



**"Population structure of the jack mackerel, *Trachurus murphyi*, in the South Pacific Ocean: proposal for discussion for a regional joint research"**

**Draft proposal prepared by Chile for discussion in the Science Working Group (SWG) during the 3<sup>rd</sup> International Meeting of the SP-RFMO.**

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# **Population structure of the jack mackerel, *Trachurus murphyi*, in the South Pacific Ocean: proposal for discussion for a regional joint research.**

## **Background**

The Science Working Group (SWG) of the RFMO agreed during the meeting held in Hobart, Tasmania, in the 2<sup>nd</sup> International Meeting, that the uncertainty in the stock structure of this species is a key question to be addressed for future fisheries management. If yields are to be effectively estimated for this species for management purposes, an understanding of the biological stock structure of *T. murphyi* is required. In order to reduce the risk of localized depletion if, as assumed multiple stocks due exist, it is necessary to manage *T. murphyi* as a number of discrete stocks. This project will help determine the nature of those management areas and address a key information identified gap for this species.

Within this context, this research project was considered a **high** priority for the entire distribution for the species.

**The overall objective identified was to determine the stock structure of *Trachurus murphyi* to inform future fisheries management.**

The specific objectives are:

1. To determine methods for separating stocks of *Trachurus murphyi* for fisheries management purposes.
2. To define the stock structure *Trachurus murphyi*.

Following additional specific objective are recommended to the WG to be taken into account:

3. To define the discrete stock boundaries
4. To determine mixing rates among relevant stocks

The proposal should contain a comprehensive study with samples from throughout the range of the species (see Figure 1). It was indicated that a multiple methods approach is needed to resolve the stock structure of this species with acceptable degree of certainty, like genetic techniques, otolith microchemistry, gene tagging and a broad range of classical approaches.

The SWG also indicated that a detailed proposal including sampling design and budget elements should be presented to the 3<sup>rd</sup> International meeting for its consideration (SWG conveners to co-ordinate).

Therefore, in Chile was found important to call for a task force to develop a general discussion paper that could help the SWG in the 3<sup>rd</sup> International Meeting, to be held in Chile from 25 to 27 April.

The participants in the working group were:

- Cristian Canales - Stock assessment, IFOP
- Luis Cubillos - Fisheries Biologist, UDEC
- Ricardo Galleguillos - Population Genetist, UDEC
- Marcelo Oliva - Parasitologist, UANT
- Ciro Oyarzún - Biologist, UDEC
- Renato Quiñones - Fisheries Oceanographer, UDEC
- Aquiles Sepúlveda - Fisheries Biologist, INPESCA
- Rodolfo Serra\* - Fisheries Biologist, IFOP

IFOP: Instituto de Fomento Pesquero; UDEC: Universidad de Concepción; UANT: Universidad de Antofagasta; INPESCA: Instituto de Investigación Pesquera.

\* Group Coordinator.

## Methodological approach

The overall objective would be achieved integrating different methods in a multi-methodological approach. This approach was applied in the HOMSIR project (Horse Mackerel Stock Identification Research). Waldman (1999) and Begg and Waldman (1999) also discuss this approach, which represents “the state of the art” for stock identification. Due that different circumstances related to each stock identification technique and that the scale at which stocks can be identified vary depending on the situation, the application of multiple stock identification techniques may confirm a particular stock structure first detected by a single procedure used in isolation. Overlaying all available information from a range of techniques will enable a generalized and definitive pattern of stock structure to be developed in accordance with the needs of fishery management (Begg and Waldman, 1999).

To differentiate stocks following techniques have been utilized: genetic tags, microchemistry of otoliths, parasites, morphometry and life history patterns. In addition biochemical techniques were also suggested to be included.

For the integration of the results obtained by different researchers, that have employed a same technique and for samples taken from different locations, it is a necessary condition that careful standardization and intercalibration procedures and selection of statistical

analysis needs to be agreed upon. This requisite obligates to agree upon a protocol for sampling and analysis procedures.

## General sampling scheme

Samples for the different techniques necessarily need to be taken from throughout the range of the species (see Figure 1). To learn about the intra and inter stock variability periodic samples should be collected, for example one sample per quarter and sampling location. However the general recommendation that samples should be taken immediately before and during the spawning season to assure the discreteness of the stocks and to minimize the mixing, to learn about the seasonal variability of the markers the samples taken out of the spawning season necessarily will be taken on the feeding ground, because of trophic and spawning migrations to the feeding and spawning ground in the jack mackerel. This opens the probability for stock mixing, which is an aspect that asks for further analysis and final decision.

A two year sampling program is recommended to learn about the stability of the markers finally selected. In **figure 1** an example of sampling locations throughout the jack mackerel distribution is shown. The dark and empty circles indicate spawning and feeding grounds.

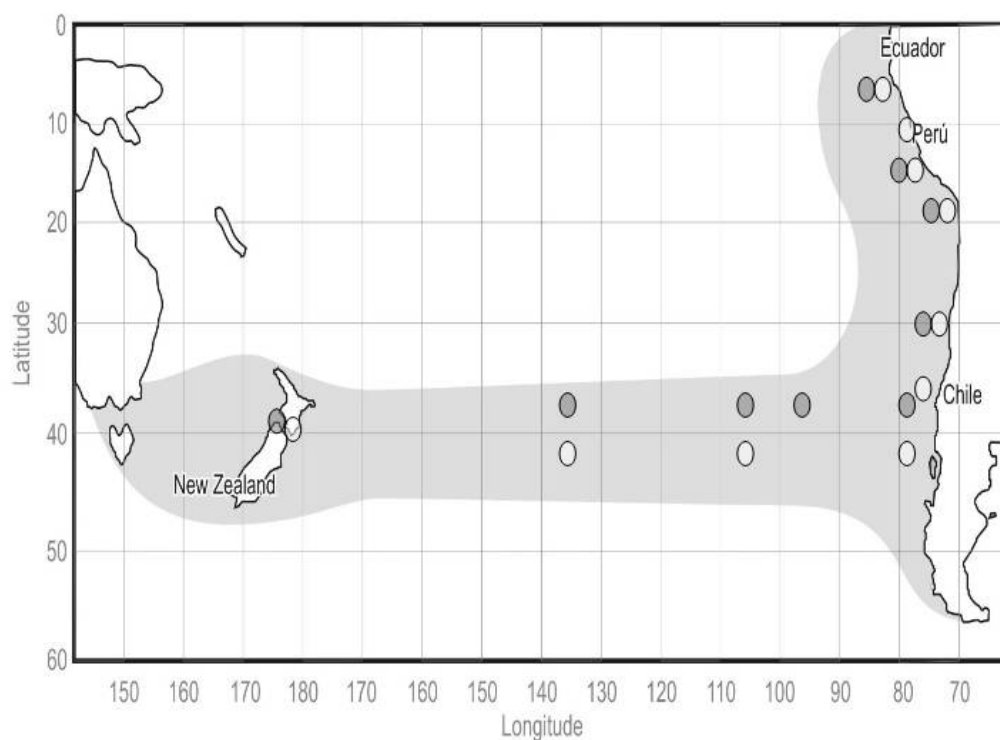


Figure 1. Distribution of the jack mackerel (*Trachurus murphyi*) and tentative sampling locations in the South Pacific Ocean. Dark circles: spawning ground; empty circles: feeding ground.

## **Discussion of potential techniques to be utilized**

Different techniques used to investigate the population structure of fish have been already applied in Chile to the jack mackerel and others have potential utility. The concept here is to briefly review these applications to identify improvements that could be introduced in a multidisciplinary regional project. Furthermore, some potential techniques to be applied on the jack mackerel will also be discussed like otolith chemistry and biochemical techniques. This information could also be useful for the discussion of an eventual prioritization process of the different techniques.

### **Genetic markers for *Trachurus murphyi* population structure studies.**

#### **Introduction**

Many different genetic markers are available for research of genetic differentiation in populations of living marine resources. Each method has some advantages and disadvantages in relation for any particular question. Protein electrophoresis was introduced in the mid-1960s and remains a simple and powerful molecular tool for generating Mendelian nuclear marker in many ecological and evolutionary applications. This represent the most extensive data bases for questions related to genetic identity, parentage and relationships among conspecific populations and application to the study of intermediate taxonomic levels (Avisé, 2004; Koljonen and Wilmot, 2005). Genetic markers isolated from the DNA molecule are available for studies in population genetics. The mitochondrial DNA (mtDNA) is a small, double stranded circular DNA molecule. The molecule is around 16.000 base pairs long, and in animals contains 13 genes coding for proteins, and one noncoding control region, called D-loop in vertebrates. Mitochondrial DNA is a effective molecules to use for analysis of intraspecific genetic variation an genealogy. The nuclear DNA markers as a single copy and repetitive Sequence Markers are very useful for population genetics studies. Nuclear DNA genes that encodes for protein products are single copy. The noncoding areas in the DNA molecule associated with genes are loci with highly repetitive DNA sequences termed microsatellites and minisatellites. Microsatellites loci are sequences between 1 and 6 base pairs in tandem repeat motif. The minisatellites locus typically shows 10 to 40 base pairs in length. This loci has high levels of genetic variability in terms of allelic diversity and heterozygosity. Microsatellite analysis is one of the favored genetics approach for the analysis of stock structure in fishes considering the amount of polymorphism in this loci, (Magoulas, 2005).

#### **Population genetic studies on the jack mackerel**

The first study done on jack mackerel (*Trachurus murphyi*) was in 1986 been also the first time that a study using this techniques was applied in Chile on an important fish stock. In this study 25 loci were analyzed, corresponding 19 to enzymes. The polymorphic systems analyzed to differentiate stocks correspond to four enzymes (L-glycerol pphosphate dehydrogenase, Phosfoglucomutase, Phosfoglucoisomerase and Esterase). Samples from different locations of the EEZ were analyzed like Chiloé Island (42°34'S; 73°43'W),

Iquique (20°12'S; 70°13'W) and Juan Fernandez Island (33°30'S; 79°39'W) (Galleguillos and Torres, 1988).

A second study was done in 1994 and its main goal was the jack mackerel stock structure between 20°12'S-70°13'W and 45°20'S-73°37'W. A total of 23 loci were analyzed consequently with the analysis of 15 enzymatic systems. Six polymorphic systems made the comparative study between stocks possible. No differences were found among the samples taken from northern to southern Chile (FIP-IT/ 94-19).

A third study was done in 1996 employing for the first time molecular techniques based on nuclear DNA. In this study with the PCR-RFLP (Restriction fragment length polymorphism) technique the ITS-2 zone of a ribosomal gene was analyzed with a total of 600 pb. 11 restriction enzymes were utilized of which 7 permitted to identify the cutting places in the ITS-2 zone. The samples taken in Mocha Island (38°21'S-73°52'W), Juan Fernandez Island (33°30'S-79°39'W) and Iquique (20°12'S-70°13'W) off Chile, and in New Zealand and Tasmania did not show differences in the genotypes that were obtained from the ITS-2 with the PCR-RFLP technique. In addition, the control region DLOOP and the ATCO gene from mtDNA was standardized for future investigations (FIP-IT/ 96-15).

The fourth and last study was done in 2002 in which samples taken in Talcahuano, San Antonio and Iquique in Chile. The molecular techniques employed correspond to mtDNA and msDNA. The results show a lack of genetic structuration in *T. murphyi* when different haplotypes found in the DLOOP region and 4 loci of the microsatellites are compared (Ojeda and Poulin, 2002). Similar results were obtained in 2004 which include samples taken also off New Zealand (Poulin et al., 2004)

### **Population genetic approach**

Different molecular approaches might be employed to study the population structure of *Trachurus murphyi* : allozyme electrophoresis, microsatellite DNA, and molecular markers sequencing from mitochondrial DNA.

#### Allozyme electrophoresis.

This technique provides a great amount of data from polymorphic loci, been possible to compare this results with the historical data base obtained from four polymorphic loci (Galleguillos y Torres 1988). This technique allows analyzing a great number of individuals, in a short time. The combination of alleles in a particular gene locus determines the genotype of an individual. We can establish for multilocus genetic mark the composition of gene frequencies in every sample and compared the grade of homogeneity of the genetic information. The application of statistical methods could explain if the amount of genetics differentiation is important to describe some genetics structure in the species.

The tissues to collect for proteins electrophoresis are white muscle and liver, kept under 20° C. The matrix to run the proteins is a starch solution at 12.5%. Each enzyme is stained by specific methods.

### MicrosatellitesDNA.

Microsatellites loci are found in all prokaryote and eukaryote genomes. Due to very high levels of genetics variation has become a very useful tool in stock identification studies. The functional significance of microsatellite is considered neutral markers. At least 4 to 10 loci microsatellite are screened in populations studies, analysis of a large number of loci provide more information about the evolutionary history of the populations.

One of the problems to apply microsatellites is to develop PCR primer in *Trachurus murphyi*. This is the first step for microsatellite analysis. It is possible to get PCR primer information from closely related species (heterologous primer). Other way to found the PCR primer is creating a genomic DNA library and then is necessary to follow a methodology that could take three or four months, although commercial vendors are available. Once the primer is created, a large number of individuals can be genotyped by sequencing the microsatellite. At least five microsatellite loci could be used and according with its variability not less than 100 individual needs to be screened.

### Mitochondrial DNA.

The Dloop region in the mtDNA has been selected in studies of population structure in several species. The amplified region can be studied by sequencing and is possible to screen an important number of individuals. In *Trachurus murphyi* the primers useful to amplified the Dloop region (TTCCACCTCTAACTCCCAAAGCTAGTAG) (CCTGAAGTAGGAACCAGATG) are described (Lee et al. 1995).

In accordance with the variability of the genetic marker the number of individual screened for every sample can be determined, in agreement with the statistical test.

### **Parasites technique**

Since the pioneer paper by (Herrington et al., 1939), parasites have been successfully used as biological tag in population studies not only in marine and freshwater fish, but also in mollusks, crustacean and mammals (see references in Oliva and Sanchez, 2005). Under a fishery perspective, parasites demonstrated their value as tag for understand migratory movements and population structure, but also to identified the habitat occupied by a fish (Oliva et al. 2004).

As pointed out by MacKenzie and Abaunza (2005) the basic principle underlying the use of parasites (both metazoan and protistan) as biological tags is that fish can become infected with a particular parasitic species only when they come within the endemic area of that parasite. The host parasite association implies two kinds of parasites: those specific to the host that in turn represent and evolutive system and generalist parasites, close associated with an ecological process. In the first case we have an association in an evolutive scenario that can give important clues about population structure; in the last case we can get

information on migratory movements. Other approach implies that if infected fishes are found outside the endemic area of the parasite, we can infer that these fish had been within that area at some time in their past history (MacKenzie and Abaunza, 2005). In this case is mandatory to know the exact extension of that parasite species, a problem not easy to solve. The use of parasites as biological tags require to meet some criteria well described and explained by Mckenzie and Abaunza (2005).

Parasites have been used successfully in the identification of population structure in some marine fishes from Chile like the hake *Merluccius gayi* (George -Nascimento 1996, Oliva and Ballon 2002), *Merluccius australis* (Gonzalez y Carvajal, 1994), the red rockfish *Sebastes capensis* (Oliva and Gonzalez, 2004), the anchoveta *Engraulis ringens* (Valdivia et al, 2007), the tail hake *Macruronus magellanicus* (Oliva, 2001) and the jack mackerel *Trachurus murphy* (George Nascimento 2000; Aldana et al 1995; Oliva 1999), among others. The problem of local variability in the parasite fauna of anchoveta was clarified by Chavez et al. (2007).

With regard to the jack mackerel, parasitological information is a little confused because the analytical procedures used by different authors, do not follow a common pattern. The most compressive studies are those by Oliva (1999) and George Nascimento (2000). Both studies analyzed metazoan parasites along the Chilean and Peruvian coast (Oliva, 7 localities: From Paita to Talcahuano) and from Iquique to Valdivia (6 localities, George Nascimento 2000). The data of Oliva (1999) strong suggest two stocks (Central - Northern Perú and a unique stock along the chilean coast) based on univariate analyses. George Nascimento suggests two ecological stocks in Chilean waters: a northern and a southern stocks, based in a multivariate analyses, unfortunately, George Nascimento (2000) pooled the northern and southern localities and do not analyzed a potential latitudinal gradient. A re-analyses of Oliva data, based on multivariate analyses using not only parasites as explanatory variable but also fish length, shows that discrimination is largely due to the fish length but not parasites.

This discrepancy strongly suggest the need of a re-evaluation of the parasitological information based in new information, in order to close the information gap in Oliva (1999). This new sampling protocol must includes samples from both the oceanic fraction associated to the Pacific jack mackerel belt and coastal Peruvian localities at southern Peru: Ilo-Mollendo (ca 16-17°S) and northward Callao : Chimbote (ca 9°S), Pimentel (ca. 7°) in addition to the localities sampled by Oliva (1999), Callao and Paita in Peru and Arica, Iquique, Antofagasta, Coquimbo, Valparaiso and Talcahuano in Chile. Ideally an additional sampling locality (Puerto Montt) will ensure to sample the coastal latitudinal range of distribution of the jack mackerel. The oceanic fraction (at least 1000 nm from the coastal line in Chile) will be sampled at least at the latitude of Talcahuano, Coquimbo and Antofagasta.

A sample size of ca 60 specimens needs to be taken from each locality. Under a statistical approach this sample size ensure the presence of metazoan parasites with a prevalence equal or larger than 5% (parasites present in no less than the 5% of the population) (McDaniel 1975). The sampling strategy should be focused in specimens larger than the size at the first maturity, i.e. fishes that have been in contact with the adult fraction of the

population. Samples are recommended to be taken on a seasonal basis. The sampling strategy must be replicate at least once, thus two samples should be taken from each locality and each season separated by one year

## **Life history patterns approach**

### **Introduction**

A fish stock may exhibit differences in one or more life history parameters compared to other stocks of the same species. According with Begg et al. (1999), vital population parameters, such as growth, survival, age-at-maturation, fecundity, distribution, and abundance, are the consequences of life history modes to which fish stocks have evolved. Differences in these parameters have long been used to identify separate management stocks assuming that phenotypic variation is due to genotypic and environmental controls. In addition, differences in life history parameters are considered as an evidence of discrete stock units for management purposes (Ihssen et al., 1981). Also other life history traits like size structure, maturity and fecundity express the interaction between the genetic background and environmental influences, and provide evidence for stock structure (Begg, 2005).

Serra (1991) based on the distribution of the abundance, seasonality (abundance, catch), spawning and size structure concluded on the existence of two self-sustaining sub-population of jack mackerel within the Southeast Pacific Ocean, one located off Peru and the second off Chile extending in the high sea (see also parasites section method).

With the goal of identifying the stock structure of jack mackerel, in this proposal a set of life history traits could be considered to study the spatial and temporal variability in the selected parameters and to discuss their contribution to the definition of the population structure. Biological information of jack mackerel related with the distribution of abundance and size-structure, otolith mass growth rate, fecundity and other reproductive indices could be analyzed along its entire area of distribution. The patterns in the distribution of the abundance and the size structure can provide further evidence in terms of recruitment and mortality processes; the otolith mass growth rate should provide further evidence of growth rate patterns variability, while batch fecundity, relative fecundity, and other indices such as gonadosomatic, hepatosomatic, and condition factor, can provide evidence for population discreteness within holistic approaches for identification of stock structure.

### **Material and methods**

#### **Patterns in the distribution of abundance and size structure**

The abundance and reproductive distributions could be considered. At present, a series of surveys and fishery-dependent information is available and it would be necessary to put this

information in the context of spatial patterns that can help to verify the results found by Serra (1991).

In order to identify spatial patterns of distribution in abundance, spawning ground, etc., a compilation of surveys in different part of the Southeast Pacific needs to be done. Abundant information is available in IFOP, INPESCA, IMARPE (Peru), and other institutions that have explored the distribution of the abundance of the jack mackerel, like Russia and New Zealand.

On the other hand, time series of length-frequency data or catch-at-length data are basic to compare size structure between areas and seasons. This kind of data allows estimating average length, size range, juvenile fraction, adult fraction in order to compare satisfactorily with modern techniques such as generalized additive models and generalized linear models. In addition, total mortality rate can be estimated and compared according with Begg et al. (1999) procedures.

#### Otolith mass growth rate

Araya et al. (2001) showed evidence for the relationship between otolith weight and age for jack mackerel off central southern Chile. A progression of modes was observed in otolith weight frequency distribution of individuals during 1996. The numbers of modes in the otolith weight frequency data were analyzed using MIX, founding an average otolith mass growth rate of  $8.3 \text{ mg yr}^{-1}$ , under the assumption that modes in the data represent year classes. A statistical comparison between geographic areas of the average otolith mass growth rate of jack mackerel is recommended to be carried out in looking for significant differences within the entire distribution of the species. The methodological aspects are found in Araya et al. (2001) and Pino et al. (2004).

#### Reproductive indices and others

A compilation of biological data, such as fork length, total weight, eviscerated weight, ovary weight, liver weight, parameters of the length-weight relationship, fat contents, macroscopic and histological maturity stages will allow estimation of organ ratios like gonadosomatic index, hepatosomatic index, condition factor, and muscle mass (e.g. Kjesbu et al. 1998, Ganas et al. 2007). Alternatively, oocyte size within ovary as well as egg size in the plankton could help in looking for significant differences within the entire distribution of the species.

### **Morphology of the jack mackerel body and otoliths**

#### **The Chilean experience using phenotypic and genotypic differentiation**

There are some studies done to attempt the stock differentiation in the Chilean jack mackerel based on genetic and phenotypic characteristics. George-Nascimento and Arancibia (1992) described differences in parasites and morphometric features from

individuals of three different fishing zones along the Chilean coast. Arancibia *et al.* (1995) conclude the existence of differences in the body morphometry of jack mackerel, comparing individuals collected from local fisheries from the north (Iquique) and the central zone of Chile.

Hernández *et al.* (1998), found significant differences in the body shape and meristic counts of jack mackerel sampled in 5 localities along the Chilean coast during 1995 and 1996. However, simultaneous studies based on genetic tracers did not show any evidences of different populations between localities along the Chilean coast (Alay *et al.*, 1996). In another study (Sepúlveda *et al.*, 1998) there no differences was found in the DNA of jack mackerel from different localities off the Chilean coast and individuals collected in other regions of the Pacific (New Zealand and Australia).

Differences in morphological characteristics between putative stocks indicate that the stocks have spent some periods of their lives in different environments (Begg *et al.*, 1999; Cadrin, 2000) and therefore have the potential to develop different life history characteristics. But there is also a possibility that a high grade of interchange or heterogeneity exist that could be affected by environmental changes or life history trends that can affect management strategies used over stock units that finally are not discrete and some interdependence exist with adjacent or even distant units. An example of this can be recognized observing interannual differences in morphological characteristics of Chilean jack mackerel along the Chilean coast, when individuals from one locality in central-south Chile show significant differences with a locality in the north of Chile during years close to the El Niño event 1997-98, but no significant differences were found between morphometric characteristics of individuals from the localities in the north of Chile sampled 3 to 4 years before the occurrence of this event (Hernández *et al.*, 2007).

### **Otoliths morphology**

Variations in morphological characteristics of otoliths have been defined as population-specific (Messieh 1972, Postuma 1974, McKern *et al.* 1974, Neilson *et al.* 1985, Smith 1992) and successful stock discriminations by means of otolith shape analyses were reported for a range of temperate marine fishes (e.g., Bird *et al.*, 1986; Castonguay *et al.*, 1991; Casselman *et al.*, 1981; Smith, 1992; Campana and Casselman, 1993; Friedland and Reddin, 1994; Rätz, 1994; Colura and King, 1995; Begg *et al.*, 2001; Smith *et al.*, 2002). In similar species like Chilean jack mackerel, a recent work considering morphological characteristics of otoliths of horse mackerel from the Mediterranean has been done by Turan (2006).

The use of otolith morphology as indicator in stock separation studies can be divided in three categories. According to recent studies (Bergenious *et al.* 2006) the first, includes the traditional one-dimensional linear measurements of size-related attributes, such as otolith length and width (e.g., Begg and Brown, 2000; Bolles and Begg, 2000) and distances between specific features on the otolith (e.g., Turan, 2000). Internal otolith measurements, such as nucleus length (e.g., Messeih, 1972; Neilson *et al.*, 1985) and width of hyaline bands or increments (e.g., Begg *et al.*, 2001) also fall within this category. The second category comprises two-dimensional size measurements, such as area, perimeter (e.g.,

Campana and Casselman, 1993; Begg and Brown, 2000; Bolles and Begg, 2000) and different shape indices, including circularity and rectangularity (e.g., Friedland and Reddin, 1994; Begg and Brown, 2000; Bolles and Begg 2000, Tuset et al., 2003). A third, more recent morphological technique examines the two-dimensional outline of otolith shape using Fourier analysis (e.g., Bird et al., 1986; Smith, 1992; Campana and Casselman, 1993; Begg and Brown, 2000; Smith et al., 2002).

## **Objectives**

- Evaluate the existence of geometrical morphometric differences in jack mackerel body and otoliths from different regions of the Pacific, inside the distribution range of this species.
- In case of differences, determine the stability and persistence of these differences in time and space.
- Determine levels of mixing of different stock units of this species.

## **Methods**

This methodology will focus on the application of the geometric morphometrics on the jack mackerel body and otoliths, as a tool for the identification of intraspecific variation from different areas of the South Pacific.

For testing body and otolith shape differences among areas, multivariate statistical techniques can be applied.

Morphometric and body shape procedures has been used in Chilean jack mackerel (George-Nascimento and Arancibia, 1992, Hernández et al. 1998 and Hernández et al. 2007).

## **Sampling**

Samples should be provided from commercial or research vessels with special reference of the spatial locations (latitude and longitude) where individuals are collected. The sampling protocol should be oriented to adult individuals (size range from 30 to 40 cm FL) and samples should be taken during the spawning season to assure stock discreteness.

To study effects of intra- and inter regions variability, periodic samples should be taken by quarters of the year during at least two years, to evaluate temporal stability of the morphological features of jack mackerel.

Samples of individuals in good conditions should be preserved frozen and at least body weight (BW) and fork length (FL) should be measured on the sampling sites. The potential use of digital photographs taked on individuals during the sampling should be studied and needs of inter-comparison experiments of measurements (direct versus digitalized) are required.

## Morphometric measurements

Morphometric measures are based in an interlaced net of 14 morphometric traces defined for 12 corporal marks (Figure 1), according to the methodology used by Winans (1987). There are also meristic counts that can be considered in the analysis.

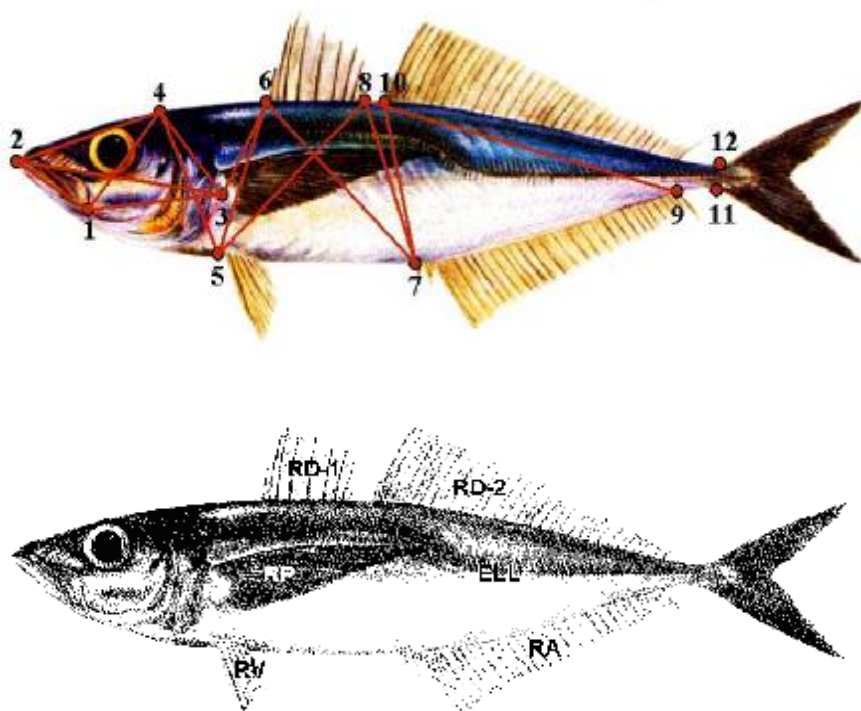


Figure 1. Marks of morphometric measurements and meristic counts in Chilean jack mackerel (after Hernández et al. 1998).

Hernández et al. (1998) determined that fish size can affect body shape in Chilean jack mackerel and they recommend the use of a function to remove the effect of fish size over the morphometric measurements. This transformation was applied by Schaefer (1991):

$$\hat{Y}_i = \log(Y_i) - b(\log(FL_i) - \log(FLa))$$

Where  $\hat{Y}_i$  represent the transformed measure for the  $i$ -th individual,  $Y_i$  is the original measure,  $FL_i$  represent the fork length of the  $i$ -th individual,  $FLa$  is the average fork length, and  $b$  represent the coefficient of regression between  $Y_i$  and  $FL_i$ .

Multivariate analysis techniques like Principal components analysis (PCA) should be used to reduce and describe body shape measurements of different regions based on the first three PCA functions. Analysis of Covariance (ANCOVA) can be utilized to evaluate sources of variation due to effects like locality, year and season of the year, using as

covariable the fork length (FL). Finally, discriminant analysis should be used to differentiate morphometric characters between regions.

### **Otoliths morphology to indicate the stock structure of jack mackerel**

Otoliths should be measured by one- and two-dimensional measures (otolith length, width, area, perimeter, circularity, and rectangularity), as well as by Fourier analysis to capture the finer details of otolith shape. Variables will be compared among different regions and the variability inside regions should be also studied. To compare the temporal stability in otolith structure in different fully recruited cohorts, samples from different years should be considered. It is recommended to follow otolith sampling during many years to determine specific features and variability between regions and to evaluate the possible effects of large oceanographic events (i.e. El Niño) in the distribution and structure of the population in the South Pacific.

It is also possible to explore the use of well preserved otoliths available in historical collections of otoliths from the South Pacific.

### **Morphological analysis**

A microscope image (10x magnification) will be projected onto a computer screen by using a video camera. From each otolith whole otolith area, length, perimeter, and width and two shape indices (circularity and rectangularity) should be registered by using an image analysis system (i.e. OPTIMAS, IMAGE PRO). Rectangularity is calculated as the area of the otolith divided by the area of its minimum enclosing rectangle, and circularity as the perimeter of the otolith squared divided by its area. The perimeter of the otolith can be traced in a counter clockwise direction and digitized into more than hundred x-y equidistant coordinates by using the distal edge of the otolith rostrum as a common starting point for the coordinates.

Fourier analysis produces a series of cosine and sine curves from the coordinates of a traced outline which, when added together, describe the outline of the traced form. The cosine and sine curves can be defined mathematically in a series of Fourier descriptors and used as variables to compare otolith shapes among individuals or potential stocks (Christopher and Waters, 1974; Younker and Ehrlich, 1977). The resultant set of complex numbers or descriptors should be subsequently normalized for differences in otolith position. The harmonics can be used in combination with the other morphological variables and shape indices to compare otoliths between cohorts and among regions and subareas within regions. The main features of the otolith shape, however, are generally captured by the first 10-20 harmonics (e.g., Campana and Casselman, 1993; Friedland and Reddin, 1994). The minimum number of Fourier descriptors required to explain at least 90% of the recorded shape of the otoliths should be calculated similarly to the range-finding procedure of Smith et al. (2002). More than hundred descriptors will be collected from two randomly selected otoliths from each subarea and cohort and then will be normalized for position, size and rotation as described above. The shape of each otolith should be reconstructed (by computing the inverse FFT). These descriptors, therefore, will be used in the statistical analyses to compare the spatial and temporal patterns in otolith shape of *T. murphyi*.

## Statistical methods

As a general procedure, the Fourier transformation can be performed using EFAwin. Differences in Fourier coefficients can be tested by covariance analysis and allocated to groups by discriminant analysis. The assumption of normality and homogeneity of variance for each morphological should be examined and some available transformations can be selected.

A relationship between otolith shape and otolith growth rate (assumed to be correlated to fish length) may confound spatial or temporal differences in otolith shape (Campana and Casselman, 1993). To minimize effects of this potential correlation there are two recommendations: 1) include only fish in a very restricted range of fork length (FL) and same age (e.g. 5 years old), and 2) standardizing morphological variables by fish FL where a significant relationship existed between the variable and FL before further analyses. The effect of FL on each morphological variable will be examined by analysis of covariance (ANCOVA; Winer et al., 1991). The primary interests in these analyses are 1) to test whether morphological variables differed with FL for any group of samples; and 2) if so, to test whether the slopes of regressions of morphological variable on FL are homogeneous among groups. If a significant regression is detected and homogeneous among groups, the effect of FL is removed from each measurement.

Multivariate analysis of variance (MANOVA; Tabachnick and Fidell, 1983) is used to investigate the effects of sex (females, males) on otolith shape and to test for spatial and temporal differences in otolith shape.

A principal component (PC; Tabachnick and Fidell, 1983) analysis is done first on the combined data set of both the shape variables and Fourier harmonics to reduce the number of variables to be incorporated in the MANOVA. Wilk's lambda criterion is used to test for group differences. A posteriori univariate analysis of variance (ANOVA) is used to explore patterns for each of the PCs separately when significant effects are indicated in the MANOVA. The communalities and variable loadings of the PCs are subsequently examined.

Two forward stepwise canonical discriminant analyses (CDA) are computed by using the shape variables and Fourier harmonics to examine the otolith shape of *T. murphyi* in multivariate space and to investigate whether otolith shape could be used to classify samples to spatial scale and cohort of origin. The factor used as a separating variable in the CDA depended on the significant effects determined in the MANOVA (i.e., cohort, region, or subarea, or any interactions between these factors). The CDA is used in this way as a confirmatory technique.

## **Otolith chemistry**

Otoliths appear to be ideal natural marker for fish populations. Unlike bones are metabolically inert; once deposited otolith material is unlikely to be reabsorbed or altered (Campana and Gagné, 1995). Otoliths are predominantly composed of calcium and trace elements that are derived from the waters inhabited by the fish. Because water bodies often differ in the concentrations of trace elements, stock may often be distinguished by the chemical signature retained in otoliths (Begg and Waldman, 1999). A further advantage of the use of otoliths is that by analyzing selected portion of it, the trace elements signals can be associated with particular growth stages that can potentially be used to reconstruct migration patterns (Elsdon and Gillanders, 2003). This last requires intensive experimental studies to learn how environmental and biological factors (temperature, salinity, exposure time, ontogeny) affect the otolith chemistry for a proper interpretation of the fish migratory history, influences that seems to be species specific. Campana and Gagné (1995) found that otoliths elemental fingerprinting has the potential to become an effective and accurate means of stock identification for cod; Ashford, Arkhipkin and Jones (2006) validated this technique for examining population structure in Patagonian toothfish, demonstrating that otoliths nucleus chemistry can discriminate between stocks in fully marine environment.

Stock mixing can also been investigated with this technique but first it must be shown that elemental fingerprints differ among stocks. Campana et al. (2000) demonstrated its effectiveness on cod.

Otoliths chemistry has been identified as a useful technique specially in cases where the genetics techniques has shown homogeneity while other techniques like life history patterns and parasites have suggested the existence of different stocks, for example in the Patagonian toothfish (Ashford, Arkhipkin and Jones, 2006).

The use of trace elements signature as natural tags makes three central assumptions (Campana et al. (2000):

- There are characteristic and reproducible markers for each group
- All possible groups contributing to the group mixture have been characterized
- Markers remain stable over the interval between characterization and mixing

## **Sampling**

The sample program should be designed for adults (size range from 30 to 40 cm FL) and taken during the spawning or near spawning season to assure stock discreteness. This implies some hypothesis on the stock structure and assumes that stock mixing is minimal at the time of spawning. Present knowledge on spawning areas should be used for this purpose.

To learn about the intra and inter stock variability periodic samples should be taken, for example one sample per quarter. In the case of the jack mackerel because of trophic and spawning migrations to the feeding and spawning ground the samples taken out of the

spawning season necessarily will be taken on the feeding ground. This opens the chance for stock mixing, which is an aspect that asks for further analysis and final decision.

A two year sampling program is recommended to learn about the stability of the trace elements and therefore on the certainty of the results obtained.

To avoid contaminations the sagittal otolith pair has to be removed from the fish using plastic forceps and stored in vials. All otoliths need to be carefully treated before elemental analysis, to minimize the possibility of contamination. Detailed description of treatment procedures can be found in Campana and Gagné (1995), Campana et al. (2000) and Ashford et al. (2006).

### **Analysis**

Two approaches exist for otolith analysis: use of whole otoliths dissolved in acid and diluted; and the use of laser ablation on the otolith nucleus. The sample obtained is analyzed with a mass spectrometer.

### **Fatty Acid as Natural Marks for Stock Identification**

There is information about the differences in fatty acid profile on the species level that can be detected easily. However, fish and many other marine animals are present in distinct population, and it is a challenge for the chemometric approach to detect systematic differences in fatty acid profiles on this level (see review by Grahl-Nielsen, 2005). Many fish species has been studied with this approach looking for possible stock differences (striped bass, herring, mackerel, cod, redfish, salmon).

According with Grahl-Nielsen (2005), we should keep the workup procedure as simple and quick as possible, avoiding extraction and fractionation of the lipids prior to analysis. It means subject the tissue samples to direct methanolysis, wich extracts and converts all fatty acid in a sample to methyl esters. In order to reduce the influence of diet triacylglycerids, it is recommended to use heart tissue.

### **Biochemical profiles: Blood chemistry of fishes.**

Basically, blood consists in a plasmatic fluid containing formed elements (cells). In fishes, plasma correspond to a clear liquid that dissolve minerals, substances uptake by the gut, waste products, proteins, hormones, enzymes, antibodies, dissolved gases, xenobiotics, etc. All this substances or its monomers are in a dynamical (active) balance (steady state) between environment and the body compartment which comprising the use, metabolism and waste of the substances (materials). Depending of the kinds of substances that can be measured in plasma, it is possible to characterize the environment and the resources used by a population through the time and it could be a biochemical profile of that population. Lipids profiles in fish (triacylglycerids, cholesterol, poly unsaturated fatty acids and so on), seems to be effected by water temperature, salinity, pressured and the diet of the fish. More

complete biochemical profiles as calcium, phosphorus, proteins, glucose, urea, heavy metals, xenobiotics and so on could reflect the environment composition (biological and chemistry composition) of a stock. The sample collection, transport and analysis it is very simple and reliable for a biochemical profile of a population.

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