Science Policy

NIWA

*Taihoro Nukurangi*

# Stock relationships of orange roughy, black oreo and smooth oreo in New Zealand waters

**Final Research Report for**
**Ministry of Fisheries Research Project DEE9701**
**Objective 2**

**National Institute of Water and Atmospheric Research**

**August 1998**

**Final Report to Ministry of Fisheries**

| | | |
|---|---|---|
| 1. | **Date** | August 1998 |
| 2. | **Contractor** | National Institute of Water & Atmospheric Research Limited |
| 3. | **Project Title** | Stock relationships of orange roughy, black oreo and smooth oreo in New Zealand waters |
| 4. | **Project Code** | DEE9701 |
| 5. | **Project Leader** | Peter Smith |
| 6. | **Duration of Project** | |
| | Start date | October 1997 |
| | Expected finish date | September 1998 |

## 7. EXECUTIVE SUMMARY

Definitions of stocks in the fisheries literature are reviewed. There is no universal definition of a stock but most definitions have the common elements of spatial and temporal isolation, and in many cases reproductive isolation. Stock discrimination depends upon an amalgam of techniques which include both ecological and genetic approaches. An understanding of the life history, in particular length of larval life and dispersive juvenile stages, can provide critical information for selecting the appropriate tools for identifying stocks. Ecological approaches, based on phenotypic and acquired characters, provide a measure of stock relationships, but because of sensitivity to environmental parameters, need to be assessed for temporal as well as spatial variation. Genetic methods, in particular micro- and mini-satellite DNA, provide a powerful tool for estimating reproductive isolation and a significant genetic difference is a sufficient but not necessary condition for separate stock management.

The management units currently applied to orange roughy and oreos within the EEZ are reviewed. It is concluded that no single method is ideal for stock discrimination of orange roughy, rather stock discrimination is dependent upon an amalgam of methods utilising different approaches. Orange roughy are subdivided into a number of discrete regional stocks around Australia and New Zealand based on evidence from a wide range of independent studies based on allozymes, mtDNA, parasites, otolith microchemistry, morphometrics and biological data. Orange roughy have a continuous, low density distribution along much of the 1000m contour, with localised peaks of abundance. Some small isolated fisheries, such as Cook Canyon and Waitaki, are based on discrete stocks, but in other areas there is uncertainty about stock discreteness, for example along the north Chatham Rise, where there are three spawning groups. Temporal variation within regions has been reported in

morphometric, genetic and parasite studies and must be considered in future stock discrimination studies.

Only limited stock discrimination studies have been carried out on black and smooth oreo, but indicate genetic differences between black oreos from Tasmania and the Chatham Rise, and meristic differences among smooth oreo around Australia. Black oreo and smooth oreo have different biological properties and geographical distributions within the New Zealand EEZ and therefore could be managed as separate species and the management areas reviewed.

## 8. OBJECTIVE

To determine stock relationships for orange roughy, black oreo, and smooth oreo within the New Zealand EEZ.

Objective 2. To review and evaluate methods and criteria for defining stock or management units for orange roughy, black oreo, and smooth oreo.

## 9. METHODS

This objective was sub divided into two sections on methods and criteria for defining stocks in marine fishes and for defining stocks in orange roughy and black and smooth oreos. The scientific literature was reviewed by searching the data bases Aquatic Sciences & Fisheries Abstracts, Current Contents, and Fish & Fisheries World-wide with key terms. In addition the review draws on information in Fisheries Assessment Research Documents on orange roughy and oreos and on discussions held by the Deepwater Stock Assessment Working Group.

## 10. RESULTS

**Index** *Page*

## 1. Review and evaluation of methods and criteria for defining stocks in marine fisheries

### Introduction

The idea that marine fish species are subdivided into stocks is a basic tenet of fisheries management. While the stock concept is attractive and simple, its implementation for practical management has not been easy, in part because there are relatively few barriers to restrict movement of marine fishes. Considerable scientific effort has gone into stock discrimination of marine species and the development of increasingly sophisticated technical and statistical methods over the past century. The methods applied to stock discrimination of marine fish species were reviewed in objective 1 and are very briefly summarised in Table 1. No one method has emerged as ideal for identifying stock units; significant area differences have been reported with all methods for some species, and if nothing else reinforce the idea that marine fishes are sub divided into discrete stock units. That no one method appears as ideal is not surprising given the different dispersal potentials among marine fishes, and that the stock identification methods measure different characters, the expression of which is determined by either one or a combination of genetic and environmental components. Further confusion arises with the range of stock definitions in the literature some of which are method specific and which have changed in emphasis over time.

The technical methods applied to stock identification fall into five categories, those that measure:

- **phenotypic** characters that are modified by the environment experienced by the individual. Meristic and morphometric characters and life history traits have a genetic base but expression of the character is determined by the physical and biotic environment experienced by individuals,
- **acquired** characters, such as accumulation of metal ions and elements or parasites during an individuals life,
- **genotypic** characters such as allozymes and DNA,
- **movement** of adults and sub adults by physical tagging, and
- **biological** descriptors, such as distribution of spawning areas and adults.

The biological descriptor approach provides a description of stock relationships by drawing on information from a variety of sources, such as egg and larval surveys and fishery statistics, and illustrates that an integrated approach to stock discrimination is required. Much of this biological information is available prior to undertaking a specific stock identification study and forms the basis of the stock structure questions to be addressed.

Methods that measure characters that are determined or influenced by the environment provide an ecological approach to discriminating stocks. Only direct genetic methods provide an estimate of reproductive isolation, while mechanical tagging measures exchange of adults between regions. Genetic methods have been used extensively over the past twenty years, in part because the focus of stock discrimination has been on defining reproductive isolation and because a range of new and more sensitive genetic tools have become available with the rapid developments in molecular biology. Table 1 highlights some differences among the stock identification methods. The methods differ considerably in laboratory costs: microchemistry, parasite and genetic approaches require specialist skills, and for microchemistry and genetics specialist facilities. Other methods can be undertaken in a standard biological laboratory; although developments in image analysis and digitising methods increase the efficiency of undertaking morphometric measurements. With the exception of tagging, most sampling can be undertaken without a dedicated research vessel. There are established methods for statistical analysis of the different data sets, and the inputs are similar, based on scientific hours. However there are differences in analytical approaches. Most methods use a multivariate classification method to address the question "which stock is this specimen closest to ?". This is different to the genetic approach in which genotypic frequencies from two, or more, data sets are used to test for differences and where accuracy and precision of allele frequencies are related to sample size and grouping within the data sets (Saila & Martin 1987). There are two approaches to classification: discrimination and clustering. The discrimination approach begins with data derived from two or more groups (a priori distinctions) while clustering techniques use a class description to find structure in the data (a priori selection of a measure of similarity).

A common finding with most stock discrimination methods has been temporal variation in the measured characters. Sampling strategies must include replication to establish significant repeatable differences within regions. Temporal variation in genetic markers, especially allozymes may indicate that some markers are under selection. An early study of allozyme markers in the eelpout *Zoaraces viviparous* found some loci with similar frequencies across the range of samples, while other loci showed significant clinal variation. Selective neutrality would not account for both observations and so either directional or balancing selection must be acting on some loci (Christiansen & Frydenberg 1974). In the killifish *Fundulus heteroclitus* the physiological properties of lactate dehydrogenase alleles correlate with water temperature (Place & Powers 1979). In the American eel *Anguilla rostrata* there are significant differences among residents and recruits derived from a single spawning population (Williams *et. al.* 1973). The issue of temporal stability will be discussed further for orange roughy in section 2 of this objective.

**Table 1: Summary of different methods applied to stock discrimination of marine fishes. Sample collection: o = sample can be collected via observer programme or other seagoing project, r = requires dedicated vessel. Laboratory analyses: estimate of technical skill and facilities required x = low, xxx = high, – = not dependent on laboratory analyses**

| Method | Sample collection | Laboratory analyses | Limitations |
|---|---|---|---|
| Meristic | o | x | Determined during larval development, modified by environment,. |
| Morphometric | o | x | Influenced by growth rate and environment |
| Otolith morphology | o | x | Display ontogenetic changes |
| Microchemistry | o | xxx | Dependent upon regional differences in seawater composition, sensitive to otolith storage and preparation techniques |
| Parasite | o | xxx | Dependent upon regional differentiation of parasite and/or intermediate hosts |
| Life history | o | x | Influenced by fish density |
| Biological | o | – | Dependent on collation of fishery statistics or other biological data |
| Tagging | r | – | Restricted to adults/large juveniles from shallow water. |
| Genetics | o | xxx | Low levels of gene flow inhibit divergence at neutral markers |

## Evaluation of methods used for defining stocks in marine fishes

An evaluation and comparison of different approaches to stock discrimination can be made when two or more methods have been tested on the same samples or samples collected at the same time period. Studies applying two or more approaches to stock discrimination are not common in the marine stock discrimination literature and are summarised in Table 2; in approximately half of the comparisons different stock structures are reported with different stock discrimination methods (Table 2). Most studies comparing two or more approaches have utilised allozymes, a genetic method, and morphometrics or meristics, phenotypic methods. There have been few direct comparisons with approaches covering acquired characters and biological descriptors.

The Atlantic herring *Clupea harengus* has been central to the stock discrimination debate with numerous stocks described based on location and spawning time. The regional differentiation revealed by early studies on morphometric, meristic and life history traits (Heincke 1898, Cushing & Burd 1957, Postuma 1974) was supported by initial allozyme studies (Lush 1969, Lewis & Ridgway 1972). More recent genetic studies indicate high genetic diversity with little regional differentiation for both allozymes (Ryan *et.al.*1984, Kornfield et al 1982) and mtDNA (Kornfield & Bogdanowicz 1987), except in Norwegian fjords where allozyme data show two very divergent stocks (Jorstad *et.al.* 1991). Comparative studies applying allozymes, mtDNA RFLPs, vertebral number and spawning behaviour showed that some fjord

stocks in Norway are similar to the Pacific herring *Clupea pallasi* (Jorstad *et.al.*1994). Safford & Booke (1992) found no significant differences among spawning groups in the north-west Atlantic with allozyme markers and while morphometric characters exhibited between site differences in one year, there was greater variation among years within spawning groups. Ryman *et.al.* (1984) also found a lack of correspondence among allozyme and morphological variability in herring stocks in the North Atlantic.

The Pacific sardine *Sardinops sagax caerula* shows low genetic diversity and little regional differentiation whereas the anchovy *Engraulis mordax* shows high genetic diversity and significant between population differences over the same geographical range (Hedgecock *et.al.*1989). However the Pacific sardine does show significant clinal variation in life history traits which lead Hedgecock *et.al.*(1989) to conclude that life history traits and growth rates are to a large extent environmentally and not genetically determined, but that these traits could be used as markers to define area specific fishery stocks.

In the Spanish sardine *Sardinella aurita* populations in the coastal waters off the south east United States showed low levels of genetic diversity measured with allozymes, and little variation in allele frequencies among regions (Kinsey *et.al.*1994). Morphological and meristic analyses showed that different forms of sardine exist in embayments and the open ocean (Kinsey *et.al.*1994), and that these differences were greatest among the smallest size classes. It was concluded that juvenile fish spend their early life in continental shelf water or in oceanic water where differences develop; thus the morphometric differences reflect habitat effects and not geographic isolation (Kinsey *et.al.*1994). Grant & Utter (1984) also concluded that morphological data might be more useful than allozyme data in detecting short term environmentally induced variation in clupeids. Ryman *et.al.*(1984) suggested that the lack of correspondence between allozyme and morphological data sets on herring was due to gene flow, sufficient to prevent differentiation of recently isolated stocks, and that the morphologic divergence was to a large extent environmentally induced.

**Table 2: Summary of studies applying two or more stock discrimination techniques to marine fishes**

| Species | Methods | Results | Reference |
|---|---|---|---|
| menhaden *Brevoortia tyrannus* | | | Epperly 1989 |
| | meristic | two populations | |
| | morphometric | two population | |
| | allozyme | two populations | |
| herring *Clupea harengus* | | | Safford & Booke 1992 |
| | morphometric | no differences | |
| | allozyme | no differences | |
| herring *Clupea harengus* | | | Ryman *et.al.* 1984 |
| | meristic | regional stocks | |
| | allozyme | no differentiation | |
| anchovy *Engraulis encrasicolus* | | | Bembo *et.al.* 1996 |
| | morphometric | two stocks | |
| | allozyme | two stocks | |
| anchovy *Engraulis encrasicolus* | | | Junquera & Perez- Gandaras 1993 |
| | morphometric | northern & southern groups | |
| | meristic | no differentiation | |
| sardine *Sardinella aurita* | | | Kinsey *et.al.* 1994 |
| | morphometric | inshore & offshore stocks | |
| | meristic | inshore & offshore stocks | |
| | allozyme | no differentiation | |
| sardine *Sardinops sagax caerula* | | | Hedgecock *et.al.* 1989 |
| | life history | cline in size at age | |
| | morphometric | size related cline | |
| | allozyme | no differentiation | |
| capelin *Mallotus villosus* | | | Roby *et.al.* 1991 |
| | morphometric | two stocks | |
| | allozymes | two stocks | |
| grey mullet *Mugil cephalus* | | | Crosetti *et.al.* 1993 |
| | chromosome | no variation | |
| | mt DNA RFLPs | four groups | |
| Atlantic cod *Gadus morhua* | | | Pepin & Carr 1993 |
| | vertebral number | northern & southern groups | |
| | morphometric | two groups | |
| | mtDNA | no differentiation | |
| | otolith structure | coastal & Arctic types | Larsen *et.al.* 1997 |
| | parasite | no differences between types, but differences between fjords | |
| tilefish *Lopholatilus chamaeleonticeps* | | | Katz *et.al.* 1983 |
| | meristic | two groups | |
| | morphometric | two groups | |
| | allozymes | three groups | |

**Table 2:** (*continued*)

| Species | Methods | Results | Reference |
|---|---|---|---|
| anglerfish *Lophius vomerinus* | | | Leslie & Grant 1990 |
| | meristic | area differences | |
| | morphometric | area differences | |
| | allozyme | no differences | |
| Atlantic halibut *Hippoglossus hippoglossus* | | | Haug & Fevolden 1986 |
| | morphometric | no differences | |
| | meristic | no differences | |
| | allozyme | no differences | |
| Greenland halibut *Reinhardtius hippoglossoides* | | | Riget *et.al.*1992 |
| | meristic | fin ray numbers no differentiation vertebral no. significant within & between area heterogeneity | |
| | allozyme | significant differences among 3 areas | |

In the European anchovy *Engraulis encrasicolus* both allozyme and morphometric analyses have shown the presence of two isolated groups in Adriatic waters (Bembo *et.al.* 1996). Likewise in the capelin *Mallotus villosus* both allozyme and morphometric methods differentiated fish samples into western and eastern groups (Roby *et.al.* 1991). Truss analysis of morphometric characters gave a better discrimination than conventional morphometric analyses (Roby *et.al.*1991). In the chub mackerel *Scomber japonicus* a combined plankton survey and genetic analysis showed that there are two major spawning sites in the northwest Pacific Ocean and that samples from the spawning areas differ in frequencies of an esterase gene (Belyaev & Ryabov 1987).

In the cod *Gadus morhua* otolith types and parasites were compared in samples from the Barents Sea, a semi-closed fjord and an open fjord (Larsen *et.al.*1997). Cod were first identified as type, Arctic or coastal, according to otolith structure and then tested for infections of 4 parasite species. Coastal cod identified from otoliths dominated in the fjords and the Barents Sea in spring, while the Arctic cod dominated in the Barents Sea in autumn. The fjord samples were much more heavily infected than the Barents Sea sample, but there were no differences in infection between the two otolith types within fjords. It was concluded that the use of two independent techniques showed that there is a component of coastal cod that migrates between fjords and off shore waters, and that some Arctic types are resident in the fjords – conclusions that would not have been possible using otolith or parasite methods alone (Larsen *et.al.*1997). An integrated approach combining allozymes and scale patterns has been used to discriminate the origin of Atlantic salmon *Salmo salar* in high seas fisheries – allozyme frequencies at seven loci were first used to distinguish North American from European fish, followed by scale characteristics (Reddin *et.al.*1990).

Samples of juvenile cod from the Newfoundland Shelf showed no consistent pattern of variation with morphological, meristic and mtDNA sequence methods (Pepin & Carr 1993). MtDNA sequence variation in the mitochondrial cytochrome *b* gene found homogeneity within and among sites, but in retrospect this is not surprising as this

region of the mitochondrial genome appears conservative and is finding greater application in taxonomic rather than population studies. Morphometric characters in juvenile cod showed significant differences among four regions, but less than 50% of reclassifications into region of origin were correct, while vertebral counts showed differences among southern and northern groups (Pepin & Carr 1993).

Samples of tilefish *Lopholatilus chamaeleonticeps* from the east coast of the United States were compared with meristic, morphometric and allozyme methods (Katz *et.al.*1983). Only one meristic character, gill raker number, showed differences among samples. Morphometric characters revealed two groups: east coast and Gulf of Mexico, while two allozyme markers further divided the east coast group into mid-Atlantic and southern groups (Katz *et.al.*1983). In the Atlantic menhaden *Brevoortia tyrannus* spawning occurs throughout the year with peaks in spring/early summer and autumn. Analysis of meristic, morphometric and allozyme markers showed significant differences among spring and autumn spawners, but because of overlapping movement patterns the populations could not be separated in the fishery (Epperly 1989).

***Summary of multiple methods applied to stock discrimination***

Of the 15 studies listed in Table 2, comparing two or more methods applied to stock discrimination, approximately half (7/15) produced similar results when different methods were applied to the same samples. For those studies producing dissimilar results with different methods, then frequently allozyme methods showed no evidence for differentiation, while morphometric/meristic methods provided evidence for regional differentiation. For clupeids it has been suggested that the lack of correspondence between allozyme and morphological data sets is due to gene flow which is sufficient to prevent differentiation of recently isolated stocks, and the morphologic divergence is due to environmentally induced variation. The dissimilar stock structures resulting from the application of different methods are explored more fully in the next section.

***Genetic and ecological approaches to stock discrimination***

Lack of consistent patterns among meristic, morphometric and allozyme data appears more common in marine than anadromous species (Pepin & Carr 1993). The contrast may result from different processes in the early life history stages – anadromous species tend to have isolated spawning and nursery sites, whereas many marine species spawn over wide areas and have greater potential for larval drift among sites due to fewer barriers to dispersal (Pepin & Carr 1993). The extensive egg and larval drift in marine species may limit the opportunity for genetic isolation, but the discrete juvenile nursery areas promote differences in life history traits (Pepin & Carr 1993). For several species no significant differences have been detected with allozyme markers, but there are significant morphological differences between regions (Winans 1980, Ryman *et.al.* 1984). Differences between genetic and morphometric methods have been interpreted as due to low levels of gene flow between regions, but also indicate that the observed morphometric differences are based on environmental modification and not inheritance of morphometric traits. The separate morphological units reflect differences in post-settlement habitat quality, and hence production of the stock, and thus are relevant to short term management goals. However stocks that are

environmentally defined may not be genetically or reproductively isolated and thus may not be stable over time. A genetic difference, at selectively neutral markers, provides evidence of reproductive isolation, but morphometric differences need to be demonstrated over years before the ecological units could be managed as discrete stocks. Attempts to define populations based on meristic or morphological features must be verified by genetic evidence if the aim is to test for reproductive isolation rather than environmental differences (Pepin & Carr 1993). Cushing (1975) concluded that the traditional methods of establishing differences between fish stocks such as meristic and morphometric characters and parasite infestations, were only of value when genetic differences could not be discovered.

In evaluating different methods used to define stocks it is useful to consider how differences arise between groups of fish. There are several mechanisms that produce differences:

- genetic drift, or the random fluctuations in gene frequencies between generations;
- genetic selection, through a differential mortality on genotypes;
- different mutations arising and maintained in isolated populations;
- environmental modification of traits due to differences in the physical environment, such as temperature and salinity;
- environmental modification of traits due to differences in the biotic environment, such as food availability;
- accumulation of different chemicals or parasites due to differences in the environment experienced by individuals.

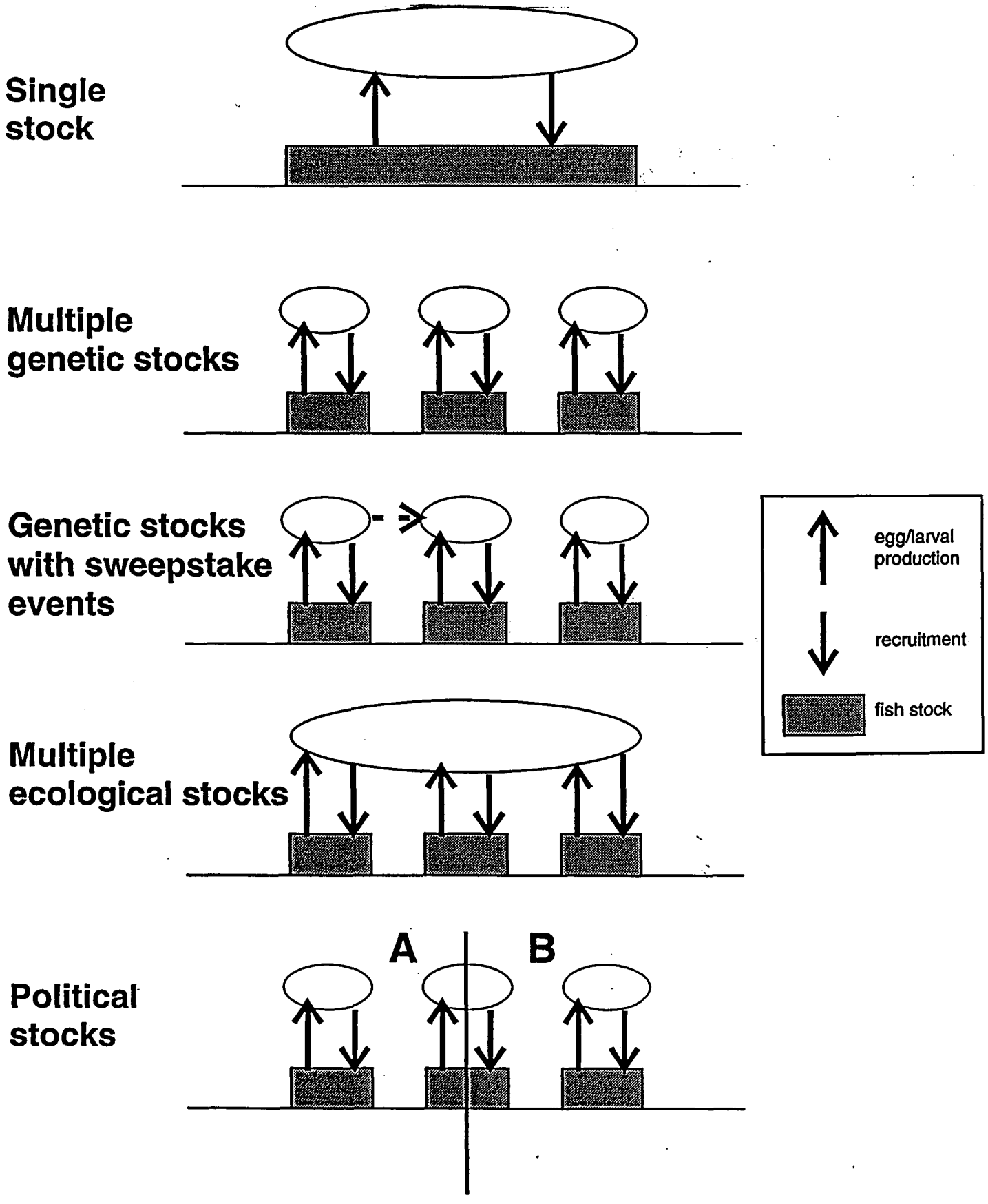
These mechanisms fall into two categories: genetic and ecological, hence the use of different approaches to stock discrimination will result in the description and definition of different biological units (Fig 1). The stock definitions will be explored further in section 2 of this objective, but indicate that it is important to address both the genetic and ecological relationships among groups of marine fish in order to determine the stock structure.

The developments in molecular biology have produced a range of tools for testing genetic diversity in fishes, and coupled with the extensive use of allozyme methods in the 1970s and 1980s, provide a large data base describing genetic relationships among groups of fish. Theoretical, and initial practical studies with mtDNA, suggested that mtDNA polymorphism would be a more sensitive genetic tool than allozyme polymorphisms due to the higher rate of nucleotide substitution than in nuclear DNA (Brown 1983, Rand 1994). Examples of greater discriminating power of mtDNA were seen in initial applications of this method to marine organisms (eg Avise 1994). However applications of allozyme and mtDNA markers in yellowfin tuna *Thunnus albacares* and orange roughy *Hoplostethus atlanticus* found greater genetic sub division with allozyme than mtDNA markers (Ward *et.al.*1994, Smith *et.al.*1997). Recently developed nuclear DNA based methods, such as micro- and mini-satellite DNA, show promise as more sensitive genetic tools for stock discrimination. Populations are likely to diverge much more quickly at the regions of non-coding micro- and mini-satellite DNA, than regions of DNA encoding for allozymes, due to their higher mutation rate (Wright & Bentzen 1994).

Genetic methods provide a test of reproductive isolation and, with selectively neutral markers, measure differences accumulated over an evolutionary time scale. When there are significant differences among regions then this is powerful evidence for limited exchange and that groups are effectively reproductively isolated. Conversely the lack of genetic differences among regional samples does not provide evidence for current interbreeding among regional populations. The lack of differences may be due to recently diverged populations that have had insufficient time to evolve differences, or there may be low levels of gene flow between the "two stocks" such that genetic divergence at neutral markers does not occur. Alternatively there may be periodic exchange every decade or century (= sweepstake events) due to unusual climatic events shifting patterns of larval dispersal or adult movement and preventing evolution of discrete stocks at selectively neutral markers.

Molecular methods are unique among the stock discrimination methods because expression is unaffected by environmental variation and hence are the only methods that directly measure genetic differences among groups. However it is possible for two stocks to be indistinguishable by molecular methods, yet be adapted to their respective environments. Hence an observed molecular difference is a sufficient, but not necessary condition for two groups of fish to be genetically differentiated (Ihssen 1981) and managed as discrete stocks.

Figure 1. Genetic and ecological stocks in marine fishes.



Genetic models indicate that exchange rates as low as a few individuals per generation maintain the same genes in populations, but not necessarily at the same frequencies (Speith 1974). Long distance tag returns show that there is movement between widely separated groups of fish, such as cod *Gadus morhua* on both sides of the Atlantic, and which theoretically should lead to genetic homogeneity. Certainly some allozyme markers show similar frequencies throughout the range of the Atlantic cod (Mork *et.al.*1985), but other loci show very different frequencies (Jamieson & Birley 1989), either these loci are under strong selection, or the migrants do not interbreed. Differences at other genetic markers which are theorteically neutral also provide evidence for genetic differences (Pogson *et.al.*1995, Bentzen *et.al.*1996, Ruzzante *et.al.*1996).

Morphometric, meristic, parasite and chemical methods measure aspects of the life histories of individual fish, and the differences detected with these methods may be dependent upon environmental conditions during an individual's life. While life history traits, such as age or length at maturity, and meristic characters such as number of vertebrae, and possibly even chemistry of the otolith, have a genetic base, the expression is influenced by the environment. Inter year environmental differences could affect the phenotypic and acquired characters, so that measured differences are due to year class variation rather than stock structure. In addition life history traits may change in response to fish density and fishing pressure and thus require a time series to establish differences among regions. Thus the characters provide a measure of short term ecological rather than genetic relationships.

Tagging is the only method that provides direct estimates of the exchange rate of adult and sub adult fish, but is dependent upon fishing across the range of the species to determine relationships between regions. The method cannot provide evidence of larval exchange and hence gene flow. There could be genetic exchange between two regions through larval drift, but little or no movement post recruitment, for example many molluscan fisheries are based on species which can only exchange material through egg and larval drift as the adults are sessile. Tagging adult Dover sole *Microstomatus pacificus*, has shown little intermingling among adult stocks off the west coast off Canada and California, yet there is extensive larval exchange (Westrheim *et.al.*1992).

Gyllensten (1987) suggested that marine species show less spatial genetic differentiation than anadromous and freshwater species, due to the fewer barriers to gene flow in the marine environment. This observation was supported by a more extensive species comparison of 57 marine, 49 freshwater, and 7 anadromous fishes by Ward *et.al.*(1994a). In spite of the low genetic divergences observed in many marine fishes, there are examples of population differentiation, as outlined in the review section on stock discrimination methods. Ward *et.al.*(1994a) suggested that more than 60% of marine fishes studied had shown genetically differentiated populations. Many of the marine species showing differentiation are coastal and inshore, where life history strategies and habitat preferences may restrict gene flow.

Fish and marine invertebrates with pelagic eggs and long periods of planktonic larvae are less likely to show genetic differentiation than species with low dispersal capabilities (Burton 1983, Waples 1987, Hunt 1993). Some invertebrate species with high dispersal potential show genetic differentiation and it is possible that steep

temperature and salinity gradients in estuarine plumes act as effective barriers to dispersal along coastlines (Burton 1983). A comparison of dispersal capabilities and genetic variation in 10 species of marine fishes in California showed a negative relationship between genetic sub division and dispersal potential (Waples 1987). The marine and estuarine catfish *Cnidoglanis macrocephalus* shows high genetic differentiation between estuarine systems off the west and south coasts of Western Australia (Ayvazian *et.al.* 1994). Reef species lacking a pelagic larval stage also have genetically differentiated populations (Doherty *et.al.*1994). However a survey of 8 species of reef fishes from the Caribbean suggested that although length of larval life contributes to levels of genetic differentiation, other traits, such as larval behaviour, may restrict gene flow (Shulan & Bermingham 1995). Likewise a survey of reef fish from New Caledonia found significant genetic differentiation in 2 out of 3 species (Planes *et.al.*1998).

**Criteria used for defining stocks in marine fisheries**

There are a large number of definitions and uses of the word stock in the fisheries literature, from separate stocks of female and male plaice in the North Sea (Beverton 1964) to multispecies stocks in tropical waters (FAO 1985). The stock concept has had a long history in fisheries research and management and is generally traced back to Heincke and Matthews last century, and to Hjort and Schmidt in the early 1900s, who recognised that many species of fishes consisted of distinct regional populations (Sinclair & Solemdal 1988, Gauldie 1991). Different populations or stocks are likely to have different productivities and react differently to harvesting.

At the beginning of the century typological thinking dominated the biological sciences and species were viewed as consisting of numerous similar individuals. The replacement of the typological species concept by the polytypic species concept was perhaps the greatest conceptual revolution that occurred in the biological sciences (Dobzhansky 1968). The shift from a typological species concept to population concept occurred early in fisheries science (see extensive review by Sinclair & Solemdal 1988). Fishers and scientists had recognised that fish, such as herring, exhibited a large amount of variability among individuals, based on morphometric characters used by taxonomists to define species. However the typological concept was only transferred down one level from species to stocks. The fisheries population concept was not the same as the genetic population concept discussed by Dobzhansky (1968) or Mayr (1982) in which the population consists of large numbers of individuals of different genotypic constitution. The fisheries population concept was one of typological stocks which were regarded as a **relatively homogeneous** group of individuals (Table 3, ICNAF 1957). The typological stock concept appears in the terms used to describe stocks of herring as "pure" and "mixed" (Rosenberg & Palmen 1982, Burd 1985). The application of molecular genetic methods to stock discrimination studies have shown that a stock consists of numerous genotypes, most of which are found in other stocks, so that a stock is not a homogeneous group of uniform individuals. Most often stocks differ in frequencies of shared alleles and rarely possess unique alleles. Nevertheless the typological concept persists in some of the fishery management literature, for example Pawson concludes that "an appropriate method for stock discrimination would allow individuals sampled from landings to be assigned to specific stocks" (Pawson 1995, Pawson & Jennings 1996). Parson (1993)

discussing management of marine fisheries in Canada defined stock as "a group of fish that can be treated as a homogeneous and independent unit".

There have been many workshops and conferences devoted to the stock concept and its application in fisheries management (eg STOCS 1981, Kumpf 1987, Carvalho & Pitcher 1994) over decades (eg Anon 1929, Symposium 1948, 1963, Marr 1957, ICNWF 1958, de Ligny 1971). In spite of this long history and scientific debate there is no universal definition of a stock, or consensus on key methods for determining stock relationships. Even for well studied species like the Atlantic cod *Gadus morhua* there is no generally accepted definition of a stock (Jakobsen 1987) inspire of years of research applying a wide range of techniques. Some widely cited definitions of stock are given in Table 3.

The stock concept developed with biological studies on demersal and pelagic species in the North Atlantic fisheries. Support for the subdivision of marine fish species into stock units came from independent observations on tag returns, meristic and morphometric data, parasite infestations, and life history traits during the first half of this century. Cushing (1968, 1975) built up a description of the unit stock based on location of spawning, time of spawning, the larval drift to a nursery ground, and migration of the adults from feeding areas to spawning areas. Larval drift appeared as the most important period in the life history of the stock as this phase provides the geographic base and maintains or erodes the differences between stocks. Adult migrations are against the prevailing currents and return the adults to the natal spawning site. The unit stock structure, contained within hydrological systems, has been summarised as a triangle of movement between spawning ground to nursery ground to feeding ground to spawning ground (Harden Jones 1966). Templeman (1983) reviewing studies with a focus on Atlantic cod concluded that a marine fish stock "has its own spawning area with patterns of egg and larval drift and migration contained within current systems. It may be genetically different to adjacent stocks if the barriers to migration of adults and drift of larvae are great enough. The degree of genetic difference is an indication of the length of the period of stock separation" (Templeman 1983). Templeman also described a stock complex as a group of adjacent stocks which at periods other than spawning intermingle or overlap greatly and are different in migratory behaviour from adjacent stock complexes.

Iles & Sinclair (1982) extended Cushing's (1968, 1975) hypothesis that larval retention areas provide the critical isolating factor for many marine fish stocks. In Atlantic herring, *Clupea harengus*, 20–30 stocks have been described in the northwest Atlantic Ocean, far more than occur for example in mackerel *Scomber scombrus* over the same geographical range (Iles & Sinclair 1982). The number of herring stocks is determined by the number of distinct geographically stable larval retention areas. The larval retention hypothesis recognises that more than one spawning location can contribute to the larval gene pool and that a knowledge of hydrography is essential for determining relationships among spatially isolated spawning groups. Iles & Sinclair (1982) also showed that for herring the size of the larval retention area determines the size of the stock, and implies that stock abundance is largely independent of reproduction and growth, but determined by behavioural characteristics of the larvae and the physical structure of the environment. Small stocks are associated with small hydrographic features and large stocks with large hydrographic features (Iles & Sinclair 1982).

**Table 3: Commonly used definitions of stock applied to marine fisheries**

| Term | Definition | Reference |
|---|---|---|
| Unit stock | A relatively homogeneous and self contained population whose loses by emigration and accessions by immigration, if any, are negligible in relation to the rates of growth and mortality | ICNAF1957 |
| Stock | A large population of fish that is distinct from neighbours, and has a single spawning ground to which adults return year after year, and contained within a current system | Cushing 1968 |
| Stock | Recognisable unit with area occupying and migratory patterns whose spawning is separate from that of other stocks | Templeman1979 |
| Stock | Intraspecific group of randomly mating individuals with temporal or spatial integrity | Ihssen 1981 |
| Population stock | Group of animals that share a common space and interbreed | Marine Mammal Protection Act 1972 (USA) |

An international symposium on the stock concept, focused on Great Lakes fisheries, produced much discussion and several definitions of a stock (STOCS 1981). Kutkuhn (1981) extended Cushing's definition of a stock and defined a unit stock as "one consisting of randomly interbreeding members whose genetic integrity persist whether they remain spatially and temporally isolated as a group or whether they alternatively segregate for breeding and otherwise mix freely with members of other unit stocks of the same species. It therefore represents not only an aggregation that is genetically discrete and breeds true but also one that ultimately demands quick, easy and accurate delineation if its innate productivity is to be most effectively protected". Booke (1981), at the same conference, defined a fish stock as "a species group or population of fish that maintains and sustains itself over time in a definable area." He went on to define two types of stock: genotypic and phenotypic. The genotypic stock was defined as a population of fish maintaining and sustaining Castle-Hardy-Weinberg equilibrium and the phenotypic stock as a group or population of fish maintaining characteristics which are expressed in one or more ways depending on the type of environment of domicile (Booke 1981). Much earlier in the literature Marr (1957) also made a distinction between genotypic and phenotypic stocks when he defined a subpopulation as a fraction of a population that is itself genetically self sustaining and is the smallest natural self-perpetuating unit (Marr 1957). A stock was defined as a population or portion of a population all members of which are characterised by similarities which are not heritable, but are induced by the environment; and may or may not include members of several different subpopulations (Marr 1957). The key distinction between the subpopulation and the stock was that members of a subpopulation segregate at spawning time whereas members of a stock need not. The partial barriers to gene flow among subpopulations included isolation in time and space and ecological isolation. Marr (1957) went on to conclude that "whereas most fisheries biologists have been interested in defining subpopulations they have in fact most frequently defined stocks".

Gauldie (1988, 1992) discussed the origins of the stock concept and the limitations of genetic and phenotypic methods in discriminating stocks and defined a harvest stock as "locally accessible fish resources in which fishing pressure on one resource has no effect on the abundance of fish in another contiguous resource" (Gauldie 1988) and specifically avoided defining stock based around a specific method.

Dizon *et.al.*(1992) took a phylogenetic approach to the stock concept and produced a hierarchical classification of four evolutionary significant units from allopatric populations with significant genetic differences to contiguous populations with extensive genetic interchange. In reality this was an attempt to provide a formal description for populations, in particular marine mammals, most in need of protection. The phylogenetic approach draws together independent evidence for stock discreteness and formalises what happens in the stock assessment process.

Legal definitions of a fish stock provide no criteria, only a circular argument for defining stock or management units, even though an aim of fishery acts has been the sustainable management of fish resources. For example the New Zealand Fisheries Act (1996) defines stock as "any fish, aquatic life, or seaweed of one or more species that are treated as a unit for the purposes of fisheries management". Likewise the Magnuson Fishery Conservation and Management Act (US) specified that an individual stock of fish shall be managed as a unit throughout its range, and interrelated stocks of fish shall be managed as a unit or in close co-ordination. The act defined a stock of fish as "a species, subspecies, geographical grouping or other category of fish capable of management as a unit".

### *Salmon*

There is a large literature base on salmonid stock discrimination and population sub-division. Salmon have a different biology to marine fishes, but the stock discrimination methodologies and philosophy applied to salmonids have relevance to marine species, Salmonid species have been sub-divided into a large number of stock units for management purposes and some key points are briefly summarised below:

- **homing.** Salmon, in particular Pacific salmon *Oncorhynchus* spp., are known to return to their natal stream to spawn. The basis for homing was established by marking juvenile salmon as they left their natal stream. A large scale tagging study on chinook salmon *Oncorhynchus tshawytscha* from the Cowlitz hatchery on the Columbia River system, based on 41 085 returns, showed that 98.6% of recovered fish were in the natal stream, and the rest in neighbouring streams (Quinn & Fresh 1984). Numerous other tagging studies have shown that homing rates range from 93–100% in *Oncorhynchus* and from 80–100% in Atlantic salmon and trout, *Salmo* (see summary in Quinn & Tallman 1987). In addition parasite and allozyme studies of wild populations of sockeye salmon *O. nerka* have indicated that straying between river systems is rare (Quinn *et.al.*1987). However straying must have allowed the establishment of salmonid stocks in the North Pacific and North Atlantic Oceans as the glaciers retreated over the past 8–15 000 years. *Oncorynchus* spp. are invading new habitat in Alaska as glaciers retreat, and *O. tshawytscha* has invaded South Island rivers following introduction of salmon into the Waitaki River at the beginning of the century (Thorpe 1994).

- **straying and immigration.** A distinction should be made between straying and immigration, as strays may not interbreed with the local population (Gharrett 1994). Straying can be detected with physical tagging whereas immigration is determined from genetic markers. A comparison of straying-immigration rates from both marking and allozyme studies in chum salmon, *O. keta,* suggested that gene flow was substantially lower than the rate of straying estimated from mark-recapture studies on the same population (Tallman & Healey 1994). Straying occurred between populations that spawned in the same season, but there was little straying between populations that were temporally isolated (Tallman & Healey 1994).

- **salmon stocks.** A combination of genetic, life history and tagging data has been used to develop a model for Atlantic salmon in which each river, and within large river systems each tributary group, is considered as a distinct reproductive group (Verspoor, University of Aberdeen, pers. com.). No upper or lower limits on straying have been used to define stocks, rather genetic and mechanical tagging have been used in a general sense to develop the discrete river stock model. The current focus on Atlantic salmon stock identification is the application of microsatellite DNA to detailed studies of gene flow and subdivision within and among river systems (Verspoor, University of Aberdeen, pers. com.). In Norway as many as 2000 salmon stocks have been recognised for conservation and management. Some stocks have been described by genetic methods, using allozymes and more recently microsatellite DNA, but in general stocks are recognised on a river by river basis. In major river systems salmon spawning in different tributary streams are recognised as separate stocks (Schei, Directorate for Nature Management, Norway, pers. com.). A similar situation exists in North America where up to 6000 stocks of Pacific salmon are recognised (Harvey, World Fisheries Trust, British Columbia, pers. com.). The Pacific salmon stocks are separated on an amalgam of information based on species, spawning tributaries, spawning year, and genetic data. In sockeye salmon *Oncorhynchus nerka* around 17% of genetic diversity measured with allozymes is associated with geographic sub division, and 83% with variation among individuals; the nursery lake is the primary geographic unit for discrete populations of sockeye salmon (Wood & Holtby 1998). Current research is aimed at determining units for conservation and restoration of wild populations. Often these units are considerably smaller than marine conservation units and may consist of just a few hundred spawning adults.

- **stock definitions.** A number of terms have been used to define salmonid stocks and populations. Recently Woods & Holtby (1998) have reviewed these terms in relation to conservation units and proposed some definitions that distinguish genetic from phenotypic groups:
  *Phenodeme*: an interbreeding group distinguished by phenotypic characters
  *Genodeme*: the smallest detectable population genetic unit. Gene flow between genodemes is large so that drift and/or migration preclude local adaptation.

In addition Woods & Holtby (1998) proposed 3 levels of populations, defined around $N_em$ (the number of migrants exchanged per generation) estimates derived from genetic data:

*subpopulation*: a group comprising one or more genodemes that is partially isolated from other such groups, $N_em > 10$;

*population*: a group comprising one or more sub populations that are relatively isolated from other such groups, $N_em < 10$;

*closed population*: a group comprising one or more populations that is almost completely isolated from other such groups $N_em < 1$. This is the smallest group for conservation.

***Biological stocks and management units in marine fishes***

Most uses of the term marine fish stock can be merged into two broad categories:

- Biological stocks which are self perpetuating intraspecific units and isolated spatially and/or temporally from other units (Jamieson 1974, Ihssen *et.al.*1981, Waldman *et.al.* 1988, Smith et al 1990, Carvalho & Hauser 1994, Pawson 1995).
- Fishery stocks or management stocks which represent a group of fish exploited in a specific area or by a specific method (Gauldie 1991, Smith *et.al.*1990, Pawson 1995). Fishery stocks are defined on an area basis and several different biological stocks could be exploited within one fishery area, alternatively one biological stock might straddle two or more fishery areas.

The differences between the biological and fishery stock concepts can be illustrated with an extreme example of Anguillid eels. The European and American eels *Anguilla anguilla* and *A. rostrata* spawn in discrete oceanic areas and the larvae drift back to occupy rivers and lakes over northwest Europe and the east coast of North America respectively. Once recruited there is little movement of adult eels between river systems. For each species there is one biological stock, but numerous fishery management stocks. Overfishing one fishery stock in a river system will have no short term impact on other fishery stocks in different river systems, although there could be a long term impact on the size of the spawning stocks and hence recruitment, if some fishery stocks were severely reduced in abundance. Genetic methods and phenotypic methods based on the characters determined early in the life history, such as meristic and morphometric markers and chemistry of the otolith nucleus, would be expected to be similar in eel samples throughout the range of the species, although there is an allele frequency cline in allozyme markers in *A. rostrata* along the east coast of North America (Williams *et.al.*1971). Selectively neutral genetic markers such as mtDNA show no differentiation throughout the species range. Characters determined later in the life history, such as microchemistry of the outer otolith and accumulation of parasites, would potentially show differences among adult stocks.

An alternative is to consider the differences between the biological and management stocks from a genetic and ecological perspective (Fig 1). Genetic stocks have continuity over time; larvae and juveniles recruit back to their birth stock and remain discrete to other stocks over time. Ecological stocks may recruit from a common larval pool but undergo differentiation in the nursery sites due to environmental differences. Political stocks are divided by arbitrary lines based on national and international fishery management zones that often have no relevance to biological parameters.

There are some common elements in many of the stock definitions. Reproductive isolation is explicit (interbreed, random mating) or implicit (losses by emigration and accessions by immigration if any) in many definitions, as is spatial isolation (Table 3). Although isolation is a critical component of the stock concept, the level of isolation is rarely defined. Genetic isolation implies that the stocks do not interbreed on a regular basis, whereas recruited adults could be ecologically isolated from other groups of adults, yet be derived from a common spawning area.

Spatial isolation of adults is relatively easy to determine from fishery statistics that document area and seasonal distributions. However reproductive isolation is more difficult to determine, and this has been the focus of much of the research into stock discrimination. Few of the methods measure reproductive isolation, only genetic and tagging approaches allow an estimate of the reproductive isolation and exchange rate. Other methods are based on non-inherited characters (parasites, chemistry) or inherited characters that are modified by the environment (morphometric and meristic, life history traits).

A conservative and simplistic approach would assess all spawning areas as discrete stocks. Such an approach would function for short lived or annual species, like squid, but for long lived species there is an unknown level of interaction through exchange of individuals, either as larval drift or movement of adults between spawning periods. Defining management units based on spawning areas also breaks down when spawning sites are separated by distances less than limits of larval drift, for example orange roughy spawning sites in the Bay of Plenty are separated by a distance of 25–40 kms, yet on the Chatham Rise the highest concentrations of juveniles are found 50–175 kms downstream from the spawning site (Zeldis *et.al.*1994). In addition the non-spawning fisheries have to be linked to the spawning fisheries. This can be achieved by finding characters that distinguish spawning stocks which can then be applied to non-spawning groups.

As data have been gathered and inferences made on stock structure, the fishery management question has shifted from that of reproductive isolation to consider short term relationships between spatially isolated groups. These groups may not be separate genetic groups with long term isolation, but groups that remain as cohesive units for much shorter time frames, even within a generation (Fig 1: ecological and genetic stocks). This shift in emphasis has rarely been explicitly stated in the literature although Gauldie (1988) draws attention to the limitations of the biological concept and its relevance to fisheries management. Setting boundaries and identifying stocks is in effect determining what part of the total species population is going to be assessed and managed (Skillman 1988). For example in the Dover sole *Microstomatus pacificus* off the west coast of Canada and California, tagging has shown that there are several geographically discrete stocks of adult sole with limited intermingling, but it is likely that there is considerable genetic mixing of progeny due to an extended larval period of up to one year in offshore waters (Westrheim et al 1992). Therefore the one biological and genetic stock of Dover sole is subdivided into several management units.

In contrast to Marr's (1957) statement that fisheries biologists have been interested in defining sub populations (= biological stocks) but have most frequently defined (fishery) stocks, the situation is now reversed. The current focus is on defining management units, yet most attempts at stock discrimination have defined biological stocks. Short term management is concerned with the impact of fishing on adult stocks, not on the long term genetic contribution of adults to the evolutionary structure of the population, although there is an increasing need for management decisions to take account of long term effects of fishing on populations (Stokes *et.al.*1993).

Pawson and Jennings (1996) concluded that much of the recent debate on the stock concept has focused on the degree of reproductive isolation. They sardonically indicated that the debate has produced numerous sub definitions of a stock many of which have little relevance to fishery management problems, and elaborated that this was because the majority of marine fisheries in the north east Atlantic are monitored and regulated in political areas which do not have logical or consistent relationships with biological processes or fish movements (Pawson & Jennings 1996). In the northwest Atlantic, catch statistics are reported by 30' latitude x 30' longitude squares. Biological definitions are often impractical in the management of multispecies fisheries in the northwest Atlantic (Almeida 1987) when for example a large proportion of the annual catch is taken as by-catch in another target fishery or by another fishing method. The pragmatic fishery biologist has taken any method that shows a difference as evidence for isolation and separate management. However in practice management decisions are rarely based on data from one method, but on an amalgam of information drawn from a variety of stock discrimination methods and biological and hydrological data.

## 2. To review and evaluate methods and criteria for defining stock or management units for orange roughy, black oreo, and smooth oreo

### Stock relationships of orange roughy

Several stock discrimination studies have been carried out on orange roughy. Results from the New Zealand studies have been presented in FARDs and reports (Clark 1990, Clark & Tilzey 1996, Francis *et.al.* 1995, Smith 1997a, 1997b) and in the scientific literature (Baker et al 1992,1995; Smith 1986, Smith & Benson 1997, Smith *et.al.* 1996, 1997). Orange roughy stock structure was initially based on geographical separation and spawning periods of the then known spawning areas (Clark 1990), but as more spawning grounds were discovered, some within the potential range of larval drift and adult movement (eg East Cape and Ritchie Bank separated by a distance of less than 200 km) then these criteria for stock discreteness became questionable.

The first genetic studies showed high variability but limited genetic divergence between widely separated stocks (Smith 1986). More recently DNA techniques have been applied to orange roughy and show regional differences and in some cases temporal variation (Smolenski *et.al.*1993, Smith *et.al.*1996, 1997). This section reviews specific stock discrimination studies on orange roughy.

*Allozymes*

The first allozyme survey of orange roughy showed a high level of genetic variation (heterozygosity 0.11, average heterozygosity for 106 marine fishes 0.06) but little genetic differentiation among samples from the Challenger Plateau and Chatham Rise (Smith 1986). One locus, *Idh-2**, revealed significant heterogeneity among samples from the east coast and Challenger Plateau (Smith 1986). More recent surveys have found significant spatial and temporal genetic heterogeneity among samples from the east coast and Chatham Rise (Francis *et.al.*1995, Smith *et.al.*1997), especially with the *Idh-2** marker (Smith & Benson 1997).

Evidence for discrete stocks off South Australia and eastern Australia based on an allozyme survey (Black & Dixon 1989) was not supported by a larger scale study (Elliott & Ward 1992), which found no heterogeneity among six populations sampled over 3 000 kms from New South Wales to Western Australia (Elliott & Ward 1992). The study by Black & Dixon (1989) produced some anomalous results. The electrophoretic data were unusual in that the gel phenotypes at 3 loci (*IDH, MDH, ME*) did not agree with the expected gel phenotypes from the quarternary structure of the enzymes; thus it is possible that the observed electrophoretic variation does not have a genetic basis (Elliott & Ward 1992). Black & Dixon (1989) also re analysed the data of Smith (1986) and concluded that there were significant differences at 13 out of 15 comparisons of New Zealand samples. However the probability levels were not adjusted for multiple tests, reducing the number of significant results. In addition Black & Dixon's (1989) analyses did not take account of differences due to sampling error. Their reported $G_{ST}$ (Nei's gene diversity statistic, Nei 1973) differences (Black & Dixon 1989) are no greater than those due to sampling error, with the exception of the *Idh-2** locus, which was reported as showing significant regional differences by Smith (1986).

The overall genetic differentiation among populations can be estimated with Nei's gene diversity statistic (Nei 1973). For Australian populations of orange roughy the $G_{ST}$ averaged over 11 loci is 0.0045 (Table 4), in other words less than 0.45% of the total genetic variation is due to differentiation among samples with the remaining 99.55% within samples. Such a low level of differentiation is no greater than that due to sampling error (Elliott & Ward 1992). A comparison of pooled Australian and New Zealand samples gives a $G_{ST}$ of 0.0022, which is significantly greater than $G_{STnull}$ (Elliott & Ward 1992). A comparison of the pooled Australian samples with a sample from the North Atlantic also showed a small, but significant $G_{ST}$ (Elliot et al 1994). An earlier analysis showed significant heterogeneity between North Atlantic and New Zealand samples at three enzyme loci (Smith 1986). Measures of $G_{ST}$ averaged over several loci may be less sensitive for stock discrimination than methods such as the $\chi^2$ test on single loci.

More recent allozyme surveys have found significant spatial and temporal genetic heterogeneity among samples collected along the Chatham Rise (Francis *et.al.*1995, Smith & Benson 1997). A comparison of eleven polymorphic allozyme loci showed a significant heterogeneity for 4 loci among samples from 4 spawning sites: Ritchie Bank, Chatham Rise (Box), Waitaki and Puysegur (Smith *et.al.*1997). There were significant differences among all pairwise comparisons except for Ritchie Bank and the Box, indicating the presence of three genetic stocks: Chatham Rise & Ritchie

Bank, Waitaki, and Puysegur (Smith *et.al.*1997). A larger survey of the same 11 loci in 5 east coast sites and 9 Chatham Rise sites found heterogeneity at two loci. All of the Chatham Rise sites were sampled on two occasions: summer 1994–95 and winter 1995 to test for short term temporal variation (Smith & Benson 1997). There was no heterogeneity among the east

**Table 4: Nei's gene diversity statistic ($G_{ST}$) estimates derived from allozyme data for marine fishes and population samples of orange roughy. $G_{ST}$ estimates are sensitive to the number of populations sampled and the extent of the geographic range sampled; the lowest estimate is for two populations of flatfish *Pleuronectes platessa* (0.001) sampled from the Irish Sea, a small area of the species total range, while the highest is among 5 populations of *Floridichthys polommus* (0.291) (Ward *et.al.*1994a)**

| **Group** | **No. species** | **$G_{ST}$ mean** | **$G_{ST}$ range** | **Reference** |
|---|---|---|---|---|
| Marine | 7 | 0.042 | 0.007–0.181 | Gyllensten 1985 |
| Shore fishes | 9 | 0.009 | 0.0–0.032 | Waples 1987 |
| Marine | 57 | 0.062 | 0.001–0.291 | Ward *et. al.*1994a |
| **orange roughy** | **No. populations** | **$G_{ST}$ mean** | | **Reference** |
| Australia | 6 | 0.005 | | Elliott & Ward 1992 |
| Australia/NZ | 7 | 0.002 | | Elliott & Ward 1992 |
| East coast NZ | 5 | 0.008 | | Smith & Benson 1997 |
| Chatham Rise | 9 | 0.012 | | Smith & Benson 1997 |
| East coast & Waitaki | 4 | 0.020 | | Smith *et.al.*1997 |
| Australia /North Atlantic | 2 | 0.010 | | Elliot *et.al.*1994 |

Coast samples, indicating that the samples had been collected from the same genetic stock. However there was heterogeneity among the Chatham Rise samples at two loci, *Idh-2** and *Ldh-1**. The *Idh-2** marker showed significant within site differences between the two sampling periods at 3 of the 9 Chatham Rise sites. In addition there was evidence for isolation by distance along the Chatham Rise, based on pairwise comparison of $G_{ST}$ values after Slatkin (1993), but no clear stock boundaries.

Additional tissue samples were collected along the north Chatham Rise and off Kaikoura and in Cook Strait during 1996 and tested for the *Idh-2** marker (Smith 1997a). Combining all the *Idh-2** data there is:

- a significant genetic difference between the east coast (Kaikoura) and the northwest Chatham Rise that is consistent over two years of sampling, 1994, 1996;
- a significant genetic difference between the northwest Chatham Rise and the Graveyard that is consistent over two years of sampling, 1994, 1996;
- a significant genetic difference between the Graveyard and the Box in 1994, but not 1996 due to temporal variation within the Box.

These data are summarised in Figure 2 and Table 5.

Half (7 out of 14) of the samples along the north Chatham Rise exhibit a significant departure from Hardy-Weinberg (HW) equilibrium (Smith 1997a), and 5 out of these 7 samples are clustered on the northwest Chatham Rise (178 °E) and the Graveyard

(Figure 3). The *Idh-2** locus was the only one out of 9 loci showing a significant departure from HW equilibrium in Chatham Rise samples (Smith & Benson 1997). Homozygous excess can arise through several mechanisms such as selection against heterozygotes, assortative mating, inbreeding and null alleles, but these seem unlikely explanations for large natural populations and would have to differ between regions of the Chatham Rise. Technical errors seem unlikely as samples from the east coast show no departure from HW equilibrium. One possible explanation is stock mixing, when two or more stocks with different allele frequencies mix. Combining samples from the Wairarapa and the northwest Rise, both of which are in HW equilibrium, produces a mixed sample with an excess of homozygotes.

The same *Idh-2** marker has been tested in samples of orange roughy from the Challenger Plateau and Lord Howe Rise (Smith 1997b). A sample from the northwest Challenger Plateau was significantly different to samples from the southwest Challenger Plateau and the Lord Howe Rise. Given the temporal variation found with this marker on the Chatham Rise additional samples are required from the Challenger Plateau before conclusions can be made about the genetic relationships among these areas (Smith 1997b).

***Mitochondrial DNA***

Statistically significant differences in haplotype frequencies among samples has been taken as evidence for limited genetic exchange and the presence of discrete stocks (see Objective 1). Most fisheries applications of mtDNA haplotypes have used a randomisation test to detect significant differences among samples. Often mtDNA data sets consist of a large number of observed haplotypes and a randomisation test overcomes the problem of a large number of low frequency haplotypes.

An analysis of restriction enzyme digests of mtDNA in Tasmanian samples of orange roughy were interpreted as showing low levels of gene flow between the east and west coasts of Tasmania, based on distribution of rare haplotypes in two small samples, n= 23 + 26 (Ovenden *et.al.*1989). A larger survey over a much wider area, and based on similar techniques, showed no significant regional differences in southeastern Australian waters (Smolenski *et.al.*1993). Application of a finer resolution technique, using four-base restriction enzymes to cut the DNA, provided an indication of genetic subdivision within areas (Smolenski et al 1993). One hundred and seven orange roughy from 7 sites (South Australia 1988, 1989, east Tasmania, west Tasmania, New South Wales, South Africa and New Zealand) revealed 104 different haplotypes, so that the majority of haplotypes were represented by just one individual (Smolenski *et.al.*1993). Breaking down the data by individual restriction enzymes revealed 33, 45, and 46 morphs per enzyme and these showed some regional variation, with differences between New South Wales, South Australia, and Tasmania. The four-base survey also revealed temporal differences between years of sampling, both between regions and within South Australia (Smolenski *et.al.*1993).

Around New Zealand a survey of samples from six spawning sites found three genetic groups using six-base restriction enzymes. The common fragment in northern (Ritchie, Box, southwest Challenger and Cook Canyon) samples was absent in samples from the Puysegur and Waitaki fisheries indicating the presence of two genetic groups: northern and southern (Smith *et. al.*1996). In addition unique

restriction fragments were found only in some Challenger fish, and this difference was repeatable over two years of sampling, showing that the southwest Challenger fish are isolated from neighbouring samples collected from the Cook Canyon and Chatham Rise (Smith *et. al.*1996).

Restriction enzyme digests of amplified fragments of mitochondrial DNA showed no significant genetic differences between samples from Ritchie Bank and the Box (Smith *et.al.*1997). The same technique showed significant differences between Waitaki and Puysegur and between Waitaki and Ritchie Bank (Smith *et. al.*1997), but not among samples from the Tasman Sea, including northwest and southwest Challenger Plateau, Lord Howe Rise, New South Wales and Tasmania (Smith 1997b). Results are summarised in Table 5.

Sequencing the cytochrome *b* gene of the mitochondrial genome found high levels of genetic variation, but no differences between small samples from 4 sites around New Zealand, n=24, from Australia, n=4, and South Africa n=4 (Baker *et. al.*1995). The cytochrome *b* gene is a conservative region of the mitochondrial DNA which is finding greater application in taxonomic studies than population discrimination.

***Nuclear DNA (DNA fingerprinting and RAPDs)***

DNA fingerprinting found high levels of genetic variation but no significant differences between two small samples from the Chatham Rise and Challenger Plateau (Baker *et. al.* 1992). The rapid advances made in this area of DNA technology, in particular the development of specific single locus probes for detecting genetic variation, and the preliminary results for marine fishes (Table 6, Objective 1), suggest that further laboratory effort in DNA micro- and mini-satellite DNA could provide informative genetic markers for orange roughy.

Use of random amplified polymorphic DNA showed no significant genetic differences between samples from Ritchie Bank and the Box, but differences between these sites and Waitaki and Puysegur (Smith *et. al.*1997). However there were technical problems with RAPDs, and Smith *et. al.*(1997) recommended use of RAPDs only when other genetic methods failed to reveal polymorphisms. Techniques such as PCR-RFLP of mtDNA, or allozymes, yield fewer polymorphisms per unit of laboratory time than RAPDs, but still produced sufficient polymorphisms to detect population structure in orange roughy (Smith *et.al.*1997).

Fig 2: *Idh-2* c* allele frequencies (± se) in orange roughy samples from the central east coast and north Chatham Rise collected between 1994–1996 (Smith 1997a). See Fig 3. For location of sample sites. Ritchie = Ritchie Bank, Wair = Wairarapa, Cook = Cook Strait, Kaik = Kaikoura, NW = Northwest Chatham Rise, Grav = Graveyard (approx $180^0$), East = East Chatham Rise, Box = spawning box north of Chatham Islands

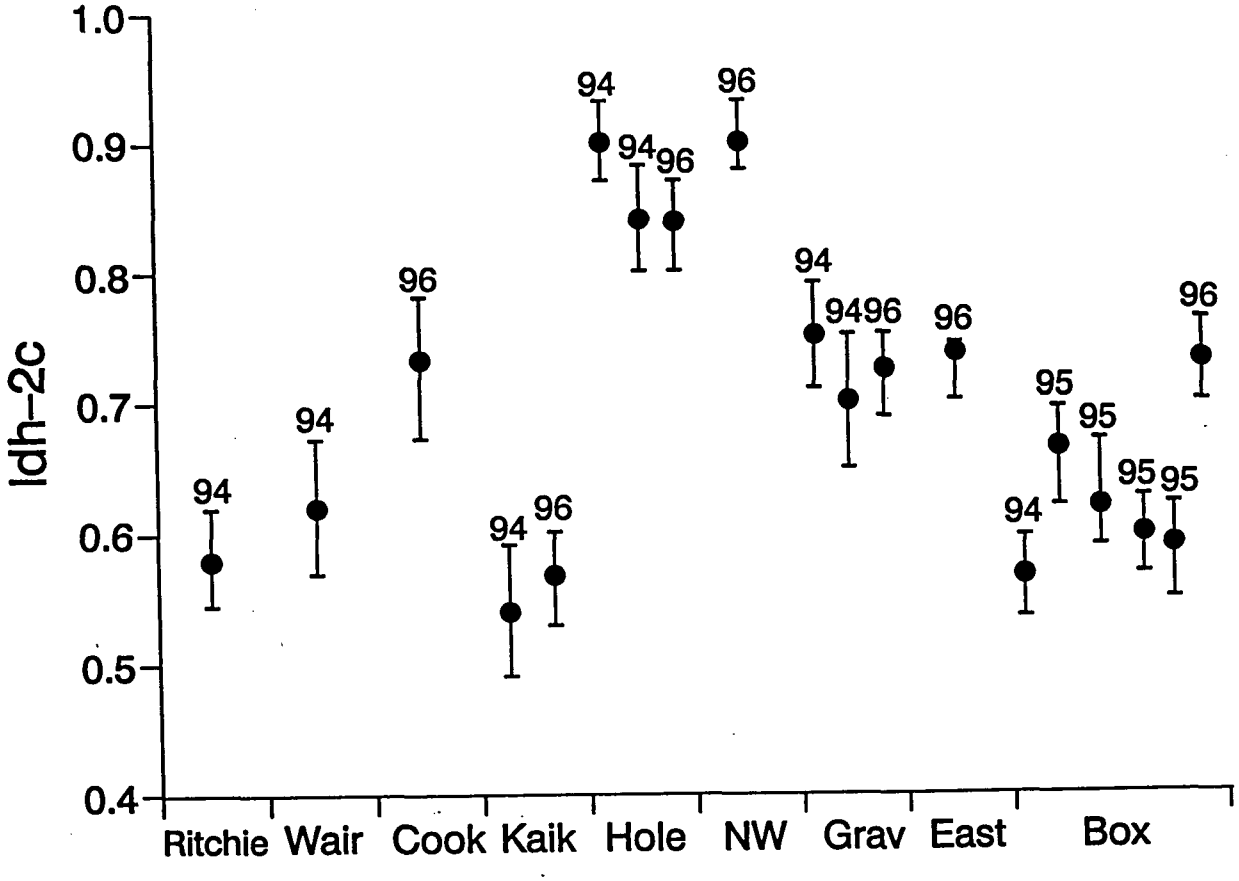
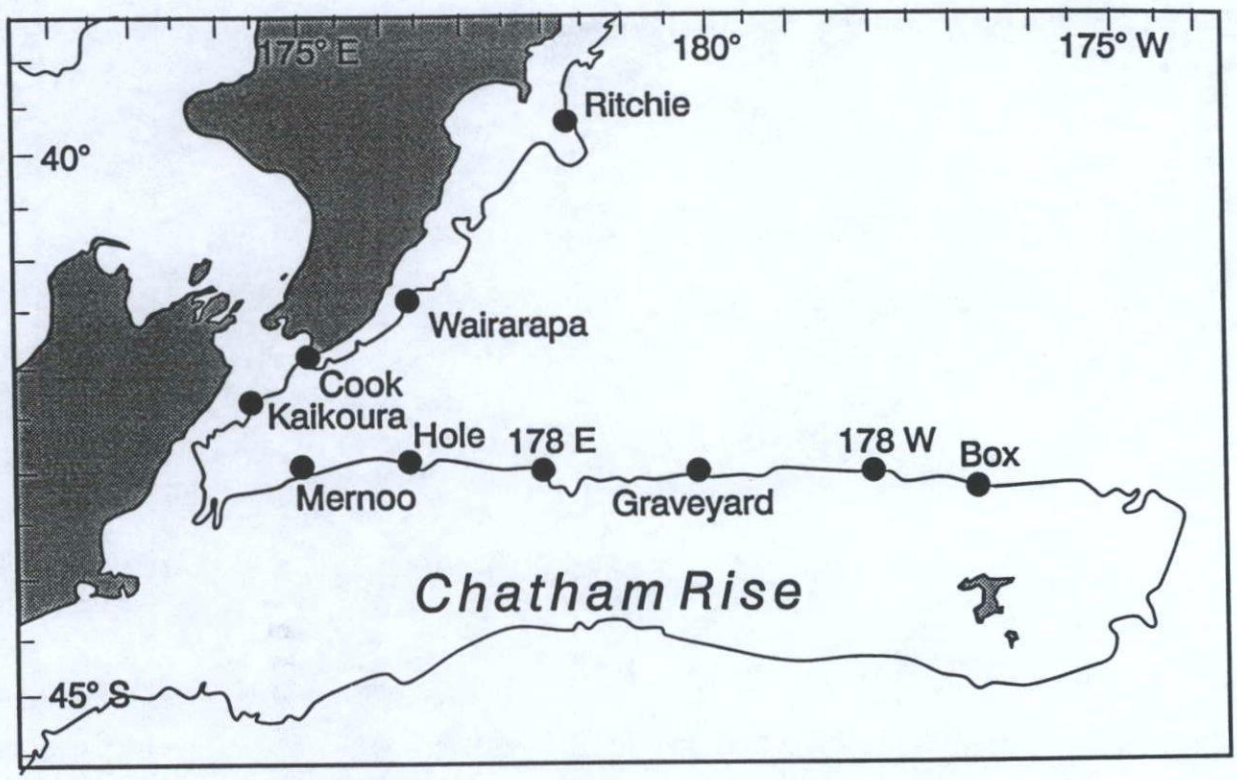


Fig 3: Locations of orange roughy genetic samples collected off the east coast and north Chatham Rise between 1994–96 (from Smith 1997a)



*Morphometrics and meristics*

Thirty-nine morphometric characters were measured in 10 population samples of orange roughy from Tasmania to Western Australia (Elliott *et.al.* 1995). Data were standardised with sexes combined to remove size effects. There was considerable morphometric variability within and among samples, with the most significant difference between temporal samples within sites, either between years of sampling or between months of sampling (Elliott *et.al.*1995). It is possible that within site differences between seasons are produced by differences in shape between spawning and spent fish, although this would not account for between year differences. There were also significant differences between regions leading the authors to conclude that there were at least seven stocks: Western Australia, Great Australian Bight, New South Wales, southern Tasmania, Cascade Plateau (south east Tasmania), east Tasmania, and St Helens Hill, within the eastern Tasmania stock (Elliott *et.al.*1995). However the observed within site variation must question a simple interpretation for regional stocks, especially as many regions were based on a single sample. Either there are temporally, as well as spatially isolated stocks, or there is considerable within stock variability produced by year class differences or artefacts of the technique, such as body change due to feeding/spawning condition. The morphometric data alone cannot be used to show that there are discrete regional stocks.

**Table 5: Summary of genetic results for New Zealand orange roughy populations**

| Location | Result | Reference |
|---|---|---|
| **Spawning areas** | | |
| Challenger Plateau-Cook Canyon | sig.difference mtDNA | Smith *et.al.*1996 |
| Cook Canyon-Puysegur | sig.difference mtDNA | Smith *et.al.*1996 |
| Puysegur-Waitaki | sig.difference mtDNA | Smith *et.al.*1997 |
| | sig.difference *Est-1** | Smith *et.al.*1997 |
| Waitaki-Box | sig.difference mtDNA | Smith *et.al.*1996 |
| | sig.difference allozymes | Smith *et.al.*1997 |
| Box-Ritchie Bank | no difference mtDNA | Smith *et.al.*1997 |
| | no difference allozyme | Smith & Benson 97 |
| **Chatham Rise stock complex** | | |
| Kaikoura-northwest Chatham Rise | sig.difference *Idh-2** | Smith & Benson 97, Smith 1997a |
| NW Chatham Rise-Graveyard | sig.difference *Idh-2** | Smith 1997a |
| Graveyard-Box | temporal difference *Idh-2** | Smith 1997a |
| **Challenger Plateau-Lord Howe Rise** | | |
| SW Challenger-NW Challenger | sig.difference *Idh-2** | Smith 1997b |
| NW Challenger-LHRise | sig difference *Idh-2** | Smith 1997b |

A smaller morphometric and meristic comparison was made for just two samples from Puysegur Bank (n= 99) and the Lord Howe Rise (n=100), separated by 1200 km (Haddon & Willis 1995). Counts were made of 8 meristic characters and measurements made of 17 body characters; sexes were treated separately and linear regression used to overcome size differences. Eight characters showed differences between sites: head length, snout length, orbit diameter, maxilla width, premaxilla length, caudal peduncle, gill raker count and anal fin count, leading the authors to conclude that fish from the two areas are relatively discrete (Haddon & Willis 1995).

No attempt was made to consider temporal variation, although fish from Puysegur were derived from two tows, between which there was no discrimination. Lack of replicate and geographically intermediate samples precludes any conclusions about stock structure. A preliminary analysis of fin ray counts (dorsal, pectoral, pelvic and anal) showed little variation and no differences between samples from the Chatham Rise (Box) and Ritchie Bank spawning fisheries (Smith unpublished data).

There is considerable variation in morphology of the orange roughy otolith but an early study found no differences among samples from the Challenger Plateau and Chatham Rise (Linkowski & Liwoch 1986). Two approaches to otolith morphometrics were evaluated in a study of orange roughy otoliths from the Tasmanian fishery (Report 1995). Measurements of four lengths and two widths, along with otolith depth, area and circularity were made on two sets of otoliths from the southern and eastern management zones and tested with analysis of variance for area and sex effects. There were significant area and sex effects with age or length as the co-variate. A Fourier shape analysis undertaken on similar samples showed no significant differences between areas indicating that the overall shape is similar between areas (Report 1995). There was a significant difference in otolith weight between fish of the same size from the two areas, and because otolith weight is linearly related to age this indicates a difference in growth rate between the two areas. Thus it was concluded that the differences found between areas for otolith morphology were not due to shape but were a function of age differences between the two areas (Report 1995). The two sites were sampled at different time periods, winter spawning fish in the eastern zone and summer non-spawning fish in the southern; thus further area samples are required from the same season (Report 1995).

Fourier analyses of otoliths from the eastern and southern zones off Tasmania were also undertaken with a more comprehensive set of samples (Robertson et al in prep, in Bax 1997). Samples from the southern summer and eastern winter (spawning) were similar, but southern summer and southern winter were different, indicating that some orange roughy move from the southern zone to the eastern zone in winter, with a residual group that does not move.

***Otolith microchemistry***

Trace element composition of whole otoliths has been tested in samples from three areas off South Australia, east and west Tasmania. Discriminant analyses of 10 elements showed that the samples were taken from three different group (Edmonds *et. al.*1991). There was also a difference between males and females from western Tasmania, although this was based on a small sample size, 7 males and 16 females (Edmonds *et. al.*1991). The orange roughy otoliths differed to some other species in concentration of some elements; for example strontium concentrations are higher and potassium and magnesium concentrations lower than those of other teleosts tested by the same method (Edmonds *et. al.*1991). The regional differences were based mostly on sodium and magnesium, which is surprising as these elements are physiologically important and concentrations are likely to be strictly controlled (Edmonds *et. al.*1991). Nevertheless the results suggest that there is little short term exchange of fish between the three sites off Australia.

A second study compared concentrations of 6 elements (sodium, strontium, calcium, potassium, sulphur and chlorine) with an electron probe from 5 areas around Australia from Western Australia to New South Wales and an outgroup sample from the North Atlantic (Report 1995). Three scans were done on each otolith, the primordial, along the posterior growth axis, and on the posterior margin. In addition a subsample of otoliths was analysed by proton microprobe for 11 trace elements. Groupings of sites and individuals were tested by linear discriminant function analysis. Five of the six macro elements differed significantly among samples, and 4 of the trace elements differed significantly among samples. Removal of the North Atlantic sample, which had significantly higher concentrations of lead and zinc, produced only one trace element, selenium, showing differences around Australia (Report 1995). However the concentrations of selenium were at the conservative limits of detection and so may be a sampling artefact (Report 1995).

The macro elements suggest that the samples were taken from at least three stock groups, based on scans at or near the primordium: Western Australia, New South Wales, and the Great Australian Bight and Tasmania. The latter group may be further sub-divided into two groups, Great Australian Bight and Tasmania, based on evidence from the otolith margin (Report 1995). Differences at the otolith primordium reflect differences in nursery sites, while differences at the margin reflect differences among adults, after larval dispersion. Thus the data indicate that although the orange roughy from the Great Australian Bight and Tasmania could be derived from the same spawning-nursery site, the adults are relatively sedentary following recruitment (Report 1995). There is also evidence for a difference between the eastern, spawning area, and southern non-spawning area at the otolith margin, which may indicate that some southern orange roughy move to the eastern spawning site leaving a different non-migratory group in the southern region; alternatively different fish might move into the southern area to spawn (Report 1995). Further sampling is required to address the regional and seasonal differences between the eastern and southern areas.

Currently the technique is being applied to orange roughy samples collected in the Tasman Sea. Preliminary results indicate a difference between pooled Australian and New Zealand samples, but little differentiation among New Zealand samples from the Challenger Plateau and Lord Howe Rise (Thresher pers. com.).

***Parasites***

One parasite study has shown significant differences between roughy samples from the Chatham Rise, Cook Canyon, and Challenger Plateau (Lester *et. al.*1988), but Jones (pers. com.) found significant within site heterogeneity on the Chatham Rise. Canonical multivariate analysis of larval nematodes and cestodes discriminated 5 Australian and 3 New Zealand stocks (Lester *et. al.*1988). The data were broken into three fish-size classes: small, medium, and large. The multivariate analyses of the data from the medium and large size classes discriminated more areas than analysis of data from the small fish, because more parasites were present in larger fish (Lester *et. al.*1988). In total five groups were discriminated around Australia: Great Australian Bight, Cascade Plateau (South of Tasmania), northeast Tasmania, New South Wales, and South Australia/west Tasmania. The five groups were based on single samples except for South Australia/west Tasmania which was based on 4 samples (Lester *et. al.*1988). Three area samples were compared from New Zealand spawning areas:

Challenger Plateau, Cook Canyon and the Box. Multivariate analyses on small, medium and large fish discriminated three regional groups in each size class (Lester *et. al.*1988). Duplicate samples taken in the same month from both the Cook Canyon and the Box were the same within sites but different between sites (Lester *et. al.*1988). The parasites that produced the greatest discriminating powers within New Zealand were *Anisakis* type 3 for small fish and an unidentified larval spirurid for the medium and large fish.

Samples of small (20–29 cm standard length), medium (30–37 cm) and large (38–45 cm) orange roughy were collected around Tasmania. Summer and winter samples were collected from 1 area east, and 2 areas west, of Tasmania, and *Anisakis* and *Callitetrahynchus* sp. counted (Lester & Gorman 1989). Parasite faunas did not differ between season in the two areas to the west of Tasmania suggesting that spawning aggregations are from local fish. Large fish from the east of Tasmania showed similarities with a sample from the south of Tasmania, suggesting a northward movement of fish to the spawning area (Lester & Gorman 1989). However the study has been criticised because of small sample size (20 fish per size class) and the small number of parasite species counted which reduce the chance of finding significant differences among samples. In particular the two parasite species that discriminated regional samples, *Terranova* sp. and spirurid sp. (Lester *et. al.* 1988), were excluded (Doonan pers. com.).

Jones & Gibson (1993) extended the approach of Lester *et. al.*(1989) to New Zealand fisheries, and compared parasite species and numbers in 1088 orange roughy from nine areas. There was significant variation between areas in number of parasite species per fish and number of species per area (Jones & Gibson 1993). In particular fish samples from the Lord Howe Rise were different to other area samples, likewise samples from the Ritchie Bank were different to those from the Chatham Rise and Kaikoura. However significant sampling problems were identified. There was variation in parasite numbers between tows within areas due to variation in host length. Given the uneven sample sizes and different size ranges of fish, the Deepwater Working Group decided that it was premature to draw conclusions about stock relationships based on this data set (Jones pers. com.).

***Mechanical tagging***

A tagging experiment was proposed to mark orange roughy on the St Helen's Hill spawning population off Tasmania using baited break-away hooks on a long line, but the project was not funded (Report 1995). Preliminary hook tagging trials were carried out by Hvid and Gauldie on the Ritchie hills in the early 1980s; only sharks and eels were caught on more than 2000 hooks and so the trials were abandoned (Hvid pers. com.). It was concluded that orange roughy are unlikely to take a bait and hence hook tagging is not feasible (Hvid pers. com.).

***Biological and life history data***

Data on length frequencies and size and age at spawning are presented in objective 1. Differences in length frequencies provide evidence that there is little short term movement of adult fish between the spawning areas on the Lord Howe Rise, northwest Challenger and southwest Challenger Plateau (Clark & Tilzey 1996).

Length frequency distributions on the south west Challenger Plateau also differ to those for the Box (Chatham Rise), Ritchie Bank and Cook Canyon (Clark 1990). On the Louisville Ridge size structure differs with larger fish in the north, but the data are limited to two years of observations (Clark 1998b).

Age at first maturity shows significant differences among the major fishing areas for both mean length and age at maturity, with the smallest and youngest fish on the Challenger Plateau, a second group comprising fish from the Chatham Rise, Ritchie Bank and Puysegur, and a third group maturing at a larger size in the Bay of Plenty (Horn et al in prep). Bell *et. al.*(1992) also reported that length at maturity varied among spawning sites off Australia. There are differences in growth rate between the eastern and southern management zones around Tasmania (Report 1995).

The genetic basis for regional differences in life history traits is unknown, but there is a strong environmental component in other species. In the orange roughy fishery to the east of Tasmania length frequencies have shown a shift toward smaller fish (Bax 1997). There has been a decline in the age structure from modal age 55 in 1992 to 40 in 1995 in eastern zone winter fish and from 60 to 35 in the southern summer zone fish around Tasmania (Bax 1997). One explanation is that orange roughy are maturing earlier and recruiting into the fishery earlier, as occurs in other heavily fished stocks of marine fishes (Bax 1997). If orange roughy are responding to fishing pressure then life history traits such as age or size at first reproduction may not be indicators of stock discreteness, but indirect indicators of fishing pressure.

***Summary of stock discrimination studies on orange roughy***

1. Evidence from a wide range of independent methods (allozymes, mtDNA, parasites, otolith microchemistry, morphometrics and biological data) demonstrate that orange roughy are subdivided into a number of discrete regional stocks around Australia and New Zealand. Characters separating the major spawning groups around New Zealand are shown in Fig 4. Orange roughy have a continuous, low density distribution along much of the 1000m contour, with localised peaks of abundance. Some small isolated fisheries, such as Cook Canyon and Waitaki, are based on discrete stocks, but in other areas there is uncertainty about stock discreteness, for example along the north Chatham Rise where there are three spawning groups, between the east coast spawning sites, and between the Challenger Plateau and Lord Howe Rise spawning sites.

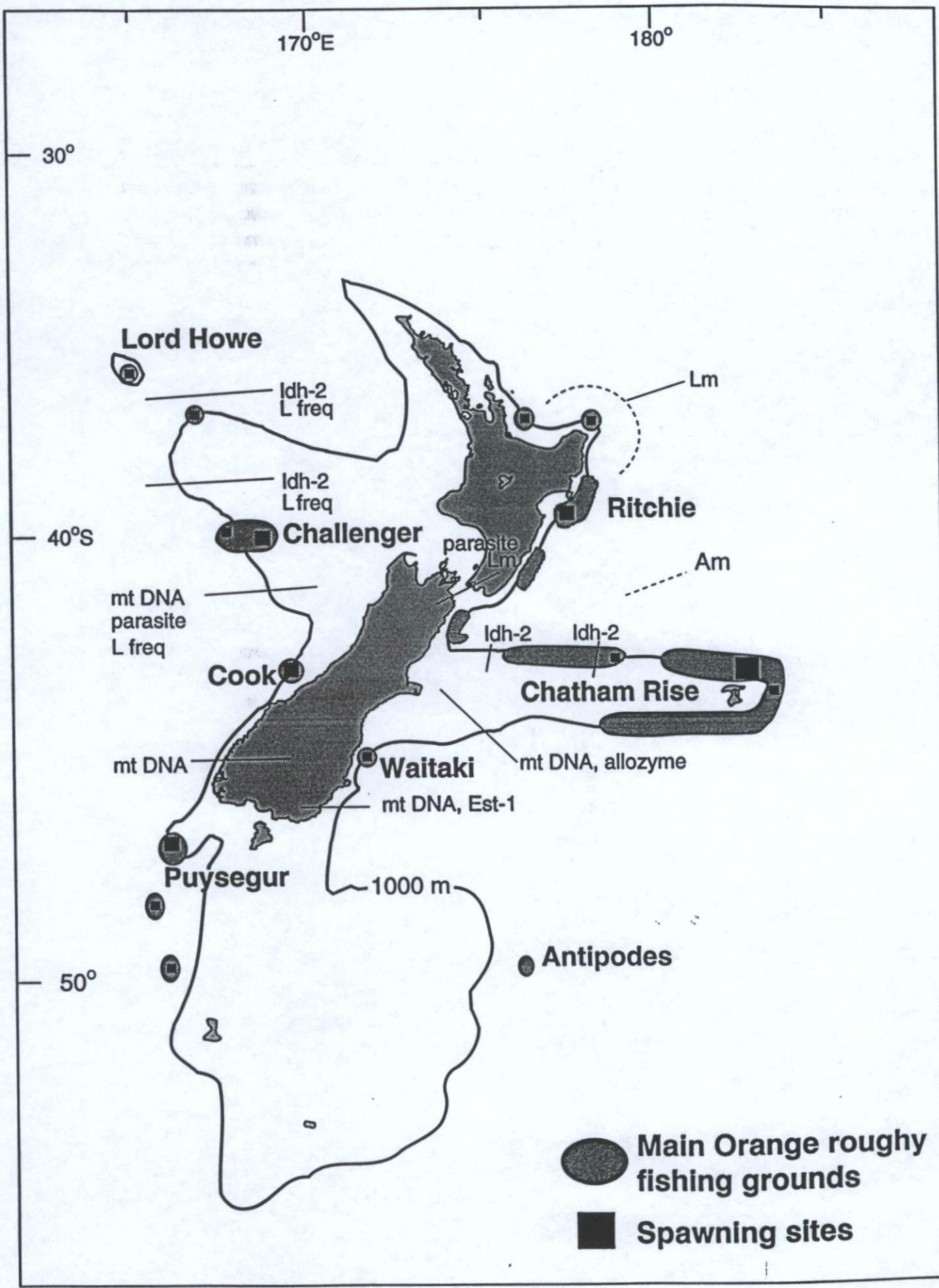
2. Temporal variation within regions has been reported in morphometric, genetic and parasite studies. There are morphometric differences between seasons and between years in samples collected off Tasmania (Elliott *et. al.*1995). Analyses of mtDNA have shown distinct genetic groups off South Australia in consecutive years (Smolenski *et. al.*1993), and between years in the spawning population in the Box on the Chatham Rise (Smith 1997a). At the allozyme marker, *Idh-2**, there are significant differences among season and year of sampling at some sites on the Chatham Rise (Smith & Benson 1997, Smith 1997a). Parasite data have indicated significant differences between tows on the Chatham Rise (Jones & Gibson 1993). Clearly temporal variability must be considered in future stock discrimination analyses of orange roughy.

3. Finding temporal variation with independent markers and in different spawning regions suggest that orange roughy have a more complex population structure than simple spatially isolated stocks. Orange roughy are thought to be long lived species which may be an adaptation for surviving in a low productivity environment that has low and irregular recruitment (Leaman & Beamish 1984). In a low productivity environment individual fish will not spawn annually and so spawning concentrations may consist of different groups of fish from year to year on the same grounds. Bell *et. al.*(1992) have shown that up to 45% of post mature females did not develop oocytes and suggested that the scarcity of food, coupled with the energetic cost of migrating to the spawning ground, precluded fish from annual spawning.

4. If roughy are not spawning each year then different hill complexes may contribute to the spawning population in different years or in different proportions each year. Productivity on individual hills may vary due to local biological and physical conditions, with more productive hills producing a faster growth rate and hence more frequent spawning, than less productive hills.

5. Genetic studies have provided evidence for stock isolation between widely separated spawning groups, but have not distinguished stocks along the east coast of the North Island from the Bay of Plenty to Kaikoura. Preliminary data on length at maturity discriminates fish samples from the Bay of Plenty and Ritchie Bank and the method should be applied to samples from the East Cape spawning site. Likewise age at maturity discriminates samples from the Box and Ritchie Bank, which current genetic methods do not, and should be applied to other spawning areas on the north Chatham Rise and off the east coast. Because age and length at maturity change in response to fishing pressure in other species, then these traits should be monitored over time. Parasite markers applied by Lester *et. al.*(1988) clearly discriminated widely separated groups of orange roughy and could be applied to samples collected over smaller geographic distances, taking into account the findings of Jones (unpublished).

Fig 4: Orange roughy fishing areas and spawning grounds around New Zealand and characters separating neighbouring populations



The northern North Island, from west of Wellington around the west coast and the north-east coast to Cape Runaway was treated as one QMA, ORH 1, and landings were small until 1994 when a fishery developed in the western Bay of Plenty, in an area known as the Mercury-Colville Box. In 1996 orange roughy were also caught off the northwest coast on the Tauroa knolls. From 1995–96 ORH 1 was subject to a five year adaptive management plan with a limit of 1 000 t applied to the western Bay of Plenty and 190 t to the rest of the area. For 1996–97 an additional 800 t was allocated to designated areas, with catch limits on any one area (Annala & Sullivan 1997). Special permits are issued to fishers with one condition being that not more than 100 tonnes (greenweight) of orange roughy is taken from any single topographic feature, defined as a prominent and definable undersea geographic unit elevated from the surrounding seabed and similar in shape to a mountain or large hill, including all the area within a 10 nautical mile radius of the shallowest point.

The Challenger Plateau (ORH 7A) has been treated as single stocks that occurs both inside and outside the EEZ (Annala & Sullivan 1997). Most fishing took place on the southwest Rise, but in the late 1980s small fisheries developed on the northwest Challenger Plateau and on the Lord Howe Rise, outside the New Zealand EEZ (Clark & Tilzey 1996). Biological data indicates that the geographically isolated fisheries may be discreet stocks (Clark & Tilzey 1996), although length frequency differences between the Lord Howe Rise and northwest Challenger have changed over time (Clark 1998b). A joint Australia-New Zealand project is using a variety of approaches to determine stock relationships among these fisheries.

A fishery developed on the Louisville Ridge, to the east of New Zealand and about 600 km outside the EEZ. Fishing occurs over a wide area from 30°–45° S and appears to be clustered into 3 areas: north, central, and south. Limited biological data on length frequencies and gonad indices indicate potential stock differences between the 3 areas (Clark 1998c). The geographical isolation of this area would suggest that it is a separate stock to those on the Chatham Rise.

A conservative and low risk policy is to manage each spawning aggregation as a separate stock. The key questions are do individual fish return to the same hill after spawning or move among hills between spawning events? And do fish spawn on different hills in different years? Most stock discrimination methods will not address these questions. If there is movement between hills then characters are homogenised. Most discrimination tools cannot distinguish between lack of differences due to movement, or due to similar conditions among isolated hills. Only mechanical tagging would permit a test of short term movement between hills, which to date has not been evaluated with orange roughy (Report 1995).

**Stock relationships of black oreo, and smooth oreo**

There have been far fewer stock discrimination studies on black and smooth oreos, than with orange roughy. Genetic studies on oreos have focused on phylogeny; and found that oreos have relatively high levels of genetic variation measured with allozymes (Lowry *et. al.*1996). One study tested three approaches to stock discrimination of four species of Australian oreo and included allozyme, mtDNA and meristic analyses of black and smooth oreo (Ward *et. al.*1996). The genetic data on allozymes and mtDNA on three species were also presented in Ward *et. al.*(1998 in

press). Ward *et. al.*(1996) pointed out the limitations of meristic data in long lived species like oreos, where differences among samples may be due to among year class rather than spatial differences.

***Black oreo***

Two samples of black oreo from Tasmania (n = 200) and the Chatham Rise (n = 90) showed no significant differences at 8 polymorphic allozyme loci; an additional sample from the South Tasman Rise (n = 40) was tested for 4 polymorphic muscle loci, at which it was not significantly different to the samples from Tasmania and New Zealand, but the samples were in poor condition and could not be scored for liver specific allozymes (Ward et al 1996). A sub set of these samples was tested for variation in mtDNA, using RFLPs. One mtDNA haplotype was present in small numbers of fish (6/96) from Tasmania, but absent in the New Zealand sample (0/76); the sample from the South Tasman Rise could not be scored for mtDNA (Ward *et. al.*1996).

Meristic counts on three samples of black oreo from Western Australia (n = 9), Tasmania (n = 55) and New Zealand (n = 9) found significant differences for lateral line scale counts and number of pyloric caeca, although samples sizes were small (Ward *et. al.* 1996). Fish from southern Tasmania had higher mean counts of lateral line scales than fish from New Zealand and Western Australia, while fish from New Zealand had higher mean counts of pyloric caeca than fish from southern Tasmania and Western Australia (Ward *et. al.*1996). Pectoral fin ray, dorsal fin ray and spine, anal fin ray and spine, and gill raker counts showed no significant differences among the same samples (Ward *et. al.* 1996).

***Smooth oreo***

Genetic and meristic studies were carried out on smooth oreo samples from Western Australia, Tasmania, New Zealand and the Lord Howe Rise (Ward et al 1996). There were no significant differences in allele frequencies at 11 polymorphic allozyme loci among samples from southern Tasmania (n = 200), New Zealand (n = 99) and Western Australia (n = 90). Likewise there were no significant differences in mtDNA haplotype frequencies among samples from New Zealand (n = 91), southern Tasmania (n = 95), Western Australia (n = 91) and Lord Howe Rise (n = 15) (Ward et al 1996).

Meristic counts on pectoral fin rays, dorsal fin rays and spines, anal fin rays and spines showed no significant differences among samples from the Chatham Rise (n = 9), Western Australia (n = 57), southern Tasmania (n = 53), and Lord Howe Rise (n = 15). Lateral line scale counts showed significant differentiation among Australian samples but could not be counted in the New Zealand fish (Ward *et. al.*1996). Gill raker counts on the right hand side showed no significant differences, but gill raker counts on the left hand side showed significant differentiation, possibly due to the small size of the New Zealand sample (Ward *et. al.*1996), and if nothing else indicate the limitations of meristic counts for stock discrimination. The number of pyloric caeca showed no significant differentiation among the Australian samples (Ward *et. al.*1996).

## Management units of black and smooth oreos

Three species of oreo, black oreo, smooth oreo and spiky oreo *Neocyttus rhomboidalis* are managed together under a combined quota. There are five management areas:

- the North Island and the west and south coasts of the South Island (OEO 1)
- east coast of the South Island and the west Chatham Rise (OEO 3A)
- east end of the Chatham Rise (OEO 4)
- sub-Antarctic including the Campbell and Bounty Plateaus (OEO 6)
- Kermadec area (OEO 10).

The Chatham Rise management areas were first used for oreos in 1982–83 and were based on the old EEZ management boundary areas. Areas C and D were equivalent to OEO 3A and OEO 4. Quotas were based on the approximate historical catch from the 1981–82 fishing year. When the ITQ system was introduced in 1986–87 quotas for the other areas were also based on historical catch.

The Chatham Rise has supported the main oreo fisheries in the New Zealand EEZ. The oreo fisheries started in the early 1970s with catches reported by Soviet vessels and rose to a peak of about 25 000 t in 1981 (Annala & Sullivan 1997). There are two fisheries on the Chatham Rise, separated by about 100 nautical miles (Doonan et al 1995). The western fishery is a target fishery for black and smooth oreo which are caught in approximate equal quantities, with a minor by catch of orange roughy. The eastern fishery is a target fishery for orange roughy with smooth oreo and black oreo, the latter in much smaller quantities, taken as by catch.

Fishing for orange roughy in OEO 4 was limited by the amount of oreo quota available as by catch prior to the 1994–95 orange roughy TACC reductions. The limitations of oreo quota in OEO 4 led to calls from fishing industry representatives to remove the management line between OEO3A and OEO 4 (176° E), to allow more oreo quota for orange roughy fishing on the eastern Chatham Rise, but this was not supported by the Deepwater Assessment Group. Other small oreo fisheries are carried out on the Puysegur/Macquarie Ridge (OEO 1) and on the Pukaki and Bounty slopes (OEO 6).

Black oreo and smooth oreo have different growth rates, natural mortalities (Table 9, Objective 1), depth and geographical distributions (Figs 2 & 3, Objective 1) and probably different population sizes and therefore could be managed as separate species. There is little information on spiky oreo but it appears to have low abundance around central and northern New Zealand and is not the target of commercial fishing. If black and smooth oreos were managed as separate species then the management areas could be reviewed.

## 11. CONCLUSIONS

1. Stock definitions. There is no universal definition of a stock but most definitions have the common elements of spatial and temporal isolation, and for many reproductive isolation.
2. Stock discrimination. No one method is ideal for discriminating stocks of marine fish. Stock discrimination depends upon an amalgam of techniques which include both ecological and genetic approaches. An understanding of the life history, in particular length of larval life and dispersive juvenile stages, can provide critical information for selecting the appropriate tools for identifying stocks. Ecological approaches, based on phenotypic and acquired characters, provide a measure of stock relationships, but because of sensitivity to environmental parameters, need to be assessed for temporal as well as spatial variation. Genetic methods, in particular micro- and mini-satellite DNA, provide a powerful tool for estimating reproductive isolation and a significant genetic difference is a sufficient but not necessary condition for separate stock management.
3. Orange roughy stock units. Orange roughy are subdivided into a number of discrete regional stocks around Australia and New Zealand based on evidence from a wide range of independent methods (allozymes, mtDNA, parasites, otolith microchemistry, morphometrics and biological data). Orange roughy have a continuous, low density distribution along much of the 1000m contour, with localised peaks of abundance. Some small isolated fisheries, such as Cook Canyon and Waitaki, are based on discrete stocks, but in other areas there is uncertainty about stock discreteness, for example along the Chatham Rise, where there are three spawning groups.
4. Temporal variation in orange roughy. Temporal variation within regions has been reported in morphometric, genetic and parasite studies, and must be considered in future stock discrimination studies. Finding temporal variation with independent markers and in different spawning regions suggest that orange roughy have a more complex population structure than simple spatially isolated stocks. Orange roughy are long lived and individuals probably do not spawn annually, which may be an adaptation for surviving in a low productivity environment. Spawning concentrations may consist of different groups of fish from year to year on the same grounds. Productivity on individual hills may vary due to local biological and physical conditions, with more productive hills producing a faster growth rate and hence more frequent spawning, than less productive hills.
5. Oreo stock units. Limited stock discrimination studies on black and smooth oreo in Australia indicate genetic differences between black oreos from Tasmania and the Chatham Rise, and meristic differences among smooth oreo around Australia. Black oreo and smooth oreo have different biological properties and geographical distributions and therefore could be managed as separate species in the New Zealand EEZ. If black and smooth oreos were managed as separate species then the management areas should be reviewed.

## 12. REFERENCES

References for this objective are listed at the end of objective 4.

## 13. PUBLICATIONS

Report to the Deepwater Fishery Assessment Working Group, February 1998.

## 13. DATA STORAGE

Electronic copy of report stored at Greta Point .