

**BIOCHEMICAL GENETICS CHARACTERIZATION OF LARGE PELAGIC STOCKS  
(*THUNNUS THYNNUS*, *THUNNUS ALALUNGA* AND *SARDA SARDA*) IN THE MEDITERRANEAN**

SCRS/1994/171

Col.Vol.Sci.Pap. ICCAT, 44 (2) : 393-397 (1995)

Plá<sup>1</sup>, C., J. Viñas<sup>1</sup>, J.M. Pujolar<sup>1</sup>, J.A. Levy<sup>1,2</sup>

<sup>1</sup>Laboratori d'Ictiologia genètica, Departament de Biologia, Universitat de Girona,  
Plaza Hospital 6, 17071 Girona, Spain

<sup>2</sup>Laboratorio de Bioquímica Marinha, Departamento de Química, Fundação Universidade do Rio Grande,  
C.P. 474, 96200 Rio Grande, RS, Brazil

**SUMMARY**

This paper presents the preliminary results on the study of the genetic structure of three large pelagic species (*Thunnus thynnus*, *Thunnus alalunga* and *Sarda sarda*) along their Mediterranean distribution. Samples of different localities in the Mediterranean Sea have been examined based on the analysis of protein electrophoresis and sequencing of the D-loop region in mitochondrial DNA. This work is part of the European Project EEC/XIV-1/MED/91-012 on "Characterization of large pelagic stocks in the Mediterranean".

**RESUME**

Le présent document fait état des résultats préliminaires de l'étude de la structure génétique de trois espèces de grands pélagiques (*Thunnus thynnus*, *Thunnus alalunga* et *Sarda sarda*) dans toute leur aire de distribution en Méditerranée. Des échantillons provenant de différents points de la Méditerranée ont été examinés par l'électrophorèse des protéines et l'analyse séquentielle de la région D-loop de l'ADN mitochondrial. Ce travail fait partie du Projet Européen CEE/XIV-1/MED/91-012 sur la "Caractérisation des stocks de grands pélagiques dans la Méditerranée".

**RESUMEN**

Se presenta los primeros resultados acerca del estudio sobre la estructura genética de tres especies de grandes pelágicos (*Thunnus thynnus*, *Thunnus alalunga* y *Sarda sarda*) en su distribución mediterránea. Muestras de diferentes localidades mediterráneas han sido examinadas por medio de la electroforesis de proteínas y análisis secuencial de la región D-loop del ADN mitocondrial. Este trabajo forma parte del Proyecto CEE/XIV-1/MED/91-012 sobre "Characterization of large pelagic stocks in the Mediterranean".

## Introduction

An important component of fishery management is knowledge of the genetic population structure of the species in question. In the absence of such information, a particular fishery is at risk of being improperly regulated. If a species is genetically subdivided but is managed for maximum sustained yield as a panmictic unit, the weaker populations suffer over-harvest and the stronger ones are under-harvested. These problems persist even if a genetic structuring is assumed to exist but is inadequately known (Allendorf *et al.*, 1987).

Previous studies on this field have been carried out in different pelagic species. About tuna, several morphometric studies have been carried out on its west and east distribution. Rivas and Mather (1975) suggest that it may exist some subpopulations structure between the eastern and western Atlantic bluefin tuna. Concerning genetic information, Edmuns and Sammons (1971, 1973), Utter (1975) and Thompson and Contin (1979) surveyed several bluefin samples from western and eastern Atlantic Ocean by protein analysis. Barlett and Davidson (1991) sequenced 290 bp of *cyt b* gene in the mtDNA and found six haplotypes in 33 individuals of bluefin from the northeast Atlantic Ocean. Chow and Inoue (1993) examined also the *cyt b* by RFLPs, but the inferred groups are distinct that it found by Block *et al.* (1993) from direct sequencing *cyt b*.

This paper present the preliminary work carried out to examine the genetic variation of bluefin tuna (*Thunnus thynnus*), albacore (*Thunnus alalunga*) and bonito (*Sarda sarda*) along its Mediterranean distribution, caught in waters under Spain, French, Italian and Greece jurisdiction. Fish were captured by trawl in geographically diverse areas within the proposed study area and, are being analyzed by protein electrophoresis and DNA sequencing in order to know the genetic structure of each sampled area.

## Materials and methods and Results

We are sampled several areas along the Mediterranean Sea: Alborán Sea, in Spain; Ligurian and Adriatic Seas in Italy and Aegean Sea in Greece, during the 1993 and 1994 fishing seasons. For bluefin tuna we also have a sample from Bay of Biscay, in the northeastern Atlantic Ocean, to be used as reference versus Mediterranean samples. For each area, 50 individuals were caught to be used in this study by commercial fishing vessels. Samples of processed fish were immediately frozen and maintained at low temperature (-30°C) until its transportation to our laboratory, where it are kepted at -80°C prior to electrophoretic and molecular analysis. Just now, we are done the analysis of bluefin tuna, albacore and bonito samples of the 93 season and we will start the analysis of the samples of this year that we just are receiving.

## Protein electrophoresis

Tissue extraction, electrophoresis and procedures for visualizing proteins generally followed the methods outlined in Aebersold *et al.* (1987). Extracts from tissues including liver (L), heart (H) and skeletal muscle (M) were electrophoretically screened for resolution and activity with buffer systems. Genetic interpretations of the banding patterns produced by enzym systems followed principles outlined in Utter *et al.* (1987). Genetic nomenclature followed the suggestions of Shaklee *et al.* (1990). A summary of the enzym systems analyzed in these studies and its nomenclature are: AAT (Aspartate aminotransferase); ACP (Acid phosphatase) ADH (Alcohol dehydrogenase); AH (Aconitate hydratase); CAT (Catalase); EST (Esterase); FUM (Fumarate hydratase); G3PDH Glycerol-3-phosphate dehydrogenase); GAPDH (Glucose dehydrogenase); GLUDH (Glutamate dehydrogenase); GPI (Glucose-6-phosphate isomerase); IDH (Isocitrate dehydrogenase); LDH (Lactate dehydrogenase); LGL (Lactoyl glutathione lyase); MDH (Malate dehydrogenase); ME (Malic enzyme (NAD+)); MEL (Malic enzyme (NADP+)); MPI (Mannose-6-phosphate isomerase); PEP (Peptidase); PGDH (Phosphogluconate dehydrogenase); PGM (Phosphoglucomutase); PK (Pyruvate Kinase); SOD (Superoxide dismutase) and XDH (Xantine dehydrogenase).

Allelic and genotypic data will be analyzed by the Biosys Program (Swofford and Selander, 1981). For each locus in the different populations, desviations from Hardy-Weinberg proportions will be tested using the independence chi-square test and exact probabilities test. Allele frequency differences among samples were tested by contingency chi-square analysis. With these data, standard genetic distance values will be calculated according to Nei (1972) and a dendrogram will be constructed using the unweighted pair-group method with arithmetic average (UPGMA; Sneath and Sokal, 1973).

## Bluefin tuna

From 26 different enzymatic systems that have been studied to be a reflection of 41 genetic loci. Ten of these loci have showed polymorphism in some of the 4 sampled locations, although only nine of these polymorphic loci have showed a good resolution with an interpretable genetic pattern to be used in the study. A summary of this data is represented on Table 1 that shows the enzym systems that produced distinct banding patterns, loci reported and best tissue and buffer used for each locus.

## Albacore

From 26 different enzymatic systems that have been studied to be a reflection of 39 genetic loci. Twelve of these loci have showed polymorphism in some of the 4 sampled locations, although only nine of these polymorphic loci have showed a good resolution with an interpretable genetic pattern to be used in the study. A summary of this data is represented on Tables 2 that shows the enzym systems that produced distinct banding patterns, loci reported and best tissue and buffer used for each locus.

## Bonito

From 26 different enzymatic systems that have been studied, only 25 showed a good activity to be a reflection of 38 genetic loci. Five of these loci have showed polymorphism in some of the 4 sampled locations and showed a good resolution with an interpretable genetic pattern to be used in the study. A summary of this data is represented on Tables 3 that shows the enzyme systems that produced distinct banding patterns, loci reported and best tissue and buffer used for each locus.

### DNA extraction, PCR amplification and sequencing

Total DNA extraction was obtained of frozen tissue from skeletal muscle using the protocol described in Martin *et al.* (1992) with minor modifications. Amplification of the mitochondrial control region D-loop was carried out by PCR using the protocol outlined in Thomas *et al.* (1990) with some modifications. Two specific primers of this region (Levy *et al.*, 1994) were used to amplify the entire control region about 1,000 bp. The L-strand primer PRO is (5'-CCC AAAGCTAAAATTCTAA-3') and the H-strand primer PHE is (5'-GCTTTAGTTAAGCT ACG-3'). PCR cycling has an initial denaturation step at 95°C for 5 min plus 5 min on ice followed by 25 cycles of denaturation at 92°C for 1 min, annealing at 50°C for 1 min and extension at 72°C for 2 min. Amplification products were purified by Wizard PCRpreps (Promega). Sequencing reactions of the doubled stranded DNA using both PRO and PHE primers were performed by automatic sequencing apparatus (model 373A, Applied Biosystems). Sequencing of the central domain were performed by same way, previous digestion of the PCR product by EcoRI and EcoRV and subsequent cloning in the vector pTZ19U.

## Bluefin

Until now, the DNA sequence analysis has been carried out in bluefin. 10 individuals from the sample caught in Bari (Italia) have been analyzed by DNA sequencing of the D-loop region. Table 4 shows the complete consensus sequence for the D-loop region in this sample. Complete length sequence is 921 bp long including both primers. Excluding the primers the complete sequence is 885 bp long.

### Future directions

Concerning the question about whether the western and eastern Atlantic bluefin tuna are or not one or two stocks in The North Atlantic Ocean, our opinion is that there is few genetic information to resolve if the bluefin population comprise only one Mendelian unit. Some information is consistent with the hypothesis that western and eastern stocks are the same population and have a considerable "mixing" between both stocks. But the sensitive enzymes involved in this studies are few and only one sample from the Bay of Biscay (in the northeastern Atlantic Ocean) has been analyzed. No bluefin Mediterranean samples analyzed I found until now. Requirements for further investigation should include a broad temporal and geographic sampling of bluefin in the spawning areas and along the main areas of its west and east distribution followed by an extensive genetic and morph-meristic analysis of these samples. Technologies involving analyses of mitochondrial and nuclear DNA should be applied within areas appearing to be genetically homogeneous on the basis of extensive surveys of electrophoretically detected variants of protein-coding loci. Following the collection and analyses of such full sets of genetic data, a firmer understanding of the population structure of this valuable resource will provide a sounder biological basis for its management. In this sense, we have planned to continue this project by sequencing different regions of mtDNA like control region of D-loop, cytochrom b and c, and the use of new techniques like analysis of microsatellites to be applied to population genetic studies.

### Acknowledgments

This work is part of the European project on "Characterization of large pelagic stocks in the Mediterranean", supported by the European Economic Community (Project EEC/XIV-

I/MED/91-012) and that it involves several departments and institutions from Spain, French, Italy and Greece. I thank to all people of this centers involved in this project that have provided me swordfish samples from the different locations.

## References

- Aebersol P.B., G.A. Winans, D.J. Steel, G.B. Milner y F.M. Utter, 1987. Manual for Starch Gel Electrophoresis: a method for Detection of Genetic Variation. U.S. Dep. Commer., NOAA Tech. Rep. NMFS, 61.
- Allendorf, F.W. ; N. Ryman and F.M. Utter. 1987. Genetics and Fishery Management: Past, present and future. En: Population genetics and fishery management, eds. N. Ryman y F.M. Utter. University of Washington Press. Seattle and London. Pp 1-19.
- Bartlett, S.E. and W.S. Davidson. 1991. Identification of Thunnus tuna species by the polymerase chain reaction and direct sequence analysis of their mitochondrial cytochrome b genes. Can.J.Fish.Aquat.Sci., 48: 309-317.
- Block, B.A.; J.R. Finnerly; A.F.R. Stewart and J. Kidd. 1993. Evolution of endothermy in fish: Mapping physiological traits on a molecular phylogeny. Science, 260: 210-214.
- Chow, S. and S. Inoue. 1993. Intra and interspecific restriction fragments length polymorphism in mitochondrial genes of Thunnus tuna species. Bull.Nat.Res.Inst.Far Seas Fish., 30: 207-225.
- Edmunds, P.H. and J.I. Sammons. 1971. Genic polymorphism of tetrazolium oxidase in bluefin tuna, *Thunnus thynnus*, from the western North Atlantic. J. Fis. Res. Bd. Can. 28: 1053-1055.
- \_\_\_\_\_. 1973. Similarity of genic polymorphism of tetrazolium oxidase in bluefin tuna from the Atlantic coast of France and the western North Atlantic. J.Fis.Rse.Bd.Can., 30:1031-1032.
- Levy, J.A.; A. Sánchez and C. Pla. 1994. Secuenciación de la región D-loop del ADN mitocondrial en poblaciones de atún rojo, *Thunnus thynnus*, mediterráneas. 29 Jornadas de Genética Luso-Españolas. Lleida, España.
- Martin, A.P.; R. Humphreys and S.R. Palunbi. 1992. Populations genetics structure of the Armorhead, *Pseudopentace wheeleri*, in the North Pacific Ocean. Application of the polymerase chain reaction to fisheries problems. Cand. J. Fish. and Aquat. Sci., 49: 2386-2391.
- Nei, M. 1972. Genetic distances between populations. American Naturalist 106:283-292.
- Rivas, L.R. and F.J. Mather. 1975. A comparison of eastern and western Atlantic bluefin tuna (*Thunnus thynnus*) with reference to stock differences.
- Shaklee J.B. / F.W. Allendorf / D.C. Morizot / G.S. Whitt, 1990. Gene Nomenclature for protein - coding loci in Fish. Trans. Am. Fish. Soc., 119:2-15.
- Sneath, P.H. y R.R. Sokal. 1973. Numerical Taxonomy. W.H. Freeman, San Francisco.
- Swofford D.L. y R.B. Selander. 1981. Biosys -1: a FORTRAN program for the comprehensive analysis of electrophoretic data in population genetics and systematics. Heredity, 72: 281-283.
- Thomas, W.K.; S. Paabo; F.X. Villablanca and A.C. Wilson. 1990. Spatial and temporal continuity of Kangaroo rat populations shown by sequencing mitochondrial DNA from museum specimens. J. Mol.Evol., 31: 101-112.
- Thompson H.C. and R.F. Contin. 1979. Electrophoretic studies of Atlantic bluefin tuna (*Thunnus thynnus*) from the eastern and western North Atlantic Ocean. ICCAT, SCRS/79/96.
- Utter, F.M. 1975. A biochemical genetic survey of bluefin tuna. unpublished report. NMFS. Environmental Conservation. NWAFC. Seattle. WA. 7pp.
- Utter, F.M.; P. Aebersold and G. Winans. 1987. Interpreting genetic variation detected by electrophoresis. In: Population Genetics and Fisheries Management. N. Ryman and F. Utter (eds.). University of Washington Press.

Table 1. Enzymatic locus abbreviations, coding commission, best tissue, buffer conditions and allele characteristics on *T.thynnus*

| LOCUS   | ECC       | BUFFER  | TISSUE  | ALLELE        |
|---------|-----------|---------|---------|---------------|
| AAT-1   | 2.6.1.1.  | 1, 2    | L, M    | M             |
| AAT-2   |           |         |         | M             |
| AHP     | 3.1.3.2.  | 3, 4    | all     | M             |
| ADH-1   | 1.1.1.1.  | 1       | L       | M             |
| ADH-2   |           |         |         | M             |
| AH      | 4.2.1.3.  | 1       | L       | M             |
| CAT     | 1.1.1.6.  | 2, 4    | L       | P (85/100)    |
| EST-1   | 3.1.1.1.  | 2       | H, L    | M             |
| EST-2   |           |         |         | M             |
| FUM     | 4.2.1.2.  | 4       | H       | M             |
| G3PDH-2 | 1.1.1.8   | 1       | L       | M             |
| G3PDH-1 |           |         | M       | M             |
| GAPDH-1 | 1.2.1.12  | 6, 1, 2 | H       | M             |
| GAPDH-2 |           |         |         | M             |
| GDA     | 3.5.4.3.  | 7, 1    | L       | M             |
| GDH     | 1.1.1.47. | 1, 4    | L       | P ?           |
| GLUDH   | 1.4.1.3.  | 1, 4    | L, H    | M             |
| GPI-1   | 5.3.1.9.  | 1, 2    | H, L, M | M             |
| GPI-2   |           |         |         | M             |
| GPI-3   |           |         |         | M             |
| IDH-1   | 1.1.1.42. | 1       | H,L     | M             |
| LDH-1   | 1.1.1.27. | 1, 4    | M       | M             |
| LDH-2   |           |         | H       | M             |
| LGL     | 4.4.1.5.  | 1, 2, 3 | all     | M             |
| MDH-1   | 1.1.1.37. | 1       | H       | P (100/190)   |
| MDH-2   |           |         | H       | P (100/135)   |
| ME-1    | 1.1.1.39. | 2, 3    | H       | P (100/110)   |
| ME-2    |           |         | H       | M             |
| ME-3    |           |         | L       | P (95/100)    |
| MEL     | 1.1.1.40. | 1, 4    | H       | M             |
| MPI     | 5.3.1.8.  | 7       | H       | M             |
| PEP-LG  | 3.4.13.-  | 2       | H       | M             |
| PEP-LGG | 3.4.13.-  | 2       | H       | M             |
| PEP-PAP | 3.4.13.-  | 2       | H       | M             |
| PGDH    | 1.1.1.44. | 5, 3    | H       | P (90/100)    |
| PGM     | 5.4.2.2.  | 1, 4    | H,L     | P (100/110)   |
| PK-1    | 2.7.1.40  | 1       | H       | P (-175/-100) |
| PK-2    |           |         |         | M             |
| SOD-1   | 1.15.1.1. | 1       | L, H    | M             |
| SOD-2   |           |         |         | M             |
| XDH     | 1.2.3.27  | 2, 3    | L       | P (100/105)   |

BuLLers: 1 AC, 2 TC/LB, 3 TBE, 4 POULIK, 5 TC, 6 TBE+NAD, 7 TP  
 Tissue: L, liver; H, heart; M, muscle.  
 Allele: M: monomorphic; P: polimorphic; bd, bad resolution

Table 2. Enzymatic locus abbreviations, coding commission, best tissue, buffer conditions and allele characteristics on *T.alalunga*

| LOCUS   | ECC       | BUFFER  | TISSUE  | ALLELE         |
|---------|-----------|---------|---------|----------------|
| AAT-1   | 2.6.1.1.  | 1, 2    | L, M    | P (-100/-130)  |
| AAT-2   |           |         |         | M              |
| AHP     | 3.1.3.2.  | 3, 4    | all     | M              |
| ADH     | 1.1.1.1.  | 1       | L       | M              |
| AH      | 4.2.1.3.  | 1       | L       | P (90/100)     |
| CAT     | 1.1.1.6.  | 2, 4    | L       | P (85/100/115) |
| EST-1   | 3.1.1.1.  | 2       | H, L    | M              |
| EST-2   |           |         |         | M              |
| FUM     | 4.2.1.2.  | 4       | H       | M              |
| G3PDH-2 | 1.1.1.8   | 1       | L       | M              |
| G3PDH-1 |           |         | M       | ?              |
| GAPDH-1 | 1.2.1.12  | 6, 1, 2 | H       | P ?            |
| GAPDH-2 |           |         |         | M              |
| GDA     | 3.5.4.3.  | 7, 1    | L       | M              |
| GDH     | 1.1.1.47. | 1, 4    | L       | P ?            |
| GLUDH   | 1.4.1.3.  | 1, 4    | L, H    | P ?            |
| GPI-1   | 5.3.1.9.  | 1, 2    | H, L, M | M              |
| GPI-2   |           |         |         | M              |
| GPI-3   |           |         |         | P(100/1300)    |
| IDH-1   | 1.1.1.42. | 1       | H,L     | M              |
| LDH-1   | 1.1.1.27. | 1, 4    | M       | M              |
| LDH-2   |           |         | H       | M              |
| LGL     | 4.4.1.5.  | 1, 2, 3 | all     | M              |
| MDH-1   | 1.1.1.37. | 1       | H       | M              |
| MDH-2   |           |         | H       | M              |
| ME-1    | 1.1.1.39. | 2, 3    | H       | M              |
| ME-2    |           |         | H       | M              |
| ME-3    |           |         | L       | P (90/100/110) |
| MEL     | 1.1.1.40. | 1, 4    | H       | M              |
| MPI     | 5.3.1.8.  | 7       | H       | M              |
| PEP-LG  | 3.4.13.-  | 2       | H       | M              |
| PEP-LGG | 3.4.13.-  | 2       | H       | M              |
| PEP-PAP | 3.4.13.-  | 2       | H       | M              |
| PGDH    | 1.1.1.44. | 5, 3    | H       | P (90/100)     |
| PGM     | 5.4.2.2.  | 1, 4    | H,L     | P (100/110)    |
| PK-1    | 2.7.1.40  | 1       | H       | P (-175/-100)  |
| PK-2    |           |         |         | M              |
| SOD-1   | 1.15.1.1. | 1       | L, H    | M              |
| SOD-2   |           |         |         | M              |
| XDH     | 1.2.3.27  | 2, 3    | L       | P (95/100/105) |

Buffers: 1 AC, 2 TC/LB, 3 TBE, 4 POULIK, 5 TC, 6 TBE+NAD, 7 TP  
 Tissue: L, liver; H, heart; M, muscle.  
 Allele: M, monomorphic; P, polimorphic; m.r. bad resolution

Table 3. Enzymatic locus abbreviations, coding comission, best tissue, buffer conditions and allele characteristics on *S.sarda*

Buffers: 1 AC, 2 TC/LB, 3 TBE, 4 POULIK, 5 TC, 6 TBE+NAD, 7 TP  
 Tissues: L, liver; H, heart; M, muscle  
 Allele: M, monomorphic; P, polymorphic; bd, bad resolution

| LOCUS   | ECC       | BUFFER  | TISSUE  | ALLELE          |
|---------|-----------|---------|---------|-----------------|
| AAT-1   | 2.6.1.1.  | 1, 2    | L, M    | M               |
| AAT-2   |           |         |         | M               |
| AHP     | 3.1.3.2.  | 3, 4    | all     | M               |
| ADH-1   | 1.1.1.1.  | 1       | L       | bd              |
| CAT     | 1.1.1.6.  | 2, 4    | L       | M               |
| EST-1   | 3.1.1.1.  | 2       | H, L    | M               |
| EST-2   |           |         |         | M               |
| FUM     | 4.2.1.2.  | 4       | H       | M               |
| G3PDH-2 | 1.1.1.8   | 1       | L       | P(55/70/85/100) |
| G3PDH-1 |           |         | M       | M               |
| GAPDH-1 | 1.2.1.12  | 6, 1, 2 | H       | M               |
| GAPDH-2 |           |         |         | M               |
| GDA     | 3.5.4.3.  | 7, 1    | L       | M               |
| GDH     | 1.1.1.47. | 1, 4    | L       | M               |
| GLUDH   | 1.4.1.3.  | 1, 4    | L, H    | M               |
| GPI-1   | 5.3.1.9.  | 1, 2    | H, L, M | M               |
| GPI-2   |           |         |         | M               |
| GPI-3   |           |         |         | P(100/110)      |
| IDH-1   | 1.1.1.42. | 1       | H, L    | P(-75/-100)     |
| LDH-1   | 1.1.1.27. | 1, 4    | M       | M               |
| LDH-2   |           |         | H       | M               |
| LGL     | 4.4.1.5.  | 1, 2, 3 | all     | M               |
| MDH-1   | 1.1.1.37. | 1       | H       | P(-100/-60)     |
| MDH-2   |           |         | H       | M               |
| ME-1    | 1.1.1.39. | 2, 3    | H       | M               |
| ME-2    |           |         | H       | M               |
| ME-3    |           |         | L       | M               |
| MEL     | 1.1.1.40. | 1, 4    | H       | M               |
| MPI     | 5.3.1.8.  | 7       | H       | M               |
| PEP-LG  | 3.4.13.-  | 2       | H       | M               |
| PEP-LGG | 3.4.13.-  | 2       | H       | M               |
| PEP-PAP | 3.4.13.-  | 2       | H       | M               |
| PGDH    | 1.1.1.44. | 5, 3    | H       | M               |
| PGM     | 5.4.2.2.  | 1, 4    | H, L    | M               |
| PK-1    | 2.7.1.40  | 1       | H       | M               |
| PK-2    |           |         |         | M               |
| SOD-1   | 1.15.1.1. | 1       | L, H    | M               |
| SOD-2   |           |         |         | M               |
| XDH     | 1.2.3.27  | 2, 3    | L       | P(95/100/105)   |

Table 4. Complete mitochondrial DNA sequence of D-loop region from bluefin tuna.

```

10      20      30      40      50      60
1  CCCAAAGCTA AAATTCATAA TTAAACTATT CTTTGTTCCTA CCGTGCCGGC ATATTTCAAT
61 ATGTCCTGCGC ATGTACATAT ATGTAATTAC ACCATATTCA TATATAGACC ATATATAATA
121 ATGTTTTAGG ACATATATGT ATTAAAACCA TTACTAGTAT TAAACCATTC ATATGTCAAT
181 AAATAATGAA GATTTACATA AACCATACAA ATAAACCTCA ACATTCATCT TGAATTCAGG
241 CGATTAAACG AGATTAAAGA CCTAACATAA ATCTAAATCG TCTAAGCCAT ACCAAGTCTC
301 CTCATCTCTG ACATCTCGTA AACTTAAGCG CAGTAAGAGC CTACCATCCA GTCCATTTCT
361 TAATGCATAC GGTATTGAA GGTGAGGGAC AATAATTGTG GGGCTAACAC TTAGTGAATT
421 APTCCCTGGCA TCTGGTTCCT ACTTCAGGGC CATAGCTTGG TAAACATCCC CATTCCTTCA
481 TTGACGCTTG CATAAGTTGT TGGTGGAGTA CATGAGATTC ATTAAGCCAC ATGCCGGGGC
541 TTCTCTCTAG GGGGTCAGGT TATTTTTTTC TCTCCTCCTT TTCATTTGAC GTCTCACAGT
601 GCAATGCAAC AATGATCAAC AAGGTAGAAC ATTTTCTTGC TTGCAGGTA AATAGCCTGC
661 ATGGCTTAAT TCCTATTACC TAAATAACCA CATAAGAGGA TATCATGAGC ATAATGATAA
721 TATTACCCGT AAAATATCTA AGACACCCCC TCTCGGCTTT TCGCGTTAA ACCCCCTAC
781 CCCCCTAAAC TCGTGATATC ATTAACACTC CTGTAAACCC CCGTAAACA GGAAAATCTC
841 GAGTGGGGTA TTTTATGGCC CAAAACGTAT CTATTTACAT TATGTAAAT ATTACCACC
901 CTAGCGTAGC TTAACATAAG C

```

Número total de bases: 921  
 Composición secuencia ADN: 289 A; 207 C; 143 G; 282 T;