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CHEMICAL VARIABILITY AND STOCK VARIATION IN NORTHERN ATLANTIC BLUEFIN TUNA

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SUMMARY

Samples of bluefin tuna from the eastern Atlantic (EA) and from the western Atlantic (WA) were compared. Proton-induced X-ray emission spectroscopy (PIXE) and accelerator based methods of determining oxygen isotopes were used to characterize the composition of elements and isotopes within .9mm sections of vertebrae. Transects of contiguous analyses were obtained between the center and the outer edge of the vertebrae. Ratios of elements such as Ca, Sr, P, S, Cl, Br, and Zn were found to vary cyclically and seasonally.

While seasonal variation was evident, regions of the vertebrae formed during the first winter differed depending upon whether fish were taken in the EA or WA. Differences were used to measure immigration. Juveniles from the coast of Virginia, Bay of Biscay, and Gulf of Lion were studied. The chemistry of vertebrae from these locations is different, and differences between the EA and WA were more pronounced than were differences between locations in the EA. Of the 95 juveniles captured in the WA, 5.3 percent had patterns similar to fish from the EA. None of the 131 fish in the EA had patterns similar to the WA fish.

Similar differences were found in the vertebrae of giant bluefin. Results from the first of two comparisons indicate that of 71 WA adults studied, 12.7 percent have patterns similar to the EA fish. Of 105 fish captured in the east, 12.4 percent had patterns similar to the west. The results from the second set of samples revealed that 9.9 percent of the 91 giants captured in the WA had patterns similar to those from the east, whereas 2.6 percent of the 190 fish in the EA samples had patterns similar to the WA fish. Monte Carlo techniques were used to evaluate the differences observed. While differences between the EA and WA bluefin are significant, the simulations also show heterogeneity within the EA. The results of the isotope studies support results obtained using PIXE.

RESUME

Des échantillons de thon rouge en provenance de l'Atlantique est (EA) et de l'Atlantique ouest (WA) ont été comparés. La spectroscopie par émission de rayons X par induction protonique (PIXE) et les méthodes, basées sur l'emploi d'accélérateurs, de détermination des isotopes d'oxygène ont été utilisées pour définir la composition en éléments et isotopes d'une coupe de vertèbre de .9 mm. Des transects d'analyses contiguës ont été obtenus du centre à la périphérie de la vertèbre. Il a été observé que la teneur en éléments tels que Ca, Sr, P, S, Cl, Br et Z varie de façon cyclique et saisonnière.

Bien que la variation saisonnière soit évidente, la portion de la vertèbre formée au cours du premier hiver diffère selon la provenance du poisson, EA ou WA. Les différences ont été utilisées pour calculer le taux d'immigration. Des juvéniles des côtes de Virginie, du golfe de Gascogne et du golfe du Lion ont été étudiés. La composition chimique des vertèbres des poissons de ces régions diffère, les différences étant plus accusées entre EA et WA qu'entre divers secteurs EA. Sur les 95 juvéniles capturés en WA, 5,3 % montrent des modes semblables à ceux pris en EA. Aucun des 131 poissons en provenance de la zone EA ne montre de mode semblable à ceux des poissons du secteur WA.

Des différences similaires ont été observées dans les vertèbres de thon rouge géant. Les résultats d'une première comparaison indiquent que, sur les 71 adultes WA étudiés, 12,7 % montrent des modes semblables à ceux du poisson EA. Sur les 105 poissons pris dans l'est, 12,4 % ont des modes semblables à ceux de l'ouest. Les résultats concernant un deuxième jeu d'échantillons a révélé que 9,9 % des 91 thons rouges géants capturés dans la zone WA montrent des modes semblables à ceux de l'est, tandis que 2,6 % des 190 spécimens dans les échantillons EA ont des modes semblables à ceux de l'ouest. Des techniques Monte Carlo ont été employées pour évaluer les différences observées. Bien que les différences entre le thon rouge de la zone EA et celui de la zone WA soient significatives, les simulations montrent également un certain degré d'hétérogénéité entre échantillons EA. Les résultats des études sur les isotopes appuient ceux qui sont obtenus par la méthode PIXE.

RESUMEN

Se comparan muestras de atún rojo procedentes del Atlántico Este (EA) con otras procedentes del Atlántico Oeste (WA). Se utilizó la espectroscopia por emisión de rayos X inducida por protones (PIXE) y métodos basados en el empleo de acelaradores de determinación de isotopos de oxígeno, para definir la composición de los elementos e isotopos en secciones de vértebras de 9 cmm. Se obtuvieron cortes transversales de análisis contiguos entre el centro y el borde exterior de las vértebras. Se observó que las tasas de elementos tales como el Ca, Sr, P, S, Cl, Br y Zn, varian por ciclos y temporadas.

Si bien la variación por temporadas era evidente, la parte de las vértebras formada en el curso del primer invierno era diferente, dependiendo de que el pez hubiese sido capturado en el EA o en el WA. Estas diferencias se aplicaron para medir la inmigración. Se estudiaron juveniles procedentes de la costa de Virginia, Golfo de Vizcaya y Golfo de León. La composición química de las vértebras de estos peces es diferentes y las diferencias entre el EA y el WA eran más pronunciadas que las observadas entre zonas del EA y el WA. De los 95 ejemplares de juveniles capturados en el WA, el 5,3% mostraban estructuras similares a las de los peces del EA. Ninguno de los 131 peces del EA tenía estructuras similares a las observadas en los peces del WA.

Diferencias similares se encontraron en las vértebras del atún rojo gigante. Las conclusiones de la primera de la dos comparaciones establecidas, indican que de los 71 adultos del WA estudiados, 12.7% tienen estructuras similares a las de los peces del EA. De los 105 peces capturados en el Este, el 12.4% tenian estructuras similares a las del Oeste. Las conclusiones resultantes de la comparación del segundo grupo de muestras evidenciaban que el 9.9% del atún rojo gigante capturado en el WA tenía estructuras similares a las observadas en el Este, mientras que el 2.6% de los 190 peces de la muestra del EA tenía estructuras similares a las observadas en los peces del WA. Se aplicaron técnicas Montecarlo con el fin de evaluar las grandes diferencias observadas. Si bien las diferencias entre el atún rojo del EA y el del WA son importantes, las simulaciones muestran tambien heterogeneidad en el EA. Las conclusiones del estudio de isotopos apoyan los resultados obtenidos del empleo de PIXE.

I. INTRODUCTION

In 1979, the Inter-American Tropical Tuna Commission (IATTC) and the National Marine Fisheries Service (NMFS) entered into a joint program directed at exploring the feasibility of utilizing trace chemical element differences to detect the origin of bluefin tuna Thunnus thynnus (L.) caught in the North Atlantic Ocean and Mediterranean Sea. The overall objective of this investigation has been to determine if pelagic marine organisms contain a form of "Natural Tag", a code that can be used to extract useful management information. The specific task undertaken is to employ these tags to determine the rate of exchange, if any, of juvenile (school fish) and adult (giant) bluefin tuna that occur in the EA and WA Ocean. The working hypothesis is that organisms acquire a form of chemical fingerprint in the skeletal tissue (vertebrae) indicative of specific properties of their micro-niche and micro-habitat, i.e. a form of ecological record. As such, an ex post facto regime of analyses can be expected to yield useful ecological data.

The program was carried out in two stages. The objectives of the first phase was to assemble suitable electronics, and to evaluate the hypothesis that bluefin tuna from different nursery areas could be distinguished on the basis of variation in the chemistry of hard parts, i.e. spines and vertebrae. Various methods of carrying out these analyses were reviewed and an innovative system of characterizing the chemistry of vertebras was designed and constructed specifically for the proposed tasks. Also, during the first phase of the program, tests were carried out to determine the sensitivity and reliability of the equipment, and two pilot studies were carried out on bluefin tuna vertebrae.

These results were judged promising and a second phase of the program was started in the fall of 1981. The objective there has been to assemble and analyze representative samples of juvenile (schoolfish) and adult (giant) bluefin from fisheries in the WA, EA, and Mediterranean.

This report deals primarily with the results obtained using X-ray spectroscopy. Some effort was directed to the task of measuring isotopes in the same region of the bone. New techniques were developed. The isotopes of 18_0 and 16_0 are stable and both occur in nature. The ratio of the concentration of these two isotopes in calcareous material is not constant, but has been shown to be dependent on such factors as the salinity and temperature of the environment when the material was formed (Epstein, et. al., 1953). The ratio of these isotopes are expected to differ in the bones of bluefin occupying the EA and WA. Some effort was devoted to measuring the isotopes on small areas of vertebrae as a means of testing the validity of the PIXE results.

Funding for earlier stages of this program has been shared jointly by IATTC and NMFS, Contract 81-ABC-00243. The second phase of the program was funded almost entirely by NMFS, contract 84-WC-C-06106. The costs associated with the collection and shipping of European samples during this latter phase of the program has been shared by NMFS and the International Commission for the Conservation of Atlantic Tunas (ICCAT). Indirect costs of research and development and the maintenance of the accelerators used and support staff have been provided by National Science Foundation Grants to Princeton University and the California Institute of Technology.

As per contract, year-end and quarterly progress reports were submitted to NMFS (Calaprice 1983, 1985). Those results are assembled in this document. The purpose is to bring together relevant aspects of the study, and to provide greater access to the findings. Some discussion of statistical methods is included to aid readers with technical features of this study.

II. METHODS

Select fisheries were sampled in the WA, EA and Mediterranean operating on both juvenile and adult bluefin tuna. Juveniles were taken from three different geographic areas and form the basis for one comparison. There are two separate collections of adults. Each collection is composed of samples from the EA and WA. Samples were cataloged and a section of the posterior region including the caudal peduncle and 4 adjacent vertebrae were removed, wrapped in individual bags, frozen, and shipped to La Jolla, California. Two vertebrae were cleaned, sectioned, dried, and stored for future analyses. Samples were transported to the Kellogg Radiation Laboratory at the California Institute of Technology where each sectioned vertebrae was irradiated one or more times. The data were returned to La Jolla where analyzed. Both standard statistical packages and specialized computer routines were developed and employed. Details follow.

A. Collection of Samples

A sampling protocol specifying how the samples were to be collected and stored was prepared and circulated to each of the different agencies. The location where samples were collected, size and numbers of fish irradiated, and individuals responsible for collections are listed in Table 1. The acquisition of adequate samples has been a difficult and limiting task, and as indicated, a number of individuals from different organizations have cooperated both in the planning and in the collection of samples. The locations where samples were obtained are illustrated in Figure 1.

B. Preparation of Samples

Using a surgical saw and scalpel, vertebrae anterior to the ural complex were separated. Flesh and debris were removed from the 34th and 35th vertebrae, these were cleaned with a nylon brush and with water filtered through both an ultra-pure filter and an "organic removal filter". Vertebrae were labelled, sectioned, and placed in a drying oven overnight (40°-50°C for 16-17 hours). Sectioning was carried out with diamond tipped saws. A number of cuts were made such that each vertebrae yielded one 6 mm wide rectangular wedge of bone, containing the origin (earliest growth) and the distal edge (latest growth). Sections were measured, individually labelled, packaged, and identified (Figure 2-A.)

C. Irradiation of Samples

Sections of vertebrae were mounted and irradiated for approximately 6 minutes. Illustrated in Figure 2b is a sample holder containing cross-sections of irradiated vertebrae. The surface of the vertebra is discolored during the analysis. The spots near the center of the wedge are track marks which serve to define the size of the area irradiated and its location. The left side is the center section of bone deposited during the first year. The apparatus used is one designed specifically to allow X-ray patterns and isotope measurements to be obtained from a small cross-section of the bone, approximately .9 mm in diameter.

Obtaining X-ray Spectra. "Proton induced X-ray emission spectroscopy" (PIXE) is a specialized form of X-ray spectroscopy, one that requires a nuclear particle accelerator (Mangelson et. al. 1977). Other than that the methods are similar to those used in previous studies (Calaprice, 1971; Calaprice et al., 1971, 1975). X-ray spectra are obtained by irradiating the specimen with a beam (190 nA) of 3 million electron volt protons. The apparatus uses an array of magnets to focus and steer the beam; a set of collimators that defines the geometry of the beam; a chamber to house the specimen while it is being irradiated; a silicon-lithium drifted X-ray detector system, capable of rapidly counting and measuring the energy of fluorescent X-rays and a pulse height analyzer, where the pulses are stored as an array of numbers of X-rays of specific energy. Within the chamber, a rotating arm with a copper target allows for the normalization of samples, approximately 95% of the time the beam is directed on the sample, 5% of the time the beam is directed to the copper target. Normalization involves adjusting the data as if the number of protons hitting the copper was the same for each sample. All samples were analyzed while in 1/6th atmosphere of helium. An Apple II+ computer was programmed to monitor the analyses, store data, and position and change samples relative to the beam. As time passed, the Apple was assigned a number of other routine tasks allowing equipment to operate without intervention (Figure 3).

Measuring Oxygen Isotope Ratios. The standard method of measuring oxygen isotopes involves dissolving the sample and employing a mass-spectrometer, a method precluded by the heterogenous nature of the surface of the vertebrae. Various means of carrying out these analyses using nuclear techniques were examined and an apparatus based on a combination of methods suggested by staff at California Institute of Technology was assembled and tested. The initial studies utilized existing equipment and involved a "forward looking spectroscopic magnet" and a silicon solid state detector. In many respects the methods are similar to the method of X-ray spectroscopy, e.g. both utilize accelerated particles. In the $^{18}O/^{16}O$ studies, the surface of the vertebrae is irradiated with a beam of "one million electron volt" protons. Characteristic Alpha particles are produced. A portion of the protons lose energy and are back scattered. The total oxygen concentration is determined from the spectrum of back scattered protons and the ^{18}O concentration is resolved using the spectroscopic magnet. Ratios are obtained and while not numerically equivalent are a function of the $^{18}O/^{16}O$ ratio and are expected to change with differences in temperature and salinity. As the method and equipment are being upgraded, no details are given at this time. Studies are underway to reduce background, improve repeatability, and reduce exposure time. Currently samples must be exposed for approximately 10 times longer than the X-ray analysis.

D. Statistical Analyses

X-ray spectra are acquired in the form of a string of 2048 numbers, each is the total number of X-rays of a specific energy. The device used to acquire these data, a "pulse height analyzer" (PHA) is a computer-based system, utilizing two micro-processors. As such, the device was readily interfaced to an "Apple II" microcomputer. The Apple was programmed to control the experiment, to acquire data during the analysis, to perform statistical tests, and to display and update intermediate results. In the event equipment malfunctioned, warning sounds were generated and diagnostics displayed. After a vertebrae was irradiated, the contents of the P.H.A. memory were acquired by the Apple, additional diagnostics and analyses carried out, intermediate results printed out, and the spectrum and related information stored on floppy disk. Thus the first statistical analyses were carried out as the data were collected. The contents of floppy disks were transferred to magnetic tape and each spectrum was plotted. Overlays were made with a standard to insure that the data were transferred properly.

These tapes containing the spectra from vertebrae of fish from different areas and of different ages were carried to La Jolla where the following more detailed statistical analyses were carried out using BMD Statistical software packages, Dixon, et. al, (1981) as indicated, and packages written for the Apple and Vax.

Data description and comparison. The data were plotted and analyses of variance were carried out on each variable. The variables were plotted by area and coefficients of variation were computed and the results examined for inconsistencies (BMD-P7D).

Ratios of the elemental peaks of chlorine and strontium. A typical spectrum is given in Figure 4. The area of peaks were determined by subtracting the backgrounds. The resultant two areas indicated here were ratioed and compared.

Factor analyses. This is a form of transformation of the data. The spectra are complex and contain a great deal of redundancy. Factor analysis was used to generate a new set of uncorrelated variables with statistical properties suitable for multivariate analyses (BMD-P4M). Factor scores were obtained from orthogonal rotation of the principal components. Only factor scores with eigenvalues greater than one were retained and used in subsequent analyses.

Discriminant function analyses. A multivariate statistical technique in which all of spectrum (all factors) are used. The technique provides a multivariate test of whether groups are significantly different, derives functions which can be used to classify individual spectra as to area of affinity, and illustrates the similarity and differences between individual spectra (BMD-P7M).

Cluster analyses. This hierarchical technique groups the samples (spectra) according to similarity. As with the discriminant function analyses, factor scores are the variables used (Ward 1963.)

^{18}O/oxygen ratios. These are simple ratios of a portion of the elastic back scattering spectrum (oxygen) and the ^{18}O counts as determined from counters.

Simulation studies. Monte Carlo techniques were used to evaluate number of statistical assumptions and one hypothesis of stock structure. Details of the methods are given in the text (Hammersley and Handscomb ,1964.)

III. RESULTS

A. Variation in X-ray Patterns

In these studies a small set of juveniles from the EA and WA were compared. Individual vertebrae were irradiated one or more times as illustrated in Figure 2-B.

1. Geographic variation

Thirty six juveniles from the EA and 39 juveniles from the WA were irradiated once, near the center of the vertebrae. The spectra of fish from these two areas are readily distinguished. The height of specific elemental peaks and the slope of the background areas within spectra differ geographically. Typical spectra of fish from the two geographic regions are illustrated in Figure 5. Individual spectra were plotted and overlays made. Differences among spectra were found to be consistent. The Y-axes in Figure 5 are logs of the number of counts. Linear plots suggest that differences exist in the less abundant elements as well as those indicated in Figure 5.

Stepwise discriminant function analyses were carried out on the strings of data. In this case adjacent variables in the spectrum were summed in order to reduce the number of variables. These preliminary studies were carried out on a small computer (PDP 11/34). Because of limitations in computer memory, the spectra were compared in sections, each section except the last contained 40 variables. Variables chosen in the stepwise process were combined into a single analysis. The results (Table 2) indicate that statistical differences exist between the spectra from each side of the Atlantic. The fact that such differences were found in each of the 40 variable sections analyzed demonstrates that the basis for classifications are differences in a number of chemical elements, and components of the background. The results also indicate that it is possible to derive equations that can be used to classify individuals as to area of origin, and that such functions should utilize variables scattered throughout the spectra.

2. Temporal variation

Each of a small set of vertebrae was irradiated a number of times. Sequential analyses were carried out forming a linear transect starting at the center of the vertebrae and extending to the outer edge. Vertebrae were systematically moved across the beam of protons at .9 mm intervals. Spectra from each point were analyzed. At each point, the background area was subtracted from the peak height for a number of different elemental peaks, and ratios of different elemental peak heights were computed. The concentration of certain chemical elements changes as one moves away from the center of the vertebra. Bromine, copper, chlorine, molybdenum and sulfur increase while vanadium and zinc appear to decrease. The relative concentration of most elements examined exhibit regular fluctuations.

The data plotted in Figure 6 illustrate the nature of temporal and geographic differences in the chlorine/strontium ratios. These are two highly visible peaks in the spectra that were observed to differ between areas. It is obvious that differences are most pronounced near the center of the vertebra, notably between the 2nd and 5th location irradiated.

The phosphorus/strontium ratios plotted in Figure 7 were obtained from vertebra of three fish believed to be age 2 and vertebrae of three fish believed to be age 3. In each case the fish were collected during the summer (July). These assumed ages are based upon independent life history and tagging data and were determined from data on the size of the fish irradiated. There appears to be cyclic and somewhat regular variations in the ratio of these two elemental peak areas, suggesting a correlation between major cycles, season, and age.

B. Estimates of Mixing

Four trace element studies have been carried out. One was carried out on juveniles, and three were carried out on adults. Each allows for independent estimates of intermingling. The first systematic comparison of spectra from different regions began in the late summer of 1982. This was the first time that a complete set of both juvenile and adult fish from both sides of the Atlantic was available for analyses. A small subset of adults was analyzed as a pilot study during the period August through October of 1982. A total of 42 adults were irradiated, 21 from each side of the Atlantic. The numbers analyzed were the largest set that could be prepared, irradiated and analyzed in time for the ICCAT meeting and the close of the contract period. The analysis of the remaining samples began in January of 1983, immediately after modifications to the apparatus were completed, and a new component of the data acquisition system was interfaced. The results that follow are based on these sets of data, data collected prior to 1983 and the main body of data. Statistical analyses and estimates were carried out separately as the new electronic components and settings resulted in small but significantly different format for the data.

1. The pilot study

The results from three different types of evaluation of the data, Table 3, indicate that approximately 14% of the samples of adult fish caught in the Mediterranean and immediate vicinity originated as juveniles in the WA. The results further suggest that some 25% of the fish taken on the western side of the Atlantic originated in the Mediterranean or EA. These estimates of intermingling were arrived at by using different methods of comparison. In each case the portion of the vertebrae irradiated was that formed during the first year of life. The data are derived from analyses at step 3 in Figures 6 and 7. In the category referred to as "Visual", the basis for comparison was the data collected on juvenile school fish. As indicated above, X-ray patterns of juveniles from the two different locations are visually different and can be readily identified and classified. Differences in peak heights as well as changes in the shape of the background are evident. Similar differences can also be found in the adults, but not all adults in a particular fishery are the same. The X-ray patterns of bone formed during the first year for most of the adults caught in the WA are similar to juveniles caught there. A small percentage have X-ray patterns similar to juveniles from the other side. The working hypothesis used is that these dissimilar fish are immigrants. This hypothesis is the basis for estimating mixing.

The second analysis given in Table 3 under the heading "Visual" is essentially a combination of statistical and visual methods. In this case only the X-ray patterns from two elemental peaks are compared, those from chlorine and strontium atoms. As illustrated in Figures 4 and 6, background counts are subtracted from these two peaks in the spectra and a ratio of the area under each peak (Cl/Sr) is calculated. This was done for juveniles and adults. Ratios differ significantly and are either high or low depending upon the side of the Atlantic where the juveniles were captured. The distribution of the values of these ratios calculated from adult samples are polymodal. Again, the majority of adults from a particular side of the Atlantic are similar to the juveniles from that side. As before, those fish with scores that appear similar to juveniles from the other side are hypothesized to be immigrants and used to assess mixing. The similarity in the two estimates is striking, but not unexpected. There are many differences between spectra from the two sides and the same individual fish show up as anomalies, i.e. immigrants.

The third category, labelled "statistical", is a form of multivariate statistics. In this instance, all the data are used. A factor transformation is carried out on the data and discriminant analysis is used to derive functions between EA and WA adults. After the functions are derived, the equations are used to classify the spectra. Fish are then classified as belonging to either the EA or WA samples. The percentage of individuals within each collection that are classified as belonging to the other group (misclassifications) is assumed to reflect the amount of mixing. Again there is good agreement among the estimates of mixing made by three different methods. It is reasonable to assume the latter method is more dependable as there is no human bias in the classification of samples and all of the data are used in deriving the estimates. Confidence limits for these estimates are broad as can be expected because the sample sizes in this pilot study are small (Goldstein, 1964).

The similarities between individuals irradiated in the above pilot study is illustrated in Figure 8. The results of the hierarchical cluster analyses show that individual fish fall into natural groupings. (Individuals that are most similar chemically are paired first). Note that individual fish from opposite sides of the ocean have been paired early in the stepwise process.

2. Discriminant analyses of juveniles (3 groups)

Factor transformation of the data resulted in a total of 79 new variables. The juvenile (school fish) data were arranged in three groups, WA, EA, and Mediterranean. The results of the discriminant analysis are summarized in Table 4 and a plot of the data in discriminant space is given in Figure 9.

The first part of Table 4 is a summary of the discriminant function analyses; 12 of the 79 factor variables were used in deriving the functions. The probability that the differences observed are due to chance alone is less than one in a thousand. The second part of Table 4 lists two classification matrices, each based upon related statistical methods, but differing in assumptions. The second method, the jackknife procedure is preferred because in these data the number of variables is large relative to the number of objects classified. The diagonals of the classification matrices are the numbers of fish that were classified correctly. Thus of the 88 fish captured in the EA 8 fish, or approximately 9%, were classified as Gulf of Lion (Mediterranean) fish. Of the 95 U.S. fish captured in the WA 5, or approximately 5%, were classified as Bay of Biscay fish. Of the Mediterranean school fish, 3 out of 36, or 8% appear as Bay of Biscay fish. The zeros in the off-diagonals are interesting as they suggest no movement or rather less frequent movement in a particular direction. While it is possible to estimate mixing using both methods of calculation, the jackknife method is used in the remainder of the comparisons.

Figure 9 shows the distribution of samples in discriminant space and is included to illustrate the clear distinction among groups.

3. Discriminant analyses of adults (2 groups in each of 2 years)

As previously indicated, the adult bluefin data base is divided into two sets of bluefin captured in the EA and in the WA. Obtaining samples of giants from different geographic locations and through a number of different organizations has been difficult. Samples were processed as they arrived in La Jolla. Comparisons were made as soon as individuals from both sides of the Atlantic were available. Preliminary estimates of mixing were made at various times during the study as requested. The data have been organized so as to provide two studies of differences between fish from the EA and WA, and the amount of net interchange. Each of the two comparisons is used to provide an independent measure of intermingling. Since the collections were made one or more years apart, some indication of annual variation is provided. Care must be taken in interpreting the differences between years as the second data set was exposed to 30% more radiation. The analyses that follow were carried out on data sets that were irradiated the same amount of time and under the same conditions.

Each vertebra was irradiated three times. This produced three data sets. Eastern and western samples were compared using each data set separately. Three different discriminant analyses were performed and three estimates of intermingling made for each of the years (Table 5). The results indicate that there was good agreement with the majority of fish, i.e. they were either classified as residents or immigrants each of the three times. There were individuals that were classified twice as residents and once as immigrants, and visa versa.

Using averaged probabilities. Individual spectra are classified immigrants on the basis of probabilities. There were cases in which two of the three values gave either very high or very low probabilities and one value close to .5 but indicating membership in the opposite group. In that case, it seemed like a more realistic index of group membership should be based on the joint probabilities of group membership. One such index was arrived at by merely averaging the three related probabilities, (Table 6-A and 6-B.) Intermingling estimates based on averages are less than when based upon each of the individual analyses taken alone.

Using a combined data base. A more direct approach is to combine the raw data, carry out the factor transformations, and then compare the different geographic areas using multivariate methods. This was what was done, except that the three sets of factor scores were combined into one data array. This short cut was necessary because of the limited computer facilities available.

The results of the discriminant function analyses of the first comparison are also summarized in Table 6-A, and the results of the second comparison are given Table 6-B. The results of the first comparison indicate that samples of adult bluefin tuna (giants) from the WA differ from those of the EA. The probability of observing such differences by chance alone is less than one in a thousand. Nineteen of the original 278 variables were chosen in the stepwise process. Of the 71 adults from the WA used in the comparison, 9 are classified as immigrants and mixing is computed to be 12.7%. Of the 105 adults from the EA group 13 are classified as immigrants and mixing is computed to be 12.4%. The results of the second comparison, like the first, indicate that samples of adult bluefin tuna from the WA differ from those of the EA. The probability of observing such differences by chance alone is less than one in a thousand. Thirty-one of the 266 variables were selected in the stepwise process. Of the 91 adults from the WA, 9 are classified as immigrants and mixing is computed to be 9.9%. Of the 190 adults from the eastern Atlantic-Mediterranean, 5 are classified as immigrants and mixing is computed to be 2.6%.

Adult bluefin from the two different geographic areas are separated much better when all variables (factor scores) from the three independent analyses are combined into one data base and one set of functions. The "p" values for each fish compared are plotted in Figure 10 for the first comparison and Figure 11 for the second comparison. Here we see that the groups are better separated in the second comparison. This is most likely related to the fact that samples were irradiated for a longer (30%) period of time. Also noteworthy is the fact that individual fish in the first comparison are much better separated when classification is based upon combined data sets and one set of functions rather than using average "p" values, (compare Figure 11 with Figure 13-A). The estimates of mixing using different methods, average "p" values and combined data are comparable.

C. Evaluating Statistical Procedures and Assumptions

The X-ray spectrometric methods applied yield a relatively large amount of information. Spectra obtained are reflections of the chemical composition and physical structure of the region irradiated. This information is acquired in the form of a string of 1024 variables for each spot irradiated. Another spectrum of 1024 variables is obtained from a standard irradiated at the same time. This data is used to normalize the X-ray data. The vertebra from one adult bluefin is irradiated three times, each in a different location yielding 3072 variables, Considering the 3072 variables used to normalize the spectra, there are 6144 variables per fish. These values range between 0 and 300,000 depending upon the frequency with which X-rays of a given energy are counted. Factor analysis is used to reduce redundancy. The resulting factor scores have a mean of zero, and a standard deviation of one; the scores are also independent variables. This has the effect of reducing the variables used in the above comparisons to less than 5% of the original number. While greatly reduced, the numbers of variables remaining are large in a statistical sense and conventional multivariate analyses, such as those carried out above, can be misleading. This problem is purely statistical, and its importance is a function of the number of variables, the number of groups, and the number of samples (vertebrae) per group. Monte Carlo methods are used to evaluate the inferences drawn. Inferences drawn from randomized data are compared with those results obtained from the actual data as a means of evaluating the probability that such inferences can be explained by chance variation alone. Results of substituting random variables for the numbers in spectra, and conducting multivariate statistics on this data base are given in the following sections.

1. Evaluating the significance of geographic differences

The first evaluation dealt with the inference that differences in the chemical composition of vertebrae of bluefin from different geographic regions are statistically significant and recognizable. The statistic employed to evaluate differences between the EA and WA samples is the "F" statistic, one that is calculated when deriving discriminant functions. The "F" values observed are always highly significant when these are compared with tabulated values using the computed degrees of freedom. The objective is to determine if the results are meaningful.

In order to evaluate the amount of bias, the real data set from the second comparison i.e., the factor scores, was replaced with the same number of random variates. As with the real data, the mean of each variable was made equal to zero and the standard deviation equal to one. Discriminant functions and "F" statistics were calculated on either 50 or 25 different sets of random variates. The results of three different scenarios are illustrated in Figure 12.

In the first scenario, all 266 variables were used to derive the discriminant functions. The distribution of "F" values is given in part A of Figure 12. Vertical lines indicate the positions where the tabulated 5%, 1%, and .5% critical values should be located. The rectangle on the right is the position of the observed "F". The "F" statistic is distributed as it should be, and the observed "F" is much farther away from the center of the distribution to be explained by chance alone. This supports the conclusion that there are statistical differences between samples of adult bluefin from the two different geographic areas.

The second scenario, part B of Figure 12, is similar to part A, except discriminant functions were derived in a stepwise fashion. The calculated "F" statistic using random numbers was statistically significant from 1 in each of the 25 simulations carried out! This result reveals the presence of a bias. The value of the "F" derived from analyses of X-ray data is far greater than any of the "F" values from random data, indicating as before, that real differences exist among the samples from the two geographic areas.

The third scenario differs from the first two in that the values of the random variates were adjusted so that their means and standard deviations correspond to the observed data. This scaling was carried out to compare the observed with the expected distribution of probabilities of group membership, assuming that samples are drawn from two homogeneous populations. The simulated values and the value observed from the real data are plotted in Figure 12-C. Here one finds that the observed "F" statistic is again much larger than those "F" values computed when scaled random variates are used. This in turn suggests that the observed data are distributed differently than one would expect to find if one were sampling from two multivariate, multinormal distributions that overlap.

2. Evaluating the validity of mixing estimates

The results given in the preceding section evaluate the probability that differences in the group mean vectors observed are chance deviations, i.e., the null hypothesis that all vectors are derived from the same statistical population. The results demonstrate that this is not the case; the samples are drawn from at least two populations. In the following sections, distributions of the "p" values, individual probabilities of group membership for each vertebra (fish), are examined in detail. The specific purpose is to evaluate the probability that individuals classed as immigrants are but individuals at the tails of each parent population. Such factors as statistical overlap, heterogeneity within the samples from each geographic region, and inappropriate classifications can serve to inflate the estimates presented. The estimates given are based upon the assumption of two groups of bluefin in the Atlantic, and homogeneity of variances within each group. The results of Monte Carlo simulations given in the following sections provide a basis for evaluating these assumptions. The first study was conducted early in the year and is based upon averages of the the three probabilities of group membership. The second study is based on probabilities computed from one pooled data set.

Mean probability of group membership. The averaged data presented in Table 5-8 is reproduced and presented with the averages of 50 randomized observations (Figure 13). The series of graphs on the left are the actual data and the series on the right are averages of randomized data. Distributions of the observed data are obviously more complex. The distribution of observed data points are in each case multimodal, whereas the distribution of the expected is unimodal. This is strong evidence for heterogeneity.

Joint probability of group membership. Values simulated here are not averages as before but individual probabilities of group membership obtained by combining the three data bases and deriving one classification function. As before, the expected distribution of "p" values within areas, assuming two overlapping homogeneous populations, was obtained by replacing the real data with scaled random numbers. These were tailored such that the variances were equal to one and the means of each variable matched values observed in the real data. The process was repeated 50 times, each time with different random numbers, and re-analyzed. The distribution of both the observed values and the expected values are given in Table 7.

The observed and expected distribution of "p" values differs for both the EA and WA samples. Chi-square for the WA samples with 4 degrees of freedom = 19. The probability of observing a larger value is less than one in a thousand. Similarly, the chi-square for the EA samples is 17.9 with 3 degrees of freedom, the probability is less than .005. In each case, classes with less than five observations per cell were pooled in order to carry out the comparisons. The nature of the deviations from expected are interesting. In each case there are many more samples with higher probabilities of group membership than was expected. Obviously, the simple explanation of two groups with some overlap must be rejected. There is either some component of mixing and/or heterogeneity within areas. Considering the results of both simulations, both factors are probably occurring.

D. Measures of Repeatability and Accuracy

Repeated measurements on the same sample provide a measure of the consistency with which individuals are classified. This provides some measure of precision of the methodology. This is usually measured by analyzing the same sample more than one time. Accuracy is another matter. Here one is concerned with whether or not inferences are correct. This concern is usually evaluated by some other methodology, or some independent test. Both of these factors are evaluated in the following.

1. The Result of analyzing the same sample twice

a. Juveniles. A subset of the juveniles irradiated early in the program were irradiated a second time. The ratios of the elements chlorine and strontium were calculated for each specimen. The results of the first irradiation are contrasted with the results from the second exposure (Figure 14). All points do not fall on a line with slope 1 and an intercept of zero. The scatter reflects experimental error. Ratios obtained from the second analyses appear to be lower suggesting that some form of degradation of the sample has occurred. The separation of points along the diagonal line of Figure 14 should be noted. Geographic differences are greater than differences caused by repeated analyses of the same specimen.

b. Adults. In as much as each individual adult was irradiated three times the first large-scale test of repeatibility is inherent in the data base. The results listed in Table 6 were given earlier. Two other tests of repeatability were carried out, one on a random set of samples and the second on a set of samples identified as immigrants.

The random group consists of 32 samples from the WA collected in 1980. The correspondence between pairs of analyses of the same fish are illustrated in Figure 15-A. For the most part, concordance is good; most points fall in the lower left quadrant of the graph. Some individuals, four out of 32, are classified as immigrants; however their probabilities of membership in the EA set of samples are not particularly high. Recall that the same samples were irradiated over one year apart, and exposed to a great deal more abuse, radiation damage and exposure.

Immigrants. The pattern of concordance of individuals classified as immigrants, Figure 15-B, is not good. There are individuals found in every quadrant of the graph. Four out of 14 WA samples were classified as immigrants twice and 3 out of 11 EA samples were classified as immigrants twice. Samples in this latter experment showed severe signs of radiation damage, the first and second analyses were carried out at the exact same location. In addition, each analyses was given a great deal more radiation than was used in other studies.

2. Geographic variation in the oxygen isotopes

Several new methodologies were explored. The simplest method involved making temporary modifications to existing equipment. These were made, and a limited number of isotope analyses were carried out on the same cross-sections of bones used in the PIXE work. A small set of juvenile bluefin from each side of the Atlantic were analyzed. As before the portions of the vertebrae formed during the first year were irradiated.

Modifications were made to the apparatus during the interval that analyses were carried out, but conditions were held as constant as possible during a particular run. Given in Table 8 are the results of several runs on juvenile and adult fish. It is apparent that, within a run, the concentration of ^{18}O isotope is higher on the western side of the Atlantic; this is true for zero age fish as well as for school fish. Two individual vertebrae were irradiated a number of times. The distances given in Table 8 were determined after samples were irradiated from the position of discolored marks. The discolored marks provide a "beam track"; these tracks were rectangular in shape and varied between 1.02 and 1.30 mm in width and 1.80 and 2.0 mm in height.

A typical backscattering spectrum is complex and there are many methods of calculating the denominator in the ratio, each depending upon integrating a segment of the curve. Another method is to assume that the composition of bone is essentially the same for fish of different geographic areas and then to make comparisons based upon the number of ^{18}O particles counted per unit of charged particles striking the surface of the bone in a second. These results are summarized in Table 9. Data on the zero age fish are not shown as care was not taken in the beginning to insure against sparking in the chamber, a process that makes determination of the total charge unreliable. The results of runs 3 and 4 are comparable and suggest as before that the values differ with area as predicted. The differences between runs appear to be less in magnitude, but still exist, e.g. the values in runs 3 and 4 are greater than in runs 5, 6, 7, and 8. Although the data in Table 8 and 9 parallel each other, differences between runs are much less when "counts per unit charge" are used. Differences within the same bone, the results of runs 7 and 8 are interesting and imply that there is some form of cyclic variation and a dramatic decrease in temperature between the first and second year of life given that age is estimated as discussed earlier.

Since obtaining these early results considerable effort has been devoted to reduce the variability between runs. Source of the problems were traced to malfunctions in the magnetic spectrometer, specifically the "NMR" unit. The equipment is complex and no longer in use. Attempts to repair and maintain the apparatus proved frustrating. A second method of making these measurements was designed and tested. While the results are much more consistent, the sensitivity was marginal. Lack of time and funds required that additional work be postponed.

E. The Nature of Differences Separating Groups

The statistical analyses carried out above indicate that the groups of samples from the EA and WA differ with respect to their group mean vector of factor scores. One would like to understand the chemical basis. While this topic has not been addressed directly, the techniques used do in fact provide an indication of the nature of these differences, but only as a kind of "statistical fallout".

1. Key factors used in differentiating groups

The key factors separating groups are obtained from the variables chosen, and from the order in which these are incorporated in the stepwise analysis. The results of the stepwise process for the juveniles and for the adults of the first comparison are summarized in Table 10. These results are obtained from only one position on the bone, step 3 in figure 6. The variables chosen first are the most important for discrimination. The results indicate that the chemical differences are complex, involving many elements and general properties of the spectra associated with background. These properties include density of the bone and so on. While the presence of many elements can be detected with these methods, the equipment is most efficient at measuring chemical elements with atomic numbers between those of phosphorus and strontium. The results suggest that there are differences in the heavy metals as well but these are difficult to resolve without subjecting the specimens to much more radiation. Many of the same chemical elements that discriminate among juveniles, also discriminate among adults. The order of importance is altered somewhat and fewer variables are chosen. It would appear that while the same form of geographic variation exists among juveniles and adults, differences at the center of the vertebra are less varied as the fish grows older.

2. Factor scores and chemical variation

Factors are derived measurements that represent the original data in a form in which 1) the axes of the factor space are orthogonal, 2) the original data are presented in the minimum number of dimensions needed to account for the variance, 3) the data are made more reliable, and 4) normality has been increased. In terms of the X-ray spectra, one elemental peak is represented by many adjacent variables, and one element can be represented by more than one peak. In addition to this form of redundancy, two or more elements may be positively or negatively correlated with each other. Elemental peaks are observed to be evident on a relatively continuous background of X-rays, in this case "Bremsstrahlung" radiations. This background is influenced by the density of the vertebra and composition (average atomic number) of the matrix. Thus, there is a relatively large number of variables that are correlated and contain information on physical structure. Thus, factor scores may be used to evaluate geographic differences. No attempt was made to interpret all of these factors. Only a few of the most important factors are analyzed in detail in order to illustrate the complexity involved.

The value of the "factor loadings" provide information about those variables that contribute most to discrimination. Given in Figures 16-A, 16-B and 16-C are graphs of factor loadings together with averaged spectra. These are examples of how specific peaks contribute to specific factors in the second comparison. The data in Figure 16-A show that a number of the variables associated with the elements phosphorus and sulfur account most for the separation between the EA and WA samples. The second most important factor is explained by differences in strontium (Figure 16-B). The fifth factor is explained by a combination of chlorine and bromine (Figure 16-C). It is impractical to show the loadings of each variable on each factor. All have been plotted as in the above examples. Results indicate that differences between areas involve physical factors that generate variation in two components of the "Bremsstrahlung" background, and in the concentrations of such elements as strontium, zinc, phosphorus, sulfur, chlorine, bromine, calcium, copper, niobium, molybdenum, iron, and chrome, some singly and some elements in combination.

IV. DISCUSSION

A. Background. The basic premise in these studies is that a form of ecological record exists in the composition of the skeleton of pelagic species and that analytical procedures can be employed to derive information useful in studies of life history and population biology. This premise was forged in part from earlier studies carried out on salmon and other marine species. There it was found that sockeye salmon, *Ocorhynchus nerka*, of different river systems could be distinguished by a method similar to that used in this study, i.e., statistical analyses of X-ray fluorescence patterns (Calaprice, 1971). It was also found that such methods could be used to distinguish between different species; to determine the age of organisms (Calaprice, *et. al*., 1971); and to identify the origin of adult sockeye taken in a mixed fishery (Calaprice *et. al*., 1975). This latter work was repeated in part, confirmed, and extended using related methods of analysis. In one study, X-ray fluorescence patterns of images were obtained from a scanning electron microscope (fish scales) (Lapi and Mulligan 1981). In a second study, tube excited X-ray fluorescence patterns were obtained from pulverized vertebrae, (Mulligan, *et. al*., 1983).

The possibility that such techniques could be used to study oceanic species was suggested by calculations and deduction. It is easy to demonstrate that salmon from different geographic areas acquire new or reinforce existing differences in trace element patterns while at sea. The possibility that such techniques might prove useful with tunas was examined in a pilot study. Dorsal spines of juvenile yellowfin tuna were collected from vessels fishing north and south of the eastern Pacific thermal equator (~5°N). These spines were pulverized, pressed into pellets, and X-ray spectra obtained using a tube based X-ray spectroscopy system. Dorsal spines from a small set were cross-sectioned and trace element patterns of portions of individual spines were acquired using a scanning electron microscope. The results of the study on pulverized spines were encouraging as the X-ray spectra of juveniles (recent recruits to the fishery) from these two different geographic areas were readily distinguishable (Anon., 1980). The results of analyses of individual segments of a spine were less encouraging; eight hours of exposure were required to obtain a single spectrum.

B. Geographic and Temporal Variation in Bluefin. A method of acquiring trace element patterns in small (<1mm diameter) cross-sections of vertebrae was designed and constructed specifically for this study. The techniques are more complex than those used previously. More equipment is used, and there are more hazards. The advantage is the method yields high resolution spectra in a short period of time. Spectra with reasonably well-defined peaks were obtained in 6 minutes rather than 8 hours. This is a significant achievement as it will be possible to irradiate even smaller cross-sections and carry out studies on species smaller than bluefin tuna.

Although several specimens of adults from each area were irradiated, the first phase of the program dealt primarily with juveniles. The results of these studies are straightforward: it is reasonable to conclude that the composition of vertebrae from the two geographic areas are dissimilar. Specifically, the composition of the vertebra formed during the first year of growth differs depending on whether fish were taken in the EA or WA. Spectra are readily distinguishable on the basis of differences in the height of elemental peaks, i.e. differences in the concentration of specific elements, or by comparing the entire spectra, including the background, i.e. multivariate statistics. As these differences involve many sections of the spectrum and components of the background, one is led to believe that the basis for distinguishing groups involves both differences in the concentration of specific elements and differences in the physical structure of vertebrae.

The results of carrying out adjacent analysis on the same vertebra, a form of line transect of vertebrae deposited during different times of life are also interesting. The primary objective was to determine the manner in which chemical components changed with time. Results relevant to the overall discussion have been summarized in this report. The peaks of chlorine and strontium in spectra are prominent, well defined, and the areas are easily calculated. These elements are also members of the set that contribute to geographic differences in bone. The ratio of these two elements was found to differ between geographic areas and with position along the vertebra. Geographic variation appears most pronounced in the region of the vertebra between 1.5 and 5 mm and again between 8 and 9 mm from the center.

Bluefin tuna are often aged by noting the size and date of capture, and growth from tagged fish that are recaptured. Dr. José Cort, a visiting bluefin specialist from Spain, was asked if he could provide an estimate of the ages of the samples of juvenile bluefin irradiated. He identified certain specimens as most likely 2+ and 3+ years of age. It was noted earlier that the relative peak heights of a number of elements varied as one moved away from the center of the vertebra. As the fluctuations were periodic, this was thought to be related in some manner to seasonal effects. The pattern of change of different elemental peaks of 2+ and 3+ year old fish were compared. While most ratios showed some signs of regular fluctuation, differences in the phosphorus/strontium ratios were most pronounced and are used to illustrate the effect and the location along the vertebra where peaks and valleys occur.

As both 2 and 3 year old fish were captured in early summer, it was reasonable to conclude that such differences in peak heights are seasonal in nature and might be used as a basis for estimating age. It was also possible to derive discriminant functions between peaks and valleys so identified, and to use these functions of many elemental and matrix differences to greatly enhance the graphical presentation of seasonal effects. The possibility that ages or organisms could be determined by such techniques was suggested earlier (Calaprice, et. al., 1971). These studies support that conclusion. Studies needed to refine a method of aging using this or related techniques are underway but have not been concluded. The diameter of the proton beam must be reduced and fish collected at different times of the year should be studied. A small set of bluefin believed to be ages 2-10 were irradiated in a subsequent study. Good agreement was found between the ages of younger fish estimated from life history data and that obtained using this technique. However, it would appear that ages of older fish are either underestimated, using life history data, or overestimated using this technique. In any event, it is reasonable to conclude that while seasonal variation is present and pronounced, there are specific regions in the vertebra formed during the first year and again during the second year where geographic differences are greater in magnitude. Samples were irradiated in this region of maximum variation and the spectra used to study mixing.

C. Mixing Estimates

1. General. There are a number of methods one could use to characterize spectra of fish from different geographic regions. These include graphical and statistical methods that use either part of or the entire set of variables. Three methods were used to evaluate data in the pilot study. These results indicate that, given the above assumptions, between 14 to 16% of fish captured in the European fisheries appear to have originated in the WA. Between 22% and 29% of fish caught in the U.S. fishery appear to have originated in the EA. While the estimates made using each method are close, some methods are more objective than others. Estimates based on visual comparison of spectra are both subjective and biased. The viewer tends to focus on that portion of the spectrum that is most varied, neglecting peaks that may be different but not as pronounced. Similarly, comparisons based upon the ratios of specific elements are biased, again the viewer must select some subset of the peaks to make comparisons. Choice is usually based on the relative ease with which peaks can be defined and in which the contribution of background X-rays can be removed. As this is a time consuming process, often only a portion of the possible peaks that could be used is analyzed. Forms of multivariate analyses, ie. factor analysis, discriminant function analysis and cluster analysis, while more complex and esoteric are considered better suited for these and related studies because all of the data are used and the data can be processed relatively rapidly.

For the most part, individuals characterized by each of the methods from a particular geographic region had similar chemical patterns. There are a number of individuals within each sample that have patterns that are more similar to fish taken on the opposite side of the ocean. This fact is evident regardless of the statistical method used for comparison. The frequency of occurrence of these anomalies is greater in the adults than in the juveniles. Inasmuch as these analyses have been obtained from regions of the bone deposited during the first year of life, those individuals found in one geographic area with patterns similar to the majority of the fish from a second part of the ocean are assumed to be immigrants. Considering the various studies carried out, including the simulation studies, this is considered to be a viable working hypothesis, one not refuted by the tests carried out to date. This hypothesis forms the basis for estimates of mixing.

There are two related subjects that should be assessed separately but considered together. The first concerns whether the samples from the two sides of the Atlantic differ, and the second is, given differences exist, what is the incidence of migration if any. While both topics have different biological and management implications, the topics are merged together in the following discussion.

2. Juveniles. The first full-scale comparisons made were of juveniles and included samples that were taken in the WA (Coast of Virginia), the EA (Bay of Biscay) and the Mediterranean (Gulf of Lion). Statistical differences were found between samples from the east and west. Also noted were differences between fish from Bay of Biscay and those captured in the Gulf of Lion. The results of the discriminant function analysis indicate that between 3 and 5% of the sample of schoolfish captured in the WA are similar to those captured in the EA. No schoolfish captured in the EA were classified as originating in the west. This would indicate that movement of fish in the direction of east to west is low, it also indicates that movement in the opposite direction, if it exists at all, is lower. As indicated, differences were found between bluefin from two different eastern fisheries, one operating in the Bay of Biscay and the other operating in the Gulf of Lion. Differences are not as great as those between east and west. The results suggest that immigration is between 8 and 9%. It is interesting to note that tagging studies were carried out during the time that fish were collected. The results of tagging studies are in accord with the above findings. Movement was found to be primarily from east to west and of the order of 7% (Cort 1982).

3. Adults (giants). As with the juveniles, adults have been sampled from fisheries operating in western Atlantic, eastern Atlantic, and Mediterranean. Two different sets of samples have been taken in each of the above areas. Collections were made over a year apart. Adults taken in each of two different geographic areas at two different time intervals have been irradiated, and the results of extensive analyses are presented.

Differences between eastern and western samples Two major conclusions are warranted. First, there are consistent and recognizable differences between fish from the two major fisheries, the EA and WA. This observation holds for comparisons made in each of the two different time frames. No effort has been made to contrast fish from within the Mediterranean and those from the EA (Barbate). The latter were caught in traps near the entrance to the Mediterranean. Monte Carlo methods were used to evaluate the potential bias in the statistical tests employed and to evaluate the observed differences. The results demonstrate that it is extremely unlikely that such differences could be due to chance alone. There are differences in amounts of specific chemical elements and differences in the physical structure of that portion of the bone deposited during the first year. Geographic differences between the vertebrae of juvenile fish are similar to differences in the vertebrae of adult fish.

There is also evidence of heterogeneity within samples from the same geographic region. This is evident in the polymodal distribution of indices of group membership, and in the results of simulation studies. The simulation studies demonstrate that the simple concept that samples are drawn from two multivariate, multinormal populations with the observed small amount of overlap must be rejected. The biological explanation for these observations is more complex; such heterogeneity can be due to mixing, i.e., immigration and emigration, different groups within the east and west, and some unidentified factor such as sex or age. The latter two explanations seem unreasonable as there is no apparent relationship between probabilities of group membership and size of fish or with the sex of fish. While other biological differences not included in this study could in some manner account for the variation observed, the most likely explanation is that the observed heterogeneity is due to a combination of within area variability, and trans-Atlantic movement.

Migration. The first conclusion is that mixing is relatively low. The means of samples compared are significantly different. It would be difficult to observe this result if fish moved freely across the oceans. The second conclusion is that there is evidence of mixing in the samples from each of the different areas evaluated. This is obvious regardless of the graphic or statistical methods used. The simplest evidence for this comes from comparing spectra. Most of the spectra of fish taken from within a geographic area are similar, whereas there are substantial differences between areas. While the method is subjective, it is possible to recognize individuals within an area that have spectra that appear like the spectra of fish from the opposite area. Similar results are obtained using a less subjective form of classification, cluster analyses. Stepwise hierarchical cluster analyses techniques used generate a dendogram in which individuals of varying degrees of similarity are paired in a stepped process. The most similar fish are paired first and finally groups of fish that are the least similar are paired last. The results of these analyses and those of subsequent studies not illustrated show that fish from the opposite side of the Atlantic cluster early in the stepped process. This is explained by considering those fish to have originated in the same area, even though they were captured in different sides of the Atlantic. These small numbers of fish in the sample have been referred to as immigrants.

A second, multivariate approach employs discriminant functions. In this case samples are divided into two groups, each from a different geographic area. Linear discriminant functions are derived such that the distance between the group mean vectors is maximized. In two dimensions, this would be the equation of a straight line positioned such that when each point is projected onto the line, the distance between the means of each of two groups is maximized. Given this equation and the derived parameters, the probability of each observation belonging to a particular group can be calculated. It is easy to visualize this concept in two dimensions, i.e., when two variables are used, but less obvious when over two hundred variables (factors) are used. While over two hundred variables were available, 19 variables were used in the first comparison and 31 variables in the second comparison. Recall that each variable used is a factor score and is composed of linear functions of a number of components of a spectrum. Equations were derived and all of the fish classified. As with the other methods most of the fish from a specific geographic area were classified as belonging to that group. A small number on the other hand were classified as belonging to the alternative group. As the groupings are geographic areas, as the analyses are on regions of the vertebrae deposited during the first year of life, and as there is a similarity between the chemistry and structure of juvenile and adult bone, the assumption is made that these specimens classified as belonging to the other group are immigrants. The results of classifying each individual fish are summarized in Table 11.

Assuming that the fisheries sampled caught a representative subset of the fish, and that samplers have a representative sample of the fishery, one can infer from the data that immigration in the WA is in the neighborhood of between 10% and 13% and immigration in the EA is between 3% and 12%. As with the juveniles, movement from west to east is less than movement in the opposite direction. The differences between the two estimates for an area are in part explained by annual variation (the areas were sampled 1 or more years apart), by better resolution in the spectra (spectra used in the second comparison were irradiated longer), and by sampling variation. The most reasonable explanation is that each factor is operating; however, no attempt has been made to isolate these alternative factors.

D. Reliability and Accuracy of Estimates

General. There are many factors that could affect the reliability of estimates. The main biological assumption is that the differences in the vertebrae of juveniles persist and/or are reinforced as the fish increase in age and size. Evidence that this is reasonable comes from the results of a factor analysis carried out on spectra from juveniles and adults. These are the same data from which estimates of mixing were derived. The results demonstrate that the key factors that formed the basis for classifying adults are also those that form the basis for classifying juveniles. As more factors are used in separating juveniles, the results suggest a partial degradation of this "natural tag". The fact that not only elemental differences, but differences in the matrix of vertebrae form the basis for differentiating groups, leads one to argue that the character of the bone persists with time. While elemental concentrations may change as a function of the "biological half life" of constituents, it is difficult to envision how the physical structure would change and yet retain the pattern of rings one sees on the surface of the vertebra. There are many similarities in the spectra obtained from the center of vertebrae of juveniles and adults from the same general area. These similarities are apparent to the "naked eye". Aside from the statistical evidence one is left with a strong impression that the chemical structure of vertebra as characterized by these techniques are in fact conservative, i.e. are not lost with time. While an important assumption, it isn't critical. It is possible that behavior patterns, and the oceanic environments of bluefin on each side of the Atlantic differ. Such differences can be expected to impose a new or enhance old "natural tags". In retrospect classifications should be made at different stages along the vertebra.

Another assumption made is that annual variation within habitats is less important than geographic variation. The samples of juveniles studied in this respect are of different age groups. Annual variation does not appear to be a problem. The adults compared are also of different age groups. The possibility exists that in some years variation is greater or extreme. As we are dealing with organisms (adults) that may live 20 years, this possibility cannot be readily dismissed. The obvious way of checking this assumption would be to repeat the study with samples of different year classes.

Characterizing samples. The ability to resolve samples is highly influenced by the period and level of exposure to protons. Samples that differ by large amounts require a relatively smaller amount of radiation than do samples that differ by small amounts. The adequacy of the experimental parameters was established using samples of juveniles. There it was shown that while experimental error was present, geographic differences were more pronounced. Some sample degradation also occurred, a factor that can greatly influence repeatability. There is considerable evidence that differences between adults are less pronounced than differences between juveniles. This was noted after the first analyses of adults, and exposure times were increased to better resolve the chemical spectra. Inasmuch as each sample was irradiated three times, the results of the three different spectra were combined into one set of values. This has the net effect of tripling exposure time without tripling sample degradation. Estimates of mixing based on these combined data sets are lower than on each of the individual estimates as expected. This result suggests that other analyses with even longer exposure times might result in even lower estimates. This is a possibility. It is unlikely however that all immigration can be accounted for in this manner. Increase in exposure not only reduced the number classified as immigrants, it also had the effect of increasing bimodality in the samples and making more distinct the differences between individuals that are classified as residents and those that are classified as immigrants.

The results of repeated analyses on the same sample are less than optimum but not unreasonable considering the results of other findings. The methods are expected to be non-destructive only with inert samples. Sample degradation does occur, it was noted in the juveniles, and is expected considering the nature of differences separating the eastern and western samples. The most significant difference between the eastern and western samples is related to the presence of sulfur and phosphorus in the sample. This implies that the ratio of protein to calcareous material varies. This was supported with the results from the backscattering experiments where differences were also noted in the concentration of nitrogen. Repeated exposures to heating and irradiation is expected to alter the matrix of the bone, reducing protein differences. In retrospect a better test would be to section the bone into pieces and to compare results of different analyses on the same location of vertebrae. In any event these data suggest that the estimates of mixing be best considered upper limits.

Statistical methods. The power and validity of the statistical methods used has been studied elsewhere (Calaprice, et. al., 1975). Here statistical methods were studied by fabricating sets of samples with different chemical composition and subjecting the data to multivariate statistical analyses similar to those used in this study. More recently, methods of analyzing spectra similar to those used here are being used in the analysis of complex organic compounds (Windlg 1983) and Meuzelaar, et. al, 1984). Readers may wish to consult this work for assurance that the methods are suited for this purpose. Lapi and Mulligan (1981) and Mulligan, et. al., (1983) advocate using elemental peak heights as the data base, and non-parametric statistical methods. Peak stripping techniques are time consuming and costly in manpower and computer time. Spectra are complex and many peaks "overlap". It is possible to readily determine the height above background of some peaks, and we have done so in this study. While the results are easier to interpret, the data base is biased and not fully representative of the spectrum. The reasons for this were given earlier. Non-parametric methods are not as "powerful" as parametric methods when the underlying assumptions are satisfied, i.e. statistical parameters are known and in accord with the assumptions of that particular method. The raw data are normally distributed and factor transformations further serve to satisfy the necessary assumptions of the tests used in this study. In any event the various methods appear to give the same general result. The simulation studies carried out evaluate inferences drawn from the data. The implications have been discussed earlier and will not be repeated here.

Accuracy. The purpose of this study has been to estimate that proportion of the fish in the sample that originated on the opposite side of the Atlantic. The inferences drawn pertain to specific fisheries in that area, and insomuch as the samples are representative of the fisheries, it is reasonable to assume that the estimates made are "the best estimates that can be made given the constraints under which the study was conducted". No confidence limits for these estimates are given. They are easily calculated; it would be wrong to assume they would be meaningful. Samples were collected as the opportunity arose. They can hardly be considered random. On the positive side, a large number of bluefin have been examined suggesting that the estimates should not be far off, unless there is some serious bias in the sampling method.

The oxygen isotope measurements made suggest that is should be possible to check on these estimates of mixing and also to test various of the above biological assumptions. So far the results are in accord with what is expected given our knowledge of the thermal structure of different breeding and nursery areas. A considerable amount of effort has gone into improving the methods used to measure oxygen isotopes. The method holds much promise. At this stage resolution is marginal. It is not possible to irradiate an individual and to ascertain with 95% confidence the area of origin from the ratio of isotopes. As the methods are still being refined, and as they are destructive, prudence dictates that this exercise be postponed.

V. CONCLUSIONS

This study utilizes chemical and physical variation in hard parts to obtain ecological information of a practical nature. Both temporal and geographic variation exists in vertebrae. Temporal variation is interesting and there are excellent reasons for believing that age- and growth- related data can be obtained. Minor modifications to the equipment are needed to better define periodicity in vertebrae, especially in the older fish where increments between seasons are not as distinct. Better field collections are needed to identify specific parts of a season. Most recent studies indicate that such age determinations can be made in intervals measured in seconds, once samples are prepared. Sample preparation is minimal, requiring only drying.

There are geographic differences; of this there is no question. Whether this variation has been characterized in the most efficient manner, remains to be seen. There is also evidence that bluefin from the eastern and western Atlantic intermingle. There are fish found on both sides of the ocean that have patterns similar to fish from the other side; the implication here is that there is some mixing. The results obtained in this study suggest that the amount of mixing is low. Movement is also not symmetrical, there is more movement from east to west.

The statistical analyses conducted so far make it difficult to associate specific chemical differences with components of the environment. Some information is presented here, other information can be obtained from the data. The oxygen isotope studies provide more possibility. Given these results, it should be possible to state with calculated certainties that a particular fish originated either in the Gulf of Mexico, the Mediterranean or elsewhere in the Atlantic. That information can be obtained not just for bluefin but other species where a lack of such information is also hampering management. More studies are needed, and they need not be costly. Other ratios of isotopes may be measured in much the same manner, and soon it should be fairly easy to determine something of the diet of specimens from hard parts. Workers should save the hard parts of tagged fish that made trans-Atlantic movement for future studies.

The gathering of this data has been carried out in concert with the development of techniques and equipment. Progress has not always been as quick as desired. Most delays have been associated with assembling representative samples and development. Much has been learned; some time consuming steps need not be followed in the future; and other steps have been taken to reduce costs greatly. The information obtained here should shed additional light on the applicability of present concepts of bluefin stock structure in the N. Atlantic. In the process, much has been achieved in exploring the possibility that tuna-like organisms contain a form of ecological record coded in their hard parts.

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Table 1.

Description of samples analyzed in this study

Fish class	Location where samples were collected	Year collected	Number of individuals Pilot Study	Number of individuals Mixing Study	$\bar{x}$ and standard deviation of fork length	Source of samples
Juveniles (school fish)	Bay of Biscay (Eastern Atlantic)	1980	36	88	mean = 86.1 cm, s.d. = 10.2 cm, n=88	Jose Cort (Spain)
	Gulf of Lion (W. Mediterranean)	1982	0	36	mean = 102. cm, s.d. = 13. cm, n=36	1 H.B. Liorzou (France)
	Coast of Virginia (Western Atlantic)	1980	39	95	mean = 94.9 cm, s.d. = 12.7 cm, n=94	N.M.F.S. (Miami)
Adults (giants)	Eastern Atlantic and Mediterranean					
first comparison	• Gibraltar (Barbate)	1982	10	53	mean = 187. cm, s.d. = 37. cm, n=48	2 Peter Miyake (I.C.C.A.T.)
	• Ionian Sea	1982	1	21	mean = 209.4 cm, s.d. = 29.2 cm, n=18	Maurizio Sara (Italy)
	• Tyrrhenian Sea	1982	10	31	mean = 227.5 cm, s.d. = 15.0 cm, n=29	Maurizio Sara (Italy)
second comparison	• Gibraltar (Barbate)	1983	0	94	mean = 222.1 cm, s.d. = 18.2 cm, n=94	2 Peter Miyake (I.C.C.A.T.)
	• Tyrrhenian Sea	1983	0	96	mean = 225.0 cm, s.d. = 45.8 cm, n=96	Maurizio Sara (Italy)
	Western Atlantic					
first comparison	• Coast of Massachusetts	1980	21	71	mean = 280. cm, s.d. = 20. cm, n=57	3 N.M.F.S. (Miami)
second comparison	• Coast of Massachusetts	1982	0	67	mean = 249.7 cm, s.d. = 28.2 cm, n=51	N.M.F.S. (Miami)
	• Gulf of Mexico	1983	0	24	mean = 218.2 cm, s.d. = 39.9 cm, n=10	N.M.F.S. (Miami)

Notes

1) Fork lengths calculated using a weight vs. length regression formula with the Bay of Biscay samples as the standard.

2) Fork lengths calculated using a vertebrae centrum section length vs. fork length regression formula using the Ionian and Tyrrhenian Sea samples as the standard.

3) Fork lengths calculated using a round weight vs. fork length regression formula with the Massachusetts 1982 samples as the standard.

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TABLE 2

SUMMARY TABLE
(Bluefin Tuna)

Stepwise discriminant function analysis of
PIXE SPECTRA in 40 variable sections (see text)

Section No	Variable numbers	Energy range (keV)	# Variables chosen	Variable No. selected	Statistics F.	d.f.(N/D)	P	% correct
1	1-40	0 - 3.2	6	35; 29; 40 7; 27; 25	95.2	6/65	<.001	100
2	41-80	3.2 - 6.7	3	71; 62; 79	8.1	3/68	<.001	72
3	81-120	6.7 - 10.2	2	99; 104	10.8	2/69	<.001	78
4	121-160	10.2 - 13.7	2	140; 145	19.4	2/69	<.001	83
5	161-200	13.7 - 17.3	2	178; 161	8.3	2/69	<.001	61
6	201-240	17.3 - 20.8	3	230; 220; 229	7.5	3/68	<.001	72
7	241-280	20.8 - 24.3	3	263; 265; 254 286; 288; 291	9.9	3/68	<.001	76
8	281-320	24.3 - 27.8	5	315; 304	6.9	5/66	<.001	82
9	321-360	27.8 - 31.3	1	347	8.4	1/70	<.005	64
10	361-400	31.3 - 34.8	1	374	6.6	1/70	<.025	60
11	401-440	34.8 - 38.3	2	406; 434	9.1	2/69	<.001	70
12	441-480	38.3 - 41.8	1	442	8.7	1/70	<.005	64
13	481-512	41.8 - 44.6	3	506; 483; 488	9.1	3/68	<.001	76
Combined (all 13 runs)								
	1-512	0.0 - 44.6	8	62; 40; 71 265; 35; 29 220; 27	89.6	3/63*	<.001*	100
Combined (runs 2 - 13)								
	41-512	3.2 - 44.6	12	140; 483; 263 145; 254; 288 434, 406; 506 62; 71	20.1	8/63*	<.001*	96

* Approximate degrees of freedom and probability as the analysis was carried out on selected variables from a larger set.

TABLE 3 - Inferred nursery areas of giant bluefin tuna taken in the western Atlantic and eastern Atlantic - Mediterranean fisheries. Data are X-ray patterns of vertabrae formed during the first year of life.

CLASSIFICATION OF GIANT BLUEFIN

METHOD (Values are percents)

AREA TAKEN	AREA OF ORIGIN	VISUAL Patterns	VISUAL Cl/Sr ratios	STATISTICAL Discriminant functions
MED. AND E. ATLANTIC (21 fish)	E. ATLANTIC (Med)	85.7	85.7	85.7
	W. ATLANTIC (Gulf)	14.3	14.3	14.3 (3%-37%)95%
WESTERN ATLANTIC (21 fish)	W. ATLANTIC (Gulf)	71.4	71.4	76.2
	E. ATLANTIC (Med)	28.6	28.6	23.8 (11%-53%)95%

TABLE 4. - Multivariate analysis of three groups of juveniles (school fish). Summary of stepwise discriminant function analysis and classifaction matrix. Variables are factor scores. (see text)

A) SUMMARY TABLE

step number	variable entered	F value to enter or remove	number of variables included	U-statistic	approximate F-statistic	degrees of freedom	
1	X(7)	314.4307	1	0.2557	314.431	2.0	216.0
2	X(14)	20.7587	2	0.2143	124.727	4.0	430.0
3	X(8)	12.7973	3	0.1914	91.720	6.0	428.0
4	X(13)	10.5988	4	0.1741	74.381	8.0	426.0
5	X(3)	8.0896	5	0.1617	63.032	10.0	424.0
6	X(53)	8.5707	6	0.1496	55.762	12.0	422.0
7	X(2)	6.7128	7	0.1406	50.011	14.0	420.0
8	X(5)	5.3016	8	0.1338	45.296	16.0	418.0
9	X(9)	6.6600	9	0.1257	42.062	18.0	416.0
10	X(58)	5.0130	10	0.1199	39.071	20.0	414.0
11	X(49)	5.7268	11	0.1136	36.831	22.0	412.0
12	X(37)	4.2757	12	0.1091	34.644	24.0	410.0

B) CLASSIFICATION MATRIX

group	percent correct	number of cases classified into group		
		SPAIN	FRANCE	USA
SPAIN	93.2	82	6	0
FRANCE	91.7	3	33	0
USA	96.8	3	0	92
TOTAL	94.5	88	39	92

Jacknifed classification

group	percent correct	number of cases classified into group		
		SPAIN	FRANCE	USA
SPAIN	90.9	80	8	0
FRANCE	91.7	3	33	0
USA	94.7	5	0	90
TOTAL	92.7	88	41	90

TABLE 5

Estimates[1] of adult bluefin immigration derived from spectra of three separate locations on the same vertebrae. All locations are within a region of the vertebrae which is formed during the first year of growth. A. First set of collections (1980 Mass. primarily and 1982 Mediterranean). B. Second set of collections (1982 Mass. and 1983 Gulf[2] primarily and 1983 Mediterranean). See text.

A. Samples analyzed in 1983

Distance from the center of vertebra	# Fish compared Med., U.S., total			Probability of observing such differences by chance alone	Estimated percentage of trans-Atlantic movement: Fish caught in the E. Atlantic and Med. of western origin	Fish caught in the W. Atlantic of eastern origin
1.2 mm	106	113	219	p<.001[F(12,206)= 10.9]	21.7	21.2
3.2 mm	106	113	219	p<.001[F(8,210)= 18.8]	14.2	18.6
5.3 mm	106	113	219	p<.001[F(11,207)= 14.4]	15.1	20.4

B. Samples analyzed in 1984

Distance from the center of vertebra	# Fish compared Med., U.S., total			Probability of observing such differences by chance alone	Estimated percentage of trans-Atlantic movement: Fish caught in the E. Atlantic and Med. of western origin	Fish caught in the W. Atlantic of eastern origin
1.2 mm	190	116	306	p<.001[F(16,289)= 20.11]	8.4	24.1
3.2 mm	190	116	306	p<.001[F(17,288)= 14.91]	14.2	20.7
5.3 mm	190	116	306	p<.001[F(9,296)= 24.07]	16.8	22.4

1. Estimates obtained from discriminant function analyses derived using only two groups of adult bluefin: 1) eastern Atlantic and Mediterranean, and western Atlantic.

2. Gulf and Gulf of Mexico fish that are believed to have been landed near the Dry Tortugas.

TABLE 7

Monte Carlo evaluations of observed and expected probabilities of group membership. The observed data are summarized in figure11 and table 6-B. Expected are obtained from random variables adjusted to observed differences between means. Chi-square and probability values illustrate how well the observed distributions fit the expected (see text).

	E. ATLANTIC AND MEDITERRANEAN				W. ATLANTIC			
Interval	#	portion	Expected	Observed	#	portion	Expected	Observed
0.00- 0.04	149	.0160	3.04	1	1854	.4158	37.84	66
0.04- 0.08	120	.0129	2.45	0	472	.1059	9.64	2
0.08- 0.12	119	.0125	2.43	0	319	.0715	6.51	3
0.12- 0.16	97	.0104	1.98	0	217	.0487	4.43	1
0.16- 0.20	117	.0126	2.39	1	177	.0397	3.61	2
0.20- 0.24	115	.0124	2.35	0	119	.0267	2.43	4
0.24- 0.28	124	.0133	2.53	0	103	.0231	2.10	0
0.28- 0.32	98	.0105	2.00	0	115	.0258	2.35	0
0.32- 0.36	106	.0114	2.17	1	88	.0197	1.79	0
0.36- 0.40	104	.0112	2.13	0	77	.0173	1.57	3
0.40- 0.44	109	.0117	2.22	1	79	.0177	1.61	0
0.44- 0.48	134	.0144	2.74	1	67	.0150	1.37	0
0.48- 0.52	145	.0156	2.96	0	67	.0150	1.37	1
0.52- 0.56	129	.0139	2.64	3	57	.0128	1.16	1
0.56- 0.60	139	.0149	2.83	1	79	.0177	1.61	1
0.60- 0.64	157	.0169	3.21	0	60	.0135	1.23	0
0.64- 0.68	163	.0175	3.33	1	57	.0128	1.16	0
0.68- 0.72	153	.0164	3.12	1	49	.0110	1.00	1
0.72- 0.76	221	.0237	4.50	2	64	.0144	1.31	1
0.76- 0.80	256	.0275	5.23	1	62	.0139	1.26	2
0.80- 0.84	354	.0380	7.22	3	46	.0103	1.03	0
0.84- 0.88	421	.0452	8.59	7	45	.0101	0.92	1
0.88- 0.92	563	.0605	11.50	4	58	.0130	1.18	0
0.92- 0.96	1014	.1089	20.69	10	51	.0114	1.04	1
0.96- 1.00	4203	.4515	85.79	152	77	.0173	1.57	1

TABLE 6

Estimates of adult bluefin immigration from discriminant function analyses. Basis for classification are jackknife probabilities of group membership. All three independent data sets used in one set of functions. Values in parenthesis obtained from averaged "p".

A. First Comparison

1. F with 19 and 199 degrees of freedom = 16.04, p is less than .001. Nineteen variables chosen in analysis.

2. Classification matrix

	Number in sample	Number immigrants	Estimated % mixing
a. Western Atlantic (1980)			
Massachusetts	71	9	(16.1) 12.7
b. Eastern Atlantic (1982)			
Gibraltar	53	6	11.3
Mediterranean			
Tyrrenhian	31	4	12.9
Ionian	21	3	14.3
total	105	13	(10.4) 12.4

B. Second Comparison

1. F with 31 and 249 degrees of freedom = 24.35, p is less than .001. Thirty-one variables chosen in analysis.

2. Classification matrix

	Number in sample	Number immigrants	Estimated % mixing
a. Western Atlantic			
Massachusetts (1982)	67	8	11.9
Gulf of Mexico (1983)	24	1	4.2
total	91	9	(8.8) 9.9
b. Eastern Atlantic (1983)			
Gibraltar	94	3	3.2
Mediterranean			
Tyrrenhian	96	2	2.1
total	190	5	(6.3) 2.6

TABLE 8 - Ratio of oxygen mass 18, to oxygen compounds in vertebrae. (values are the ratio of ^{18}O counts to area of backscatter curves)

A) Analysis of individual fish

	size of fish	origin of samples	
		(Gulf of Mexico)	(Mediterranean)
run 1	zero age fish	3.70	2.69
run 2	"	2.50	2.25
		(Coast of Virginia)	(Bay of Biscay)
run 3	school fish	2.06	1.87
		*	1.92
run 4	school fish	1.74	1.32
		1.84	1.41
		(Western Atlantic)	(Mediterranean)
run 5	giant	.88	--
run 6	"	--	.99

B) Multiple measurements on individual vertabrae (school fish)

run 7 (Coast of Virginia)

distance from center of vertebrae (mm)	2.3	4.5	6.4	8.9	11.0	13.6	16.0
value of ratio	.73	.85	.79	.85	.78	.88	.78

run 8 (Gulf of Lion)

distance from center of vertebrae (mm)	1.5	3.9	5.0	9.1	10.5	12.7
value of ratio	.72	.74	.85	.82	.91	.95

-- no data

* analysis aborted

TABLE 9 - Number of alpha particles emmited from ^{18}O atoms in the vertebrae, normalized to the amount of charge and time (counts/nano-amper/second)

A) Analysis of individual fish

	size of fish	origin of samples	
		(Gulf of Mexico)	(Mediterranean)
run 1	zero age fish	--	--
run 2	" "	--	--
		(Coast of Virginia)	(Bay of Biscay)
run 3	school fish	3.76	2.84
		*	2.88
run 4	" "	3.15	2.43
		3.38	2.88
		(western Atlantic)	(Mediterranean)
run 5	giants	1.56	1.12

B) Multiple measurements on individual vertebrae (school fish)

run 7 (Coast of Virginia)

distance from center of vertebrae (mm)	2.3	4.5	6.4	8.9	11.0	13.6	16.0
counts/nano-amp/sec. (NC^{-1})	1.31	1.27	1.30	.71	.82	.47	.73

run 8 (Gulf of Lion)

distance from center of vertebrae (mm)	1.5	3.9	5.0	9.1	10.5
counts/nano-amp/sec. (NC^{-1})	1.39	1.49	1.44	1.54	1.01

-- no data

* analysis aborted

TABLE 10 - Sources of chemical variation in vertebrae that enable separation.

Variable chosen in the discriminant function analysis: Step#	Factor	Inferred source of X-rays that are important contributors to factor chosen
Juveniles		
1	7*	The K_α X-rays of Bromine, Chlorine Iron , Potassium and Manganese
2	14	The K_α X-rays of Potassium
3	8*	The K_β X-rays of Iron and Manganese
4	13	The K_β X-rays of Mangañese
5	3	Complex of many channels, background on lower portion of curve
6	53	Does not appear to be any dominant region of the spectrum
7	2	A number of regions load on this variable and appear to define the shape of the background curve
8	5*	The K_α and K_β X-rays of Zinc
9	9	The K_α and K_β X-rays of Strontium
10	58	Difficult to interpret does not appear to be a prominant region of the spectrum
11	49	" "
12	37	" "
Adults		
1	5*	see above
2	7*	"
3	8*	"
4	6	This is a background variable, includes peaks and valleys and the major elements such as Calcium

* - Factors chosen that are common to both juveniles and adults

TABLE 11

BLUEFIN MIXING
(estimates of immigration)*

SIZE CLASS	LOCATION OF FISHERY	NUMBER IN SAMPLE	AREA OF ORIGIN: E. ATLANTIC (MED) %	AREA OF ORIGIN: W. ATLANTIC (GULF OF MEXICO) %
JUVENILES (SCHOOL FISH)				
	F. ATLANTIC (1980)	88	100	0.0
	MED. (1982)	38	100	0.0
	W. ATLANTIC (1980)	95	*5.3	94.7
ADULTS (GIANTS)				
FIRST COMPARISON				
	W. ATLANTIC (1980)	71	*12.7	87.2
	E. ATLANTIC (1982)			
	GIBRALTAR	53	88.7	*11.3
	MED.	52	86.5	*13.5
	TOTAL	105	87.6	*12.4
SECOND COMPARISON				
	W. ATLANTIC (1982)	91	*9.9	90.1
	E. ATLANTIC (1983)			
	GIBRALTAR	94	96.8	*3.2
	MED.	96	97.9	*2.1
	TOTAL	190	97.4	*2.6

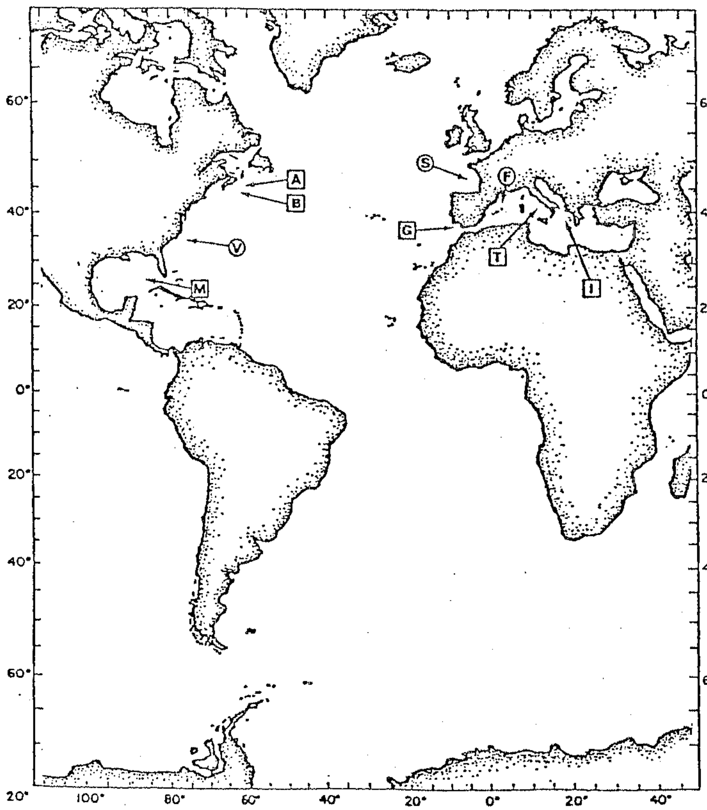
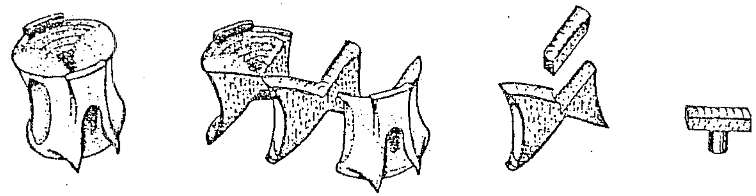


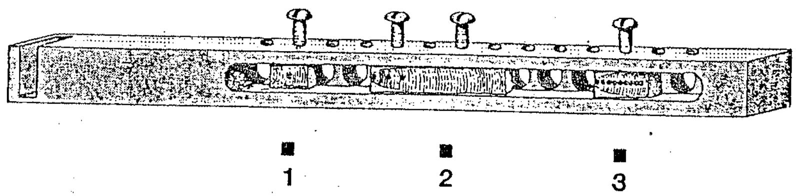
FIGURE 1. General locations where samples were collected.

□ - Adults: A-Mass.(1980), B-Mass.(1982), I-Ionian Sea, T-Tyrrhenian Sea, G-Gibraltar, M-Gulf of Mexico.

O - Juveniles: V-Virginia, S-Bay of Biscay, F-Gulf of Lion.



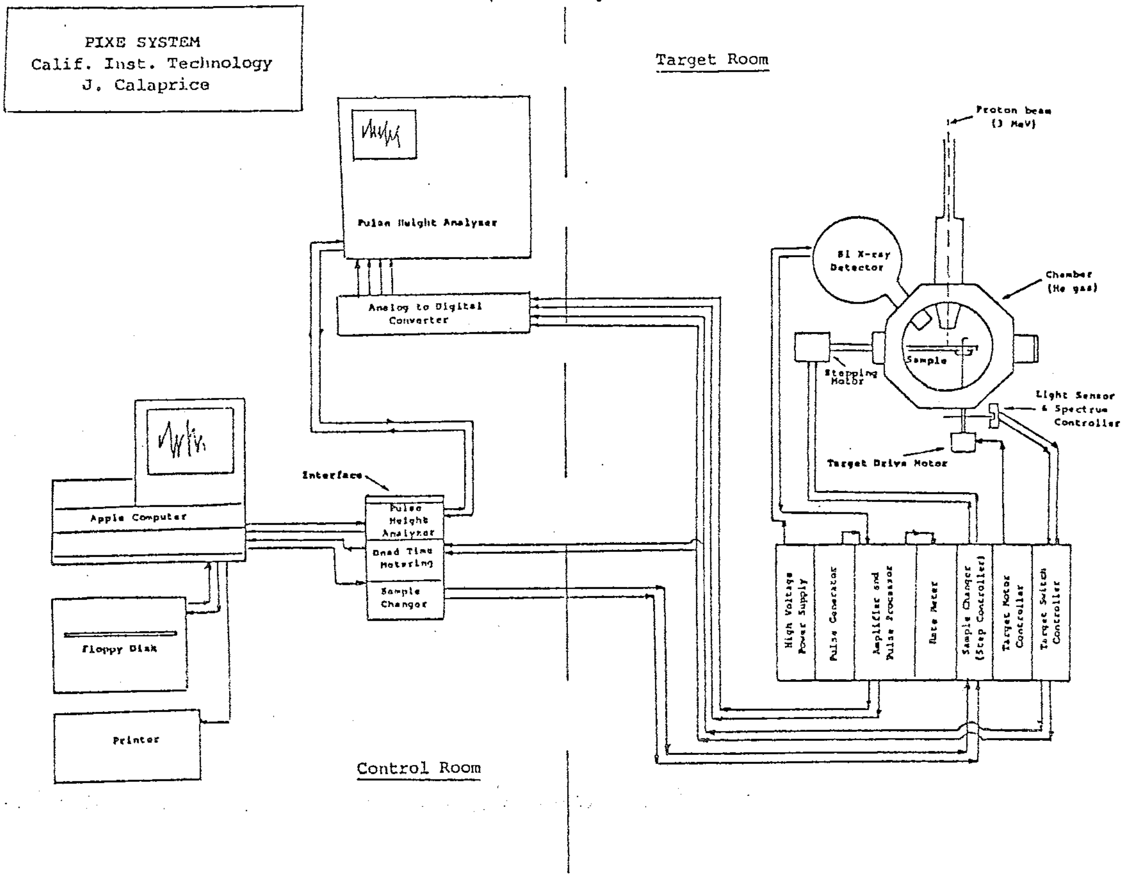
A. Sections cut using diamond blade saw.



B. Sections mounted on plexiglass holder.

FIGURE 2. Method of sectioning (A) and mounting posterior side of 35th vertebra. 1) pattern produced by irradiating juvenile,2) adult, 3) juvenile used in study of temporal variation.

FIGURE 3 · Schematic of the PIXE control and data acquisition system.



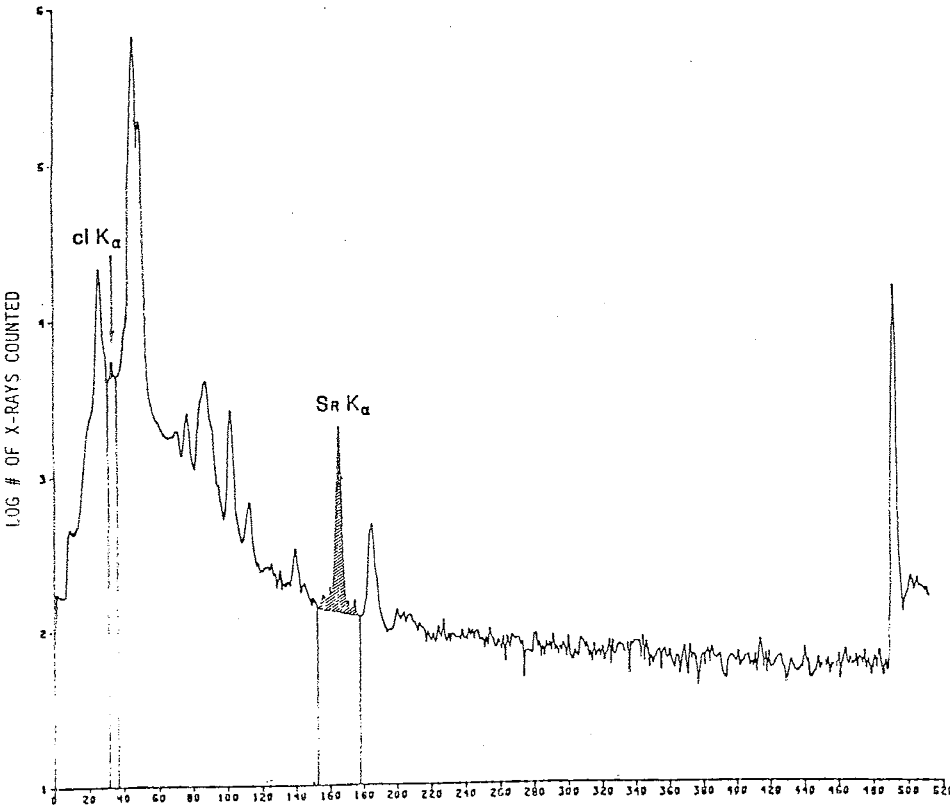


FIGURE 4 Spectrum of .9mm cross-section of vertebra near center. X-ray energy = -.35 + .088 (channel number). Areas for the chlorine and strontium peaks are shaded. Histograms under these peaks are background areas. High peaks at left are calcium peaks. Large peak at left of chlorine peak is phosphorus K-alpha.

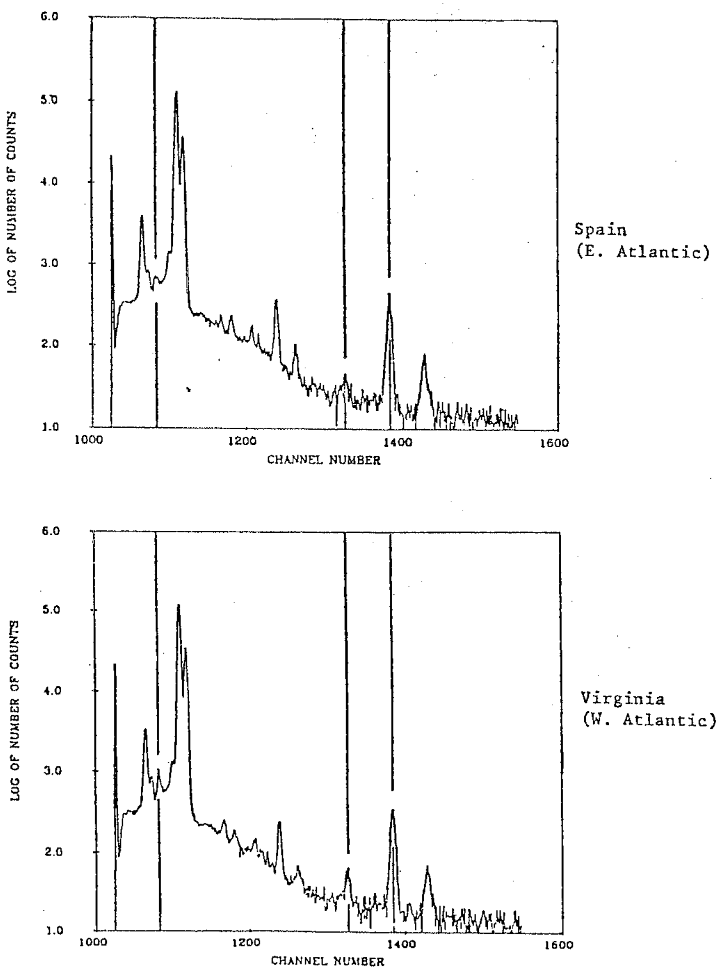


FIGURE 5 - Typical spectra of vertebrae from two sides of the Atlantic Ocean. Vertical lines are drawn through peaks that show consistent differences between locations. X-ray energy = -38.645 + .038 (channel number).

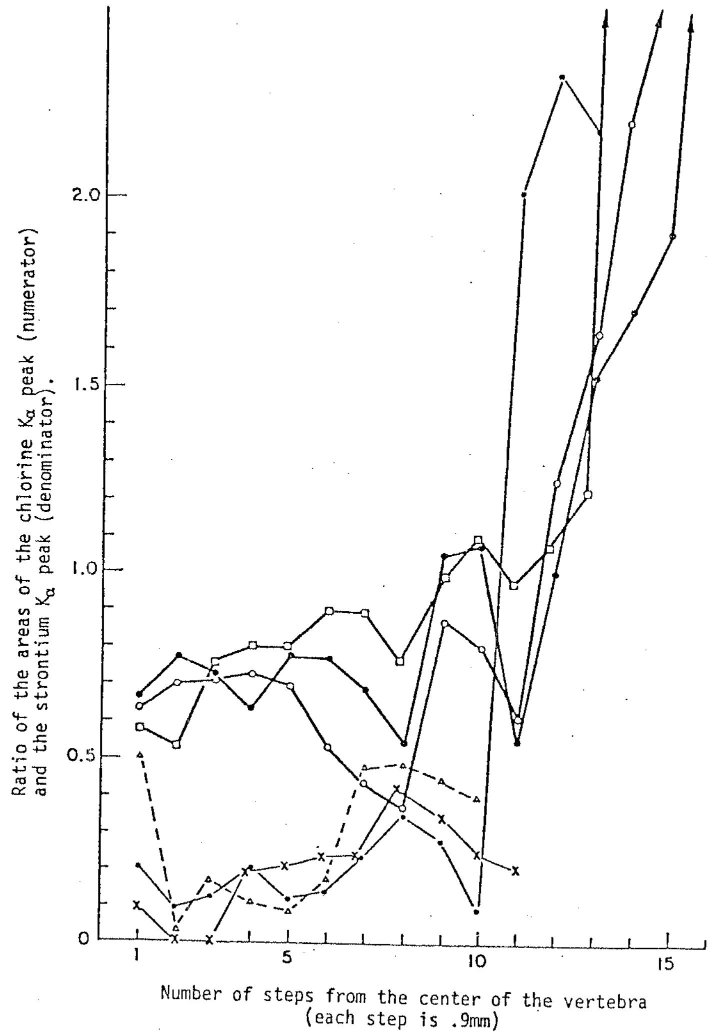


Figure 6 - Sequential analyses of individual vertebrae from age 2 and 3 bluefin from the eastern (bold lines) and western Atlantic.

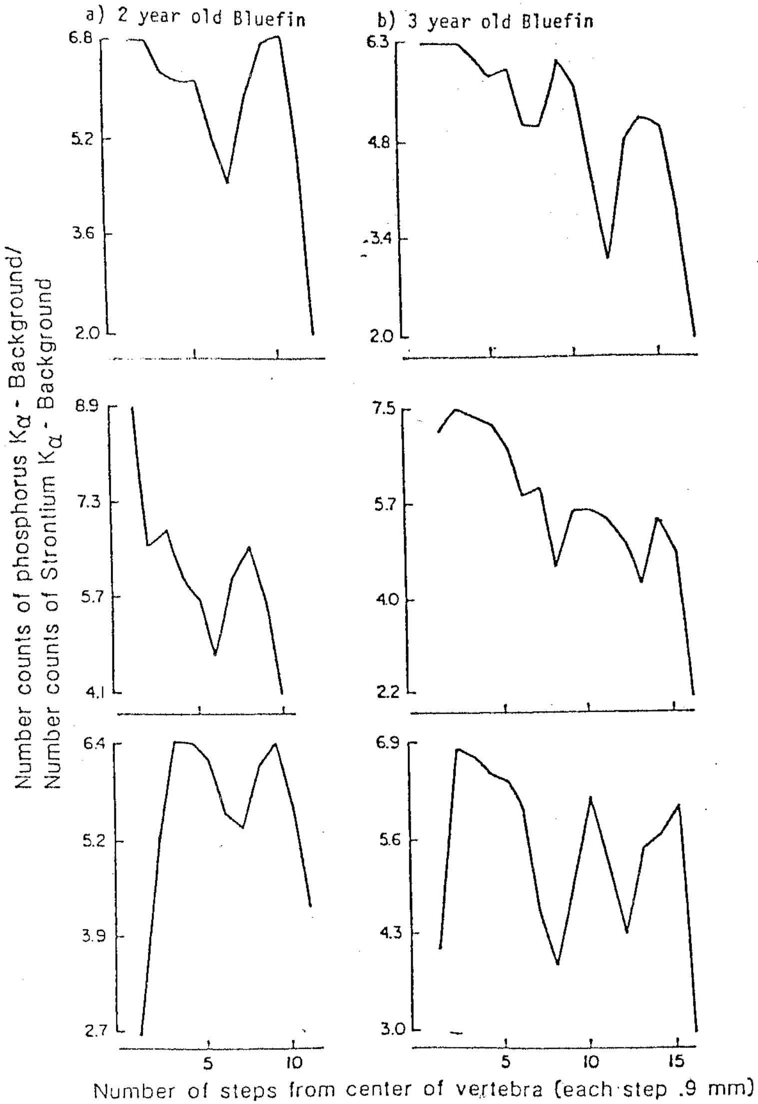


Figure 7 · Sequential analyses of individual vertebrae from 2 and 3 year old Bluefin tuna.

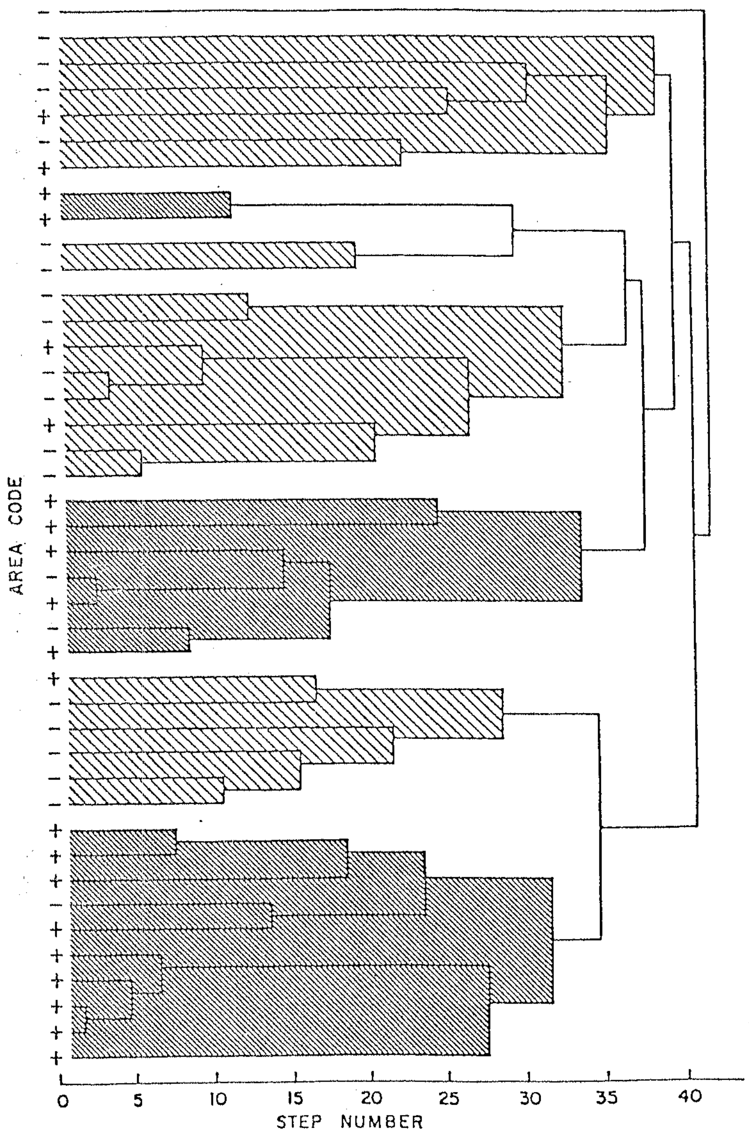


Figure 8 - Dendograms of X-ray spectra from the eastern Atlantic (+) and western Atlantic (-). Variables used are factor scores selected in the stepwise discriminant analyses. Different shaded areas are defined by predominance of one or the other groups.

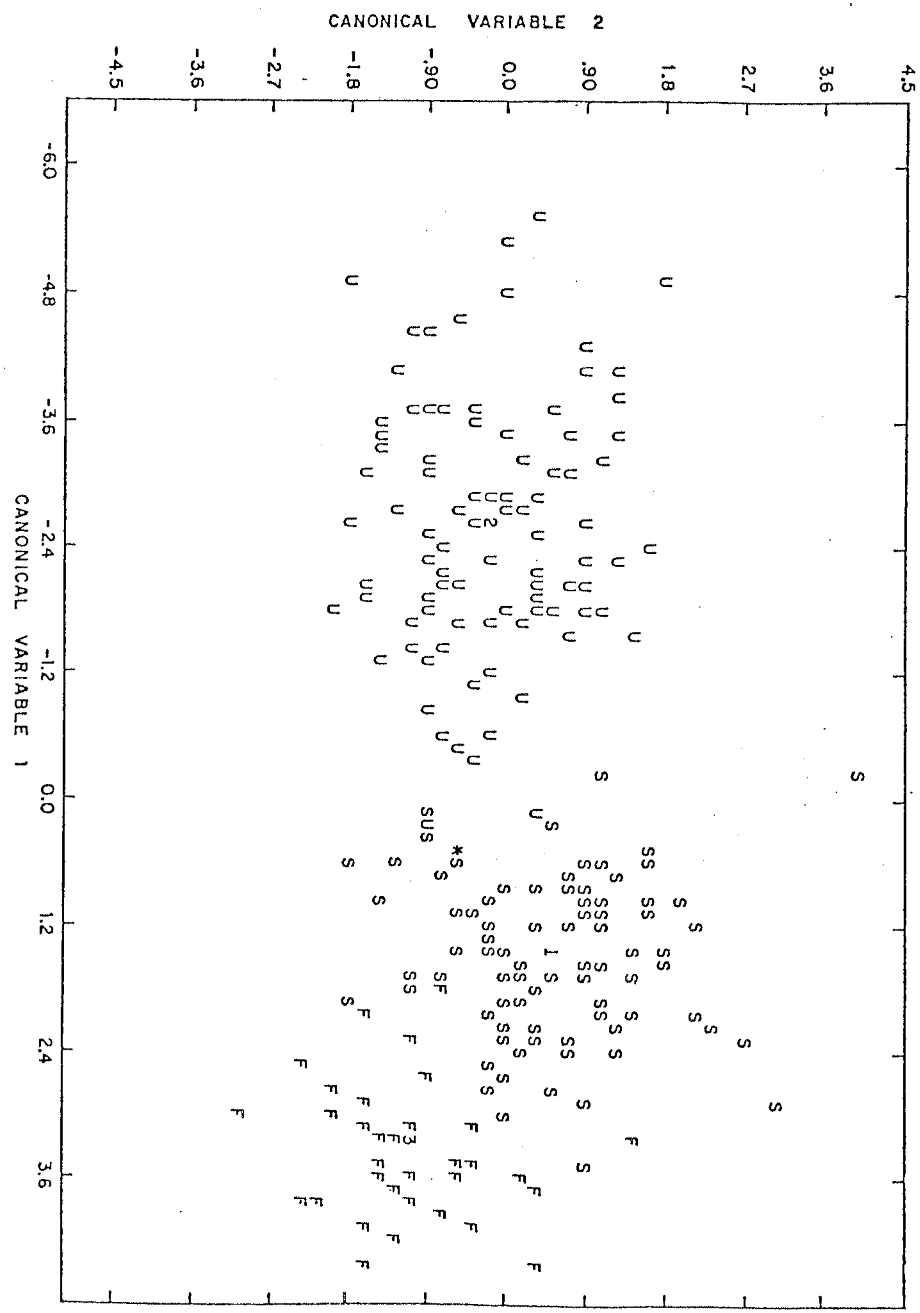


Figure 9 · School fish. Location of individual fish in discriminant space (first two canonical axes). Variables are factor scores. (U = U.S. Fishery, Virginia, S = Spanish fishery, Bay of Biscay, F = French fishery, Gulf of Lion. Overlap of different groups indicated by a *. The numbers are centers of distribution).

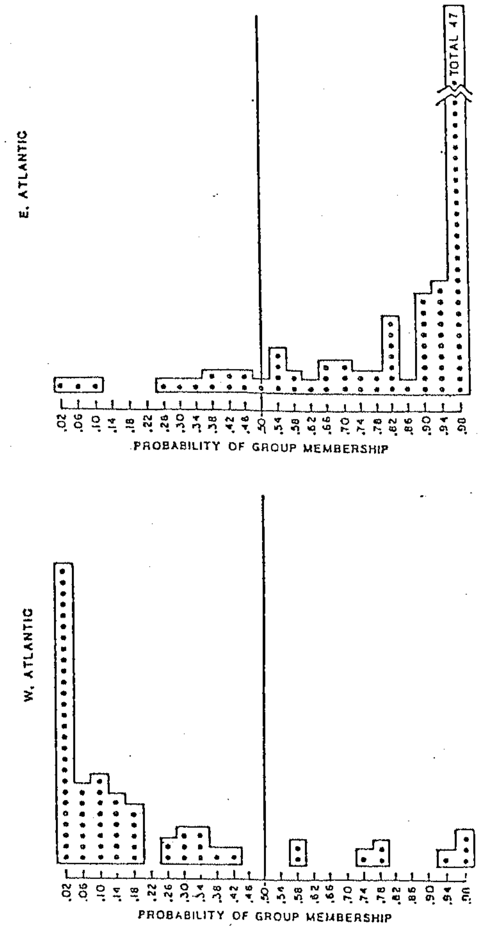


FIGURE 10· Distribution of joint probabilities. The values are the probability that individual vertebrae belong to the eastern Atlantic set of samples (jackknife method). Data obtained in first comparison (see text).

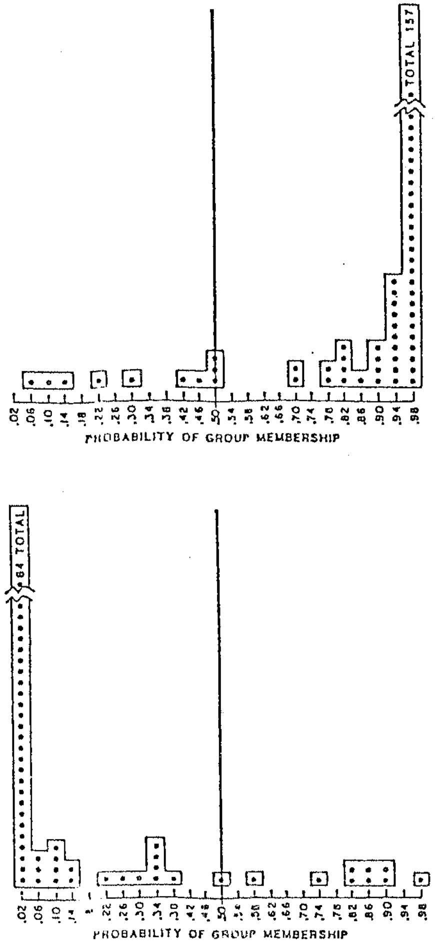


FIGURE 11. Distribution of joint probabilities. The values are the probability that individual vertebrae belong to the eastern Atlantic set of samples (jackknife method). Data obtained in second comparison (see text).

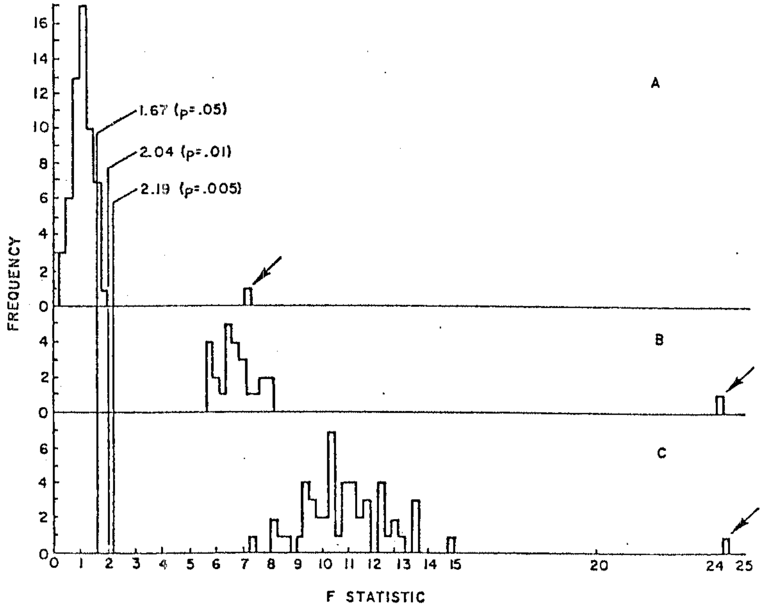


FIGURE 12. Monte Carlo evaluation of observed F statistic. Illustrated are the probabilities of obtaining observed F values (arrows) by chance alone. A - All variables (factor scores) used to derive functions. B - Using stepwise process. C - Random variables adjusted to correspond to observed differences.

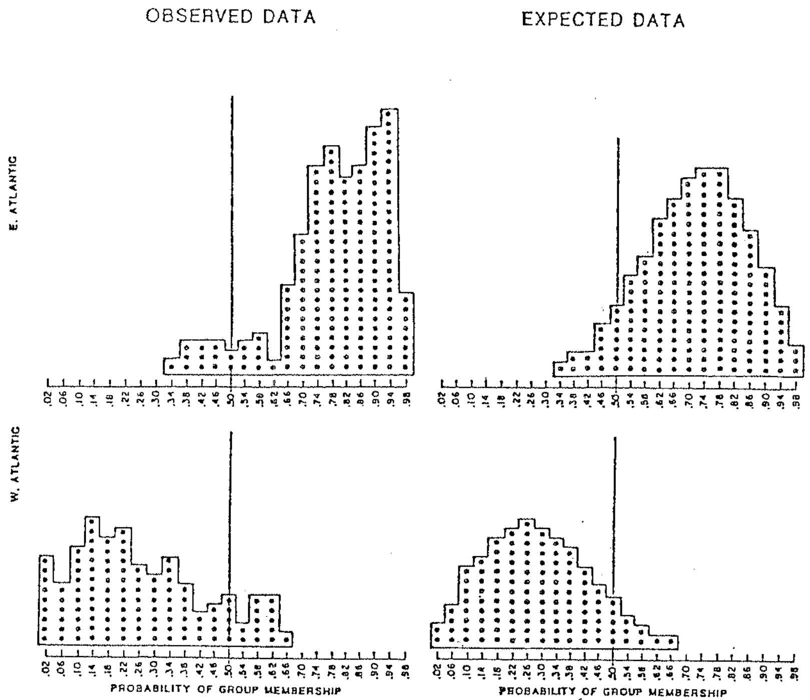


FIGURE 13. Mean probability of group membership.

Distribution of observed data and expected under the assumption of two homogeneous and overlapping distributions. Means in each case obtained by averaging three values of each vertebra. Observed data are from second comparison. Random values are means of 150 random points (50 replicates of 3 point averages).

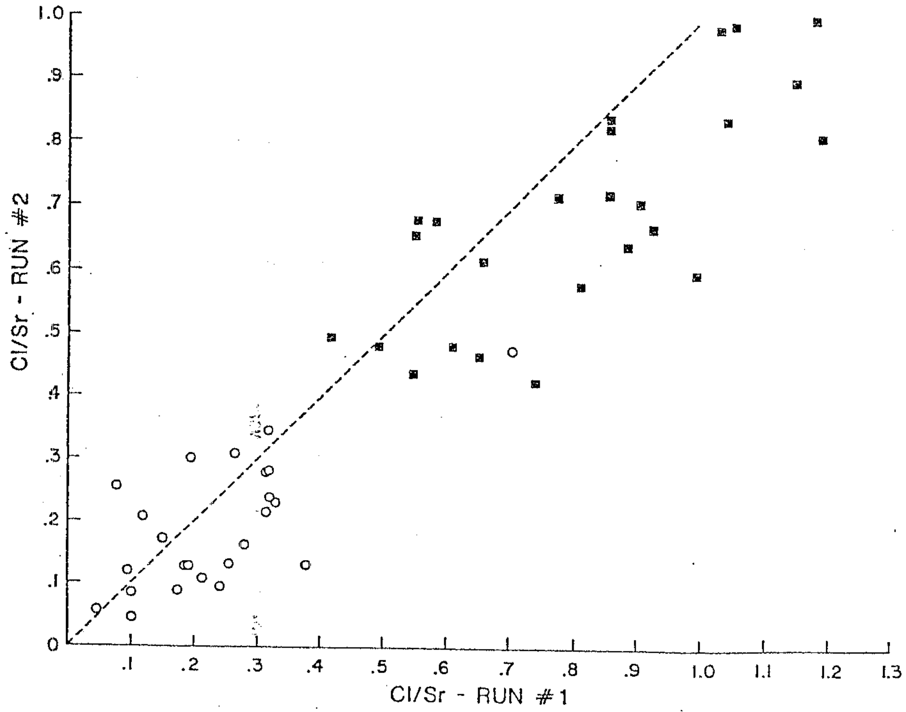


FIGURE 14. Ratios of chlorine and Strontium obtained from same location on vertebrae irradiated on two different occasions. Circles are juveniles from eastern Atlantic and squares are from western Atlantic.

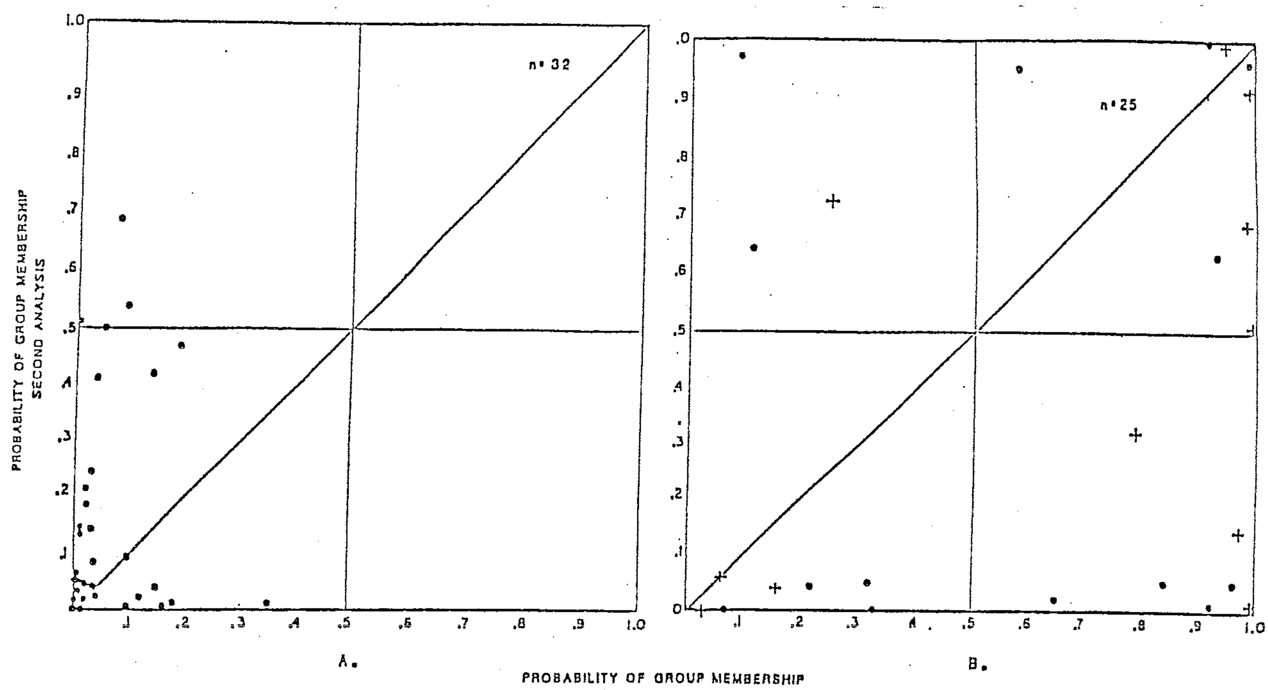


FIGURE 15. Concordance between two sets of analyses on the same vertebrae. A. Random samples of vertebrae, irradiated on different occasions and classified using different functions. B. Individuals identified as immigrants in preliminary study and irradiated a second time.

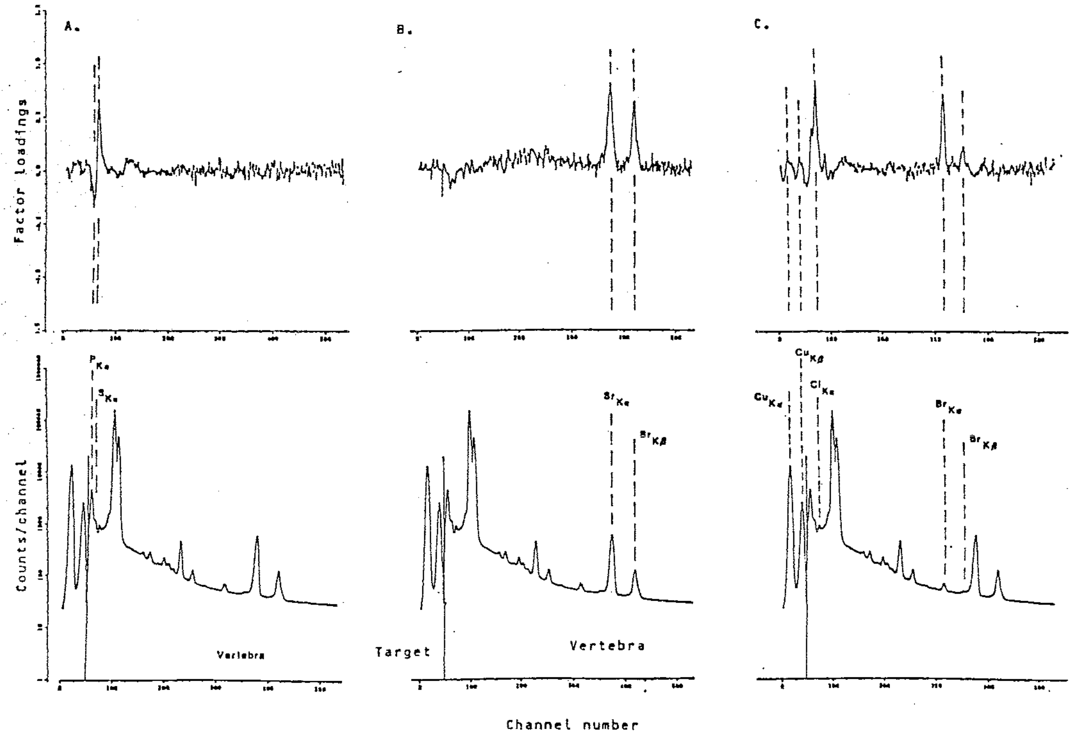


FIGURE 16. Relationship between factor loadings and peaks in spectrum. Spectrum in lower portion of graph obtained by averaging all samples. Upper figures are factor loadings for most important factor (A), second most important factor (B), and fifth most important factor. (See text)