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USE OF LECTINS TO CHARACTERISE GENETIC VARIABILITY AND GEOGRAPHIC DIFFERENTIATION IN NATURAL POPULATIONS OF *Thunnus alalunga* (Bonn. 1788)

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SUMMARY

New tools from cell and molecular biology can be used to implement the knowledge of fish biology and fisheries. In this sense, we suggest that the use of lectins can be an understandable procedure to study population structure of fishes. Lectins are proteins or glycoproteins of non-immune origin that agglutinate cells and/or precipitate complex carbohydrates. Several lectins behave like blood group antibodies and can be used as specific gene markers. We characterise blood groups of Thunnus alalunga worldwide populations under a population genetics point of view using lectins. Fishes from several populations of Mediterranean Sea, Atlantic, Pacific and Indian Oceans were captured and 5 mL blood was obtained from each fish. Each fish was treated with 8 FITC-labelled lectins. The binding activity of the lectins was detected using a fluorescence assay. Several statistical procedures of populations genetics were employed to analyse genetic variability, geographic differentiation, and genetic distance showing that: i) there is genetic variability within Thunnus alalunga populations, ii) we detect highly significant heterogeneity among populations, and considerable geographic differentiation, iii) Mediterranean population is quite distant of North Atlantic population, iv) North and South Atlantic populations are extremely distant, v) Pacific population is the most distant from the others , vi) Gulf of Guinea and South Atlantic populations are distant, vii) Gulf of Guinea and North Atlantic are proximate, viii) Indian and South Atlantic populations are also proximate.

RÉSUMÉ

De nouveaux moyens fournis par la biologie cellulaire et moléculaire peuvent servir à appliquer les connaissances sur la biologie et la pêche du poisson. A cet égard, nous suggérons que l'utilisation des lectines peut constituer un processus raisonnable pour étudier la structure des stocks de poisson. Les lectines sont des protéines ou des glycoprotéines d'origine non-immunitaire qui agglutinent les cellules et/ou précipitent les carbohydrates complexes. Plusieurs lectines se comportent comme des anticorps de groupes sanguins et peuvent servir de marqueurs génétiques spécifiques. Nous identifions les groupes sanguins des populations globales de Thunnus alalunga du point de vue de la génétique des populations au moyen de lectines. Des poissons de plusieurs stocks de la Méditerranée, de l'Atlantique, du Pacifique et de l'océan Indien ont été capturés, et 5 ml de leur sang a été prélevé. Chacun de ces poissons a été traité avec des lectines dénommées 8 FITC. L'activité agglutinante des lectines a été détectée au moyen de tests de fluorescence. Plusieurs processus statistiques de génétique des populations ont été employées pour analyser la variabilité générique, la différentiation géographique et l'écartement génétique, d'où: i) il existe une variabilité génétique su sein des populations de Thunnus alalunga, ii) nous détectons un degré très significatif d'hétérogénéité entre les populations, et une différentiation géographique considérable, iii) la population méditerranéenne se démarque assez de la population nord-atlantique, iv) les populations du nord et du sud de l'Atlantique sont extrêmement distinctes, v) la population du Pacifique est celle qui se différencie le plus des autres, vi) les populations du

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golfe de Guinée et de l'Atlantique sud sont distinctes, vii) les populations du golfe de Guinée et de l'Atlantique nord sont proches, viii) les populations de l'océan Indien et de l'Atlantique sud sont également proches.

RESUMEN

Las nuevas herramientas de biología molecular y celular se pueden utilizar para implementar el conocimiento de la biología de los peces y de la pesca. En este sentido, proponemos la utilización de lectinas como procedimiento comprensible para estudiar la estructura de población de los peces. Las lectinas son proteínas o glucoproteínas de origen no inmune que aglutinan células o precipitan carbohidratos complejos. Muchas lectinas se comportan como anticuerpos de grupos sanguíneos y pueden utilizarse como marcadores genéticos específicos. Hemos identificado grupos sanguíneos de poblaciones de Thunnus alalunga de todo el mundo desde el punto de vista de genética de poblaciones mediante lectinas. Se capturaron peces de varias poblaciones del mar Mediterráneo y de los océanos Atlántico, Pacífico e Índico y se les extrajo 5 mL de sangre a cada uno. Se trató a cada pez con lectinas marcadas con FITC 8. La actividad aglutinante de la lectina se detectó utilizando fluorimetrías. Se emplearon varios procedimientos estadísticos de genética de poblaciones para analizar la variabilidad genética, diferenciación geográfica y distancia genética, que mostraron que: i) existe una variabilidad genética dentro de las poblaciones de Thunnus alalunga; ii) detectamos una heterogeneidad muy significativa entre las poblaciones y una considerable diferenciación geográfica; iii) la población mediterránea se distancia bastante de la población del Atlántico Norte, iv) las poblaciones del Atlántico norte y sur son extremadamente distantes; v) La población del Pacífico es la que presenta una mayor distancia con respecto a las demás; vi) las poblaciones del Atlántico Sur y del Golfo de Guinea son distantes; vii) las poblaciones del Golfo de Guinea y el Atlántico norte son próximas; viii) las poblaciones del Índico y el Atlántico sur también son próximas.

KEY WORDS

Lectins, population structure, geographic differentiation, genetic distance.

INTRODUCTION

1.1.- Worldwide albacore fisheries

Albacore (*Thunnus alalunga*, Bonn. 1788) is a temperate tuna widely distributed throughout the Pacific, Indian and Atlantic Oceans, including the Mediterranean Sea.

In the North Atlantic it is exploited by surface and longline fisheries. Surface fisheries that target juvenile and preadult fish (50 to 90 cm FL) include baitboat, trolling, driftnets and pelagic trawling operating mainly in the Bay of Biscay, Azores islands and adjacent waters. Longline is targeting preadult and adult fish (60 to 120 cm FL) in the northwest and central Atlantic Ocean (Anon. 2001).

There are some minor catches of adult albacore (Anon. 2001) made by the purse seine fleet targeting tropical tunas in the tropical Atlantic area.

In the South Atlantic, longline fleets operating all over the southern temperate region account for the majority of the catches, but there are also some baitboats operating near the southern African coast.

The information about catches in the Mediterranean is scarce. The main fishing gears operating in the Mediterranean are driftnets, longline, handline and trolling (Anon. 2001)

The albacore catches in the Indian Ocean come primarily from longline fisheries operating in all the southern temperate region from Madagascar and almost till Australia.

In the Pacific, longline operates all along the temperate region of both hemispheres. Baitboat catches are distributed, in general, in higher latitudes and more coastal waters than longline, existing some common areas for baitboat and longline in the northwest Pacific.

1.2. Worldwide albacore stock structure

It is assumed that there are two separate stocks in the Atlantic ocean, the northern and the southern stock, separated at 5ºN (ICCAT 1978; [, 2001 #473]), allthough there may be some little interchange of individuals between them (Bard 1978; Beardsley 1969; González-Garcés 1997; Koto 1969; Le Gall 1974; Le Gall et al. 1975; Yang 1970).

Colouration studies (Aloncle and Delaporte 1974), colouration, tagging, size frequency and parasite studies (Aloncle and Delaporte 1979; Hue 1980), electrophoretic studies (Hue 1979; Hue 1980b), and colouration, electrophoretic, size frequency and hard part studies (Hue 1980b) indicate some heterogeneity within the northern Atlantic stock, concluding that there may exist more than one subpopulation in it (González-Garcés 1997).

The northern stock is considered to be independent from the Mediterranean stock (Bard 1981), based on the existence of an independent spawning zone in the Mediterranean (Duclerc et al. 1973; Lalami et al. 1973; Dicenta et al. 1975; Dicenta 1978; Dicenta and Piccinetti 1978), different morphometrics (Bard 1978; Bard 1981; GFCM/ICCAT 1990), different growth rates and age of first maturity (Arena et al. 1980), serological differences (Keyuanfar 1964) and tagging and larvae distribution (Fao Gen. Fisheries Counc. for the Mediterranean 1994). Nevertheless, Di Natale (1991) relates the presence of large albacore (more than 30 kg) in the Mediterranean with their possible income from the Atlantic, and some Atlantic-Mediterranean interchange of individuals has been recorded through tagging experiments (Aloncle and Delaporte 1976; González-Garcés 1997; Ortiz de Zárate and Cort 1998; Arrizabalaga et al. 2001). Viñas J. (1998) found no genetic differentiation among samples from several locations on the northeast Atlantic and western Mediterranean. Only significant differences were found when the Alboran Sea locality had been included in the Atlantic group and the Balearic islands was the only one in the Mediterranean group. It was suggested that this observation should be confirmed increasing the sample size (only 24 fish were analysed in their experiment).

Very little work is done to identify the stock structure of albacore in the Indian Ocean. According to Stequert et al. (1989) there are two separate and distinct larvae concentration zones, one in the Eastern and the other in the Western Indian Ocean, from November to April. Yeh et al. (1996), based on morphometric and genetic studies, also believe that it is possible to have two albacore stocks delimited by the 90ºE meridian in the Indian Ocean, and Penney et al. (1998), based on morphometric studies, also showed dissimilarities between eastern and western regions.

In the Pacific Ocean, it is accepted an hemispheric stock structure for albacore (SPC 2001)

Allthough some scientists maintain a two stock hypothesis in the North Pacific Albacore (Holts and Bartoo, 1985; Laurs and Lynn 1991), it is considered to be a unique stock in the whole Northern Pacific, based on length frequency, spawning ground and tag recapture information (Nakano 1996), and several genetic studies have indicated that there are no differences among the north Pacific albacore (Graves, 1985; Graves and Dizon 1989; Chow et al., 1993).

Based on tagging information, it is assumed that there is one single albacore stock in the South Pacific Ocean (SPC 2000).

Usually, the population structure of commercial fishes currently assumed on several international organizations for management purposes is based mainly on commercial fisheries data. On the contrary, and in addition to other genetic procedures that are also in use, new tools from cell and molecular

biology can be used to implement the knowledge of fish biology and fisheries. In this sense, we suggest that the use of lectins can be a understandable procedure to study population structure of fish.

Lectins are proteins or glycoproteins of non-immune origin that agglutinate cells and/or precipitate complex carbohydrates (reviewed by Liener et al 1986, and Slifkin & Doyle 1990). Due to the fact that many different membrane proteoglycans and glycolipids are present, the cell surface can be precisely characterised using lectins (Slifkin & Doyle 1990). Lectins are isolated from a wide variety of natural sources including seeds, plants, fungi, bacteria, seaweed, sponges, corals, molluscs, fishes, invertebrates and mammalian.

Several lectins behave like blood group antibodies and agglutinate various blood groups of erythrocytes (Nance 1986). These lectins (called phytohemaglutinins) can be used as specific gene markers. For example, the lectin of *Dolichus biflorus* reacts specifically with A1 erythrocytes from animals with A1 allele (Issit 1985, Nance 1986). Lectins have great potential as tools to distinguish between cell types, and consequently, lectins have widely been used as cell markers (Slifkin & Doyle 1990). Recently new procedures that allows the preparation of an unlimited number of novel lectins with diverse specificities has been developed using genetic engineering (Yim et al 2001).

In this work we characterise frequencies of blood groups of *Thunnus alalunga* worldwide populations using lectins. Frequencies of blood groups in natural populations have constituted a useful tool to analyse genetic structure of natural populations, differentiate races, and interpret migration rates. As examples, the seminal Cavalli-Sforza´s papers on genetic structure of human populations are based on blood groups (Cavalli-Sforza 1963, 1981; Edwards & Cavalli-Sforza 1964), and many work on phylogenies and races of domestic animals was also performed with blood groups (Nicholas 1987).

2. MATERIAL AND METHODS

139 fishes from populations of Mediterranean, North Atlantic, Gulf of Guinea, South Atlantic, Indian Ocean and Pacific Ocean were obtained by scientific observers aboard Spanish fishing vessels operating in the different areas (Table 1, Figure 1). Bearing in mind the schooling behaviour of albacore tuna (and tunas in general), sampling was done along several days and geographic locations during each fishing cruise in order to sample individuals from several schools of the same population avoiding the “familiar effect” problem described by Viñas et al. (1998)

From each fish 5 mL blood was obtained from branchia artery using heparynised vaccutainers. Bleed were conserved at 4ºC until its study in laboratory. Only bleed with living erythrocytes was used. From each fish aliquots of 50 µL blood were washed in isotonic PBS, and treated with eight FITC-labelled lectins (Table 2) for 1h at 20ºC in accordance to manufacturer´s recommendations as employed in previous studies (Gonzalez de Chabarri et al. 1994, Costas & López-Rodas 1994). After the incubation period, the erythrocytes were washed three times in PBS, and the washed erythrocytes were observed in a Zeiss axiovert microscope with a FITC filter set for epifluorescence to detect positive (or negative) lectin binding. All the tests were read "blind"; that is, the person reading the test did not know the identity of the sample. At least two different persons observed each sample.

The heterogeneity and geographic differentiation among populations was tested by a *x2* test as well as a G test based on likelihood-ratio (Sokal & Rohlf 1995). Distances among populations were estimated based on *x2* statistics according to Balakrishnan & Sanghvi (1968) procedure. A clusters analysis was performed employing the Euclidian distances of a complete linkage procedure (Hartigan 1975).

3. RESULTS AND DISCUSSION

Lectins seem to be useful tools to rapid and precise characterisation of erythrocytes surface moieties in fishes. The optical examination of the quality of lectin binding demonstrated that all the erythrocytes of a fish show similar lectin binding pattern; they presented bright stain, or they are not

bind at all, but mixed patterns of stained and unstained erythrocytes were not observed. The lectin binding pattern (blood groups) of a great number of fishes can be easily analysed. Consequently, the frequency of lectin binding pattern can be analysed under a population point of view, to detect variability within populations and geographic differentiation among populations.

Figure 2 is a descriptive Radar/Spider plot for the percentage of negative binding patterns of lectins PWM, Con A, SBA, WGA, ECA, VVA in the different *Thunnus alalunga* populations, showing different shapes for each population studied, and which gives an idea of the similarities-disimilarities among them.

There is substantial genetic variability within all the populations of *Thunnus alalunga* analysed (Table 3). Most of the lectins are able to detect variability within populations (PWM, Con A, SBA, WGA, ECA, VVA). Only two lectin (TPA which detect the human blood h, and PEA) were useless in *T. alalunga*. In addition, we detect highly significant heterogeneity and considerable geographic differentiation among *T. alalunga* worldwide populations (Table 3). Some lectins (PWM, Con A, and SBA) are a useful tool to detect significant differences among populations of *T. alalunga*.

A cluster analysis representing the Euclidian distances among the analysed populations using a complete linkage procedure (Figure 3) as well as the genetic distances (GD) based on the *x2* analysis show that: i) Mediterranean population is quite distant of North Atlantic population (GD = 0.021), ii) North and South Atlantic populations are extremely distant (GD = 0.023) , iii) Pacific population is the most distant from the others , vi) Gulf of Guinea and South Atlantic populations are distant (GD = 0.0191), v) Gulf of Guinea and North Atlantic are proximate (GD = 0.002), vi) Indian and South Atlantic populations are also proximate (GD = 0.006).

There is a great agreement between other genetic and tagging studies and our population analysis of blood groups using lectins. For instance, recently reviewed migrations of *T. alalunga* based on conventional tag release-recapture show that none of the 23,777 albacore released in the North Atlantic and the Mediterranean have been recaptured in the South Atlantic (Arrizabalaga 2001). Our analysis of blood groups frequency using lectins also suggest that North Atlantic and South Atlantic constitutes two genetically differentiated populations.

According to the present northern and southern stock separation criteria (the parallel 5ºN), the purse seine catches in the equatorial area are supposed to be from the southern stock (Anon., 2001). Koto (1969), based on Japanese longline catch statistics, first suggested that some intermingling of adult albacore may occur between the Northern and Southern stocks. Ortiz de Zárate and Cort (1998) also agreed that the understanding of the up to date accepted stock structure is complicated because of these purse seine catches, as no information is yet available on the origin of these albacore. The results presented in this paper are the first genetic analyses done with albacore from equatorial origin (caught by tropical purse seiners in the Gulf of Guinea), suggesting that they are much more closer to the northern stock than to the southern one, which is not corresponding to the present stock structure assumed in ICCAT.

The northern stock is considered to be independent from the Mediterranean stock (Bard 1981; Dicenta and Piccinetti 1978;. Recently reviewed conventional tagging data reveals that only two albacores tagged in the North Atlantic have been recaptured in the Mediterranean, and two albacores released in the Mediterranean have been recaptured in the North Atlantic (Arrizabalaga 2001). Our results are also in agreement with these tagging data and the assumed stock structure in ICCAT: the Mediterranean population is genetically different from the Atlantic population.

It is also assumed that there may be some transfer of individuals from the Atlantic to the Indian ocean and vice versa. Based on catch statistics of the Japanese longline fishery, and considering that the areas with high hooking rates appear to be continuous between southern waters of the Atlantic and the Indian Ocean through off South Africa, Koto (1969) suggests that some intermingling of immature fish between both oceans may occur during the southern hemisphere summer. Penney et al. (1998)

suggest that, although there are morphometric differences between Indian and Atlantic albacore stocks, the Aghulas current acting as a superficial frontier between them, there may be some limited and sporadic interchange of adult albacore in deep waters. (Yeh et al. 1998) showed differences between DNA sequences from South Atlantic and the eastern Indian Ocean, but no genetic samples from the western Indian ocean were collected, and it was suggested that the catch statistics of Taiwanese longline seemed to show a continuous catch rate distribution from the Atlantic crossover to the Indian Ocean, this implying that the stock structure of the Indo-Atlantic albacore nearby South Africa is complicated. Our results are in agreement with those discussions in favor of the possible interchange of individuals from one ocean basin to the other, which makes them be proximate from a genetic point of view.

Graves and Dizon (1989) found mitocondrial DNA similarity of south Atlantic and North Pacific albacore tuna, suggesting recent isolation of Atlantic and Pacific albacore or, more likely, at least a small amount of migration between the two ocean basins via the Indian Ocean, even if this intermingling rate is not important for fishery managing purposes. They also recommended to conduct more studies with larger sample size. In this sense, Chow and Ushiama (1995) made a global genetic study based on a much larger sample size from the Atlantic (north and south), Pacific (north and south) and Indo-Atlantic (Cape of Good Hope) regions, in order to clarify the worldwide stock structure of albacore. The results showed that highly significant heterogeneity was evident between the Pacific and Atlantic-Cape of Good Hope samples, which coincides with serological studies made by Suzuki (1962). They proposed that the Atlantic and Pacific Oceans may be separated by a boundary, or simply the gene flow may be restricted by the distance between the ocean basins. Our results are in total agreement with these observations from Chow and Ushiama (1995), as the Pacific population is the most distant one.

The population analysis of blood groups using lectins is more rapid and effortless than other procedures. Consequently, it is a tool with great potential for the characterisation and delimitation of *T. alalunga* populations.

4. CONCLUSIONS

i) Lectins are interesting tools to obtain data on geographic differentiation (and genetic distances) between fish population.

ii) The analysis of geographic differentiation of *Thunnus alalunga* worldwide populations using lectins suggest that:

- North and South Atlantic are extremely distant populations.
- North Atlantic and Mediterranean are quite differentiated populations.
- Pacific population is the most distant from the others.
- Indian and South Atlantic populations are proximate.
- Gulf of Guinea and North Atlantic populations are similar.
- Gulf of Guinea and South Atlantic populations are different.

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REFERENCES

ALONCLE, H. and F. Delaporte 1974. Données nouvelles sur le germon Thunnus alalunga Bonnaterre 1788 dans le nord-est Atlantique (2ª partie). Rev. Trav. Inst. Pêches marit. 38(1): 5-102.

ALONCLE , H. et F. Delaporte. 1976. Marquages de germons par l'ISTPM, 1967-1974. ICCAT. Col. Vol. Sci. Pap. 5: 216-220.

ALONCLE, H. et F. Delaporte. 1979. Nouvelles remarques sur la structure du stock de germons (T. alalunga) dans le nord-est Atlantique. ICCAT. Col. Vol. Sci. Pap. 8(2): 261-264.

ANON., 2001. Informe de la Sesión del SCRS de ICCAT de Evaluación del Stock de Atún blanco (Madrid, España, 9 a 15 de octubre de 2000." (2001). Collect Vol Sci Pap Iccat; Recl Doc Sci Cicta; Colecc Doc Cient Cicaa 52(1): 1283-1390.

ARENA, P., A. Potoschi e A. Cefali. 1980. Risultati prelimiari di studi sull'etá, l'accrescimento a la prima maturitá sessuale dell' alalunga Thunnus alalunga (Bonn., 1788) del Tirreno. Mem. Biol. Mar. Ocean. 10(3): 71-81.

ARRIZABALAGA, H., V. López-Rodas, V. Ortiz de Zárate, E. Costas, A. González-Garcés. 2001 Study on the migrations and stock structure of albacore (Thunnus alalunga) from the Atlantic Ocean and the Mediterranean sea based on conventional tag release recapture experiences. SCRS/01/70 In Press.

BARD, F.X. 1978. Etude des stocks de germon atlantique. ICCAT. Col. Vol. Sci. Pap. 7(2): 258-265.

BARD, F.X., 1981. Le thon germon (Thunnus alalunga Bonnaterre 1788) de l'Ocean Atlantique. De la dynamique des populations à la stratégie démographique. Thèse de Doctorat d'État. Université de Paris: 333p.

BALAKRISHNAN V. and D Shanghvi. 1968. Distance between populations on the basis of the attributed data. Biometrics 25: 859-865.

BEARDSLEY, G. L. 1969. Proposed migrations for albacore, Thunnus alalunga, in the Atlantic Ocean. Transactions of the american fisheries society 98(4): 589-598.

CAVALLI-SFORZA, L.L 1963. Genetic drift for blood groups. In: Genetics of Migrants and Isolated Populations. E. Goldschmidt Ed. Baltimore.

CAVALLI-SFORZA, L.L. 1981. The Genetics of Human Populations. Freeman and Company. San Francisco.

CHOW, S. and H. Ushiama 1995. Global population structure of albacore (Thunnus alalunga) inferred by RFLP analysis of the mitochondrial ATPase gene. Mar. Biol. 123(1): 39-45.

COSTAS E. and López-Rodas V. 1994. Identification of marine dinoflagellates using fluorescent lectins. J. Phycol. 30: 987-990.

DI NATALE, A. 1991. Buefin tuna (Thunnus thynnus L.) And albacore (Thunnus alalunga Bonn.) Fishery in the Southern Tyrrhenian Sea: 1985 - 1989 surveys. ICCAT. Col. Vol. Sci. Pap. 33: 128-134.

DICENTA, A. 1978. L'ichthyoplancton pour l'évaluation des stocks de thons en Méditerranée. Actes de colloques du C.N.E.X.O. 8: 135-140.

DICENTA, A. y C. Piccinetti. 1978. Desove de atún (Thunnus thynnus L.) en el Mediterráneo occidental y evaluación directa del stock de reproductores, basado en la abundancia de sus larvas. ICCAT. Col. Vol. Sci. Pap. 7(2): 389-395.

DICENTA, A., C. Piccinetti y G. Piccinetti Manfrin. 1975. Observaciones sobre la reproducción de los túnidos en las Islas Baleares. Bol. Inst. Espa. Oceanogr. 204: 27-37.

DUCLERC, J., J. Sacchi, C. Piccinetti, G. Piccinetti-Manfrin, A. Dicenta et J.M. Barrois. 1973. Nouvelles données sur la reproduction du thon rouge (Thunnus thynnus L.) et d'outres espèces de thonidés en Méditerranée. Rev. Trav. Inst. Pêches marit. 37 (2) 163-176.

EDWARDS, A.W.F. and L.L. Cavalli-Sforza. 1964. Reconstruction of evolutionary trees. In: Phenetic and Phylogenetic Classification. Heywood and McNeil eds. London

FAO Gen. Fisheries Counc. for the Mediterranean, R. (1994). Expert Consultation on Stocks of Large Pelagic Fishes in the Mediterranean Area. Iraklion (Crete) Greece, 17-23 September 1992. Fao Fish. Rep. Fao Rapp. Peches.

GFCM/ICCAT. 1990. Consulta de expertos sobre evaluación se stocks de grandes peces pelágicos en la zona del Mediterráneo. ICCAT. Col. Vol. Sci.Pap.33: 199p.

GONZÁLEZ-GARCÉS, A. 1997. Contribución al conocimiento de la dinámica de la población del atún blanco (Thunnus alalunga, Bonn. 1788) del Atlántico Norte. Tesis Doctoral. 201 pp.

GONZÁLEZ DE CHÁVARRI E., Aguado J. A., Navarro M., López-Rodas V., Costas E. (1994). The use of lectins to characterize and separate living canine spermatozoa. Theriogenology 41: 356-369.

GRAVES, J. E. and A. E. Dizon. 1989. Mitochondrial DNA sequence similarity of Atlantic and Pacific albacore tuna (Thunnus alalunga). Can. J. Fish. Aquat. Sci. 46(5): 870-873.

HARTIGAN J. A. 1975. Clustering algorithms. Wiley. New York.

HUE, S.B. 1979. Recherches sur l'hétérogénéité du stock de germon T. alalunga du NE Atlantique par électrophorese. ICCAT. Col. Vol. Sci. Pap. 8(2): 265-271.

HUE, S.B. 1980a. New knowledge on the migration of albacore (T. alalunga) in the Northeast Atlantic. ICCAT. Col. Vol. Sci. Pap. 9(2): 344-352.

HUE, S.B. 1980b. Summary of the study on the heterogeneity of the stock of albacore (T. alalunga) in the Northeast Atlantic. ICCAT. Col. Vol. Sci. Pap. 9(2): 353-355.

ICCAT, Ed. 1978. Manual de Operaciones para las Estadísticas y el Muestreo de Tunidos y Especies Afines en el Oceano Atlántico. 2ª Ed. Madrid.

ISSIT, P.D. 1985. Applied blood group serology. 3rd ed. Montgomery Scientific Publications. Miami.

KEYUANFAR, A. 1964. Sérologie et immunologie de deux espèces de thonidés (Germo alalunga et Thunnus thynnus) de l'Atlantique et de la Méditerranée. Rev. Trav. ISTPM. 26(4).

KOTO, T. 1969. Studies on the albacore - XIV. Distribution and movement of the albacore inthe Indian and the Atlantic Oceans based onthe catch statistics of Japanese tuna longline fishery. Bull. Far Seas Fish. Res. Lab 1: 115-129.

LALAMI, Y., S. Tallai, J.M. Barrois, C. Piccinetti et G. Piccinetti-Manfrin. 1973. Observations sur les œufs et larves des thonides des cotes algériennes. Pelagos 4 (2): 54-65.

LAURS, R. M. and R. J. Lynn 1991. North Pacific albacore ecology and oceanography.

LE GALL, J.Y. 1974. Expose synoptique des données biologiques sur le germon Thunnus alalunga (Bonaterre, 1788) de l'Océan Atlantique. Synopsis FAO sur les pêches, 109: 70p.

LE GALL, J. Y., A. Laurec, F. X. Bard and J. C. Dao 1975. Etude de l'etat du stock nord-atlantique de germon (Thunnus alalunga) par l'analyse de cohortes. Collect Vol Sci Pap Iccat; Recl Doc Sci Cicta; Colecc Doc Cient Cicaa 4: 109-127.

LIENER, I.E., Sharon, N. and Goldstein, I.J. Ed. 1986. The Lectins: properties, functions and applications in Biology and Medicine. Academic Press. London.

NAKANO, H. 1996. Review of biology and fisheries on albacore in the North Pacific Ocean. Collect. Vol. Sci. Pap. Iccat Recl. Doc. Sci. Cicta Colecc. Doc. Cient. Cicaa. 43: 269-272.

NANCE, S.T. 1986. Serology of the ABH and Lewis blood group systems. In: Blood group systems: ABH and Lewis. Wallace and Gibbs ed. p. 57-81.

NICHOLAS F. V. 1987 Veterinary Genetics. Clarendon Press. Oxford. 580 pp.

ORTIZ DE ZÁRATE, V. y J.L. Cort. 1998. Albacore (Thunnus alalunga, Bonnaterre) stock structure in the Atlantic Ocean, as inferred from distribution and migration patterns. ICCAT. Col. Vol. Sci. Pap. 50(1): 251-260.

PENNEY, A. J., S. Y. Yeh, C. L. Kuo and R. W. Leslie 1998. Relationships Between Albacore (Thunnus alalunga) stocks in the southern atlantic and indian oceans. ICCAT Tuna Symposium, Pta. Delgada. Azores. Coll. Vol. Sci. Pap. 50(1), 261-271.

SLIFKIN, M. and Doyle, R.J.1990. Lectins and their application to clinical microbiology. Clin. Microb. Rev. 3: 197-218.

SPC. 2001. http://www.spc.org.nc/OceanFish/Html/TEB/index.htm#stock

SPC. 2000. Thirteenth meeting of the Standing Committee on tuna and Billfish, Noumea, New Caledonia, 5-12 July 2000.

STEQUERT, B., F. Marsac and R. Fao Fisheries Dep 1989. Tropical tuna -- surface fisheries in the Indian Ocean. Fao Fish. Tech. Pap.

SOKAL R. R. and Rohl F.J. 1995. Biometry. Freeman. San Francisco.

SUZUKI, A. 1962. Serological studies of the races of tuna. VI. Bigeye-3 antigen occurred in the albacore. Rep Nankai Reg Fish Res Lab 16: 67-70.

VIÑAS, J., J. Santiago y C. Pla. 1999. Genetic characterisation and Atlantic-Mediterranean stock structure of albacore (Thunnus alalunga). ICCAT. Col. Vol. Sci. Pap. 49(4): 188-190.

YANG, R. T. 1970. Studies of age and growth of Atlantic albacore an da critical review on the stock structure. China Fish. Monthly 213: 3-16.

YEH, S. Y., C. F. Hui, T. D. Treng, C. L. Kuo. 1996. Indian Ocean albacore stock structure studies by morphometric and DNA sequence methods. Proceedings of the sixth Expert Consultation on Indian Ocean Tunas Colombo, Sri Lanka, 25 29 September 1995: 258-263.

YEH, S. Y., T. D. Treng, C. T. Hui and A. J. Penney 1998. Mitochondrial DNA sequence analysis on albacore, Thunnus alalunga, meat samples collected from the waters off western south africa and the eastern indian ocean. ICCAT Tuna Symposium, Pta. Delgada. Azores. Coll. Vol. Sci. Pap. 50(1), 272.

YIM, M., Ono, T. And Irimura, T. 2001. Mutated plant lectin library useful to identify different cells. Proc. Natl. Acad. Sci. USA 98: 2222-2225.

Table 1. *Thunnus alalunga* blood sample collecting information.

Area	Latitude	Longitude	Number of individuals	Date
W Mediterranean	36º N	1º-2º W	41	Nov 99
NE Atlantic	45º N	3º-5º W	20	Aug 96
Gulf of Guinea	1º N	15º-16ºW	23	Nov 99 – Jan 00
Central South Atlantic	19º-24º S	10º-24ºW	28	Nov99- Feb 00
SW Indian Ocean	27º-29º S	38º-58º E	10	May 98
Central Indian Ocean	2º -4º S	66º - 72º E	10	Jan 98
W of S America	37S	80º-81ºW	7	Apr-May 00

Table 2. Lectins used in this study.

Lectin	**abbreviaton**	**specificity** sugars	**on:** human blood group
Phytolacca americana	(PWM)	(glcNAc)3	
Concanavaline A	(Con A)	α-man, α-glc	
Glycina maxima	(SBA)	galNAc	
Triticum vulgare	(WGA)	(gluNAc)2, NeuNAc	
Erythrina cristagalli	(ECA)	β-gal(1->4)glcNAc	
Vicia villosa	(VVA)	galNAc	At+Tn
Pisum sativum	(PEA)	α-man	
T*etraglonolobus purpureas*	(TPA)	α-l-fuc	H

Table 3. Lectin binding pattern (blood groups) obtained in different populations of Thunnus alalunga. + = number of positive binding fishes; = number of negative binding fishes. Differentiation among populations was tested by a x2 test and a G test. Abbreviations as in Table 1.

	PWM		Con A		SBA		WGA		ECA		VVA		PEA		TPA	
	+	-	+	-	+	-	+	-	+	-	+	-	+	-	+	-
Mediterranean	0	41	0	41	1	40	23	18	0	41	1	40	0	41	0	41
North Atlantic	0	20	5	15	2	18	9	11	1	19	1	19	0	20	0	20
Gulf of Guinea	0	23	5	18	1	22	9	14	1	22	0	23	0	23	0	23
Souh Atlantic	1	27	1	27	6	22	8	20	0	28	0	28	0	28	0	28
Indian Ocean	0	20	0	20	6	14	8	12	0	20	0	20	0	20	0	20
Pacific Ocean	4	3	0	7	2	5	5	2	0	7	0	7	0	7	0	7
x2	61.76		20.61	14.14	7.06		4.56		3.22							
G	24.88	21.70	14.61	7.73		4.77		3.59								
significance	p<0.001		p<0.001		p<0.05		p>0.05		p>0.05		p>0.05					

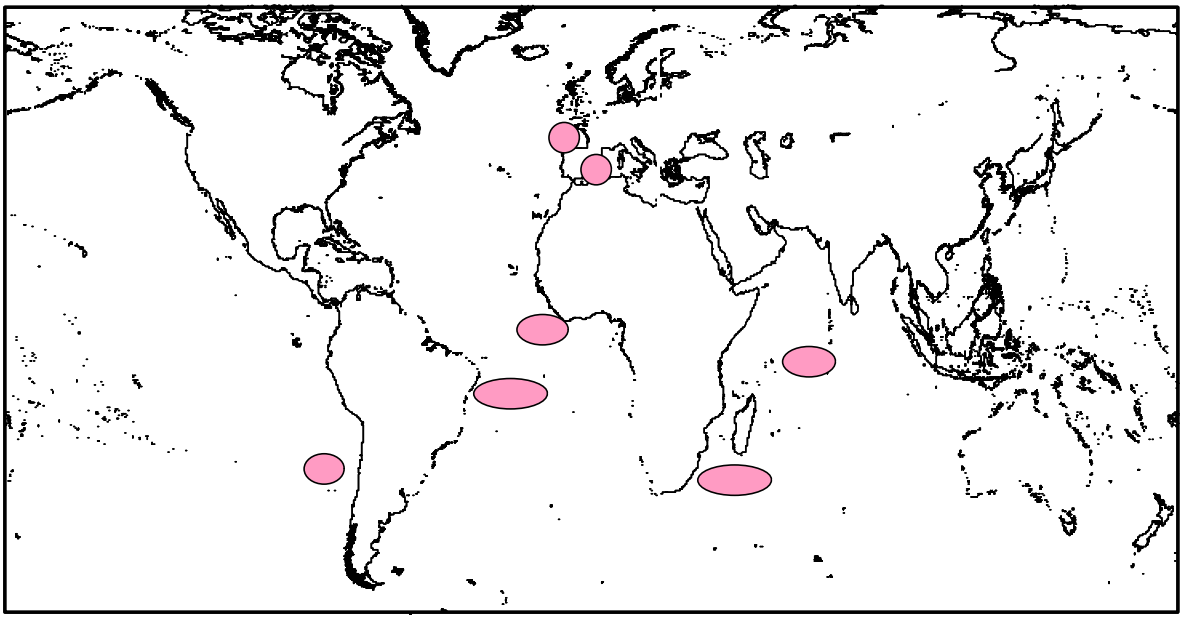

Figure 1. Blood sampling locations of *Thunnus alalunga* .

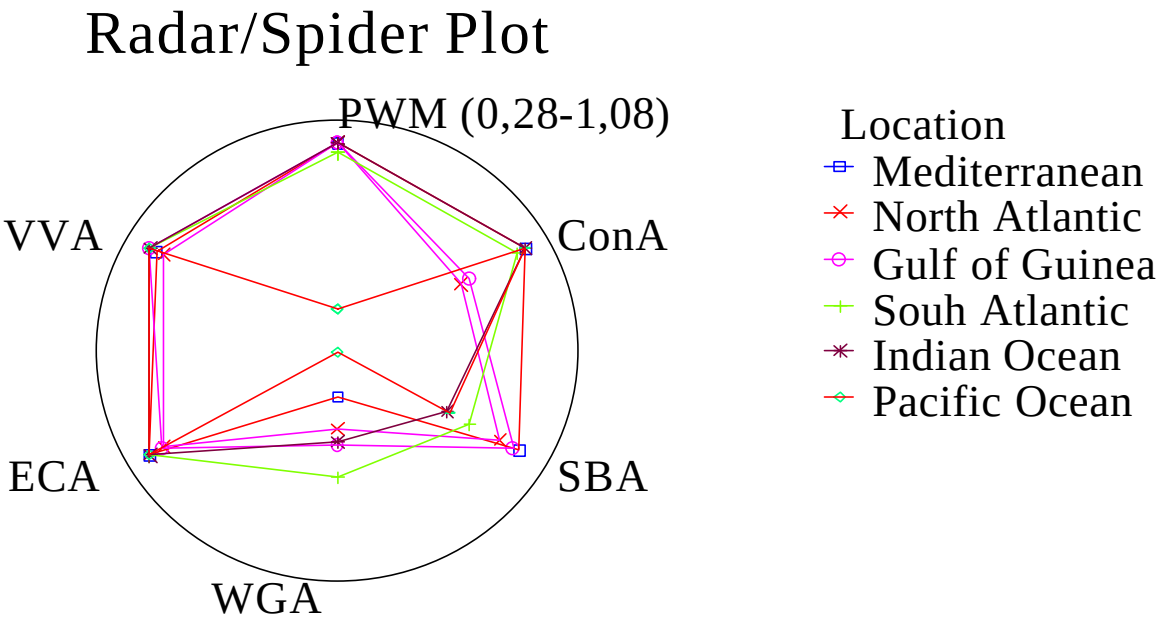

Figure 2. Radar/Spider plot for the percentage of negative binding patterns of six lectins in the different Thunnus alalunga populations studied (the center of the circle represents 0% of negative binding for each lectin).

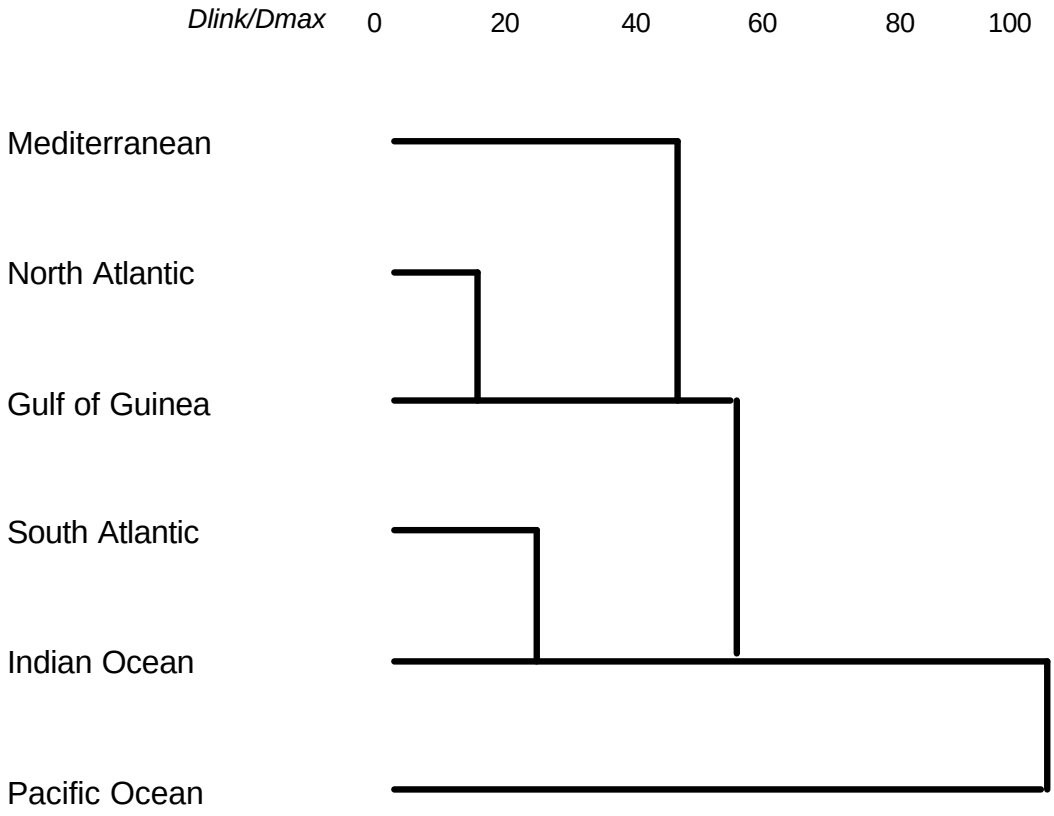

Figure 3. Cluster analysis (Euclidian distances of a complete linkage procedure) summarising distances among populations.