

ELECTROPHORETIC STUDY OF ATLANTIC BLUEFIN TUNA (THUNNUS THYNNUS)
FROM THE EASTERN AND WESTERN NORTH ATLANTIC OCEAN

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SUMMARY

The study was initiated to determine whether Atlantic bluefin tuna (Thunnus thynnus) from the eastern and western North Atlantic Ocean constitute a single breeding population or two different populations. We used the biochemical technique, polyacrylamide gel electrophoresis for population differentiation, employed with specific staining procedures.

This study was conducted in two phases. Phase I was a survey of 12 fish, six each from the eastern and western North Atlantic. From each of the 12 fish, four tissues (heart, liver, white muscle, and red muscle) were analyzed for 23 different enzymes. These enzymes were: glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphohexose isomerase, phosphogluconutase, tetrazolium oxidase, lactate dehydrogenase, esterase, carbonic anhydrase, alkaline phosphatase, α -glycerophosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, leucine aminopeptidase, peroxidase, glutathione reductase, malate dehydrogenase, amino aspartate transferase, acid phosphatase, glutamate dehydrogenase, xanthine dehydrogenase, malic enzyme, succinate dehydrogenase, and alcohol dehydrogenase. The Phase I survey provided the data necessary to make an appropriate selection as to which tissues and enzymes should be studied for comparison of eastern and western North Atlantic samples. Phase II of the study involved

the analysis of the remaining tunas from both the eastern and western North Atlantic with emphasis on those tissues and enzymes indicated by data from Phase I.

Only one-year old fish were used for the study in order to insure that the fish were spawned in their respective sides of the ocean. Sixty-five fish collected off Cape May, N.J., constituted the western North Atlantic sample. For the eastern Atlantic, two samples were obtained: seventeen fish from off Casablanca, Morocco, Africa, and fifty-seven fish from the Bay of Biscay. The Moroccan tunas could not be obtained in sufficient numbers to constitute a statistically valid sample.

We concluded that there is no genetically based variation in any of the 23 enzyme systems that were examined with the exception of the tetrazolium oxidase system. This system has previously been reported as a genetically based variant in Atlantic bluefin tuna (Edmunds and Sammons, 1971, 1973). However, their data on both eastern and western North Atlantic tunas clearly indicates that frequency distributions of the tetrazolium oxidase phenotypes in samples from the two areas do not differ significantly. Also the frequency distribution of phenotypes in their combined sample does not differ significantly from a Hardy-Weinberg distribution. Our data as well as Edmunds' and Sammons' data strongly support the hypothesis that bluefin tuna from the eastern Atlantic (Bay of Biscay) and the western North Atlantic Ocean belong to the same breeding population rather than to two geographically separate populations.

RESUME

Cette étude a été mise en route dans le but de déterminer si le thon rouge de l'Atlantique (*Thunnus thynnus*) des secteurs est et ouest de l'Atlantique nord constitue une population de géniteurs unique, ou deux populations distinctes. Nous avons utilisé une technique biochimique, l'électrophorèse par gel de polyacrylamide destinée à la différenciation des espèces, avec des procédés spéciaux de coloration.

Cette étude a été effectuée en deux stades. Le premier stade consistait à examiner 12 poissons, six de chaque secteur. Quatre sortes de tissus ont été prélevés (cœur, foie, chair blanche et chair rouge), et analysés à la recherche de 23 enzymes: gluco-6-phosphato-déshydrogénase, 5-phosphogluconato-déshydrogénase, phosphohéxose-isomérase, phosphoglucomutase, tétrazolium-oxydase, lactico-déshydrogénase, estérase, anhydrase carbonique, phosphatase alcaline, α -glycérophosphato-déshydrogénase, glycéro-3-phosphato-déshydrogénase, isocitrico-déshydrogénase, leucine-aminopeptidase, peroxydase, glutathion-réductase, malato-déshydrogénase, amino-aspartatofranfêrase, acido-phosphatase, glutamato-déshydrogénase, xanthine-déshydrogénase, enzyme malique, succinato-déshydrogénase, alcool-déshydrogénase. Ce premier stade a fourni les données nécessaires pour effectuer un choix pertinent quant aux tissus et enzymes qu'il convient d'étudier pour comparer les échantillons des secteurs est et ouest. Le deuxième stade consistait à analyser le reste des thonidés prélevés dans les deux secteurs, en portant l'accent sur les tissus et enzymes isolés grâce aux données du premier stade.

L'étude n'a porté que sur des poissons de 1 an, afin de garantir que les individus provenaient bien du secteur dans lequel ils avaient été prélevés. L'échantillon de l'ouest se composait de 65 poissons pêchés au large du Cap May (New Jersey) A l'est, deux échantillons avaient été prélevés: 17 poissons au

large de Casablanca (Maroc) et 57 dans le golfe de Gascogne. L'échantillon marocain ne comportait pas un nombre suffisant d'individus pour constituer un échantillon statistiquement valable.

Nous en avons conclu qu'aucun des 23 systèmes enzymatiques étudiés, exception faite de la tétrazolium-oxydase, ne présentait de variation d'origine génétique. Le système qui fait exception avait déjà été signalé par Edmunds & Sammons (1971, 1973) comme étant sujet, en ce qui concerne le thon rouge de l'Atlantique, à des variations d'origine génétique. Cependant, les données présentées par ces chercheurs sur les thonidés des secteurs est et ouest montrent clairement que la distribution de fréquence des phénotypes de la tétrazolium-oxydase ne diffère pas sensiblement entre les échantillons de ces deux secteurs. La distribution de fréquence de ces phénotypes dans un échantillon combiné ne s'écarte pas non plus beaucoup de la distribution type de Hardy-Weinberg. Nos données, tout comme celles de Edmunds & Sammons, étayaient fortement l'hypothèse selon laquelle le thon rouge de l'est (golfe de Gascogne) et celui de l'ouest de l'Atlantique nord appartiennent à une même population de géniteurs, plutôt qu'à deux populations distinctes.

RESUMEN

Se inició el estudio para determinar si el atún rojo (*Thunnus thynnus*) del Este y Oeste del Océano Atlántico Norte, constituye una población individual o dos poblaciones diferentes. Nosotros utilizamos la técnica bioquímica electroforesis de gelatina poliacrilamida, para diferenciación de la población, empleada con sistemas específicos de decoloración.

Este estudio fue llevado a cabo en dos fases. La primera fase, fué una prospección de 12 peces, seis del Este y otros seis del Oeste del Océano Atlántico Norte. De cada uno de los 12 peces, se analizaron 4 tejidos (corazón, hígado, músculo blanco y músculo rojo) para 23 encimas diferentes. Estas encimas fueron: glucosa-6- fosfato dehidrogenasa, 6-fosfogluconato dehidrogenasa, fosfatasa alcalina fosfoglucomatosa, tetrazolium oxidasa, lactasa dehidrogenasa, esterasa, anhídrido carbónico, fosfatasa ácida y glicerosfato dehidrogenasa, gliceraldeido-3- fosfato dehidrogenasa, y socitrato dehidrogenasa, leucino aminopepsidasa, peroxidasa, glutamato reductasa, molato dehidrogenasa, elaminoaspártico transferasa, fosfatasa ácida, glutamato dehidrogenasa, xantina dehidrogenasa, encima malico, succinasa dehidrogenasa y alcohol dehidrogenasa. La prospección de la fase I, suministra los datos necesarios para hacer una selección apropiada sobre qué tejidos y encimas se deberían estudiar para comparar muestras del Este y Oeste del Atlántico Norte. La fase II del estudio, compara los análisis de los túnidos restantes del Este y Oeste del Atlántico Norte prestando más atención sobre los tejidos y encimas indicados por los datos de la fase I.

Solo se estudiaron peces de 1 año de edad, para el estudio, con el fin de garantizar que éstos habian desovado en sus zonas respectivas del Océano. 65 peces recogidos frente a la costa de Cabo May N.J. constituyeron la

muestra del Oeste del Atlántico Norte. Para el Atlántico Este, se obtuvieron 2 muestras: 17 peces de las costas frente a Casablanca, Marruecos y Africa y 57 peces del Golfo de Vizcaya. Los túnidos de Marruecos no pudieron ser obtenidos en número suficiente, para constituir una muestra estadística válida.

Hemos llegado a la conclusión de que no hay variación basada en la genética en ninguno de los 23 sistemas de encimas que fueron examinados, a excepción del sistema Tetrazolium oxidase. Se ha informado de este sistema como variante de base genética en el atún rojo del Atlántico por (Edmunds y Sammons 1971, 1973). Sin embargo, sus datos sobre los túnidos del Este y Oeste del Atlántico Norte, indican claramente que la frecuencia de las distribuciones del tetrazolium oxidase de fenotipos en muestras de las dos zonas, no difieren significativamente. Asimismo, la distribución de frecuencias de fenotipos en sus muestras combinadas, no se diferencian, significativamente, de una distribución de Hardy-Weinberg. Nuestros datos así como los de Edmunds y Sammons, apoyan fuertemente la hipótesis de que el atún rojo del Este (Golfo de Vizcaya) y del Oeste del Océano Atlántico Norte, pertenecen a la misma población, más que a dos poblaciones geográficamente separadas.

INTRODUCTION

The Atlantic Bluefin Tuna Program of the Miami Laboratory (Southeast Fisheries Center, National Marine Fisheries Service, NOAA) develops scientific data that aids in formulating more effective management regulations on the Atlantic bluefin tuna fishery. One of the critical factors concerning the effective management of the tuna fishery is to know how many populations compose the total stock, because different management schemes may need to be applied to different populations. Consequently, this study was initiated to determine whether Atlantic bluefin tuna (*Thunnus thynnus*) from the eastern and western North Atlantic Ocean constitute a single breeding population or two discrete populations.

We used the biochemical technique of polyacrylamide gel electrophoresis for population differentiation. If employed with specific staining procedures, this technique may be an effective tool for the analysis of specific enzymes. The usefulness of an enzyme system as a genetic marker in differentiating between widely geographically separated populations is determined by its variability, i.e., displaying either or both different positioning or numbers of bands on the gel. If an enzyme does not show variability, then its isozyme pattern cannot be used for comparison among populations. By analysis of data, it can be determined whether specimens are from the same or from different breeding populations. Enzyme variability is the basic premise upon which this study was based. Several successful applications of biochemical genetic methods for the identification of fish populations have demonstrated the usefulness of electrophoresis as a technique for fisheries management (de Ligny, 1971, 1972; Utter et al., 1974; Allendorf and Utter, in press).

MATERIALS AND METHODS

This study was accomplished in two phases. In Phase I we electrophoretically surveyed 12 fish, six each from the eastern and western North Atlantic. From each of the 12 fish, four tissues (heart, liver, white muscle, and red muscle) were analyzed for 23 different enzymes in duplicate. These enzymes were: glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase, phosphohexose isomerase, phosphoglucomutase, tetrazolium oxidase, lactate dehydrogenase, esterase, carbonic anhydrase, alkaline phosphatase, α -glycerophosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, isocitrate dehydrogenase, leucine aminopeptidase, peroxidase, glutathione reductase, malate dehydrogenase, amino aspartate transferase, acid phosphatase, glutamate dehydrogenase, xanthine dehydrogenase, malic enzyme, succinate dehydrogenase, and alcohol dehydrogenase. The Phase I survey provided the data necessary to make an appropriate selection as to which tissues and enzymes should be studied for comparison of eastern and western North Atlantic samples. In Phase II the remaining tunas from the eastern and western North Atlantic were analyzed with emphasis on those tissues and enzymes indicated from Phase I data.

Bluefin Tuna Samples

Only 1-year-old (12-14 months, Farber and Baglin, 1979) fish were used for this study. We assumed that these tunas were spawned in their respective sides of the ocean since no tagged 1-year-old bluefin has ever been recaptured in the same year class on the opposite side of the Atlantic from which it was tagged. This indicates that 12-14 month old bluefin have not yet undergone transoceanic migration. Sixty-five 1-year-old bluefin tunas collected off Cape May, New Jersey, U.S.A., during the summer of 1976 constituted the sample from the western North Atlantic. For the eastern Atlantic two samples were obtained: 17 1-year-old bluefin tunas from off Casablanca, Morocco, Africa, in August 1977 and 57 1-year-old bluefin tunas from the Bay of Biscay near the border between France and Spain, during the fall of 1977. Tunas could not be obtained from off Morocco in sufficient numbers to constitute a statistically valid sample. All samples were placed on ice soon after collection, transported to a freezer for freezing, and then shipped to the laboratory and held in frozen storage at -20°C until analyzed. All manipulations involving tissue homogenization, extraction, centrifugation, filtration, and electrophoresis were performed at 4°C .

Sample Preparation

Tissue extracts were prepared by homogenizing 0.4 g of tissue in 1.2 ml of a pH 7.0 solution containing 5×10^{-3} M Na_2HPO_4 , 1×10^{-3} M EDTA and 2×10^{-3} M β -mercaptoethanol. Homogenates were transferred to 50 ml polyethylene centrifuge tubes and centrifuged in a DuPont-Sorvall RC-5¹ refrigerated centrifuge equipped with an SS-34 motor at 18,000 rpm (39,079 x g) for 30 min. After centrifugation, only liver tissue extracts required filtration through a 13-mm Millipore filter holder fitted to a standard 2-ml glass syringe with a Luer-type fitting. The filter paper (Millipore # HAWP 01300) used was 13 mm diameter of $0.45 \mu\text{m}$ pore size. Filtration was necessary for liver tissue extracts due to excessive lipid material, which precipitated on the top of the gels causing severe protein streaking. Thirty microliters of supernatant were used for each electrophoretic determination.

Methodology

All enzyme analyses were accomplished in a Buchler Polyanalyst apparatus using polyacrylamide disc gel electrophoresis, according to the methods of Davis (1964) and Brewer (1967, 1970). Modifications and adaptations of their methods included the following:

- 1) omitting the sample gel;

¹Reference to trade names does not imply endorsement by the National Marine Fisheries Service, NOAA.

- 2) adding 1 drop of a 0.01% bromphenol blue solution in 50% aqueous sucrose to the total tissue extract supernatant to form a density gradient, preventing mixing of the cathode buffer and tissue extract during addition of cathode buffer to the gel tubes as well as during application of current to the system;
- 3) pre-electrophoresis of the separating gel using 2 ma current for 1 h to remove the ammonium persulfate catalyst;
- 4) electrophoresis of all samples for 30 min, using 2 ma current/gel then increasing the current to 4 ma/gel until the bromphenol blue tracking dye reaches the bottom of the gel tube;
- 5) increasing the percentage of *n,n'*-methylene-bis-acrylamide from 0.184% to 0.35% in gels used for analyses of all the dehydrogenases, tetrazolium oxidase and acid phosphatase since this produced better separation of isozymes; and
- 6) casting gels in precision bore ($+ 1.016 \times 10^{-2}$ mm i.d.) glass tubes 6 mm i.d. x 92 mm long. Specific enzyme staining was accomplished according to methods outlined by Brewer (1967, 1970).

Although analyses for malic enzyme (ME) and lactate dehydrogenase (LDH) were performed using standard methodology, problems involving lack of consistent resolution of ME and LDH isozymes in the western North Atlantic and eastern Atlantic (Bay of Biscay) tunas were encountered. The following modifications were employed for ME determinations: 1) The stacking gel pH was lowered to 5.1. 2) A two-fold increase in the concentration of acrylamide and *n,n'*-methylene-bis-acrylamide was employed in the stacking gels. 3) The buffer system recommended by Clayton and Tretiak (1972) was utilized (stock solution of 0.04 citric acid adjusted to pH 6.1 used undiluted in buffer compartments and diluted 1:20 for gels). Each modification was tried separately and in combination, except the one three combination. The following modifications were employed for LDH determinations: 1) The pH of separating gels was adjusted to 8.9. 2) Six percent acrylamide was incorporated into the separating gels. 3) The discontinuous buffer system recommended by Edmunds (1972) was employed. Each modification was tried separately and in combination.

Data Recording

Data for all enzyme determinations were recorded as graphic representations on millimeter lined graph paper to record exact positions of bands. Black and white photographs were also made of gels suspended in water in a clear Plexiglas tray. Intensities of bands were recorded by visual evaluation by the senior author.

RESULTS

Of the 23 enzymes in each of the four different tissues surveyed in each of six tunas from the eastern and western North Atlantic, five enzyme systems appeared to display variation. With one exception, these five enzymes were confined to liver tissue. The enzyme, tetrazolium oxidase (TO), displayed variation in all four tissues examined. The enzyme TO, however, is the only one from Atlantic bluefin tuna that has previously been reported to be a genetically based variant system by other researchers (Edmunds and Sammons, 1971, 1973; Utter, 1975). The other four enzymes appearing to be variable in liver tissue were glucose-6-phosphate dehydrogenase (G6PD), acid phosphatase (AP), ME, and LDH.

The preliminary findings from the Phase I analyses indicated that the enzymes G6PD, TO, AP, LDH, and ME in liver tissue warranted a more extensive investigation. The liver tissues from the remaining 51 eastern and 59 western North Atlantic tunas were analyzed for these five enzyme systems except TO, since it previously had been identified as a variable system by others. The G6PD analyses of the liver tissues of the western North Atlantic tunas revealed 25 fish with a six-banded isozyme pattern and 40 fish with a seven-banded pattern. For eastern Atlantic (Bay of Biscay) tunas, G6PD analyses had 10 fish exhibiting the six-banded pattern and 47 fish the seven-banded pattern (Table 1). The AP analyses of liver tissues from the western North Atlantic tunas showed that 61 fish displayed a four-banded pattern and four fish a one-band pattern. The eastern Atlantic (Bay of Biscay) tunas also displayed a one- and four-banded pattern for AP with 36 fish showing four bands and 21 fish showing one band (Table 1). The 17 Moroccan tunas were all analyzed for the five enzyme systems that appeared to be variable in liver tissue as indicated by Phase I data. No variation was observed among any of these 17 fish for any of the systems analyzed, with the exception of TO. The AP isozyme pattern was four-banded for all 17 fish and the G6PD pattern was seven-banded for all 17 fish (Table 1). Although conventional methodology was employed for the determination of ME and LDH, their isozyme patterns could not be consistently resolved in the eastern (Bay of Biscay) and western North Atlantic samples.

DISCUSSION

Our data and Edmunds' and Sammons' (1971, 1973) data strongly support the hypothesis that bluefin tuna from the eastern Atlantic (Bay of Biscay) and the western North Atlantic belong to the same breeding population rather than to two geographically separate populations.

We observed the three phenotypes for TO described by Edmunds and Sammons (1971, 1973) and Utter (1975). TO determinations were performed on Phase I samples as well as the Moroccan samples simply to verify the existence of the phenotypes observed by Edmunds and Sammons (1971, 1973). The variant TO data presented by Edmunds and Sammons (1973) was genetically based. Their data on eastern and western Atlantic tunas clearly indicates that the frequency distributions of the TO phenotypes in samples from the two areas do not differ significantly. Also the frequency distribution of phenotypes in their

combined samples does not differ significantly from a Hardy-Weinberg distribution.

After analysis of our data and through consultation with colleagues (Utter, Koehn, and Sharp, personal communications),² we concluded that no logical genetic basis exists for either the AP or G6PD differences in numbers of bands for the eastern and western North Atlantic samples. These differences are artifactual due to the condition (freshness) of the samples.

Various modifications--changes in gel composition, gel pH, use of different buffer systems--were employed to try to solve the ME and LDH resolution problem. None of these modifications provided any consistency in resolution of the isozymes of these two systems. Based on these observations, we concluded that the condition (freshness) of the liver samples played a very important role in the resolution of these enzyme systems. The ME and LDH analyses of the remaining western Atlantic tunas were not completed until the summer of 1977. These samples had been in frozen storage for approximately one year by the time analysis of samples had progressed to this stage, while the eastern Atlantic samples had been in frozen storage for approximately four months before the ME and LDH analyses were performed. The point needs to be made that the ME and LDH analyses of the Moroccan tunas were completed within one month of sample acquisition. There were no problems with achieving consistent resolution of isozyme patterns of ME and LDH in Moroccan tunas as was encountered in the western North Atlantic and eastern Atlantic (Bay of Biscay) samples that had been in frozen storage for an extended period. Some enzymes are more labile under the rigors of extended periods of frozen storage than others--AP, G6PD, ME, and LDH are perhaps four of those.

The discovery of only one genetically based variant enzyme system out of 23 examined in Atlantic bluefin indicates a small degree of genetic variation compared to other organisms reviewed by Lewontin (1973). The 23 enzyme systems we examined had a high probability of showing genetically based variation because the majority of these systems have been observed to vary in other organisms. We cannot explain this low degree of genetic variation on the basis of existing data.

Selander and Kaufman (1973) observed that smaller relatively immobile organisms (most invertebrates) were subjected more to the whims of their environments than the large, more mobile organisms (most vertebrates). The relatively immobile organisms existed in what they called "coarse-grained environments," while the mobile organisms existed in what they called "fine-grained environments." The organisms existing in coarse-grained environments consistently had greater amounts of genetic variability. This was suggested to represent an adaptive strategy to the environmental parameters. The size

²F.M. Utter. July 1978. for affiliation and address, see references; R.K. Koehn. Department of Ecology and Evolution, State University of New York, Stony Brook, New York. 11794. July, 1978; G.D. Sharp. Food and Agricultural Organization of the United Nations, Viale de Terme di Caracalla, Rome, Italy. July, 1978.

and mobility of Atlantic bluefin tuna clearly indicates that bluefin exist in a fine-grained environment. The relative lack of genetic variation in bluefin is possibly a reflection of its mobility.

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Table 1. Location of sample collection and distribution of isozymes for the G6PD and AP systems from Atlantic bluefin tunas.

Location	N _a	G6PD isozyme patterns _b		AP isozyme patterns _b	
		6-banded	7-banded	1-banded	4-banded
Western North Atlantic (Cape May, N.J)	65	25	40	4	61
Eastern Atlantic (Bay of Biscay)	57	10	47	21	36
Eastern Atlantic (Morocco, Africa)	17	0	17	0	17

^aNumber of fish constituting sample from designated area.

^bNumber of fish from each sample displaying indicated isozyme pattern.