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# REPORT ON THE ELECTROPHORETIC AND MORPHOMETRIC STUDIES CONDUCTED AT THE INTER-AMERICAN TROPICAL TUNA COMMISSION FROM 1969 TO 1978

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# INTRODUCTION

Knowledge of the population structure of an exploited stock is necessary for the effective management of a fishery. For this reason the Inter-American Tropical Tuna Commission (IATTC) has sponsored numerous genetic, morphometric, and migration studies of yellowfin, <u>Thunnus albacares</u>, and skipjack, <u>Katsuwonus</u> <u>pelamis</u>, in a concerted effort to determine the structure of these stocks in the eastern Pacific Ocean.

This report focuses on studies conducted by two Commission scientists, Drs. Sharp and Robert C. Francis, from 1969 to 1978, which involved the Gary D. collection and analysis of electrophoretic (Sharp) and morphometric (Francis) data. The initial analyses, results, and conclusions of these studies have been reported in IATTC bi-monthly and annual reports (1970-1977) and in publications by Sharp (1969, 1972, 1973), Sharp and Francis (1976), and Sharp and Pirages (1978). In these reports the data were presented in abbreviated formats summarized by sample or area, and as a result of numerous manipulations and reanalyses of these data over the years their original character has been obscured. Consequently, recent attempts to verify earlier results and reanalyze conclusions have been hampered by incomplete data sets and apparent inconsistencies among data in different reports. The purpose of this report is to describe, as clearly as possible, the procedures involved in the collection, analysis, and storage of the electrophoretic and morphometric data and to reconstruct the data base in its entirety from the original electrophoretic and morphometric records so that these data will be readily accessible to other researchers.

This report has been divided into three principal sections, History, Materials and Methods, and Data. The History section is an historical outline of the samples collected and the analyses performed. The Materials and Methods section is a detailed description of the techniques employed for the collection of specimens and data and the laboratory analyses of the blood and tissue samples. Descriptions of the various types of data which are available are contained in the Data section. The reconstructed data base consists of two data files, one for yellowfin and one for skipjack. Each data file contains the genetic, morphometric, and collection (area, date, gear, etc.) information for each sampled fish. Because the data files are too large to be included in this volume the genetic data have been summarized by sample in Appendices 6 and 7. The complete data files are available on request from the IATTC in either a printed format or on computer magnetic tape. The information reported here was extracted directly from the electrophoretic gel records and original collection data. In all cases where inconsistencies existed in the data or where information was unclear, a number of sources were consulted.

#### GLOSSARY OF TERMS

Blood (sera) sample: Four to 5 ml of whole blood taken from a fish using a plastic Peel-A-Way blood sampler. Each sampler was labeled with a sample code and either the length of the fish or an identification number.

<u>Blood or tissue survey:</u> A procedure where a number of blood or tissue samples are electrophoretically analyzed for a number of specific enzymes in a search for polymorphic enzyme products.

Cores and drains: Samples of blood taken from fish which had been frozen.

<u>Data file:</u> A collection of the genetic and morphometric data for each sampled fish and a sample index to identify each sample. There are two data files, one for yellowfin and one for skipjack.

<u>Electrophoresis:</u> The separation and identification of protein (enzyme) bands using an induced electric field and histochemical staining.

<u>Electrophoretic gel (starch gel or gel):</u> A horizontal glass plate filled with hydrolyzed starch used in the electrophoretic process as a matrix for the separation of proteins.

<u>Electrophoretic gel notebooks:</u> A collection of eight notebooks and one folder containing the electrophoretic gel records.

<u>Electrophoretic gel records</u>: The records of the phenotypes assigned to the pattern of enzyme bands of blood or tissue samples after electrophoretic analysis.

- <u>G code:</u> A coding system used to identify samples which have associated morphometric data. The code consists of the letter G and a three-digit number.
- <u>Green cruise logbooks:</u> Green bound books containing a daily record of the scientific observations and experiments of the IATTC staff aboard a chartered fishing vessel or tagging cruise.
- <u>Morphometric data sheets:</u> Formatted records of the measurement of external body dimensions collected from yellowfin and skipjack. These sheets have been partially categorized by year in the morphometric notebook.
- <u>Morphometric notebook:</u> A black three-ring binder labeled "Morphometric sheets" containing the original morphometric data sheets.
- <u>Phenotypic score:</u> Code assigned to a band or group of protein bands which appear on a starch gel after a tissue or blood sample has been electrophoresed and the proteins have been selectively histochemically stained. It is assumed that the phenotypes (protein banding patterns) recorded represent genetic (DNA) products.
- <u>Sample:</u> A collection of typically 200 blood samples and/or 50 sets of morphometric measurements taken from fish captured in one purse-seine set or during one baitboat stop.
- <u>Sample Code:</u> Two to six characters used to identify a sample. Lists of sample codes and sample collection data are given in the sample indices (Appendices 3 and 4).

<u>Sample (collection) data:</u> Data concerning the location, date, and gear employed in the capture of the fish used for a blood and/or morphometric sample.

- <u>Sample folder:</u> A manila folder containing sample data, copies of the electrophoretic gel records, and morphometric data sheets used to compile the data. Folders are categorized by year of capture and may contain from one to four samples.
- <u>Sample index</u>: A table of sample codes, sample data, morphometric codes, and the dates of electrophoretic analyses used to cross reference samples that have been reported earlier. There are two sample indexes, one for yellowfin (Appendix 2) and one for skipjack (Appendix 3).
- <u>Sample summary:</u> A summary of the phenotypic scores of each sample. There are two sample summaries, one for yellowfin (Appendix 6) and one for skipjack (Appendix 7).
- <u>Sampling instructions:</u> Detailed instructions given to waterfront and at-sea personnel describing the techniques and areas that were to be employed in blood and morphometric sampling. A collection of these instructions can be found in Appendix 2.

Standard reference sample: A collection of four samples (CAC FF, CAC GG, CAC HH, CAC II) of yellowfin blood collected in one small area over a 4-day period.

<u>Summary sheets:</u> A summary created by Sharp from the electrophoretic gel records of the distribution, by size of fish, of the phenotypic frequencies of a sample.

#### HISTORY

# Yellowfin sampling in the eastern Pacific

# 1969

Due to the expansion of the yellowfin surface fishery into offshore areas in 1968, the IATTC staff felt a need to reevaluate and extend earlier population studies using genetic (blood typing and immunological) and morphometric techniques. The purpose of these studies was to elucidate the population structure of eastern Pacific yellowfin. The proposed null hypothesis was that yellowfin in the eastern Pacific, both within the IATTC's Yellowfin Regulatory Area (CYRA) and in the offshore areas, belong to one homogeneous population. This hypothesis was to be tested using gene frequencies determined from electrophoretic studies and standard numerical population genetic techniques. It was assumed in these studies that samples whose allelic frequencies closely approximated the expectations of a Hardy-Weinberg equilibrium distribution were from similar origins and belonged to the same population, and samples whose allelic frequencies diverged from the expected were assumed to be the product of a mixture of fish from different genetic units or subpopulations.

The initial program consisted of the collection and electrophoretic analysis of yellowfin tissues for polymorphic loci and the determination of the variation and frequency of occurrence of these allelic characters in several geographic regions of the eastern Pacific Ocean. The following enzymes were assayed for polymorphisms:

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1)Lactate dehydrogenase (LDH)
2)Glucose-6-phosphate dehydrogenase (G6PDH)
3)Glutamate dehydrogenase (GDH)
4)Na-alpha-glycerophosphate dehydrogenase (alpha-GDH)
5)Phosphoglucomutase (PGM)
6)Alcohol dehydrogenase (ADH)
7)Transferrins (TFN)
8)Esterases (EST)
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The samples employed in these early surveys were collected on two simultaneous IATTC tagging cruises during October and November 1969. Cruise 1054 was aboard the baitboat Mary Carmen, and Cruise 1055 was on the 400 purse-seiner Connie Approximately yellowfin blood samples <u>Jean</u>. representing 5 distinct schools were collected, along with blood samples from 36 black skipjack, Euthynnus lineatus, and assorted other scombroid species taken on jig lines trolled behind the vessels. The techniques employed in the collection of blood samples are described in the Materials and Methods section of this report. The exact number of tissue samples taken during these cruises It can be assumed, however, that most, if not all, of the 400 is unknown. yellowfin used in the blood collection were also used as a source of tissue samples, because the sex is recorded for each fish and both sex determination and tissue sampling require dissection. Heart, muscle, liver, and a number of stomachs were dissected from the freshly-caught fish, and stored at -10°C. Morphometric measurements were collected from 76 yellowfin. For each of these fish 11 measurements were recorded. In order to examine the variability between measurers, a number of the fresh fish were measured independently by two people. To determine the effect of the freezing process on the morphometric measurements

The laboratory analysis of the blood and tissue samples began in December 1969. The micro starch gel method of Tsuyuki <u>et al.</u>(1966) was used to separate the proteins electrophoretically. The zones of activity for each enzyme were then determined by a specific stain or, in the case of transferrins, by the autoradiographic technique of Barrett and Tsuyuki (1967). A complete listing of the stains and buffers with references is given by Sharp (1972: Addendum 1) (Appendix 1 of this report).

each fish was measured twice, once immediately after capture and again during

unloading after being frozen in brine aboard the vessel.

The results and conclusions of the early enzyme surveys were published in the IATTC bi-monthly report series. In the January-February 1970 issue it was reported that reproducible electrophoretic banding variants had been observed in yellowfin red blood cell LDH. Analysis of this enzyme system in yellowfin and other scombrids continued until July 1970, when it appeared that the observed variation was the result of the random expression of several tissue-specific LDH isozymes. Because the necessary facilities were not available to do a complete biochemical analysis, the LDH system was considered to be of no further value as a population discriminator. Similarily, the four remaining dehydrogenases and the mutase were eliminated as useful enzyme systems because they were too complex (GDH, alpha-GDH), monomorphic (ADH, G6PDH), or the resolution of the banding patterns was poor (PGM). This left only transferrins and esterases, which had previously been shown to be polymorphic by Barrett and Tsuyuki (1967) and Fujino and Kang (1968a, 1968b), as possible useful enzyme systems.

Many difficulties were encountered in the early stages of the analysis for serum esterases. Variability which could not be fully accounted for on a genetic basis was observed using the techniques of Fujino and Kang. These difficulties were resolved by consulting with Dr. Fujino in March 1970, and the results of the esterase analysis of the initial 400 blood samples were reported in the IATTC May-June 1970 bi-monthly report. Details of the esterase system are given in the Materials and Methods section of this report. In 1971 these blood samples were reanalyzed to check for scoring reliability and the stability of the esterase enzyme. The phenotypic scores of both analyses can be found in the yellowfin sample summary (Appendix 6). The 1970 results are coded with a number 1, (CNJ A1, MRC B1, MRC C1, CNJ D1 and CNJ E1), while the 1971 scorings are coded with a number 2 (CNJ A2, MRC B2, MRC C2, CNJ D2 and CNJ E2). A more detailed description of these samples and the scoring records can be found in the Data section of this report.

No difficulties were encountered in the characterization of serum transferrins using the standard techniques of Barrett and Tsuyuki (1967). Although they could uncover transferrin zones using an amido black stain, Barrett and Tsuyuki did not employ this method in their study because of their limited knowledge of the allelic structure of the transferrin system, and because precise definition of the bands was not always possible using the micro starch gel techniques. Given the superior resolution of the Fujino techniques, it was possible in 1970 to abandon the costly radiographic process for a simplified double-staining procedure. After electrophoresis each gel was stained twice. First, the gel was stained for esterases. These bands formed within 10 minutes and could easily be scored. After recording the esterase phenotypes the gel was stained with an amido black solution. After a few minutes the stain was removed, the gels were cleared, and the transferrin phenotypes recorded. The details of this process can be found in Sharp (1972: Addendum 1)(Appendix 1 of this report).

# <u>1970</u>

From 1970 to 1974 Sharp collaborated with the staff of Dr. N.O. Kaplan's laboratory at the University of California at San Diego (UCSD). Difficult-to-assay enzymas, particularly LDH and isocitrate dehyrogenase, were examined using gel electrophoresis, electrofocussing, and column chromatography. No polymorphisms were encountered. Additionally, Drs. S. Y. Yang and M. Ε. Soule of the UCSD genetics department were consulted during the course of these investigations. £

The results of the initial screening limited the materials to be collected to whole bloods because only the serum for population discrimination transferrins and esterases appeared to be useful in this respect. Dr. Fujino was not optimistic in discussions concerning the use of these enzymes to describe yellowfin subpopulations because, in his analyses of transferrin gene frequencies of samples taken from various areas of the Pacific, he had found no significant heterogeneity in either intra-area or inter-area comparisons for eastern Pacific, Hawaiian, or Line Islands yellowfin (IATTC March-April 1970 bi-monthly report). These discussions were to lead to the development of the 1970 sampling program. The goal of this program was to determine the statistical sampling requirements to define rigorously the levels of gene variation within the sampled population(s), particularly gene frequency frequency variations with size-age. A major component of this sampling program would involve the collection of a 2000-fish "standard reference sample." It was hypothesized that the reference sample (collected in 1971) would represent the true population gene frequency for the sampled area. This sample would then be used to set optimum sampling requirements for the discriminate analysis of fish in time and space. A complete description of the experimental design and its assumptions and limitations is given by Sharp, Mobrand, and Francis (1970). A summary of the hypotheses and the equations employed is given by Sharp (1972: Addendum 2) (Appendix 1 of this report).

It was hoped that this avenue of research would lead to the description of separate areal groups for yellowfin. A total of 25 samples was collected from June to December 1970. Three purse-seine cruises were involved, two aboard the <u>Anne M.</u> (Cruises 1057 and 1058) and one aboard the <u>Marietta</u> (Cruise 1059). These samples are coded ANM (B through K) and MRT (L1 through XN) in Appendices 3 and 6. Whole blood samples were taken from as many fish as possible over a wide range of lengths. Lengths were recorded in either centimeters or millimeters and kept in bound books referred to in this report as green cruise logbooks. Each fish and each blood sampler was given an identification number so that the lengths and genetic data could be correlated.

Upon arrival at the laboratory blood samples were analyzed using the double-staining technique, and the esterase and transferrin phenotypes were recorded. As these data were collected each fish in each sample was alloted a computer card (1971 computer cards) on which was recorded the sample code, the date and location of capture, the length of the fish (usually to the nearest centimeter), sex (if known), and the electrophoretic phenotypes. To facilitate further analyses, the data from the 400 blood samples collected for the initial survey in 1969 were also recorded on computer cards in 1971. Regrettably, it is now impossible to match with certainty a computer card to an individual fish because the fish identification numbers were not included on the computer cards. For this reason the computer cards, although available, were not used to compile the yellowfin data. The data used in the yellowfin sample summary (Appendix 6) and the yellowfin data file were taken from the original electrophoretic gel records, recorded on loose-leaf paper, using the fish identification numbers to correlate the genetic data with the lengths recorded in the green cruise logbooks.

In subsequent analyses of the above data it became apparent that the complexity of the data (the level of heterogeneity in gene frequencies both within and among samples) would not allow the description of "separate areal groups" of yellowfin. This discovery resulted in the restructuring of the sampling program for the following year.

# 1971

The 1971 sampling program was devised to provide samples which would contain large numbers of fish recruited to the fishery during one quarter of the year from areas believed to be relatively isolated with respect to stock mixing. This study was based on the hypothesis that samples of fish hatched in the same quarter of the year would have similar esterase and transferrin gene frequencies. Because most of the sampling was done in November it was decided that all fish in the samples would be given a relative age standardized to November 15 and adjusted by 3-day intervals to 10ths of months. In this manner fish were assigned to one of four quarterly entry groups by their lengths. Quarter Q1 was from February to April, Q2 from May to July, Q3 from August to October, and Q4 from November to January. A computer program, POP-DIF, was developed for aging the fish based on the growth curves created by Davidoff (1963) from time-dependent modal progressions observed in length-frequency data. This computer program performed a Chi-square analysis of each sample by size groups. The program first divided the sample into size groups, and then computed the allelic frequencies for each size group and for the entire sample. After determining the frequencies, it would test the fit of these frequencies against those expected from a Hardy-Weinberg equilibrium. Finally, the total, pooled, and heterogeneity Chi-squared values were determined. A major part of Sharp (1972) deals with the analysis of these quarterly entry data, and for further details one should consult that report.

From April to November 1971 a series of large samples was collected in an attempt to insure size homogeneity. Sampling was directed at fish less than 2 years of age, and maximum effort was expended in areas where traditionally large numbers of small "school-fish" (fish not associated with porpoise) were encountered. Three purse-seine tagging cruises were involved. On the <u>Pacific Tradewinds</u> (Cruise 1062) seven samples were collected, (PTW 10, 12, 27, 37, 42, 45, and 52). Each sample was coded by the number of the net set from which the sample was taken. Samples PTW 10 and PTW 12, which were composed of fish taken from three sets (10, 11, and 12), were combined into one sample in many of the analyses. During Cruise 1063 of the J.M. Martinac five samples were collected

which have been labeled, JMM AA, JMM BB, JMM CC, JMM DD, and JMM EE. During Cruise 1064 aboard the <u>Cachita</u> five more samples were added (CAC FF, CAC GG, CAC HH, CAC II, and CAC JJ). Four of the five <u>Cachita</u> samples (CAC FF, CAC GG, CAC HH, and CAC II) were collected in a "small time-area stratum" (September 9-13 at approximately 23°N-113°W). These fish represented in nearly equal proportions "age cohorts" from the first two quarters of the year, Q1 and Q2. This set of four samples was the most homogeneous (in size and time) group of samples collected, and when combined into a single sample was referred to as "the standard reference sample."

In October and November 1971 a series of small samples was collected from unloadings at Manta , Ecuador, and during Cruise 1066 aboard the Marco Polo. Samples ECU XA, ECU XB, ECU XX, and ECU JC were collected at the Inepaca Samples ECU XA and ECU XB are samples of approximately 28 fish, while cannery. ECU JC is a combination of several small samples. Sample ECU XX was collected by Sharp at the Inepaca cannery, and because cannery samples came from mixed schools of fish he grouped this sample with ECU XA and ECU XB into one large sample labeled "XX." Many of the small samples collected during Cruise 1066 were combined into a single large sample labeled "WB" (Sharp, 1972). In the yellowfin data file these samples have, where possible, been separated into their component parts, resulting in four samples for Cruise 1066 (MP A1, MP BC, MP D1, and MP E1). Most of the data collected and the analyses performed during 1971 were described in the IATTC September-October 1971 bi-monthly report. This report contains, the sample collection dates, locations, the number of fish, and the age group composition by quarters of each of the samples.

#### 1972-1973

In 1972 Sharp and Francis initiated an integrated program for collecting and analyzing several biological parameters simultaneously. This program was to include: 1) blood sampling for genetic analysis (particularly transferrins, as the esterase system did not contain enough variability), 2) morphometric data, 3) basic biological data (collections of gonads for reproductive studies and stomachs for food studies), and 4) migration data as determined from tagging studies. It was hoped that with this collection of data the structure of the yellowfin population(s) in the eastern Pacific could be better defined. Sampling was to be conducted in three general areas which supported continuous fisheries; the northern grounds, fished by San Diego- and Ensenada-based baitboats; the southern areas, fished by Ecuadorian vessels based in Salinas and Manta; and the Central American grounds, fished from vessels based in Puntarenas, Costa Rica. To these were to be added any samples which could be collected opportunistically on tagging cruises.

This new program was designed to evaluate the following concepts:

1) Discrete subpopulations have discrete morphological and genetic characters. 2) The level of genetic frequency variability both among and within samples is of the same order. This was based on the statistical analyses (heterogeneity Chi-square) of samples collected in 1969-1971.

3) Individual yellowfin have only one spawning period per year. The two observed yearly spawning peaks were hypothesized to be the result of two genetically isolated semestral units, A and B. (Although Sharp's A and B semestral units coincide in some cases with Hennemuth's (1961) division of yellowfin into X and Y cohorts, the former is based on genetic considerations and the latter on modal progressions. Consequently, each system of subdivisions should be considered independently.)

4) Sampling narrow length classes (10 to 15 cm) should increase the chance of genetic relatedness in samples.

5) Statistical sensitivity to small-frequency differences would be enhanced if samples were large (200 or more fish per sample).

6) Samples from different subpopulations which have similar gene frequencies can be differentiated using morphometric techniques.

7) As more data are collected the "sample variance" for both morphometric and genetic data should decrease and take on a central tendency if all the yellowfin in the eastern Pacific belong to one population.

In order to evaluate and further refine these concepts a sampling scheme designed to increase the probability of genetic relatedness. Samples were was to consist of 200 fish whose lengths corresponded to one of a number of 10- to 15-centimeter length modes. A list of expected modes for each month had been tabulated based on modal progressions and given to each person sampling. Morphometric samples were to be composed of a subset of 50 fish from the blood sampling. Nine external body dimensions were to be recorded for each fish. (Appendix 2 contains a collection of sampling instructions, and the Materials and Methods section gives further details on morphometric sampling.) Sampling from tagging cruises was slightly modified from the above in that only 70 fish were taken. Samples of blood and morphometrics during this experimental period were identified using a three-digit code preceded by the letter G. The first sample taken was labeled G150, and the remainder of these samples can be found in the yellowfin sample summary (Appendix 6) by referring to the G codes and associated sample codes in the sample index (Appendix 3).

Initially both blood samples and morphometrics were taken from fresh fish (G150-152), but this procedure was changed for samples G153-157 and G160-161. For these samples bloods were taken from fresh fish as before, but then numbered plastic flags were attached to each caudal peduncle and the fish were frozen in brine. The morphometrics for these fish were collected when the fish were thawed at the cannery just before butchering. The sampling procedure was again changed for samples G159, 163, and 164, and an electrophoretically unscorable sample from the <u>Vikingo</u>. For these samples both blood and morphometrics were taken from thawed fish at the cannery. The details for taking blood from frozen fish, "cores and drains," are given in the Materials and Methods section.

In 1973 the sampling program was altered to take advantage of the possibility of obtaining help from participants in the U.S. National Marine Fisheries Service (NMFS) porpoise observer program, as well as an abundance of small fish being caught in the Gulf of Panama and off Costa Rica during that year. As before, 200 yellowfin bloods and 50 morphometrics were taken from fish thawed at the cannery just before butchering, but only eight morphometric measurements were taken at that time. Each sample was to represent a single purse-seine set or baitboat stop. To aid in the identification of these fish NMFS porpoise observers had been equipped with plastic flags to mark groups of uniformly-sized fish which they encountered. A total of 16 samples, labeled G200-215, were collected in this manner. Additionally, two small samples were collected from Cruises 1070 and 1071. The sample from Cruise 1071 is of particular interest because the 70 fish in that sample were measured twice. Morphometrics were taken at sea on the freshly-caught fish and at the cannery just before butchering. This was to prove useful in explaining some of the variability seen in the morphometric data. Further details on Sharp's analysis of these data and his conclusions are given by Anonymous (1973,1974).

During this period Francis was analyzing the morphometric data. In order the morphometrics of fish of different sizes comparable, to the make measurements for each fish were adjusted to those which would be expected to occur on fish of a standard total length (600 mm). The variance-covariance structure of the adjusted morphometric data was examined by means of a multivariate statistical technique. Then the within-sample variance-covariance structure was examined by means of a principle component analysis. Finally, a series of analyses were performed on three sets of replicate morphometric samples taken at sea and subsequently at the cannery from thawed fish. A11 three samples were taken during tagging cruises aboard the purse-seiner Marietta, two in 1970 (Cruise 1059) and one in 1973 (Cruise 1071). These data can be found in the morphometric notebook, and the analyses of these data were From these analyses it was concluded that described by Anonymous (1974). morphometrics had taken place during the significant changes in the freezing-thawing process, and the magnitude of those changes required that all future measurements be taken from fresh fish to avoid additional random as well as systematic errors.

# <u>1974–1976</u>

The simultaneous collection of yellowfin blood and morphometric samples continued for three more years (1974-1976). The sampling design, underlying concepts, and assumptions first expressed in 1972 were retained, the only difference from the earlier studies being the source of the blood and morphometric samples. Because of the variability believed introduced by the freezing-thawing process, frozen fish were no longer used for blood sampling or for the collection of morphometric data. During this period a total of 56 samples of blood and morphometrics was collected from three distinct areas of the fishery. Samples were coded for the area of capture by using G codes. Samples collected off Baja California by San Diego- and Ensenada-based baitboats were labeled with a G300 series, Ecuadorian collections were labeled by a G500 series, and samples taken in the central and offshore areas of the fishery were labeled by a G400 series. A complete listing of the samples is given in the Materials and Methods section under yellowfin morphometric samples. An additional six samples were collected on tagging cruises where only blood samples were taken.

All of the blood samples collected were analyzed electrophoretically in the same manner as the fresh fish samples collected in 1972. After the electrophoretic phenotypes were determined for each blood sample, each sample was analyzed for its genetic homogeneity based on the frequency of the serum transferrin A allele. When the frequency of the allele in a sample was below .660 in the south or below .705 in the north the sample was considered to be a "homogeneous low-frequency" sample. Similarly, when the sample frequency was greater than .780 the sample was considered to be a "homogeneous high-frequency" sample. Samples with intermediate frequencies were viewed as being potential composites or mixtures of genetically high- and low-frequency groups. As in 1972, it was believed that, based on the sample frequency of the transferrin A allele, the genetically heterogeneous samples should exhibit more within-sample morphometric variability than the genetically homogeneous samples.

Francis continued his analysis of the morphometric data, and in 1976 he "There is no evidence that morphometric characters presently concluded that, being examined have any relationship to the serum transferrin gene frequencies being employed to assess subpopulation structure" (Anonymous, 1977). Following a reanalysis of the data in 1981, Francis elaborated on his earlier findings in a memorandum to Dr. John R. Calaprice (Appendix 5). The only systematic differences which Francis was able to detect in the morphometric data relate to levels of allometry among different areas of the eastern Pacific. He determined that, given the limitations of the present analytical techniques, fish length, not area of capture, was a better discriminator of morphometric relatedness. After numerous manipulations of the data and comparing the analyses of transformed and untransformed data sets, he further concluded that the stepwise canonical analysis, as performed by the computer program BMD07M (Dixon 1974), presented more questions than answers, and that another avenue of analysis might be more appropriate.

Part of the concomitant sampling of yellowfin involved the collection of gonads for reproductive studies. Much of this material was analyzed by Knudsen (1977a, 1977b). Sharp had proposed to Knudsen that, based on several IATTC length-frequency studies, he believed there were at least two dominant yellowfin reproductive periods annually in the north and central portions of the eastern Pacific fishery and only one in the south. Sharp could not characterize these semestral units by esterase or transferrin frequency groups but he contended that some type of temporal distinction could be shown for different genetically distinguishable components based on the semestral cycling of transferrin gene frequencies which he had observed in the data. Knudsen addressed the questions of temporal and spatial isolation of the two semestral units and concluded that the spawning data collected could not support the hypothesis that separate subpopulations were being maintained by temporal differences in the spawning of X and Y cohorts because spatial isolation of the cohorts did not appear to occur. She based this conclusion on the observation that fish of both cohorts spawned during all times of the year and in all areas of the fishery for which she had data. She was, however, unable to comment further, because the genotypes of the spawning fish which she studied were unknown, and it could not be determined whether separate genetic components were segregating among areas or perhaps returning to specific sites within the areas to spawn.

# PGI Analysis 1976-1978

In 1976 a new genetic polymorphic enzyme, phosphoglucose isomerase (PGI), was detected in yellowfin sera. A two-locus, four-allele genetic system was proposed, using blood samples collected from the Gulf of Guinea in the Atlantic Ocean. Inter- and intra-ocean comparisons were made using Atlantic and Pacific Ocean materials. All alleles and phenotypes observed had electrophoretically identical counterparts in both oceans and in all areas. A more complete description of this enzymatic system can be found in the Materials and Methods section of this report. Because this system was polymorphic, and the phenotypic frequencies fit the expectations of an Hardy-Weinberg population, it was decided to reevaluate all of the previously collected blood samples in the hope that this new system might be helpful for subpopulation discrimination. The PGI A or fast PGI enzyme system proved to be unusually stable, for it was possible to electrophorese and score phenotypes from blood samples that had been repeatedly frozen and thawed over a 7-year period. Regrettably, due to protein overloading and streaking of the protein bands, it was not possible to analyze the "cores and drains" collected from frozen fish in 1973. Reanalysis for the fast PGI system continued until its completion in May 1978. The results of this analysis can be found in the yellowfin data file by fish and sample, and summarized by sample in the yellowfin sample summary (Appendix 6).

# Yellowfin Sampling in the Atlantic and Western Pacific

From 1975 to 1978 a number of samples of yellowfin and skipjack bloods and morphometrics were collected from the central, western, and south Pacific. These samples came from Cruises 5001 and 5002 to the Marquesas Islands, exploratory fishing cruises (Paramount in 1975, Mary Elizabeth and Zapata Pathfinder in 1976), and joint collections involving the IATTC and the staff of New Zealand's Fishery Research Division (FRD) and Papua New Guinea's Fisheries Division (FD). The purpose of these collections was to determine the range and the origin of the genetic groups of yellowfin that had been proposed for the eastern Pacific, and to determine if the yellowfin and skipjack of the south and central Pacific belonged to the same exploitable stock as those of the eastern Pacific fishery. For an inter-ocean comparison, a small number of blood samples from Atlantic yellowfin and bigeye, Thunnus obesus, were made available to the IATTC. These blood samples were analyzed electrophoretically using the same techniques employed in earlier studies. Esterase, transferrin, and PGI alleles were compared to eastern Pacific material to determine if any unique area-specific alleles or allelic frequencies could be found. No unique alleles were discovered, but variability in allelic frequencies between areas was noted. As was the practice, all phenotypic scorings were recorded on electrophoretic gel records which were arranged in electrophoretic gel notebooks. Many of the starch gels of the Atlantic blood specimens were photographed so that a permanent copy of the scoring procedure and resulting phenotypes are available. These photos can be found with the other electrophoretic gel records in the electophoretic gel notebooks.

# Skiplack Sampling

In 1975 a cooperative sampling program with FRD and FD was initiated to evaluate the population structure of skipjack from the western, south, central, and eastern Pacific. A total of 21 samples of blood and morphometrics were collected from different fisheries of the eastern Pacific, and 21 samples of blood and 1 sample of morphometrics were collected from the south and central Pacific. The blood samples were analyzed by the IATTC laboratory for their esterase and transferrin phenotypes, using the standard procedures developed by Fujino and Kang (1968a, 1968b). (The details are given in the Materials and Methods section.) Results of these analyses were to be compared with the work of Fujino, who had analyzed numerous skipjack blood samples from Hawaii, Japan, and the south and western Pacific. By 1977 the IATTC was no longer involved in the analysis of the south Pacific material, because the South Pacific Commission's (SPC) skipjack survey and assessment group had initiated its own genetic sampling and analysis program. The laboratory analyses were conducted by Dr. Barry Richardson at the Australian National University Research School of Biological Sciences. The IATTC continued to collect and analyze eastern Pacific material and to exchange data with the SPC's project participants until 1978, when it ceased collecting and analyzing blood samples. For further details concerning the analyses performed by the SPC the reader is referred to Anonymous (1980 and 1981).

#### MATERIALS AND METHODS

# Collection of Samples

# Fresh blood samples

Whole blood samples of 4 to 5 ml were taken by direct heart puncture with a 1 1/2-inch 18-gauge needle on a heparinized 10-ml Peel-A-Way blood sampler. Each sampler contained 5 ml of a red blood cell preservative (Cushing <u>et al.</u> 1957), consisting of a 40:60 mixture of glycerol and a 5% sodium citrate solution. Each sampler was immediately sealed, the contents were thoroughly mixed, and the samplers were placed in a freezer for storage at approximately  $-10^{\circ}$ C (Sharp, 1969). This technique remained essentially the same for all subsequent whole blood samplings with one modification. Beginning in 1970 blood samplers were stored in a refrigerator for 4 to 8 hours, after which an equal volume of preservative was added to each, agitated to insure mixing, and frozen as above.

# Frozen blood samples ("cores and drains")

Blood samples were taken in 1973 from commercially-caught fish (<u>i.e.</u> fish from vessels on which at-sea sampling was not conducted) at the canneries just prior to butchering. The fish were first thawed, and then a boring tool (a laboratory cork borer with a removable plunger) was inserted into the heart region of the fish ("In the isthmus between opercula, in line with the back of the opercula") (Fujino, 1966). The boring tool was removed and approximately 2 ml of the liquid blood were collected in a labeled plastic vial. Care was taken to remove any heart or muscle tissue from these blood samples. The vials were then frozen at  $-10^{\circ}$ C until analyzed.

#### Labeling of the blood samples

Each of the blood samplers or vials was labeled with a code to designate the sample and either an identification number or the length of the fish. The codes used varied from time to time to accomodate certain aspects of the sampling scheme employed. The following is a general outline of the types of codes used during the different sampling periods. 1) In 1969-1970 the majority of the blood samplers was labeled with a number. Each sample consisted of a sequence of numbers (e.g. 45 to 247). Often the numbers were prefixed by a code letter for a series of numbers (e.g. an X series numbered 1 to 1000, a number only series from 1 to 1000, a (..) series, and an LL series). The corresponding length for each numbered fish was recorded in a green cruise logbook along with the date and location of capture of the fish.

2) From 1970 to 1976 samples of fresh blood, for which morphometric data were not collected, were labeled with a sample code and the length of the fish, usually in centimeters. After the laboratory analysis was completed and the blood samplers were refrozen it was no longer possible to match a phenotype on an electrophoretic gel record to the blood sampler from which it came.

3) For samples of fresh blood collected from 1970 to 1977, for which morphometric data were taken, the first 50 to 52 samplers which had corresponding morphometric data were labeled 1 to 50 or 1 to 52, often with an M preceding the number to designate morphometric samples. The remainder of the blood samplers were labeled with a sample code and the length of the fish in certimeters. The corresponding measurements for the first 50 to 52 samplers were recorded on the morphometric data sheets which can be found in the morphometric notebook. After electrophoretic analysis many of these samples were given G codes based on the locations where the fish were caught. Details on the G codes are given in the section on morphometric sampling.

#### Yellowfin morphometric samples

Yellowfin external body dimensions were determined using the measurements and calipers described by Marr and Schaefer (1949). Over the years a number of different sets of morphometric measurements have been collected, and the following is a description of the samples, their respective measurements, and how each sample was labeled for analysis. For additional reference five sets of sampling instructions issued to the scientific staff have been included in Appendix 2.

In 1970 it was reported that during Cruise 1054 a series of 11 characters was measured from 76 yellowfin. It was also reported that in an attempt to assess the variability between measurers, a number of these fresh fish were measured independently by two people, and to determine the variability introduced by the freezing-thawing process these fish were measured twice, once when they were caught and again when they were unloaded at the cannery after being frozen in the vessel's hold (Anonymous, 1970). Some of these morphometric can be found in the morphometric notebook under the tab labeled data "Fresh-Frozen." These data were extracted from the original data, and only 6 measurements from approximately 60 fish were recorded. Because the original records of the experiment have been lost the exact number of fish sampled and the number of measurements taken are now unknown.

A program was started in 1970 for the morphometric sampling of the commercial catch. Approximately 50 fish, selected at random, from a single set were sampled from vessels unloading at the canneries. The following nine measurements, described by Marr and Schaefer (1949), were taken from these fish as they were thawing prior to butchering: Total length (TL)
 Snout to first dorsal (S-1D)
 Snout to second dorsal (S-2D)
 Snout to anus (S-A)
 Snout to ventral (S-V)
 Head length (HL)
 First dorsal to second dorsal (1D-2D)
 Ventral to anus (V-A)

9) First dorsal to anus (1D-A)

Fifteen samples were collected in 1970 and eleven more were added the following year, eight from within the CYRA and three from the area west of the CYRA, but east of  $150^{\circ}W$ .

A morphometric computer card was punched for each fish; this contained the morphometric data plus the date of capture, the morphometric area (described in Appendix 2), location of capture (using the codes of Shimada and Schaefer, 1956), gear (baitboat or purse-seiner), measurer, and preservation (fresh or frozen fish). Sampling of the commercial catch continued into 1972, but the data for some of those samples have been lost. A listing of the morphometric computer cards, the formats for the data, and some of the initial analysis of the 1970-1971 sampling can be found in a large green computer binder labeled "1970-1971 Morphometrics." In addition, the 1972 morphometric data sheets that are available can be found in the morphometric notebook.

In 1972 Francis and Sharp began a sampling program to collect samples of morphometrics and blood simultaneously. In most cases blood samples were taken from fresh fish at sea. A flag was attached to each fish, and they were frozen in the vessels' wells with the remainder of the catch. Morphometric data were then collected from these fish after they were unloaded at the canneries and while they were thawing prior to butchering. As in previous samplings, nine measurements were recorded for each fish, and infrequently sex was determined. For identification samples with both morphometric and genetic data were assigned a G code for identification. A total of seven samples were collected in the above manner, G150, and G153-G158, and two additional morphometric samples, G151 and G152, were taken from fresh fish. The phenotypic scorings for the fish in these samples can be found in the yellowfin data file and the yellowfin sample summary by referring to the G codes and sample codes in the sample index (Appendix 3). Regrettably, only the morphometric data collected from fresh fish (G151 and 152) are now available, and they can be found in their respective sample folders and in the morphometric notebook. (Additional information on these samples is given by Anonymous (1973).

A total of 16 samples of blood and morphometrics, G200-G215, was collected from the commercial catch in 1973. In contrast to the previous years, both bloods and morphometrics were taken from frozen fish. Each 200-fish sample of bloods and each 50-fish morphometric subsample was to represent a single purse-seine set or baitboat stop. To aid in the identification of these fish NMFS porpoise observers had been equipped with flags to mark large groups of uniformly sized fish. In 1973 two additional samples were collected during Cruises 1070 and 1071. On Cruise 1070, 80 bloods and morphometrics were taken from fresh fish. However, as noted in the green cruise logbook, the fixed part of the calipers had become misaligned so that the accuracy of those measurements was suspect. Seventy fresh fish were sampled for blood and morphometrics on Cruise 1071. This sample is of particular importance because these fish were measured twice, once at sea and again when they were thawed at the cannery. These data, along with those of Cruise 1059, were used to determine the effect of the freezing-thawing process and to explain some of the variability observed in the morphometric data.

In 1973 only eight morphometric measurements were recorded. The two measurements involving the ventral fin used in earlier studies were eliminated, and the distance from the insertion of the second dorsal fin to the insertion of the anal fin was measured, rather than computed as had been done in the past. This collection of measurements was to remain unchanged for the remainder of the morphometric study. Records of the genetic data collected during this period can be found in the yellowfin sample summary (Appendix 6) by sample and samples can be cross-referenced to their G codes using the sample index (Appendix 3).

The following 56 samples of blood and morphometrics were collected from fresh fish between 1974 and 1976.

Northern area 1974 G (300-302) 1975 G (305-312, 320, and 1 unnumbered sample) 1976 G (313-316, 321) <u>Central area</u> 1974 G (400-406) 1976 G (420-425) <u>Ecuador</u> 1974 G (500-510) 1975 G (520-528, 530) 1976 G (531-534)

Morphometric data sheets for these samples are available in the morphometric notebook and copies of the sheets were included in each sample folder. Seven boxes and one metal tray of computer cards employed by Francis in his analyses are also available. The metal tray, labeled "IT 13, Francis, Master file," contains all of the original measurements, and the boxes contain a collection of programs and manipulated data used in his analyses. The morphometrics recorded in the yellowfin data file were taken directly from the morphometric data sheets.

# <u>Skipjack morphometric samples</u>

In 1975 and 1976 blood and morphometrics were collected from skipjack using the same techniques employed for yellowfin. The following 17 samples were collected in the eastern Pacific:

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Northern area KA, KB, MK A1, MK A2, MK C, MK D, TAR B1, TAR G2 <u>Central area</u> SC SG <u>Ecuador</u> ECB, ECC, ECD, ECE, ECF, ECG, ECH, ECI

Only three samples of skipjack morphometrics (PNG NH, S218, and S223) were collected from the south Pacific. The first sample was taken from 40 fish near Papua New Guinea, and the other two came from the inshore waters off New Zealand. In all cases the same eight characters used in the yellowfin study were measured.

# Laboratory Analysis

Between 1969 and 1978 the following people were involved in the analysis of blood and tissue samples at the IATTC laboratory. Scientist in charge: 1969-1978 Gary Sharp

Laboratory assistants: 1970 Dorothy Normark 1970-1971 James Cravens 1971-1973 John De Beer 1973-1974 Robert Olson 1974-1975 Suellen Pirages 1975 Deborah Jo Czapinski 1975-1978 William Kane

For the esterase and transferrin analyses, each person was instructed by Sharp in sample preparation, electrophoretic techniques, and staining procedures. Except for Suellen Pirages and William Kane, the other assistants were initially unfamiliar with electrophoretic procedures.

The electrophoretic gels were prepared in two groups per day, usually by Sharp prior to 1975 and by Kane thereafter. The resulting phenotypes were scored either by Sharp or by the experienced assistants (Pirages and Kane). Many of the gels analyzed by Sharp and Pirages and most of the pre-1976 analyses of Sharp and Kane were checked for reading errors. First one person would read the gels and record the phenotypes. Then the other person would read the same gel (without referring to the previous reading) and compare his scores to the ones that had been recorded. The blood samples with scoring discrepancies were reanalyzed, and questionable scores in the gel records were marked with the symbol "RR." These blood samples were reanalyzed until a satisfactory scoring was possible, or the blood sample was deemed unscorable. A blood sample was usually considered unscorable when enzyme breakdown products (satellite bands or excessive streaking) were observed upon repeated analysis. Serum enzyme breakdown was commonly due to the contamination of a blood sample by stomach fluids resulting from inserting the needle too deeply when the blood sample was taken, improper storage, or heating of the blood samples during transit from the collection site to the laboratory. In addition, due to inconsistent handling of the materials and techniques by some of the inexperienced assistants, or to obvious technical problems, an entire gel or series of gels were marked with an "RR" and later reanalyzed.

The following is a general outline of how the blood and tissue samples were analyzed:

. For electrophoretic analysis the blood samples in vials or samplers were arrayed while thawing in racks by 1) numerical order, 2) numerical order for the morphometric fish and length for the remainder, or 3) length. For fresh blood samples, aliquots of each sampler were dispensed onto a plastic plate having 100 depressions in a 10 X 10 array. Whatman No.3 filter paper wicks  $(3 \times 5 \text{ mm.})$  were then placed in the depressions, or into the vials in the case of frozen blood samples, until they were saturated. The excess was removed by blotting on filter paper, and the wicks were placed in the previously prepared gel. (For diffuse blood samples which had been overdiluted with preservative the proteins were reconcentrated using Sephadex granules to absorb the excess liquid.)

Initially (Dec. 1969-Feb. 1970) the micro starch gel method of Tsuyuki, et al. (1966) was used to separate the proteins electrophoretically. The zones of activity for each enzyme were determined by using a specific histochemical stain or, in the case of transferrins, by the autoradiographic technique of Barrett and Tsuyuki (1967). In March 1970, after conferring with Dr. Fujino, Sharp adapted a "modified" Fujino method for the separation of proteins because it gave improved resolution over the micro starch gel technique. Also adopted an amido black staining procedure to replace the at this time Was autoradiographic method for assaying for transferrins. With this staining procedure it was possible to perform a double-staining process. After completion of the protein separation the gels were first stained for serum esterases using alpha-napthyl propionate and alpha-napthyl acetate as substrates and scored for the resulting phenotypes in 10 minutes. After recording the esterase phenotypes the gel could then be stained with an amido black solution for transferrins. This double-staining process did not affect the resolution of either of the two enzyme systems. A list of stains and buffers with references is given in Sharp (1972: Addendum 1) (Appendix 1 of this report).

The electrophoretic gels used to evaluate the phosphoglucose isomerase (PGI) phenotypes were made using 35.0 g starch (Connaught Laboratories, Inc.) in 330 ml 0.0025 M Tris-citrate pH 8.00 gel buffer. This volume was sufficient to fill two gel molds of approximately  $21.5 \times 14 \times 0.6$  cm. Fifteen whole blood samples were then applied to each gel via Whatman No. 3 filter paper wicks. A discontinuous buffer system was employed to process the gels. The cathodal chamber was filled with 2 l of a 0.05 M Tris solution buffered to pH 8.0 with 0.05 M citric acid. The anodal chamber contained 0.3 M borate and 0.06 M NaOH at pH 8.0. A current of 50 ma was maintained across each gel for 4 hrs by periodically adjusting the voltage (ranging from 160 to 200 V). To maintain an even temperature electrophoresis was performed in a refrigerator set at approximately 4°C.

The gels were stained for PGI activity by applying a wash of 0.01 M Tris-HCl pH 8.0 containing 50 mg of the substrate fructose-6-phosphate, 100 units of the coenzyme glucose-6-phosphate dehydrogenase, 20 mg NADP, 40 mg nitro blue tetrazolium (NBT), 20 mg thiozolyl blue (MTT), and 10 mg phenazine methasulfate (PMS). The gels were then incubated at approximately 40°C for 20 minutes and scored for the fast PGI (A) phenotypes. To enhance the resolution of the slow PGI (B) bands many of the gels were incubated overnight (approximately 12 hours). But even given the longer incubation time, PGI (B) phenotypes were not resolvable in most gels.

#### Description of the Enzyme Systems

# Esterase

Nonspecific serum esterases for yellowfin, skipjack, albacore, <u>Thunnus</u> <u>alalunga</u>, and bigeye were first characterized by Fujino and Kang (1968a). Six esterase bands were recognized in skipjack, three in albacore, and two each in bigeye and yellowfin. The relative mobilities of these bands were described in two figures and, based upon the standard Hardy-Weinberg analysis, the bands were assumed to represent codominant genes of multiple-allele esterase genetic systems. With the data available it was possible to test the skipjack allele frequencies for sex and size relationships (none were found), but insufficient data were available to perform these tests on yellowfin, albacore, or bigeye.

The IATTC electrophoretic techniques and scoring systems for yellowfin, bigeye, and skipjack were adapted from those of Fujino with slight modifications. For yellowfin and bigeye the serum esterases were labeled such that the most rapidly migrating common allele was given the lowest value (EST 1) and slower-migrating products higher values (EST 2 and EST 3). A rare, rapidly migrating allele which was encountered after the numbering system had been adopted was labeled EST 4.

Skipjack esterases were labeled in a similar manner, resulting in Fujino's E 1sj, E 2sj, and E 3sj being renamed EST 2, EST 3, and EST 4 respectively. EST 1, the fastest-migrating esterase product, was not encountered by Fujino in his early studies. Faint or otherwise unclear phenotypes were re-electrophoresed with samples of known phenotypes and restained. This process was repeated as many as four times, until either a clear, easily scored result was obtained or the serum sample was considered unscorable.

# Transferrin

Barrett and Tsuyuki (1967) were the first to describe polymorphic serum transferrins for scombrid fishes. Yellowfin, skipjack, bluefin, <u>Thunnus</u> <u>thynnus</u>, albacore, and bonito, <u>Sarda chiliensis</u>, sera from various areas of the eastern Pacific were analyzed using a micro starch gel electrophoretic technique followed by an autoradiographic process to detect transferrin activity. Genetic control of the banding patterns observed was assumed, based upon an analysis of the frequency of the observed phenotypes and the expected values for a population in Hardy-Weinberg equilibrium. A combination of two bands resulting in three phenotypes was reported for yellowfin and bonito. Six phenotypes were used to describe a codominant three-allele system for skipjack and albacore. Bluefin transferrins did not show any allelic variation.

In 1968 Fujino and Kang (1968b), using horizontal starch gels and an amido black staining procedure, described polymorphic transferrins in three species, skipjack, yellowfin, and southern bluefin, <u>Thunnus maccovii</u>, from the Atlantic and Pacific Oceans. As in the earlier work of Barrett and Tsuyuki, six phenotypes were observed in skipjack sera. The numbering system and the amido black staining procedure were adopted, for the IATTC laboratory, with the addition of a rare, slowly migrating allele TFN 4. Fujino and Kang were also able to test the skipjack transferrin system for independence, and found no relationship between the TFN phenotypes, the esterase phenotypes, and the sex or size of the fish.

Fujino and Kang (1968b), in their study of yellowfin transferrins, adopted the lettering nomenclature of Barrett and Tsuyuki. The fast-migrating band was labeled "A" and the slow-migrating band "B." In addition to the previously described alleles, Fujino recognized a faint band that migrated faster than the "A" allele and labeled this "a." This gave a total of five phenotypes for yellowfin. As in earlier studies, a comparison of Hardy-Weinberg equilibrium frequencies was used to verify a codominant autosomal allelic interpretation of the observed banding patterns. Fujino also reported at that time that the yellowfin transferrins were independent of the esterase system, and the size or the sex of the fish. Although the same electrophoretic and staining procedures employed by Fujino were used at the IATTC, no "a" alleles were encountered. As a result, the "a" allele was not included in the nomenclature used at the IATTC. The relative mobilities of the yellowfin and skipjack transferrins are shown in Figure 1.

#### Phosphoglucose isomerase

In late January 1976, 26 serum samples each from yellowfin, skipjack, wahoo, Acanthocybium solandri, and black skipjack were electrophoresed using a standard Ashton buffer. This buffer system, described by Sharp (1972: Addendum 1) (Appendix 1 of this report), was routinely used to assay for esterase and transferrin activity. The resulting gels were histochemically stained for esterase, transferrin, glutamate-oxaloacetate-transaminase (GOT), and phosphoglucose isomerase (PGI). Esterase and transferrin bands were easily assayed under these conditions, giving a collection of bands for yellowfin, skipjack, Jahoo, and black skipjack. The Ashton buffer did not provide good separation of the GOT enzyme system, although some multiple banding was noted. In the case of PGI, the separation was good, but distinct bands were not formed. Changing the buffer system to Tris-citrate pH 8.0 (labeled "LDH" in the electrophoretic gel records) improved the PGI resolution dramatically, and a polymorphic PGI enzyme system for yellowfin and bigeye was described in February 1976. A total of 294 yellowfin and 63 bigeye blood samples collected in the Atlantic Ocean were used for this survey. Photographs of the gels used in the survey are available in an envelope in the electrophoretic gel notebook for 1976.

Upon staining the following zones of PGI activity were noted: (1) a strongly staining fast-migrating group of bands, later designated the PGI (A) system; (2) a broad area from the origin up to the fast-migrating system where activity was observed but no distinct bands or patterns could be discerned; and (3) an area of cathodally migrating bands where activity in yellowfin was sporadic. (In yellowfin only two bands were observed in 26 samples, compared to skipjack which had distinct cathodal bands. Photographs of these gels are available in the electrophoretic gel record attached to the page for January 29, 1976. Initially a lettering system was used to describe the phenotypes This nomenclature was soon abandoned in favor of a numbering system, observed. so that scores for transferrins and PGI could be easily differentiated. Details on the relative mobilities and the nomenclature employed are shown in Figure 2 for yellowfin and bigeye and Figure 3 for skipjack.

Agreement with Hardy-Weinberg equilibrium frequencies was used to verify that the PGI (A) system was genetic in nature. Samples from different regions of the eastern Pacific were then analyzed and compared to each other and to the Atlantic material. In a G test of the allelic frequencies of different areas of the Pacific it appeared that inter-area gene frequencies were statistically different. At this point it was decided to reanalyze all previously collected yellowfin blood samples for the PGI (A) system, in the hope that they would be useful for subpopulation discrimination.

Resolution of the diffuse area below the fast-PGI bands did not improve with a change in buffers, and for many blood samples enzymatic activity in that area was negligible. It was hypothesized that this activity was due to some form of tissue-specific PGI, and because it was only marginally resolvable in whole blood it was not considered usable at that time. In May 1976, tissue surveys for PGI were conducted on 14 species using frozen red, white, and heart muscle tissue. These tissue samples had been collected over a period of years, and many were showing signs of deterioration due to storage. The PGI (A) enzyme system was detectable in all three tissues, but showed the greatest activity in the heart muscle tissue. This observation was true for skipjack, albacore, bluefin, wahoo, sierra, <u>Scomberomorus sierra</u>, frigate tuna, <u>Auxis thazard</u>, black skipjack, and yellowfin. Many of the gels used in this survey were photographed; a permanent record is available in a folder in the 1976 electrophoretic gel notebook. The slower-migrating PGI activity noted earlier was either very faint or in most cases totally absent in these gels.

In order to determine if there were any low-level polymorphisms in skipjack and to check that nothing had been overlooked in the earlier survey, 51 skipjack blood samples were analyzed for fast PGI in April 1976. Twenty-six of these blood samples were from New Zealand and 25 from the eastern Pacific. Only one of the New Zealand blood samples was electrophoretically distinct. This specimen displayed a typical three-banded heterozygous pattern. Otherwise, the PGI electrophoretic bands were identical for both areas.

In April 1977, during routine analysis for the PGI (A) system, it was noted that in some of the recently acquired yellowfin samples from the south Pacific the slower-migrating activity of the gel could be resolved into distinct bands. From April 1977 until the end of the study approximately 430 yellowfin blood samples were scored for both fast and slow PGI systems. Because of the sporadic nature of the slow PGI system, it was not possible to test this system of bands adequately for its genetic nature or linkage to the PGI (A) system. Based upon a recent review of the PGI literature, it is now believed that the slow PGI system may not be independent of the fast system, and may be a collection of heterodimeric bands of a fast PGI muscle system and a slow PGI liver system. This type of condition has been observed in many teleosts (Avise et al. 1973: Dando 1974; Allendorf and Utter 1979). This hypothesis could be tested by analyzing a number of fresh tissue samples (e.g. white, red, and heart muscle, liver, pyloric caeca, sera, eye lens, and red blood cells) for specific PGI activity. Eye lens tissue in particular would be a good tissue to study because a polymorphic slow (liver-type) PGI system from the eye lens of albacore was recently described using techniques similar to the ones employed here (Dr. John Graves, personal communication).

A total of approximately 18,000 yellowfin blood samples was analyzed for the fast PGI enzyme system. Where possible, these data have been cross referenced to individual fish. The PGI phenotypic scores were recorded in columns 45 and 46 in the yellowfin data file and were used to compile the yellowfin sample summary (Appendix 6). For many of the blood samples collected before 1975 it is impossible to match blood samples to individual fish. Consequently, a separate listing of PGI phenotypic scores and associated lengths are supplied in the yellowfin data file. Because of the questionable nature of the slow PGI system, these data were not included in this data report. However, a separate folder labeled "PGI data (slow)" in the PGI workup and photographs notebook contains copies of all the electrophoretic gel records for the slow system plus some photographs.

Approximately 144 skipjack were analyzed and scored for the PGI (A) enzyme system, 26 from New Zealand, 25 from the eastern Pacific, and the remainder from the Marquesas Islands. The phenotypic distribution was 132 "2-2," 6 "2-3," 3 "1-2," and 3 unscorable. Of the total, only 15 of the Marquesas blood samples were scorable for the hypothesized slow-PGI (B) system. Eleven were homozygous slow "SS," two homozygous fast "FF," and two were heterozygous "FS." As is true for yellowfin, the exact genetic nature of this group of bands is unknown, and more work must be conducted to determine their usefulness. It should also be noted that skipjack blood possesses a strong cathodally migrating group of PGI bands, and this area also deserves further attention.

Recently Fujino, Sasaki, and Okumura (1981) described a PGI enzymatic system found in whole skipjack blood and the hemolysate of red blood cells. Using a Tris-citrate pH 5.8 buffer, nine phenotypes were observed, formed from nine anodally migrating bands, the most common being PGI 6, with a frequency greater than 0.970. No mention is made of any cathodally migrating bands in either the text or the accompanying diagrams. The apparent differences between this system and the one described here may be due to the different pH values employed, 5.8 for Fujino and 8.0 in this study.

Sixteen bigeye blood samples from Papua New Guinea and 63 from the Atlantic Ocean were analyzed for PGI. Only fast-migrating PGI (A) bands were discernable, and the corresponding alleles were indistinguishable from those of the yellowfin PGI (A) system.

# Tissue Surveys

Samples of heart muscle, white and red lateral muscle, and liver were excised from fresh and frozen fish and placed in individual Whirl-Pak bags, labeled, and stored at  $-20^{\circ}$ C. In many cases tissues were stored for as long as 4 years before being analyzed. To prepare tissue samples for analysis, small segments of approximately 1 g were homogenized in either 0.1 M phosphate and glycerol solution (60:40) pH 7.0 or a distilled water and glycerol (60:40) solution. A motorized ground glass mortar and pestle in ice was used for homogenization. The samples were then centrifuged at 200 g for 10 minutes. Supernatants were collected by pipettes and stored at  $-20^{\circ}$ C.

A number of tissue surveys were conducted at the IATTC laboratory. In 1969 and 1970 Sharp employed the Tsuyuki micro starch gel technique to separate proteins. He then histochemically stained these gels in the original survey for possible polymorphic yellowfin enzymes. In 1970 and 1973 Sharp performed additional tissue surveys using the horizontal starch gel techniques of Fujino. The following notebooks are available to describe the analyses performed: 1) "Esterase and transferrin early records December 1969-June 1970," 2) "Esterase and transferrin scoring records, tissue survey, photographs of standards, March-April 1971," and 3) "Cetacea and yellowfin enzymes 1971-1973." Poloroid photographs of many of the gels are available, either attached to the notebooks or at random in a box marked "photos."

In 1973 Sharp conducted an electrophoretic survey of 20 yellowfin, 40 to 44 cm in length, from a single school to measure the amount of intra-school genetic variability. Blood, heart muscle, liver, white and red lateral muscle, and eye tissue were sampled from each fish and analyzed for esterase, transferrins and 14 other enzyme-protein systems. The results of this survey were reported in Table 7 of Anonymous (1974). Records of the gels can be found in the electrophoretic gel notebook for December 6, 1973, to January 8, 1974.

Sharp and Pirages (1978) performed a number of tissue surveys in 1974-1975. They surveyed 14 species for polymorphisms using a number of enzymes, the standard horizontal starch gels of Fujino, and the staining recipes of Shaw and Prasad (1970). Records of the gels used for this study can be found in the 1974 electrophoretic gel notebook. Many of the gels were photographed and can be found as figures in that paper or in the box marked "photos."

A small number of tissue surveys were conducted by Sharp and Kane from 1975 to 1978. The electrophoretic gel records and photographs of the gels can be found in the electophoretic gel notebooks for those years and in the notebook marked "PGI work up and photos."

# DATA

# Sources of Data

At the outset of this project there was no reliable source of information on the electrophoretic analyses that had been performed at the IATTC. The data which were available were in the form of published and unpublished reports and sample summaries, none of which represented all of the data. In order to eliminate any transcription errors that, over the years, may have been incorporated into the data and to be as complete as possible the original electrophoretic gel records have been used in this report to compile the data files and produce the sample summaries. There are four principal sources of data available at this time, 1) green cruise logbooks, 2) bridge logbook abstracts (described below), 3) morphometric data sheets, and 4) electrophoretic gel records. The quantity and quality of information available from each of these sources varies due to the large number of people involved in the collection and analysis of the data. Consequently, it was necessary to check all entries to insure their accuracy. The following is a detailed description of the data and how they were used to compile the yellowfin and skipjack data files.

# Green cruise logbooks:

Green cruise logbooks were kept by the scientific staff aboard each chartered tagging vessel. These logbooks were designed to be a daily record of all fishing and scientific activities, but because there was no set format for logbook entries the amount of data recorded varied considerably, depending on who was responsible for keeping the logbook. For this report logbooks were used to supply details on dates, locations, and size composition of the samples. Additionally, some of the early (1969-1971) logbooks were employed as a source of raw data (usually lengths for numbered samplers). The green cruise logbcoks, identified by cruise number, are stored at the IATTC headquarters with the tagging records. When discrepencies in sample dates and locations were noted between the published records and green cruise logbook data, the bridge logbook extracts were consulted to check the information. Bridge logbooks are kept by the navigator of each vessel, and abstacts of these logbooks are made by the IATTC staff at the end of each fishing trip so that catch and effort statistics may be computed. The bridge logbook abstracts, which are confidential, are stored at the IATTC headquarters with the statistical records.

# Morphometric data sheets:

Several different formats were used to record the morphometric data.

1) Initially forms consisting of 6 to 9 columns and 50 rows were constructed by the person sampling. There was no particular order for the columns, so that one must note the column headings in each case.

2) Starting in 1972 a form entitled "Field Data Sheet-Tuna Morphometrics" was employed to record data. Nine measurements were recorded for each fish, along with information on the morphometric area (described in Appendix 2), location of capture (using the codes of Shimada and Schaefer, 1956), gear, measurer, preservation, and infrequently sex. This form was constructed to allow key punching of the data directly from the sheets.

3) From 1973 onward, a new form entitled "Morphometric Sample Sheet" was used. Eight measurements were recorded, as well as flag number (or fish number), measurer, vessel, location and date of capture, and location and date sampled. Later, G codes and location of capture codes were added to the top of each form. In the 1973 samples it is common to see the esterase and transferrin scores written in the far right column of each morphometric sheet.

All of the original morphometric data now available are contained in a large black looseleaf binder marked "Morphometric Sheets." Photocopies of the morphometric data sheets used to create the data files can also be found in each sample folder. It should be noted that from 1970 to 1973 many morphometric samples were taken that were not associated with genetic samples. Many of these samples were collected in port on frozen fish, and have since been lost. The samples that are available can be found in the morphometric notebook.

# Electrophoretic gel records:

1) From the initiation of sampling in 1969 until December 1970, 400 blood samples were analyzed and summary sheets were made from the electrophoretic gel records. Only an incomplete set of the original electrophoretic gel records is now available, so a collection of summary sheets prepared by Sharp was used as a source of data for these samples.

2) From December 1970 till February 1971 the electrophoretic gel records were recorded in random order on looseleaf pages. From these pages a collection of summary sheets (8 1/2 by 14-inch yellow paper) were transcribed by Sharp. Later, the data on the summary sheets were used for key punching the 1971 computer cards. For the yellowfin data file the loose-leaf data were matched to the lengths recorded in the green cruise logbooks. These summary sheets can be found in the <u>Marietta</u> 1970 sample folder. 3) A brown folder entitled "Esterase and Transferrin Scoring Records" contains photographs of the gels containing enzyme standards and records of the blood samples analyzed from March to June 1971. The records of the second analysis of the original 400 blood samples collected in 1969 and some of the early tissue surveys can also be found there.

In 1972 all of the existing genetic data were computerized. Although it is impossible to match all of the computer cards to individual fish, most of these data were key punched in the same order as they appeared in the electrophoretic gel record sheets. As a result, it is possible to compare some of the phenotypic scores recorded in the electrophoretic gel records with the data recorded on the computer cards.

After the yellowfin data file was compiled from the original electrophoretic gel records, this information was compared to a 1972 computer printout of the computer cards. When scorings in these two sources did not match, the original documentation was rechecked and any discrepencies were noted on the computer printouts that can be found in the sample folders for these samples.

4) From June 1971 until 1978 the electrophoretic gel records were recorded on daily work sheets. These work sheets are arranged by year in four black looseleaf binders (electrophoretic gel notebooks). These electrophoretic gel records were used by Sharp to create summary sheets that described the phenotypic frequency distribution of each sample by 1-cm length groups. Each summary sheet was divided into length groups to analyze the shifts in gene frequencies as a function of length. During this analysis Sharp removed fish with lengths in the extreme tail ends of the length distribution to make the samples more size homogeneous. Once removed, these fish were not considered in any future analyses.

5) A separate folder was used to record the details of the early PGI analysis. This notebook, labeled "PGI Work Up and Photos," contains photographs of all the gels used to define that genetic system.

# <u>Construction of the Data Files</u>

The following is a list of the steps employed in preparing the data files. 1) For quick reference a 3- by 5-inch index card was created for each sample containing information collected from, published and unpublished reports, manuscripts and green cruise logbooks. These cards were used as a reference only, and were not intended as a summary of all the information available for a sample.

2) The electrophoretic gel records were then photocopied, and the photocopies were cut up, sorted into their respective samples, and placed into the appropriate sample folders.

3) Data for each sample were arranged on formatted sheets for key punching. (The key-punch sheets are available in each sample folder.) In instances where the blood samples were numbered in the electrophoretic gel records but the blood samples were not analyzed in numerical order, the photocopies were put into numerical order before key punching.

4) A listing of the new computer cards was made and checked for accuracy against the original documentation.

5) The new computer cards were read into files on computer discs, (14 files for yellowfin and 3 files for skipjack).

6) Key-punching errors were corrected in a two-step process. First, the data for each fish was punched on a card, and then the cards were repunched to verify the information. When errors involved only a small number of cards new cards were punched. When errors involved a large number of cards these errors were corrected on the computer disc. When corrections were made on the computer disc the corresponding computer cards were not repunched. Consequently, the computer cards at that point were no longer an accurate record of the data.

7) The data on the disc were rechecked for errors, primarily spacing errors, and changes were made to some of the codes used for sample identification. Most of these changes involved vessel type, and were made so that the skipjack and yellowfin codes were compatible.

8) The 14 yellowfin and 3 skipjack files were condensed into two large data files.

9) The two large data files were indexed by sample code for faster retrieval and used to print copies of the data and to create the sample summaries (Appendices 6 and 7).

# Organization of the Data files

The yellowfin and skipjack data files are listings of the data associated with each blood sample. To aid in the identification of samples, two sample indices (Appendices 3 and 4) have been included as cross-reference keys. Through the use of these indices each sample code can be matched to the vessel, cruise number, and date and location of the collection. Also included in the sample indices are the dates on which samples were analyzed electrophoretically, the approximate number of blood samples collected, and the G codes. This was done so that a person can reference the original data in one of the electrophoretic gel notebooks.

The genetic, morphometric, and sample collection data for each fish have been coded so that they can be accessed using a computer routine. A key as to how the information was coded is included at the end of this section. Except for blood samples which were analyzed more than once, each entry in a data file represents one fish. Many of the pre-1976 blood samples have two sets of data. This is a consequence of the way each blood sampler was marked when it was collected. The first set of data includes sample collection information, morphometric data, and the results of the esterase, transferrin, and PGI analyses for any fish that was used for morphometric studies. The second set of data contains sample collection data and the results of the PGI analysis for the remainder of the fish in the sample. The first 50 fish in a sample were often used as a source of morphometric data, so each sampler was assigned a unique number. Using these numbers, it was possible to match the morphometric samples with their corresponding esterase, transferrin, and PGI phenotypes when compiling the yellowfin data files. The second sample set usually starts at fish number 51 and continues to fish number 200. These samplers were marked with a sample code and a length, and when the PGI analysis was performed it was impossible to match these samplers and their phenotypic scores with the phenotypic scores of the earlier esterase and transferrin analysis. Consequently, for some of the yellowfin samples in the data file, there are two sets of data with fish numbered from 51 to 200. The first set has esterase and

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transferrin data, the second has PGI data, and the fish numbers and fork lengths in one set do not correspond to those in the other. Samples arriving at the laboratory after the PGI system was described were analyzed simultaneously for esterase, transferrin, and PGI. There is only one set of data for each of these samples, and it contains information on all of the enzymatic systems. Thus the problem is only with those samples that had to be reanalyzed for PGI after this polymorphic enzyme system was discovered in February 1976. These samples can be identified using the date of analysis found in the sample index.

In order to check that the blood samples analyzed for PGI were correctly matched to the esterase and transferrin data, any questionable samples were sorted by length and the number of fish in each length category in each analysis was compared. Several discrepencies were noted in 1971 samples (Sample MP I is a good example). These inconsistencies are believed due to a change in the way some of the lengths were rounded when millimeters were converted to centimeters. In 1971 some of the samples were rounded down (e.g. 75-cm fish were fish with lengths from 750 to 759 mm.). In 1975 and later analyses all millimeter records were rounded so that 75-cm fish would represent fish having lengths from 745 to 754 mm. In this report lengths have been recorded in millimeters whenever possible to eliminate rounding problems.

In many samples the number of fish scored for esterase, transferrin, and FGI phenotypes are not identical. This is due to 1)samples being scorable for only one or two of the systems, 2) blood samples being removed after the esterase and transferrin analyses to a collection of "standards" if unusual phenotypes were evident, or 3) blood samples becoming either lost or unidentifiable after freezer storage.

A number of the yellowfin and skipjack samples are represented twice in the data files and sample summaries. This is due to these samples having been analyzed on more than one occasion for the same enzyme system(s). The following is a description of these duplicate samples and the details pertaining to their analyses.

1) In Sharp (1972) five samples are used as examples of the stability and reproducibility of the esterase and transferrin systems. In this report Sharp stated that the original 400 blood samples were reanalyzed two years after collection and found to be invariant, i.e. no time dependent loss of resolution was observed. Both the initial analyses of 1970 and the reanalyses of 1971 have been included in this report. The scoring records of the 1970 analyses are labeled CNJ A1, MRC B1, MRC C1, CNJ D1, and CNJ E1, and the phenotypic scores assigned in the 1971 reanalyses are labeled CNJ A2, MRC B2, MRC C2, CNJ D2, and CNJ E2, respectively. In both cases the modified Fujino technique was employed to separate the proteins, and many of the individual blood samples were analyzed and then reanalyzed on different days and on different gels when initial scorings were questionable. These scores were not considered to be "altered scores" by the investigator because the resulting phenotypic scores were used in a number of reports. (The summarized 1970 esterase scores were described in the IATTC May-June 1970 bi-monthly report and versions of the 1971 esterase and transferrin analyses were reported in Sharp, 1972).

It should be noted that only an incomplete set of the original 1970 scorings is available. Consequently, it was necessary to abstract some of these data from summary sheets created by Sharp from the original electrophoretic gel records. It is also important to note that early in 1970 Sharp reported having reproducibility problems with the esterase system (IATTC March-April 1970 bi-monthly report). These problems were reported solved by consulting with Dr. Fujino, however, and the results of the esterase analysis were reported in the IATTC May-June 1970 bi-monthly report. These esterase scores and their associated transferrin scores were used for the 1970 scorings in the yellowfin data.

If a comparison is made between the esterase and transferrin phenotypes recorded for the 1970 and 1971 analyses, several discrepencies are evident. Of the approximately 400 fish used in these analyses, a total of 46 esterase and transferrin scores do not coincide in both analyses, and 17 of the fish that were scorable in 1970 were no longer scorable, either for esterase or transferrin, in 1971. It is not known why there are disparate scores in these two analyses when the two analyses had been reported to be invariant.

Sharp (personal communication, 1983) has responded to these statements saying that, "Anyone trying to work with the data from 1969 through 1970 would have to have been there to follow the series of minor numbering shifts that occurred in the samples, or in the ordering for the analysis in order to sort out how these were juggled in order to arrive at the phenotypes. The original series were often mis-queued. There are several ways in which the tables and numbers on samples could and did get mixed up by lot and series. The moment of application of the sample numbers in the early samples was confused by having four or more people involved in the sampling, and often either numbers were duplicated or poorly written on the sample vials, ..., These samples were used in only setting up the systems employed."

It is possible that the 1971 analysis produced better resolution, or the person analyzing the gels was more confident of the scoring procedure after consulting with Fujino. But whatever the cause of the disparity, the 1970 and 1971 analyses are not good examples of protein stability or the scoring reliability of the esterase and transferrin systems.

2) The only example of the reanalysis of a yellowfin sample for the esterase and transferrin systems involving a large time interval (approximately 5 1/2 years) is the <u>Gemini K samples.</u> Sample GEM K1 was analyzed and scored by Sharp in September 1972. The same group of blood samples was reanalyzed by Kane for esterase and transferrin activity in March 1978 and for PGI activity in The 1978 analysis was later labeled GEM K2 to distinguish it February 1978. from the initial analysis. Neither of these two analyses is well suited for determining scoring reliability because many of the blood samples have no corresponding lengths and the samplers were not numbered individually so that one can not compare scorings for individual blood samples. However, if each analysis is taken as a whole, there is a considerable difference between the number of transferrin "AA" and "AB" phenotypes and a smaller difference in the number of esterase phenotypes recorded for these analyses. In the early transferrin analysis there were 117 "AA," 69 "AB," and 14 "BB," and in the early esterase analysis there are 9 "12" and 2 "13." In the 1978 analyses there are 104 "AA," 83 "AB," 13 "BB," 11 "12," and 2 "13" recorded. The disparity in the scores could be due to simple reading errors or to some combination of protein degradation and subsequent reading errors. It is possible that after repeated freezings and thawings and long storage the single banded "AA" transferrin phenotype may have become fuzzy due to the presence of breakdown products. This fuzzy banding pattern could then be mistaken for the heterozygous "AB" phenotype. Whatever the reason for the disparity, these two analyses raise some

questions about the reproduciblity and stability of the esterase and transferrin systems as they have been analyzed here.

In reference to the <u>Gemini</u> K samples and the apparent dissimilarity in the analyses, Sharp (personal communication, 1983) commented that, " The entire Gemini K sample series was confused because of tape recorder failure and sample tube marking problems. Besides that the fish sizes were variable, hence of little value to anyone looking at the data beyond its original intent - to see if Non-CYRA material differed distinctly from CYRA samples. It did not, except the fish tended to be bigger and mixed sized."

3) In the skipjack sample summaries and data files three <u>Taurus</u> samples (Cruise 1075) have been duplicated. Samples Taur B, Taur G, and Taur G2 were analyzed and scored in September 1975 by Sharp and Czapinski. This was the first large skipjack sample analyzed at the IATTC, and there is no record of how the banding patterns were scored. Sharp (personal communication, 1982) has commented that: "The numbering system used was the fastest to slowest esterase bands and were numbered 1, 2, and 3 respectively. As only one fast band was observed as a heterozygote, no 'standard' was available to evaluate these against Fujino's labels. Later, after analyzing several samples from various locations in the Pacific, and discovering the fourth allele, the labeling of the Taurus samples was changed to conform to the described labeling system." If the above were accurate, then the Taurus samples should have been renumbered to coincide with the scoring procedure reported here by increasing all the previously assigned allele numbers by 1. Thus allele 1 would become 2, 2 would become 3, and 3 would become 4 to make room for the newly discovered fast allele which would be labeled 1. However, this is not the case. In Sharp's summary sheets for these samples the esterase alleles 1, 2, and 3 that were first recorded have been changed to 4, 3, and 2, respectively. An additional allele labeled 1 was added, but was not observed in these samples. The same kind of transformation was applied to the transferrin alleles. Transferrin allele 2 was relabeled 3, allele 3 was relabeled 2, and no transferrin 1 alleles were observed in these samples. In the skipjack sample summaries and data file the scorings of the 1975 analysis have been recorded and labeled TAUR B, TAUR G, and The transformed scores used in subsequent publications (transformed TAUR G2. using the procedure outlined in Sharp's summary sheets) are labeled TAUR B1, TAUR G1, and TAUR G3, respectively.

There are two examples where the validity of these transformations may be scrutinized.

A) Sample TAUR B was first analyzed on September 2, 1975, and either Sharp read the gels or he checked the scoring records of the gels. On January 8, 1976, eight of the blood samples from TAUR B were reanalyzed and scored by both Sharp and Kane. With one exception (B34 was first recorded as a 22, was transformed to a 33, and then was recorded as a 22 on January 8) the transformation of the September 2 transferrin phenotypes matches those recorded for January 8. With the esterase scores there are two blood samples where the transformed scores do not coincide with the January 8 analysis. Fish B34 and B38 are both listed as esterase 12 in the original scoring. This scoring would normally be transformed to a 34 phenotype, but on January 8 these two blood samples were scored as 23.

B) TAUR G is a sample of 21 fish. This sample was first analyzed on September 4, 1975, and then fish 1, 2, 6, 10, 11, and 21 were reanalyzed and

scored by Sharp on September 5, 9, and 22. These blood samples were reanalyzed by Sharp and Kane on January 6 and 8, 1976. It should be noted that the scoring procedure employed on January 6 and 8 is the same procedure or labeling of phenotypes shown in Figure 1 and used for the majority of the skipjack samples analyzed. The phenotypes of these samples and the transformed phenotypes are depicted in Table 1. The transformed scores of the transferrin analyses are consistent with the January analyses with one exception. Fish 6 was recorded as a 11 on September 4, and there is no precedent for transforming this score. For the esterase system there is no consistent way to transform the scores to coincide with the January scorings. Also, some of the samples scored on September 4 and 5 do not coincide with the scores recorded for September 9 and 22. These two sets of scores should be the same if the phenotypes were scored consistently.

When the inconsistencies in the data were brought to Sharp's attention he responded (personal communication, 1983) saying, "The Taurus samples (skipjack) were analyzed without reference standards and were only originally scored to see if we could achieve the resolution needed to do the comparative studies which came after." "Rescoring a 3-4 as a 2-3 or the original 1-2 as a 2-3, or a single band 2-2 as a 3-3 during the first few scoring sessions was not novel or unexpected. That is why we reran them time and again - to get consensus for the long series to be run later. I doubt that we had really settled on the scoring system by 8 January 1976, as we were just getting started through the samples of skipjack from PNG, the October '75 cruises."

The following comments of Sharp (personal communication, 1983) are included in this report in order to clarify some of his positions and opinions concerning the above discusion of the data, and the data base as a whole:

"Anyone who would be interested in the data should be clearly warned that there is not any reason to utilize samples with great size variance, or those which have large numbers of non-scorable samples due to their probable deteriorated state. They served their purpose, to prove that they were not adequate for definitive work due to their being internally heterogeneous with respect to size-age and gene frequencies. That should never be gone through again, it is too exhausting and will prove little beyond what is already stated."

"I do not hesitate to state that scoring errors are part of the problem in any analytical technique, but in selecting and utilizing odd phenotype combinations as standards over the years and in normal situations where bloods were collected from fresh caught fish, quality deterioration was negligible. The quality problems were big in the frozen fish sampling due to stomach fluid contaminations. These were readily recognizable due to 'flaring' of the samples rather than banding; and you will find innumerable reruns during this period, including Sephadx, etc. in order to cope with these problems.

"If someone wants to study sample stability it can quickly be done from the cache of 'standards'. These were shared with Lewis and Richardson and no one ever complained."

And in summary,

"The better data sets are those from the collections of uniform fish, analyzed in sequence in that these have internal standardization, hence little question about scores and resulting frequencies. Their utility is quite clearly outlined in all of the early reports, in spite of typos and editorial disasters. The other possible analysis from the data have been done and I am not sure what will ever come of them. But the results are not different than I stated in 1977-1978."

	<u>TAUR G</u> 9/4/75		TAUR G TAUR GI			REANALYSES										
			Transform of 9/4/75		9/5/75		9/9,	9/9/75		9/9/75		9/22/75		1/6/76		1/8/76
Sampler	EST	TFN	EST	TFN	EST/TFN		EST/TFN		EST/TFN		EST/TFN		EST/TFN		EST/TFN	
#1	13	23	24	23	13	23	13	23	11					23	13	23
#2	23	23	23	23							23	23		23		
#6	34	23	12	23	34	23	22	23	22		22	23		23	34	23
#10	23	11	23	?										33	23	33
#11	24	23	13	23	24	23	34	23	34		34	23		23		23
#21	34	33	12	22	34	33	34	23	34		34	33			12	22
	TAUR BI 9/2/75		Trai	R B2 1sform 9/2/75	1/	8/76										
#33	23	23	23	23	22	23										
#34	12	22	34	33	23	22										
#35	22	33	33	22	33	22										
#36	23	33	23	22	23	22										
#37	22	33	33	22	33	22										
#38	12	33	34	22	23	22										
#39	23	33	23	22	23	22										
#40	22	23	33	23	33	23										

TABLE 1.

Key for Coded Data:

Spaces (1-6)Sample code: Usually an abbreviation of the vessel name used to identify each sample. (7-8)Species: YF Yellowfin TT Thunnus tonggol SJ Skipjack GS <u>Gymnosarda</u> sp. PS Porpoise sp. (It should be noted that the yellowfin and skipjack data files contain some data entries for other species.) (9-14)Date: (9-10)Month (January = 01, February = 02, etc.) (11-12)Day of the month (13 - 14)Last two digits of the year (15-26)Location: (15) Always contains a zero (16 - 19)Latitude in degrees and minutes Hemisphere (N or S) (20) $(2^{1}-25)$ Longitude in degrees and minutes (26)Hemisphere (E or W) (27)Gear: Type of gear used in capturing fish. (1) Purse seine (bolichero) (2) Baitboat (3) Composite purse seine (more than 1 set) (4) Composite baitboat (more than 1 stop or day) (5) or () Unknown (28)Condition of the sample and how it was taken: (1) Morphometrics and blood taken from fresh fish (2) Blood only taken from fresh fish (3) Morphometrics taken from frozen fish: blood taken from fresh fish (4) Morphometrics and blood taken from frozen fish (5) Blood only taken from frozen fish (29)Sex: (1) Male (2) Female () Unknown (0) or (3) Indeterminable (30 - 32)Fish number: If each blood sample was given a number when it was taken and the blood samples were analyzed in numerical or random order, then the number on the sampler or vial was used as the fish number. If each blood sample was given a number and the samples were analyzed in order of increasing or decreasing length, then the number assigned to the blood sample in the

gel record sheet was used for the fish number. (For analysis samplers

were arranged in racks and dispensed onto a 10X10 matrix depression plate, either by length or by sample number.)

# (33-36) <u>Length:</u>

Most of the lengths are recorded in centimeters, and the remainder in millimeters. A 75-cm fish was recorded as " 75 ", a 750-mm fish was recorded as " 750", and a 1002-mm fish was recorded as "1002." The code " NFL" means that no length was available for that fish.

(37) This space is blank.

# (38-39) Esterase score:

The esterase phenotype judged to be the most reliable was recorded here. Alternate (conditional or questionable) esterase scores were recorded in col. 49-50.

(40-41) <u>Transferrin score:</u>

The transferrin phenotype judged to be the most reliable was recorded here. An A-B allele numbering system was used for yellowfin and a 1-4 system for skipjack. Alternate (conditional or questionable) transferrin scores were recorded in col. 51-52.

# (42,43,47)<u>Repeats (R1, R2, R3)</u>

These three spaces record the number of times that each enzyme system was scored. R1 was for esterases, R2 was for transferrins, and R3 was for PGI. The rules used for assigning values to R1, R2, and R3 were as follows:

(0) Unscorable

a) The blood sample was analyzed once and was not scorable for the system.

b) The blood sample was analyzed, a conditional score was or was not assigned, and the electrophoretic gel record was marked "RR." The blood sample was then reanalyzed for the system in question and the score was again indeterminable. In these cases the columns for the most reliable score would be blank, and, if a conditional score had been assigned, it would be recorded in the appropriate alternate score columns.

c) In cases where blood samples were analyzed and scored three or more times, and each time the blood sample was analyzed the banding pattern was unclear or scorings varied, this was considered the same as unscorable.

- (1) The blood sample was analyzed and scored once.
- (2) The blood sample was analyzed twice for the same system.

a) The blood sample was analyzed twice, and the same phenotype was obtained both times.

b) the blood sample was analyzed, given a conditional score, and the electrophoretic gel record was marked "RR." The blood sample was reanalyzed, and the score assigned was the same as the conditional score.

c) The blood sample was analyzed, given a conditional score, and the electrophoretic gel record was marked "RR." The blood sample was

reanalyzed, and the score assigned was not the same as the conditional score. In this case the conditional score was recorded in the alternate score column, and the second scoring was recorded in the most reliable column.

- (3) The blood sample was analyzed for the same system three times.
  a) The blood sample was analyzed three times for the same system and the same phenotype was obtained in all cases.
  b) If a blood sample was analyzed three times, twice obtaining one score and once obtaining another score, then the score that was assigned once would be recorded in the alternate score column and the score that was assigned twice would be recorded in the most reliable column.
- (4, 5, etc.) The blood samples were analyzed more than three times. a) The blood samples were analyzed for the same system four or more time, and the same phenotype was obtained in all cases. These samples are usually those that had been used as standards in a number of different electrophoretic gels. It was not a common practice to analyze a sample more than three times if there was some ambiguity in the scoring. All samples that were analyzed three or more times without arriving at a consistent score were considered unscorable.
- (44) This space is blank.
- (45-46) <u>PGI score:</u> The PGI phenotype judged to be the most reliable was recorded here. Alternate (conditional or questionable) PGI scores were recorded in col. 53-54.
- (47) See 42,43.
- (48) This space is blank.

(49-54) <u>Alternate scores:</u> These scores were tentatively assigned when there was some ambiguity or difficulty reading the starch gels.

Υ.

- (49-50) Alternate esterase scores
- (51-52) Alternate transferrin scores
- (53-54) Alternate PGI scores
- (55) This space is blank.
- (56-76) <u>Morphometric measurements:</u>
- (56-58) Snout to first dorsal
- (59-61) Snout to second dorsal
- (62-64) Snout to anus
- (65-67) Head length
- (68-70) First dorsal to second dorsal
- (71-73) First dorsal to anus
- (74-76) Second dorsal to anus

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## APPENDICES

- 1) Addendum 1 and 2 from Sharp (1972).
- 2) Sampling instructions.
- 3) Yellowfin sample index.
- 4) Skipjack sample index.
- 5) Memorandum from Robert Francis to John R. Caliprice.
- 6) Summary of the phenotypic frequencies for each yellowfin sample.
- 7) Summary of the phenotypic frequencies for each skipjack sample.

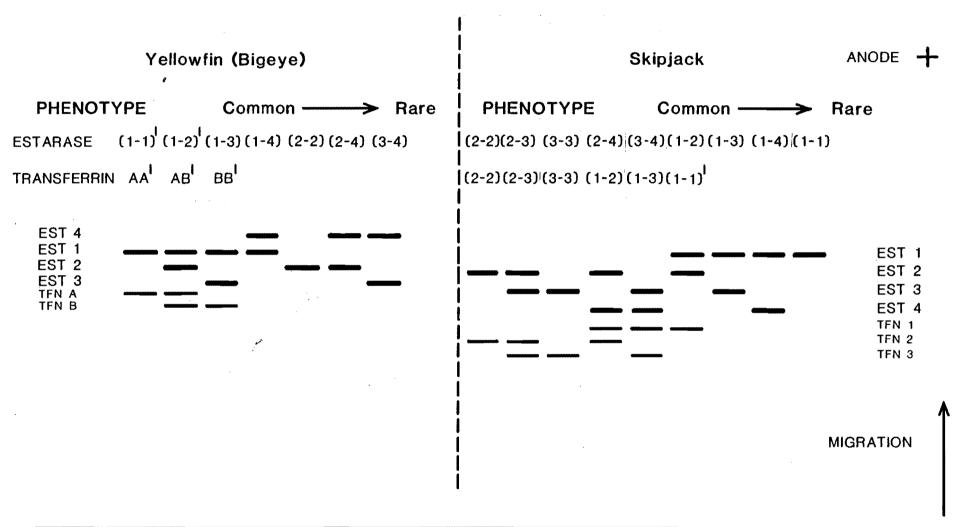


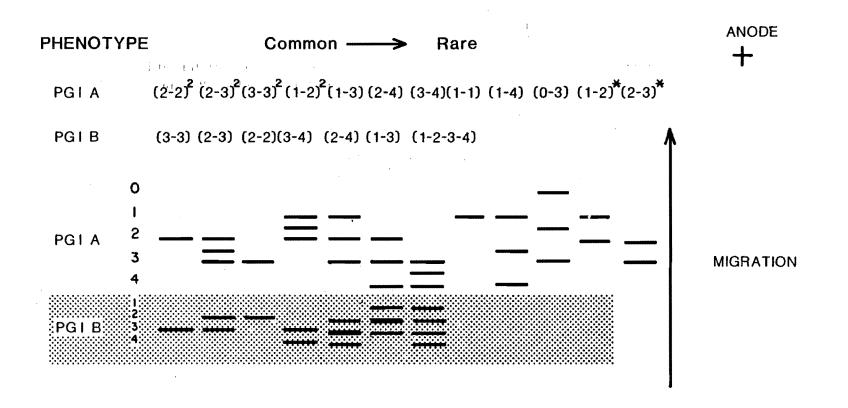
FIGURE 1. Esterase and transferrin phenotypes of yellowfin, bigeye, and skipjack.

# ORIGIN

I - Observed in both yellowfin and bigeye.

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FIGURE 2. PGI phenotypes of yellowfin and bigeye.

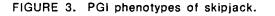


THIS AREA SHOWED ACTIVITY BUT WAS ALWAYS INDISTINCT

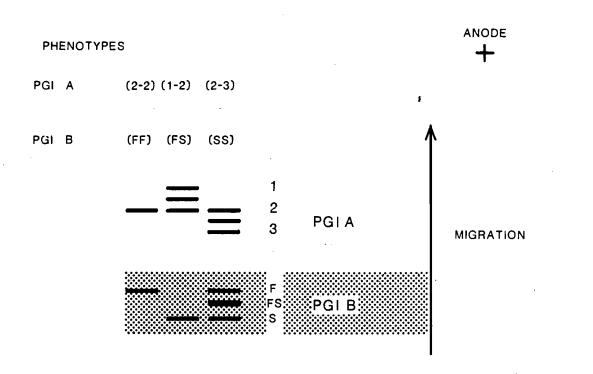


ORIGIN

- \*- Exhibited no intermediate heterozygous band.
- 2 Observed in both yellowfin and bigeye.



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THIS AREA SHOWED ACTIVITY BUT WAS INDISTINCT

ORIGIN

AREA OF DISTINCT CATHODAL BANDING

APPENDIX 1.

Addendum 1 and 2 from Sharp (1972).

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## Ι.

# A. Dehydrogenase methods

Dehydrogenase Stock buffer, pH = 7.8

TRIS (SIGMA 7-9)

Citrate

.H20

to 1 liter

121 gm

51 am

Tank buffers 1:3 of stock buffer, pH 7.8

Gel buffer 1:60 of stock buffer

Dilutions are made up with distilled water

Gels are made up with 300-330 ml of gel buffer and 34.5 grams of prehydrolysed starch (Connaught).

# B. Modified Fujino method:

Tank and gel stock buffers

- 1) 0.3 M Borate, pH = 8.2 (adjusted with NaOH)
- 2) 0.018 M LiOH and 0.19 M Borate, pH = 7.8

3) 0.0054 M Citrate and 0.04 M TRIS (Sigma 7-9) pH = 7.2 Buffer number one is used in the anodal tank. Buffer number two is used in the cathodal tank. The gel is made up of 250 ml of buffer number two and 80 ml of buffer number three and 34.5

grams of prehydrolysed starch (Connaught).

The 300 ml of gel buffer makes up two gels (147 x 212 x 5mm). The starch is suspended in 100 ml of the buffer solution and the remaining 200 ml of the buffer solution is brought to near boiling temperature. The warmed buffer is added to the starch 65

suspension and brought to a slow boil. Care is taken to evenly heat the starch solution by vigorous agitation of the solution while heating. Negative pressure is applied to the solution with an aspirator. This degasses the solution and the gels are then poured into the forms. After a short period at room temperature the gels become slightly opaque. They have a firm consistency and are then transferred to a 4°C refrigerator to set and equilibrate to the 4°C running conditions. After the gels have reached 4°C, they are fairly firm and resilent. Thirteen sample slots are made in each gel with a square tipped spatula. The samples are applied to the gels on individual 3 x 5 mm pieces of Whatman number 3 filter paper. The gels are subjected to electrophoresis for from three to six hours, depending on the resolution and separation required, at 4°C in a humidity saturated environment. The milliamperage for each gel should not exceed 50 at any time and the maximum voltage applied should be determined by the relation mA x V  $\stackrel{<}{=}$  9.000 m Watts, below which value there generally is not overheating. A typical gel is run at 45 mA and 200 volts for three hours and gives a migration of the frontal material of approximately five centimeters. Longer runs tend to yield diffuse band patterns but greater separation. The gels are stained by immersion into staining solutions. The staining processes are generally carried out in the dark due to the effect of light on the precipitation

66

reactions characteristic of each stain. After staining is obtained, the gels are washed and fixed in methanol,  $H_2^0$  and acetic acid solution (4.5:4.5:1) and photographed or stored for future reference.

40 mg

40 mg

# C. Dehydrogenase Developer Buffers

- 1) 0.5 M TRIS-HCL, pH 7.1
- 2) 0.5 M TRIS-HCL, pH 8.8

Stain solutions:

Nitro blue tetrazoliam

DPN (NAD<sup>+</sup>)

Phenazine methosulfate (PMS)10 mgMgCl (Saturated solution)0.5 ml

Appropriate developer buffer 100 ml

- 2 M Substrate (or 95% ethanol) 4 ml
- (a) Alcohol DH, ∝-glycerophorphate DH, 15 ml 0.1 M KCNUse developer buffer #l. Incubate overnight.
- (b) Glutamate DH, Lactate DH, Malate DH Use developer buffer #2. Incubate for 1 hour at 37°C in dark.

11.

# A. Esterase Developer Buffer, pH 6.55

10.5 gm NaH2P04	10.5 gm
8.95 gm Na <sub>2</sub> HPO <sub>4</sub>	8.95 gm
H <sub>2</sub> 0	to 1 liter

Substrate solution:

	l‰a-napthyl ester	2 m]
	Fast garnet	40 mg
	Fast blue in Esterase developer buffer	40 mg
	Incubate for 15-60 minutes in the dark	at 37°C
Β.	Leucine Aminopeptidase, pH 6.0	
	Stain buffer stock solutions	
	1) TRIS (Sigma 7-9)	24.2 gm
	Maleic acid	23.2 gm
	H <sub>2</sub> 0	to l liter
•	2) 0.2 M NaOH	
	Stain buffer	
	Solution 1	40 m]
	Solution 2	26 m]
	H <sub>2</sub> 0	to 200 ml
	Stain solution:	
	Stain buffer	50 ml
	Black K salt	50 mg
	L-leucyl-B-napthylamide	20 mt
	H <sub>2</sub> 0	50 m]
	Incubate at 37°C for one hour.	

# ADDENDUM 1 - TABLE 1

# **REFERENCES TO TECHNIQUES**

Transferrin

- 1. Fe<sup>59</sup> labeling technique as described by Barret, 1967.
- Amido black staining as used by Fujino's laboratory.

# Esterases

1. Shaw and Prasad, 1970. a-napthyl-acetate 2. Fujino. 1968 (*a*-napthyl-acetate only). -propionate -caprylate -laurate a-glycerophosphate 1. Shaw and Prasad (1970). 2. Nyman (1967). 1. Morrison and Wright (1966). Lactate DH 2. Sensabaugh and Kaplan, 1972. Malate DH 1. Shaw and Prasad, 1970. 2. Sensabaugh (personal communication). Glutamate DH 1. Smith, 1968. 2. Shaw and Prasad, 1970. Alcohol DH 1. Smith, 1968. 2. Shaw and Prasad, 1970. Leucine-amino-1. Smith, 1968. peptidase 2. Shaw and Prasad, 1970.

# **ADDENDUM 2**

Consider the 2 x 2  $\chi^2$  contingency table and the hypothesis of homogeneity

$$H_0 : P_1 = P_2 = P$$

against the alternative hypothesis  $H_A : P_1 = P + (\frac{C}{\sqrt{N}})$ and  $P_2 = P - (\frac{C}{\sqrt{N}})$ . Then under  $H_A$  the limiting distribution of the usual  $\chi^2$  test statistics is the noncentral  $\chi^2$  distribution with 1 degree of freedom and noncentrality parameter:

$$\lambda = \frac{4 C^2 n_1 n_2}{P - (1 - P)N^2}$$
(1)

(Chapman and Nam, 1968 and Meng and Chapman, 1966).

A statistic having this distribution is described by  $\chi^2$ ,  $(\lambda)$ . The resulting power of the test is given by 1- $\beta$  where  $\beta$  = Probability  $\chi^2_1(\lambda) > \chi^2_{1,\alpha}$  where  $\chi^2_{1,\alpha}$  is the critical value of the  $\chi^2$  distribution with 1 d.f. corresponding to the significance level.

If  $H_A$  is written  $P_1 = P_2 + \Delta$ , (1) becomes

$$\lambda = \frac{\Delta^2 n_1 n_2}{P(1-P)N}$$
(2)

Values of  $\lambda$  are tabulated in the literature for various combinations of  $\alpha$  and  $\beta$  levels. The requisite sample sizes required for given values of  $\alpha$ ,  $\beta$ , P and  $\Delta$  are obtained from equation (2). 70

APPENDIX 2.

Instructions issued to persons taking blood and morphometric samples.

		Page
1)	1972	2-2 to 2-5
2)	1972–1973	2-6 to 2-7
3)	1973	2-8 to 2-11
4)	1974	2-12 to 2-13
5)	1974-1976 Eastern Tropical Pacific	2-14 to 2-16
6)	1976 Western Pacific expedition	2-17 to 2-19

C

Morphometrics and Blood Sampling in the Pacific Ocean. (1972)

- 1. No fish over 105 cm is to be sampled.
- 2. Samples will consist of 200 fish in a range of 10-12 cms based on the occurrence of a mode or abundance of smaller fish. The enclosed form is to be used as a guide for collecting the 200 animal samples. The samples will be taken in the months of July, August or September. Under the appropriate column heading a series of approximations of lengths are given.

These groups are assumed to be relatively homogeneous. The 200 fish samples should be collected within one of these ranges when possible.

Occasionally a sample is encountered where the mode is centered on or near the size indicating a division of two of these intervals. When this is apparent, the sample should be collected assumming that 10-12 centimeters is the maximum range of overall sizes to be collected, and handled in a similar manner to the more usual method. A note should be made in the record book concerning the size interval sampled for every sample. The date, location and sample data should be recorded in the provided record book along with the well data and morphometric and tagging I.D. (set # or whatever).

3. Two sets of blood sampled fish should be flagged for morphometric use. The flags and fasteners are provided, prenumbered, and should be placed onto each fish tail and placed into a single well when possible. The flags are numbered from 1-200 and the syringes for blood sampling should be labeled correspondingly. Flag 1 should be placed on the fish from which blood # 1 was collected and so forth.

The two sets of morphometric fish should be of different size composition if possible.

4. Seven size intervals are given for use in sampling. The optimum sampling scheme would fill each of the seven intervals. This is rarely possible when fishing in a single area, and more commonly the size composition tends to be very similar until large areal shifts are made. If it appears that only 2 or 3 intervals are going to be sampled, repeated sampling can be done fifteen days after the initial sampling of a given interval. Gross area changes (> 100 miles) will make resampling an interval legitimate. An attempt should be made to sample at all the intervals, even though an area change has been made.

Page 2-2

July	Aug.	Sept.	Oct.
30-49	33-42	36-45	30-39
40-49	43-52	46-55	40-49
50-58	53-62	56-65	50-58
59-69	63-73	<b>66-7</b> 9	59-69
70-84	74-87	80-91	70-84
85-95	88-98	92-102	85-95
96-105	99-105	103-105	96-105

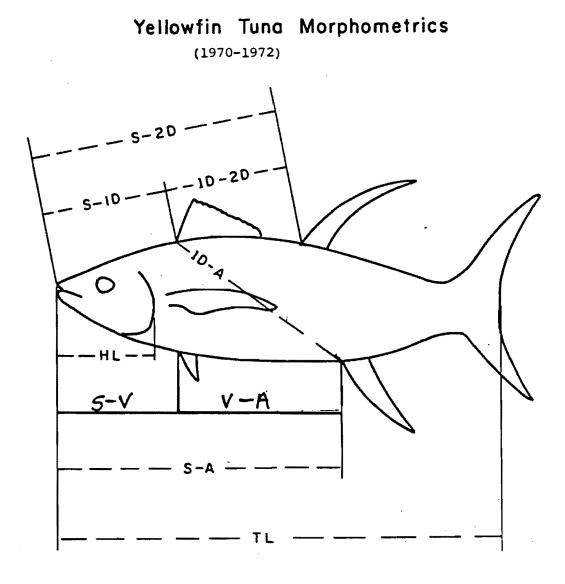
For use in collecting blood samples in the Pacific Ocean.only

	(Month of or	igin)						1. A.			*	,		0
<i>.</i>	Age in Nov.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	NOV.
	Nov. 0					6	5	· .				33	36	40
•	Oct. 1					• •	1. C				33	36	40	43
S	Sept. 2					Y.K	*			33	36	40	43	46
dn	Aug. 3					l ? ^		ŀ	33	36	40	43	46	50
groups	July 4							33	36	40	43	46	50	53
	June 5						33	36	40	43	46	50	53	56
signing	May 6					33	36	40	43	46	50	53	56	59
ц Ц	Apr. 7				33	36	40	43	46	50	53	56	59	63
318	Mar. 8			33	36	40	43	46	50	53	56	59	63	66
920	Feb. 9		33	36	40	43	46	50	53	56	59	63	66	70
	Jan. 10	33	36	40	43	46	50	53	56	59	63	66	70	74
ti I	Dec. 11	36	40	43	46	50	53	56	- 59	63	66	70	74	80
used	Nov. 12	40	43	46	50	53	56	59	63	66	70	74	80	85
sn	Oct. 13	43	46	50	53	56	59	63	66	<b>70</b> ·	74	80	85	88
	Sept.14	46	50	53	56	5	63	66	70	74	80	85	88	92
ston	Aug. 15	50	53	56	5	63	66	70	74	80	85	88	92	96
SO S	July 16	53	56	5	63	63 66	<b>70</b> ·	74	80	85	88	92	96	99
progre	June 17	56	518	63	66	70	74	80	85	88	92	96	99	103
0	May 18	59	63	66	70	74	80	85	88	92	96	99	103	106
Id	Apr. 19	63	66	70	74	80	85	88	92	96	99	103	106	109
L L	Mar. 20	66	70	74	80	85	88	92	96	99	103	106	109	112
moda1	Feb. 21	70	74	80	85	88	92	96	99	103	106	109	112	114
	Jan. 22	74	80	85	88	92	96	99	103	106	109	112	114	116
the	Dec. 23	80	85	88	92	96	99	103	106	109	112	114	116	121
	Nov. 24	85	88	92	96	99	103	106	109	112	114	116	121	123
of	Oct. 25	88	92	96	99	103		. 109	112	114	116	119		1
	Sept.26	92	· 96	99	103	106	109	112	114	116	119	121		
ф 1	Aug. 27	96	99	103	106	109	112	114	116	119	121	123		
example	July 28	99	103	106	109	112	114	116	119	121	123	126		
ez	June 29	103	106	109	112	114	116	119	121	123	126	128		
an •	May 30	106	109	112	114	116	119	121	123	·	128	130		
12.	Apr. 31	109	112	114	116	119	121	123			130	132		
ts 1972	Mar. 32	112	114	116	119	121	123				132			
s d	Feb. 33	114	116	119	121	123					134			
(This in	Jan. 34	116	119	121	123						136 137			
Ċ	Dec. 35	119	121	123							1.57			
	Nov. 36	121	123			l								
	Oct. 37	123				l					l			
	Sept.38	126			!						ł			ļ .
	Aug. 39	128				ľ								
	July 40	130												
	June 41	132									•			
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The following morphological characteristics were measured on each fish (Fig. 19): 1) total length; 2) snout to insertion of first dorsal fin; 3) snout to insertion of second dorsal; 4) snout to insertion of anal; 5) snout to insertion of ventral; 6) head length; 7) insertion of first dorsal to insertion of second dorsal; 8) insertion of ventral to insertion of anal; 9) insertion of first dorsal to insertion of anal.

## TAGGING CRUISE INSTRUCTIONS (1972-1973)

1. Flagging fish with morphometric and blood sampling:

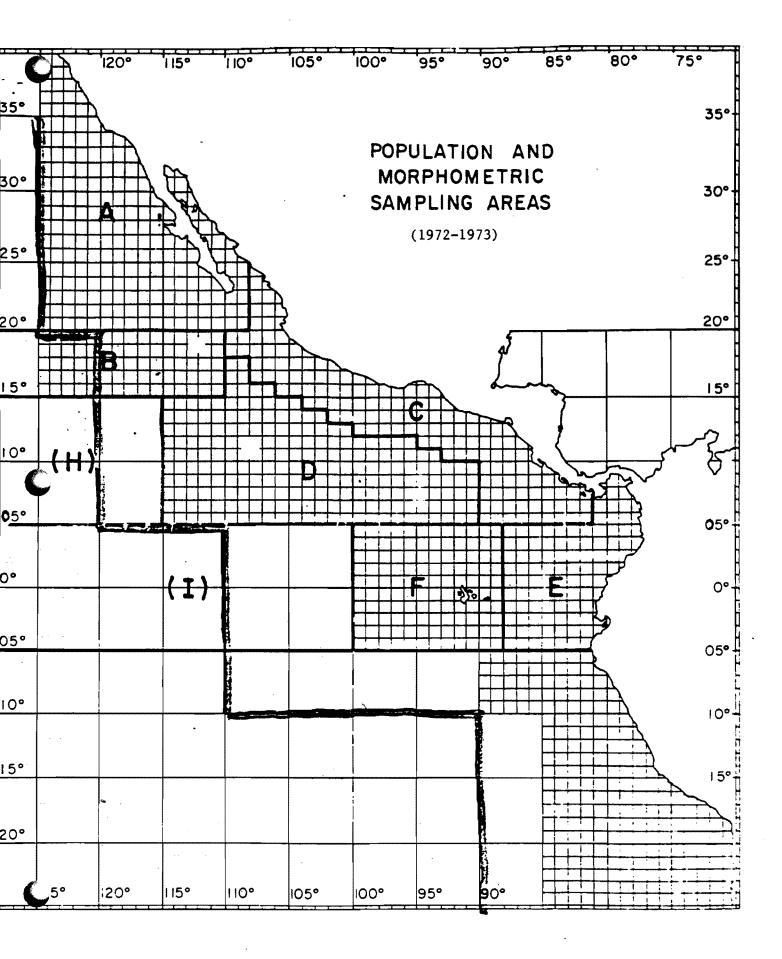
Select 200 fish ( $\sim$  1-2 tons of fish 50-70 cm long) within a 12 cm length interval. Blood samples of 2 ml should be drawn from 70 of these fish. As each fish is successfully sampled a flag should be attached above the caudal peduncle. The flags are numbered and the appropriate number should be written on the blood sample vial. When 70 blood samples have been collected, the fish from which the bloods were drawn should have morphometric measurements taken and each data set should be appropriatly numbered so that the flagged fish and blood samples can be related. If possible, get the crew to flag the remaining 130-140 animals in the sample and put them into the well. The well number should be recorded and position, date of capture, etc, recorded in the daily log. Two samples (one from large ( 85cm) and two consecutive from small ( 85cm) fish) are to be collected in this fashion.

2. Flagging fish:

Sufficient flags (3 sets of 210) are provided to flag 3 sets of fish, preferable from 3 distinct areas. If it appears that the trip is going to only visit one general area (e.g., local Banks, Mexican coast or Revillagigedos) a replicate sample, a sample of fish similar in length to the sample of fish from which bloods and morphometrics were sampled should be flagged, and if possible the third set of flags should be used on another size class of animals than that previously sampled. If three areas are worked, ther is best to flag animals of similar length intervals to the blood-morphometric sample.

- 3. One hundred blood sample vials are provided, seventy of which are to be used on the flagged fish. Sufficient RBC preservative is supplied to provide for wastage. Each of the yellowfin in the flagged sample should have <u>only</u> 2 ml drawn. An equal volume of RBC preservative should be drawn into the blood, mixed well and the samplers sealed and stored in the meat freezer.
- 4. There will be thirty extra samplers available for opportunistic sampling of various fish species which may be encountered during the trip. Samples of wahoo, sierra mackerel, skipjack and black skipjack are to be given priority for this project. Up to 5 ml of blood should be collected from each individuals of these species. Sample as many wahoo and sierra mackerel as are available, but no more than five individuals of any of the other species. These samples should be preserved and stored as described in section 3. The samples need only be identifiable to species. Code the vials and log the code and species in the day log.

Page 2-7



## (1973)

- Take blood samples and morphometric measurements from 200 yellowfin from each sampling area shown on the enclosed map, for each month (month of capture) of the year. The fish must be unbutchered and must be thawed out before working on them.
- 2. Each 200-fish sample must come from a well containing yellowfin from a single set or stop, except that samples should be taken also from baitboats landings from islands or banks where fishing was continuous and in which one or more wells of island or bank fish are available from any single location, but not necessarily from a single stop or day.
- 3. Fish sampled must be under 110 cm long, preferably from within a 10-15 cm size range and from one modal groups (\* See note on last page of these instructions).
- 4. After thawing the fish take morphometric measurements from 50 fish in good condition (no broken tails, etc.) over the complete size range. (See attached sheet showing which morphometric data are required).
- 5. Next, take blood samples from the <u>same</u> 50 fish used for morphometrics, in the same order. The boring tool should be inserted into the heart region and a core removed. Two ml of fluid blood are then drained into a vial. The boring tool must be rinsed between uses. Care should be taken not to contaminate the vials with slime or other debris when collecting blood.
- 6. The blood sample vials for these 50 fish must be marked so that we can later tell which blood sample belongs to which set of morphometric data. For example, when you draw blood from fish #35, mark the vial with "35 M" (the letter "M" stands for "Morphometric") so that we will know that that particular blood sample came from fish # 35 in the morphometric sample of 50 fish.
- 7. Next, take blood samples in the same way from the remaining 150 fish. However, these sample vials need only be marked with the length (nearest cm) of each fish.
- 8. Bag the blood vials in sample lots and freeze them as soon as possible. Keep them frozen until carried or shipped to La Jolla.
- 9. Send morphometric data and blood samples to La Jolla about once a month along with information about the origin of the samples. Use information sheets provided to record this information. Samples from T.I. should be hand-carried to La Jolla. Samples from Mayaguez should be hand-carried to San Juan and put aboard a direct flight to San Diego.

\* Note: If time permits, more than one sample should be collected if the length

#### Page 2-9

## Instructions for Blood Samples and Morphometrics (Cont.)

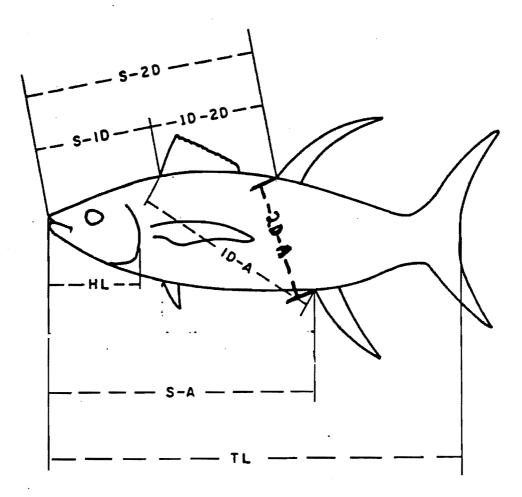
interval of the available fish has not been previously sampled within the capture month. For example, for capture month February in Area C, if one sample of 200 fish within the length interval 60-75 cm has been collected and another sample of 200 from 80-90 cm is available, it should be taken. Two samples of the same length interval, however, are unnecessary.

#### INSTRUCTIONS FOR YELLOWFIN TUNA MORPHOMETRICS

- Total length (TL). The distance from the tip of the snout (most anterior point on upper jaw), with jaws closed, to the cartilaginous median part of the caudal fork.
- 2) Snout to insertion of first dorsal fin (S-1D). The distance from the tip of the snout to the insertion of the first dorsal fin. The insertion of the first dorsal is the intersection of the anterior margin of the first dorsal spine, when the fin is held erect, with the contour of the back. This point is identical with the most anterior point of the first dorsal fin slot.
- 3) Snout to insertion of second dorsal fin (S-2D). The distance from the tip of the snout to the insertion of the second dorsal fin. The insertion of the second dorsal is the intersection of the anterior margin of the second dorsal with the contour of the back when the fin is held erect.
- 4) Snout to insertion of anal fin (S-A). The distance from the tip of the snout to the insertion of the anal fin. The insertion of the anal fin is determined in the same way as the insertion of the second dorsal.
- 5) Head length (HL). Distance from the cip of the snout to the most posterior point on the margin of the subopercle (depressing the fleshy flat extending posteriorly).
- 6) Insertion of first dorsal fin to insertion of second dorsal fin (1D-2D). -The distance from the insertion of the first dorsal fin (defined in 2) to the insertion of the second dorsal fin (defined in 3).
- 7) Insertion of first dorsal fin to insertion of anal fin (1D-A). The distance from the insertion of the first dorsal fin (defined in 2) to the insertion of the anal fin (defined in 4).

8) Insertion of second dorsal fin to insertion of anal fin (2D-A). - The distance from the insertion of the second dorsal fin (defined in 3) to the insertion of the anal fin (defined in 4).

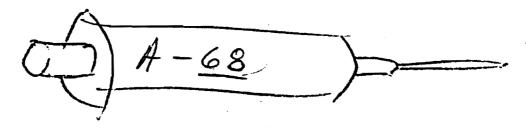




#### BLOOD SAMPLING TECHNIQUES

(1974)

- 1. Each sampler (syringe) should be maintained sterile until just prior to its use.
- 2. A sterile needle with protective cap should be placed on each syringe just prior to its use.
- 3. 4 m. of blood should be collected from the heart or gill area of each sample animal. Care should be taken to sample each fish only once.
- 4. The length of each fish sampled should be written on the sampler as shown below:



so that all numbers read from the right of the sample identifications letter (A-1). See FRANCIS FOR SAMALER F.D. M. M. Momentuc. 5. Discreet sample identification is necessary - Every sampler should have a length and sample identification letter.

- 6. Red Blood Cell Preservative (RBC preservative) is provided. After each lot has been collected an equal volume (4 m. of the preservative should be drawn into sampler, the sampler sealed, shaken well and frozen. Samples should not be allowed to thaw after this has been accomplished.
- 7. The samplers should be placed into the provided shipping containers in a freezer. On the day of shipping, dry ice should be included into the containers. Any encess room in the shipping containers should be utilized to ship empty REC preservative bottles or data cards to the Commission headquarters for refilling and recording.
- B. Plastic sheets are provided for the location data for each sample lot. Example:

Date of C	collections and I.D.	Place sample collected	Total
2/27/71 3/01/71	Sample A Sample B	Manta embarcadero 1°20'N, 92°47'W	200 200
otc.			

This information should be collected and returned with each lot of samples to the Commission's headquarters as soon as possible after the sample total of 200 blood samples have been collected..

9. The shipping containers should be transported by the most direct air route to Commission headquarters.

- 10. Samples should consist of 200 animals and be collected from one school or small boat, if possible. No animals over 84 cm should be sampled.
- 11. For on board samples on opportunistic cruises -

Lots of 200 fish from approximately 40 - 80 cm should be collected from each general fishing area visited, areas should be separated by at least 1 one-degree square or by a 15 day interval within an area, until all samples are filled. Do not return empty samplers - use then up!

12. Also get some secret duplicate blood samples.

#### BLOOD SAMPLING INSTRUCTIONS

(1974-1976 Eastern Tropical Pacific)

- Each sample should consist of 200 yellowfin tuna and be collected from one school if possible.
- 2) Each sample should come from a single modal (age) unit (approx. 15 cm. range in fork length).
- 3) No fish larger than 100 cm. fork length should be sampled.
- 4) Each sample should have an identification letter (a, B, C., .....)
- 5) For each sample, a random subsample of 50 fish should be selected. The following procedure should be followed for each of these fish.
  - 1) Assign a number (1 50) to fish.
  - 2) Write the assigned number (along with the sample ID letter)

on the blood sample vial and then draw the blood from the fish.

3) Make the following 8 measurements on each fish.

a) Total length (TL) - distance from the tip of the snout (most anterior point on the upper jaw), with jaws closed, to the cartilaginous median part of the caudal fork.

b) Snout - 1st Dorsal (PDL) - distance from the tip of the snout to the insertion of the first dorsal (most anterior point of the first dorsal fin slot).

c) Snout - second dorsal (S-2D) - distance from the tip of the snout to the anterior insertion of the second dorsal.

d) Snout - Anal - (S-A) - distance from the tip of the snout to the anterior insertion of the anal.

e) Head Length - (HL) - distance from the tip of the snout to the most posterior point on the margin of the subopercle.

#### Page 2-15

f) First dorsal - Second dorsal - (1D-2D) - distance from the anterior insertion of the first dorsal to the anterior insertion of the second dorsal.

g) First dorsal - Anal - (ID-A) - distance from the anterior insertion of the first dorsal, cross body to the anterior insertion of the anal.

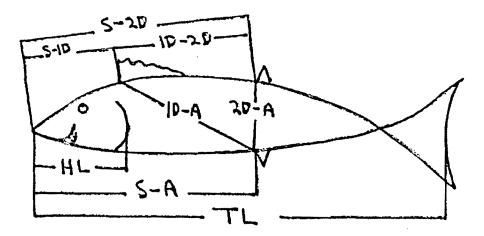
h) Second dorsal - Anal - (2D-A) - distance from the anterior insertion of the second dorsal to the anterior insertion of the anal.

4) Record these measurements along with the number assigned to fish.

All morphometric measurements should be made to the nearest millimeter.

6)

For the remaining 150 fish in the sample, blood should be collected and the length (PDL or FL in cm) should be written on the sampler as shown below, along with the sample identification letter, the length should be underlined.



- 7) Each sampler (syringe) should be maintained sterile until just prior to its use.Do not remove the attached cap until the sample is in the vial.
- A sterile needle with protective cap should be placed on each syringe just prior to its use.

2 - 4 ml. of blood should be collected from the heart of each sample fish. For most fish the needle can be inserted into the soft spot along the midline just anterior of the ventral fins.

Insert needle here Ventral Viene

Seal vial with attached cap and dispose of needle. Store the samples away from heat or in refrigerator until preservative is added. Care should be taken to sample each fish only once. Do not freeze yet.

- 10) Discreet sample identification is necessary - every sampler should have a sample indentification letter and a length (or subsample number in the case of the 50 fish morphometric subsample).
- 11) Red Blood Cell Preservative (RBC preservative) is provided. After each 200 fish sample has been collected an equal volume (2-4 ml.) or the preservative should be drawn into the sampler, the sampler sealed, shaken well and frozen. Samples should not be allowed to thaw after this has been accomplished.

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## COMISION INTERAMERICANA DEL ATUN TROPICAL INTER-AMERICAN TROPICAL TUNA COMMISSION

c/o Scripps Institution of Oceanography La Jolla, California 92038

#### WESTERN PACIFIC EXPEDITON

#### SAMPLING INSTRUCTIONS FOR BLOOD STUDY

#### (1976)

- A. Information necessary for processing these samples is: catch date, catch area (to nearest 1°), gear type, associated catch in tons (tons of YF and tons of skipjack for that set that day), sample date, personnel involved in sampling and location of sampling.
- B. A representative sample of the major size group (contribution in numbers) in the catch should be collected at approximately three week intervals. A replicate sample should be collected within a week of the first sample date and examples of secondary contributions (size range outside the initial 15 cm sampled) should be collected whenever they are available but not to exceed one sample of each secondary size range for each three week sampling period.
- C. Samples should be collected from one set where possible and composite samples should be clearly labeled as such when they occur. A composite sample is one where due to sparodic or marginal fishing conditions, two or more sets are sampled to fill out the sample requirements. This type of sampling should be kept to a minimum unless the area of capture or size range of the sample makes this sample of interest.
- Samples should be shipped to San Diego via Panama or Los Angeles. Avoid Miami connections if possible. Each shipment should be initiated from Sunday through Wednesday only, and a telegram or radio message should indicate date of departure, number of packages, the waybill number and airline carrier.
- E. Measurment apparatus should be kept dry and in sufficiently good repair as to avoid loss of sampling opportunities. The sampling materials are in boxes which are adequate for shipment of the samples in lots of 400 if the boxes are kept dry. The individual cartons of one hundred samplers should be kept and used to contain units of approximately 100 samples which are to be bagged and sealed in two lots of fifty inside these cartons. This insures adequate security of the samples so as to reduce sample leakage and subsequent weakening of the shipping boxes. The lots of \_ 400 samples should be returned in the larger box which is to be wrapped in plastic and sealed. They should also be securely tied so that rough handling will not result in severe damage. The boxes should be clearly labled as blood samples and the shipping clerks should note this on the handling forms.

The shipping address hould be as follows:

GARY D SHARP / CLIFFORD PETERSON Inter-American Tropical Tuna Commission 8604 La Jolla Shores Drive La Jolla, California 92038

Tel: 453-2820

Telegrams should be sent to:

TUNACOM, San Diego, California

## COMISION INTERAMERICANA DEL ATUN TROPICAL INTER-AMERICAN TROPICAL TUNA COMMISSION

c/o Scripps Institution of Oceanography La Jolla, California 92038

## BLOOD SAMPLING TECHNIQUES

- 1. EAch sampler (syringe) should be maintained sterile until just prior to its use.
- 2. A sterile needle with protective cap should be placed on each syringe just prior to its use.
- 3. 4 m. of blood should be collected from the heart or gill area of each sample animal. Care should be taken to sample each fish only once.
- 4. The length of each fish sampled should be written on the sampler as shown below:

St Claim ann Corrésponding F.L. Sample otolith Sample Number

so that all numbers read from the right of the sample identifications letter (A-1). For the first 20 fish, the blood and otolith samples should be from the same fish, and this noted on the syringes.

- 5. Discreet sample identification is necessary Every sampler should have a length and sample identification letter.
- 6. Red Blood Cell Preservative (RBC preservative) is provided. After each lot has been collected an equal volume (4 m.) of the preservative should be drawn into sampler, the sampler sealed, shaken well and frozen. Samples should not be allowed to thaw after this has been accomplished. THEY MAY NEVER GET SCLO, THAT is OK.
- 7. The samplers should be placed into the provided shipping containers in a freezer. On the day of shipping, dry ice should be included into the containers. Any excess room in the shipping containers should be utilized to ship data to the Commission headquarters for recording.

8.			location data for each sample lot.	
	Example:			
	Date of Co	llections and I.D.	Place sample collected	Total
	2/27/71	Sample A	Manta embarcadero	200
	3/01/71	Sample B	1 <sup>0</sup> 20'N, 92 <sup>0</sup> 47'W	200

This information should be collected and returned with each lot of samples to the Commission's headquarters as soon as possible after the sample total of 200 blood samples have been collected.

## c/o Scripps Institution of Oceanography La Jolla, California 92038

- 9. The shipping containers should be transported by the most direct air route to Commission headquarters.
- 10. Samples should consist of 200 animals and be collected from one school or small boat, if possible.
- 11. For on board sample on opprtunistic cruises. Lots of 200 fish should be collected from each general fishing area visited; areas should be separated by at least 1 one-degree square or by a 15 day interval within an area, until all samples are filled. Do not return empty samplers - Use them up !

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APPENDIX 3.

Yellowfin sample index (list of yellowfin samples).

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SAMPLE		APPENDIX	CRUISE	G		MONTH						NUMBER	NUMBER	DAIE	ANALYZI	ED
CODE	VESSEL	PAGE	NUMBER	COQE	SET	DAY	YEAR	LAI	Г	L	OG	HORPHS	BLOODS	EST	TEN	PGI
AKS	AK STROM	6-1		212		04/13		007 1					200	07/19/73		
ANC 2	ANTONINA C	6-1		208		04/15		005 2					207	07/11/73		
ANC 5	ANTONINA C	6-1		209		05/01	73	008 1					171	07/09/73		
ANM	ANNE M	6-2		211		05/02		008 2					198	06/20/73		
ANM B	ANNE M	5-2	1057	000		08/05 06/24		010 1					100 27	01/12/71		 
ann f Ann g	ANNE M ANNE M	6-2 6-3	1057 1057	000 000		06/28	70 70	003 4					27 91	00/00/71		11
ANM H	ANNE M	5-3 5-3	1058	000	29	10/21	70	012 1					89	01/29/71		
ANN I	ANNE M	6-3	1058	000	1	09/10	70	010 (					247	02/03/71		/ /
ANN J	ANNE N	6-4	1058	000	2	09/11	70	009 4					87	02/02/71		11
ANH K	ANNE H	6-4	1058	000	21	10/03	70	010 (					219	02/03/71		
APL	APOLI.O	6-4		210		05/09	73	008 3					200	07/17/73		
ATL A		6-5		000		10/31	75	000 5	50 S	001	25 8	0	24	02/19/76	02/19/76	02/09/76
ATL B		6-5		000		11/04	75	001 3	30 S	005	45 E	E 0	47	02/19/76	02/19/76	02/09/76
ATL C		6-5		000		11/07	75	002 2	20 S	001	15 8	E 0	8	02/20/78		
ATL D		6-6		000		11/08	75	001 <i>'</i>					11	02/23/76		
ATL E		6-6		000		11/11	75	001 1					27	02/23/76		
ATL F		6-6		000		11/14		004 (					72	02/23/76		
ATL 6	BOLD GOUTENS	6-7		000		11/15		005 1					105	02/24/76		
BC CAC EC	BOLD CONTEND	6-7 (-7		205		04/05 04/09	73 72	006 3					111 181	07/16/73 04/26/72		
	CACHITA CACHITA	<del>Տ-</del> 7 6-8		155 154		04/03		008 (					201	04/23/72		
	CACHITA	5-8	1064	000		09/07	71	023 0					199	10/28/71		
	CACHITA	5-9	1054	000		09/09	71	023 (					250	10/21/71		
	CACHITA	6-9	1054	000		09/10	71	023 (					199	10/12/71		
	CACHITA	6-9	1054	000		09/13		023 (					100	10/26/71		
CAC JJ	CACHITA	6-9	1064	000		10/10	71	009 5	50 N	085	55 \$	ΙΟ.	141	10/14/71	10/14/71	02/17/78
CNJ A1	CONNIE JEAN	6-10	1053	000	9	10/31	69	014 4	41 N	098	5 <b>9</b> (	4 0	199	00/00/70	00/00/70	1 1
	CONNIE JEAN	6-10	1055	000	9	10/31	69	014 4					199	04/22/71		1.1
	CONNIE JEAN	6-10	1055		19	11/07		010 (					50	00/00/70		
	CONNIE JEAN	5-11				11/07		010 0					50	04/14/71		
	CONNIE JEAN	6-11				11/16							41	00/00/70 · 04/20/71 ·		
	CONNIE JEAN CHRISTINA C	5-11 5-12		000 000		11/16 05/24							41 64	04/20/71		
	CHRISTINA C	6-12 5-12		000		05/28							155	07/01/75		
	CHRISTINA C	6-12		320		07/31							154	08/29/75		
	CHRISTINA C	6-13		000	•	07/30		020 1					11	09/25/75		
CUA	CUAUHTENOC	6-13		202		01/02		001 4					193	11	02/27/73	11
CYV	CYVONES	6-13		206	BB	04/25		024 (	N 0(	109	00 V	50	50	06/00/73	06/ <b>00</b> /73	1 1
ECU A	SANTA FE	6-14		500		05/14		001 (					31	96/21/74	06/21/74	1 1
ECU B	MARIA EMILIA			501		05/19		001 (					200	06/14/74		
ECU C	MARIA DE LOU	5-14		502	BB	05/23	74	000 (	00 5	081	00 1	1 50	171	06/19/74	06/19/74	09/01/77

Code for SET column.

C

C

BB = Sample taken from one baitboat stor.

a Number = Indicates the purse-sein set from which the sample was taken.

C = Composite set. The sample was taken from more than one purse-sein set or baitboat stop.

CODE         VESSEL         PAGE         NUMBER         CODE         SET         DAY         YEAR         LAT         LOB         MEPHS         BLOODS         EST         TFM         PCI           ECU B         BARONCHO         6-15         503 C         07/07         74         001 40 S         009 38 H         50         200         07/23/74         07/22/74         07/22/74         07/21/74         02/22/74         02/21/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/22/74         02/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/74         12/21/77         12/21/74         12/21/77         12/21/74         12/21/77         12/21/74         12/21/77         12/21/74         12/21/77         12/21/77         12/21/74         12/21/77         12/21/75         12/21/77	SAMPLE	APPENDIX	CRUISE	6		MONTH						HUMBER	NUMBER	DAI	E ANALYZE	0
ECU:         NARCLA         6-15         504         C         0.07         74         001         0.0	CODE VESSEL	PAGE	NUMBER	CODE	SET	DAY	YEAR	LAT		1.09		MORPHS	RLOODS	EST	TFN	PGI
ECU:         NARCLA         6-15         504         C         0.07         74         001         0.0																
EEU F         APOLLO II         6-15         505         58         09/25         74         000         40 H 081         93         10/21/74         10/21/74         12/11/																
ECU 6         MARIA         6-16         506         C         10/2         74         000         04 N 081         13         N         50         200         10/18/74         10/1					-											
ECU H         M ENILIA         6-16         507         C         19/2         7         101         22/3         7         101         3         508         10         9         7         7         11/12/7         12/13/77           ECU J         R03         BABEL         6-17         509         C         11/30         7         100         131         7         12/13/71         2/13/77           ECU J         R03         BASEL         6-17         500         C         11/30         7         003         04         50         200         7         12/10/71																
ECU I         RID         JUBDNES         6-16         508         C         10/27         74         001         34         S 081         40         4         50         200         /         /         11/14/74         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/14/75         12/					-											
ECU J. ROSA ISABEL       6-17       509       C       11/30       74       003       04       5       01       1       1       1       1       1/10/71 <t< td=""><td></td><td></td><td></td><td></td><td>-</td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td><td></td></t<>					-											
EEU JC         6-17         000         C         10/00         71         001         05         081         00         4         45         12/10/71         12/10/71         1/10/71																
ECU K PERLA DEL PA       6-17       510       12/01       74       002       30       S 081       16       M       50       200       7       7       12/20/74       01/10/78         ECU L       MCNTE       6-18       520       C       01/11       75       001       28       082       50       50       200       7       02/10/75       01/10/78         ECU H       MU DUVIA       6-18       522       01/17       75       000       08       08       50       200       7       02/10/75       01/10/75         ECU P       SAN PABLO       6-19       523       BB       05/17       75       000       00       N81       00       0       09       76       06/05/75       06/26/75       06/2					-									•		
ECU L         JACKTE         6-18         520         C         01/15         75         001         28         8.98         50         200         /         /         02/16/75         01/07/75           ECU H         MU CLUIA         6-18         521         01/15         75         000         0.8         0.8         0.0         50         200         /         02/14/75         01/07/75         04/07/75					L							-				
ECU H         HV OLIVIA         6-18         521         01/15         75         001         00         S 083         00         50         200         //         20/14/75         91/0777         91/0775         91/0777         91/0775         91/0777					r											
ECU N         DE LORES N         6-18         522         05/13         75         000         24         N 08         6         N         50         200         06/03/75         06/03/75         06/03/75         06/03/75         06/03/75         06/03/75         06/03/75         06/05/75         06					U											
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ECU Ø PRINCESA PAC       6-19       524       1       08/01       75       000       12       S 083       15       N       50       200       /       /       08/27/73       10/20/77         ECU R       MARTELENA       6-20       525       BB       08/24       75       000       15       N       01       15       10/20       12/03/75       12/03/75       04/21/75       04/21/75       04/21/75       04/21/75       04/21/75       04/21/75       04/21/75       03/04/75       05       01       35       N       50       200       12/03/75       04/23/75       04/23/75       04/20/77       04/20/75       03/04/75       04/21/75       04/21/75       03/04/75       05       01       35       N       50       200       12/05/75       03/04/75       05/01/75       04/20/71       04/20/75       04/20/77       01/12/76       04/20/77       01/12/76       04/20/77       01/12/76       04/20/77       01/12/76       04/20/77       01/12/76       04/20/77       04/20/77       10/10/05       08/05       10/0       0       0       04/07       04/07       04/07       04/07       04/07       04/07       04/07       04/07       04/07       04/07       04/07					BB											
ECU R       MARIELENA       6-20       525       BB       08/24       75       003       16       N 078       44       9       199       12/01/75       12/01/75       04/21/75         ECU S       RIGARDITO       6-20       526       BB       09/19       75       000       15       N 08       53       %       50       200       12/03/75       04/21/75       04/21/75         ECU U       LUCY       6-21       528       B       09/20       75       001       15       081       31       %       50       200       12/05/75       03/04/75       04/21/75         ECU U       LUCY       6-21       533       10       12/07       75       001       15       082       0       10       04/11/76       04/17/76       04/20/76       04/17/76       04/20/76       06/11/76       06/11/76       05/01/76       06/11/76       06/11/76       06/11/76       05/01/76       06/11/76       06/11/76       06/07/76       07/27       10       00       0       10       05       10       0       0       12/05/71       10/12/71       1/12/07/11       /       /       0/11/27       10       00       05       10																
ECU S       RICARDITO       6-20       526       BB       09/19       75       000       15       N 081       53       V       200       12/03/75       12/03/75       04/23/76         ECU T       FIPO       6-20       527       BB       09/20       75       001       35       N 081       53       V       200       12/03/75       12/03/75       04/23/76         ECU V       LUCY       6-21       538       5       001       15       081       31       V       50       200       12/07/75       03/04/76         ECU V       LUCY       6-21       532       12       04/11       76       000       15       N 081       20       V       /       12/07/71       04/29/76         ECU V       LUCY       6-22       531       13       04/01       76       000       15       N 081       20       V       00       36/08/76       06/11/76       05/07/76         ECU X       LUCY       6-22       000       07/27       71       001       05       081       04       0       198       10/15/71       0/17/77       0/17/77         ECU X       LUCY       6-23       533 <td>ECU R MARIELENA</td> <td>6-20</td> <td></td> <td></td> <td>BB</td> <td></td> <td>75</td> <td></td> <td></td> <td></td> <td></td> <td>50</td> <td></td> <td></td> <td></td> <td></td>	ECU R MARIELENA	6-20			BB		75					50				
ECU U       LUCY       6-21       528       5       09/28       75       001       35       S       01       35       S       01       15       S       020       /       /       12/09/7;       04/29/76         ECU V       LUCY       6-21       530       30       12/07       75       001       15       S       020       12/28/77       12/28/77       01/12/78         ECU W       LUCY       6-21       532       12       04/31       76       000       5       N E1       04       50       133       06/11/76       06/08/76       05/11/76         ECU X       LUCY       6-22       000       07/27       1       001       05       081       04       0       24       10/20/71       10/20/71       /       /         ECU X       6-23       000       10/07       71       001       05       081       04       0       145       03/28/77       03/23/77       04/11/77       6/11/75         ECU X       6-23       533       09/17       76       001       55       081       04       0       03/28/77       03/23/77       04/11/77       04/11/77       04/11/77 <t< td=""><td>ECU S RICARDITO</td><td>5-20</td><td></td><td>526</td><td>BB</td><td>09/19</td><td></td><td></td><td></td><td></td><td></td><td>50</td><td></td><td></td><td></td><td></td></t<>	ECU S RICARDITO	5-20		526	BB	09/19						50				
ECU U       LUCY       6-21       528       5       09/28       75       001       35       S       011       15       S       020       /       /       12/09/7%       04/29/7%         ECU V       LUCY       6-21       530       30       12/07       75       001       15       N       50       200       12/28/77       12/28/77       01/12/78         ECU X       LUCY       6-21       532       12       04/31       76       000       05       N       80       200       163       06/11/76       06/11/76       06/11/76       06/11/76       06/11/76       06/11/76       06/11/76       06/10/77       1001       05       081       00       0       24       10/20/71       01/29/71       /       /         ECU X       6-23       000       10/07       71       001       05       081       00       0       198       10/15/71       10/20/71       /       /         ECU X       6-23       533       0       001       05       081       00       0       198       10/15/71       10/15/71       //       //       //         ECU X       America       6-23	ECU T PIPO	5-20		527	BB	09/20	75	001 34	S 08	81 55	¥	50	200			
ECU W         LUCY         6-21         532         12         04/31         76         000         05         N 081         20         W         50         163         06/11/76         06/11/76         05/07/76           ECU X         LUCY         6-22         531         13         04/01         76         000         15         N 081         20         9         0         06/08/76         06/08/76         05/01/76         05/01/76           ECU XA         6-22         000         07/27         71         001         05         081         00         0         24         10/20/71         10/20/71         /           ECU XB         6-23         000         10/07         71         001         05         081         0         198         10/15/71         10/15/71         /         /           ECU X         6-23         533         C         08/17         76         000         581         081         3         50         200         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/23/77         03/2	ECU U LUCY	5-21		528	5	09/28	75	001 35	S 08	31 31	W	50	200	11	12/09/75	04/29/75
ECU X         LUCY         6-22         531         13         04/01         76         000         15         N         081         20         950         200         06/08/76         06/08/76         05/11/75           ECU XA         6-22         000         07/27         71         001         05         081         00         0         24         10/20/71         10/20/71         /           ECU XA         6-23         000         10/07         71         001         05         081         00         0         28         10/20/71         10/20/71         /           ECU X         AMERICA         6-23         533         C         08/17         76         005         65         081         33         50         200         03/23/77         03/21/77         04/15/77           ELU Y         AMERICA         6-24         213         06/10         73         023         00         N111         09         52         192         06/27/73         04/15/77           EL SINDRE         6-24         215         07/26         73         007         40         N 825         9         000         03/17/78         03/10/78         03/10/78	ECU V LUCY	6-21		530	30	12/07	75	001 10	S 08	82 20	Ц	50		12/28/77		
ECU XA       6-22       000       07/27       71       001       00       S 081       00       0       24       10/20/71       10/20/71       /         ECU XB       6-22       000       08/05       71       001       00       S 081       00       0       28       10/20/71       10/20/71       /       /         ECU XX       6-23       000       10/07       71       001       00       S 081       00       0       198       10/15/71       10/15/71       /         ECU Y       AMERICA       6-23       533       C       08/17       76       000       56       S 081       33       50       200       03/23/77       04/11/77         ELU LUCY       6-23       534       09/17       76       001       25       S 081       40       50       145       03/28/77       04/15/77         ELS       ELSINDRE       6-24       213       06/10       73       023       00       110       0       52       120       05/20/77       0/20/21       03/28/77       04/15/77       0/20/21       07/26/73       0/20       09/14/72       0/20       09/00/72       0/20       0/9/00/72       0/20<	ECU W LUCY	6-21		532	12	04/31	76	000 05	N OS	31 20	W	50	163	06/11/76	06/11/76	05/07/76
ECU XB       6-22       000       08/05       71       001       00       S 081       00       0       28       10/20/71       10/20/71       /         ECU XX       6-23       000       10/07       71       001       00       S 081       00       0       198       10/15/71       10/20/71       /       /         ECU XX       6-23       533       C       08/17       76       000       56       S 081       33       9       50       200       03/23/77       03/23/77       04/11/77         EU Z       LUCY       6-23       533       C       08/17       76       001       25       81       00       3/23/77       03/23/77       03/23/77       04/15/77         ELS ELSINDRE       6-24       213       06/10       73       027       00       N 110       08       50       100       /	ECU X LUCY	6-22		531	13	04/01	76	000 15	N 01	B1 20	¥	50	200	05/08/75	06/08/76	05/11/76
ECU XX       6-23       000       10/07       71       001       00       5       081       00       198       10/15/71       10/15/71       7         ECU Y       AMERICA       6-23       533       C       08/17       76       000       36       5       081       33       %       50       200       03/23/77       03/23/77       04/11/77         EU Z       LUCY       6-23       534       09/17       76       001       25       081       40       %       50       145       03/28/77       03/28/77       04/15/71       //         ELS       ELSINORE       6-24       213       06/10       73       023       00       110       04       52       192       06/27/73       06/27/73       04/15/71       //       03/17/	ECU XA	6-22		000		07/27	71	001 00	S 08	B1 00	Į,	0	24	10/20/71	10/20/71	11
ECU Y       AMERICA:       6-23       533       C       08/17       76       000       36       \$ 081       33       #       50       200       03/23/77       03/23/77       04/11/77         ECU Z       LUCY       6-23       534       09/17       76       001       25       \$ 081       40       \$ 50       145       03/23/77       03/23/77       04/15/77         ELS       ELSINORE       6-24       213       06/10       73       023       00       N 111       00       \$ 52       192       06/27/73       06/27/73       / / / / / / / / / / / / / / / / / / /		5-22		000		08/05	71					0	28	10/20/71	10/20/71	11
ECU Z       LUCY       6-23       534       09/17       76       001       25       081       40       50       145       03/28/77       03/28/77       04/15/77         ELS       ELSINDRE       6-24       213       06/10       73       023       00       N 111       00       W       52       192       06/27/73       06/27/73       /       /         ETP       ENTERPRISE       6-24       215       07/26       73       007       40       N 082       55       9       100       /<						10/07	71	001 00	S 08	B1 00	W	0	i <b>9</b> 8	10/15/71	10/15/71	11
ELS       ELSINORE       6-24       213       06/10       73       023       00       N 11       00       52       192       06/27/73       06/27/73       /         ETP       ENTERPRISE       6-24       215       07/26       73       007       40       N 082       55       ¥       50       100       /       /       /       /         GEM       K1       GEMINI       6-24       1069       000       09/06       72       012       00       140       28       9       200       69/00/72       09/00/72       03/05/78         GEM       GEMINI       6-25       1069       100       09/06       72       012       00       140       28       9       200       03/17/78       03/17/78       03/06/78         GEM       GEMINI       6-25       1069       161       09/27       72       009       03       141       32       9       200       03/17/78       03/17/78       03/16/77       03/06/78         GEM       GEMINI       6-25       1069       161       09/27       2       003       141       32       9       210       200       03/14       90       210					C							50	200			
ETP         ENTERPRISE         6-24         215         07/26         73         007         40         N 082         55         M         50         100         /         /         /         /           GEM         K1         GEMINI         6-24         1069         000         09/06         72         012         00         N 140         28         0         200         09/00/72         09/00/72         03/05/78           GEM         K2         GEMINI         6-25         1069         000         09/06         72         012         00         N 140         28         0         200         03/17/78         03/17/78         03/16/78           GEM         GEMINI         6-25         1069         160         08/18         72         012         15         N 133         05         4         0         218         12/26/72         12/26/72         09/12/77           GEM         GEMINI         6-26         1069         000         C         10/9         72         011         12         N 133         5         W         0         210         /         01/04/73         01/04/73         /////7         //////7         10/21/27         /																04/15/77
GEM K1       6-24       1067       000       09706       72       012       00       140       28       9       0       0970072       0370778       0370778         GEM K2       GEMINI       6-25       1067       000       09706       72       012       00       140       28       9       0       007072       0370778       0370777       07072       0710777       0370777       0070       071777       0370777       0105773       0970777       0370777       0370777       0370777       056       1777       0370777       03707777       0370777       0370777       03707777       0071077       0370777       037097777       0170777       057       01704773       01707777       070777       07077       070777       070777       070777       0707777       0707777       0707777       0707777       0707777       0707777       07077777										-						
GEM K2 GEMINI       6-25       1069       000       09/06       72       012       00       N 140       28       0       200       03/17/78       03/17/77       03/17/77       03/17/77       03/17/77       03/17/77       03/17/77       03/17/77       03/17/77       03/17/77       03/17/77			44/0													
GEM L       GEMINI       6-25       1069       160       08/18       72       012       15       N       133       05       4       0       200       09/14/72       09/14/72       02/29/78         GEM H       GEMINI       6-25       1069       161       09/27       72       009       03       N       141       32       W       0       218       12/26/72       02/14/72       09/14/72       09/14/72       09/12/77         GEM N       GEMINI       6-26       1069       000       11/20       72       003       14       5 081       40       W       210       /       01/05/73       09/07/77         GEM S       GEMINI       6-26       1069       000       C       10/09       72       011       12       N       133       50       W       0       210       /       01/04/73       01/04/73       /       /       /       06/09/72       /       /       06/09/72       /       /       06/09/72       /       /       06/09/72       11/09/72       11/09/72       11/09/72       11/09/72       11/09/72       11/09/72       01/25/78         N       N       N       N												-				
GEN H       GENINI       6-25       1069       161       09/27       72       009       03       N       141       32       W       0       218       12/26/72       12/26/72       09/12/77         GEN N       GEMINI       6-26       1069       000       11/20       72       003       14       5       914       0       210       /       01/05/73       09/07/77         GEN Z       GEMINI       6-26       1069       000       C       10/09       72       011       12       N       133       50       W       0       210       /       01/04/73       01/04/73       /         HST       HISTORIC       6-26       000       06/02       72       025       29       N       133       50       W       0       196       11/09/72       1/       /       IN04/73       01/04/73       1/04/74       0       100       1/09/72											-	-				
GEN N       GEMINI       6-26       1069       000       11/20       72       003       14       5       81       40       0       210       /       01/05/73       09/07/77         GEM Z       GEMINI       6-26       1069       000       C       10/09       72       011       12       N       133       50       W       0       57       01/04/73       01/04/73       /         HST       HISTORIC       6-26       000       06/02       72       025       29       N       113       35       W       184       200       /       06/09/72       /       /         IDP       INDEPENDENCE       6-27       159       C       10/31       72       010       C0       N       04       0       196       11/09/72       11/09/72       /       /         JNV       INVADER       6-27       1067       153       BB       07/21       72       018       00       159       08/09/72       08/02/76         JHN F       PARAHOUNT       6-27       000       12/02       75       003       08       150       46       E       0       213       06/17/76       06/17/76 <td></td>																
GEM Z       GEMINI       6-26       1069       000       C       10/09       72       011       12       N       133       50       W       0       57       01/04/73       01/04/73       /         HST       HISTORIC       6-26       000       06/02       72       025       29       N       113       35       W       184       200       /       /       06/09/72       /       /         IDP       INDEPENDENCE       6-27       159       C       10/31       72       010       C0       N       104       00       W       0       196       11/09/72       11/09/72       1//25/78         JHN       F       PARAHOUNT       6-27       1067       153       BB       07/21       72       018       50       N       111       00       W       0       159       C8/09/72       08/09/72       01/25/78         JHN       F       PARAHOUNT       6-27       000       12/02       75       003       08       5150       46       E       0       218       06/17/76       08/02/76       08/02/76         JHM AA       JH MARTINAC       6-28       1062       000       <																
HST       HISTORIC       6-26       000       06/02       72       025       29       N       113       35       W       184       200       /       /       06/09/72       /       /         IDP       INDEPENDENCE       6-27       159       C       10/31       72       010       C0       N 104       00       H       0       196       11/09/72       11/09/72       /       /         INV       INVADER       6-27       1067       153       BB       07/21       72       018       50       N 111       00       W       0       159       C8/09/72       01/25/78         JHN F       PARAMOUNT       6-27       000       12/02       75       003       08       5       150       46       E       0       218       06/17/76       06/17/76       08/02/76         JHN F       PARAMOUNT       6-28       1063       000       6       06/17       71       014       29       N 094       31       W       0       100       07/22/71       0/22/71       /       /         JHM AA       JH MARTINAC       6-28       1062       000       15       06/23       71       <					r											
IDP         INDEPENDENCE         6-27         159         C         10/31         72         010         C0         104         00         H         0         196         11/09/72         11/09/72         /           INV         INVADER         6-27         1067         153         BB         07/21         72         018         50         N         111         00         W         0         159         C8/09/72         08/09/72         01/25/78           JHN F         PARAMOUNT         6-27         000         12/02         75         003         08         5         150         46         E         0         218         06/17/76         06/17/76         08/02/76           JMH AA         JM HARTINAC         6-28         1063         000         6         06/17         71         014         29         N 095         20         W         0         95         07/20/71         07/20/71         /         /           JHM BB         JH MARTINAC         6-28         1062         000         15         06/23         71         011         20         N 095         50         07/20/71         07/20/71         /           JHM CC         JM			1001		5											
INV         INVADER         6-27         1067         153         BB         07/21         72         018         50         N         111         00         W         0         159         C8/09/72         08/09/72         01/25/78           JHN F         PARAHOUNT         6-27         000         12/02         75         003         08         5         150         46         E         0         218         06/17/76         06/17/76         08/02/76           JHN F         PARAHOUNT         6-28         1063         000         6         06/14         71         014         29         N         094         31         W         100         07/22/71         0//22/71         /           JHN BB         JM MARTINAC         6-28         1062         000         C         06/17         71         014         02         N         095         07/20/71         07/20/71         /           JHM BB         JM MARTINAC         6-28         1062         000         15         06/23         71         011         20         N         095         07/20/71         07/20/71         /         /           JHM CC         JM MARTINAC         6-29					r											
JHN F       PARAHOUNT       6-27       000       12/02       75       003       08       5       150       46       E       0       218       06/17/76       06/17/76       08/02/76         JHN AA JH HARTINAC       6-28       1063       000       6       06/17       71       014       29       N       094       31       W       C       100       07/22/71       0//22/71       /         JHH AA JH HARTINAC       6-28       1062       000       C       06/17       71       014       29       N       094       31       W       C       100       07/22/71       0//22/71       /       /         JHH BB JH MARTINAC       6-28       1062       000       C       06/17       71       014       02       N       095       07/20/71       07/20/71       /       /         JHH CC JH MARTINAC       6-28       1062       000       15       06/23       71       011       20       N       095       07/20/71       07/20/71       /       /         JHH DD JH MARTINAC       6-29       1062       000       17       07/02       71       024       47       N       114       29       <			1067													
JMH AA JH HARTINAC       6-28       1063       000       6       06/14       71       014       29       N       094       31       W       0       100       07/22/71       0//22/71       /         JMH BB       JH MARTINAC       6-28       1062       000       C       06/17       71       014       02       N       095       07/20/71       0//22/71       /       /         JHH BB       JH MARTINAC       6-28       1062       000       C       06/17       71       014       02       N       095       07/20/71       07/20/71       /       /         JHH CC       JH MARTINAC       6-28       1062       000       15       06/23       71       011       02       N       095       07/20/71       07/20/71       /       /         JHH DD       JH MARTINAC       6-29       1062       000       17       07/02       71       024       47       N       114       29       W       0       100       /       07/22/71       02/28/78         JHH EE       JH MARTINAC       6-29       1063       000       07/03       71       024       51       N       114       29					**											
JHH BB JH MARTINAC         6-28         1062         000         C         06/17         71         014         02         N         095         07/20/71         07/20/71         /           JHH CC JH MARTINAC         6-28         1062         000         15         06/23         71         011         20         N         095         20         W         0         95         07/20/71         07/20/71         /         /           JHH CC JH MARTINAC         6-28         1062         000         15         06/23         71         011         20         N         095         54         0         89         07/20/71         07/20/71         /         /           JHH DD JH MARTINAC         6-29         1062         000         17         07/02         71         024         47         N         114         29         W         0         100         /         07/22/71         02/28/78           JHH EE JM MARTINAC         6-29         1063         000         07/03         71         024         51         N         114         29         W         0         138         /         07/23/71         02/28/79           JNC         JEANETTE C			1063		6											
JHH CC JH MARTINAC       6-28       1062       000       15       06/23       71       011       20       N 094       55       W       0       99       07/20/71       07/20/71       /         JHH DD JH MARTINAC       6-29       1062       000       17       07/02       71       024       47       N 114       29       W       0       100       /       07/22/71       02/28/78         JHH EE JH MARTINAC       6-29       1063       000       07/03       71       024       51       N 114       29       W       0       100       /       07/23/71       02/28/78         JHH EE JH MARTINAC       6-29       1063       000       07/03       71       024       51       N 114       29       W       0       138       /       07/23/71       02/28/78         JNC       JEANETTE C       6-29       214       06/30       73       007       00       N 082       00       W       50       60       11/28/73       11/28/73       /         JNC       JEANETTE C       6-30       1078       305       BB       11/17       75       019       00       N 112       100       12/11/75       12																
JHH DD JH MARTINAC         6-29         1062         000         17         07/02         71         024         47         N         114         29         N         0         100         /         07/22/71         02/28/78           JHH EE JH MARTINAC         6-29         1063         000         07/03         71         024         51         N         114         29         N         0         138         /         07/23/71         02/28/78           JNC         JEANETTE C         6-29         214         06/30         73         007         00         N         082         00         11/28/73         11/28/73         /           KRM KD KAREN HARY         6-30         1078         305         BB         11/17         75         019         00         N         12         100         12/11/75         12/11/75         03/10/76																
JNC         JEANETTE C         6-29         214         06/30         73         007         00         N         082         00         ¥         50         60         11/28/73         11/28/73         /           KRM KD KAREN MARY         6-30         1078         305         BB         11/17         75         019         00         N         122         100         12/11/75         12/11/75         03/10/76	JHH DD JH HARTINAC															
KRM KB KAREN MARY 6-30 1078 305 BB 11/17 75 019 00 N 112 00 W 52 100 12/11/75 12/11/75 03/10/76		6-29	1063	000		07/03	71	024 51	N 13	14 29	y	0	138	11	07/23/71	02/28/79
						06/30	73	007 00	N 08	32 00	Ņ	50	60	11/28/73	11/28/73	11
KRM KS KAREN MARY 6-30 1078 000 BB 11/02 75 018 50 N 111 00 4 0 145 12/15/75 12/15/75 03/11/76					-								100			
	KRM KS KAREN MARY	6-30	1078	000	BB	11/02	75	018 50	N 11	1 00	Ч	0	145	12/15/75	12/15/75	03/11/76

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SAMPLE	APPENDIX	CRUISE	G		MONTH							NUMBER	NUMBER	BAIE	ANALYZEI	1
CODE VESSEL	PAGE	NUMBER	CODE	SET	Day	YEAR	Lf	¥T	ł	.06		MORPHS	8LOODS	EST	TEN	PGI
MB MARY BARBARA	6-30		203		03/08	73	005	05 1	1 082	2 15	¥	52	133	04/30/73	04/30/73	1.7
ME B MARY ELIZABE	6-31		000	7	08/05		000					0	200		04/01/77	
ME E MARY ELIZABE				11	08/13	76	001					0	199	03/30/77		
ME F MARY ELIZABE	6-31			19	08/21	76	001					0	112	05/03/77		
MK B MARY K	6-32	1079		BB	06/15	76	019					50	199	07/03/76		
MK B2 NARY K	6-32	1082		BB	10/13	76	019					50	500	06/03/77		
MK C MARY K	5-32	1079		BB	05/14	76	019					24	24	11	/ /	07/25/76
HK E MARY K	6-33	107 <del>9</del>		BB	06/19	76	021					50	200		07/12/75	
MK F MARY K	6-33	1079		BB	06/20	76	022					50	121	07/15/76		
NP A MARCO POLO	6-33	1073	404		10/29	74	010					50	168		11/20/74	08/15/77
MP A1 MARCO POLO	5-34	1066	000	C	11/11	71	007					0	205	12/02/71		/ /
MP B MARCO POLO	6-34	1073	405		11/07	74	009					50	54	11/25/74		11
MP BC MARCO POLO	6-34	1066	000	C	11/19	71	009					Ú	311	11/29/71		1.7
MP C MARCO POLO	6-35	1073	406		11/08	74	009					50	189	11/25/74		11
MP D1 MARCO POLO	6-35	1066		23	11/22	71	009					0	8	12/10/71		11
MP E1 HARCO POLO	6-35	1065		24	11/23	71	009					0	34	11/30/71		11
MP 5 MARCO POLO	6-36	1072		6	04/18	74	012					52	152	06/28/74		
MP I MARCO POLO	6-36	1073	403		10/23	74	009					50	173		11/18/74	
MP O MARCO POLO	6-36	1072	401	19	04/25	74	012					50	199	06/25/74		
NP P MARCO POLO	<del>5-</del> 37	1072	402	45	05/13	74	009					50	134		06/21/74	
HRA H MARY ANTOINE		1068		1	07/28	72	800					0	200	08/31/72		
MRA I MARY ANTOINE		1068	157	20	08/14	72	018					0	204		08/28/72	
MRA X MARY ANTOINE		1068	000		08/04	72	009					0	176	08/31/72		
MRC B1 MARY CARMEN	6-38	1054		BB	10/29	69	015					0 0	16		00/00/70	
MRC B2 MARY CARMEN	6-38	1054		BB	10/29	69			1 099			0	16	04/22/71		
MRC C1 MARY CARMEN	6-39	1054	000	BB	11/05	69			109			C A	100		00/00/70	
HRC C2 MARY CARMEN	6-39	1054		BB	11/06	69			109			0	100	04/21/71		/ /
NRQ 1 TANUI	6-39			0	09/00				5 140				35		03/21/78	
MRQ 2 MARY K	6-40			0	03/00		009						70	05/16/78		
MRT MARIETTA	6-40	1071		11	10/15	-	800			-		70	70 705	11/28/73		/ /
MRT L1 MARIETTA	6-40				11/19									01/18/71		
MRT L2 MARIETTA	6-41			4	11/15		009						168	01/18/71		
MRT M1 MARIETTA	6-41			5	11/08		009						131 18	01/25/71		· / /
HRT M2 MARIETTA	6-41	1059 1059		6 7	11/09 11/09	70 70	009 009					0 0	16 154	12/28/70		1 1
MRT N MARIETTA MRT P MARIETTA	చ−42 6−42			10	11/14		014					0	93	12/28/70		
MRT Q MARIETTA	6-42	1057		11	11/19	70	008					õ	268	12/15/70		
MRT R1 MARIETTA	6-43	1057			41/19		009					0	200 69		12/16/70	
MRT R2 MARIETTA	5-43	1057	000	13	11/20	70	009					õ	174	12/21/70		
NRT R3 MARIETTA	6-43	1057	000	14	11/20	70	009					0	140	12/15/70		
HRT R4 MARIETTA	5-44	1057	000	15	11/21	70	010					ò	16	01/05/71		
MRT R5 MARIETTA	5-44	1059	000	16	11/21	70	010					Ő	32	01/05/71		
MRT S MARIETTA	6-44	1059		2	10/30	70	016					õ	155	01/13/71		
MRT T NARIETTA	6-45	1057	000	4	11/27	70	015			•			55		01/27/71	
MRT U MARIETTA	5-45	1057	000	20	11/29		016						126	01/05/71		
MRT V MARIETTA	5-45	1059			11/30		019						220	01/05/71		
UNI A THUTTIN	0-40	1031	VVV	£1	21/20	70	V17	100	. 101	10	(3	v	46V	41100111	~., ~., I	- V/ V// / /

PAGE 3-3

SAMPLE	APPENDIX	CRUISE	G		HONTH						NUMBER	NUMBER	DAI	E ANALYZE	0
CODE VESSEL	PAGE	NUMBER	CODE	SET	DAY	YEAR	LAT		LC	)G	MORPHS	8LOODS	EST	TEN	PGI
NRT W MARIETTA	6-46	1059	000	22	12/02	70	022 15	N	109	33 6	i 0	23	01/13/71	01/13/71	/ /
HRT XN MARIETTA	6-45	1059	000	8	11/10	70	009 26					37		01/25/71	11
PNG 1 KAISEI MARU	6-46		000	-	10/28	75	003 28					20			03/15/76
PNG 2	6-41		000	C	11/11	75	011 00					30	01/23/76	01/23/76	03/15/76
PTW 10 PACIFIC TRDW	6-47	1062	000	12	04/29	71	006 57					107	06/17/71	06/17/71	11
PTW 10 PACIFIC TRDW	6-47	1062	000	C	04/29	71					0	188	11	11	09/21/77
PTW 12 PACIFIC TRDW	6-47	1062	000	13	04/29	71	006 58	N	092	53 1	0	<b>39</b>	06/17/71	06/17/71	11
PTW 27 PACIFIC TRDW	6-48	1062	000	27	05/07	71	007 38	N	092	25 1	0	200	06/30/71	95/30/71	09/22/77
PTW 37 PACIFIC TRDW	6-48	1062	000	37	05/17	71	008 47	N	088	16 6	0	201	06/24/71	06/24/71	09/23/77
PTW 42 PACIFIC TRDW	6-48	1062	000	42	05/20	71	009 45	N	085	40 1	1 0	178	06/28/71	06/23/71	09/28/77
PTW 45 PACIFIC TRDW	6-49	1062	000	45	05/22	71	009 15	N	085	10 5	0	199	07/02/71	07/02/71	10/03/77
PTW 52 PACIFIC TRDW	6-49	1062	000	52	05/29	71	015 19	N	096	30 V	0	134	06/22/71	05/22/71	10/24/77
SEP SEA PREME	6-49		204		03/31	73	006 30	N	078	35 6	50	99	05/02.173	05/02/73	11
SEQ SEA QUEST	6-50		201	C	01/21	73	007 00					107	03/01/73	03/01/73	11
SNC A SANDRA C	6-50	1080	420	8	10/14	76	015 05					200			06/17/77
SNC B SANDRA C	6-50	1080	421	23	10/28	76	009 48					200			\$6/20/77
SNC C SANDRA C	6-51	1080	422	33	11/09	76	013 24					130			05/23/77
SNC D SANDRA C	5-51	1080	423	41	11/19	76	010 00					200			05/21/77
SNC E SANDRA C	6-51	1080	424	45	11/22	76	006 45					130			06/27/77
SNC F SANDRA C	6-52	1080	425	56	11/28	76	013 30					200			06/24/77
SNL A SAN LUCAS	6-52		300	BB	04/04	74	021 00					130		06/26/74	/ /
SNL AB SAN LUCAS	6-52		000	BB	C4/04	74	021 00					96			03/10/78
SNL B SAN LUCAS	6-53		301	BB	04/04	74	021 00					65			03/10/78
SNL C SAN LUCAS	6-53	4070	302	RB	04/07	74	021 55					135			03/01/79
STA SANTA ANITA STI SANTA ISABEL	6-53	1070	000	BB	06/26	73	022 05					70		07/12/73	
STI SANTA ISABEL STS 1 SOUTH SEAS	6-54 6-54		200 163		01/17	73 72	019 00					200 192		01/31/73 01/17/73	
STS 2 SOUTH SEAS	5-54		164	С	01/15	73	016 52					22	11	01/17/73	11
TAR A TAURUS	6-55	1075	306	BB	06/27	75	020 35					155			10/27/27
TAR C TAURUS	6-55	1075	307	RB	07/03	75	019 20					41		08/07/75	1 1
TAR D TAURUS	6-55	1075	308	RR	07/07		023 58					199		08/07/75	11/04/77
TAR E TAURUS	5-56	1075	309	BB			024 03	N	113	43 6	50	200			10/28/77
TAR E1 TAURUS	6-56	1075	000	BB	07/11		023 51					61	11	08/14/75	11/07/77
TAR F TAURUS	6-56	1075	310	BB	07/12	75	024 05	N	113	29 1	1 50	180	11	08/18/75	11/09/77
TAR H TAURUS	6-57	1075	311	88	07/18	75	019 00	N	112	00 6	50	74	11	08/20/75	11
TAR I TAURUS	6-57	1075	312	BB	07/21	75	022 03	N	110	20 1	50	200	08/20/75	03/20/75	11/08/77
TRD TRINIDAD	6-57		207		04/13	73	006 42	N	081	22	50	198	07/02/73	07/02/73	11
XX	6-58		000	C	07/18	72	002 05	S	081	00 1	0	349	07/29/72	07/29/72	01/25/78
XXY	6-58		000		07/18	72	002 05	S	081	00 6	51	51	97/27/72	07/27/72	01/26/78
XXZ	6-58		000	C	07/18	72	002 05	S	081	00 V	51	51			01/28/78
ZP A1 ZAPATA PATHF	6-59		000	5	08/02	75	002 04					53			05/1//77
ZP A2 ZAPATA PATHF	6-59		000	8	08/15	76	000 28					130			05/17/7"
ZP D1 ZAPATA PATHF	6-59		000	15	09/18	76	002 31					56			05/18/77
ZP B2 ZAPATA PATHF			000	16	09/19	76	002 03					112			05/19/77
ZP H ZAPATA PATHE			000	25	10/09		003 38					199			05/20/77
ZP I ZAPATA PATHE			000	27	10/12		001 45					109			15/23/77
ZP J ZAPATA PATHF	5-61		000	29	10/16	/5	002 15	N	154	47 E	. 0	127	08/02/77	06/02/77	05/24/77

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APPENDIX 4.

Skipjack sample index (list of skipjack samples).

SAMPLE		APPENDIX	CRUISE	6		HONTH						NUMBER	NUMBER	BATE	ANCLYZEB
CODE	VESSEL	PAGE	NUMBER	CODE	SET	DAY	YEAR	LAT		L	DG	HORPHS	BLOORS	EST	TEN
AP A	APOLLO	7-1		000		09/18	75	002 05	S	141	22 E	0	172	03/14/77	7 03/14/77
CRC C	CHRISTINA C	0-0	1077	000		10/29	75	004 59	N	103	28 V	0	20		
EC B	LUCY	7-1		000	14	09/30	75	001 25	S	081	10 0	50	200	01/06/78	5 01/05/75
EC C	LUCY	7-1		000	6	11/17	75	002 05	S	082	30 \	50	200	09/09/78	5 09/09/76
EC B	LUCY	7-2		000	14	11/19	75	002 05	i S	081	55 เ	50	200	09/13/76	5 09/13/76
EC E	LUCY	7-2		000	4	03/27	75	000 45					200	02/10/70	7 01/10/77
EC F	LUCY	7-2		000	7	03/28	75	000 40					200		7 02/15/77
EC G	JACKIE	7-3		000	BB	05/21	76	000 19	S	081	48 1	1 50	<b>?0</b> 0	09/17/78	5 09/17/75
EC H	LUCY	7-3		000		09/09	75	000 50	S	081	20 1	1 50	200	02/08/77	7 02/08/77
EC I	VIVIENNE II	7-3		000	BB	04/12	77	003 30					200		7 08/02/77
John F	PARAMOUNT	7-4		000		04/19	76	002 00					137	06/21/78	5 06/21/75
KA	KAREN MARY	7-4	1078	000	BB	10/30	75	024 12					153	01/08/78	5 01/08/75
KB	KAREN MARY	7-4	1078	000	BB	11/01	75	019 00					162		5 01/13/28
KC	KAREN HARY	7-5	1078	000	BB	11/17	75	019 00					136		5 10/15/76
HE 6	MARY ELIZABE	7-5		000	6	08/04	76	000 00					200		01/26/77
HE D	MARY ELIZABE	7-5		000	9	08/09	76	003 14					198	-	7 02/01/77
ME G	MARY ELIZABE	7-6		000	21	09/11	76	004 30					100		7 02/04/77
MK A1	NARY K	7-6	1079	000	BB	06/09	76	021 18					200		5 05/28/76
MK A2	MARY K	7-5	1082	000	BB	10/07	76	025 41					198		7 01/20/77
MK C	NARY K	7 <b>-7</b>	1082	000	BB	10/14	76	019 00					200		7 01/24/77
MK D	MARY K	7-7	1079	000	BB	06/17	76	021 16					199		5 07/01/76
HRQ	Mary K	7-7	1079	000	C	03/00	78	010 00					93		3 05/17/78
NZ A	KERRY H	7-8		000	C	02/00	76	034 00					199		5 04/01/76
NZ B	KERRY M	7-8		000	C	02/00	76	034 00					199		5 04/01/75
NZ00 1		7-8		000		12/00	75	035 00					126		5 04/15/76
NZ00 2		7-9		000		12/00	75	035 00					70		5 04/15/76
NZ00 3		7-9	·	000		12/00	75	035 00					30		5 04/15/76
PNG A	NV SHINEI H3	7-9		000	BB	10/26	75	003 55					147		5 12/18/75
PNG B	MV TAISIN M6	7-10		000	BB	10/27	75	003 55					163		5 12/18/75
PNG NH		7-10		000		06/18	76	002 45					180		7 03/21/77
PNG Y		7-10		000		11/13	75	011 52					149		6 01/20/76 6 01/20/76
PNG Z		7-11		000		11/12		011 18					187		5 01/21/76
S218		7-11		000		03/06	76	037 40					124		5 04/06/76
S223		7-11	4400	000		03/08		037 40					200		5 04/13/76
	SANDRA C	7-12	1080	000	58 DD	12/01		009 36					201		7 01/04/77
	TAURUS	7-12	1075	000	BB	07/01		020 05					157		5 09/02/75
	TAURUS	7-12	1075	000	BB	07/01		020 05					157		5 09/02/75
	TAURUS	7-13 7-17	1075	000	BB	07/12	75 75	024 15					21		5 09/04/75 5 09/04/75
	TAURUS	7-13 7-17	1075	000	BB	07/12	75 75	024 15					21		5 09/04/75 5 09/04/75
	TAURUS	7-13 7-14	1075	000	BE	07/20	75 75	021 26					200		5 09/04/75
THK 03	TAURUS	7-14	1075	000	BB	07/20	75	021 26	N	110	4/ 1	1 JV	200	V77V4773	5 09704770

Code for SET column.

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C

BB = Sample taken from one baitboat stor.

a Number = Indicates the purse-sein set from which the sample was taken.

C = Composite set. The sample was taken from more than one purse-sein set or baitboat stop.

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SAMPLE		APPENDIX	CRUISE	G		нонтн						NUMBER	NUMBER	DAIE	ANALYZED
CODE	VESSEL	PAGE	NUMBER	CODE	SET	DAY	YEAR	LAT		L06		MORPHS	BLOODS	EST	tfn
ZP B	ZAPATA PATHF			000	6	<b>08</b> /07							198		03/02/77
ZP C	ZAPATA PATHE	7-14		000	11	08/31	76	005 10	N	123 1	5 E	0	189	13/08/77	03/08/77
ZP EA	ZAPATA PATHF	7-15		000	19	09/23	76	001 16	S	139 52	? E	0	36	02/17/77	02/17/77
ZP EB	ZAPATA PATHF	7-15		000	22	09/29	75	003 13	N	149 00	) ε	Ģ	94	02/17/77	02/17/77
ZP F	ZAPATA PATHF	7-15		000	21	09/29	75	003 01	N	148 57	? E	0	200	02/23/77	02/23/77
ZP G	ZAPATA PATHF	7-16		000	24	10/08	76	003 21	N	148-19	ΡE	0	201	02/25/77	02/25/77

Memorandum from Robert C. Frances to John R. Calaprice regarding Yellowfin morphometrics.

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To:John CalapriceFrom:Bob FrancisSubject:1974-1976 Yellowfin Tuna Morphometrics

This project has had two main objectives. The first was to gather together and organize the yellowfin tuna morphometrics data taken on fresh fish during the years 1974, 1975 and 1976. The second was to examine the statistical method of stepwise canonical analysis (sometimes referred to as stepwise discriminant analysis) as to its basic discriminatory properties. In particular I was interested in determining what the effect of different fork length (FL) distributions was on the discriminatory properties of the statistical method. My basic condition is that in order to use this method samples should be standardized to a common fork length before being subjected to the canonical analysis. If this is impossible (which it very well may be, [as] I did not investigate the presence or absence of allometry in the samples) then some other statistical technique should be used.

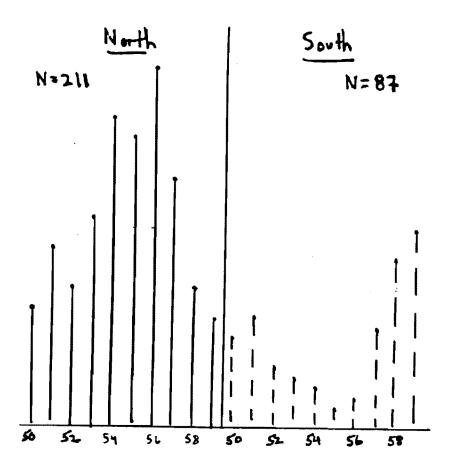
Table 1 gives a listing of all pertinent information about the samples on the master file which I left in La Jolla with you. As you can see there are several samples for which I have morphometrics which are not identifiable in Sharp's [1978] table 2 (307, 311, 313, 403, 405, 500, 550) [Sample 550 is a skipjack sample, W.P. Kane]. All 300 samples are from the northern area (N of 15 N), all 400 samples from the middle area (5 N to 15 N) and all 500 samples are from the southern area (S of 5 N). As I told you when I was in La Jolla, Gary [Sharp] mentions several morphometric samples in his paper that I cannot find in Table 2 (e.g. San Lucas A - I would guess that to be G300 although I cannot be sure [confirmed by W.P. Kane].

In order to investigate some of the properties of stepwise canonical analysis [computer program] (BMD 07M) [Dixon 1974] as they relate to the underlying size distributions of the animals in the sample, I proceeded in two directions.

First I compared two <u>simulated</u> groups of animals, drawn from the <u>same</u> population, with the only difference between them being the distributions of total length and the sample sizes. The idea arose as a result of comparing the pooled 1975 samples ranging from 500-599 mm between the north and south areas. What arose was the following classification table:

		Number	of Cases	Classified	into Groups
			Into		
		N		S	
From	N	184		27	
	S	14		73	

Thus 13% of the N animals were misclassified and 16% of the S animals were misclassified. The underlying FL distribution of the two groups was as follows:



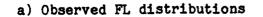
The basic question that I hoped to answer was; how much of the apparent difference between the two samples was due to the different underlying FL distributions? I therefore wrote a program which would, assuming that the N and S samples were taken from the same underlying population (null hypothesis):

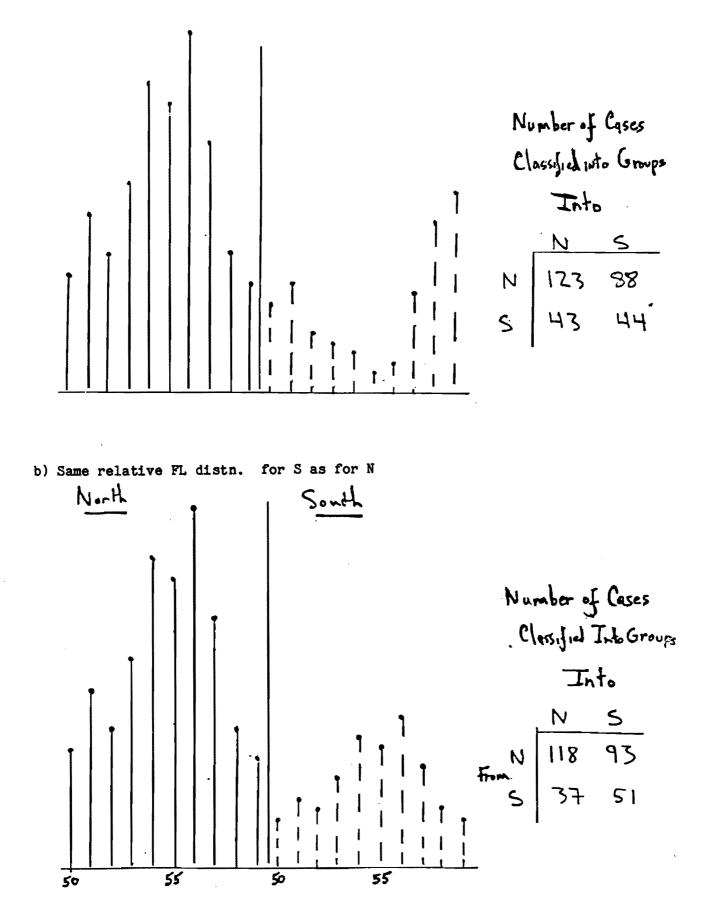
a) Estimate the mean value of the 7 other morphometrics (S-1D, S-2D, S-A, HL, 1D-2D, 1D-A, 2D-A) for each 1-cm FL class from 50 to 59 cm.

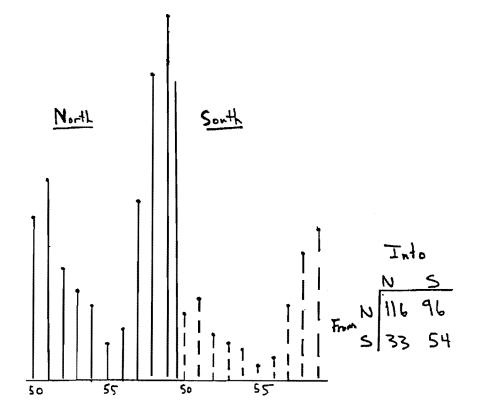
b) Estimate the <u>within-class</u> variance-covariance matrix for the 7 other morphometrics

c) Generate multivariate normal random vectors of morphometrics with the desired distributions of FL.

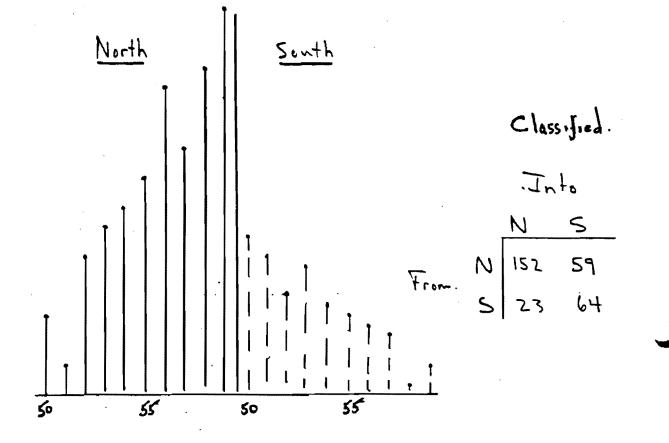
The following cases were simulated and compared using BMD 07M. Remember that the only differences between the N and S samples are the <u>sample sizes</u> and <u>distributions of FL</u>:







d) N skewed to left, S skewed to right.



It is quite obvious from this analysis that even if samples are taken from as small as 10 cm. fork length range, the actual size distributions of the samples within that range can have a significant impact on the resolution of the canonical analysis.

Finally a second set of canonical analyses were made with FL excluded from the set of variables. Virtually the same results were obtained.

In the next set of analyses I took all samples that had <u>at least</u> 20 animals in the 10 cm. FL ranges -50-59 cm, 60-69 cm, 70-79 cm - and classified them within those ranges using stepwise canonical analyses (BMD 07M). For each sample I then examined the animals that were <u>not</u> classified back into that sample to see if:

a) they tended to be classified into samples from the same region (N=North, M=Middle, S=South)

b) they tended to be classified into samples with similar mean fork lengths.

In order to examine b) above samples were categorized as  $S=\underline{small}$  if their mean fork length occurred in the lower third of the 10 cm. range,  $M=\underline{medium}$  if their mean fork length occurred in the middle third of the 10 cm. range, and  $L=\underline{large}$  if their mean fork length occurred in the upper third of the 10 cm. range. The observed classification frequencies are compared with those that one would expect to occur if classification was at random.

Tables 2 and 3 show the results of this analysis for samples with at least 20 animals in the fork length range of 50-59 cm. Thus if <u>misclassifications</u> were made at random into the 18 samples being compared, one would expect 125  $(9/17 \times (136+30+71))$  of the northern animals to be misclassified into the north, 30 of the northern animals to be misclassified into the middle and 71 of the northern animals to be misclassified into the south (Table 2), and one would expect 14  $(3/17 \times (38+36+3))$  of the <u>small</u> animals to be misclassified into S, 54 of the small animals to be misclassified into M and 9 of the small animals to be misclassified into L [Table 3]. Looking at the Chi square comparisons of observed from expected frequencies under random misclassifications, it appears that classification was, on the average, more strongly influenced by <u>size</u> than by <u>area</u> in the case of animals ranging from 50-59 cm. fork length.

Tables 4 and 5 show the results of similar analyses for samples with at least 20 animals in the fork length range of 60-69 cm. Again there is no indication that misclassifications were more strongly influenced by area than by size.

Tables 6 and 7 show similar results for samples with at least 20 animals in the fork length range of 70-79 cm. The only difference is that the <u>small</u> (S) category contains subsamples whose mean fork length is less than 75 cm. and the <u>large</u> (L) category contains subsamples whose mean fork length is greater than 75 cm. Again size seems to have a stronger influence on classification than area.

These tables (2-7) only give an indication of the relative importance of size and area in classification of yellowfin by stepwise canonical analysis on morphometrics. Obviously size and area are confounded and no attempt, here, was made to partition their effects independently. However, I think the trend is clear. It is quite obvious that the size distribution of <u>uncorrected</u> samples does have an effect on the classification procedure. And it <u>appears</u> to have a stronger effect than the area in which the animals were caught. If northern, middle or southern animals tended to show a distinctive "shape" or relative growth pattern, then one would expect animals caught in one of those areas to have a greater chance of being misclassified into another sample from that area than into a sample from another area.

My overall feeling, after manipulating these data for a while, is that stepwise canonical analysis on <u>uncorrected</u> morphometrics presents more questions than answers. If I were to seriously delve into the morphometrics on a long-term basis I would look for other avenues of analysis. I think that this could provide a very good project for an M.S. or Ph.D. student with interests in biostatistics. TABLE 1.

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Sample	Sharp's Table 2	Date	Location		FL Range (cm)
G300	San Lucas B?	4-04-74 [1]		50	46-62
G301	San Lucas?			49	49-60
G302	San Lucas C			49	51-60
G306		6-27-74 [3]		50	45-77
G307		7-03-75		41	73-87
G308		7-07-75		50	48-59
G309		7-10-75		49	50-60
G310	Taurus F	7-12-75		50	50-78
G311	[Taurus H]	7-18-75		50	69-99
G312	Taurus I	7-21-75		50	47-88
G320	Christina C-A	7-30-75 [4]		54	77-95
G305	K D	11-17-75		51	46-69
G313	[MKC]	6-14-76		24	59 <b>-</b> 76
G314	MKB	6-15-76		50	48-77
G315		6-19-76		50	42-68
G316		6-20-76		50	48-69
G321	Mary K B2			49	43-74
				50	52 68
G400	Marco Polo G			50	
G401	Marco Polo O	4-25-74		50	
G402	Marco Polo P			50	
G403	[Marco Polo I]			50	
G404	Marco Polo A		·	50	70-91
G405	[Marco Polo B]			50	65-91
G406	Marco Polo I			50	60-78
G420		10-14-76		49	79-103
G421	Sandra C B	10-28-76		49	59-82
G422	Sandra C C	11-09-76		50	
G423	Sandra C D	11-19-76		50	66-85
G424		11-22-76		50	65-87
G425	Sandra C F	11-28-76		50	43-63
G500	[A]	5-14-74		28	80-110
G501	В	5-18-74		50	56-77
G502	C	5-23-74		50	53-59
G503	D	7-08-74		47	57-68
G504	E	7-09-74		48	58-68
G505	F	9-25-74		46	54-70
G506	G	10-02-74		49	63-71
G507	H	10-28-74		50	60-73
G508	I	10-29-74		50	56-71
G509	J	12-01-74 [6]		49	67-79
G510	K	12-10-74 [7]		50	63–78

		TABLE 1.	(continued)			
G520 G521 G522 G523 G524 G525 G526 G527 G528 G530 G532 G531 G550 G533 G533 G534	L M N P Q R S T U V W X G Y Z	$1-11-75 \\ 1-15-75 \\ 5-13-75 \\ 5-29-75 \\ 8-01-75 \\ 8-24-75 \\ 9-19-75 \\ 9-20-75 \\ 9-28-75 \\ 12-07-75 \\ 3-31-76 \\ 4-01-76 \\ 6-21-76 [8] \\ 8-17-76 \\ 9-17-76 \\ 9-17-76 \\ $		50 55 50 50 50 50 50 50 50 50 50 50 50 5	35-45 40-49 41-51 87-109.7 44-55 52-58 53-59 39-53	
[2] true [3] date	sample should	name San Lucas A name San Lucas B be 6-27-75 name Christina C-AD, data	e should be '	7-31	-75	
[6] date [7] date [8] This	should should sample	name Marco Polo C be 11-30-74 be 12-01-74 is a skipjack sample, no lied by William P. Kane	t a yellowfi	n sa	mple.	

300 301 302 306 308 309 310 305 316 321	20 18 9 27 20 17 15 9 15	8 13 15 14 20 26 13 7 11	10 5 4 1 1 1 1 0	9 11 20 5 1 1 3	
302 306 308 309 310 305 316 321	9 9 27 20 17 15 9	15 14 20 26 13 7 11	4 4 1 1 1 0	20 5 1 1 3	
306 308 309 310 305 316 321	9 27 20 17 15 9	14 20 26 13 7 11	4 1 1 1 0	5 1 1 3	
308 309 310 305 316 321	27 20 17 15 9	20 26 13 7 11	1 1 1 0	1 1 3	
309 310 305 316 321	20 17 15 9	26 13 7 11	1 1 0	1 3	
310 305 316 321	17 15 9	13 7 11	1 0	3	
310 305 316 321	15 9	7 11	0		
305 316 321	15 9	7 11	_		
316 321	9	11	4	10	
321			1	4	
		9	3	7	
Total N	159	136	30	71	
xpected und			_		3.12
andom miscl	assification	125	28 <b>-</b>	84	
<b>b</b> 22	10	6	0	16	
420	14	9	I	11	
Total M	33	15	1	27	
					14.93
andom miscl	assification	25	3	15	الله فاله بلية وله من بين بلية منه وي الله
500	20	16		4 h	
		-			
221	10	20	D	14	
Total S	90	68	15	61	
rpected und	er	-			12.23
andom miscl	assification	85	17	42	
	andom miscl 422 425 Total M xpected und andom miscl 502 522 523 530 532 531 Total S xpected und	andom misclassification           422         19           425         14           Total M         33           xpected under         33           andom misclassification         33           502         20           522         21           523         13           530         17           532         9           531         10	andom misclassification       125         422       19       6         425       14       9         Total M       33       15         xpected under       33       15         andom misclassification       25         502       20       16         522       21       7         523       13       3         530       17       4         532       9       18         531       10       20         Total S       90       68         xpected under       68	andom misclassification       125       28         422       19       6       0         425       14       9       1         Total M       33       15       1         xpected under       33       15       1         andom misclassification       25       3         502       20       16       0         522       21       7       0         530       17       4       5         530       17       4       5         532       9       18       3         531       10       20       6         Total S       90       68       15         xpected under       15       15	andom misclassification       125       28       84         422       19       6       0       16         425       14       9       1       11         Total M       33       15       1       27         xpected under       20       16       0       14         andom misclassification       25       3       15         502       20       16       0       14         522       21       7       0       3         523       13       3       1       10         530       17       4       5       0         532       9       18       3       20         531       10       20       6       14         Total S       90       68       15       61         xpected under       5       61       5       61

TABLE 2. 50-59 cm. FL classified by area.

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Size	Sample	Classified into itsel		Misclassified M	l into L	x <sup>2</sup>
	in منه منه منه ۲۰ منه منه (in and in a sec in a s	******			و جهان مواند برواند برواند موان بروان بروان بروان موان بروان بروان بروان بروان بروان بروان بروان بروان بروان ب	
Small	306	9	4	17	0	
50-53 cm	530	17	6	3	0	
mean FL.	422	19	16	8	2	
	425	14	12	8	.1	
	Total	S 59	38	36	3	
Exp	ected un	der				51.14
		lassification	14	54	9	
Medium	300	20	13	13		
54-56 cm	301	18	7	10	5	
Mean FL.	302	9	6	22	8	
	502	20	0	21	9	
	308	27	3	18	1	
	309	20	3	24	1	
	310	17	1	14	2	
	305	15	0	. 9	5	
	316	9	1	12	3	
	321	15	4	13	.2	
	532	9	6	22	13	
	531	10	9	27	4	
	Total	M 189	53	205	54	
Exp	ected un	der				13.33
		lassification	73	202	37	
Large	522	21	0	10	0	و حال چین فلن باید این وی این این و
57-59	523	13	2	6	6	
Mean FL		• • •	-		<u>,</u>	
_	Total		2	16	6	
	ected un					27.73
ran	dom misc	lassification	6	17	1	
					TOTAL X <sup>2</sup> (5)	92.20

TABLE 3. 50-59 cm. FL classified by size

Area	Sample Clas	sified itself	N	Classified into	S	x <sup>2</sup>
	1nt(	) 103011 	N 	M 		ه ۱۸۰ یور بید مد مد س
N	305	14	0	0	6	
	314	6	6	3	18	
	316	12	3	4	5	
	Total N	32	9	7	29	
	Expected under					4.65
	random misclass	sification	5	11	29	
M	400	14	2	18	3	
	400	14	0	18	3	
	401	16	1	10	11	
	421	10	3	0	11	
	Total M	54	6	46	28	
	Expected under					88.79
	random misclass	ification	14	14	52 	الله الله الله عنه عنه منه <b>الله ا</b>
S	500	7	3	1	11	
0	503	14	1	10	17	
	504	14	2	10	18	
	505	10	5		23	
	506	30	5 1	2	13	
	507	9	7	2	26	
	508	12	7	3 2 3 8	20	
	510	11	4	õ	12	
	523	16	2	ĩ	4	
	533	29	1	2	16	
	534	23	6	1	11	
	Total S	175	39	41	171	
	Expected under	X				9.63
	random misclass	ification	44	59	148	
	ی هی میں اور				OTAL X <sup>2</sup> (5)	103.07

TABLE 4. 60-69 cm. FL classified by area.

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Size	Sample	Classified into itself	S	Misclassified M	into L	x <sup>2</sup>
Small	400	14	23	1	0	
60-63 cm.		14	21	1	3	
Mean FL.		16	21	2	Ō	
	503	14	12	10	õ	
	504	14	18	9	3	
	508	12	16	19	õ	
	305	14	3	2	.1	
	523	16	3	3	1	
	Metel	o esh	4.477	ha	0	
Run	ected un	S 114 der	117	47	8	54.05
		lassification	71	71	30	24103
• al.			, I 	, , 	~.	
Maditum	500	7	7	4	- 4	
Medium	500	7	7			
64-66 cm.		10	10	12	10	
mean FL.		9	21	11	4	
	314	6	3	12	9	
	316	12	3	7	2	
	421	10	6	3 6	3	
	533	29	2	6	11	
	Total		52	55	43	
	ected un					16.28
ran	dom misc	lassification	71	53	26	
-			_	16	1.	
	506	30	0	12	4	
67-69 cm		11	1	9	6	
Mean FL.	534	23	3	12	3	
	Total		4	33	13	
	ected un					31.69
ran	dom mise	lassification	24	21	6	
		الله بيه الله بيه عنه علماً من يك يك الله هي عن علم بين	440 440 440 440 4 <u>46</u> 480 480 4	و هر ها بن بين ها بي	TOTAL X <sup>2</sup> (5	

TABLE 5. 60-69 cm. FL classified by size.

Area		Classified into itself	Misclassi M	fied into. S	x <sup>2</sup>
M	403	6	15	20	
	404	19	4	3	
	405	11	15	3 8	
	406	13	10	12	
	423	10	13	7	
	424	8	9	5	
	Total M	67	66	55	
	Expected unde	r			1.00
	random miscla		60.5	60.5	
S	500 509 510 520 521 Total S Expected unde	11 2 7 15 9 44 r	6 23 9 15 22 75	3 17 7 16 17 60	1.11
	random miscla		81	54 TOTAL X <sup>2</sup> (1)	2.11

TABLE 6. 70-79 cm. FL classified by area.

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PAGE	5-14	
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ze	Sample	Classified into itself	Misclassif: S	ied into L	x <sup>2</sup>
all	403	6	26	9	
'5 cm	405	11	12	11	
an FL.	406	13	15	7	
	500	11	5	4	
	509	2	34	6	
	510	7	12	4	
	520	15	25	6	
	521	9	26	13	
<b>R</b>	Total S bected unde	74	155	60	0.45
		ssification	150.5	64.5	0.45
rge	404	 19	6	1	
5 cm.	423	10	11	11	
an FL.	424	8	7	7	
Err	Total L Dected under	37 r	24	19	15.72
		ssification	34.4	8.6	

TABLE 7. 70-79 cm. FL classified by size.

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Summary of the phenotypic scores of the yellowfin samples. The samples are listed with the Ecuador samples first, and the remainder are in reverse chronological order.

SAMPLE TFN-SCORE AKS AA 98 200 AB 83 BB 17	EST-SCORE       PGI-SCORE         1-1       192       1-1       0         1-2       6       1-2       0         1-3       0       1-3       0         1-4       0       1-4       0         2-2       1       2-2       0         2-3       0       2-3       0         2-4       0       2-4       0         3-3       0       3-3       0         3-4       0       3-4       0         4-4       0       4-4       0	
TOTAL FISH SCORED	TFN EST	PGI
	198 199 2 1 A)0.705 (1) 0.980 (1) B)0.295 (2) 0.020 (2) (3) 0.000 (3) (4) 0.000 (4)	0.000
SAMPLE TFN-SCORE ANC 2 AA 123 207 AB 73 BB 11	EST-SCORE       PGI-SCORE         1-1       193       1-1       0         1-2       9       1-2       0         1-3       0       1-3       0         1-4       2       1-4       0         2-2       2       2-2       0         2-3       0       2-3       0         2-4       0       2-4       0         3-3       0       3-3       0         3-4       0       3-4       0         4-4       0       4-4       0	
TOTAL FISH SCORED	TFN EST	PGI
	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0.000 0.000
SAMPLE TFN-SCORE ANC 5 AA 98 171 AB 64 BB 9	EST-SCORE       PGI-SCORE         1-1       164       1-1       0         1-2       5       1-2       0         1-3       1       1-3       0         1-4       0       1-4       0         2-2       0       2-2       0         2-3       0       2-3       0         2-4       0       2-4       0         3-3       0       3-3       0         3-4       0       3-4       0         4-4       0       4-4       0	
TOTAL FISH SCORED	TFN EST 171 170	PGI O
	0 1 A)0.760 (1) 0.982 (1) B)0.240 (2) 0.015 (2) (3) 0.003 (3) (4) 0.000 (4)	0 0.000 0.000 0.000

$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	
TOTAL FISH SCORED       TFN       EST       PGI         198       197       0         NUMBER UNSCORABLE       0       1       0         ALLELE FREQUENCY       (A)0.818       (1)       0.944       (1)       0.000         (B)0.182       (2)       0.056       (2)       0.000         (3)       0.000       (3)       0.000         (4)       0.000       (4)       0.000	
SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         ANM B       AA       46       1-1       89       1-1       0         100       AB       48       1-2       9       1-2       0         BB       6       1-3       0       1-3       0         1-4       1       1-4       0       2-2       0       2-2       0         2-2       0       2-2       0       2-3       0       2-3       0         2-4       1       2-4       0       3-3       0       3-3       0         3-3       0       3-3       0       3-3       0       3-4       0         4-4       0       4-4       0       4-4       0       4-4       0	
TOTAL FISH SCORED       TFN       EST       PGI         100       100       0         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY       (A)0.700       (1)       0.940       (1)       0.000         (B)0.300       (2)       0.050       (2)       0.000         (3)       0.000       (3)       0.000         (4)       0.010       (4)       0.000	
SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         ANM F       AA       11       1-1       27       1-1       0         27       AB       14       1-2       0       1-2       0         BB       2       1-3       0       1-3       0         1-4       0       1-4       0       2-2       0       2-2       0         2-2       0       2-3       0       2-3       0       2-3       0         2-4       0       2-4       0       2-4       0       3-3       0         3-3       0       3-3       0       3-3       0       3-4       0         4-4       0       4-4       0       4-4       0       4-4       0	
TOTAL FISH SCORED       TFN       EST       PGI         27       27       0         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY       (A)0.667       (1)       1.000       (1)       0.000         (B)0.333       (2)       0.000       (2)       0.000         (4)       0.000       (4)       0.000	

SAMPLE TFN-SCORE ANM G AA 43 91 AB 44 BB 3	EST-SCORE       PGI-SCORE         1-1       84       1-1       0         1-2       6       1-2       0         1-3       0       1-3       0         1-4       0       1-4       0         2-2       1       2-2       0         2-3       0       2-3       0         2-4       0       2-4       0         3-3       0       3-3       0         3-4       0       3-4       0         4-4       0       4-4       0	
TOTAL FISH SCORED	TFN EST	PGI
NUMBER UNSCORABLE	90 91 1 0	0 0
	A)0.722 (1) 0.956 (1	
(	B)0.278 (2) 0.044 (2 (3) 0.000 (3	
	(4) 0.000 (4	) 0.000
	EST-SCORE PGI-SCORE	
ANM H AA 49 89 AB 34	1-1 84 1-1 0 1-2 4 1-2 2	
BB 5	1-3 0 1-3 2	
	1-4 0 1-4 0 2-2 0 2-2 0	
	2-3 0 2-3 7	
	2-4 0 2-4 0 3-3 0 3-3 7	
	3-4 0 3-4 0	
	4_4 0 4_4 0	· · · ·
TOTAL FISH SCORED	TFN EST	PGI
NUMBER UNSCORABLE	88 88 1 1	18 9
ALLELE FREQUENCY (	A)0.750 (1) 0.977 (1	) 0.111
(	B)0.250 (2) 0.023 (2 (3) 0.000 (3	
	(4) 0.000 (4	
SAMPLE TFN-SCORE	EST-SCORE PGI-SCORE	
ANM I AA 144 247 AB 92	1-1 230 1-1 0 1-2 12 1-2 0	
BB 11	1-3 2 1-3 0	
	1-4 2 1-4 0 2-2 1 2-2 0	
	2-3 0 2-3 0	
	2-4 0 2-4 0 3-3 0 3-3 0	
	3-4 0 3-4 0	
	• • •	
	4-4 0 4-4 0	
TOTAL FISH SCORED	TFN EST	PGI
NUMBER UNSCORABLE	<b>TFN EST</b> 247 247 0 0	0 0
NUMBER UNSCORABLE ALLELE FREQUENCY (	TFN EST 247 247 0 0 A)0.769 (1) 0.964 (1	0 0 ) 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY (	<b>TFN EST</b> 247 247 0 0	0 0 0.000 0.000

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SAMPLE ANM J 87	TFN -SCORE AA 54 AB 28 BB 4	EST-SCORE 1-1 81 1-2 5 1-3 0 1-4 0 2-2 0 2-3 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE         1-1       0         1-2       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         3-3       0         3-4       0         4-4       0	
	SH SCORED	<b>TFN</b> 86 1	EST 86 1	PGI O O
	REQUENCY (	•	) 0.971 (1) ) 0.029 (2) ) 0.000 (3)	0.000 0.000 0.000 0.000
SAMPLE ANM K 218	TFN-SCORE AA 107 AB 90 BB 21	EST-SCORE 1-1 205 1-2 10 1-3 2	PGI-SCORE 1-1 1 1-2 10 1-3 24	
		1-4 1 2-2 0 2-3 0	1-4 0 2-2 16 2-3 60	
		2-4 0 3-3 0 3-4 0 4-4 0	2-4 0 3-3 55 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN	EST	PGI
		B)0.303 (2 (3	218 0 ) 0.970 (1) ) 0.023 (2) ) 0.005 (3) ) 0.002 (4)	0.307 0.584
SAMPLE APL 200	TFN-SCORE AA 122 AB 71	EST-SCORE 1-1 189 1-2 11	1–1 0	
200	BB 6	1-3 0 1-4 0	1-3 0 1-4 0	
		2-4 0	2-2 0 2-3 0 2-4 0	
			3-3 0 3-4 0 4-4 0	
TOTAL FI	SH SCORED	<b>TFN</b> 199	EST 200	PGI O
		1 A)0.791 (1 B)0.209 (2 (3	0 0.973 (1) 0.027 (2) 0.000 (3) 0.000 (4)	0 0.000 0.000 0.000

SAMPLE TFN-SCORE ATL A AA 6 24 AB 15 BB 3	1-1 23 1-2 1 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 0 1-2 1 1-3 0 1-4 0 2-2 10 2-3 12 2-4 0 3-3 1 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (. ()	B)0.437 (2 (3	24 0 ) 0.979 (1) ) 0.021 (2) ) 0.000 (3) ) 0.000 (4)	0.687 0.292
	1-1 46 1-2 1 1-3 0 1-4 0 2-2 0 2-3 0	1-3 1 1-4 0	
TOTAL FISH SCORED	TFN 47	EST 47	PGI 47
NUMBER UNSCORABLE Allele Frequency (. ()	0 A)0.649 (1 B)0.351 (2 (3	Ō	0 0.011 0.543 0.447
SAMPLE TFN-SCORE ATL C AA 2 8 AB 5 BB 1	1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 5 2-3 2 2-4 0 3-3 1 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN 8	EST 8	PGI 8
NUMBER UNSCORABLE ALLELE FREQUENCY (/	0 A)0.562 (1 B)0.437 (2 (3	0	0 0.000 0.750 0.250

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SAMPLE ATL D 11	TFN - S AA AB BB	SCORE 1 6 4	EST-5 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	SCORE 11 0 0 0 0 0 0 0 0 0 0 0	PGI-SO 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	CORE 0 2 0 5 4 0 0 0 0 0 0	
TOTAL F	ISH SCO	DRED		7N	ESI		PGI
NUMBER ALLELE		ICY (		36 (2) (3)	11 (0) 0.000 0.000 0.000	(1) (2) (3)	
SAMPLE ATL E		SCORE 9	EST-3 1-1	SCORE 27	PGI-SC	ORE 0	
AIL E 27	AA AB	12	1-2	0	1-2	1	
	BB	6	1-3 1-4		1–3 1–4	1 0	
			2-2 2-3		2-2 2-3 1	9 3	
			2-4 3-3	0	2-4	0	
			3-4	0 0	3-3 3-4	3 0	
			4-4	0	4-4	0	
TOTAL F	ISH SCO	RED		<b>7N</b> 27	ESI 27		PGI 27
NUMBER	UNSCOR	BLE	ä	27 0	27 0		27 0
	UNSCOR	BLE ICY ()	4)0.55	27 0 56 (1) 44 (2)	27 0 1.000 0.000	(1) (2)	27 0 0.037 0.593
NUMBER	UNSCOR	BLE ICY ()	4)0.55	27 0 56 (1) 44 (2) (3)	27 0 1.000	(1) (2) (3)	27 0 0.037 0.593 0.370
NUMBER	UN SCOR <i>I</i> FREQUEN	IBLE ICY (/	2 A)0.55 B)0.41	27 0 56 (1) 44 (2) (3) (4)	27 0 1.000 0.000 0.000 0.000	(1) (2) (3) (4)	27 0 0.037 0.593 0.370
NUMBER ALLELE SAMPLE ATL F	UN SCOR A FREQUEN TFN - S A A	BLE ICY (/ () SCORE 21	A)0.55 B)0.4 B)0.4 ST-S 1-1	27 0 56 (1) 44 (2) (3) (4) SCORE 68	27 0 1.000 0.000 0.000 0.000 PGI-SC 1-1	(1) (2) (3) (4) ORE 0	27 0 0.037 0.593 0.370
NUMBER ALLELE	UN SCOR <i>I</i> FREQUEN TFN – S	ABLE ICY (/ SCORE 21 36	A)0.55 B)0.44 EST-5 1-1 1-2 1-3	27 0 56 (1) 44 (2) (3) (4) SCORE 68 3 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3	(1) (2) (3) (4) ORE 0 0	27 0 0.037 0.593 0.370
NUMBER ALLELE SAMPLE ATL F	UN SCORA FREQUEN TFN - S A A AB	ABLE ICY (/ SCORE 21 36	EST-5 1-1 1-2 1-3 1-4 2-2	27 0 56 (1) 44 (2) (3) (4) SCORE 68 3 0 1 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4 2-2 2	(1) (2) (3) (4) ORE 0 0 0 0 29	27 0 0.037 0.593 0.370
NUMBER ALLELE SAMPLE ATL F	UN SCORA FREQUEN TFN - S A A AB	ABLE ICY (/ SCORE 21 36	A)0.55 B)0.44 EST-5 1-1 1-2 1-3 1-4	27 0 56 (1) 44 (2) (3) (4) SCORE 68 3 0 1 0 0	27 0 1.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4	(1) (2) (3) (4) ORE 0 0 0 0 29	27 0 0.037 0.593 0.370
NUMBER ALLELE SAMPLE ATL F	UN SCORA FREQUEN TFN - S A A AB	ABLE ICY (/ SCORE 21 36	EST-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3	27 0 56 (1) 44 (2) (3) (4) 5CORE 68 3 0 1 0 0 0 0 0 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4 2-2 2-3 3 2-4 3-3	(1) (2) (3) (4) ORE 0 0 0 0 29 0 0 3	27 0 0.037 0.593 0.370
NUMBER ALLELE SAMPLE ATL F	UN SCORA FREQUEN TFN - S A A AB	ABLE ICY (/ SCORE 21 36	EST-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4	27 0 56 (1) 44 (2) (3) (4) SCORE 68 3 0 1 0 0 0 0 0 0 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4 2-2 2-3 2-4	(1) (2) (3) (4) ORE 0 0 0 0 9 9 0 0	27 0 0.037 0.593 0.370
NUMBER ALLELE SAMPLE ATL F	UN SCORA FREQUEN TFN - S AA AB BB	BLE ICY (/ SCORE 21 36 15	A)0.55 B)0.44 EST-5 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	27 0 56 (1) 44 (2) (3) (4) 5CORE 68 3 0 1 0 0 0 0 0 0 0 0 0 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4 2-2 2-3 3-3 3-4 4-4 ESI	(1) (2) (3) (4) ORE 0 0 0 0 29 0 0 3 0 0	27 0.037 0.593 0.370 0.000
NUMBER ALLELE SAMPLE ATL F 72 TOTAL F NUMBER	UNSCORA FREQUEN TFN-S AA AB BB	BLE ICY (/ SCORE 21 36 15 DRED BLE	A)0.55 B)0.44 EST-5 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 TI	27 0 56 (1) 44 (2) (3) (4) SCORE 68 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4 2-2 2-3 3-3 1 3-4 4-4 ESI 72 0	(1) (2) (3) (4) ORE 0 0 0 0 0 0 0 0 0 0 0 0 0	27 0 0.037 0.593 0.370 0.000
NUMBER ALLELE SAMPLE ATL F 72 TOTAL F	UNSCORA FREQUEN TFN-S AA AB BB	BLE ICY (/ SCORE 21 36 15 DRED BLE ICY (/	A)0.55 B)0.44 EST-5 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 TI A)0.54	27 0 56 (1) 44 (2) (3) (4) 5CORE 68 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4 2-2 2-3 3-4 3-4 3-4 4-4 ESI 72 0 0.972	(1) (2) (3) (4) ORE 0 0 0 0 9 0 0 3 0 0 0 29 0 0 0 29 0 0 0 29 0 0 0 29 0 0 0 29 0 0 0 29 0 0 0 29 0 0 0 20 20 20 20 20 20 20 20 20 20 20	27 0.037 0.593 0.370 0.000 PGI 72 0.000
NUMBER ALLELE SAMPLE ATL F 72 TOTAL F NUMBER	UNSCORA FREQUEN TFN-S AA AB BB	BLE ICY (/ SCORE 21 36 15 DRED BLE ICY (/	A)0.55 B)0.44 EST-5 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 TI A)0.54	27 0 56 (1) 44 (2) (4) 500RE 68 3 0 1 0 0 0 0 0 0 0 0 0 0 0 0 0	27 0 1.000 0.000 0.000 0.000 PGI-S0 1-1 1-2 1-3 1-4 2-2 2-3 3-3 1 3-4 4-4 ESI 72 0	(1) (2) (3) (4) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	27 0.037 0.593 0.370 0.000 PGI 72 0.000 0.611 0.389

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TOTAL FISH SCOREDTFNESTPGI105105104NUMBER UNSCORABLE001ALLELE FREQUENCY(A)0.567(1)0.981(1)0.010(B)0.433(2)0.019(2)0.611(3)0.000(3)0.370(4)0.000(4)0.010	· ·
SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         BC       AA       54       1-1       102       1-1       0         111       AB       41       1-2       7       1-2       0         BB       16       1-3       0       1-3       0         1-4       2       1-4       0         2-2       0       2-2       0         2-3       0       2-3       0         2-4       0       2-4       0         3-3       0       3-3       0         3-4       0       3-4       0	
TOTAL FISH SCOREDTFNESTPGI1111111110NUMBER UNSCORABLE000ALLELE FREQUENCY(A)0.671(1)0.959(1)0.000(B)0.329(2)0.032(2)0.000(3)0.000(3)0.000(4)0.000	
SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         CAC EC       AA       100       1-1       166       1-1       2         361       AB       71       1-2       13       1-2       12         BB       10       1-3       0       1-3       24         1-4       2       1-4       0         2-2       0       2-2       15         2-3       0       2-3       53         2-4       0       2-4       0         3-3       0       3-3       74         3-4       0       3-4       0	
TOTAL FISH SCORED       TFN       EST       PGI         181       181       181       180         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY       (A)0.749       (1)       0.959       (1)       0.111         (B)0.251       (2)       0.036       (2)       0.264         (3)       0.000       (3)       0.625         (4)       0.006       (4)       0.000	

SAMPLE CAC FC 398	TFN-SCORE AA 98 AB 88 BB 15	EST-SCORE 1-1 193 1-2 7 1-3 0 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 3 1-2 11 1-3 34 1-4 0 2-2 13 2-3 86 2-4 0 3-3 50 3-4 0 4-4 0	
	ISH SCORED	<b>TFN</b> 201	EST 201	PGI 197
		0 A)0.706 (1) B)0.294 (2) (3) (4)	0.000 (3)	0.312
SAMPLE CAC FF 388	TFN-SCORE AA 98 AB 81 BB 20	EST-SCORE 1-1 189 1-2 9 1-3 0 1-4 0 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 1 1-2 7 1-3 28 1-4 0 2-2 13 2-3 58 2-4 0 3-3 82 3-4 0 4-4 0	
	ISH SCORED	<b>TFN</b> 199	EST 199	PGI 189
		0 A)0.696 (1) B)0.304 (2) (3) (4)	0.028 (2)	0 0.098 0.241 0.661 0.000
SAMPLE CAC GG 443	TFN-SCORE AA 125 AB 98 BB 27	EST-SCORE 1-1 240 1-2 6 1-3 0 1-4 3 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 5 1-2 11 1-3 40 1-4 0 2-2 14 2-3 62 2-4 0 3-3 60 3-4 1 4-4 0	

TOTAL FISH SCORED	TFN		EST		PGI
	250		250		193
NUMBER UNSCORABLE	0		0		0
ALLELE FREQUENCY	(A)0.696	(1)	0.978	(1)	0.158
	(B)0.304	(2)	0.016	(2)	0.262
		(3)	0.000	(3)	0.578
		(4)	0.006	(4)	0.003

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SAMPLE CAC HH 398	TFN-SCORE AA 99 AB 85 BB 14	EST-SCORE 1-1 189 1-2 6 1-3 0 1-4 3 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 2 1-2 12 1-3 26 1-4 0 2-2 24 2-3 53 2-4 1 3-3 81 3-4 0 4-4 0	
TOTAL FI	SH SCORED	<b>TFN</b> 198	EST 198	PGI 199
	NSCORABLE Requency (	1	1 1) 0.977 (1)	0
		B)0.285 (2 (3	2) 0.015 (2) 3) 0.000 (3) 4) 0.008 (4)	0.286 0.606
SAMPLE CAC II	TFN-SCORE	EST-SCORE	PGI-SCORE	
201	AB 40 BB 7	1-2 1 1-3 2	1–2 7 1–3 9	
		1-4 2 2-2 0	1-4 0 2-2 8	
		2-3 0 2-4 0 3-3 0	2-3 39 2-4 0 3-3 35	
		3-4 0 4-4 0	3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN	EST	PGI
NUMBER U Allele F	NSCORABLE	100 0 (A)0 730 (1	100 0 1) 0.975 (1)	98 3 0.082
RELEE F		B)0.270 (2 (3	2) 0.005 (2) 3) 0.010 (3) 4) 0.010 (4)	0.316 0.602
SAMPLE CAC JJ	TFN-SCORE AA 79	EST-SCORE	E PGI-SCORE 1-1 3	
279	AB 54 BB 8	· · · <del>·</del>		
		1-4 0 2-2 0	1-4 0 2-2 13	
		2-3 0 2-4 0 3-3 0	2-3 49 2-4 0	
		3-4 0 4-4 0	3-3 55 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN	EST	PGI
	NSCORABLE	141 0	141 0	138 0
ALLELE F		B)0.248 (2 (3	1) 0.975 (1) 2) 0.025 (2) 3) 0.000 (3) 4) 0.000 (4)	0.297 0.616

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CNJ A1 A 200 A	EFN-SCORE LA 115 LB 70 BB 11	2-4 0 3-3 0	1-1 0 1-2 0 1-3 0 1-4 0	
TOTAL FISH NUMBER UNS ALLELE FRE	SCORABLE QUENCY (A	(2) (3)	EST 196 4 0.957 (1) 0.043 (2) 0.000 (3) 0.000 (4)	0.000
CNJ A2 A 200 A	<b>TFN - SCORE</b> LA 111 LB 68 BB 9		PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH Number Uns Allele Fre	CORABLE QUENCY (A	(2) (3)	EST 192 7 0.958 (1) 0.042 (2) 0.000 (3) 0.000 (4)	0.000
CNJ D1 A 50 A	FN-SCORE A 22 AB 25 AB 2	EST-SCORE 1-1 47 1-2 2 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH NUMBER UNS ALLELE FRE	SCORABLE SQUENCY (A	3)0.296 (2)	EST 49 1 0.980 (1) 0.020 (2) 0.000 (3)	0.000

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(3) 0.000 (3) 0.000 (4) 0.000 (4) 0.000

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SAMPLE TFN-SCORE EST CNJ D2 AA 20 1-1 50 AB 14 1-2 BB 0 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
NUMBER UNSCORABLE ALLELE FREQUENCY (A)O.	TFN         EST         PGI           34         40         0           16         10         0           794         0.975         0.000           206         (2)         0.013         (2)         0.000           (3)         0.000         (3)         0.000         (4)         0.000
SAMPLE         TFN-SCORE         EST           CNJ E1         AA         18         1-1           41         AB         20         1-2           BB         3         1-3         1-4           2-2         2-3         2-4           3-3         3-4         4-4	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$
NUMBER UNSCORABLE ALLELE FREQUENCY (A)O.	TFN         EST         PGI           41         41         0           0         0         0           683         (1)         0.878         (1)         0.000           317         (2)         0.122         (2)         0.000           (3)         0.000         (3)         0.000           (4)         0.000         (4)         0.000
SAMPLE TFN-SCORE EST CNJ E2 AA 16 1-1 41 AB 19 1-2 BB 6 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$
NUMBER UNSCORABLE ALLELE FREQUENCY (A)O.	TFN         EST         PGI           41         41         0           0         0         0           622         (1)         0.976         (1)         0.000           378         (2)         0.024         (2)         0.000           (3)         0.000         (3)         0.000           (4)         0.000         (4)         0.000

	SAMPLE TFN-SCOR CRC A AA 42 128 AB 18 BB 4	1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 0 1-2 3 1-3 9 1-4 0 2-2 11 2-3 15 2-4 0 3-3 26 3-4 0 4-4 0	
	TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY	(A)0.797 (1) (B)0.203 (2)	0.048 (2) 0.000 (3)	0.312 0.594
£	SAMPLE TFN-SCOR CRC A1 AA 80 251 AB 64 BB 10	1-3 0 1-4 1 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 1 1-2 6 1-3 14 1-4 0 2-2 13 2-3 36 2-4 0 3-3 25 3-4 0 4-4 0	·
	TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY	(B)0.273 (2) (3)		0.358 0.526
	SAMPLE TFN-SCOR CRC AD AA 86 257 AB 58 BB 9	1-1 135 1-2 16 1-3 1 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 1 1-2 12 1-3 17 1-4 0 2-2 9 2-3 51 2-4 0 3-3 63 3-4 0 4-4 0	
	TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY	153 1 (A)0.752 (1) (B)0.248 (2) (3)		0.265 0.634

SAMPLE CRC D 11	TFN-SCORI AA 8 AB 3 BB 0	1-1 10 1-2 1 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	E PGI-SCORE 1-1 0 1-2 0 1-3 3 1-4 0 2-2 0 2-3 3 2-4 0 3-3 5 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN 11	EST 11	PGI 11
		0 (A)0.864 ( (B)0.136 (2 (1)	0 1) 0.955 (1) 2) 0.045 (2) 3) 0.000 (3) 4) 0.000 (4)	0 0.136 0.136 0.727
SAMPLE CUA 193	TFN-SCOR AA 84 AB 81 BB 18	1-1 0	1–3 0	-
TOTAL FI	SH SCORED	TFN 183	EST O	PGI 0
		10 (A)0.680 (1 (B)0.320 (1 (1)	193 1) 0.000 (1) 2) 0.000 (2) 3) 0.000 (3) 4) 0.000 (4)	0 0.000 0.000 0.000
SAMPLE CYV 50	TFN-SCORI AA 21 AB 17 BB 6	E EST-SCORI 1-1 21 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	E PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN 44	EST 21	PGI O
		6 (A)0.670 (* (B)0.330 (* (*	29 1) 1.000 (1)	0 0.000 0.000 0.000

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SAMPLE ECU A 31	TFN-SCORI AA 0 AB 2 BB 0	E EST-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	1–1 0	
TOTAL FI	SH SCORED	TFN 2	EST 0	PGI
		29 (A)0.500 (1 (B)0.500 (2 (3	31	0.000
SAMPLE ECU B 347	TFN-SCOR AA 91 AB 82 BB 15		PGI-SCORE 1-1 2 1-2 12 1-3 24 1-4 0 2-2 15 2-3 73 2-4 0 3-3 68 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN	EST	PGI
		B)0.298 (2) (3	196 3 ) 0.985 (1) ) 0.008 (2) ) 0.008 (3) ) 0.000 (4)	0.296
SAMPLE ECU C 292	TFN-SCORE AA 68 AB 72 BB 18	2-4 0 3-3 0	1-1 2 1-2 11 1-3 15 1-4 0 2-2 17 2-3 59 2-4 0 3-3 57 3-4 0	
NUMBER U		B)0.342 (2	EST 168 3 ) 0.976 (1) ) 0.015 (2) ) 0.003 (3)	0.323

<sup>(3) 0.003 (3) 0.584</sup> (4) 0.006 (4) 0.000

SAMPLE TFN-SCORE ECU D AA 91 270 AB 49 BB 14	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	GI-SCORE         -1       1         -2       7         -3       19         -4       0         2-2       11         2-3       57         2-4       0         3-3       58         3-4       0         3-3       58         3-4       0         3-4       0			
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (A	TFN 154 6 A)0.750 (1)	EST 160 0 0.997 (1)	PGI 153 7 0.092		
	(3) (4)	0.003 (4)	0.281 0.627 0.000		
SAMPLE TFN-SCORE ECU E AA 116 348 AB 74 BB 8	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	GI-SCORE -1 3 -2 10 -3 29 -4 0 -2 21 -3 55 -4 0 -3 79 -4 0 -4 0 -4 0			
TOTAL FISH SCORED	TFN 198	EST 199	PGI 197	,	
NUMBER UNSCORABLE ALLELE FREQUENCY (1 (1	3)0.227 (2) (3)	1 0.995 (1) 0.003 (2) 0.000 (3) 0.003 (4)	0.272 0.614		
SAMPLE TFN-SCORE ECU F AA 109 326 AB 61 BB 16	1-2       4       1         1-3       0       1         1-4       0       1         2-2       0       2         2-3       0       2         2-4       0       2         3-3       0       3         3-4       0       3	GI-SCORE -1 0 -2 11 -3 27 -4 0 -2 23 -3 56 -4 0 -3 65 -4 0 -3 65 -4 0 -4 0			
TOTAL FISH SCORED	TFN	EST	PGI		

TOTAL FISH SCORED	) TFN		EST		PGI
	186		187		182
NUMBER UNSCORABLE	: 2		1		6
ALLELE FREQUENCY	(A)0.750	(1)	0.989	(1)	0.104
	(B)0.250	(2)	0.011	(2)	0.310
		(3)	0.000	(3)	0.585
		(4)	0.000	(4)	0.000

SAMPLE	E TH	FN-SCORE	EST-	EST-SCORE		SCORE
ECU G	A	<b>103</b>	1-1	185	1–1	2
351	I AE	3 75	1-2	8	1-2	9
	BE	3 16	1-3	0	1-3	29
			1-4	0	1-4	0
			2-2	0	2-2	23
			2-3	0	2-3	60
			2-4	0	2-4	0
			3-3	0	3-3	72
			3-4	0	3-4	0
			4-4	0	4-4	0
TOTAL	FISH	SCORED	]	(FN	E	ST

TOTAL FISH SCORED	TFN		EST		PGI
	194		193		195
NUMBER UNSCORABLE	6		7		6
ALLELE FREQUENCY	(A)0.724	(1)	0.979	(1)	0.108
	(B)0.276	(2)	0.021	(2)	0.295
	•	(3)	0.000	(3)	0.597
		(4)	0.000	(4)	0.000

SAMPLE	TFN	-SCORE	EST-S	SCORE	PGI-	SCORE
ECU H	AA	108	1–1	0	1-1	2
348	AB	81	1-2	0	1-2	16
	BB	9	1-3	0	1-3	24
			1-4	0	1-4	0
			2-2	0	2-2	20
			2-3	0	2-3	65
			2-4	0	2-4	0
			3-3	0	3-3	64
			3-4	0	3-4	0
			4-4	0	4-4	0

TOTAL FISH SCORED	TFN		EST		PGI
	198		0		191
NUMBER UNSCORABLE	1		199		8
ALLELE FREQUENCY	(A)0.750	(1)	0.000	(1)	0.115
	(B)0.250	(2)	0.000	(2)	0.317
		(3)	0.000	(3)	0.568
		(4)	0.000	(4)	0.000

SAMPLE	TFN	-SCORE	EST-S	SCORE	PGI-	SCORE
ECU I	AA	111	1–1	0	1-1	3
350	AB	76	1-2	0	1-2	9
	BB	13	1-3	0	1-3	32
			1-4	0	1-4	0
			2-2	0	2-2	22
			2-3	0	2-3	79
			2-4	0	2-4	0
			3-3	0	3-3	44
			3-4	0	3-4	0
			4-4	0	4_4	0
TOTAL FI	SH S	CORED	TI	<b>FN</b>	E	ST

TOTAL F	ISH SCORED	) TFN		EST		PGI
		200		0		189
NUMBER	UNSCORABLE	: 0		200		11
ALLELE	FREQUENCY	(A)0.745	(1)	0.000	(1)	0.124
		(B)0.255	(2)	0.000	(2)	0.349
			(3)	0.000	(3)	0.526
			(4)	0.000	(4)	0.000

SAMPLE TFN-SCORE ECU J AA 65 211 AB 56 BB 10	EST-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 5 1-3 18 1-4 0 2-2 19 2-3 48 2-4 0 3-3 39 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY (/	B)0.290 (2) (3)	0 131 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4)	0.353 0.558
SAMPLE TFN-SCORE ECU JC AA 252 465 AB 175 BB 37	EST-SCORE 1-1 33 1-2 4 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency () ()	B)0.268 (2) (3)	37 428 ) 0.946 (1) ) 0.054 (2) ) 0.000 (3) ) 0.000 (4)	0.000 0.000
SAMPLE TFN-SCORE ECU K AA 108 350 AB 80 BB 12	1-3 0 1-4 0 2-2 0 2-3 0	1-1 2 1-2 13 1-3 22 1-4 0 2-2 18	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (/ ()	B)0.260 (2) (3)	0 200 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4)	0.329 0.570

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SAMPLE ECU L 350	TFN-SCORE AA 117 AB 75 BB 8	EST-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 3 1-2 15 1-3 15 1-4 0 2-2 19 2-3 85 2-4 0 3-3 61 3-4 0 4-4 0	
NUMBER	FISH SCORED UNSCORABLE FREQUENCY ( (	B)0.228 (2 (3	EST 0 200 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4)	0.348 0.561
SAMPLE ECU M 349	TFN-SCORE AA 92 AB 85 BB 23	EST-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 11 1-3 24 1-4 0 2-2 24 2-3 77 2-4 0 3-3 61 3-4 0 4-4 0	
NUMBER	FISH SCORED UNSCORABLE FREQUENCY (	B)0.327 (2 (3	EST 0 200 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4)	0.345 0.566
SAMPLE ECU N 350	TFN-SCORE AA 113 AB 73 BB 13	1-3 0 1-4 0 2-2 0 2-3 0	PGI-SCORE 1-1 1 1-2 14 1-3 24 1-4 0 2-2 18 2-3 62 2-4 0 3-3 77 3-4 2 4-4 0	
NUMBER	FISH SCORED UNSCORABLE FREQUENCY (	B)0.249 (2 (3	EST 192 8 ) 0.969 (1) ) 0.031 (2) ) 0.000 (3) ) 0.000 (4)	0.283 0.611

SAMPLE	TFN-	SCORE	EST-S	SCORE	PGI-	SCORE
ECU O	AA	31	1–1	1	1-1	0
152	AB	41	1-2	0	1-2	6
	BB	4	1–3	0	1-3	6
			1-4	0	1-4	0
			2-2	0	2-2	10
			2-3	0	2-3	34
			2-4	0	2-4	0
			3-3	0	3-3	20
			3-4	0	3-4	0
			4_4	0	4_4	0
TOTAL FI	SH SC	ORED	TH	'n	E	ST
			7	76		1
NUMBER U	NSCOR	ABLE		0		75

TOTAL FISH SCORED	TFN		EST		PGI
	76		1		76
NUMBER UNSCORABLE	0		75		0
ALLELE FREQUENCY	(A)0.678	(1)	1.000	(1)	0.079
	(B)0.322	(2)	0.000	(2)	0.395
		(3)	0.000	(3)	0.526
		(4)	0.000	(4)	0.000

SAMPLE	TFN	-SCORE	EST-	-SCORE	PGI-	SCORE
ECU P	AA	124	1-1	185	1-1	1
350	AB	64	1-2	15	1-2	15
	BB	12	1-3	0	1-3	23
			1-4	0	1-4	0
			2-2	0	2-2	27
			2-3	0	2-3	70
			2-4	0	2-4	0
			3-3	0	3-3	60
			3-4	0	3-4	0
			4-4	0	4_4	0

TOTAL FISH SCORED	TFN	EST	PGI	
	200	200	196	
NUMBER UNSCORABLE	0	0	4	
ALLELE FREQUENCY	(A)0.780	(1) 0.962	(1) 0.102	
	(B)0.220	(2) 0.038	(2) 0.355	
		(3) 0.000	(3) 0.543	
		(4) 0.000	(4) 0.000	

SAMPLE	TFN-	SCORE	EST-S	SCORE	PGI-	SCORE
ECU Q	AA	96	1-1	0	1-1	4
350	AB	85	1-2	0	1-2	14
	BB	19	1–3	0	1-3	18
			1-4	0	1-4	0
			2-2	0	2–2	16
			2-3	0	2-3	70
			2-4	0	2-4	0
			3-3	0	3-3	75
			3-4	0	3-4	3
			4-4	0	4_4	0
					_	

TOTAL FISH SCORED	TFN		EST		PGI
	200		0		200
NUMBER UNSCORABLE	0		200		0
ALLELE FREQUENCY	(A)0.692	(1)	0.000	(1)	0.100
	(B)0.308	(2)	0.000	(2)	0.290
		(3)	0.000	(3)	0.603
		(4)	0.000	(4)	0.007

SAMPLE ECU R 348	TFN-SCORE AA 121 AB 67 BB 11	1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	1-1 0 1-2 6 1-3 22 1-4 0 2-2 25	
TOTAL FI	SH SCORED	TFN 199	EST 199	PGI 198
	NSCORABLE REQUENCY (	0	0	1
		B)0.224 (2 (3	) 0.020 (2) ) 0.000 (3) ) 0.005 (4)	0.318 0.611
SAMPLE ECU S	TFN-SCORE AA 97	EST-SCORE		
350	AB 85 BB 18		1-2 7	
		1-4 0 2-2 0	1-4 0 2-2 26	
		2-3 0 2-4 0 3-3 0	2-3 74 2-4 0 3-3 66	
		3-4 0 4-4 0	3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN	EST	PGI
	NSCORABLE	200 0	200 0	200 0
ALLELE FI		B)0.303 (2 (3	) 0.983 (1) ) 0.018 (2) ) 0.000 (3) ) 0.000 (4)	0.333
SAMPLE ECU T		EST-SCORE		
350	AA 107 AB 82 BB 10	1–1 191 1–2 6 1–3 1	1-1 3 1-2 11 1-3 22	
	22 10	1-4 0 2-2 0	1-4 0 2-2 24	
		2-3 0 2-4 0	2-3 78 2-4 0	
		3-3 0 3-4 0 4-4 0	3-3 61 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TPN	EST	PGI
NUMBER U	NSCORABLE	199 1	198 2	199 1
ALLELE F		B)0.256 (2	) 0.982 (1) 2) 0.015 (2)	0.344
			) 0.003 (3) ) 0.000 (4)	

SAI	IPLE	TFN-	SCORE	EST-S	CORE	PGI-S	SCORE
ECU	JU	AA	112	1-1	0	1-1	3
	350	AB	74	1-2	0	1-2	11
		BB	14	1-3	0	1-3	29
				1-4	0	1-4	0
				2-2	0	2-2	24
				2-3	0	2-3	80
				2-4	0	2-4	0
				3-3	0	3-3	53
				3-4	0	3-4	0
				4_4	0	4_4	0

TOTAL 1	FISH	SCOREI	) TFN		EST		PGI
			200		0		200
NUMBER	UNSC	ORABLE	3 0		200		0
ALLELE	FREC	UENCY	(A)0.745	(1)	0.000	(1)	0.115
			(B)0.255	(2)	0.000	(2)	0.347
			×.	(3)	0.000	(3)	0.538
				(4)	0.000	(4)	0.000

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SAMPLE	TFN-	-SCORE	EST-	-SCORE	PGI-	SCORE
ECU V	AA	98	1-1	195	1-1	0
200	AB	87	1-2	5	1-2	14
	BB	15	1-3	0	1-3	24
			1-4	0	1-4	Ò
			2-2	0	2-2	28
			2-3	0	2-3	81
			2-4	0	2-4	0
			3-3	0	3-3	51
			3-4	0	3-4	0
,			4-4	0	4-4	0

TOTAL FISH SCORED	TFN		EST		PGI
	200		200		198
NUMBER UNSCORABLE	0		0		2
ALLELE FREQUENCY	(A)0.707	(1)	0.988	(1)	0.096
	(B)0.293	(2)	0.013	(2)	0.381
		(3)	0.000	(3)	0.523
		(4)	0.000	(4)	0.000

SAMPLE	TFN-	-SCORE	EST-	-SCORE	PGI-	SCORE	
ECU W	AA	85	1-1	152	1-1	1	
163	AB	71	1-2	8	1-2	11	
	BB	7	1-3	0	1-3	27	
			1-4	3	1-4	0	
			2-2	0	2-2	21	
			2-3	0	2-3	56	
			2-4	0	2-4	0	
			3-3	0	3-3	47	
*			3-4	0	3-4	0	
			4-4	0	4-4	0	

TOTAL FISH SCOREI	) TFN		EST		PGI
	163		163		163
NUMBER UNSCORABLE	S 0		0		0
ALLELE FREQUENCY	(A)0.739	(1)	0.966	(1)	0.123
	(B)0.261	(2)	0.025	(2)	0.334
		(3)	0.000	(3)	0.543
		(4)	0.009	(4)	0.000

SAMPLE ECU X 200	AA 102 AB 85	1-2 9 1-3 0 1-4 3 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 3 1-2 9 1-3 29 1-4 0 2-2 23 2-3 83 2-4 0 3-3 53 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN	EST	PGI
	,	B)0.277 (2 (3	200 0 ) 0.970 (1) ) 0.023 (2) ) 0.000 (3) ) 0.007 (4)	0.345 0.545
SAMPLE ECU XA 24	TFN-SCORE AA 13 AB 11 BB 0	2-4 1 3-3 0 3-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN	EST	PGI
		B)0.229 (2 (3	24 0 ) 0.833 (1) ) 0.146 (2) ) 0.000 (3) ) 0.021 (4)	0.000
SAMPLE ECU XB 28	TFN-SCORE AA 15 AB 12 BB 1	2-3 0 2-4 0 3-3 0	1-1       0         1-2       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         3-3       0         3-4       0	
TOTAL FI	SH SCORED	TFN	EST	PGI
		B)0.250 (2 (3	28 0 ) 0.982 (1) ) 0.000 (2) ) 0.000 (3) ) 0.018 (4)	0.000

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SAMPLE	TFN	-SCORE	EST-	-SCORE	PGI-	SCORE
ECU XX	AA	102	1-1	185	1–1	0
198	AB	77	1-2	10	1–2	0
	BB	16	1-3	1	1-3	0
			1-4	0	1-4	0
			2-2	0	2-2	0
			2-3	0	2-3	0
			2-4	0	2-4	0
			3-3	0	3-3	0
			3-4	0	3-4	0
			4_4	0	4-4	0

TOTAL I	FISH	SCORED	TFN		EST		PGI
			195		196		0
NUMBER	UNSC	CORABLE	3		2		0
ALLELE	FREC	UENCY	(A)0.721	(1)	0.972	(1)	0.000
			(B)0.279	(2)	0.026	(2)	0.000
				(3)	0.003	(3)	0.000
				(4)	0.000	(4)	0.000

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SAMPLE	TFN	-SCORE	EST-	SCORE	PGI-	SCORE
ECU Y	AA	108	1-1	188	1-1	4
200	AB	74	1-2	12	1-2	10
	BB	18	1-3	0	1-3	19
			1-4	0	1-4	0
			2-2	0	2-2	18
			2-3	0	2-3	68
			2-4	0	2-4	0
			3-3	0	3-3	80
			3-4	0	3-4	0
			4_4	0	4_4	0

TOTAL F	ISH SCOREI	) TFN		EST		PGI
		200		200		199
NUMBER	UNSCORABLE	C 0		0		1
ALLELE	FREQUENCY	(A)0.725	(1)	0.970	(1)	0.093
		(B)0.275	(2)	0.030	(2)	0.286
			(3)	0.000	(3)	0.621
			(4)	0.000	(4)	0.000

SAMPLE	TFN	-SCORE	EST-	-SCORE	PGI-	SCORE
ECU Z	AA	81	1-1	136	1-1	1
145	AB	46	1-2	6	1-2	16
	BB	17	1-3	0	1-3	12
			1-4	2	1-4	0
			2-2	0	2-2	17
			2-3	0	2-3	51
			2-4	0	2-4	0
			3-3	0	3-3	47
			3-4	0	3-4	0
			4-4	0	4-4	0

TOTAL FISH SCOREI	) TFN		EST		PGI
	144		144		144
NUMBER UNSCORABLE	3 1		1		1
ALLELE FREQUENCY	(A)0.722	(1)	0.972	(1)	0.104
	(B)0.278	(2)	0.021	(2)	0.351
		(3)	0.000	(3)	0.545
		(11)	0 007	( 11 )	0 000

SAMPLE TFN-SCORE ELS AA 109 192 AB 71 BB 11	EST-SCORE 1-1 186 1-2 5 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY (	191 1 A)0.757 (1	191 1 ) 0.987 (1)	0 0 0.000
	(3	<ul> <li>0.013 (2)</li> <li>0.000 (3)</li> <li>0.000 (4)</li> </ul>	0.000
		PGI-SCORE	
ETP AA O 50 AB O	1-1 0 1-2 0	1-1 0 1-2 0	
BB 0	1–3 0 1–4 0	1-3 0 1-4 0	
	2-2 0	2-2 0	
	2-3 0 2-4 0	2-4 0	
	3-3 0 3-4 0	3-4 0	
	4-4 0	4-4 0	
MORIT PTOUL COOPPE		200	PGI
TOTAL FISH SCORED	TFN	EST	
NUMBER UNSCORABLE	0 50	0 50	0
NUMBER UNSCORABLE ALLELE FREQUENCY (	0 50 A)0.000 (1 B)0.000 (2	0 50 ) 0.000 (1) ) 0.000 (2)	0 0 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY (	0 50 A)0.000 (1 B)0.000 (2 (3	0 50 ) 0.000 (1)	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( (	0 50 A)0.000 (1 B)0.000 (2 (3 (4	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4)	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE GEM K1 AA 117	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) PGI-SCORE 1-1 0	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) PGI-SCORE 1-1 0 1-2 0 1-3 0	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) FGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0 2-3 0	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) FGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) FGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) FGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	0 0 0.000 0.000 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN	0 50 0.000 (1) 0.000 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 EST	0 0.000 0.000 0.000 PGI
NUMBER UNSCORABLE ALLELE FREQUENCY ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69 BB 14 TOTAL FISH SCORED NUMBER UNSCORABLE	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN 200 0	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) FGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 EST 200 0	0 0.000 0.000 0.000 0.000 PGI 0 0
NUMBER UNSCORABLE ALLELE FREQUENCY ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69 BB 14 TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN 200 0 A)0.757 (1	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) FGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 EST 200 0	0 0.000 0.000 0.000 0.000 PGI 0 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY ( SAMPLE TFN-SCORE GEM K1 AA 117 200 AB 69 BB 14 TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (	0 50 A)0.000 (1 B)0.000 (2 (3 (4 EST-SCORE 1-1 189 1-2 9 1-3 2 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN 200 0 A)0.757 (1 B)0.243 (2 (3	0 50 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3) ) 0.000 (4) FGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 EST 200 0 ) 0.973 (1)	0 0.000 0.000 0.000 0.000 PGI 0 0.000 0.000 0.000

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	A 104 3 83 3 13	EST-SCORE 1-1 187 1-2 11 1-3 2 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 10 1-3 24 1-4 0 2-2 22 2-3 75 2-4 0 3-3 65 3-4 1 4-4 0	
TOTAL FISH	SCORED	TFN 200	EST 200	PGI 197
NUMBER UNSC Allele frec	QUENCY (A	0 1)0.728 (1) 3)0.272 (2) (3)	0 0.967 (1) 0.027 (2) 0.005 (3) 0.000 (4)	3 0.086 0.327 0.584
SAMPLE TE Gem L AA 198 AE Be	A 105 3 72 3 19	2-4 0 3-3 0	1-1 2 1-2 7 1-3 33 1-4 0	
TOTAL FISH	SCORED	TFN	EST	PGI
NUMBER UNSC Allele Frec	QUENCY (A	3)0.281 (2) (3)	196 2 ) 0.980 (1) ) 0.020 (2) ) 0.000 (3) ) 0.000 (4)	0.286 0.601
GEM M AA 434 AB	A 120 B 83	EST-SCORE 1-1 198 1-2 17 1-3 0 1-4 2 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 2 1-2 14 1-3 21 1-4 0 2-2 16 2-3 82 2-4 0 3-3 77 3-4 0 4-4 0	
TOTAL FISH	SCORED	TFN 217	EST 217	PGI 212
NUMBER UNSC Allele frec	QUENCY (A	1 1)0.744 (1) 3)0.256 (2) (3)	1 ) 0.956 (1) ) 0.039 (2) ) 0.000 (3) ) 0.005 (4)	4 0.092 0.302 0.606

SAMPLE TFN-SCORE GEM N AA 109 324 AB 84 BB 12	EST-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 1 1-2 2 1-3 14 1-4 0 2-2 12 2-3 38 2-4 0 3-3 44 3-4 0 4-4 0	
TOTAL FISH SCORED	<b>TFN</b> 205	EST 0	PGI 111
NUMBER UNSCORABLE ALLELE FREQUENCY ( (	5 A)0.737 (1) B)0.263 (2)	210 ) 0.000 (1) ) 0.000 (2) ) 0.000 (3)	3 0.081 0.288 0.631
SAMPLE TFN-SCORE GEM Z AA 29 56 AB 20 BB 7	EST-SCORE 1-1 54 1-2 1 1-3 0 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency ( (	B)0.304 (2)	) 0.009 (2) ) 0.000 (3)	0.000
SAMPLE TFN-SCORE HST AA 99 200 AB 84 BB 17	1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	
TOTAL FISH SCORED	<b>TFN</b> 200	EST O	PGI 0
NUMBER UNSCORABLE Allele Frequency ( (	0 A)0.705 (1)	200 ) 0.000 (1) ) 0.000 (2)	0 000.0 000.0

(3) 0.000 (3) 0.000 (4) 0.000 (4) 0.000

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SAMPLE TFN-SCORE IDP AA 98 196 AB 83 BB 13		PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (A (E	3)0.281 (2) (3)		0.000
SAMPLE TFN-SCORE INV AA 103 159 AB 43 BB 10	2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 0 1-2 15 1-3 16 1-4 0 2-2 14 2-3 60 2-4 0 3-3 50 3-4 0 4-4 0	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (A (E	3)0.202 (2) (3)	EST 156 3 0.978 (1) 0.019 (2) 0.003 (3) 0.000 (4)	0.332 0.568
SAMPLE TFN-SCORE JHN F AA 120 228 AB 85 BB 10	1-3 0 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	1-1 0	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (A (E	3)0.244 (2) (3)	EST 215 0 0.984 (1) 0.014 (2) 0.000 (3) 0.002 (4)	0.658 0.335

SAMPLE	TFN-S	CORE	EST-	SCORE	PGI-S	SCORE	
JMM AA	AA	49	1–1	91	1–1	0	
100	AB	38	1-2	6	1–2	0	
	BB	12	1-3	0	1–3	0	
			1-4	1	1-4	0	
			2-2	0	2–2	0	
			2-3		2-3	0	
			2-4	1	2-4	0	
			3-3	0	3-3	0	
			3-4	0	3-4	0	
			4_4	0	4_4	0	
TOTAL	FISH SCO	RED	T	FN	ES	ST	
				99	9	)9	
	UNSCORA			1		1	
ALLELE	FREQUEN	CY (1	A)0.6	87 (1)	) 0.95	55 (1)	0
		(1	3)0.3	13 (2)	) 0.03	35 (2)	0
				· (3)	) 0.00	)0 (3)	0
				(4)	0.01	10 (4)	0
SAMPLE			EST-	SCORE	PGI-S	SCORE	
JMM BB		61	1-1	87	1–1	0	
95	AB	24	1-2		1–2	0	
	BB	10	1-3	0	1–3	0	
			4 11	4	4 h	^	

TOTAL FISH SCORE	D TFN		EST		PGI
	99		99		0
NUMBER UNSCORABL	31		1		0
ALLELE FREQUENCY					
	(B)0.313				
			0.000		
		(4)	0.010	(4)	0.000

SAME	PLE	TFN-S	SCORE	EST-S	SCORE	PGI-S	CORE
MM	BB	AA	61	1–1	87	1–1	0
	95	AB	24	1-2	7	1-2	0
		BB	10	1–3	0	1–3	0
				1-4	1	1-4	0
				2-2	0	2-2	0
				2-3	0	2-3	0
				2-4	0	2-4	0
				3–3	0	3–3	0
				3–4	0	3-4	0
				4-4	0	4_4	0

TOTAL FISH SCOR	ED TFN		EST		PGI
	95		95		0
NUMBER UNSCORAB	LE O		0		0
ALLELE FREQUENC	Y (A)0.768	(1)	0.958	(1)	0.000
	(B)0.232	(2)	0.037	(2)	0.000
,		(3)	0.000	(3)	0.000
		(4)	0.005	(4)	0.000

SAMI	PLE	TFN-S	SCORE	EST-	SCORE	PGI-S	CORE
JMM	CC	AA	42	1-1	82	1–1	0
	89	AB	40	1–2	6	1-2	0
		BB	7	1-3	0	1-3	0
				1-4	1	1-4	0
				2–2	0	2-2	0
				2-3	0	2-3	0
				2-4	0	2-4	0
				3-3	0	3-3	0
				3-4	0	3-4	0
				4_4	0	4_4	0
TOT	L FIS	SH SCO	DRED	Т	FN	ES	т
			_		89	8	9

TOTAL FISH SCORED	TFN		EST		PGI
	89		89		0
NUMBER UNSCORABLE	0		0		0
ALLELE FREQUENCY					
	(B)0.303				
			0.000		
		(4)	0.006	(4)	0.000

SAMPLE JMM DD 202	AA 55	1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	1-1 1 1-2 5 1-3 15 1-4 0 2-2 16	
TOTAL	FISH SCORED	TFN 100	EST O	PGI 101
	UNSCORABLE FREQUENCY (. ()	0 A)0.750 (1) B)0.250 (2) (3)	100	1 0.109 0.327 0.564
SAMPLE JMM EE 284	TFN-SCORE AA 86 AB 44 BB 8	1-1       0         1-2       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         3-3       0	PGI-SCORE 1-1 0 1-2 7 1-3 18 1-4 0 2-2 15 2-3 53 2-4 0 3-3 53 3-4 0 4-4 0	
TOTAL	FISH SCORED	<b>TFN</b> 138	EST 0	PGI 146
	UNSCORABLE FREQUENCY (	0 A)0.783 (1) B)0.217 (2) (3)	138	0 0.086 0.308 0.606
SAMPLE JNC 50	AA O	3–3 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL	FISH SCORED	TFN O	EST O	PGI O
	UNSCORABLE FREQUENCY ()	50 A)0.000 (1) B)0.000 (2) (3)	50	0 0.000 0.000 0.000

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SAMPLE KRM KD 148	TFN-SCOF AA 51 AB 37 BB 10	$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	1-1 0	
TOTAL FI	SH SCOREI	) <b>TFN</b> 98	EST 98	PGI 99
	NSCORABLE REQUENCY	2 (A)0.709 (1 (B)0.291 (2 (3	2	1 0.141 0.212 0.646
SAMPLE KRM KS 288	TFN-SCOR AA 83 AB 48 BB 14	1-2 4 1-3 0 1-4 1 2-2 0 2-3 0 2-4 0	1-1 0 1-2 4 1-3 30 1-4 0 2-2 12 2-3 41 2-4 0 3-3 56	
TOTAL FI	SH SCORED	145	EST 145	PGI 143
	NSCORABLE REQUENCY	(A)0.738 (1 (B)0.262 (2 (3	0 0.983 (1) 0.014 (2) 0.000 (3) 0.003 (4)	0 0.119 0.241 0.640
SAMPLE MB 133	TFN-SCOF AA 65 AB 59 BB 8	1-2 4 1-3 1 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0	
TOTAL FI	SH SCORED	TFN 132	<b>EST</b> 133	PGI O
	N SCOR ABLE REQUEN CY	(A)0.716 (1 (B)0.284 (2 (3)	0 0.981 (1) 0.015 (2) 0.004 (3) 0.000 (4)	0 0.000 0.000 0.000

SAMPLE TFN-SCORE ME B AA 101 200 AB 86 BB 13	1-1 193 1-2 6 1-3 0 1-4 1 2-2 0 2-3 0	PGI-SCORE 1-1 0 1-2 5 1-3 1 1-4 0 2-2 81 2-3 91 2-4 0 3-3 22 3-4 0 4-4 0	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (A (B		) 0.015 (2) ) 0.000 (3)	0.645 0.340
	EST-SCORE 1-1 190 1-2 5 1-3 1 1-4 2 2-2 0 2-3 0		
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (A (E	3)0.268 (2) (3)	EST 198 1 ) 0.980 (1) ) 0.013 (2) ) 0.003 (3) ) 0.005 (4)	0.687 0.295
112 AB 40 BB 10	1-1 102 1-2 8 1-3 0 1-4 1 2-2 1 2-3 0 2-4 0 3-3 0	1-1 0 1-2 2 1-3 2 1-4 0 2-2 52 2-3 44 2-4 0 3-3 12 3-4 0	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (A (B	3)0.268 (2)	EST 112 0 0.951 (1) 0.045 (2) 0.000 (3)	0.670

(3) 0.000 (3) 0.312 (4) 0.004 (4) 0.000

SAMPLE TFN-SCOR MK B AA 108 198 AB 82 BB 8	1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0		
TOTAL FISH SCORED	<b>TFN</b> 198	EST 197	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY	0 (A)0.753 (1) (B)0.247 (2) (3)	1 ) 0.980 (1)	0.327 0.574
SAMPLE TFN-SCOR MK B2 AA 113 200 AB 74 BB 11	1-2 12 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	1-1 4	
TOTAL FISH SCORED		EST	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY	(B)0.242 (2) (3)		0.293 0.598
SAMPLE TFN-SCOR MK C AA O 24 AB O BB O	2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 0 1-2 2 1-3 1 1-4 0 2-2 1 2-3 11 2-4 0 3-3 9 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY	(A)0.000 (1) (B)0.000 (2) (3)		0.312 0.625

MK E AA 119 200 AB 67 BB 13	EST-SCORE       PGI-SCORE         1-1       185       1-1       2         1-2       12       1-2       10         1-3       0       1-3       32         1-4       0       1-4       0         2-2       0       2-2       18         2-3       0       2-3       61         2-4       1       2-4       0         3-3       0       3-3       76         3-4       0       3-4       0         4-4       0       4-4       0	
	TFN         EST           199         198           1         2           00.766         (1)         0.965           00.234         (2)         0.033         (2)           (3)         0.000         (3)         (4)	0.269 0.616
MK F AA 67 121 AB 43 BB 7	EST-SCORE       PGI-SCORE         1-1       116       1-1       0         1-2       1       1-2       6         1-3       0       1-3       15         1-4       0       1-4       0         2-2       0       2-2       6         2-3       0       2-3       43         2-4       0       2-4       0         3-3       0       3-3       50         3-4       0       3-4       0         4-4       0       4-4       0	
	TFN       EST         117       117         4       4         00.756       (1)       0.996       (1)         00.244       (2)       0.004       (2)         (3)       0.000       (3)         (4)       0.000       (4)	0.254 0.658
MP A AA 86 286 AB 76 BB 6	EST-SCORE PGI-SCORE 1-1 161 1-1 1 1-2 6 1-2 3 1-3 0 1-3 13 1-4 1 1-4 0 2-2 0 2-2 12 2-3 0 2-3 71 2-4 0 2-4 0 3-3 0 3-3 68 3-4 0 3-4 0 4-4 0 4-4 0	
	TFN       EST         168       168         0       0         0.738       0.979       (1)         0.262       (2)       0.018       (2)         (3)       0.000       (3)         (4)       0.003       (4)	0.292 0.655

SAMPLE TFN-SCORE MP A1 AA 115 205 AB 74 BB 16	EST-SCORE 1-1 186 1-2 14 1-3 0 1-4 4 2-2 0 2-3 0 2-4 1 3-3 0 3-4 0 4-4 0	PGI-SCORE         1-1       0         1-2       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         3-3       0         3-4       0         4-4       0	
TOTAL FISH SCORED	<b>TFN</b> 205	est 205	PGI
NUMBER UNSCORABLE Allele Frequency ( (	0 A)0.741 (1 B)0.259 (2	0 ) 0.951 (1) ) 0.037 (2) ) 0.000 (3)	0.000
SAMPLE TFN-SCORE MP B AA 34 54 AB 18 BB 2	EST-SCORE 1-1 49 1-2 5 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE         1-1       0         1-2       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         3-3       0         3-4       0         4-4       0	·
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY ( (	B)0.204 (2 (3	54 0 ) 0.954 (1) ) 0.046 (2) ) 0.000 (3) ) 0.000 (4)	0.000
SAMPLE TFN-SCORE MP BC AA 162 311 AB 128 BB 19	3–3 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequencý ( (	B)0.269 (2 (3	310 1 ) 0.979 (1) ) 0.018 (2) ) 0.002 (3) ) 0.002 (4)	0.000

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TOTAL FISH SCORED       TFN       EST       PGI         186       188       0         NUMBER UNSCORABLE       2       0       0         ALLELE FREQUENCY (A)0.715       (1)       0.968       (1)       0.000         (B)0.285       (2)       0.032       (2)       0.000         (3)       0.000       (3)       0.000         (4)       0.000       (4)       0.000	
$\begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	
TOTAL FISH SCORED       TFN       EST       PGI         8       8       0         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY       (A)0.750       (1)       0.875       (1)       0.000         (B)0.250       (2)       0.062       (2)       0.000         (3)       0.000       (3)       0.000         (4)       0.062       (4)       0.000	
SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         MP       E1       AA       18       1-1       30       1-1       0         34       AB       13       1-2       2       1-2       0         BB       3       1-3       0       1-3       0         1-4       2       1-4       0         2-2       0       2-2       0         2-3       0       2-3       0         2-4       0       2-4       0         3-3       0       3-3       0         3-4       0       3-4       0         4-4       0       4-4       0	
TOTAL FISH SCOREDTFNESTPGI34340NUMBER UNSCORABLE00ALLELE FREQUENCY (A)0.721(1)0.941(1)0.000(B)0.279(2)0.029(2)0.000(3)0.000(3)0.000(4)0.029	

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242	AA 68 AB 63 BB 12	1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	1-1 2 1-2 4 1-3 25 1-4 0	
TOTAL FIS	H SCORED	<b>TFN</b> 143	EST 146	PGI 146
NUMBER UN		3	0 0 0.979 (1)	0
1		B)0.304 (2 (3	) 0.021 (2) ) 0.000 (3) ) 0.000 (4)	0.271 0.613
SAMPLE	TFN-SCORE	EST-SCORE		
295	AB 68 BB 14		1-2 9	
	14	1-4 1 2-2 0	1-4 0	
		2-3 0 2-4 0	2-2 14 2-3 63 2-4 0	
		3-3 0 3-4 0	3-3 60 3-4 0	
		4-4 0	4 <u>4</u> 0	
TOTAL FIS	H SCORED	<b>TFN</b> 173	est 172	PGI 172
NUMBER UN		0	1 ) 0.977 (1)	0
ALLELE FR		B)0.277 (2	) 0.017 (2)	0.291
ALLELE FR	(	(2		0 602
ALLELE FR	,		) 0.003 (3) ) 0.003 (4)	
SAMPLE	TFN-SCORE	(4 EST-SCORE	) 0.003 (4) PGI-SCORE	
SAMPLE MP O 346	TFN-SCORE AA 103 AB 81	(4 EST-SCORE 1-1 186 1-2 10	) 0.003 (4) PGI-SCORE 1-1 2 1-2 7	
SAMPLE MP O 346	TFN-SCORE AA 103	(4 EST-SCORE 1-1 186 1-2 10 1-3 1 1-4 1	) 0.003 (4) PGI-SCORE 1-1 2 1-2 7 1-3 26 1-4 0	
SAMPLE MP O 346	TFN-SCORE AA 103 AB 81	(4 EST-SCORE 1-1 186 1-2 10 1-3 1 1-4 1 2-2 0 2-3 0	) 0.003 (4) PGI-SCORE 1-1 2 1-2 7 1-3 26 1-4 0 2-2 11 2-3 68	
SAMPLE MP O 346	TFN-SCORE AA 103 AB 81	(4 EST-SCORE 1-1 186 1-2 10 1-3 1 1-4 1 2-2 0	PGI-SCORE 1-1 2 1-2 7 1-3 26 1-4 0 2-2 11 2-3 68 2-4 0 3-3 82	
SAMPLE MP O 346	TFN-SCORE AA 103 AB 81	(4 EST-SCORE 1-1 186 1-2 10 1-3 1 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0	) 0.003 (4) PGI-SCORE 1-1 2 1-2 7 1-3 26 1-4 0 2-2 11 2-3 68 2-4 0 3-3 82	
SAMPLE MP O 346	TFN-SCORE AA 103 AB 81 BB 14	(4 EST-SCORE 1-1 186 1-2 10 1-3 1 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	) 0.003 (4) PGI-SCORE 1-1 2 1-2 7 1-3 26 1-4 0 2-2 11 2-3 68 2-4 0 3-3 82 3-4 0	
SAMPLE MP O 346 TOTAL FIS NUMBER UN	TFN-SCORE AA 103 AB 81 BB 14 SH SCORED	(4 EST-SCORE 1-1 186 1-2 10 1-3 1 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 3-4 0 4-4 0 TFN 198 0	) 0.003 (4) PGI-SCORE 1-1 2 1-2 7 1-3 26 1-4 0 2-2 11 2-3 68 2-4 0 3-3 82 3-4 0 4-4 0 EST	0.000 PGI 196 2
SAMPLE MP O 346 TOTAL FIS NUMBER UN	TFN-SCORE AA 103 AB 81 BB 14 SH SCORED SCORABLE EQUENCY (	(4 EST-SCORE 1-1 186 1-2 10 1-3 1 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN 198 0 A)0.725 (1 B)0.275 (2	) 0.003 (4) PGI-SCORE 1-1 2 1-2 7 1-3 26 1-4 0 2-2 11 2-3 68 2-4 0 3-3 82 3-4 0 4-4 0 EST 198 0	PGI 196 2 0.094 0.247

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TOTAL FISH SCORED       TFN       EST       FGI         184       184       184       184         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY (A)0.701 (1) 0.973 (1) 0.098       (B)0.299 (2) 0.027 (2) 0.296       (3) 0.000 (3) 0.606         (B)0.299 (2) 0.027 (2) 0.296       (3) 0.000 (3) 0.606       (4) 0.000 (4) 0.000         SAMPLE       TFN-SCORE EST-SCORE PGI-SCORE         MRA H       AA 111       1-1 195       1-1       1         400       AB 76       1-2 5       1-2 16       1         BB       13       1-3 0       1-3 23       1-4 0       1-4 0         2-2 0       2-2 2 26       2-3 0       2-3 81       2-4 0       2-4 0         2-4 0       2-4 0       2-4 0       3-3       3-3 4 0       3-4 0         4-4 0       4-4 0       4-4 0       0       0       0         ALLELE FREQUENCY (A)0.745 (1) 0.988 (1) 0.102       (B)0.255 (2) 0.013 (2) 0.373       (3) 0.000 (3) 0.525       (4) 0.000         MRA I       AA 109       1-1 190       1-1 2       20       20       22       1         405       AB 79       1-2 8       1-2 9       1       40       3-3 12       3 <t< th=""><th>SAMPLE TFN-SCORE MP P AA 92 184 AB 74 BB 18</th><th>1-1 175 1-2 8 1-3 0 1-4 0 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0</th><th>PGI-SCORE 1-1 1 1-2 12 1-3 22 1-4 0 2-2 14 2-3 69 2-4 0 3-3 66 3-4 0 4-4 0</th><th></th></t<>	SAMPLE TFN-SCORE MP P AA 92 184 AB 74 BB 18	1-1 175 1-2 8 1-3 0 1-4 0 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 1 1-2 12 1-3 22 1-4 0 2-2 14 2-3 69 2-4 0 3-3 66 3-4 0 4-4 0	
NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY (A)0.701 (1) 0.973 (1) 0.098 (B)0.299 (2) 0.027 (2) 0.296 (3) 0.000 (3) 0.606 (4) 0.000 (4) 0.000         SAMPLE       TFN-SCORE EST-SCORE PGI-SCORE         MRA H       AA 111 1-1 195 1-1 1 400 AB 76 1-2 5 1-2 16 BB 13 1-3 0 1-3 23 1-4 0 1-4 0 2-2 0 2-2 26 2-3 0 2-3 81 2-4 0 2-4 0 3-3 0 3-3 53 3-4 0 3-4 0 4-4 0 4-4 0         TOTAL FISH SCORED       TFN       EST       PGI 200 200         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY (A)0.745 (1) 0.988 (1) 0.102 (B)0.255 (2) 0.013 (2) 0.373 (3) 0.000 (3) 0.525 (4) 0.000 (4) 0.000         SAMPLE       TFN-SCORE EST-SCORE PGI-SCORE MRA I       AA 109 1-1 190 1-1 2 405 AB 79 1-2 8 1-2 9 BB 13 1-3 0 1-3 29 1-4 3 1-4 1 2-3 0 2-3 79 2-4 0 2-4 0 3-3 0 3-3 72 3-4 0 3-4 1 4-4 0 4-4 0         TOTAL FISH SCORED       TFN       EST       PGI 201 201 204 NUMBER UNSCORABLE         TOTAL FISH SCORED       TFN       EST       PGI 201 201 204 0 3-3 0 3-3 72 3-4 0 3-4 1 4-4 0 4-4 0	TOTAL FISH SCORED			
(B) 0.299 (2) 0.027 (2) 0.296 (3) 0.000 (3) 0.606 (4) 0.000 (4) 0.000 SAMPLE TFN-SCORE EST-SCORE PGI-SCORE MRA H AA 111 1-1 195 1-1 1 400 AB 76 1-2 5 1-2 16 BB 13 1-3 0 1-3 23 1-4 0 1-4 0 2-2 0 2-2 26 2-3 0 2-3 81 2-4 0 2-4 0 3-3 0 3-3 53 3-4 0 3-4 0 4-4 0 4-4 0 TOTAL FISH SCORED TFN EST PGI 200 200 200 200 NUMBER UNSCORABLE 0 0 0 0 ALLELE FREQUENCY (A)0.745 (1) 0.988 (1) 0.102 (B)0.255 (2) 0.013 (2) 0.373 (3) 0.000 (3) 0.525 (4) 0.000 (4) 0.000 SAMPLE TFN-SCORE EST-SCORE PGI-SCORE MRA I AA 109 1-1 190 1-1 2 405 AB 79 1-2 8 1-2 9 BB 13 1-3 0 1-3 29 1-4 3 1-4 1 2-2 0 2-2 11 2-3 0 2-3 79 2-4 0 2-4 0 3-3 0 3-3 72 3-4 0 3-4 1 4-4 0 4-4 0 TOTAL FISH SCORED TFN EST PGI 0 0 0 ALLELE FREQUENCY (A)0.739 (1) 0.973 (1) 0.105 (B)0.251 (2) 0.020 (2) 0.270 (3) 0.000 (3) 0.620		0	0	0
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		B)0.299 (2 (3	2) 0.027 (2) 3) 0.000 (3)	0.296 0.606
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	SAMDI P TEN_SCOPE	-		
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	MRAH AA 111	1 <b>-</b> 1 195	1–1 1	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	•	1–3 0	1-3 23	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$		2-2 0	2-2 26	
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			-	
4-4       0       4-4       0         TOTAL FISH SCORED       TFN       EST       PGI         200       200       200       200         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY (A)0.745 (1)       0.988 (1)       0.102         (B)0.255 (2)       0.013 (2)       0.373         (3)       0.000 (3)       0.525         (4)       0.000 (4)       0.000         SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         MRA I       AA       109       1-1       10         405       AB       79       1-2       8       1-2       9         BB       13       1-3       0       1-3       29       1-4       3       1-4       1         2-2       0       2-2       11       2-3       0       2-3       79         2-4       0       2-4       0       3-3       1-3       1-3       1-4       1         2-2       0       2-2       11       2-3       0       2-3       79         2-4       0       2-4       0       3-3       1       4-4       0				
$\begin{array}{cccccccccccccccccccccccccccccccccccc$			-	
NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY       (A)0.745       (1)       0.988       (1)       0.102         (B)0.255       (2)       0.013       (2)       0.373         (3)       0.000       (3)       0.525         (4)       0.000       (4)       0.000         SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         MRA I       AA       109       1-1       190         405       AB       79       1-2       8       1-2       9         BB       13       1-3       0       1-3       29         1-4       3       1-4       1       2-2       0       2-2       11         2-3       0       2-3       79       2-4       0       2-4       0         3-3       0       3-3       72       3-4       0       3-4       1         4-4       0       4-4       0       0       0         TOTAL FISH SCORED       TFN       EST       PGI         201       201       204       0       0         NUMBER UNSCORABLE       0       0       0       0		· · · · ·		
$(B) 0.255 (2) 0.013 (2) 0.373  (3) 0.000 (3) 0.525  (4) 0.000 (4) 0.000  SAMPLE TFN-SCORE EST-SCORE PGI-SCORE  MRA I AA 109 1-1 190 1-1 2  405 AB 79 1-2 8 1-2 9  BB 13 1-3 0 1-3 29  1-4 3 1-4 1  2-2 0 2-2 11  2-3 0 2-3 79  2-4 0 2-4 0  3-3 0 3-3 72  3-4 0 3-4 1  4-4 0 4-4 0  TOTAL FISH SCORED TFN EST PGI  201 201 201 204  NUMBER UNSCORABLE 0 0 0 0  ALLELE FREQUENCY (A)0.739 (1) 0.973 (1) 0.105  (B)0.261 (2) 0.020 (2) 0.270  (3) 0.000 (3) 0.620  (B)0.261 (2) 0.020 (2) 0.270  (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 (3) 0.620 \\ (3) 0.000 \\ (3$	TOTAL FISH SCORED	TFN	EST	
(4) 0.000 (4) 0.000 SAMPLE TFN-SCORE EST-SCORE PGI-SCORE MRA I AA 109 1-1 190 1-1 2 405 AB 79 1-2 8 1-2 9 BB 13 1-3 0 1-3 29 1-4 3 1-4 1 2-2 0 2-2 11 2-3 0 2-3 79 2-4 0 2-4 0 3-3 0 3-3 72 3-4 0 3-4 1 4-4 0 4-4 0 TOTAL FISH SCORED TFN EST PGI 201 201 204 NUMBER UNSCORABLE 0 0 0 ALLELE FREQUENCY (A)0.739 (1) 0.973 (1) 0.105 (B)0.261 (2) 0.020 (2) 0.270 (3) 0.000 (3) 0.620	NUMBER UNSCORABLE	TFN 200 0	EST 200 0	200 0
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	NUMBER UNSCORABLE ALLELE FREQUENCY (	TFN 200 0 A)0.745 (1 B)0.255 (2	EST 200 0 ) 0.988 (1) 2) 0.013 (2)	200 0 0.102 0.373
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	NUMBER UNSCORABLE ALLELE FREQUENCY (	TFN 200 0 A)0.745 (1 B)0.255 (2 (3	EST 200 0 ) 0.988 (1) 2) 0.013 (2) 3) 0.000 (3)	200 0 0.102 0.373 0.525
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	NUMBER UNSCORABLE ALLELE FREQUENCY ( (	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4	EST 200 0 ) 0.988 (1) 2) 0.013 (2) 3) 0.000 (3) 4) 0.000 (4)	200 0 0.102 0.373 0.525
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE MRA I AA 109	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190	EST 200 0 ) 0.988 (1) 2) 0.013 (2) 3) 0.000 (3) 4) 0.000 (4) 4) PGI-SCORE 1-1 2	200 0 0.102 0.373 0.525
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE MRA I AA 109 405 AB 79	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190 1-2 8 1-3 0	EST 200 0 ) 0.988 (1) ) 0.013 (2) ) 0.000 (3) ) 0.000 (4) ; PGI-SCORE 1-1 2 1-2 9 1-3 29	200 0 0.102 0.373 0.525
$\begin{array}{cccccccccccccccccccccccccccccccccccc$	NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE MRA I AA 109 405 AB 79	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190 1-2 8 1-3 0 1-4 3 2-2 0	EST 200 0 ) 0.988 (1) 2) 0.013 (2) 3) 0.000 (3) 4) 0.000 (4) 4) 0.000 (4) 5 PGI-SCORE 1-1 2 1-2 9 1-3 29 1-3 29 1-4 1 2-2 11	200 0 0.102 0.373 0.525
TOTAL FISH SCOREDTFNESTPGI201201204NUMBER UNSCORABLE00ALLELE FREQUENCY(A)0.739(1)0.973(B)0.261(2)0.020(2)0.270(3)0.000(3)0.620	NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE MRA I AA 109 405 AB 79	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190 1-2 8 1-3 0 1-4 3 2-2 0 2-3 0 2-3 0 2-4 0	EST 200 0 0.988 (1) 0.013 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 2 1-2 9 1-3 29 1-4 1 2-2 11 2-3 79 2-4 0	200 0 0.102 0.373 0.525
NUMBER UNSCORABLE000ALLELE FREQUENCY(A)0.739(1)0.973(1)0.105(B)0.261(2)0.020(2)0.270(3)0.000(3)0.620	NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE MRA I AA 109 405 AB 79	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190 1-2 8 1-3 0 1-4 3 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	EST 200 0 0.988 (1) 0.013 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 2 1-2 9 1-3 29 1-4 1 2-2 11 2-3 79 2-4 0 3-3 72 3-4 1	200 0 0.102 0.373 0.525
ALLELE FREQUENCY (A)0.739 (1) 0.973 (1) 0.105 (B)0.261 (2) 0.020 (2) 0.270 (3) 0.000 (3) 0.620	NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE MRA I AA 109 405 AB 79 BB 13	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190 1-2 8 1-3 0 1-4 3 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN	EST 200 0 ) 0.988 (1) 2) 0.013 (2) 3) 0.000 (3) 4) 0.000 (4) 5) 0.000 (3) 5) 0.000 (4) 5) 0.000 (4) 0.000 (4) 5) 0.000 (4) 0.000 (4) 5) 0.000 (4) 0.000	200 0.102 0.373 0.525 0.000
(3) 0.000 (3) 0.620	NUMBER UNSCORABLE ALLELE FREQUENCY ( ( SAMPLE TFN-SCORE MRA I AA 109 405 AB 79 BB 13 TOTAL FISH SCORED	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190 1-2 8 1-3 0 1-4 3 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN 201	EST 200 0 0.988 (1) 0.013 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 2 1-2 9 1-3 29 1-4 1 2-2 11 2-3 79 2-4 0 3-3 72 3-4 1 4-4 0 EST 201	200 0.102 0.373 0.525 0.000 PGI 204
	NUMBER UNSCORABLE ALLELE FREQUENCY ( SAMPLE TFN-SCORE MRA I AA 109 405 AB 79 BB 13 TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (	TFN 200 0 A)0.745 (1 B)0.255 (2 (3 (4 EST-SCORE 1-1 190 1-2 8 1-3 0 1-4 3 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN 201 0 A)0.739 (1	EST 200 0 0.988 (1) 0.013 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 2 1-2 9 1-3 29 1-4 1 2-2 11 2-3 79 2-4 0 3-3 72 3-4 1 4-4 0 EST 201 0 0.973 (1)	200 0.102 0.373 0.525 0.000 PGI 204 0 0.105

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MRAX AA 8 351 AB 6	2-4 0 3-3 0	1-1 1 1-2 9 1-3 14 1-4 0 2-2 21 2-3 75 2-4 0 3-3 53 3-4 0	
TOTAL FISH SCOP NUMBER UNSCORAD ALLELE FREQUENC	176 BLE 0 CY (A)0.702 (1 (B)0.298 (2 (3	EST 176 0 ) 0.972 (1) ) 0.026 (2) ) 0.000 (3) ) 0.003 (4)	0.364 0.564
SAMPLE TFN-SO MRC B1 AA 16 AB BB	CORE EST-SCORE 0 1-1 10 0 1-2 3 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	1-1 0 1-2 0 1-3 0 1-4 0	
TOTAL FISH SCOP NUMBER UNSCORAD ALLELE FREQUENC	0 BLE 16 CY (A)0.000 (1 (B)0.000 (2 (3	EST 13 3 ) 0.885 (1) ) 0.115 (2) ) 0.000 (3) ) 0.000 (4)	0.000
SAMPLE TFN-SO MRC B2 AA 16 AB BB	CORE EST-SCORE 0 1-1 10 0 1-2 2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCOP NUMBER UNSCORAD ALLELE FREQUENC	0 BLE 16 CY (A)0.000 (1 (B)0.000 (2	EST 12 4 ) 0.917 (1) ) 0.083 (2) ) 0.000 (3)	0.000

(3) 0.000 (3) 0.000 (4) 0.000 (4) 0.000

SAMPLETFN-SCOREEST-SCOREPGI-SCOREMRC C1AA58 $1-1$ 91 $1-1$ 0100AB $34$ $1-2$ 9 $1-2$ 0BB8 $1-3$ 0 $1-3$ 0 $1-4$ 0 $1-4$ 0 $2-2$ 0 $2-2$ 0 $2-2$ 0 $2-3$ 0 $2-3$ 0 $2-3$ 0 $2-4$ 0 $3-3$ 0 $3-3$ 0 $3-3$ 0 $3-4$ 0 $3-4$ 0 $4-4$ 0	
TOTAL FISH SCORED       TFN       EST       PGI         100       100       0         NUMBER UNSCORABLE       0       0         ALLELE FREQUENCY       (A)0.750       (1)       0.955         (B)0.250       (2)       0.045       (2)         (3)       0.000       (3)       0.000         (4)       0.000       (4)       0.000	
SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         MRC C2       AA       60       1-1       91       1-1       0         100       AB       33       1-2       9       1-2       0         BB       7       1-3       0       1-3       0         1-4       0       1-4       0         2-2       0       2-2       0         2-3       0       2-3       0         2-4       0       2-4       0         3-3       0       3-3       0         3-4       0       3-4       0         4-4       0       4-4       0	
TOTAL FISH SCORED       TFN       EST       PGI         100       100       0         NUMBER UNSCORABLE       0       0       0         ALLELE FREQUENCY       (A)0.765       (1)       0.955       (1)       0.000         (B)0.235       (2)       0.045       (2)       0.000         (4)       0.000       (4)       0.000	
SAMPLETFN-SCOREEST-SCOREPGI-SCOREMRQ 1AA16 $1-1$ 28 $1-1$ 035AB $14$ $1-2$ 3 $1-2$ 0BB1 $1-3$ 0 $1-3$ 2 $1-4$ 0 $1-4$ 0 $2-2$ 0 $2-2$ 7 $2-3$ 0 $2-3$ 16 $2-4$ 0 $2-4$ 0 $3-3$ 0 $3-3$ 5 $3-4$ 0 $3-4$ 0	
TOTAL FISH SCORED       TFN       EST       PGI         31       31       30         NUMBER UNSCORABLE       2       2       3         ALLELE FREQUENCY       (A)0.742       (1)       0.952       (1)       0.033         (B)0.258       (2)       0.048       (2)       0.500         (3)       0.000       (3)       0.467	

(3) 0.000 (3) 0.467 (4) 0.000 (4) 0.000

SAMPLE TFN-SCORI MRQ 2 AA 41 70 AB 25 BB 4	2-4 0 3-3 0 3-4 0	1-1 0 1-2 0 1-3 4 1-4 0	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY	(B)0.236 (2 (3	EST 60 10 ) 0.942 (1) ) 0.050 (2) ) 0.000 (3) ) 0.008 (4)	0.566 0.404
SAMPLE TFN-SCORI MRT AA 37 70 AB 27 BB 2	E EST-SCORE 1-1 65 1-2 1 1-3 0 1-4 0 2-2 0 2-3 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0	0.000
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY	(B)0.235 (2 (3)	EST 66 4 ) 0.992 (1) ) 0.008 (2) ) 0.000 (3) ) 0.000 (4)	0.000
SAMPLE TFN-SCORI MRT L1 AA 170 323 AB 113 BB 34	1-2 13 1-3 0 1-4 3 2-2 1 2-3 0 2-4 0	PGI-SCORE 1-1 0 1-2 2 1-3 3 1-4 0 2-2 4 2-3 14 2-3 14 2-4 0 3-3 11 3-4 0 4-4 0	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY	(B)0.285 (2	EST 317 5 ) 0.972 (1) ) 0.024 (2) ) 0.000 (3)	0.353

(4) 0.005 (4) 0.000

SAMPLE MRT L2 168	TFN-SCORE AA 83 AB 73 BB 7		PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL	FISH SCORED	TFN	EST	PGI
	UNSCORABLE FREQUENCY (/ (1	3)0.267 (2) (3)	165 3 0.976 (1) 0.021 (2) 0.000 (3) 0.003 (4)	0.000
SAMPLE MRT M1 131	TFN-SCORE AA 73 AB 48 BB 10	2-4 0 3-3 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL	FISH SCORED	TFN	EST	PGI
	UNSCORABLE FREQUENCY (/	3)0.260 (2) (3)	131 0 0.966 (1) 0.023 (2) 0.000 (3) 0.011 (4)	0.000
SAMPLE MRT M2 18	TFN-SCORE AA 7 AB 9 BB 2		PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL	FISH SCORED	TFN	EST	PGI
	UNSCORABLE FREQUENCY (/ (1	3)0.361 (2) (3)	17 1 0.941 (1) 0.059 (2) 0.000 (3) 0.000 (4)	0.000

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SAMPLE TFN-SCORE MRT N AA 90 154 AB 55 BB 7	1-3 0 1-4 1 2-2 0 2-3 0 2-4 1 3-3 0 3-4 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (. (;	B)0.227 (2 (3	152 2 ) 0.967 (1) ) 0.026 (2) ) 0.000 (3) ) 0.007 (4)	0.000
MRT P AA 44	1-2 3 1-3 1 1-4 0 2-2 0 2-3 0	1-1 1 1-2 4 1-3 9 1-4 0	
TOTAL FISH SCORED		EST	PGI
NUMBER UNSCORABLE Allele Frequency (/	B)0.289 (2 (3)	92 1 ) 0.978 (1) ) 0.016 (2) ) 0.005 (3) ) 0.000 (4)	0.267
SAMPLE TFN-SCORE MRT Q AA 153 267 AB 92 BB 15		PGI-SCORE 1-1 1 1-2 16 1-3 42 1-4 0 2-2 19 2-3 87 2-4 0 3-3 92 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN 260	EST 266	PGI
NUMBER UNSCORABLE Allele Frequency () ()	7 A)0.765 (1) B)0.235 (2 (3)	1	0.274 0.609

SAMPLE TFN-SCORE MRT R1 AA 27 69 AB 32 BB 8	EST-SCORE 1-1 65 1-2 2 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0		
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (A	B)0.358 (2) (3)	67 2 ) 0.985 (1) ) 0.015 (2) ) 0.000 (3) ) 0.000 (4)	0.254 0.631
SAMPLE TFN-SCORE MRT R2 AA 87 174 AB 67 BB 16	1-1 163	PGI-SCORE 1-1 4 1-2 5 1-3 26 1-4 0 2-2 13 2-3 57 2-4 0 3-3 58 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (1 (1	B)0.291 (2) (3)	172 2 ) 0.974 (1) ) 0.026 (2) ) 0.000 (3) ) 0.000 (4)	0.610
SAMPLE TFN-SCORE MRT R3 AA 76 140 AB 56 BB 6	EST-SCORE 1-1 132 1-2 5 1-3 1 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 3 1-2 11 1-3 12 1-4 1 2-2 10 2-3 48 2-4 0 3-3 45 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (A (B	3)0.246 (2) (3)	138 2 ) 0.978 (1) ) 0.018 (2) ) 0.004 (3) ) 0.000 (4)	0.304 0.577

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SAMPLE MRT R4 16	TFN-SCOR AA 8 AB 6 BB 1	2-4 0 3-3 0	$ \begin{array}{rrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrrr$	
TOTAL FISH SCORED		15	EST 15	PGI 13
	NSCORABLE REQUENCY	1 (A)0.733 (1 (B)0.267 (2 (3	1 1) 0.967 (1) 2) 0.033 (2) 3) 0.000 (3) 4) 0.000 (4)	3 0.154 0.308 0.538
SAMPLE MRT R5 32	TFN-SCOR AA 19 AB 10 BB 2	1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	1-1 0 1-2 1 1-3 5 1-4 0 2-2 0 2-3 12 2-4 0 3-3 13	
	SH SCORED	31	EST 31 1	PGI 31 1
		(A)0.774 (* (B)0.226 (2 (3	1) 0.968 (1) 2) 0.032 (2) 3) 0.000 (3)	0.097
SAMPLE MRT S 155	TFN-SCOR AA 77 AB 58 BB 15	1-3 0 1-4 2 2-2 0 2-3 0 2-4 0	1-1 2 1-2 11 1-3 12 1-4 0	
NUMBER U	SH SCOREI NSCORABLE REQUENCY	150 5 (A)0.707 (1 (B)0.293 (1) (3)	EST 151 4 1) 0.980 (1) 2) 0.013 (2) 3) 0.000 (3) 4) 0.007 (4)	0.313 0.586

MRT T AA 40 66 AB 20 BB 6	EST-SCORE       PGI-SCORE         1-1       62         1-2       4         1-2       4         1-3       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         2-4       0         2-4       0         3-3       0         3-4       0         4-4       0	
	TFN         EST           66         66           0         0           1)0.758         (1)         0.970           3)0.242         (2)         0.030         (2)           (3)         0.000         (3)           (4)         0.000         (4)	0.273 0.576
MRT U AA 74 126 AB 40 BB 10	EST-SCORE PGI-SCORE 1-1 120 1-1 2 1-2 4 1-2 14 1-3 0 1-3 13 1-4 1 1-4 0 2-2 0 2-2 5 2-3 0 2-3 44 2-4 0 2-4 0 3-3 0 3-3 45 3-4 0 3-4 0 4-4 0 4-4 0	
	TFN         EST           124         125           2         1           1)0.758         (1)         0.980         (1)           3)0.242         (2)         0.016         (2)           (3)         0.000         (3)         (4)         0.004         (4)	0.276 0.598
MRT V AA 116 220 AB 80 BB 14	EST-SCORE PGI-SCORE 1-1 200 1-1 2 1-2 10 1-2 11 1-3 0 1-3 29 1-4 1 1-4 0 2-2 0 2-2 19 2-3 0 2-3 74 2-4 0 2-4 0 3-3 0 3-3 77 3-4 0 3-4 0 4-4 0 4-4 0	
	TFN         EST           210         211           10         9           10.743         (1)         0.974           3)0.257         (2)         0.024         (2)           (3)         0.000         (3)         (4)         0.002         (4)	0.290 0.606

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SAMPLE MRT W 23	TFN-SCOR AA 10 AB 8 BB 5	E EST-SCORE 1-1 22 1-2 0 1-3 0 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN 23	EST 23	PGI O
	N SCORABLE REQUEN CY	0 (A)0.609 (1 (B)0.391 (2 (3	0 0.978 (1) 0.000 (2) 0.000 (3) 0.022 (4)	0 0.000 0.000 0.000
SAMPLE MRT XN 37	TFN-SCOR AA 22 AB 14 BB 1	E EST-SCORE 1-1 35 1-2 1 1-3 1 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FI	SH SCORED	TFN 37	EST 37	PGI 0
	NSCORABLE REQUENCY	0 (A)0.784 (1 (B)0.216 (2 (3	0 ) 0.973 (1)	0 000.0 000.0 000.0
SAMPLE PNG 1 20	TFN-SCOR AA 13 AB 6 BB 1	E EST-SCORE 1-1 19 1-2 1 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 8 2-3 11 2-4 0 3-3 1 3-4 0 4-4 0	
TOTAL FI	SH SCORED	<b>TFN</b> 20	EST 20	PGI 20
	N SCORABLE REQUENCY	: 0 (Å)0.800 (1 (B)0.200 (2 (3	0	0 0.000 0.675 0.325

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SAMPLE PNG 2 59	TFN-SCOR AA 15 AB 14 BB 1	E EST-SCORE 1-1 29 1-2 1 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 1 1-4 0 2-2 16 2-3 11 2-4 0 3-3 1 3-4 0 4-4 0	
TOTAL F	ISH SCORED	TFN 30	EST 30	PGI 29
NUMBER	UNSCORABLE	0	0	0
ALLELE	FREQUENCY	(A)0.733 (1 (B)0.267 (2		
		(3 (1	) 0.000 (3) ) 0.000 (4)	
	TEN CON	E EST-SCORE		0.000
SAMPLE PTW 10	AA 46	1-1 97	1–1 3	
295	AB 46 BB 9	1-2 4 1-3 0	1–2 16 1–3 21	
		1-4 0 2-2 0	1-4 1 2-2 19	
		2-3 0 2-4 0	2-3 64 2-4 0	
		3-3 0	3-3 64	
		3-4 0 4-4 0	3-4 0 4-4 0	
TOTAL F	ISH SCORED		EST	PGI
NUMBER	UNSCORABLE	101	101 6	188 0
ALLELE	FREQUENCY		) 0.980 (1) ) 0.020 (2)	
		(3	) 0.000 (3)	
SAMPLE	TEN_900E	E EST-SCORE		
PTW 12	AA 47	1-1 79	1-1 0	
89	AB 33 BB 6	1–3 0	1-2 0 1-3 0	
		1-4 1 2-2 1	1–4 0 2–2 0	
		2-3 0 2-4 0	2-3 0 2-4 0	
		3-30 3-40	3-3 0 3-4 0	
		4-4 0	4-4 0	
TOTAL F	ISH SCORED	TFN 86	EST 86	PGI O

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TOTAL FISH SCORED	TFN		EST		PGI
	86		86		0
NUMBER UNSCORABLE	3		3		0
ALLELE FREQUENCY	(A)0.738	(1)	0.953	(1)	0.000
	(B)0.262	(2)	0.041	(2)	0.000
		(3)	0.000	(3)	0.000
		(4)	0.006	(4)	0.000

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SAMPLE TFN-SCORE PTW 27 AA 108 386 AB 75 BB 16	1-3 0 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	1-1 2 1-2 12 1-3 21 1-4 0 2-2 13	
TOTAL FISH SCORED	TFN 199	EST 199	PGI 186
NUMBER UNSCORABLE ALLELE FREQUENCY ( (	1 A)0.731 (1) B)0.269 (2) (3)	1 0.962 (1)	0 0.099 0.282 0.618
	1-2       8         1-3       1         1-4       1         2-2       0         2-3       0         2-4       0         3-3       0         3-4       0	PGI-SCORE 1-1 3 1-2 19 1-3 24 1-4 0 2-2 12 2-3 66 2-4 0 3-3 71 3-4 0 4-4 0	
TOTAL FISH SCORED	<b>TFN</b> 195	EST 195	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY ( ()	6 A)0.718 (1) B)0.282 (2) (3)	6 0.974 (1)	0.279
SAMPLE TFN-SCORE PTW 42 AA 101 365 AB 81 BB 16	1-3 1 1-4 3 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 2 1-2 13 1-3 16 1-4 0 2-2 17 2-3 69 2-4 0 3-3 49 3-4 0 4-4 0	
TOTAL FISH SCORED	<b>TFN</b> 198	EST 198	PGI 166
NUMBER UNSCORABLE Allele Frequency ( ()	0 A)0.715 (1) B)0.285 (2) (3)	0 0.965 (1)	1 0.099 0.349 0.551

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SAMPLE TFN-SCORE PTW 45 AA 116 394 AB 65 BB 18		1-1 0 1-2 9 1-3 19 1-4 0 2-2 8 2-3 73 2-4 0 3-3 86	
TOTAL FISH SCORED	<b>TFN</b> 199	EST 199	PGI 195
NUMBER UNSCORABLE ALLELE FREQUENCY ( (	0 1)0.746 (1 B)0.254 (2 (3)	0 ) 0.975 (1) ) 0.025 (2) ) 0.000 (3)	0 0.072 0.251
SAMPLE TFN-SCORE PTW 52 AA 73 260 AB 50 BB 9	EST-SCORE 1-1 124 1-2 6 1-3 1 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	1-1 1 1-2 16 1-3 16 1-4 0 2-2 8 2-3 43 2-4 0 3-3 42	
TOTAL FISH SCORED	<b>TFN</b> 132	EST 132	PGI 126
NUMBER UNSCORABLE ALLELE FREQUENCY ( (	2 A)0.742 (1 B)0.258 (2 (3	2 ) 0.970 (1)	0 0.135 0.298 0.567
SAMPLE TFN-SCORE SEP AA 60 99 AB 35 BB 4	EST-SCORE 1-1 92 1-2 5 1-3 0 1-4 0 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0	
TOTAL FISH SCORED	TFN 99	EST 98	PGI 0
NUMBER UNSCORABLE Allele Frequency ( ()	0 A)0.783 (1 B)0.217 (2	1 ) 0.964 (1) ) 0.036 (2) ) 0.000 (3)	0 000.0 000.0 000.0

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SAMPLE TFN-SCORE SEQ AA 60 107 AB 41 BB 5	EST-SCORE 1-1 99 1-2 7 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCORED	<b>TFN</b> 106	EST 106	PGI O
NUMBER UNSCORABLE Allele Frequency (1 (1	1 A)0.759 (1) 3)0.241 (2) (3)	1	0 0.000 0.000 0.000
SAMPLE TFN-SCORE SNC A AA 94 200 AB 86 BB 20	EST-SCORE 1-1 188 1-2 10 1-3 1 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 2 1-2 11 1-3 23 1-4 0 2-2 19 2-3 63 2-4 0 3-3 81 3-4 1 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (1 (1	3)0.315 (2) (3)	200 0 0.970 (1) 0.025 (2) 0.002 (3) 0.002 (4)	0.280 0.623
SAMPLE TFN-SCORE SNC B AA 109 200 AB 84 BB 7	EST-SCORE 1-1 188 1-2 11 1-3 0 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	1-1 0 1-2 11 1-3 17 1-4 0 2-2 24 2-3 75 2-4 1 3-3 72	
TOTAL FISH SCORED NUMBER UNSCORABLE ALLELE FREQUENCY (1		EST 200 0 ) 0.970 (1)	PGI 200 0 0.070
	B)0.245 (2)	) 0.027 (2) ) 0.000 (3)	0.338

(3) 0.000 (3) 0.590
(4) 0.002 (4) 0.002

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TOTAL FISH SCORED TFN EST	PGI
129 129 NUMBER UNSCORABLE 1 1	130 0
ALLELE FREQUENCY (A)0.674 (1) 0.973 (1) (B)0.326 (2) 0.023 (2) (3) 0.000 (3) (4) 0.004 (4)	0.288 0.615
SAMPLE TFN-SCORE EST-SCORE PGI-SCORE	
SNC D AA 109 1-1 185 1-1 2 200 AB 80 1-2 13 1-2 17	
BB 11 1-3 0 1-3 18 1-4 2 1-4 0	
2-2 0 2-2 26 2-3 0 2-3 81	
2-4 0 2-4 0 3-3 0 3-3 56	
3-4 0 $3-4$ 0 4-4 0 $4-4$ 0	
TOTAL FISH SCORED TFN EST 200 200	<b>PGI</b> 200
NUMBER UNSCORABLE 0 0 ALLELE FREQUENCY (A)0.745 (1) 0.962 (1)	0 0.097
(B)0.255 (2) 0.032 (2) (3) 0.000 (3)	
(4) 0.005 (4)	
SAMPLE TFN-SCORE EST-SCORE PGI-SCORE SNC E AA 54 1-1 123 1-1 3	
130 AB 62 1-2 6 1-2 11	
BB 13 1-3 0 1-3 17 1-4 0 1-4 0	
2-2 0 2-2 16 2-3 0 2-3 51	
2-4 0 2-4 0 3-3 0 3-3 32	
3-4 0 3-4 0 4-4 0 4-4 0	
TOTAL FISH SCORED TFN EST	PGI
129 129 NUMBER UNSCORABLE 1 1	130 0
ALLELE FREQUENCY (A)0.659 (1) 0.977 (1) (B)0.341 (2) 0.023 (2)	
(3) 0.000 (3)	

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SAMPLE	IT S	m-s	CORE	EST-	SCORE	PGI-	SCORE
SNC F	A	A 12	20	1-1	185	1-1	1
200	A C	3 (	54	1-2	12	1-2	15
	BI	3 '	16	1-3	0	1-3	21
				1-4	3	1-4	0
				2-2	0	2-2	14
				2-3	0	2-3	73
				2-4	0	2-4	0
				3-3	0	3–3	76
				3-4	0	3-4	0
				4-4	0	4-4	0
TOTAL	FISH	SCOL	RED	TFN		EST	
				2	200	20	00

TOTAL FISH SCORED	TFN		EST		PGI
	200		200		200
NUMBER UNSCORABLE	0		0		0
ALLELE FREQUENCY	(A)0.760	(1)	0.962	(1)	0.095
	(B)0.240	(2)	0.030	(2)	0.290
		(3)	0.000	(3)	0.615
		(4)	0.007	(4)	0.000

SAMPLE	TFN-	-SCORE	EST-S	SCORE	PGI-S	SCORE
SNL A	AA	25	1–1	0	1-1	0
50	AB	22	1-2	0	1-2	0
	BB	3	1-3	0	1-3	0
			1-4	0	1-4	0
			2-2	0	2-2	0
			2-3	0	2-3	0
			2-4	0	2-4	0
			3-3	0	3-3	0
			3-4	0	3-4	0
			4-4	0	4-4	0

TOTAL FISH SCORED	TFN		EST		PGI
	50		Ő		0
NUMBER UNSCORABLE	0		50		0
ALLELE FREQUENCY	(A)0.720	(1)	0.000	(1)	0.000
	(B)0.280	(2)	0.000	(2)	0.000
÷		(3)	0.000	(3)	0.000
		(4)	0.000	(4)	0.000

SAMPLE	TFN-SCO	RE EST-	-SCOR	E PGI-	SCO	RE	
SNL AB	AA 59	1-1	93	1-1	1		
191	AB 31	1-2	0	1-2	4		
	BB 3	1-3	0	1-3	9		
		1-4	Ó	1-4	Ō		
		2-2	Ō	2-2	12		
		2-3	0	2-3	33		
		2-4	Ō	2-4	1		
		3-3	ŏ	3-3	34		
		3-4	Ō	3-4	0		
		4_4	ō	4_4	Ō		
		•••	•	•••	•		
TOTAL F	ISH SCORE	D I	FN	E	ST		
			93		93		
NUMBER	UNSCORABL	E	3		3		
	FREQUENCY		_	1) 1.0	_	(1)	0.
		(B)0.1		2) 0.0	00	(2)	0.
				~ ~ ~	~ ~	1	

	94	
	1	
1)	0.080	
2)	0.330	

PGI

(3) 0.000 (3) 0.585 (4) 0.000 (4) 0.005

	SNL B 50	TFN-SCORE AA 30 AE 16 BB 3	1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 0 1-2 2 1-3 10 1-4 0 2-2 3 2-3 17 2-4 0 3-3 18 3-4 0 4-4 0		
	TOTAL FIS	H SCORED	TFN 49	EST 49	PGI 50	
	NUMBER UN Allele Fr	EQUENCY (A	1 ()0.776 (1) ()0.224 (2) (3)	1 1.000 (1) 0.000 (2) 0.000 (3) 0.000 (4)	0 0.120 0.250 0.630	
	SNL C 221	TFN-SCORE AA 73 AB 50 BB 13	1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	PGI-SCORE 1-1 3 1-2 6 1-3 20 1-4 0 2-2 17 2-3 39 2-4 0 3-3 48 3-4 0 4-4 0 0-3 1		
	TOTAL FIS	H SCORED	<b>TFN</b> 136	EST 136	PGI 134	
	NUMBER UN Allele Fr	EQUENCY (A	0 L)0.721 (1) 3)0.279 (2) (3)	0 1.000 (1) 0.000 (2) 0.000 (3) 0.000 (4)	1 0.119	,
,	STA 79	TFN-SCORE AA 46 AB 25 BB 5	1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	• • •		
	TOTAL FIS	H SCORED	TFN 76	EST 13	PGI 0	
	NUMBER UN Allele Fr	EQUENCY (A	3 1)0.770 (1) 3)0.230 (2) (3)	66 0.962 (1) 0.038 (2)	0 0.000 0.000 0.000	

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STI 1 200	IFN-SCORE AA 96 AB 91 BB 10	EST-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0	
TOTAL FISH	H SCORED	TFN	EST	PGI
NUMBER UNS	SCORABLE	197 3	0 200	0
	EQUENCY (	A)0.718 (1) B)0.282 (2)	) 0.000 (1) ) 0.000 (2) ) 0.000 (3)	0.000 0.000 0.000
		EST-SCORE		
	AA 98	1-1 0	1-1 0	
	AB 78 BB 11	1-2 1 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	1-2       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         3-3       0         3-4       0         4-4       0	
TOTAL FISH	H SCORED	TFN 187	EST 1	PGI 0
NUMBER UNS Allele Fri	EQUENCY (	3 A)0.733 (1) B)0.267 (2 (3)	189 ) 0.500 (1) ) 0.500 (2) ) 0.000 (3) ) 0.000 (4)	0 0.000 0.000 0.000
STS 2 1 22 1	IFN-SCORE AA 11 AB 11 BB 0	EST-SCORE 1-1 0 1-2 9 1-3 0 1-4 0 2-2 0 2-3 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH	H SCORED	<b>TFN</b> 22	EST O	PGI O

 22
 0
 0

 NUMBER UNSCORABLE
 0
 22
 0

 ALLELE FREQUENCY (A)0.750 (1)
 0.000 (1)
 0.000

 (B)0.250 (2)
 0.000 (2)
 0.000

 (3)
 0.000 (4)
 0.000

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SAMPLE TFN-SCORE TAR A AA 71 260 AB 53 BB 12	EST-SCORE 1-1 149 1-2 3 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 2 1-2 12 1-3 25 1-4 0 2-2 7 2-3 51 2-4 0 3-3 48 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN 136	EST 152	PGI 145
NUMBER UNSCORABLE ALLELE FREQUENCY ( (	19	3 ) 0.990 (1) ) 0.010 (2) ) 0.000 (3)	10 0.141 0.266 0.593
SAMPLE TFN-SCORE TAR C AA 22 41 AB 16 BB 3	EST-SCORE 1-1 41 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE Allele Frequency (. (:	B)0.268 (2 (3		0.000
SAMPLE TFN-SCORE TAR D AA 105 348 AB 79 BB 11	EST-SCORE 1-1 194 1-2 1 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	PGI-SCORE 1-1 0 1-2 8 1-3 30 1-4 0 2-2 13 2-3 65 2-4 1 3-3 77 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN 195	EST 195	PGI 194
NUMBER UNSCORABLE ALLELE FREQUENCY (/	4 A)0.741 (1 B)0.259 (2 (3)	4	5 0.098 0.258 0.642

SAMPLE TFN TAR E AA 303 AB BB	82 1-2	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1 4 17 0 13 54 0 62 0	
TOTAL FISH S			ST 97	PGI 151
NUMBER UNSCO Allele Frequ	RABLE ENCY (A)0.7	3	3 87 (1) 13 (2) 00 (3)	2 0.076 0.278 0.646
SAMPLE TFN TAR E1 AA 122 AB BB	-SCORE EST- 26 1-1 31 1-2 4 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	0 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4	0 3 7 0 4 27 0 19 0	
TOTAL FISH S	CORED	FN E	ST	PGI
NUMBER UNSCO Allele Frequ	ENCY (A)0.6	0	00 (2) 00 (3)	0.317 0.600
SAMPLE TFN TAR F AA 310 AB BB	-SCORE EST- 110 1-1 64 1-2 5 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	3 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4	0 13 23 0 17 69 0 57 0	
TOTAL FISH S		rfn E 179	ST 3	PGI 179
NUMBER UNSCO Allele Frequ	RABLE ENCY (A)0.7	1 1	77 00 (1) 00 (2)	1 0.101 0.324

(3) 0.000 (3) 0.575 (4) 0.000 (4) 0.000

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SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         TAR H       AA       41       1-1       0       1-1       0         74       AB       31       1-2       0       1-2       0         BB       2       1-3       0       1-3       0         1-4       0       1-4       0       2-2       0         2-2       0       2-2       0       2-3       0         2-4       0       2-4       0       3-3       0         3-3       0       3-3       0       3-4       0         4-4       0       4-4       0       4-4       0	
TOTAL FISH SCORED       TFN       EST         74       0         NUMBER UNSCORABLE       0       74         ALLELE FREQUENCY (A)0.764       (1)       0.000       (1)         (B)0.236       (2)       0.000       (2)         .       (3)       0.000       (3)         (4)       0.000       (4)	0.000 0.000
SAMPLE       TFN-SCORE       EST-SCORE       PGI-SCORE         TAR I       AA 119       1-1 112       1-1       0         350       AB 72       1-2       6       1-2       12         BB 9       1-3       0       1-3       20         1-4       0       1-4       0         2-2       0       2-2       12         2-3       0       2-3       86         2-4       0       2-4       0         3-3       0       3-3       68         3-4       0       3-4       0	-
TOTAL FISH SCORED       TFN       EST         200       118         NUMBER UNSCORABLE       0       82         ALLELE FREQUENCY       (A)0.775       (1)       0.975       (1)         (B)0.225       (2)       0.025       (2)         (3)       0.000       (3)       (4)       0.000       (4)	0.308 0.611
SAMPLE         TFN-SCORE         EST-SCORE         PGI-SCORE           TRD         AA         92         1-1         184         1-1         0           198         AB         63         1-2         11         1-2         0           BB         15         1-3         0         1-3         0           1-4         1         1-4         0           2-2         0         2-2         0           2-3         0         2-3         0           2-4         0         2-4         0           3-3         0         3-3         0           3-4         0         3-4         0           4-4         0         4-4         0	
TOTAL FISH SCORED       TFN       EST         170       196         NUMBER UNSCORABLE       28       2         ALLELE FREQUENCY (A)0.726       (1) 0.969       (1)         (B)0.274       (2) 0.028       (2)         (3) 0.000       (3)       (4)	0.000 0.000

SAMPLE TFN-SCOR XX AA 207 694 AB 119 BB 22	1-2 22 1-3 0 1-4 5 2-2 0 2-3 0 2-4 0		
TOTAL FISH SCORED	<b>TFN</b> 348	EST 348	PGI 341
NUMBER UNSCORABLE ALLELE FREQUENCY	1	1	- 4
	(B)0.234 (2 (3	2) 0.032 (2) 3) 0.000 (3) 4) 0.007 (4)	0.302 0.598
SAMPLE TFN-SCOR XXY AA 22	E EST-SCORE 1-1 49	PGI-SCORE	
51 AB 22 BB 7	1-2 2 1-3 0	1-2 1 1-3 8	
	1-4 0 2-2 0	-	
	2-4 0	2-3 17 2-4 0	
		-3-3 18 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE	51 0 (1)0 6 hr (1	51 0	51 0
ALLELE FREQUENCY	(B)0.353 (2 (3	(1) (1) (1) (1) (1) (1) (1) (1) (1) (1)	0.275 0.598
SAMPLE TFN-SCOR XXZ AA 20	E EST-SCORE 1-1 44	PGI-SCORE	
51 AB 23 BB 8			
	1-4 0		
	2-4 0	2-3 18 2-4 0	
		3-3 18 3-4 0 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE	51 0	51 0	51 0
ALLELE FREQUENCY	(B)0.382 (2	2) 0.059 (2)	0.324
		) 0.010 (3) ) 0.000 (4)	0.588

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SAMPLE TFN-SCORE ZP A1 AA 22 63 AB 29 BB 9		PGI-SCORE 1-1 0 1-2 2 1-3 0 1-4 0 2-2 24 2-3 31 2-4 0 3-3 3 3-4 1 4-4 0	
TOTAL FISH SCORED	TFN	EST	PGI
NUMBER UNSCORABLE ALLELE FREQUENCY (/	B)0.392 (2) (3)		0.664 0.311
SAMPLE TFN-SCORE ZP A2 AA 70 130 AB 50 BB 10	3-3 0	1-1 0 1-2 2 1-3 1 1-4 0 2-2 62	
		EST	PGI
TOTAL FISH SCORED	TFN	CO1	LOT
NUMBER UNSCORABLE Allele Frequency ()	130 0 A)0.731 (1) B)0.269 (2) (3)	130 0	130 0 0.012 0.681 0.308
NUMBER UNSCORABLE ALLELE FREQUENCY (1	130 0 A)0.731 (1) B)0.269 (2) (3) (4) EST-SCORE 1-1 53 1-2 1 1-3 0 1-4 0 2-2 1 2-3 0 2-4 0 3-3 0	130 0 0.981 (1) 0.019 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 28 2-3 22 2-4 0 3-3 5 3-4 0	130 0 0.012 0.681 0.308
NUMBER UNSCORABLE ALLELE FREQUENCY (1 (1) SAMPLE TFN-SCORE ZP D1 AA 30 56 AB 22	130 0 A)0.731 (1) B)0.269 (2) (3) (4) EST-SCORE 1-1 53 1-2 1 1-3 0 1-4 0 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN	130 0.981 (1) 0.019 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 28 2-3 22 2-4 0 3-3 5 3-4 0 4-4 0 EST	130 0 0.012 0.681 0.308 0.000
NUMBER UNSCORABLE ALLELE FREQUENCY (A SAMPLE TFN-SCORE ZP D1 AA 30 56 AB 22 BB 3	130 0 A)0.731 (1) B)0.269 (2) (3) (4) EST-SCORE 1-1 53 1-2 1 1-3 0 1-4 0 2-2 1 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0 TFN 55 0	130 0.981 (1) 0.019 (2) 0.000 (3) 0.000 (4) PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 28 2-3 22 2-4 0 3-3 5 3-4 0 4-4 0 EST 55 0	130 0 0.012 0.681 0.308 0.000 PGI 55 0

 (2)
 0.027
 (2)
 0.709

 (3)
 0.000
 (3)
 0.291

 (4)
 0.000
 (4)
 0.000

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SAMPLE ZP D2 112	TFN-SCOF AA 61 AB 42 BB 8	1-3 0 1-4 1 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0	1-1 0 1-2 1 1-3 2 1-4 0 2-2 51 2-3 47 2-4 0 3-3 11	
TOTAL FI	SH SCOREI	) TFN 111	EST 111	PGI 112
NUMBER UN Allele Fi		1 (A)0.739 (1 (B)0.261 (2 (3	1	0 0.013 0.670 0.317
SAMPLE ZP H 199				
TOTAL FI	SH SCORED	) TFN 199	EST 199	PGI 199
NUMBER UN Allele Fi		(A)0.701 (1 (B)0.299 (2 (3	0	0 0.020 0.671 0.309
SAMPLE ZP I 109	TFN-SCOF AA 61 AB 40 BB 8	1-2 6 1-3 0 1-4 1 2-2 0 2-3 0 2-4 0	1-1 0 1-2 1 1-3 0 1-4 0 2-2 47 2-3 52 2-4 0 3-3 9	
TOTAL FI	SH SCOREI	) TFN 109	EST 109	PGI 109
NUMBER UN Allele Fi		CA)0.743 (1 (A)0.257 (2 (B)0.257 (2 (3	0	0 0.005 0.674 0.321

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SAM	IPL	E TI	FN-SC	ORE	EST-	-SCORE	PGI-	SCORE
ZP	J	A	A E	54	1-1	118	1-1	0
	12	7 Al	B 5	53	1-2	8	1-2	3
		B	<b>B</b> 1	0	1-3	0	1-3	2
					1-4	1	1-4	0
					2-2	0	2-2	68
					2-3	0	2-3	43
					2-4	0	2-4	1
					3-3	0	3-3	10
					3-4	0	3-4	0
					4-4	0	4-4	0
TOT	AL	FISH	SCOF	ED	1	FN	E	ST
							<u>م</u>	

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TOTAL FISH SCORED	TFN		EST		PGI
	127		127		127
NUMBER UNSCORABLE	0		0		0
ALLELE FREQUENCY	(A)0.713	(1)	0.965	(1)	0.020
	(B)0.287	(2)	0.031	(2)	0.720
			0.000		
		(4)	0.004	(4)	0.004

Summary of the phenotypic scores of the skipjack samples.

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SAMPLETFN-SCOREEST-SCOREPGI-SCOREAP A $1-1$ 0 $1-1$ 0 $172$ $1-2$ 3 $1-2$ 0 $1-3$ 0 $1-3$ 0 $1-3$ $1-4$ 0 $1-4$ 0 $1-4$ $2-2$ 82 $2-2$ 79 $2-2$ $2-3$ 68 $2-3$ 66 $2-3$ $2-4$ 0 $2-4$ 2 $2-4$ $3-3$ 18 $3-3$ $24$ $3-3$ $3-4$ 0 $3-4$ 0 $3-4$ $4-4$ 0 $4-4$ 0
TOTAL FISH SCORED       TFN       EST       PGI         171       171       0         NUMBER UNSCORABLE       1       1       0         ALLELE FREQUENCY       (1)       0.009       (1)       0.000       (1)       0.000         (2)       0.687       (2)       0.661       (2)       0.000         (3)       0.304       (3)       0.333       (3)       0.000         (4)       0.000       (4)       0.006       (4)       0.000
SAMPLETFN-SCOREEST-SCOREPGI-SCOREEC B $1-1$ 0 $1-1$ 0200 $1-2$ 1 $1-2$ 0 $1-3$ 2 $1-3$ 1 $1-3$ $1-4$ 0 $1-4$ 0 $1-4$ $2-2$ $116$ $2-2$ $29$ $2-2$ $2-3$ $72$ $2-3$ $91$ $2-3$ $2-4$ 0 $2-4$ $2$ $2-4$ $3-3$ $8$ $3-3$ $72$ $3-3$ $3-4$ 0 $3-4$ $4$ $3-4$ $4-4$ 0 $4-4$ 0
TOTAL FISH SCOREDTFNESTPGI1991990NUMBER UNSCORABLE110ALLELE FREQUENCY(1)0.008(1)0.003(1)0.000(2)0.766(2)0.379(2)0.000(3)0.226(3)0.603(3)0.000(4)0.000(4)0.015(4)0.000
SAMPLETFN-SCOREEST-SCOREPGI-SCOREEC $1-1$ $0$ $1-1$ $0$ 200 $1-2$ $2$ $1-2$ $0$ $1-3$ $0$ $1-3$ $1$ $1-3$ $1-4$ $0$ $1-4$ $0$ $2-2$ $95$ $2-2$ $30$ $2-2$ $95$ $2-2$ $30$ $2-3$ $83$ $2-3$ $87$ $2-3$ $83$ $2-3$ $87$ $2-3$ $0$ $2-4$ $1$ $2-4$ $0$ $2-4$ $1$ $3-3$ $18$ $3-3$ $69$ $3-4$ $0$ $3-4$ $0$ $4-4$ $0$ $4-4$ $0$
TOTAL FISH SCOREDTFNESTPGI1981980NUMBER UNSCORABLE220ALLELE FREQUENCY(1)0.005(1)0.003(1)0.000(2)0.694(2)0.374(2)0.000(3)0.301(3)0.596(3)0.000(4)0.000(4)0.028(4)0.000

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EC D 1- 200 1- 1- 2- 2- 3- 3- 3-	-3 85 2-3 -4 0 <u>2</u> -4	$\begin{array}{ccccc} 0 & 1-1 \\ 0 & 1-2 \\ 1 & 1-3 \\ 0 & 1-4 \\ 26 & 2-2 \\ 93 & 2-3 \\ 2 & 2-4 \\ 73 & 3-3 \\ 4 & 3-4 \end{array}$	0 0 0 0 0 0 0 0
TOTAL FISH S NUMBER UNSCO ALLELE FREQU	1 DRABLE JENCY (1) 0.4 (2) 0.4 (3) 0.3	020 (1) 0.00 598 (2) 0.30	0       0         1       0         03       (1)       0.000         59       (2)       0.000         13       (3)       0.000
EC E 1- 200 1- 1- 2- 2- 3- 3- 3-	-3 91 2-3 -4 0 2-4	$\begin{array}{cccc} 0 & 1-1 \\ 0 & 1-2 \\ 1 & 1-3 \\ 0 & 1-4 \\ 53 & 2-2 \\ 97 & 2-3 \\ 2 & 2-4 \\ 45 & 3-3 \\ 2 & 3-4 \end{array}$	0 0 0 0 0 0
TOTAL FISH S NUMBER UNSCO ALLELE FREQU	20 DRABLE JENCY (1) 0.4 (2) 0.4 (3) 0.2		0 0 0 0 02 (1) 0.000 12 (2) 0.000 75 (3) 0.000
EC F 1- 200 1- 1- 2- 2- 3- 3- 3-	-3 74 2-3 -4 0 2-4	0 1-1 0 1-2 3 1-3 0 1-4 49 2-2 91 2-3 2 2-4 52 3-3 2 3-4	0 0 0 0 0
TOTAL FISH S NUMBER UNSCO ALLELE FREQU	19 DRABLE JENCY (1) 0. (2) 0. (3) 0.	010 (1) 0.00 741 (2) 0.48	0       0         0       0         08       (1)       0.000         08       (2)       0.000         03       (3)       0.000

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SAMPLE TFN-S EC G 1-1 200 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORA Allele Frequen	CY (1) 0.027 ( (2) 0.670 ( (3) 0.303 (	2) 0.460 (2)	0.000
SAMPLE TFN-S EC H 1-1 200 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$		
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORA ALLELE FREQUEN	CY (1) 0.015 ( (2) 0.709 ( (3) 0.276 (	199 1 1) 0.005 (1) 2) 0.432 (2) 3) 0.543 (3) 4) 0.020 (4)	0.000
EC I 1-1 200 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	COREEST-SCORE0 $1-1$ 02 $1-2$ C2 $1-3$ 00 $1-4$ 082 $2-2$ $31$ 97 $2-3$ $101$ 0 $2-4$ 115 $3-3$ $64$ 0 $3-4$ 10 $4-4$ 0	$ \begin{array}{cccccc} 1-1 & 0 \\ 1-2 & 0 \\ 1-3 & 0 \\ 1-4 & 0 \\ 2-2 & 0 \\ 2-3 & 0 \\ 2-4 & 0 \\ 3-3 & 0 \\ 3-4 & 0 \end{array} $	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORA ALLELE FREQUEN	CY (1) 0.010 ( (2) 0.664 ( (3) 0.326 (	198 2 1) 0.000 (1) 2) 0.414 (2) 3) 0.581 (3) 4) 0.005 (4)	0.000

	SAMPLE       TFN-SCORE       EST-S         JOHN F       1-1       0       1-1         139       1-2       0       1-2         1-3       2       1-3       1-4         1-4       0       1-4       2-2       65       2-2         2-3       59       2-3       2-4       0       2-4         3-3       12       3-3       3-4       0       3-4         4-4       0       4-4       0       4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
	ALLELE FREQUENCY (1) 0.0 (2) 0.6 (3) 0.3	8 138 1 1 07 (1) 0.022 (1)	0.000
	2-3 69 2-3 2-4 0 2-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
• .	ALLELE FREQUENCY (1) 0.0 (2) 0.6 (3) 0.3	51 151 2 2	0.000
	SAMPLE         TFN-SCORE         EST-S           KB         1-1         0         1-1           162         1-2         3         1-2           1-3         2         1-3           1-4         0         1-4           2-2         77         2-2           2-3         60         2-3           2-4         0         2-4           3-3         19         3-3           3-4         0         3-4           4-4         0         4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	
	ALLELE FREQUENCY (1) 0.0 (2) 0.6 (3) 0.3	1 161 1 1	0.000

SAMPLE	TFN-SCO	RE EST-		PGI-SCOI	
KC		0 1-1			
136		2 1-2 0 1-3			)
	-	0 1-3		1-3 (	
	2-2 5			2-2 (	
	2-3 6		68	2-3 (	
	2-4 (	0 2-4	1	2-4 (	)
	3-3 1			3-3 (	
	+	0 3-4		3-4 (	
	4_4 (	0 4-4	0	4_4 (	)
TOTAL FI	SH SCORE		FN 36	EST 136	PGI O
NUMBER U	NSCORABLE		0	0	Ő
	REQUENCY			) 0.007	(1) 0.000
		(2) 0.			(2) 0.000
			316 (3		
		(4) 0.	000 (4	) 0.026	(4) 0.000
SAMPLE	TFN-SCO			PGI-SCOI	
ME A		0 1-1	0	1-1 (	
200		2 1-2 3 1-3		1-2 ( 1-3 (	
		5 1-3 0 1-4		1-3 (	
	2-2 9		_	2-2 (	
	2-3 8			2-3 (	
	2-4 (	0 2-4	2	2-4 (	)
	3-3 12			3-3 (	
	-	0 3-4		3-4 (	
	4_4 (	0 4-4	0	4_4 (	)
TOTAL FI	SH SCORE		FN 00	EST 200	PGI O
NUMBER I	INSCORABLI		0	0	õ
	REQUENCY		013 (1	) 0.007	(1) 0.000
			707 (2		
				) 0.347	
		(4) 0.	000 (4	) 0.007	(4) 0.000
SAMPLE	TFN-SCO	RE EST-	SCORE	PGI-SCOI	RE
MED		0 1-1			)
198		1 1-2			)
	· +	4 1–3 0 1–4			)
	2-2 8				) ·
	2-3 8				)
		0 2-4			)
	3-3 2		28		)
	+	0 3-4			
	4_4 (	0 4_4	U		)
TOTAL FI	SH SCORE		FN 95	EST 195	PGI O
NUMBER U	INSCORABLI		3	3	Ō
ALLELE H	REQUENCY				(1) 0.000
				) 0.603	
			338 (3 000 (4	) 0.379 ) 0.013	
		(4) 0.	000 (4	/ 0.013	· · · · · · · · · · · · · · · · · · ·

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ME G 1- 100 1- 1- 2- 2- 3- 3-	2 1 1-2 3 1 1-3 4 0 1-4 2 48 2-2 3 45 2-3 4 0 2-4	0 1- 2 1- 0 1- 44 2- 44 2- 44 2- 9 3- 1 3-	1 0 2 0 3 0 4 0 2 0 3 0 4 0 3 0 4 0	
TOTAL FISH S			est 100	PGI O
NUMBER UNSCO Allele Frequ	RABLE ENCY (1) 0.0 (2) 0.0 (3) 0.1	1 010 (1) 0 717 (2) 0 273 (3) 0	0 .010 (1) ( .670 (2) ( .315 (3) (	0 0.000 0.000 0.000 0.000
MK A1 1- 200 1- 1- 2- 2- 2- 2-	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0 1- 0 1- 1 1- 1 2- 98 2- 1 2- 53 3- 3 3-	1 0 2 0 3 0 4 0 2 0 3 0 4 0 3 0 4 0 3 0 4 0	
TOTAL FISH S			est	PGI
NUMBER UNSCO Allele Frequ	RABLE ENCY (1) 0. (2) 0. (3) 0.	1 008 (1) 0 691 (2) 0	.460 (2) ( .523 (3) (	0 0.000 0.000 0.000 0.000
MK A2 1- 198 1- 1- 2- 2- 3-	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	0 1- 1 1- 3 1- 1 1- 38 2- 110 2- 2 2- 37 3- 4 3-	1 0 2 0 3 0 4 0 2 0 3 0 4 0 3 0 4 0	
TOTAL FISH S			EST	PGI
NUMBER UNSCO Allele Frequ	RABLE	4 021 (1) 0	196 2 .013 (1) ( .482 (2) (	

MK C 1-1 200 1-2 1-3 1-4	0 2-4 4	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCO NUMBER UNSCORAL ALLELE FREQUEN	200 BLE 0	) 0.425 (2) ) 0.558 (3)	0.000
SAMPLE TFN-SO MK D 1-1 199 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	
TOTAL FISH SCOUNT NUMBER UNSCORAL ALLELE FREQUEN	189	) 0.484 (2) ) 0.484 (3)	0.000
MRQ 1-1	0 1-4 0 40 2-2 19 36 2-3 37 0 2-4 1 8 3-3 32 0 3-4 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	
TOTAL FISH SCO NUMBER UNSCORAI ALLELE FREQUEN(	88	) 0.418 (2) ) 0.560 (3)	0.000

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SAMPLE	TFN-S	CORE	EST-S	CORE	PGI-SC	ORE	
NZ A	1-1	0	1–1	0	1-1	0	
197	1-2		1-2		1-2	0	
	1-3		1-3	1	1-3	0	
	1-4		1-4		1-4	0	
	2-2 2-3		2-2	55 01	2-2 2-3	0 0	
	2-3 2-4		2-3 2-4	4	2-5 2-4		
		16			3-3		
		Ő			3-4		
	4_4		4-4		4-4		
TOTAL FI	SH SCO	RED	TF		EST		PGI
NUMBER U	NGUUDA	ים זמ	195	2	195 2		0 0
ALLELE F							
	1134 OBN				) 0.53		
					) 0.44		
					) 0.01		
CAMDE D	forma c		7070 0/		DAT GA	000	
SAMPLE NZ B	1-1		1-1		PGI-SC 1-1		
199	1-2		1-2		1-2	0	
	1-3		1-3		1-3	õ	
	1-4		1-4	ō	1-4		
	2-2	100			2-2		
					2-3		
	2-4				2-4		
					3-3		
		n		1	- <b>3</b> R	n	
					3-4		
			3-4 4-4		3-4 4-4		
TOTAL FI	4_4	0		0	4-4 EST	0	PGI 0
TOTAL FI NUMBER U	4_4 SH SCO	0 RED	4_4 TFN 197	0	4-4	0	PGI O O
NUMBER U	4_4 SH SCO	0 RED BLE CY (1	4-4 TFN 197 2 ) 0.01	0 17 22 13 (1	4-4 EST 197 2 ) 0.00	0	0 0 0.000
NUMBER U	4-4 SH SCO NSCORA	0 RED BLE CY (1 (2	4-4 TF1 197 2 ) 0.01 ) 0.71	0 7 2 13 (1 13 (2	4-4 EST 197 2 ) 0.00 ) 0.57	0 0 (1) 4 (2)	0 0 0.000 0.000
NUMBER U	4-4 SH SCO NSCORA	0 RED BLE CY (1 (2 (3	4-4 TF 197 2 ) 0.0 ) 0.7 ) 0.2	0 7 2 13 (1 13 (2 74 (3	4-4 EST 197 2 ) 0.00 ) 0.57 ) 0.42	0 0 (1) 4 (2) 1 (3)	0 0 0.000 0.000 0.000
NUMBER U	4-4 SH SCO NSCORA	0 RED BLE CY (1 (2 (3	4-4 TF 197 2 ) 0.0 ) 0.7 ) 0.2	0 7 2 13 (1 13 (2 74 (3	4-4 EST 197 2 ) 0.00 ) 0.57	0 0 (1) 4 (2) 1 (3)	0 0 0.000 0.000 0.000
NUMBER U	4_4 SH SCO NSCORA REQUEN	0 RED BLE CY (1 (2 (3 (4	4-4 TFN 197 2 ) 0.0 <sup>1</sup> ) 0.7 <sup>1</sup> ) 0.27 ) 0.00	0 7 2 13 (1 13 (2 74 (3 00 (4	4-4 EST 197 2 ) 0.00 ) 0.57 ) 0.42	0 (1) 4 (2) 1 (3) 5 (4)	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4_4 SH SCO NSCORA REQUEN	0 RED BLE CY (1 (2 (3 (4	4-4 TFN 197 2 ) 0.0 <sup>1</sup> ) 0.7 <sup>1</sup> ) 0.27 ) 0.00	0 7 2 13 (1 13 (2 74 (3 00 (4	4-4 EST 197 2 ) 0.00 ) 0.57 ) 0.42 ) 0.00	0 (1) 4 (2) 1 (3) 5 (4)	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2	0 RED BLE CY (1 (2 (3 (4 CORE 0 2	4-4 TFN 197 2 0.0 0.7 0.27 0.00 EST-S0 1-1 1-2	0 7 2 13 (1 13 (2 74 (3 74 (3 00 (4 CORE 0 0	4-4 EST 197 2) 0.00 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2	0 (1) 4 (2) 1 (3) 5 (4) ORE 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4-4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0	4-4 TFN 197 2 0.0 0.7 0.27 0.00 EST-S0 1-1 1-2 1-3	0 7 2 13 (1 13 (2 74 (3 00 (4 CORE 0 0 0 0	4-4 EST 197 2) 0.00 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2 1-3	0 (1) 4 (2) 1 (3) 5 (4) ORE 0 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4	0 RED CY (1 (2 (3 (4 CORE 0 2 0 0	4-4 TFN 197 2 0.0 0.7 0.27 0.0 0.27 0.0 0.0 EST-S( 1-1 1-2 1-3 1-4	0 7 2 13 (1 13 (2 74 (3 00 (4 00 (4 00 0 0 0 0 0	4-4 EST 197 2) 0.000 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2 1-3 1-4	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56	4-4 TFN 197 2 0 0.0 0 .7 0 0.27 0 0.27 0 0.27 0 0.27 1 0.27 0 0.27 1 0.2	0 7 2 13 (1 13 (2 74 (3 00 (4 CORE 0 0 0 0 37	4-4 EST 197 2) 0.00 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2	0 RED CY (1 (2 (3 (4 CORE 0 2 0 0	4-4 TFN 197 2 0.0 0.7 0.27 0.0 0.27 0.0 0.0 EST-S( 1-1 1-2 1-3 1-4	0 7 2 13 (1 13 (2 74 (3 00 (4 2007E 0 0 0 0 0 0 37 67	4-4 EST 197 2) 0.00 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4-4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58	4-4 TFN 197 2 0.01 0.71 0.07 0.00 EST-S0 1-1 1-2 1-3 1-4 2-2 2-3	0 7 2 13 (1 13 (2 74 (3 74 (3 74 (3 70 (4 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 7 67 0	4-4 EST 197 2) 0.00 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0 0 0 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4-4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58 0 10 0 0	4-4 TFN 197 2 0.0 0.7 0.27 0.0 0.27 0.0 0.27 0.0 0 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	0 7 2 13 (1 13 (2 74 (3 0 0 (4 0 0 0 0 0 0 37 67 0 22 0	4-4 EST 197 2 0.000 0.57 0.42 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	0 (1) 4 (2) 1 (3) 5 (4) 0 RE 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4-4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58 0 10	4-4 TFN 197 2 0.0 0.7 0.27 0.00 EST-S0 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3	0 7 2 13 (1 13 (2 74 (3 0 0 (4 0 0 0 0 0 0 37 67 0 22 0	4-4 EST 197 2) 0.00 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0.000 0.000 0.000
NUMBER U Allele F Sample NZOO 1	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58 0 10 0 0	4-4 TFN 197 2 0.07 0.77 0.27 0.00 EST-S0 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	0 7 2 13 (1 13 (2 74 (3 74 (3 70 (4 0 0 0 0 37 67 0 22 0 0 0	4-4 EST 197 2 0.000 0.57 0.42 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0 0.000 0.000 0.000
NUMBER U ALLELE F SAMPLE NZOO 1 126	4-4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 SH SCO	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58 0 10 0 0 RED	4-4 TFN 197 2 0.0 0.7 0.27 0.0 EST-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 TFN 126	0 7 2 13 (1 13 (2 74 (3 74 (3 70 (4 0 0 0 0 37 67 0 22 0 0 0	4-4 EST 197 2) 0.00 ) 0.57 ) 0.42 ) 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-4 4-4 EST	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0.000 0.000 0.000 PGI
NUMBER U ALLELE F SAMPLE NZOO 1 126 TOTAL FI	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 SH SCO NSCORA	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58 0 10 0 56 58 0 10 0 0 RED BLE CY (1	4-4 TFN 197 2000 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 TFN 126 () 0.00 0.07 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 () 0.00 0.07 0.00 0.07 0.00 0.07 0.00 0.07 0.00 0.07 0.00 0.07 0.00 0.00 0.07 0.00 0.07 0.00	0 13 (1 13 (2 74 (3 74 (3 0 (4 0 0 0 0 0 0 37 67 0 22 0 0 0 5 0 0 1 37 (1 1 3 (2 7 4 (3 0 0 0 0 0 0 0 0 0 0 0 0 0	4-4 EST 197 2 0.000 0.57 0.42 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 EST 126 0 0.000	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0.000 0.000 0.000 0.000 PGI 0 0.000
NUMBER U ALLELE F SAMPLE NZOO 1 126 TOTAL FI NUMBER U	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 SH SCO NSCORA	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58 0 10 0 56 58 0 10 0 0 8 LE CY (1 (2 (2) (1) (2) (2) (1) (2) (2) (1) (2) (2) (1) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2	4-4 TFN 197 2000 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.27 0.00 0.27 0.00 0.27 0.27 0.00 0.27 0.00 0.0	0 13 13 13 13 13 13 13 13 13 13	4-4 EST 197 2 0.000 0.57 0.42 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 EST 126 0 0.000 0.56	0 (1) 4 (2) 1 (3) 5 (4) 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0.000 0.000 0.000 0.000 PGI 0 0.000 0.000
NUMBER U ALLELE F SAMPLE NZOO 1 126 TOTAL FI NUMBER U	4_4 SH SCO NSCORA REQUEN TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 SH SCO NSCORA	0 RED BLE CY (1 (2 (3 (4 CORE 0 2 0 0 56 58 0 10 0 56 58 0 10 0 0 8 LE CY (1 (2 (2) (1) (2) (2) (1) (2) (2) (1) (2) (2) (1) (2) (2) (2) (2) (2) (2) (2) (2) (2) (2	4-4 TFN 197 2000 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.07 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.27 0.00 0.0	0 13 13 13 13 13 13 13 13 13 13	4-4 EST 197 2 0.000 0.57 0.42 0.00 PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4 EST 126 0 0.000	0 (1) 4 (2) 1 (3) 5 (4) 0 RE 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	0 0.000 0.000 0.000 0.000 PGI 0 0.000 0.000 0.000

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SAMPLE NZOO 2 69	$ \begin{array}{cccccccccccccccccccccccccccccccccccc$	E EST-SCORE 1-1 0 1-2 1 1-3 0 1-4 0 2-2 21 2-3 29 2-4 0 3-3 17 3-4 1 4-4 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	
TOTAL FI	SH SCORED	TFN 69	EST 69	PGI O
	(	0 1) 0.000 (1 2) 0.630 (2 3) 0.370 (3	0 1) 0.007 (1) 2) 0.522 (2) 3) 0.464 (3) 4) 0.007 (4)	0 0.000 0.000 0.000
SAMPLE NZOO 3 30	1-1 0 1-2 0 1-3 0 1-4 0 2-2 13 2-3 12 2-4 0	1-4 0 2-2 10 2-3 17 2-4 0 3-3 2	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0	
TOTAL FI	SH SCORED	TFN 29	est 29	PGI O
	(	0 (1) 0.000 (1) (2) 0.655 (1) (3) 0.345 (1)	0 1) 0.000 (1) 2) 0.638 (2) 3) 0.362 (3) 4) 0.000 (4)	0 0.000 0.000 0.000
SAMPLE PNG A 147	TFN-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 81 2-3 53 2-4 0 3-3 12 3-4 0 4-4 0	2-4 0	1-1 0 1-2 0 1-3 0 1-4 0	

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TOTAL FISH SCORED		TFN		EST		PGI
		146		146		0
NUMBER UNSCORABLE		0		0		0
ALLELE FREQUENCY (	(1)	0.000	(1)	0.007	(1)	0.000
(	(2)	0.736	(2)	0.661	(2)	0.000
(	(3)	0.264	(3)	0.332	(3)	0.000
(	(4)	0.000	(4)	0.000	(4)	0.000

SAMPLE PNG B 163	1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3	0 1 1 0 88 62 0 9	1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3	0 0 0 68 66 4 25	PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	0 0 0 0 0 0 0 0	
	4-4		4_4		4_4		
TOTAL FI	NSCORA	BLE	TFN 161	1 2	EST 163 0	} 	PGI 0 0
ALLELE F	REQUEN						0.000
					) 0.35 ) 0.01		
SAMPLE PNG NH 180	TFN-S 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	0 4 1 0 87 79 0 7	1-1 1-2 1-3 1-4 2-2 2-3 2-4	0 4 6 63 79 5 20 1	PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	0 0 0 0 0 0 0 0 0	
TOTAL FI		-	TFN		EST		PGI
NUMBER U Allele F		CY (1 (2 (3	) 0.72 ) 0.26	2  4 (1 22 (2 54 (3	178 2 ) 0.02 ) 0.60 ) 0.35 ) 0.01	28 (1) 91 (2) 94 (3)	0.000
SAMPLE PNG Y 148	1-1 1-2 1-3 1-4 2-2	0 1 62 65 0 18 0	1-1 1-2 1-3 1-4 2-2 2-3 2-4	0 0 0 62 67 2 15 0	PGI-SC 1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	0 0 0 0 0 0 0	
TOTAL FI	SH SCO	RED	TFN 146		EST 146		PGI 0
NUMBER U Allele f		CY (1 (2 (3	2 0.00 0.61 0.31	2 )3 (1 47 (2 49 (3	2 ) 0.00 ) 0.66 ) 0.33	2 10 (1) 11 (2) 12 (3)	0 0.000 0.000 0.000 0.000

PNG Z 1-1 187 1-2 1-3 1-4	0 1-2 1 4 1-3 2 0 1-4 0 90 2-2 103 72 2-3 56 0 2-4 3 13 3-3 14 0 3-4 0	E PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORAL ALLELE FREQUEN	CY (1) 0.011 (2) 0.704 (3) 0.285	179 8 (1) 0.008 (1) (2) 0.743 (2) (3) 0.240 (3) (4) 0.008 (4)	0.000
SAMPLE TFN-SO S218 1-1 124 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	2-3 0 2-4 0	
TOTAL FISH SCOL		EST	PGI
NUMBER UNSCORAL ALLELE FREQUEN	CY (1) 0.008 (2) 0.690 (3) 0.302	123 1 (1) 0.004 (1) (2) 0.553 (2) (3) 0.431 (3) (4) 0.012 (4)	0.000
S223 1-1 200 1-2 1-3 1-4 2-2 2-3 2-4 3-3	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORAI ALLELE FREQUEN	CY (1) 0.015 (2) 0.725 (3) 0.260	198 2 (1) 0.000 (1) (2) 0.588 (2) (3) 0.407 (3) (4) 0.005 (4)	0.000

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SAMPLE SC SG 201	1-1 1-2 1-3 2-2 2-3 2-4 3-3 3-4	0 2-3 0 2-4	0 2 0 39 100 2 56 2	1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	RE 0 0 0 0 0 0 0 0 0 0	
TOTAL FI	SH SCORE	D TF 20		EST 201		PGI O
	NSCORABLI REQUENCY	E	0 25 (1) 64 (2) 11 (3)	0 0.005 0.448 0.537	(2) (3)	0 0.000 0.000 0.000
SAMPLE TAR B1 157	1-1 ( 1-2 ( 1-3 ( 1-4 ( 2-2 1' 2-3 6( 2-4 ( 3-3 6) 3-4 (	6 2-3 0 2-4	0 2 3 0 32 77 0 32 0	1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4	RE 0 0 0 0 0 0 0 0 0 0	
TOTAL FI	SH SCORE	D TF		EST 146		PGI 0
	NSCORABLI REQUENCY	E 1	1 00 (1) 42 (2) 58 (3)	11 0.017 0.490 0.493	(2) (3)	0 0.000 0.000 0.000
SAMPLE TAR B2 157	1-1 ( 1-2 ( 1-3 ( 1-4 ( 2-2 6) 2-3 6( 2-4 ( 3-3 1)	6 2 <b>-</b> 3 0 2 <b>-</b> 4	0 0 0 32 77 3 32	1-1 1-2 1-3 1-4 2-2 2-3 2-4 3-3	RE 0 0 0 0 0 0 0 0	
		0 4-4	0	4-4	0	
TOTAL FI		0 4-4	N	4-4 EST 146	0	PGI 0

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	TAR G 1- 21 1- 1- 1- 2-	-1 1 -2 0 -3 0 -4 0 -2 4 -3 14 -3 14 -3 2 -4 0	1-2 0 1-3 1 1-4 0 2-2 0 2-3 11 2-4 1 3-3 6 3-4 2	GI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
	TOTAL FISH S	CORED	TFN 21	EST 21	PGI O
	NUMBER UNSCO Allele frequ	ENCY (1) (2)	0 0.048 (1) 0.524 (2) 0.429 (3)	0 0.024 (1) 0.286 (2) 0.619 (3)	0 0.000 0.000 0.000 0.000
	SAMPLE TFN TAR G1 1- 21 1- 1- 2- 2- 3- 3- 4-	2 0 3 0 4 0 2 2 3 14 4 0 3 5 4 0	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	3I-SCORE         1-1       0         1-2       0         1-3       0         1-4       0         2-2       0         2-3       0         2-4       0         3-3       0         3-4       0	
	TOTAL FISH S NUMBER UNSCO ALLELE FREQU	RABLE TENCY (1) (2) (3)	0.429 (2) 0.571 (3)	EST 21 0 0.071 (1) 0.619 (2) 0.286 (3) 0.024 (4)	0.000 0.000
	TAR G2 1- 200 1- 1- 2- 2- 3- 3- 3-	1 0 2 0 3 0 4 0 2 18 3 83 4 1 3 96 4 0	1-2 1 1-3 0 1-4 0 2-2 54 2 2-3 106 2 2-4 0 3-3 37 3 3-4 0	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	
•	TOTAL FISH S NUMBER UNSCO ALLELE FREQU	RABLE ENCY (1) (2) (3)	0.303 (2) 0.694 (3)	EST 198 2 0.003 (1) 0.543 (2) 0.455 (3) 0.000 (4)	0.000 0.000

SAMPLE TFN-S TAR G3 1-1 200 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	0 1-2 0 1 1-3 0 0 1-4 0 95 2-2 37 84 2-3 106 0 2-4 0 18 3-3 54 0 3-4 1	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORA ALLELE FREQUEN	CY (1) 0.003 ( (2) 0.692 ( (3) 0.306 (	(2) 0.455 (2)	0.000
ZP B 1-1 198 1-2 1-3 1-4	4 1-2 0 1 1-3 1 0 1-4 0 87 2-2 71 82 2-3 98 0 2-4 2 24 3-3 25 0 3-4 1	$ \begin{array}{cccc} 1-1 & 0 \\ 1-2 & 0 \\ 1-3 & 0 \\ 1-4 & 0 \\ 2-2 & 0 \\ 2-3 & 0 \\ 2-4 & 0 \end{array} $	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORA ALLELE FREQUEN	CY (1) 0.013 ( (2) 0.657 ( (3) 0.331 (	198 0 (1) 0.003 (1) (2) 0.611 (2) (3) 0.379 (3) (4) 0.008 (4)	0.000
SAMPLE TFN-S ZP C 1-1 189 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$ \begin{array}{ccccc} 1-1 & 0 \\ 1-2 & 0 \\ 1-3 & 0 \\ 1-4 & 0 \\ 2-2 & 0 \\ 2-3 & 0 \\ 2-4 & 0 \end{array} $	
TOTAL FISH SCORED TFN EST			PGI
NUMBER UNSCORA ALLELE FREQUEN	CY (1) 0.008 ( (2) 0.704 ( (3) 0.288 (	189 0 (1) 0.011 (1) (2) 0.741 (2) (3) 0.246 (3) (4) 0.003 (4)	0.000

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SAMPLE TFN-S ZP EA 1-1 36 1-2 1-3 1-4 2-2 2-3 2-4 3-3 3-4 4-4	0 1-4 0 12 2-2 19 21 2-3 11 0 2-4 1 3 3-3 3 0 3-4 0	1-1 0 1-2 0 1-3 0 1-4 0	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORA ALLELE FREQUEN	CI (1) 0.000 ( (2) 0.625 ( (3) 0.375 (	36 0 (1) 0.028 (1) (2) 0.722 (2) (3) 0.236 (3) (4) 0.014 (4)	0.000
ZP EB 1-1 94 1-2 1-3 1-4 2-2 2-3 2-4	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	PGI-SCORE 1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0 3-4 0 4-4 0	
TOTAL FISH SCO	RED TFN	EST	PGI
NUMBER UNSCORA Allele Frequen	CY (1) 0.011 ( (2) 0.717 ( (3) 0.272 (	92 2 (1) 0.011 (1) (2) 0.707 (2) (3) 0.277 (3) (4) 0.005 (4)	0.000
ZP F 1-1 200 1-2 1-3 1-4 2-2 2-3 2-4	0 1-4 0 91 2-2 93 96 2-3 90 0 2-4 0 12 3-3 16 0 3-4 1	1-1 0 1-2 0 1-3 0 1-4 0 2-2 0 2-3 0 2-4 0 3-3 0	
TOTAL FISH SCO		EST	PGI
NUMBER UNSCORA		200 0	0 0
ALLELE FREQUEN	(2) 0.697 ( (3) 0.300 (	(1) 0.000 (1) (2) 0.690 (2) (3) 0.308 (3) (4) 0.002 (4)	0.000

SAMPLE	TFN-SCORE		EST-SCORE		PGI-SCORE		
ZP G	1-1	0	1-1	0	1-1	0	
200	1-2	3	1-2	0	1–2	0	
	1-3	2	1–3	2	1-3	0	
	1-4	0	1-4	0	1-4	0	
	2-2	97	2-2	92	2-2	0	
	2-3	82	2-3	78	2-3	0	
	2-4	0	2-4	2	2-4	0	
	3-3	17	3-3	25	3-3	0	
	3-4	0	3-4	2	3-4	0	
	4-4	0	4-4	0	4-4	0	
					507		
TOTAL FI	SH SCO	RED	TF		EST		PGI
			20		201		0
NUMBER U	NSCORA	BLE		0	0		0
ALLELE F	REQUEN	CX ('			1) 0.00		0.000
		(:	2) 0.6	94 (2	2) 0.65	7 (2)	0.000
		(3	3) 0.2	94 (3	3) 0.32	8 (3)	0.000
		(1	4) 0.0	00 (1	4) 0.01	0 (4)	0.000

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