribution, there seems to be introgressive hybridization in the waters around Kii channel where *N. mitsukurii* populations are

faced with *N. albiflora* populations. This hypothesis is supported by findings of individuals with intermediate body color pattern between the two species.

The average D value between the brown pomfret *Parastromateus niger* (Bloch) and the other two pomfrets *Pampus argenteus* (Euphrasen) and *Pampus chinensis* (Euphrasen) are very high (D=10.59) and there is hardly any genetic similarity between them⁴². This shows that the separation of *P. niger* from the family Stromatidae and inclusion in another family Apolectidae seems to be justified.

The unique ability of biochemical genetic data to clarify population structure has been demonstrated both in identifying previously unknown structures as well as in falsifying previously assumed ones⁴. In the absence of such clarifying information, mismanagement of many fisheries is inevitable.

Some researchers have demonstrated the utility of isozyme techniques to describe the transport of organisms by physical processes in the ocean environment. For example, Bucklin *et al.*⁴³ used allelic isozyme markers to describe transport of a calanoid copepod in coastal filaments off the west coast of USA. Isozymes have also been used to study genetic variation within and between species of other copepods^{44,45}. In addition to zooplankton, isozymes have been useful in examining phytoplankton species and communities. For example, Gallagher⁴⁶ demonstrated that isolates of the diatom *Skeletonema costatum* could be distinguished by their allelic isozymes, and Gallagher & Alberte⁴⁷ later showed that these isolates were physiologically distinguishable as well.

Advances in genetic techniques of the 1980s have opened up vast areas of the genome to broader and deeper exploration than was previously possible. We can now look directly at fragments or actual nucleotide sequences of the DNA or RNA⁴⁸. While protein electrophoresis has limitations relative to some of the DNA technologies, it has many advantages in fishery management^{49,50}. These include relative ease of application and the capability to sample a large number of individuals and loci at moderate costs. There is an urgent need to promote rather than to de-emphasize the collection of allelic and genotype

data by protein electrophoresis in questions related to fishery management⁴. The challenge for the future is thus to implement these methods and expand their usefulness.

References

- 1 Ryman N, in *Fish gene pools*, Vol 34, edited by N Ryman, (Ecological Bulletins, Stockholm) 1981, 61.
- 2 FAO/UNEP, *FAO Fish. Tech. Pap.*, 217 (1981) pp. 37.
- 3 Ryman N, *J Fish Biol*, 39 (1991) 211.
- 4 Allendorf F W, Ryman N & Utter F, in *Population genetics and fishery management*, edited by N Ryman & F Utter, (Seattle University Press, Washington) 1987, 144.
- 5 Allendorf F W & Utter F M, in *Fish physiology*, Vol. 8, edited by W S Hoar, D S Randall & J R Brett, (Academic Press, New York) 1979, 407.
- 6 Lewontin R C & Hubby J, *Genetics*, 54 (1966) 595.
- 7 Clayton J W & D N Tretiak, *J Fish Res Board Can*, 29 (1972) 1169.
- 8 Menezes M R & Taniguchi N, *Jpn J Ichthyol*, 35 (1988) 40.
- 9 Shaklee J B, Allendorf F W, Morizot D C & Whitt G S, *Trans Amer Fish Soc*, 119 (1990) 2.
- 10 Menezes M R, *Biol Edu*, 7 (1990) 297.
- 11 Selander R K, in *Molecular evolution*, edited by F J Ayala, (Sinauer Associates, Massachusetts, USA) 1976, 21.
- 12 Nevo E, *Theor Pop Biol*, 13 (1978) 121.
- 13 Menezes M R, Martins M & Naik S, *Aquaculture*, 105 (1992) 117.
- 14 Powers D A, in *Large marine ecosystems, V: Stress mitigation and sustainability*, edited by K Sherman, L M Alexander & B D Gold, (AAAS Press, Washington) 1993, 320.
- 15 Menezes M R, *Curr Sci*, 59 (1990) 209.
- 16 Nei M, *Am Nat*, 106 (1972) 283.
- 17 Sneath P H A & Sokal R R, *Numerical taxonomy*, (Freeman, San Francisco) 1973, pp. 573.
- 18 Trewavas E, *Ann Mag Nat Hist*, 13 (1962) 167.
- 19 Menezes M R, Naik S & Martins M, *J Fish Biol*, 43 (1993) 61.
- 20 Shaklee J B & Tamaru T, *Syst Zool*, 30 (1981) 135.
- 21 Nevo E, Beiles A & Ben-Shlomo R, in *Evolutionary dynamics of genetic diversity*, edited by G. S. Mani, (Springer-Verlag, New York) 1984, 13.
- 22 Soule M, in *Molecular evolution*, edited by F J Ayala, (Sinauer Associates, Massachusetts) 1976, 60.
- 23 Menezes M R, Naik S & Martins M, *Proc Indian Acad Sci (Anim Sci)*, 99 (1990) 457.
- 24 Menezes M R, *Aust J Mar Freshwater Res*, 45 (1994) 257.
- 25 Becwitt R, *Copeia*, 1983 (1983) 691.
- 26 Menezes M R, Taniguchi N & Seki S, *Jpn J Ichthyol*, 37 (1990) 39.
- 27 Smith P J & Fijio Y, *Mar Biol*, 69 (1982) 7.
- 28 Hedgecock D, Hutchinson E S, Li G, Sly F L & Nelson K, *Fish Bull (US)*, 87 (1989) 653.
- 29 Day F, *The fishes of Malabar*, (Bernard, Quaritch, London) 1865, pp. 293.

- 30 Crow J F & Kimura M, *An introduction to population genetics theory*, (Harper, New York) 1970.
- 31 Chakraborty R & Nei M, *Evolution*, 31 (1977) 347.
- 32 Allendorf F W & Phelps S R, in *Fish gene pools*, Vol 34, edited by N Ryman, (Ecological Bulletins, Stockholm) 1981, 37.
- 33 Fetterolf C M, Jr, *Can J Fish Aquat Sci*, 38 (1981) 4.
- 34 Shaklee J B, Phelps S R & Salini J, in *Electrophoretic and isoelectric focusing techniques in fisheries management*, edited by D H Whitmore (CRC Press, Boca Raton, USA) 1980, 173.
- 35 Shaklee J B & Salini J, *Aust J Mar Freshwater Res*, 36 (1985) 203.
- 36 Utter F, Teel D, Milner G & Mcissac D, *Fish Bull U S*, 85 (1987) 13.
- 37 Menezes M R, Naik S & Martins M, *Indian J Fish*, 40 (1993) 135.
- 38 Jones S & Silas E G, in *Proc Symp on Scombroid Fishes*, (Mar Biol Assoc India, Cochin) 1962, 253.
- 39 Luther G, *Indian J Fish*, 20 (1973) 425.
- 40 Felley J D & Avise J C, *Trans Am Fish Soc*, 109 (1980) 108.
- 41 Taniguchi N, *Aquabiology*, 20 (1982) 210.
- 42 Menezes M R, *Aqua Fish Manage*, 24 (1993) 341.
- 43 Bucklin A, Rlenecker M M, & Mooers C N K, *J Geophys Res*, 94 (1989) 8277.
- 44 Frost B W, *Can J Zool*, 67 (1989) 233.
- 45 Sevigny J M, McLaren I A, & Frost B W, *Mar Biol*, 102 (1989) 321.
- 46 Gallagher J C, *J Phycol*, 18 (1982) 148.
- 47 Gallagher J C & Alberte R S, *J Exp Mar Biol Ecol*, 94 (1985) 233.
- 48 Hallerman E M & Beckman J S, *Can J Fish Aquat Sci*, 45 (1988) 1075.
- 49 Utter F M, *J Fish Biol*, 39 (1991) 1.
- 50 Menezes M R & Qasim S Z, *Indian J Fish*, 40 (1993) 142.
- 51 Menezes M R, *Mahasagar*, 27 (1994) 29.