Population structure of the Chilean jack mackerel, *Trachurus murphyi*, in the South Pacific Ocean: full proposal for discussion for an international joint research.

Draft proposal for the Jack Mackerel Sub-group of the Science Working Group (SWG), South Pacific Regional Fisheries Management Organization.

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1. Background

The Science Working Group (SWG) of the RFMO agreed during the meeting held in Hobart, Tasmania, in the 2nd International Meeting, that the uncertainty in the stock structure of *Trachurus murphyi* was a key question to be addressed for future fisheries management.

An understanding of the number and distribution of any discrete stocks within the jack mackerel population is needed if yields are to be effectively estimated and the risk of localized depletion is to be minimised. This project aims to address this key information gap and determine the nature of any discrete stocks and appropriate management areas.

It was also indicated that the proposal should contain a wide-ranging study with samples from throughout the range of the species and that a multiple technique approach was needed to resolve the stock structure question with an acceptable degree of certainty.

The Science Working Group decided during the 3rd International Meeting held in Reñaca (Chile) to develop a proposal to investigate the stock structure of the jack mackerel and Rodolfo Serra from Chile and Alexander Glubokov from Russia were nominated as the Task Team for this purpose. In Chile the following scientists collaborated in developing the proposal: Ricardo Galleguillos, Marcelo Oliva, Aquiles Sepulveda, Fransisco Cerna, Ciro Oyarzun, Juan Carlos Quiroz; Juan Carlos Saavedra, Jaime Letelier, Samuel Hormazabal and Sergio Nuñez.

2. Distribution and present understanding of its stock structure

The Chilean jack mackerel is distributed throughout the southeastern Pacific, ranging from the Galapagos Islands and south of Ecuador to southern Chile. Its current distribution also extends from south-central Chile across the Pacific Ocean, to New Zealand and Tasmanian waters (Evseenko, 1987; Serra, 1991; Elizarov et al., 1993; Taylor, 2002).

A large increase in abundance over the 20 years to 1991 has been reported (Serra, 1991; Elizarov et al., 1993) which is believed to explain its wide present distribution. Serra (op. cit.) also described a seasonal migration between coastal and oceanic waters for the Chilean subpopulation, and related this to "reproductive and trophic processes". In Chilean fisheries, large jack mackerel tend to be more common toward the south. A similar tendency for there to be larger fish in southern waters is also seen in the New Zealand fishery (Taylor, in prep.).

The existence of up to three separate stocks of Chilean jack mackerel are suggested by the existing data collected throughout the range of this species. In South America there is evidence for at least separate Peruvian and Chilean stocks based on the results of genetic studies (Koval 1996), of generalized studies using distribution, abundance, size composition, and reproductive distributions (Evseenko 1987, Serra 1991, Storozhuk et al. 1987), and of studies of parasites (Oliva, 1999). There is also evidence for at least one stock in the high seas of South West Pacific Ocean based on reproductive distributions (Evseenko 1987), and morphological and parasite information (Duran & Oliva 1983, Romero & Kuroki 1985, Kalchugin 1992, Avdeyev 1992; all in: Taylor, 2002; Kotenev et al., 2006). The independence of this stock from the South East Pacific Ocean stocks is an open question.

Evidence shows that following a strong increase in its abundance from the early 1970s, *T. murphyi* expanded its distribution along the southwestern coast of South America (Ecuador, Peru and Chile) and toward the west and crossed the Pacific Ocean along the West Wind Drift, reaching New Zealand waters in the early to mid 1980s (Bailey, 1989; Serra 1991; Elizarov et al. 1993; Taylor 2002).

Although a large population of *T. murphyi* has existed in New Zealand waters following its initial invasion sometime during the early to mid 1980s, there is little evidence to support the stock being self-sustaining. According to Taylor (2002), New Zealand waters appear to be conducive to the establishment of a self-sustaining stock, although analyses are compromised by inclusion of data for *T. declivis and T. Novaezelandiae*. The widespread distribution of prey species in New Zealand waters and the highly adaptable feeding strategy of *T. murphyi* largely preclude the possibility of food being limiting and the reproductive condition of specimens sampled in New Zealand indicates a wide geographic range of fish in maturing and spawning condition. However, few juvenile specimens have been taken during the approximately 20 years that *T. murphyi* has been present in New Zealand waters and recent monitoring shows that this species is now less abundant at the surface than during the mid 1990s (Taylor in prep).

The diverse work done does not show a clear picture in regard with the stock structure and the results from different authors are not entirely consistent. However an important conclusion is that although the results show that the population of Chilean jack mackerel is not homogeneous, the boundaries of some identified stocks units are not clear. In conclusion present knowledge of the jack mackerel stock structure deserves further investigation to fully identify the number and extent of discrete stocks of jack mackerel over its entire range.

3. Objectives of the study

The overall objective is:

To determine the stock structure of *Trachurus murphyi* to inform future fisheries management.

The specific objectives are:

1. To describe the stock structure of Trachurus murphyi.
2. To define the discrete stock boundaries
3. To determine mixing rates among relevant stocks

4. Method

The overall objective would be achieved by integrating the results of different techniques in a multi-methodological approach. This approach was applied in the HOMSIR project (Horse Mackerel Stock Identification Research; www-homsir.com). Waldman (1999) and Begg and Waldman (1999) also discuss this approach, which represents "the state of the art" for stock identification. Due to the different requirements of each technique and variability in the scale at which stocks can be identified; the application of multiple techniques for stock identification 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).

It is proposed to use the following techniques to differentiate the stocks: genetic tags, morphometry (body and otolith), parasites, life history patterns including physiological adaptations, and microchemistry of otoliths.

There is a need to develop an agreed protocol for sampling and analytical procedures. To integrate the results obtained by different techniques it is preferable that the same specimens are used whenever possible. This will reduce the influence of individual differences and facilitate the identification of immigrants from other stocks. Also, if fish samples taken from different locations and are to be processed in different laboratories then standardization and intercalibration procedures will need to be undertaken. Finally, the statistical analyses to be applied need to be agreed.

5. General sampling scheme (spatial and temporal scales)

Two scales of variability exist: geographic and temporal. Therefore the samples have to be collected from throughout the range of the species (see the approximate sampling sites

Figure 1) and on more than one occasion over at least one year. To learn about the intra and inter stock variability of the marker associated with each technique, periodic samples need to be collected, for example one sample per quarter and sampling location. However a general recommendation is that at least one set of samples should be taken during the spawning season to increase the chance of detecting discrete stocks and to minimize the influence any mixing at other times of year.

To learn about the seasonal variability of the markers and the influence of seasonal migrations, samples should also be taken out of the spawning season on the feeding grounds. Interanual variation is important and therefore a two year sampling program is recommended to learn about the stability of the markers derived from the different techniques. Chile is developing an investigation with a similar approach and these results could be used as a guide to allow refinements to the sampling strategy. Finally, the number of samples taken during the year will be function of the available funds.

For the design of potential sampling locations present knowledge of the jack mackerel distribution and stock structure was considered. In **figure 1** an example of potential sampling locations throughout the distribution of jack mackerel are shown. There are 19 sites identified which includes five sampling locations between 110 and 170º W. Sampling from all sites in all seasons would be difficult due to the large distances and the high cost involved particularly in sampling between 110 and 170º W. But it would be important to get at least one sample from these locations during the spawning period. The relative larger number of sampling locations in southern Peru and northern Chile and between 90º and 130º W is explained by presumed stock boundaries.

The sampling program should be designed to collect adults, giving preference to a size range from 30 to 45 cm FL, to minimize the potential effects of fish size on the measured parameters. However, some samples of juveniles are also needed, particularly to identify any age-related migration patterns studies with otolith microchemistry, and some sampling locations should be selected for this purpose.

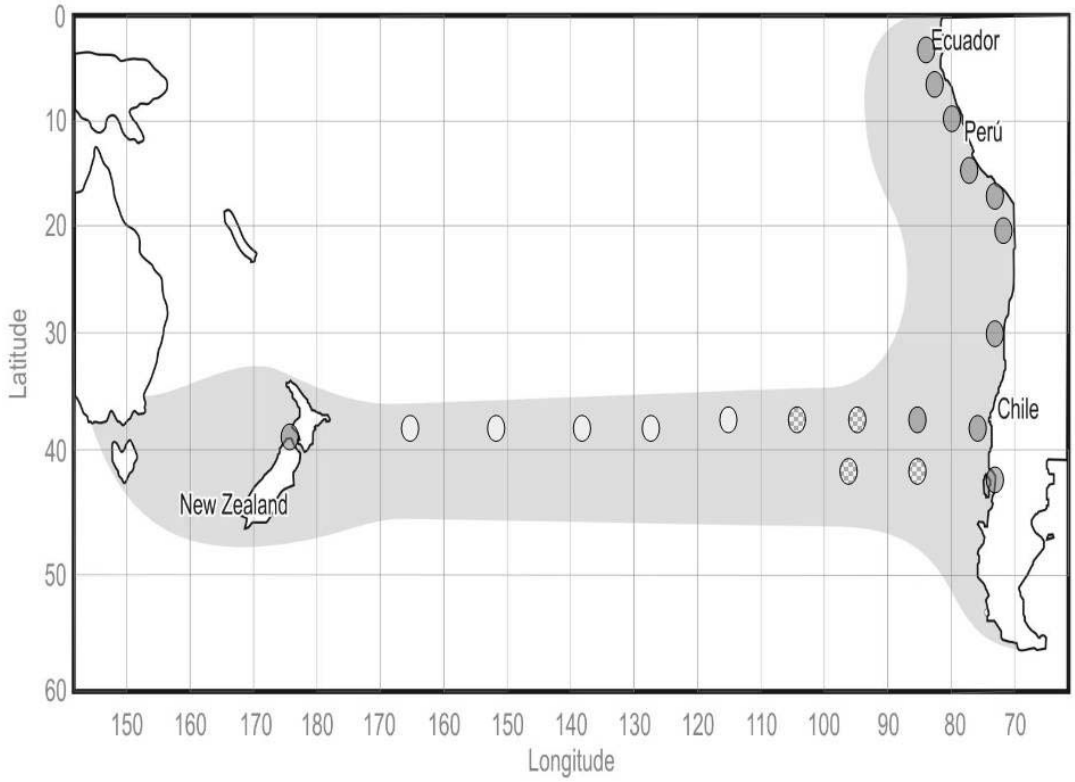

Figure 1. Distribution of jack mackerel (*Trachurus murphyi*) in the South Pacific Ocean and potential sampling locations. Darker circles indicate samples to be taken from coastal fisheries; circles with dots indicate samples from the international fleet; and empty circles indicate samples to be collected with a research vessel.

5.1 Sample size

The minimum size of each sample is 100 fish. This sample size is required for genetic analyses. For this technique and to support the results with α of 0.05 and β of 0.1 a sample size between 50 and 132 specimens per locality is needed (Richardson *et al.* 1986).

To assure 100 fish in good condition a sample of 120 adult fish of size between 30 and 45 cm FL is recommended. Jack mackerel of larger sizes exist but to minimize the size effect in the results this range is proposed.

Another complementary sample of 60 juveniles (equal and less than 20 cm FL) for microchemical analysis of otoliths is required from Central Peru, Northern Chile, South-Central Chile, mid South Pacific and New Zealand. The purpose of these sample is to allow reconstruction of life history migratory patterns.

The information for each fish sampled that should be recorded is:

- Geographical reference (name, Latitude and Longitude)
- Number of fish (sequential)

- Date
- Fork length of fish (cm)
- Total weight (gr)
- Sex
- Maturity

5.2 Samples processing

To reduce the influence of individual variability on results the different pieces of information should come from the same fish, i.e. the body morphometry, otolith morphometry, tissue for genetics, parasites, and trace elements from otoliths should be obtained from the same fish.

Otoliths are fragile and need to be stored in suitable containers to avoid breakage.

6. Techniques

The techniques to be used are: body and otolith morphometry, genetics tags (mtDNA, msDNA), parasites, microchemistry of otoliths, and life history patterns which are discussed further down. Most of these techniques have been applied already to the jack mackerel to investigate the population structure but independently from each other. Exceptions are otolith morphometry, microchemistry and organ indices (hepatic, gonadosomático).

6.1 Morphometry

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

6.1.1 Body morphometry

A few studies have been done to investigate the stock structure of the 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 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 evidences about the existence of different subpopulations between localities along the Chilean coast (Alay *et al.*, 1996).

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 exists that could be affected by environmental changes or life history trends. This could affect management strategies if assumed stock units prove not to be discrete. An example of this is the interannual differences in morphological characteristics of jack mackerel that are observed along the Chilean coast. 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).

6.1.1.1 Data

Morphometric measurements will be based on the methods proposed by Winans (1987) (**Figure 2).** But, it is suggested that the reduced set of measurements be used as selected by Arancibia *et al*. (1996) and Hernández *et al*. (1998), based on the work by George – Nascimento and Arancibia, (1992). These measurements correspond with the lines between following points: (1-2), (1-3), (1-4), (2-3), (2-4), (3-4), (3-6), (4-5), (5-6), (5-8), (6-7), (7-8), (7-10), (8-9). This lines needs to be measured with the precision of 1 mm.

This data should be obtained from a sample of 100 fish from each location.

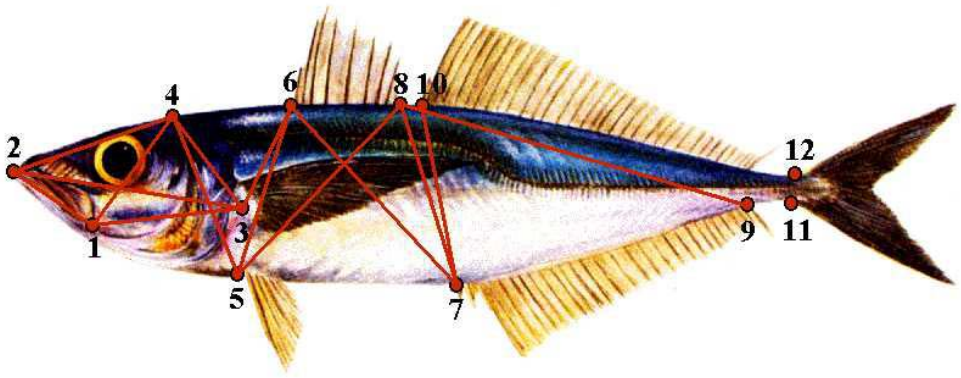

Figure 2. Morphometric measurements for *T. murphyi*.

6.1.1.2 Statistical analysis

In order to get morphometric measurements that are independent from the size of the fish transformation of the data is necessary. Both logarithmic transformation (Schaefer, 1991)

and multivariate types (Winans, 1984; Cortes et al., 1996) have been suggested but Hernandez *et al* (MS) indicates that the logarithmic transformation give better results.

Exploratory multivariate analysis will be used to find the measurements that explain the largest differences between locations, like multiple discriminatory analyses (Winans, 1987). Analysis of covariance will also be used to assess location and fish size effects

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.

6.1.2 Otolith morphology

Variations in otolith morphology 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 have been 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). An application for the horse mackerel from the Northeast Atlantic and Mediterranean also exists (Turan, 2006; Stransky, in press).

The left otoliths should be used for morphological analysis and for age determination and the right otolith saved for microchemical analysis. To standardize morphological measurements the left otolith should be examined with the external face upwards and the rostrum to the right.

The techniques applied for stock differentiation can be divided in three categories. The first is the traditional one-dimensional linear measurements of size-related attributes, such as otolith length and width (e.g., Begg and Brown, 2000; Bergenious et al., 2006; 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 include 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; Stransky et al., in press).

6.1.2.1 Morphological analysis

A microscope image (10x magnification) should be used to project onto a computer screen by using a video camera. From each otolith the area, length, perimeter, width and two shape indices (circularity and rectangularity) should be recorded by using an image analysis system (i.e. OPTIMAS, IMAGE PRO) (Bolles and Begg, 2000; Pothin et al., 2006). Circularity should be measured as the squared of the otolith perimeter divided by its area. The perimeter of the otolith can be traced against the counter clockwise direction and digitized into more than one hundred x-y equidistant coordinates by using the distal edge of the otolith rostrum as a common starting point for the coordinates. Rectangularity should be calculated as the area of the otolith divided by the area of its minimum enclosing rectangle.

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, called Fourier descriptors, 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 one 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. These descriptors, therefore, will be used in the statistical analyses to compare the spatial and temporal patterns in otolith shape of *T. murphyi*.

6.1.2.2 Statistical analysis

i) one-dimensional linear measurements

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.

ii) multi-dimensional size measurements

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.

iii) two-dimensional Fourier analysis

The Fourier transformation can be performed using Elliptical Fournier Analysis (EFA, Rohlf and Archie, 1984). Differences in Fourier descriptors can be tested by covariance analysis and allocated cross-validation into groups by discriminant analysis, specifically linear discriminant function analysis (Klecka, 1980). Otherwise, the average residuals from linear regressions of each Fourier descriptor on fish fork length can be compared in a set of multivariate technique (hierarchical cluster, detrended correspondence analysis and multidimensional scaling) to derive a detailed stock-specific comparison.

In this way, from reproduced outlines of the averages Fourier descriptors average otolith shapes stocks-specific are obtained. For all tests, the assumption of normality and homogeneity of variance for each morphological should be examined and some available transformations can be selected.

6.2 Genetic markers

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 in many ecological and evolutionary applications. These protein markers are particularly effective at addressing questions related to genetic identity, parentage and relationships among conspecific populations and application to the study of intermediate taxonomic levels (Avise, 2004; Koljonen and Wilmot, 2005). Genetic markers isolated from the DNA molecule are available for studies in population genetics. The mitocondrial 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. Mitocondrial DNA is an effective molecule to use for analysis of intraspecific genetic variation and genealogy. 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. A minisatellite locus typically shows 10 to 40 base pairs in length. This locus 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 on jack mackerel (*Trachurus murphyi*) was done in 1986. In this study 25 loci were analyzed, corresponding to 19 enzymes. The polymorphic systems analyzed to differentiate stocks correspond to four polymorphic enzymes. Samples from different locations inside the EEZ were analyzed,(Galleguillos and Torres, 1988).

Another study was done in 1994 and its main goal was the jack mackerel stock structure between 20º12'S-70º13'W and 45º20'S-73º37'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).

Nuclear DNA techniques were employed for the first time in 1996. In this study with the Restriction Fragment Length Polymorphism (RFLP) technique, the ITS-2 zone of a ribosomal gene was analyzed with a total of 600 pb. 11 restriction enzymes were trialed, of which 7 were used. The samples taken from Mocha Island (38º21'S-73º52'W), Juan Fernandez Island (33º30'S-79º39'W) and Iquique (20º12'S-70º13'W) off Chile, and in New Zealand and Tasmania did not show genetic differences using ITS-2. In addition, the control region and the ATCO gene from mtDNA were standardized for future investigations (Sepúlveda et al., 1996).

In another study no differences was found in the DNA of jack mackerel from different localities off the Chilean coast and individuals collected in New Zealand and Australia (Sepúlveda *et al*., 1998).

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 microsatellites DNA (msDNA). The results show a lack of genetic structure in *T. murphyi,* when different haplotypes found in the control 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)

6.2.1 Population genetic methods

Different molecular approaches can be applied to study the population structure of *Trachurus murphyi*: microsatellite DNA, and molecular markers sequencing from mitocondrial DNA.

MicrosatellitesDNA.

Microsatellites loci are found in all prokaryote and eukaryote genomes. Due to their inherent very high levels of genetic variation they have become a very useful tool in stock identification studies. Microsatellites are considered to be neutral markers with no functional significance. Typically at least 4 to 10 microsatellite loci are screened in populations studies, however analysis of a large number of loci provide more information about the evolutionary history of populations.

One of the problems in applying microsatellites is the need to develop PCR primers for *Trachurus murphyi*. This is the first step for microsatellite analysis. It is possible to get PCR primers information from closely related species (heterologus primer) like *T. trachurus*. Four markers are available for *T. trachurus* (Kasapidis and Magoula, 2008). But more importantly, present research done in Chile has permitted the identification of specific markers for *T. murphyi*. At present 17 polymorphic microsatellites loci specific for *T. murpyhi* are available. This was obtained from a genomic library of specimens sampled off central Chile to investigate its stock structure. The repeat motifs isolated microsatellites from *T. murphyi* are: (CA)3, (ATG)4, (CATC)6 and (TAGA)4. It has been described that tetranucleotides could be more useful to differentiate the stock structure of the jack mackerel and which are now available. At present these markers have been standardized and are ready to be used over the entire jack mackerel distribution.

Mitocondrial DNA.

The D-loop 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 a large number of individuals. In *Trachurus murphyi* the primers useful for the amplified D-loop region (TTCCACCTCTAACTCCCAAAGCTAGTAG) (CCTGAAGTAGGAACCAGATG) have been described (Lee et al. 1995).

6.2.2 Statistical analysis

From the corrected microsatellite data for null alleles, the genotypic and allelic frequencies will be estimated for each loci. The observed heterozygosity can be directly compared to expected as a measure of deviation from Hardy-Weinberg equilibrium.

To establish the differentiation level between the different localities in the study, a molecular ANOVA (analysis of molecular variation AMOVA) of the allelic frequencies within and between the populations will be done based on Excoffier et al. (1982).

To differentiate the populations the "Fixation index" from Wright (1978) (FIT, FST, FIS, Hartl, 1980) will be calculated, which are based in a model of infinite alleles based on the variance (=θ) (Weir & Cockerham, 1984); the levels of significance will be tested according to Workman & Niswander, (1970), using FSTAT (Goudet, 1995).

The genetic distance (Nei, 1978) will be estimated with a matrix of genetic distance versus geographic distance to assess the isolation by distance of the populations through the Mantel test (Mantel, 1967) using GENETIX v4.05 (Belkhir et al., 2004).

The genotypic and allelic frequencies will be compared with a test for homogeneity between the sampled locations through a contingency table. The homogeneity will be verified with G-test (Whitmore, 2000). The significance level will be calculated for contingency table with a Monte Carlo method (1000 iteration) as was proposed by Roff & Bentzen (1989).

The distribution of the genotypic frequencies in the sampled locations will be analyzed with an exact test of population differentiation, under the null hypothesis "that the genotypic frequencies are identical between the populations". For this test a contingency table is considered with a Markov Chain Monte Carlo (MCMC) procedure. The value for P is estimated to determine the significance of each comparison for all the loci, with the Fisher exact test (Raymond & Rousset, 1995).

A demographic analysis will be done using the program BOTTLENECK (Piry et al., 1999). It will be used to test the recent bottle neck events or effective decline of the population size by historical changes in the distribution of the allelic frequencies. For each loci 1000 permutations will be done to assess the two mutations models for microsatellites: IAM, infinite alleles model (Kimura & Crow, 1964); and SMM, scaled mutations model (Kimura & Ohta, 1978).

6.3 Parasites technique

Since the pioneer paper by Herrington et al. (1939), parasites have been successfully used as biological tags in population studies not only in marine and freshwater fish in order to evaluate stock discreteness, migratory movement and habitat (Moser, 1991; MacKenzie and Abaunza, 1998; Oliva 2001; Oliva and Ballon 2002; Oliva et al 2004), but also in molluscs, crustaceans and mammals (Balbuena et al., 1995; González and Kroeck, 2000; Pascual and Hochberg 1996; Thompson and Margolis 1987, Oliva and Sanchez 2005).

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 an evolutionary system; and generalist parasites, close associated with an ecological process. In the first case we have an association in an evolutionary scale that can give important clues about population structure; in the second case we can get information on migratory movements. Other approaches imply 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 it is mandatory to know the exact distribution of that parasite species, a problem not easy to solve. The use of parasites as biological tags require meeting criteria that are well described and explained by Mckenzie and Abaunza (2005).

Parasites have been used successfully to identify the population structure in some marine fishes from Chile like hake *Merluccius gayi* (George -Nascimento 1996, Oliva and Ballon 2002), *Merluccius australis* (Gonzalez and Carvajal, 1994), red rockfish *Sebastes capensis* (Oliva and Gonzalez, 2004), anchovy *Engraulis ringens* (Valdivia et al, 2007), hoki *Macruronus magellanicus* (Oliva, 2001) and jack mackerel *Trachurus murphyi* (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 confusing because the analytical procedures used by different authors do not follow a common pattern. The most comprehensive studies are those by Oliva (1999) and George-Nascimento (2000). Both studies analyzed metazoan parasites in the jack mackerel along the Chilean and Peruvian coast (Oliva, 1999) and from Iquique to Valdivia (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 on a multivariate analysis, unfortunately, George-Nascimento (2000) pooled the northern and southern localities and did not analyze a potential latitudinal gradient. Unpublished re-analyses of Oliva's data (1999), based on multivariate analyses using not only parasites as explanatory variable but also fish length, shows that the difference is a function of fish length but not parasites.

6.3.1 Statistical analysis

A sample size of about 60 specimens needs to be taken from each locality. This sample size should ensure the presence in the sample of any metazoan parasites that are present in at least 5% of the population (McDaniel 1975). The sampling strategy should be focused on adults fish. Samples are recommended to be taken on a seasonal basis. The sampling strategy must be replicated at least once, thus two samples should be taken from each locality and each season separated by one year.

The percentage of infected fishes with a particular parasite species in the sample (the prevalence of infection) and the mean number of parasite individuals of a given species in each infected host (the intensity of infection) will be estimated according to Bush et al. (1997). Differences in these measures between sexes will not be considered.

Univariate techniques

Significance in potential differences in parasite population descriptor between localities should be explored, with a significance of $\alpha = 0.05$ for all statistics as follows:

- Prevalence of infection: should be assessed using a 2 * n contingency table with the G likelihood test or chi square test. The size of the table (n) is function of the number of sampling points.

- Mean intensity of infection: should be assessed with one-way ANOVA; the number of categories is function of the number of sampling points. Because parasites are typically over dispersed, the data need be transformed to log (n+1) in order to reduce variance by re-scaling the data.

Multivariate techniques
The univariate techniques permit the assessment of the differences for each parasite species but not for the parasite community structure. To evaluate if the community structure is a good predictor of the site of capture, two multivariate techniques can be used:

- Exploratory analysis. A principal component analysis will allow us to explore potential association between different sampling points and their parasites. (Oliva and Ballon 2002).
- Multivariate discriminant analysis will used to evaluate the discriminant (locality) capacity of the parasite community. (Oliva et al. 2005)

6.4 Otolith microchemistry

Otoliths appear to be a good natural marker for fish populations. Unlike bones they 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 method requires some 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 a 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 especially 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

To avoid contamination the sagittal otolith pair has to be removed from the fish using plastic forceps, rinsed with distilled water to remove the tissue and blood, and stored in vials or paper bags. All otoliths need to be carefully treated in the laboratory before the trace element concentration analysis, to eliminate the risk of contamination. Detailed description of treatment procedures can be found in Campana and Gagné (1995), Campana et al. (2000) and Ashford et al. (2006).

A sample of 60 fish from each of 15 sampling areas and 60 juvenile fish from each of three sampling areas will be taken. Because fish from different spawning grounds may subsequently mix, large sample sizes enhance the ability to detect the underlying groups from their nucleus chemistry, and estimate the proportion occurring in each sampling area that derive from each spawning ground. This is especially important where proportions are small: because of spatial effects, fish in some areas - even though occurring in low numbers - can make disproportionately large contributions to subsequent generations.

6.4.1 Laboratory Procedures

We will use a Thermo Finnegan Element 2 double-focusing sector-field ICP-MS to examine otoliths for minor and trace element chemistry. Samples will be introduced in automated sequence (Chen et al. 2000) using a New Wave Merchantek UP-213 laser ablation system and a PFA microflow nebulizer. Ablated otolith material from the sample cell will be mixed in the spray chamber with HNO_3 aerosol introduced by the nebulizer, and the mixture carried to the ICP torch.

Laboratory calibration standards will consist of dissolved otolith reference material obtained from the National Research Council of Canada, similarly introduced to the spray chamber by the nebulizer as an aerosol before being carried to the ICP torch. Blanks of HNO_3 aerosol will also be used. To control for operational variability in the laser-ICPMS, our standard operating procedure is to use a randomized blocks design with each petrographic slide as the blocking factor, considered randomly drawn, and each sampling area considered a fixed treatment. Readings of count-rates ($counts \bullet s^{-1}$) for blanks and references will be obtained before and after random presentation of the otolith sections in each block.

Otoliths will be analysed for ^{48}Ca, ^{25}Mg, ^{55}Mn, ^{88}Sr, and ^{138}Ba, and reported as ratios to ^{48}Ca. To calculate elemental Ca ($Me \bullet Ca^{-1}$) ratios, background counts will be subtracted from otolith counts by interpolating between readings taken before and after each block of otoliths, and the corrected otolith counts will be converted to $Me \bullet Ca^{-1}$ concentrations using the references. To sample the nucleus, we will use a grid raster type with a laser beam of

diameter 20 μm traveling at 6 μm•s^{-1}, set at 60% power and frequency at 10 Hz. This will give a predicted crater depth of approximately 100 μm (Jones and Chen 2003, equation 3).

6.4.2 Statistical methods

A spatially discrete population structure implies fish in each area were spawned separately from those in other areas, and therefore were exposed to different early life environments. To test for these differences, we will apply analysis of variance (ANOVA), using separate univariate analyses to examine the behaviour of each element ratio. Multivariate outliers will be identified by plotting robust squared Mahalanobis distances of the residuals (D^2_i) against the corresponding quantiles (Q-Q plot) of the chi-square distribution, and the assumption of multivariate normality will be checked analytically using tests ($\alpha = 0.05$) based on Mardia's multivariate skewness and kurtosis measures (Khattree and Naik 1999). If variance-covariance matrices are not equal, we will use univariate ANOVA for each Me•Ca^{-1} ratio instead, testing for univariate normality using the Kolmogorov-Smirnov test ($\alpha = 0.05$) and equality of variances using the F_{max} test, with Student-Newman-Keuls (SNK) Multiple Range tests for pairwise comparisons between sampling areas, adjusted for an experiment-wise α.

Spatial heterogeneity can also result when fish, though spawned separately, then disperse and mix in different proportions between sampling areas. Therefore, to examine whether spatial heterogeneity is due to discrete populations or mixing of more than one, we will employ cluster analysis to detect fish crossing between sampling areas, using Ward's minimum variance hierarchical approach. To determine the quality of clustering, we will use root mean square standard deviation (RMSSTD), semi-partial R-square (SPRSQ), R-square (RSQ), and between-cluster sum-of-squares (BSS) to measure the loss of homogeneity through successive merging of clusters (Khattree and Naik 2000).

6.5 Life history patterns approach

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, and biological aspects such as distribution, abundance and spawning grounds are the consequences of life history modes to which fish stocks have evolved. Differences in these parameters and patterns 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 time, spawning ground and size structure, proposed 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 into the high sea. Its results were supported by Oliva (1999) examining the parasites species composition.

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, size-structure, fecundity and other reproductive indicators like eggs and larvae, reproductive season, condition factor, could be analyzed along its entire geographical 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 indicators such as gonadosomatic and hepatosomatic indexes and condition factor, can provide evidence for population discreteness within an holistic approach for the identification of the stock structure.

6.5.1 Patterns in the distribution of abundance and size structure

The abundance and spawning distributions could also 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 could help to verify the results found by Serra (1991) for the Southeast Pacific.

Time series of length-frequency data or catch-at-length data are also important to examine the size structure between areas and seasons. This kind of data allows the estimation of average length and the size range, and the identification of areas with concentrations of juveniles or adults.

In order to identify spatial patterns of distribution in abundance, spawning grounds, etc., the compilation of the information available in different countries needs to be done. This information is available in Ecuador, Peru, Chile, Russia, Ukraine, China, New Zealand, Vanuatu, Holland and Faroe Islands.

6.5.2 Reproductive indexes 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, macroscopic and histological maturity stages allows for estimation of organ ratios like gonadosmatic index, condition factor, and muscle mass (e.g. Kjesbu et al. 1998, Ganias et al. 2007). These indexes will be explored depending on the availability of the specific data in each location.

6.5.3 Statistical analysis and methodological approach

Time series of reproductive indexes, gonadosomatic index, maturity stages, condition factor will be analyzed by using Generalized Additive Models (GAM), particularly the techniques available in the package ‘mgcv’ (Wood, 2000, Wood and Augustin, 2002; Wood, 2003) for the software and language R (Ihaka and Gentleman, 1996). Similarly, length frequency data will be analyzed by using Generalized Linear Models by using the package ‘MASS’ (Venables and Ripley, 2002). These techniques will take into account location as the main

source of variation and other co-variable affecting the variable response. Similarly, ANOVA and MANOVA techniques will be applied for simple comparison of a single response variable.

6.6 Oceanographic features

An important aspect to be considered is the oceanographic description of the jack mackerel habitat, in particular in association with the stock structure to be found, since physical processes might structure sub-populations acting as barriers that prevent population exchange or due to other factors like favoring areas for reproductive or feeding purposes. This might help in the interpretation of the stock structure of the jack mackerel.

The large distribution of the jack mackerel has been well described, particularly by the research done by Russia, Chile, Peru and New Zealand (see jack mackerel species profile). In this large distribution of the jack mackerel abundance is not homogeneous as can be inferred from the catches in the different fishing grounds and present knowledge from surveys. For example, the largest concentration of the resources seems to be in front of south-center Chile, and a second main concentration off central and northern Peru. What explains the concentration of the jack mackerel in the two areas? Oceanographic processes at the meso and macro scale might help to understand this. To have previous knowledge about the stock structure of the jack mackerel would facilitate in characterizing the environment of each stock which unfortunately is not the case. But working hypothesis would help.

6.6.1 Data

The data used for this purpose would come from research and fishing surveys, satellite and oceanographic buoys. From research and fishing surveys data on temperature, salinity, oxygen, chlorophyll and zooplankton can be obtained. From satellite sea surface temperature, wind, chlorophyll and sea level can be obtained. Available climatic maps might also be used.

An inventory of the available data and data sources is a first step and to have it in a data base a second and crucial step.

6.6.2 Method

Different statistical method should be used like

a) Spatial analysis with geostatistical tools and empirical orthogonal functions.
b) Time domain analysis like autocorrelations, cross correlation, vectorial correlation and empirical orthogonal functions.
c) Frequency domain analysis like spectral analysis, phase and coherence spectra.
d) Time-frequency domain analysis like wavelet and cross wavelet (Torrence and Compo, 1998; Torrence and Webster, 1999).

7. Integrated Analysis

For an integrated analysis of the results from all the techniques a similar approach as with the HOMSIR project could be followed. For the analysis of the results obtained it is necessary to take into account the nature and the temporal scale of each technique. Some of them have an evolutionary meaning, like the genetics, in which the time and the geographic scales are very important. The case of expanded distribution colonizing new areas could be an example. However when differences are found with any technique, those are true and the difference found has to be explained. Thus is important to note that only heterogeneity and not homogeneity can be demonstrated (Waldman, 1999).

The start is to examine the results, discussing and relating the different findings, taken into consideration the time scale (microevolution and macroevolution), the geographical factor and the oceanographic information.

Other options can be discussed during the development of the project.

8. Budget

Due to the inconveniency that the Task Team takes decision about the participation of the different parties in the research some considerations for a proper understanding of the budget are:

- All the countries that fish for jack mackerel (coastal and distant flag vessels) and that are part of the RFMO negotiation process was assumed that would participate. These countries are Ecuador, Peru, Chile, New Zealand, Vanuatu, Russia, Holland, Faroe Islands and the Popular Republic of China.
- It is also considered that all countries will participate in the five techniques identified, which are genetics, morphometry, parasites, microchemistry of otoliths and life history patterns. This means five scientists per country.
- The baseline for the calculations is a sample size of 100 fish per sampling site and a sampling exercise in the whole region. The sampling exercise involve the 15 sampling locations (Ecuador, Peru, Chile and N. Zealand), plus 3 samples extra of juveniles for microchemistry and 5 samples taken with a research vessel.
- The cost of scientist and technicians was assumed that it would be financed by each country. Exceptions are additional man power for oceanography.
- Two scenarios were developed, one with one sampling (main spawning season) and another with three sampling events (autumn, winter and main spawning season).
- The cost to survey the zone between 100º and 170º W is identified separately due to its high cost and uncertainty.
- After knowing the participating countries and number of scientists per country the budget can be adjusted easily.

An overall budget is described below for the two scenarios and a detailed description by method and item is given in Annex

8.1 Scenario: One sampling exercise

	US$
Sampling	30070
Genetic technique	127500
Mprphometry: body	18000
Morphometry: otolith	141298
Parasites technique	118500
Life history pattern	
Microchemistry of otolith	130604
Oceanography	14000
Workshops (travel)	1000000
Unforseen expenses (10%)	157997
SUBTOTAL	1737969

Research vessel samples

	US$
Sampling	44800
Genetic technique	42500
Mprphometry: body	4000
Morphometry: otolith	4000
Parasites technique	5000
Life history pattern	
Microchemistry of otolith	12000
SUBTOTAL	112300

TOTAL	1732792

8.2 Scenario: Three sampling exercise

	US$
Sampling	90210
Genetic technique	382500
Mprphometry: body	36000
Morphometry: otolith	159298
Parasites technique	235500
Life history pattern	
Microchemistry of otolith	333686
Oceanography	14000
Workshops (travel)	1192700
Unforseen expenses (10%)	244389
TOTAL	2688283

For total cost in this scenario the cost of the research vessel samples is added.

TOTAL US$	2800583

For each scenarios the cost of the research vessel operation should be added

8.3 Cost of research vessel to survey area from 120º W to 170ºW

Research vessel

Research vessel cost 1	1925000
Research vessel cost 2	1284000

* Russian vessel: cost 1 or 2 depend on Russian funding for a CCAMLR survey

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ANNEX

Budget description by item

Sampling

Exercise for sampling the fisheries 1 time

	US$
Input	2500
Technician	720
Sample transport in Peru	1500
Sample transport in Chile	1000
Sample transport from abroad	6500
Flight ticket (national)	3600
Per diem (US $100 day)	3600
Unforeseen expenses	2000
SUBTOTAL	21420

Exercise for sampling fishing vessels outside EEZ.
Assume no sample transport.

	US$
Input	1000
Technician	1250
Flight ticket	4600
Per diem (US $100 day)	1800
SUBTOTAL	8650

TOTAL	30070

Exercise for sampling in research vessel

	US$
Input	2000
Technician	400
Sample transport (5 samples)	34000
Flight ticket (international)	4600
Per diem (US $200 day)	1800
Unforeseen expenses	2000
TOTAL	44800

Cost of sampling in the fisheries plus research vessel

TOTAL sampling	74870

Exercise for sampling the fisheries 3 times

	US$
Input	7500
Technician	2160
Sample transport in Peru	4500
Sample transport in Chile	3000
Sample transport from abroad	19500
Fligth ticket (national)	10800
Per diem (US $100 day)	10800
Unforseen expenses	6000
SUBTOTAL	64260

Exercise for sampling fishing vessels outside EEZ.
Assume no samples transport.

	US$
Input	3000
Technician	3750
Fligth ticket	13800
Per diem	5400
SUBTOTAL	25950

TOTAL sampling 3 times	90210

TOTAL sampling plus research vessel	135010

GENETIC METHOD

1 sampling exercise: Coastal and International Fleet samples

Genetic method	
Laboratory inputs, DNA extraction, analysis	90000
Reading	37500
Subtotal	127500

Samples from 100º to 170º W

Genetic method	
Laboratory inputs, DNA extraction, analysis	30000
Reading	12500
Subtotal	42500

TOTAL	170000

Exercise for sampling the fisheries 3 times

Genetic method	
Laboratory inputs, DNA extraction, analysis	270000
Reading	112500
Subtotal	382500

TOTAL	425000

MORPHOMETRIC METHOD

Body morphometry

Exercise for sampling the fisheries 1 time

Morphometry: body	
	US$
Inputs	4500
Photographic camera	9000
Other	4500
SUBTOTAL	18000

Samples from 100º to 170º W

Morphometry: body	
Inputs, Photographic camera	2000
Other	2000
SUBTOTAL	4000

TOTAL	22000

Exercise for sampling the fisheries 3 times

	US$
Inputs	13500
Photographic camera	9000
Other	13500
SUBTOTAL	36000

Plus samples from 100º to 170º

TOTAL	40000

PARASITES METHOD

Exercise for sampling the fisheries 1 time

	US$
Lab inputs	54000
Microscope	60000
Other	4500
SUBTOTAL	118500

Samples from 100º to 170º W

	US$
Lab inputs	4500
Other	500
SUBTOTAL	5000

TOTAL	123500

Exercise for sampling the fisheries 3 times

	US$
Lab inputs	162000
Microscope	60000
Other	13500
SUBTOTAL	235500

Plus samples from 100º to 170º W

TOTAL	240500

Otolith morphometry

Exercise for sampling the fisheries 1 time

	US$
Inputs	4500
Microscope	20000
Image Analyser*	102298
Analitical trade (0.1 mg)	10000
Other	4500
TOTAL	141298

*Considered for four countries

Samples from 100º to 170º W

	US$
Inputs	2000
Other	2000
SUBTOTAL	4000

TOTAL	145298

Exercise for sampling the fisheries 3 times

	US$
Inputs	13500
Microscope	20000
Image Analyser	102298
Analitical trade (0.1 mg)	10000
Other	13500
TOTAL	159298

Plus samples from 100º to 170º W

TOTAL	163298

MICROCHEMISTRY OF OTOLITH

Exercise for sampling the fisheries 1 time

	US$
Personnel	36571
Supplies, general expenses	6000
Otolith processing	43200
Travel	6204
Subtotal	91975
Indirect cost (42%)	38629
SUBTOTAL	130604

Samples from 100º to 170º W

	US$
Otolith processing	12000

TOTAL	142604

Exercise for sampling the fisheries 3 times

	US$
Personnel	101586
Supplies, general expenses	12000
Otolith processing	115200
Travel	6204
Subtotal	234990
Indirect cost (42%)	98696
TOTAL	333686

Plus samples from 100º to 170º W

TOTAL	345686

OCEANOGRAPHY

Calculated for four countries

	US$
Personnel (additional)	8000
1 PC	6000
TOTAL	14000

WORKSHOPS

It was considered as a minimum necessary to held five WS. These are one to initiate the project (planning, decisions on method and procedure), for progress review, one for analysis of results and write report on different methods, one to integrate the results from the different method and write report, a last to review final report.

Two additional meetings were considered necessary, one for life history patterns and the other for oceanography.

Exercise for sampling the fisheries 1 time

	US$
Flight tickets	634500
Per diem	324000
Meeting expenses	5000
SUBTOTAL	963500

Additional Workshops (LHP, Oceanography)

	US$
Flight tickets	32900
Per diem	2800
Meeting expenses	800
SUBTOTAL	36500

TOTAL	1000000

Exercise for sampling the fisheries 3 times
(plus 1 more WS)

	US$
Flight tickets	761400
Per diem	388800
Meeting expenses	6000
SUBTOTAL	1156200

Plus additional Workshops (LHP, Oceanography)

TOTAL	1192700