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A STUDY OF MEDITERRANEAN BLUEFIN TUNA (Thunnus thynnus L.) WITH REFERENCE TO STOCK IDENTIFICATION AND MANAGEMENT STRATEGIES

ANDREINA FENECH FARRUGIA

PHD

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FARRUGIA, ANDREINA FENECH

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by

ANDREINA FENECH FARRUGIA

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Abstract

The management of bluefin tuna (*Thunnus thynnus*) in the north Atlantic has been based on the assumption that there are two separate stocks (an eastern Atlantic stock, including the Mediterranean Sea and a western Atlantic stock). This hypothesis has never been scientifically confirmed. This study provides evidence of unique stock characteristics of the bluefin tuna population targeted specifically in the Mediterranean and aims at showing that it is a single stock suitable for its own management regime. This has been done through the study of the biological parameters of bluefin tunas sampled in the Mediterranean, including biometric relationships, age determination, size at first maturity and reproductive studies. The identity of the Mediterranean stock has also been examined through tagging activities, extraction and chemical analysis of the otoliths and through genetic studies. Old and new exploitation techniques within the Mediterranean have been analysed in order to identify trends in landings, existence of illegal, unreported and unregulated catches and to conclude whether or not the bluefin tuna population in the Mediterranean is being overexploited. Results obtained all lead to the conclusion that the Mediterranean stock is an independent stock confined to the Mediterranean with minimal exchange through the Strait of Gibraltar. This study provides a strong argument for the management of the bluefin tuna population in the Mediterranean as a unique stock biologically independent of the Atlantic stock.

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To my husband Adrian

and my daughters

Krista and Kyra

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At no time during the registration for the degree of Doctor of Philosophy has the author been registered for any other University award without prior agreement of the Graduate Committee.

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Relevant scientific seminars and conferences were regularly attended at which work was often presented; external institutions were visited for consultation purposes and several papers prepared for publication.

Publications:

Scientific Committee for Research and Statistics (SCRS)/99/93 Report submitted to ICCAT-SCRS on research activities about large pelagics in the Mediterranean Sea in the framework of Project COPEMED. Farrugia, A., de la Serna, J.M., Srour, A., El Tawil, M. & Hattour, A. 839 – 846

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SCRS/02/095 Description of Maltese bluefin tuna (*Thunnus thynnus* L.) fisheries. Farrugia A.

SCRS/02/096 Revision of historical catches of bluefin tuna made by Maltese longliners. Farrugia A.

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SCRS/03/049 Description of Swordfish Bycatches made with Bluefin Tuna Longlines near Malta during 2002. Fenech Farrugia, A., de la Serna, J.M. & Ortiz de Urbina, J.M.

SCRS/03/138 By-catch de la pesquería de palangre de superficie dirigido al atún rojo (*Thunnus thynnus*) en el mediterráneo centro-oriental. Farrugia, A., El Tawil, M., de la Serna, J. M. & Macías, D.

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Size and age at sexual maturity in female bluefin tuna (*Thunnus thynnus* L. 1758) from the Mediterranean Sea. Corriero, A., Karakulak, S., Santamaria, N. Deflorio, M., Addis, P., Desantis, S., Cirillo, F., Fenech Farrugia, A., Vassallo, R., de la Serna, J. M. Oray, I., Cau, A. & de Metrio, G.

Presentation and Conferences Attended:

- Since 1999 attended annually the Scientific Committee for Research and Statistics of ICCAT as the scientific representative for Malta.

- Regularly attended working groups and annual meetings of the COPEMED Large Pelagics Project since nominated National Coordinator for this project.

- Regularly attended working groups and annual meetings of the EU Project Reprodott (Fisheries) (Domestication of *Thunnus thynnus*, the bluefin tuna – strategies for European development in the context of a global market).

- Regularly attended meetings of the Ad Hoc GFCM/ICCAT working group on sustainable tuna farming/fattening practices in the Mediterranean since nominated rapporteur

- Invited as a speaker in the first ReproDott Conference held between the 4 – 8th February, 2002 in Cartagena, Spain

- Invited as a speaker in the Euro-Mediterranean conference for the marine environment held between the 12 – 14th September, 2003 in Tunis, Tunisia

External Contacts:

Dr. Victor Restrepo, Chief Scientist, ICCAT
Dr. Julie Porter, Scientific Editor, ICCAT
Papa Kebe, Chief Statistician, ICCAT
Rafael Robles, Director COPEMED
Jose Miguel de la Serna, Coordinator of FAO COPEMED Large Pelagics Project
Dr. David Macias, Consultant on Histology, IEO Malaga, Spain
Jose Maria Ortiz de Urbina, Consultant on Stock Studies, IEO Malaga, Spain
Abdellah Hattour, Chief Scientist on Large Pelagics, Tunisia
Mohammed el Tawil, Chief Scientist on Large Pelagics, Libya
Abdellah Srour, Chief Scientist on Large Pelagics, Morocco
Dr. Carles Pla, University of Girona, Spain
Dr. Peter Miyake, Consultant on Large Pelagics, Japan
Profs. Gregorio de Metrio, Consultant on Large Pelagics, University of Bari, Italy
Dr. Jay Rooker, Assistant Professor of Marine Biology, Texas A & M University at Galveston
Juan Antonio Caminas, Director IEO Malaga, Spain
Antonio Garcia, Coordinator of ReproDott Project
Isik Oray, Chief Scientist on Large Pelagics, Turkey
Dr. Jean Marc Fromentin, IFREMER, France
Alessandro Lovatelli, GFCM

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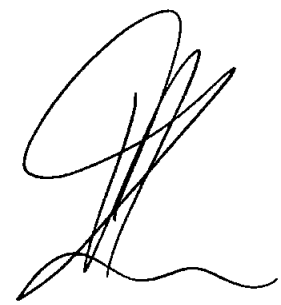


Signed:

Date: 14th November 2005

Chapter 1

INTRODUCTION

1.1 The Mediterranean Sea

The Mediterranean Sea is an intercontinental sea situated between Europe to the north, Africa to the south, and Asia to the east (Fig. 1.1). It covers an area, including the Sea of Marmara but excluding the Black Sea, of about 970, 000 square miles (2, 512, 000 square km). It has an east to west extent of some 3, 860 km and a maximum width of about 1, 600 km. Generally shallow, with an average depth of 1, 500 m, it reaches a maximum depth of 5, 150 m off the southern coast of Greece (http://www.unipv.it.).

The Mediterranean Sea is an almost completely closed basin where the continuous inflow of surface water from the Atlantic Ocean is the sea's major source of replenishment and water renewal. It is estimated that waters take over a century to be completely renewed through the Strait of Gibraltar which is only 300 m (1,000 ft) deep (Richards, 1987). The scarce inflow, coupled with the high evaporation, makes the Mediterranean more saline than the Atlantic Ocean. To the south east part, the Suez Channel, which is an artificial channel, connects the Mediterranean Sea with the Red Sea.

The low concentration of phosphates and nitrates necessary for marine primary producers, limits the food availability and thus quantity of marine life in the Mediterranean, which thus should be considered as an oligotrophic sea. In this context, over-exploitation of this sea's limited marine resources is a serious problem.

There are some exceptions to the general description. For example the Corso-Ligurian Basin and the Gulf of Lyon, are characterised by high levels of primary productivity related with upwelling of nutrients. This results in an increased density of marine organisms in these regions.

Fig 1.1 Map of the Mediterranean Sea *(http://www.unipv.it)*

1.2 General Remarks on Scombrids

The Scombridae is a family composed of 15 genera and 49 species of mostly epipelagic marine fishes, the mackerels, Spanish mackerels, bonitos, and tunas (Collette & Nauen, 1983). Fig. 1.2 represents a general overview of the classification of scombrids (FAO, 1985).

The family Scombridae is essentially confined to marine waters. Spanish mackerels (*Scomberomorus*) enter estuaries to feed and are generally restricted to coastal waters. Most species of Spanish mackerel have fairly restricted ranges, two in the eastern Pacific, four in the western Atlantic, one in the eastern Atlantic, and 11 in parts of the Indo-West Pacific (Gibbs & Collette, 1967). One species (*Scomberomorus sinesis*) moves long distances in freshwater. Bonitos (*Sarda*) and little tunas (*Euthynnus*) are also primarily coastal fishes but the distribution of individual species is more widespread. Tunas generally prefer more oceanic habitats; five of the seven species of *Thunnus* are found worldwide and are known to migrate extensively. These migrations are still being studied

in detail in order to determine their nature, i.e. whether they occur randomly or else if they follow regular annual cycles.

Scombrids are dioecious (separate sexes) and most display little or no sexual dimorphism in structure or colour pattern. Females of many species attain larger sizes than males. Batch spawning of most species takes place in tropical and subtropical waters, frequently inshore. The eggs are pelagic and hatch into planktonic larvae.

Scombrids are active predators. The mackerels (*Scomber* and *Rastrelliger*) filter plankton out of the water with their long gillrakers. The Spanish mackerels, bonitos and tunas feed on larger prey, small fishes, crustaceans and squids. The main predators of smaller scombrids are other predacious fish, particularly larger scombrids and billfishes.

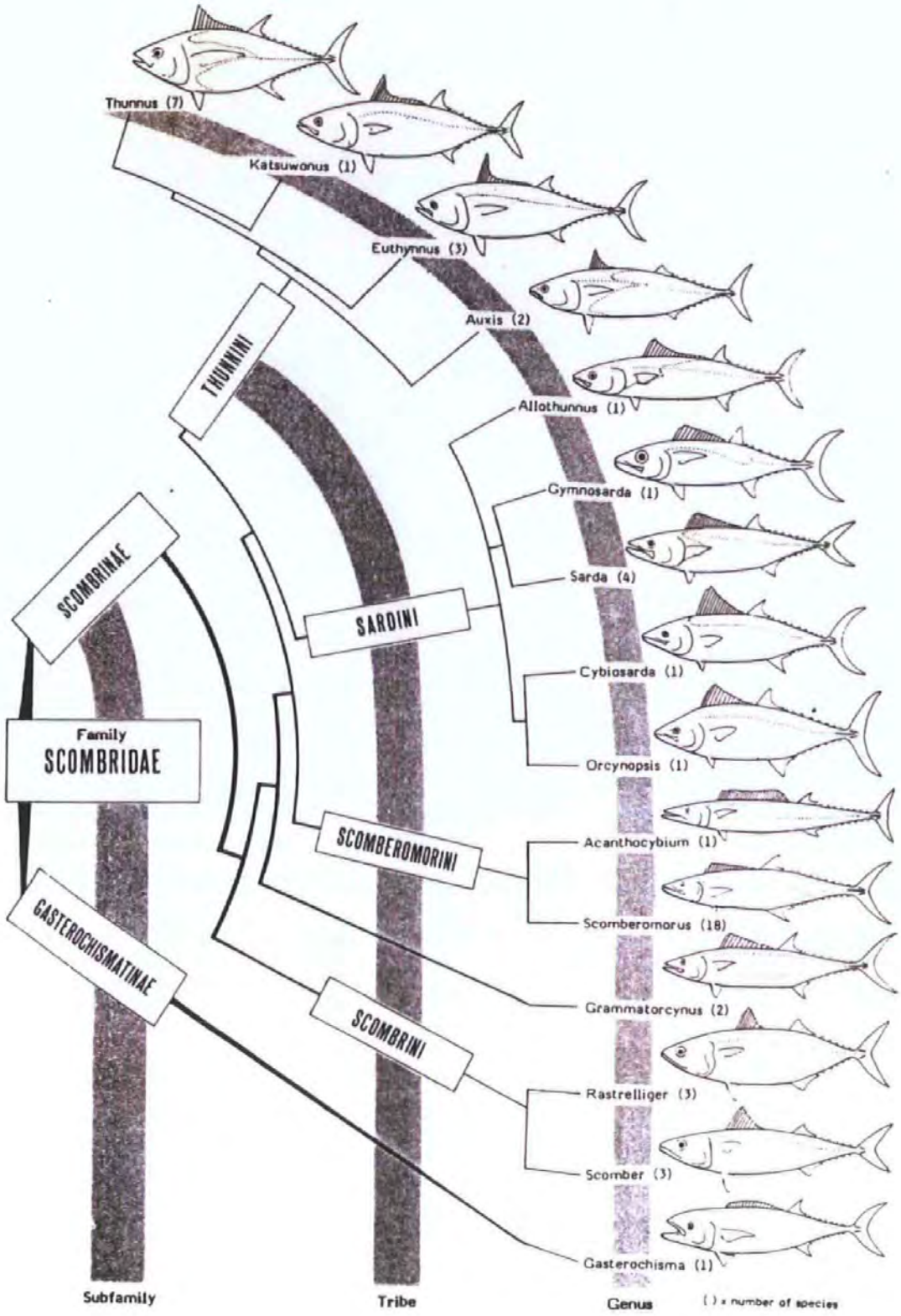


Fig. 1.2 Classification of scombrids (FAO, 1985)

Mackerels and tunas support very important commercial and recreational fisheries as well as substantial artisanal fisheries throughout the tropical and temperate waters of the world. Many species of tunas and mackerels are the target of long-distance fisheries.

The principal fishing methods used for fish schooling near the surface include purse seining, drift netting, hook and line/bait boat fishing and trolling. Recreational fishing methods involve mostly surface trolling and pole and line fishing, while numerous artisanal fisheries deploy a great variety of gear including bag nets, cast nets, lift nets, gill nets, beach seines, hook and line, hand lines, harpoons, specialised traps and fish corrals.

Virtually all scombrids are highly appreciated fish for their high quality flesh. While mackerels and Spanish mackerels are marketed fresh, frozen, canned, smoked and salted, most of the catch of tunas is canned, though they may also be marketed fresh to Asian countries. The high price paid for premium meat of bluefin tuna in the sashimi markets is continuously attracting interest for tuna fishing.

As a result of increasing fuel prices, more sophisticated spotting methods have been introduced in scombrid fisheries for the purpose of reducing expensive search time. Such methods include satellite imagery, airplane spotting and more efficient use of monthly surface temperature charts and other hydrographical information that can now more reliably be applied with our increased understanding of the correlation between environmental parameters and scombrid behaviour. With satellite imagery, sea surface data of many sorts including temperature, chlorophyll, etc. is available several times a day.

1.2.1 *Thunnus thynnus* (Linnaeus, 1758)

Fig. 1.3 shows the appearance of a bluefin tuna which is classified as follows:

Phylum	Chordata
Class	Osteichthyes
Order	Perciformes
Family	Scombridae
Genus	*Thunnus*
Species	*thynnus*

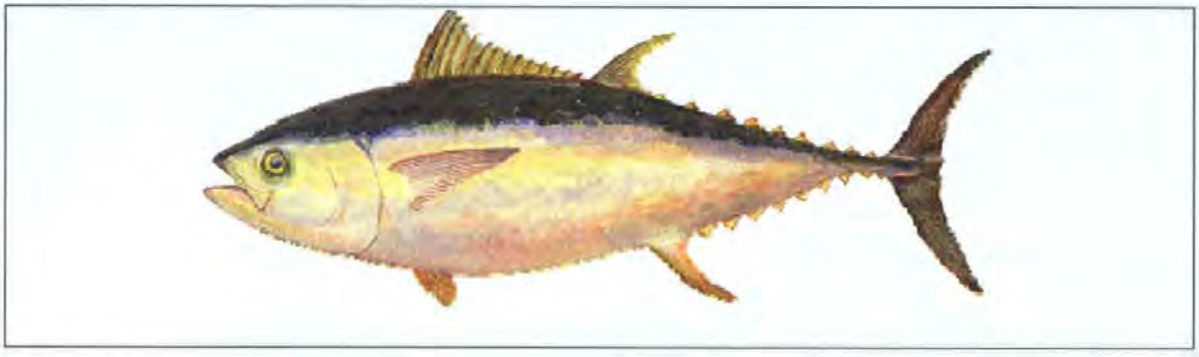


Fig 1.3 Thunnus thynnus (Linnaeus, 1758)(FAO, 1985)

1.2.2 Taxonomy and Morphology

The bluefin tuna is a very large species that commonly reaches lengths greater than 200 cm weighing several hundred kilograms (Colette & Nauen, 1983). The all tackle angling record is a 304 cm bluefin tuna weighing 679 kg caught in 1979 off Nova Scotia (IGFA, 1995).

The colour of the back of the bluefin tuna is a dark metallic blue. The lower sides and the belly are silvery white with colourless transverse lines alternated with colourless dots. The first dorsal fin is yellow or bluish, the second is reddish brown. The anal and dorsal finlets are dusky yellow edged with black whilst the median caudal keel is black in adults (ICCAT, 1978; Cort, 1980).

The bluefin tuna is deepest near the middle of the first dorsal fin base. The second dorsal fin is higher than the first one. The pectoral fins are short (17 to 21% of fork length). They never reach the inter space between the dorsal fins.

Internal distinctive features of the bluefin tuna include 34 to 43 gill rakers on the first arch. The ventral surface of the liver is striated whilst a swimbladder is absent. 39 vertebrae are present i.e. 18 precaudal plus 21 caudal (Colette & Nauen, 1983).

1.2.3 Ecology

The northern bluefin tuna is an epipelagic species, usually oceanic but seasonally coming close to shore and living in temperate waters (Colette & Nauen, 1983). Adult northern bluefin tuna tolerate a wide range of temperatures from 6°C to 30°C (Sharp & Dizon, 1978) and the highest catches are made in waters between 15°C and 22°C (Rivas, 1978).

It has been generally assumed by ICCAT (International Commission for the Conservation of Atlantic Tunas) that separate stocks inhabit the western Atlantic and the eastern Atlantic and Mediterranean Sea. Up to a size of 40 to 80 kg, northern bluefin tunas school by size, sometimes together with albacore, yellowfin, bigeye, skipjack or frigate tuna (Colette & Nauen, 1983).

Giant bluefin tuna (reaching a weight of between 200 and 600 kilograms) exhibit in some cases, a rigidly defined school structure, whose degree of organisation and rules are functions of the number of individuals in the school. According to Partridge *et al.*, (1983), the bluefin tuna schools can form a parabolic shape school when hunting that suggests co-operative predation.

1.2.4 Feeding and Predation

Bluefin tunas feed on a large variety of prey, and this variation in the food spectrum is mainly attributed to behavioural differences (Colette & Nauen, 1983).

On one hand, bluefin tunas can exhibit a chasing behaviour where preys such as small schooling fishes (anchovies, sauries, hakes) or squids are vigorously chased by either isolated fish or a structured school. On the other hand, bluefin tunas can exhibit a modified filter feeding behaviour to feed on crabs and other less agile organisms (Colette & Nauen, 1983).

Bluefin tuna are usually preyed on by killer whales, pilot whales and black fish (Colette & Nauen, 1983).

1.2.5 Growth and Natural Mortality

Atlantic bluefin tuna can grow to over 300 cm and reach 600 kg. ICCAT have reported that bluefin tuna from the western Atlantic grow more slowly but generally reach a larger maximum size (ICCAT, 1996).

The oldest age considered reliable is 30 years, based on an estimated age of 2 years at tagging and about 28 years at liberty, although it is believed that bluefin tuna may live to older ages (ICCAT, 1996).

Until 1990, annual natural mortality rate was assumed to be 0.1 for assessment purposes for western Atlantic bluefin tuna and 0.18 for eastern Atlantic bluefin tuna including the Mediterranean (Clay, 1991).

1.2.6 Reproduction

For the Atlantic bluefin tuna, only two spawning areas have been detected i.e. the Gulf of Mexico in Florida Strait area and the Mediterranean Sea (ICCAT, 1996) (Fig. 1.4). Evidence of spawning in the Gulf of Mexico has been reviewed by Richards (1976 and 1987). Larvae and juveniles are found primarily in the northern region of the Gulf, with sporadic occurrences in the Florida Straits and off the Texas coast (NRC, 1994). For the eastern Atlantic bluefin, spawning occurs in the entire western Mediterranean Sea and in the Adriatic (Richards, 1990).

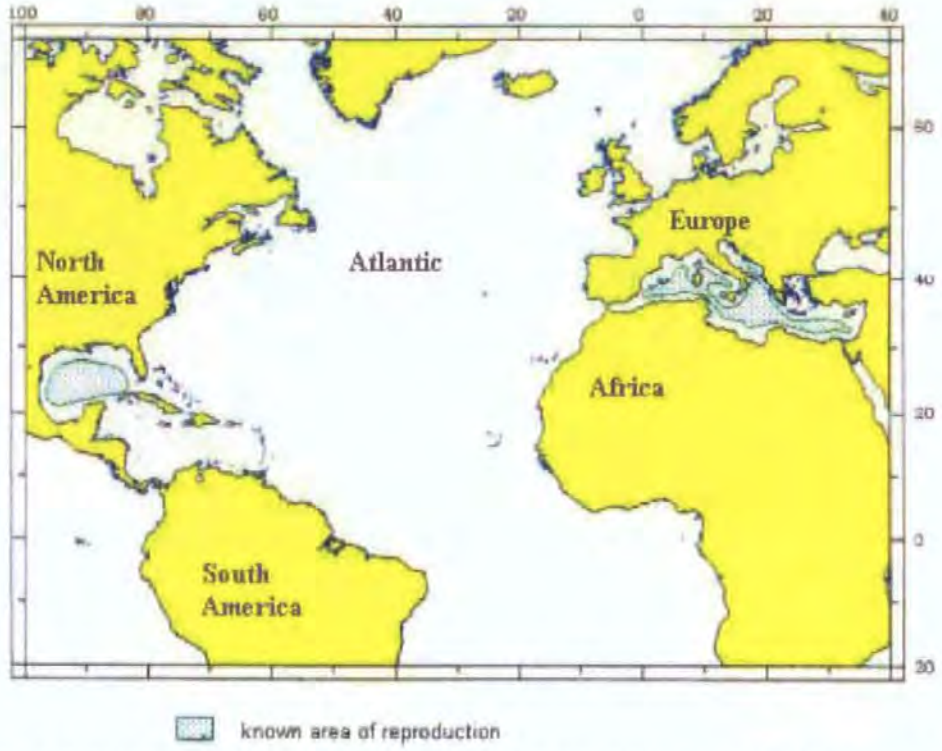


Fig.1.4 Areas of reproduction for Thunnus thynnus in the Atlantic Ocean and the Mediterranean Sea (ICCAT, 1996)

No bluefin tuna larvae have been found in the eastern Atlantic Ocean and therefore it is generally assumed that bluefin tuna do not spawn in the eastern Atlantic Ocean (Cort & Liorzou, 1990a).

Spawning in the Gulf of Mexico occurs from mid April to mid June (ICCAT, 1996). In the Mediterranean Sea, it is thought to occur from June to August (Richards, 1990). Knowing that bluefin tuna can cross the Atlantic in less than 60 days, it would therefore be theoretically possible for a bluefin to spawn in the Gulf of Mexico in April, migrate to the east, and arrive in time to spawn again in the Mediterranean Sea during the same year (NRC, 1994). No evidence has yet been reported of such behaviour.

Spawning occurs in waters of 24.9 °C to 29.5 °C in the Gulf of Mexico and in waters of 21 °C in the Mediterranean Sea (Rivas, 1954).

1.3 Stock

Adult bluefin tuna are encountered in temperate and subtropical waters. The Atlantic species is found in the western Atlantic from Labrador and Newfoundland south into the Gulf of Mexico and the Caribbean Sea. In the eastern Atlantic, it is found from the Lofoten Islands off Norway south to the Canary Islands (Colette & Nauen, 1983). Adult bluefin tuna are also found in the Mediterranean Sea (Fig.1.5). Juveniles are present throughout the shaded area since they spread away from the nursery areas in search for feeding grounds (Colette & Nauen, 1983).

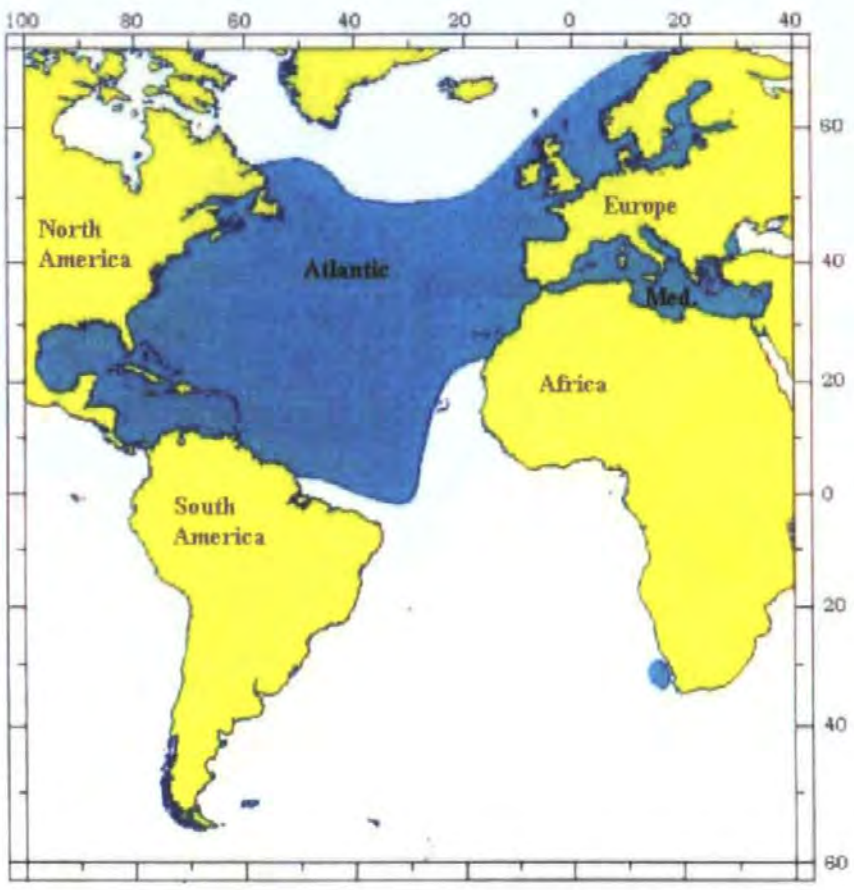


Fig 1.5 Stock (ICCAT, 2000)

In the western Atlantic, juveniles are thought to occur in the summer over the continental shelf, primarily from about 34 °N to 41 °W and offshore of that area in the winter (ICCAT, 1996).

1.4 Exploitation of Bluefin Tuna

1.4.1 Fisheries

Bluefin tuna is exploited over all its distribution range in the temperate waters of the northern Atlantic Ocean and in the Mediterranean Sea (ICCAT 1998, 1999) (Fig. 1.6).

Adult bluefin tuna are targeted by the longline fishery which is practised mainly by Japanese vessels in the western and eastern Atlantic and by some U.S. vessels in the western Atlantic. The fishery extends across the Atlantic between 30° and 50 ° to 60° N, and in the Gulf of Mexico. However the fishing grounds of the longline fishery have changed. Since the early 1980s, the Japanese have used a new winter fishing area located around 60 ° N and 20 ° W. Over the period 1985 - 1994, longline catches represented about 35% of the total catch of bluefin tuna in the north Atlantic Ocean.

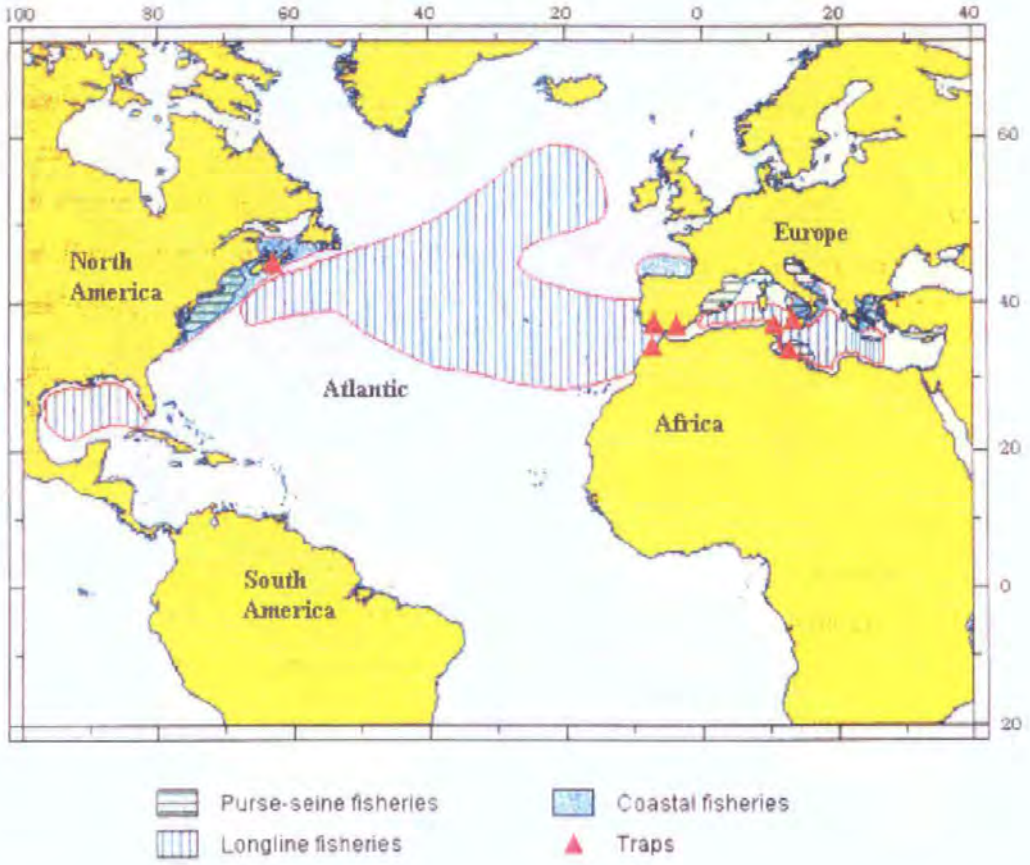


Fig. 1.6 Areas of exploitation by different fishing methods (Adapted from ICCAT, 1996)

In the Cantabrian Sea and the Bay of Biscay, bluefin tuna is targeted seasonally by Spanish, French and Portugese pole and line vessels. A few traps are still catching large bluefin tuna at the entrance of the Mediterranean Sea on both the Spanish and Moroccan sides. Bluefin tuna are also caught by French pelagic trawlers.

In the western Atlantic, a large share of the catch is taken by the American purse-seine and recreational fisheries off the north eastern coast of the U.S.A. during summer and autumn. Recently, a winter-spring fishery developed off Cape Hatteras (North Carolina). Bluefin tuna is also caught by several Canadian coastal fisheries. Due to the bluefin tuna migrations, all these fisheries are active only during winter and spring.

Two migrations have been identified in the Mediterranean. The first is known as the *forward* or *genetic* migration. This allows bluefin tuna to move into the spawning grounds and lasts from April to June. The second migration is known as the *reverse* or *trophic* movement. This migration extends from July till October and during this period the tuna is very active in search of food (COPEMED Annual Report, 2002).

1.4.2 Bluefin Tuna Catches

Because bluefin tuna come very close to shore and because of the quality of its flesh, its exploitation is very old and has been described during the period of the Roman Empire. At this time, bluefin tuna were caught in the Mediterranean Sea and around the Strait of Gibraltar by traps and by nets thrown from vessels (COPEMED Annual Report, 2000).

During the mid-20th century, the catch of spawning bluefin tuna by Atlantic and Mediterranean traps have decreased with the disappearance of the traps due to the inefficiency of this gear compared to other methods. Also, traps target tunas which move very close to land but nowadays, mainly due to climatic changes (COPEMED Annual Report, 2002), bluefin tuna is not approaching as close to the shore as before.

Purse seine, longline and pole and line fisheries, which catch more juveniles than adults, have continued to develop.

In the northeast Atlantic, purse seine fishery is predominantly used to catch bluefin tuna.

In the 1960s, the catch of the traditional fisheries for bluefin tuna in the east Atlantic fell down from about 35, 000 metric tons per year to about 25,000 metric tons and then continued to decrease to less than 12, 000 metric tons (Fig.1.7).

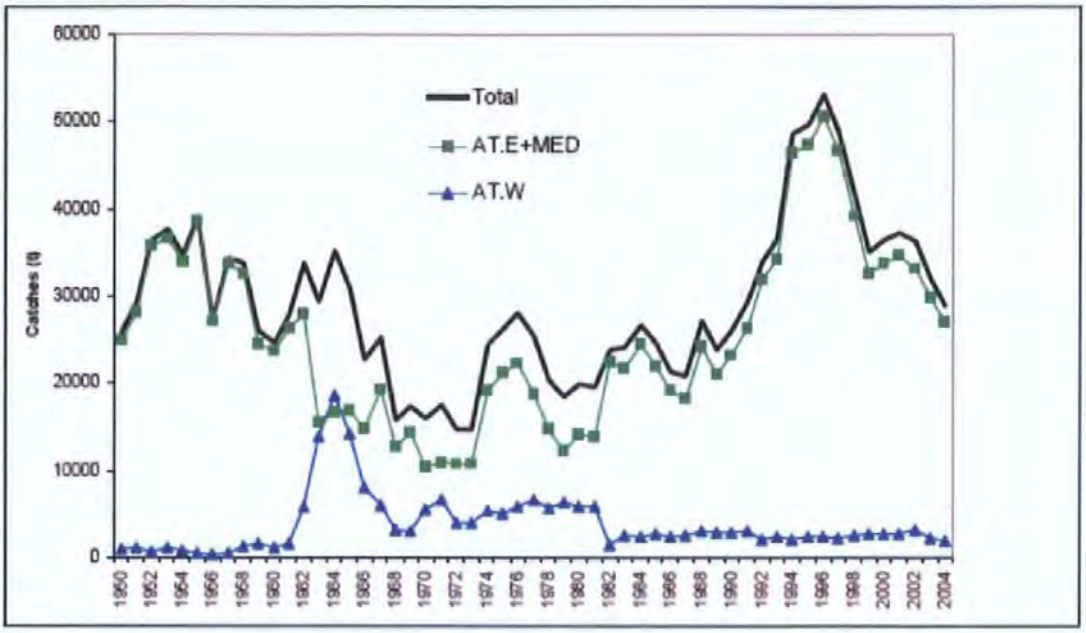


Fig. 1.7 Historical evolution of bluefin tuna catches (ICCAT, 2005)

Since then, the share of the catch realised by the longline fishery has slightly increased (about 2,500 to 3,000 metric tons since 1991). Landings by traps have remained at a low level, the amount of bluefin tuna caught by other gears (as target species or as by catch) has increased whilst the amount of bluefin tuna caught by purse seines has increased significantly.

The pole and line fishery has remained stable since 1960 at about 2,500 metric tons per year.

In the western Atlantic, catch of bluefin tuna rapidly increased with the development of the longline fishery. Bluefin catch by the surface fisheries of the western Atlantic, have remained at a similar level since 1960 (around 2,000 metric tons per year) with however, a maximum in the beginning of the 1970s.

The Mediterranean Sea is characterised by a variety of vessel types and fishing gears with many landing sites located in 17 coastal states. With such a large number of landing sites,

the statistics are difficult to obtain and their reliability sometimes questionable. Historical statistics show that there were important catches through the last millenia with the most ancient gear being the trap. Other fisheries, like the purse seine fishery emerged in the 1960s. Based on estimates of 1995 – 2000 catches, the greater proportion of the catch was from purse seine and longline for the Mediterranean. Nowadays, the purse seine fleet accounts for 60 – 80% of the Mediterranean catch (WWF, 2004).

In 2002, ICCAT carried out its most recent stock assessment. In 2001, landings for the East Atlantic and Mediterranean amounted to 34, 563 metric tons, which is less than 1998 (39, 097 metric tons) and slightly more than or similar to 1999 and 2000 (32, 454 and 37, 752 metric tons respectively).

1.5 New Types of Exploitation

A new expanding practice in the Mediterranean is tuna farming. The concept of farming is very different from that of aquaculture. In the case of aquaculture species, the whole cycle would have been closed. This means that the broodstock are able to spawn in captivity and that the larvae obtained can be grown until they reach the adult size. In the case of bluefin tuna, the cycle has not yet been closed since spawning has been achieved to some degree in captivity but not growth of the larvae to the adult size.

Tuna farming is defined as the rearing of bluefin tuna in cages in order to increase commercial value by increasing its fat content. There have been several trials of bluefin aquaculture, in which case, tuna are kept for few years (between 3 to 5 years) in a cage until they reach a good commercial size.

Tuna farming started in Canada in the late 1960s by a Japanese company (Miyake *et al.*, 2003). The farming was motivated by a highly specialised and categorised Japanese tuna market, where bluefin tuna have the highest commercial value if the meat contains proper fat contents. In the past, the highest value attached to such a bluefin tuna was about U.S. $900 per kilogram at the Tsukiji fish market (more commonly sold at U.S. $200 to 300 per kg). This tuna is sold for the preparation of *sashimi*. On the other hand, daily in the same market, a large quantity of lean bluefin tuna are sold for just a few dollars per kg. Thus the idea was to convert the lean cheap tuna into fat tuna for *sashimi* commanding the higher price.

Originally, large but lean bluefin tuna (generally post-spawning) captured by the trap fishery were kept in a cage (pen) for a few months during which period bait fish are fed to tuna to increase fat contents. Almost all these tuna were shipped to the Japanese market, particularly at the time the price is peaked (i.e. towards the end of the year). In this way, the tuna value increases more than hundred fold during a few months (Miyake *et al.*, 2002).

In the Mediterranean Sea, the tuna farming industry started in late 1970s in Ceuta (near Gibraltar). Until the 1980s, the small to medium (under 120 kg) sized tuna caught in the Mediterranean by purse seiners were sold at the local market at a price range much less than the prime large tunas which were exported to the *sashimi* market, mainly because of the lower fat contents and the less brilliant red colour of the flesh. However, it was discovered in late 1980s that even those medium sized tuna could be sold for a reasonably good price if the fat contents were high.

In the mean time, the southern bluefin tuna of the medium size caught by the Australian purse seine fishery went into the farming in the 1980s. These products established a

completely new market in Japan. Stimulated by the success of southern bluefin farming, the Japanese farms started a similar type of farming operations in the Mediterranean Sea in the mid 1990s. The concept of this new type of farming involves the transfer of live tuna caught by purse seiners into cages. Purse seiners are the only mobile gear able to capture bluefin tuna alive. This feature makes purse seiner fleets an essential factor to the tuna farming industry. This new type of farming has spread very rapidly throughout the Mediterranean and is now carried out in various countries and the quantities are increasing very rapidly as well (Miyake *et al.*, 2003).

Consequently bluefin tuna farming has a lot of socio-economic impact on the Mediterranean tuna fishing industry and the Japanese market. It also has impact on stock management.

1.5.1 Marketing of bluefin tuna

Bluefin tuna is regarded as a high grade product in Japan and is involved in an unusual marketing system, by seafood standards. Each fish is individually inspected for various attributes before being flown to Japan for the fresh tuna market. The consumption of bluefin tuna depends strongly on the specific Japanese culture. It is almost exclusively eaten raw in Japan. The four basic attributes on which fresh bluefin tuna traders rely to measure product quality are the freshness, fat content, colour and shape of the individual fish. Auction market officials in Japan grade these attributes from A - E (A representing the highest and E the lowest possible grade) to assist Japanese wholesale buyers in their purchasing (Carroll, Anderson & Martinez-Garmendia, 2001).

In 2001, Mediterranean farmed tuna for the frozen market were selling at 20 – 40 Euros per kg. Fig. 1.8 gives the average prices of fresh bluefin tuna in the Osaka market during

the months from June, 2002 to January, 2004 in Canadian dollars and Japanese yen. The prices for fresh fish are determined by auction prices in Japan, as well as airfreight charges. These prices are rather high when compared to other tuna species; e.g. big eye and yellow fin tuna whose prices range just between 3 – 6 Euros per kg.

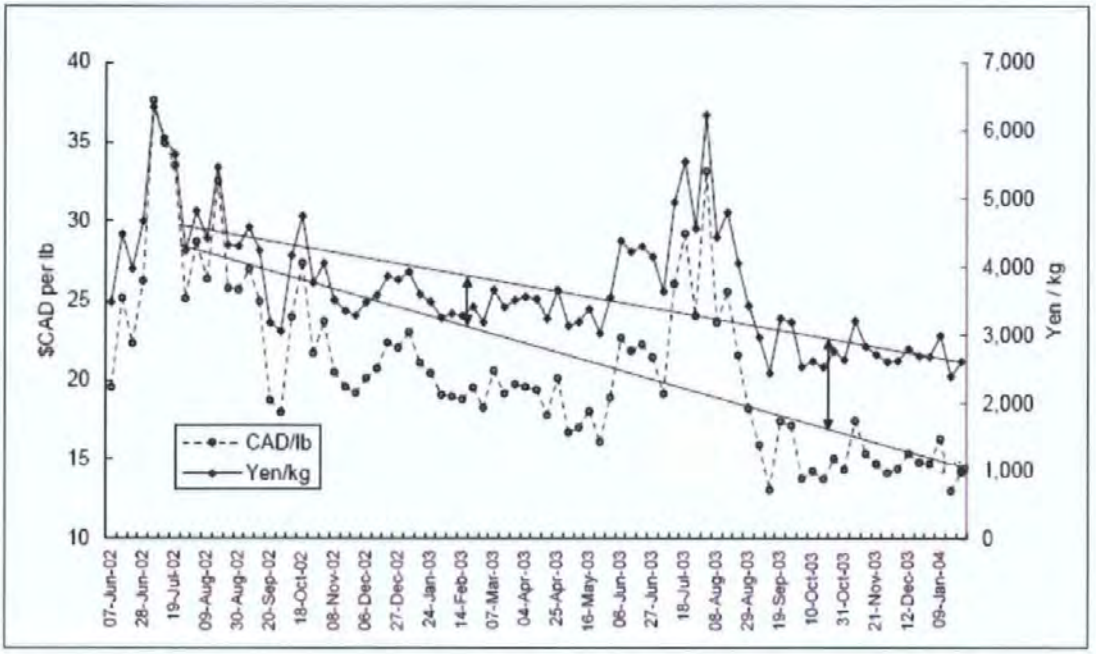


Fig. 1.8 Average prices of fresh bluefin tuna in the Osaka market, 2004 (www.fis.com)

1.5.2 Review of the distribution of bluefin tuna farms

Table 1.1 summarises the main characteristics of the bluefin tuna farms located throughout the Mediterranean Sea as at 1st January, 2005. Fig. 1.9 indicates the position of these farms.

Country	Date of establishment	No. of registered farms	Total capacity in metric tons (2004)
Spain (Tudela, 2002)	1996	13	9 000
Croatia (Katavic *et al.*, 2003)	1997	7	2 500
Italy (Miyake *et al.*, 2003)	1999	6	3 000
Malta *(pers. comm.)*	2000	5	6 350
Turkey (ATRT, 2004)	2001	6	6 000
Tunisia (ATRT, 2004)	2002	4	2 500
Greece (ATRT, 2004)	2003	1	1 000
Cyprus (ICCAT, 2004)	2004	1	500

Table 1.1 Characteristics of the bluefin tuna farms present within the Mediterranean Sea

As these farms are stocked from wild sources, it is important that sound management strategies are in place to protect the supply. If the supply diminishes, there would not be enough for all the farms and thus the farming industry would slowly start to collapse.

Breeding bluefin tuna in captivity is still in its experimental stages (REPRODOTT Symposium, 2002). On the 7th July, 2005, REPRODOTT reported for the first time the successful hormonal induction of capture bred stock to obtain eggs and sperm. In vitro fertilisation was carried out successfully and viable tuna larvae have been produced. This is a very important achievement in controlling the reproduction of the bluefin tuna as it

proves that this species is able to mature in captivity and produce viable eggs and sperm for successful fertilisation. This, in turn, is the first step at controlling the whole life cycle of the fish in captivity and more must follow to bring this technology to fruition.

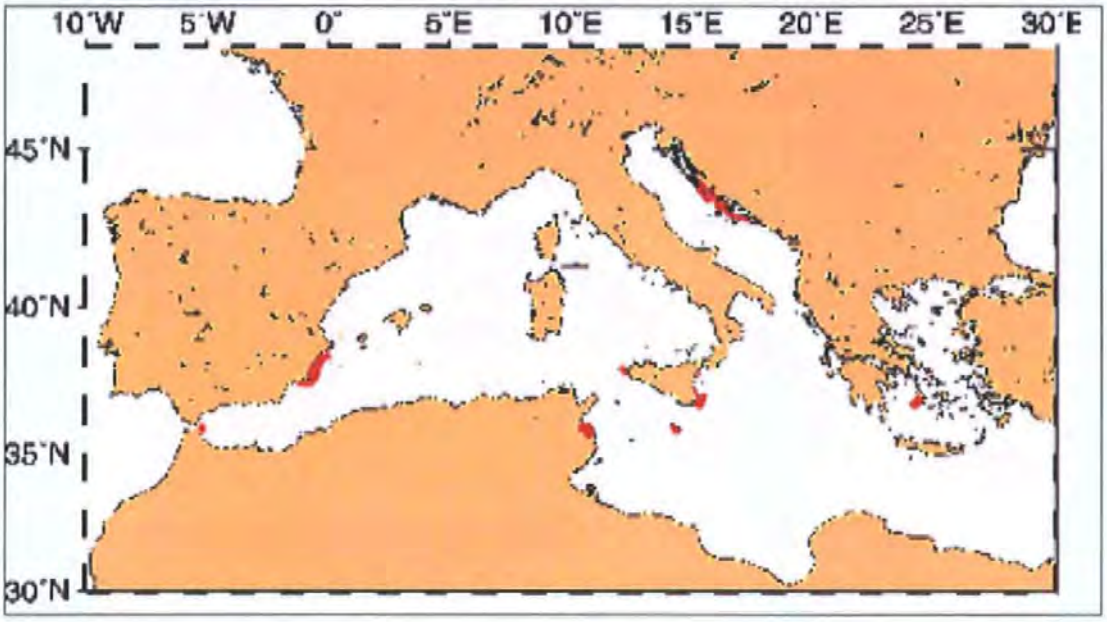


Fig. 1.9 Distribution of bluefin tuna farming sites in the Mediterranean Sea (Farrugia, A. et al., 2002)

1.6 International Commission for the Conservation of Atlantic Tunas

Industrialised tuna fisheries in the Atlantic were initiated towards the end of the 1950s, with a rapid expansion in the area fished, fleet capacity and catches during the 1960s. In the initial stages, the catch rate dropped quickly. Although this is normal for recently initiated fisheries, serious concerns were expressed about the stocks of the species being exploited by the countries concerned.

Under these circumstances, the Food and Agricultural Organisation (FAO) of the United Nations called a meeting of Plenipoteniaries, which resulted in the International Convention for the Conservation of Atlantic Tunas being opened for signature in May

1966, in Rio de Janeiro, Brazil. A Commission, the International Commission for the Conservation of Atlantic Tunas (ICCAT) was established under this Convention.

The Convention came into effect in 1969, after being ratified by seven signatory countries. The first Commission meeting was held in Rome in December, 1969, and the Secretariat was established in Madrid, at the invitation of the Government of Spain, with activities starting in 1970 (http: www.iccat.es).

While the actual objectives of the Commission are defined in the Convention, the goals of the Commission have undergone substantial changes, as have the circumstances and conditions of attaining these goals. Major factors have been advances in research and reporting techniques, improvements in fishing equipment, and the public's increasing awareness of environmental concerns.

The Commission is mainly concerned with collection of statistics, research concepts, techniques and activities and giving management advice and regulations. The enforcement of these regulations is still in the hands of the Contracting Parties and ICCAT itself does not have inspectors to carry out this work. In the case of the European Union, the enforcement is also up to the Member States but then the European Union has its own inspectors which visit the Member States to make sure that these regulations are being enforced (http:www.iccat.es).

1.6.1 Current Regulations of ICCAT

In the 1960s, the catch of the traditional fisheries for bluefin tuna in the east Atlantic fell down from about 20, 000 metric tons per year to about 2,000 metric tons. Combined with the drop in the catch in the western Atlantic, this resulted in the adoption of the first management measures by ICCAT in the 1970s.

In 1975, a minimum size of 6.4 kg with a 15 % tolerance, in number of fish, was recommended for the entire Atlantic (including the Mediterranean). The main aim of this regulation was to stop all landings of juvenile bluefin tuna which were being caught immediately after leaving the nursery grounds.

The Commission recommended in 1994 that bluefin tuna catches in the east Atlantic ocean and Mediterranean Sea should be reduced from 1993 or 1994 levels (whichever is higher) by 25 % starting in 1996 and until 1998. While this regulation could not be evaluated finally until the 1998 catches had been reported, overall the 1996 and 1997 catches were 8.4 % and 2.9 % higher, respectively than 1994 levels. It would therefore appear that this regulation had not been fully enforced by the Contracting Parties.

In 1998, the quotas regulation rules were revised according to the 1998 assessment. Quotas were fixed at 32,000 metric tons and 29,500 metric tons for the east stock in 1999 and 2000 respectively. As the European Community (EC) joined ICCAT in 1998, the quotas were fixed for the EC and no longer for the individual European countries as was done before 1998.

A regulation entered into force on 1 June 1994 which prohibits large pelagic longliners of more than 24 metres in length from fishing in the Mediterranean during the months of June and July. The objective of this regulation is to limit fishing mortality.

There is a prohibition of purse seine fishing in the Mediterranean in August and the use of airplanes or helicopters in June (entered into force on 4 August 1997). The dates chosen for this measure adopted in 1996 were not based on solid scientific information and thus alternate closure dates were proposed. A change in the dates of the closure was decided in

1998: May 1st to 31st for the Adriatic Sea and July 16th August 15th for the rest of the Mediterranean.

An ADAPT Virtual Population Analysis was developed with appropriate specifications (ICCAT, 1998). However results of this assessment differ somewhat due to an abrupt increase of the catches of the spawning fish since 1994 and also due to the revision of the catch statistics by various countries. Consequently it was decided to carry out ICCAT assessments using a constant natural mortality for all age groups and all years equal for both stocks to 0.14.

1.6.2 Delineation of Stock

ICCAT has for many years assessed bluefin tuna on the hypotheses that there are two separate stocks – the Eastern Atlantic stock, including the Mediterranean and the Western Atlantic stock (Fig. 1.10). The current boundary line that came about in 1980 was based on apparent discontinuities in the distribution of catches at that time, on the limited biological knowledge, as well as on taking more or less the midpoints, geographically, from the continents to the east and west. As indicated earlier the two presumed stocks are thought to have independent spawning grounds. Each stock is subject to different management restrictions with the most prominent being a low quota for the western fishery of 3, 000 metric tons and a higher quota for the eastern fishery dominated by European countries of 32, 000 metric tons (ICCAT, 1996).

However these hypotheses have remained untested and exchange rates, if any, between the Atlantic and the Mediterranean remain unknown. A recent review of the scientific bases for the management of Atlantic bluefin tuna recommends that the two stock hypothesis be rigorously tested (Miyake, 1998).

Now there is also an increasing literature survey evidence of the tagging experiments (Block *et al.*, 1998, 2000; Lutcavage *et al.*, 1999, 2000; De Metrio *et al.*, 2000) that there is a residing population in the Mediterranean. The extent of bluefin tuna movement within and between the eastern and western Atlantic regions including migrations through the Straits of Gibraltar and the effects these movements might have on the choice of management strategies need to be examined. These conflicting theories are causing debates between the American and Mediterranean countries concerned during the annual ICCAT management meetings (ICCAT, 2004).

Block *et al.*, (2005) analyzed the data over a nine-year period and discovered that bluefin tuna have a complex migratory life-cycle that varies depending on the season, as well as the age and body size of the fish. The study confirmed that the North Atlantic is home to at least two populations of bluefin – a western stock that spawns primarily in the Gulf of Mexico and an eastern stock that breeds in the Mediterranean Sea. It appears that some bluefin tunas from the east may feed in the Atlantic until they are old enough to become breeders, at which point they go back to the Mediterranean spawning grounds and are unlikely to move out again. Full details of these migrations and behaviour are not yet available to date.

Knowledge of the actual exchanges could affect the conclusion of future stock assessments (ICCAT, 2000) since the determination of both the extent of mixing of mature bluefin tuna and the fidelity to an Atlantic or Mediterranean spawning ground is critical for future bluefin tuna management. Barbara Block (2005) suggested that ICCAT should take into consideration the new ongoing research studies using pop up tags and consider changing fishing policy to reflect it.

Fig 1.10 Separation of bluefin tuna stocks (ICCAT, 1996)

1.7 Aims and Objectives

The report of the workshop on bluefin tuna mixing (ICCAT, 2001) points out that the stock structure of bluefin tuna has been the subject of intensive discussion by scientists since the ICCAT began its scientific work in 1971. While the management approach initiated in 1982 may have been appropriate at the time, much has changed since then; mainly the development of more efficient gears and the development of tuna farming.

Bluefin tuna has until now been managed as two separate stocks for almost 20 years but there is increasing evidence of a residing bluefin tuna population in the Mediterranean (Block *et al.*, 2005). These conflicting theories are causing debates between all parties

concerned and are not resulting in the effective management of this vulnerable bluefin tuna population.

Noting the lack of recovery of the eastern and western stock even after 20 years of management as well as the information coming from new sources, e.g. tagging; it seems that the current management procedures are not sufficient to rebuild the Atlantic and the Mediterranean stocks. The 45 ^{O}W degree management boundary is largely arbitrary and it does not express the fundamental strategy in the management of Atlantic bluefin tuna if sustainability is to be established.

ICCAT (1998) emphasised the need for increased research to redefine these boundaries while at the same time implementing immediate measures to protect the bluefin tuna population. These revised management measures will also need to take into account the new exploitation techniques which have taken over the historical exploitation techniques within the Mediterranean.

As a result the FAO-COPEMED Large Pelagics Project was set up (ICCAT, 1999). Research on biology and fisheries is better accomplished if countries with fisheries targeting bluefin tuna are involved in joint projects with a view towards providing information for a better understanding of the population biology and stock status. This Project was the principle source of funding for this thesis. It allowed the incorporation of non EC members to research activities, supported by FAO funds through COPEMED Project. The author was the co-ordinator for Malta and the rapporteur during the meetings attended. Other countries involved in the project were Spain, Morocco, Tunisia and Libya.

The aim of this thesis is to examine the hypothesis that the Mediterranean stock of bluefin tuna is largely independent of the bluefin tuna population in the Atlantic.

To meet this aim, the following objectives were set up:

- To review all literature associated with establishing the identity of stocks and the techniques available to support this (before each experiment)
- To determine biometric relationships on the Mediterranean bluefin tuna stock and establish differences for comparison with Atlantic stock/s (Chapter 2)
- To set up an age-length key for larger tunas that will provide essential information in all aspects of studies carried out on bluefin tuna and comparison with Atlantic tunas (Chapter 3)
- To perform a reproductive study to determine the level of sexual maturity of bluefin tuna within the Mediterranean stock (Chapter 4)
- To determine the age at first sexual maturity of the Mediterranean bluefin tuna stock and compare it with that of the Atlantic stock (Chapter 4)
- To carry out stock identification through:
    - tagging activities (Chapter 5)
    - chemical analysis of the otoliths (Chapter 6) and
    - genetic analysis (Chapter 6)
- To analyse new exploitation strategies in order to determine how they are affecting the bluefin tuna stocks in the Mediterranean Sea and come up with better management strategies which provide solutions that are targeted more specifically to the current situations (Chapter 7).

As international demands remain high for large bluefin tuna, intensive fishing raises concerns for the long-term sustainability of this resource. Given the large potential profits and the many competing users in the bluefin tuna fishery, conservation efforts have

sparked international disputes. It is under these conditions that this study aims to provide a basic framework for the sustainable management of the bluefin tuna population in the Mediterranean Sea integrating biology with the demands imposed by global economics and politics.

1.8 Summary

1. Bluefin tuna is exploited over all its distribution range by different countries and different gears.

2. The Mediterranean Sea is the main area where bluefin tuna is targeted.

3. Bluefin tuna is regarded as a prized table fish and as a high grade product in Japan.

4. Bluefin tuna is exploited over all its distribution range by different countries and different gears.

5. There is a strong evidence of over fishing.

6. A new expanding practice in the Mediterranean is tuna farming. This has brought a lot of socio-economic impacts on the Mediterranean tuna fishing.

7. ICCAT is concerned with the collection of statistics, research concepts, techniques and activities and giving management advice and regulations which are then enforced by the Contracting Parties.

8. Current management regime based on poor scientific evidence particularly where stock delineation is concerned.

9. Increasing evidence of a Mediterranean stock that is distinct from Atlantic stocks.

10. If above statement is true, it will require management measures specific to the Mediterranean.

11. The purpose of this thesis is to present evidence from the series of experiments listed above to show that within the bounds of probability a residing Mediterranean stock exists.

Chapter 2

BIOMETRIC RELATIONSHIPS

2.1 Introduction

Biometric relationships i.e. length distributions and length – weight relationships are crucial in the evaluation of stocks, in the study of stock structure and in comparing wild stocks present in different regions.

Following the recommendations on research by the Scientific Committee for Research and Statistics (SCRS) of ICCAT, this experiment was established to study the biometric parameters of this targeted species in order to contribute to the assessment processes periodically accomplished by the SCRS of ICCAT. This experiment was sponsored by FAO-COPEMED 'Large Pelagics Project' and all meetings were held in the Oceanographic Institute, Malaga, Spain. Results have been presented and published in the ICCAT's annual SCRS meeting of 2002 and 2003.

Before starting the experiment, a meeting was held between the author and the observers who were involved in the collection of data in the other ports of the Mediterranean, in order to standardise the methodology of sampling. The author was responsible for the organisation of the collection of data in the Maltese Islands while four other observers were involved in the collection of data in Libya, Tunisia, Morocco and Spain.

In order to ensure the validity of results, the same type of plastic calipers and scales (same manufacturer) were used in the different countries. All the data was then pooled to the author who analysed the data as detailed below.

2.2 Literature review

Several studies regarding bluefin tuna in the Atlantic and in the Mediterranean have focussed on biometric relationships: Rodriguez-Roda (1983), Rey & Alot (1987), Rey *et al.* (1987), Cort (1990) and De la Serna *et al.* (1992). The general conclusions for the aforementioned studies were that different length - weight relationships were present for the Atlantic Ocean and the Mediterranean Sea.

In fact, in the Field Manual of ICCAT (1990), different length-weight relationships are found for bluefin tuna caught in the Atlantic and bluefin tuna targeted in the Mediterranean:

East Atlantic Round weight = $2.95 \times 10^{-5} \times$ Fork Length $^{2.89}$(Rey & Cort, Unpubl.)

West Atlantic Round weight = $2.86 \times 10^{-5} \times$ Fork Length$^{2.82}$(Parrack & Phares, 1979)

Mediterranean Round weight = $1.96 \times 10^{-5} \times$ Fork Length $^{3.01}$(Beardsley, 1971)

These results are so old that a new study is required to test their validity. Further confirmation through this experiment of the different length - weight relationships for the west Atlantic, east Atlantic and the Mediterranean would help in the differentiation of stocks and would lead more to the conclusion of a permanent residing stock in the Mediterranean. The results will also contribute to the assessment processes periodically accomplished by the SCRS of ICCAT which are an essential tool in ensuring the sustainability of the species.

2.3 Materials and Methods

The individual fork length and gilled and gutted weights of bluefin tunas were recorded at the main landing ports of the countries involved in this research (Fig. 2.1) according to the agreed methodology.

Fig. 2.1 Main landing ports of the countries from where data was collected

Plastic calipers were used to measure the fork length (FL) of the tuna with each fish being placed on a flat surface in a horizontal position while being measured. The fork length is the projected straight distance from the tip of the upper jaw to the posterior tip of the shortest caudal ray (Fig. 2.2). Sometimes the fish were too large for the calipers in use or the fork length was difficult to achieve. In this case, the next best measurement to be recorded was the pre dorsal length (LD_1) which is the straight distance from the tip of the upper jaw to the insertion of the first dorsal spine.

Pre dorsal length can then be converted to fork length. The relationship between the pre dorsal length and fork length can be established for each species (e.g. bluefin tuna, albacore and bigeye tuna) and area based (e.g. west Atlantic, East Atlantic and

Mediterranean) on adequate samples, as they are quite variable. The larger the number of samples, the lower the margin of error will be.

Fig. 2.2 Length measurements of bluefin tuna

The weight recorded was the gilled and gutted weight, i.e. the individual weight of the tuna without the internal organs and the gills. A transformation (Miyake, 1994) was used to convert the gilled and gutted weight into round weight. This transformation is very reliable since it was produced from a large number of samples and has been accepted by the SCRS of ICCAT.

Round weight = Gilled and gutted weight x 1.16

The individual length and weight data collected in each country were then used to calculate the length - weight relationship for bluefin tunas caught by different gears in different fishing regions.

The relationship between fork length and round weight was analysed by the power regression method:

$$\text{Round Weight} = a\ \text{Fork Length}^{b}.$$

where a = growth index

b = exponential index

Therefore, the response variable analysed was the natural logarithm of length.

2.4 Results

In this particular experiment, a considerable effort was spent in terms of sampling coverage in order to record as many individual samples as possible and thus make the results more reliable. Table 2.1 summarises the number of samples collected from the different countries during the years 1999 – 2003.

Country	Gear	No. of Samples Collected
Malta	Longline	5 926
Libya	Longline	486
Libya	Trap	469
Tunisia	Trap	146
Tunisia	Purse seine	5 530
Spain	Purse seine	21 052
Spain	Trap	3 528
Spain	Handline	697
Morocco	Handline	211
Morocco	Trap	28

Table 2.1 Number of samples collected from each country per gear

The individual fork lengths of all the bluefin tuna observed were grouped in 5 cm intervals. This was carried out using all the data collected from around the Mediterranean.

Table 1 of the Appendix shows the number of bluefin tuna caught in each appropriate length range during the months of April, May, June and July for the whole duration of the study in the Maltese Islands. In the case of the Maltese Islands, all the bluefin tuna observed was caught by surface longlines since during the study period this was the only gear utilized by the Maltese fishermen.

During 2001, only 95 bluefin tunas were sampled. During this particular year, marketing of bluefin tuna in Malta followed a different procedure. This involved the Maltese fishermen selling their product directly from their fishing vessels onto a Japanese processing vessel. Thus the bluefin tuna were never landed in Malta and the only samples that could be collected were those where an observer on board was present on board the fishing vessel. Figures 2.3, 2.4, 2.5, 2.6, 2.7 and 2.8 are graphical representations of the raw data collected in Table 1 of the Appendix.

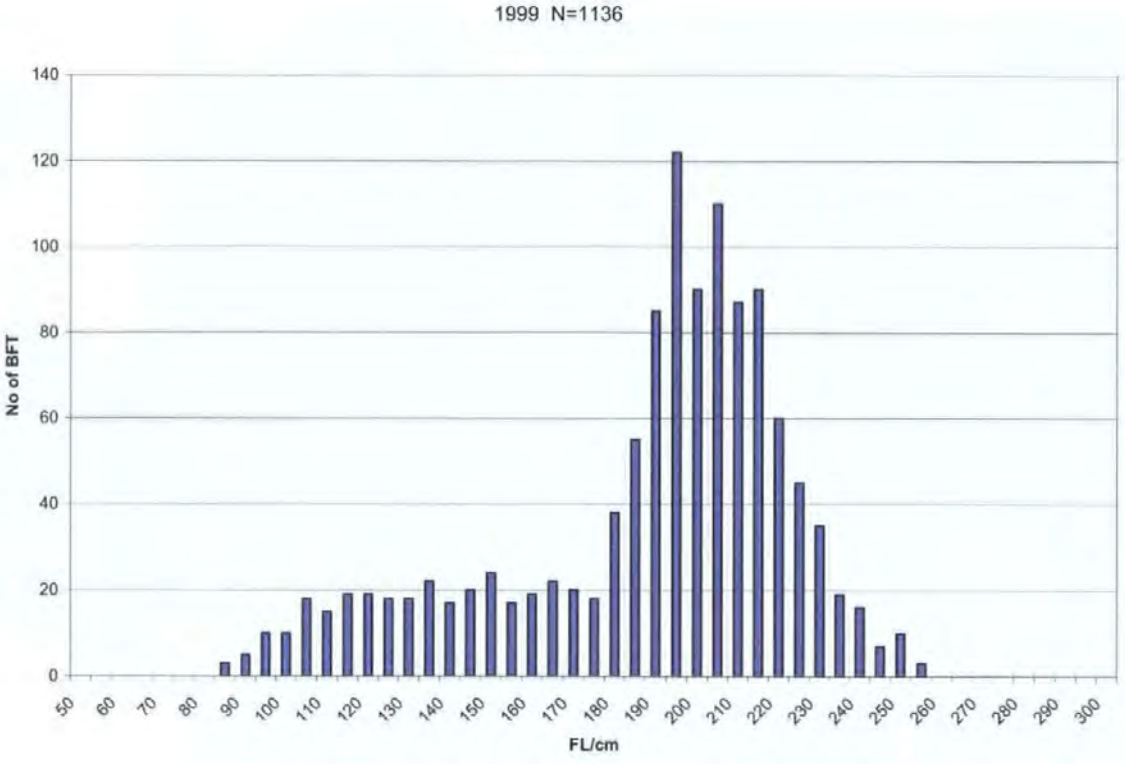


Fig. 2.3 No. of bluefin tuna caught at each length range - 1999

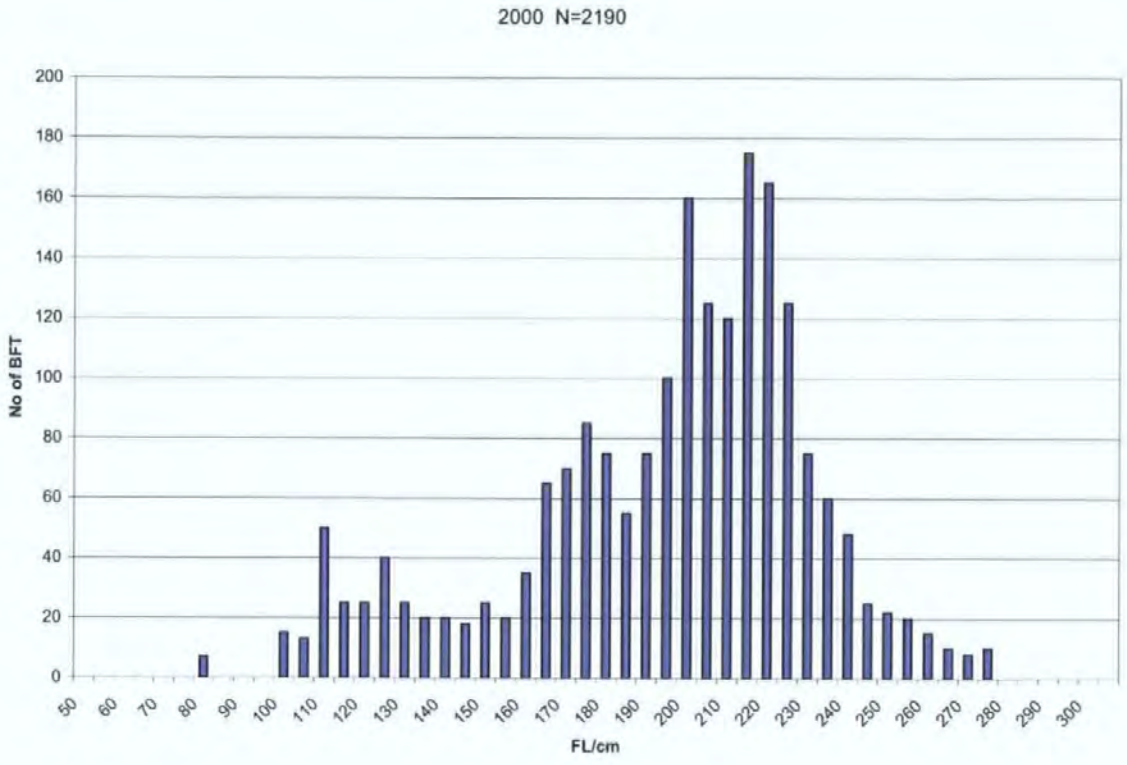


Fig. 2.4 No. of bluefin tuna caught at each length range - 2000

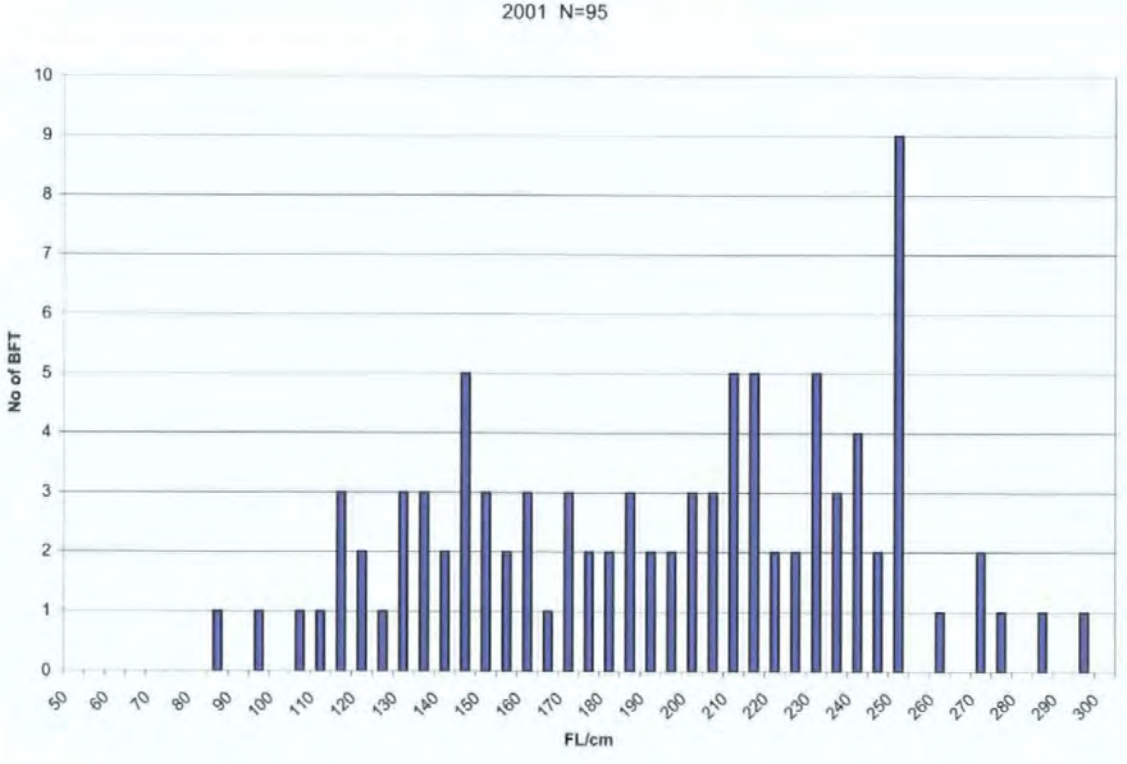


Fig. 2.5 No. of bluefin tuna caught at each length range - 2001

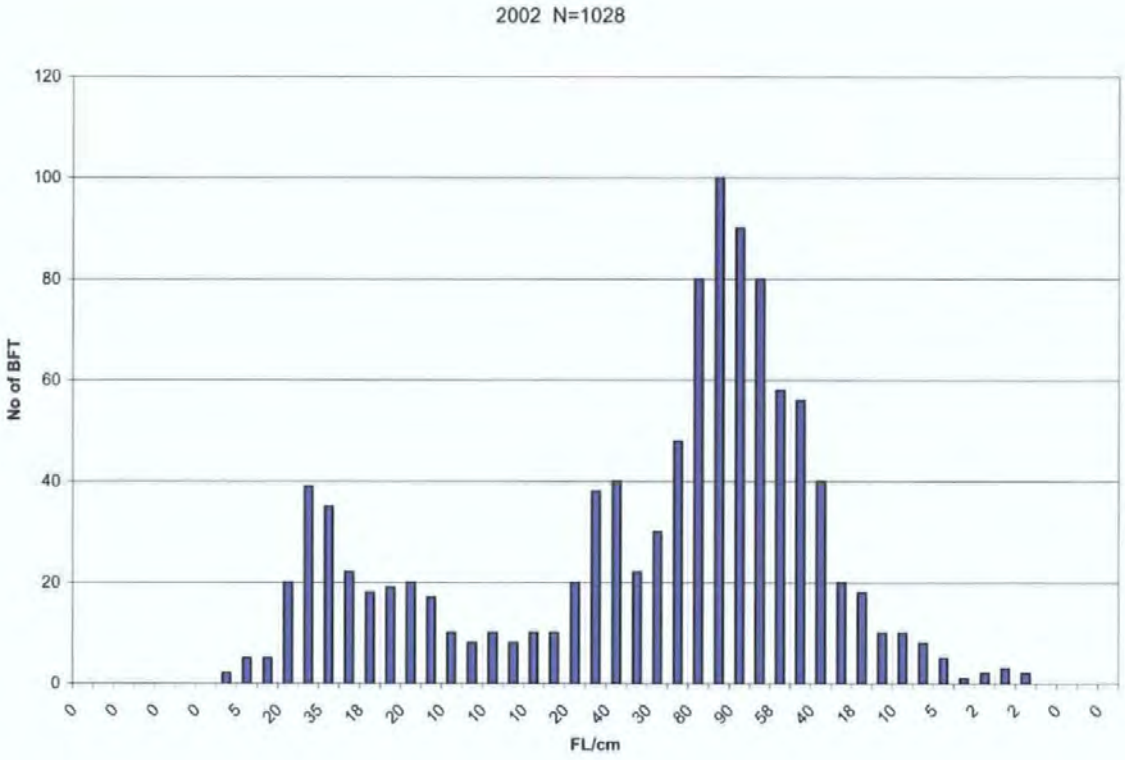


Fig. 2.6 No. of bluefin tuna caught at each length range - 2002

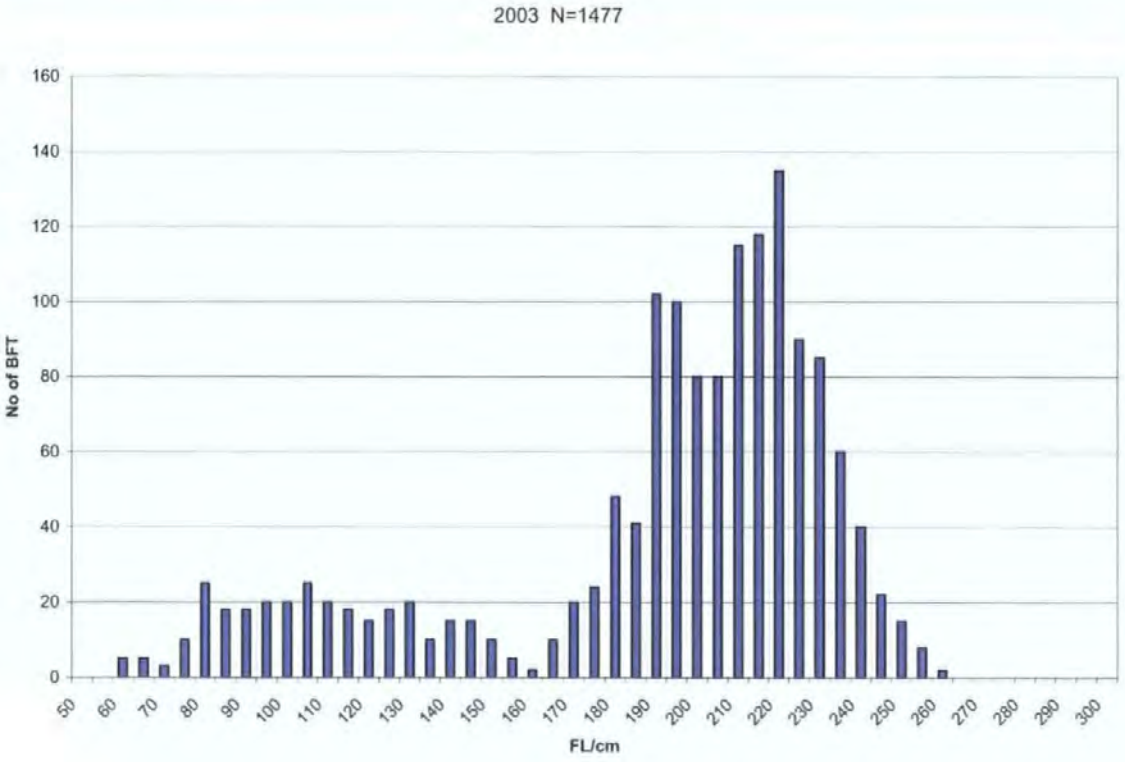


Fig. 2.7 No. of bluefin tuna caught at each length range - 2003

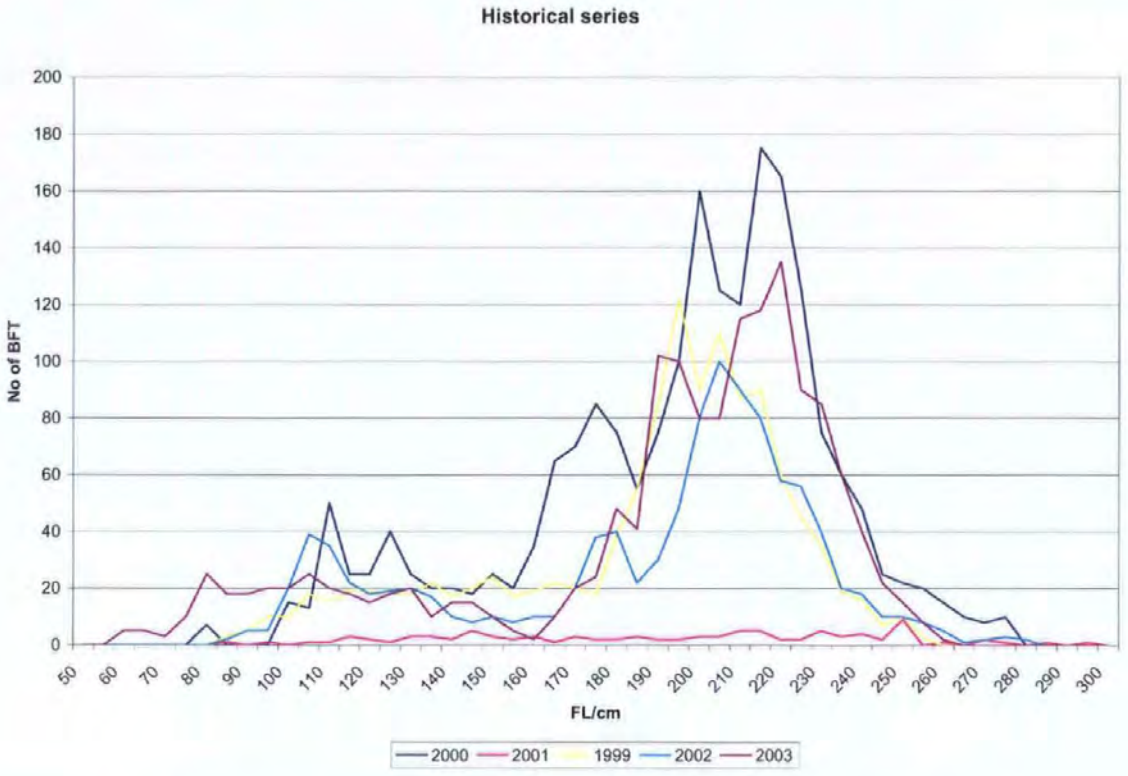


Fig.2.8 Historical series of the length classes during each year, 1999 - 2003

Table 2.2 gives the mean fork length in cm per year together with the standard deviation from the mean.

Year	Mean FL / cm	Standard Deviation
1999	193	2.5
2000	199	4.2
2001	209	5.3
2002	205	1.9
2003	219	3.2

Table 2.2 Mean FL and standard deviation per year during the whole period of study

The ANOVA test was carried out to determine whether any statistical differences are present between the mean fork lengths calculated for the different years. At the 95% confidence level, no significant difference was found between the results (0.0221).

In the other countries of the Mediterranean, the biometric measurements of bluefin tunas were recorded and processed using the same methodology.

Fig. 2.9 to Fig 2.18 show the length distributions of bluefin tuna which had been caught by the main gears (trap nets, purse seine, hand lines and drifting surface longlines) used in Malta, Libya, Tunisia, Morocco and Spain.

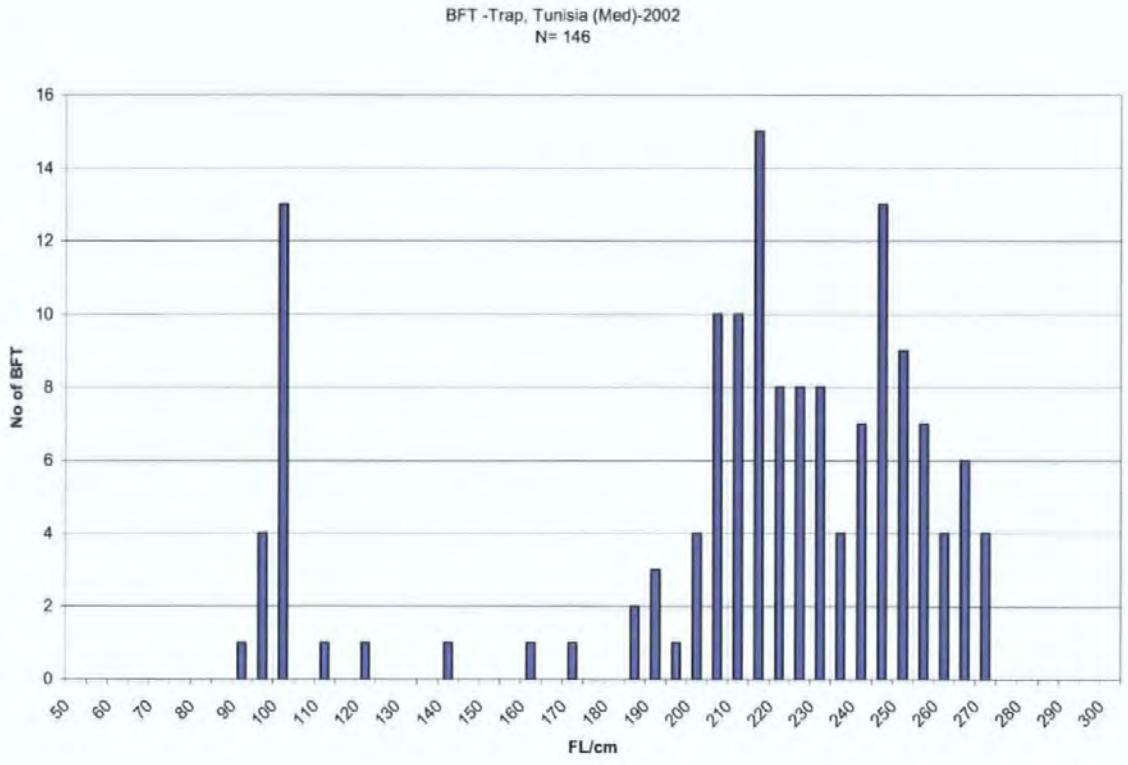


Fig. 2.9 Length distributions of bluefin tuna landed by Tunisian traps

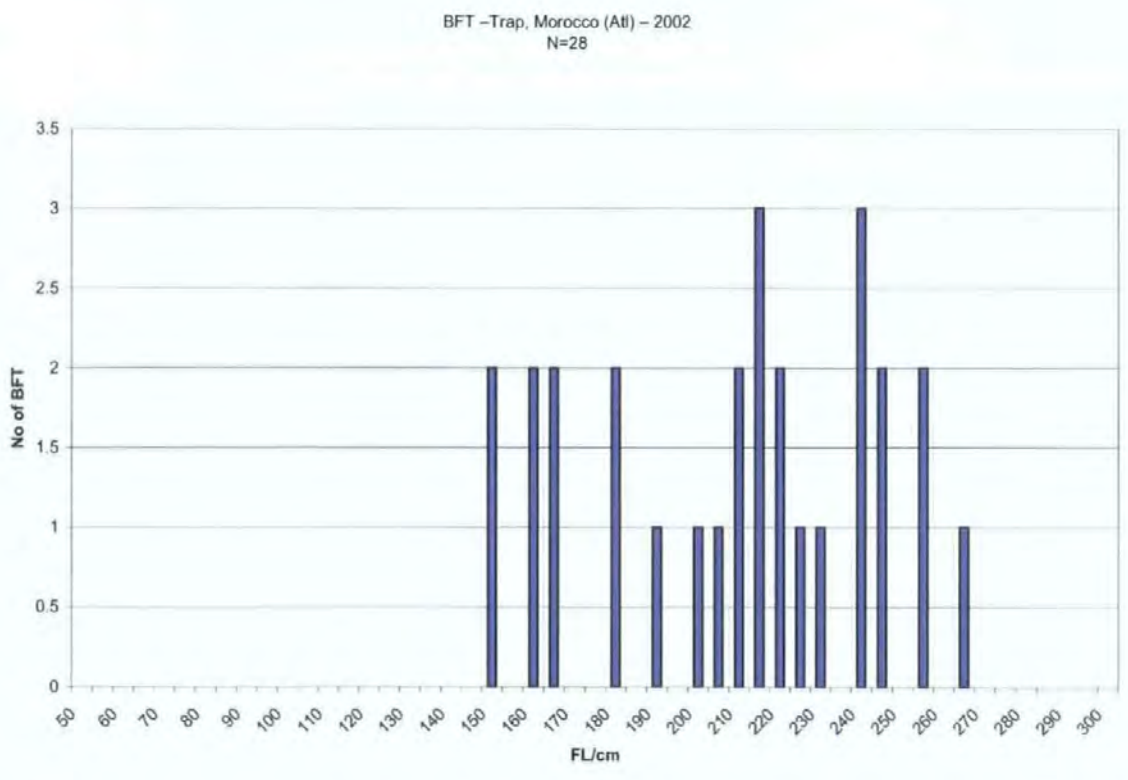


Fig. 2.10 Length distributions of bluefin tuna landed by Moroccan traps

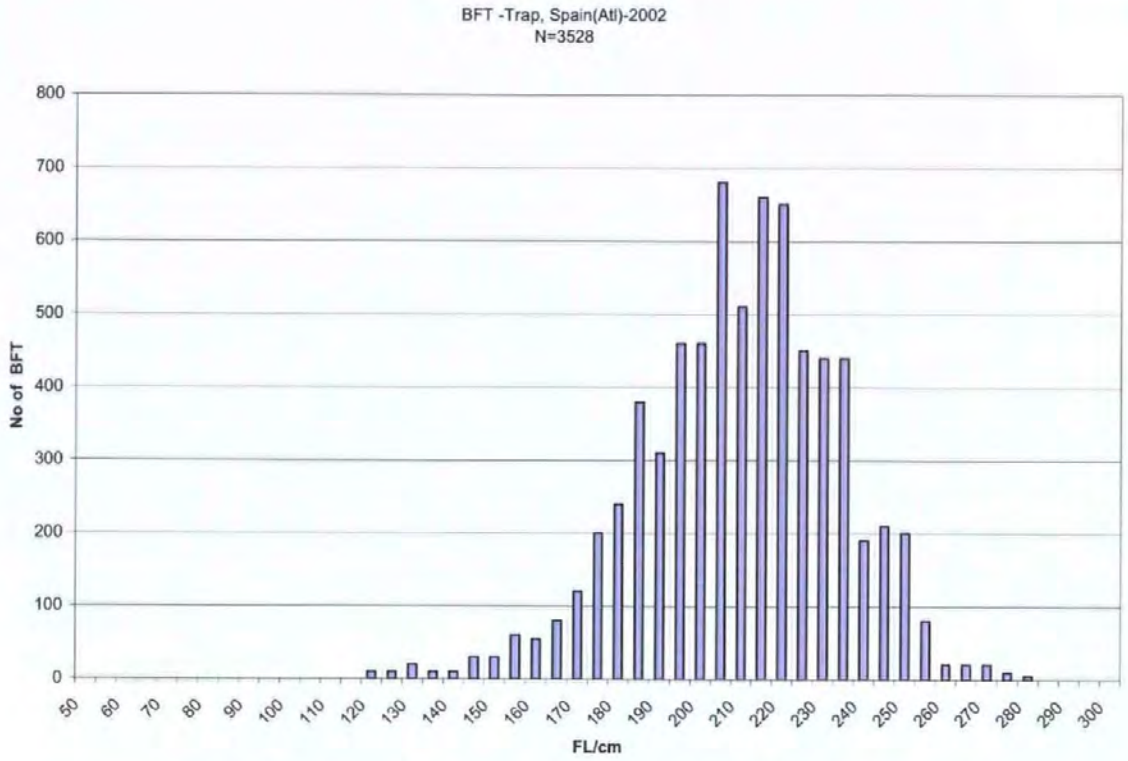


Fig. 2.11 Length distributions of bluefin tuna landed by Spanish traps

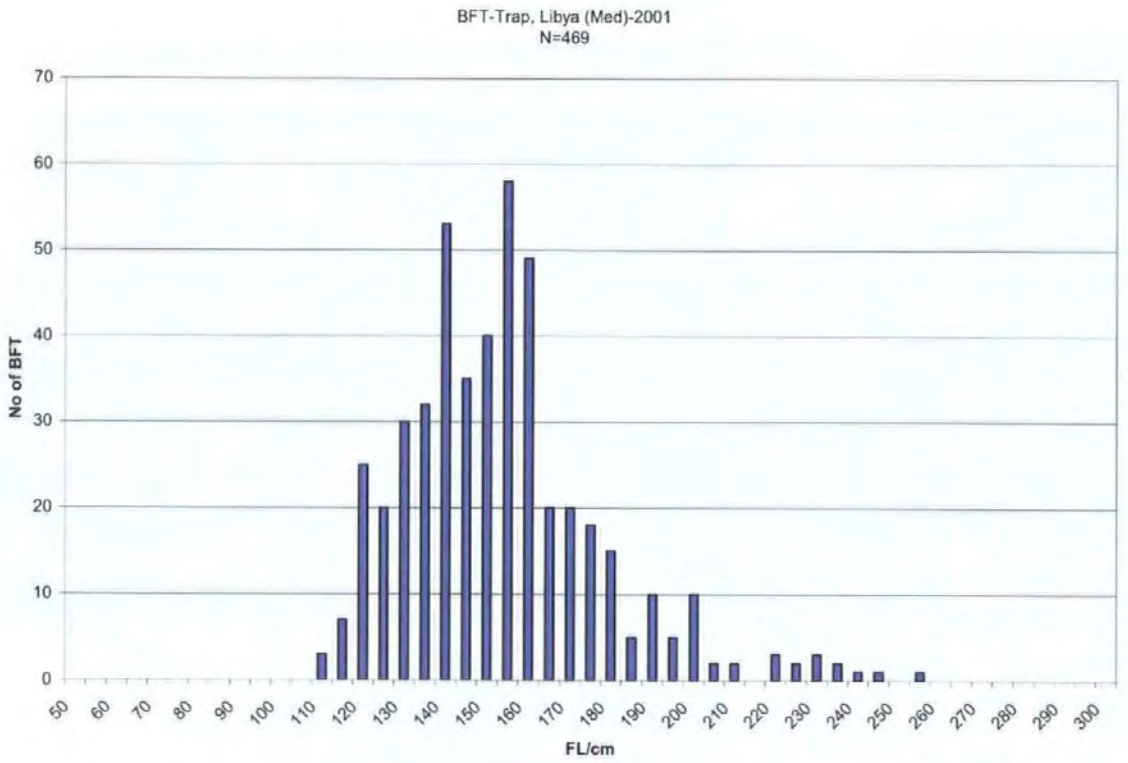


Fig. 2.12 Length distributions of bluefin tuna landed Libyan traps

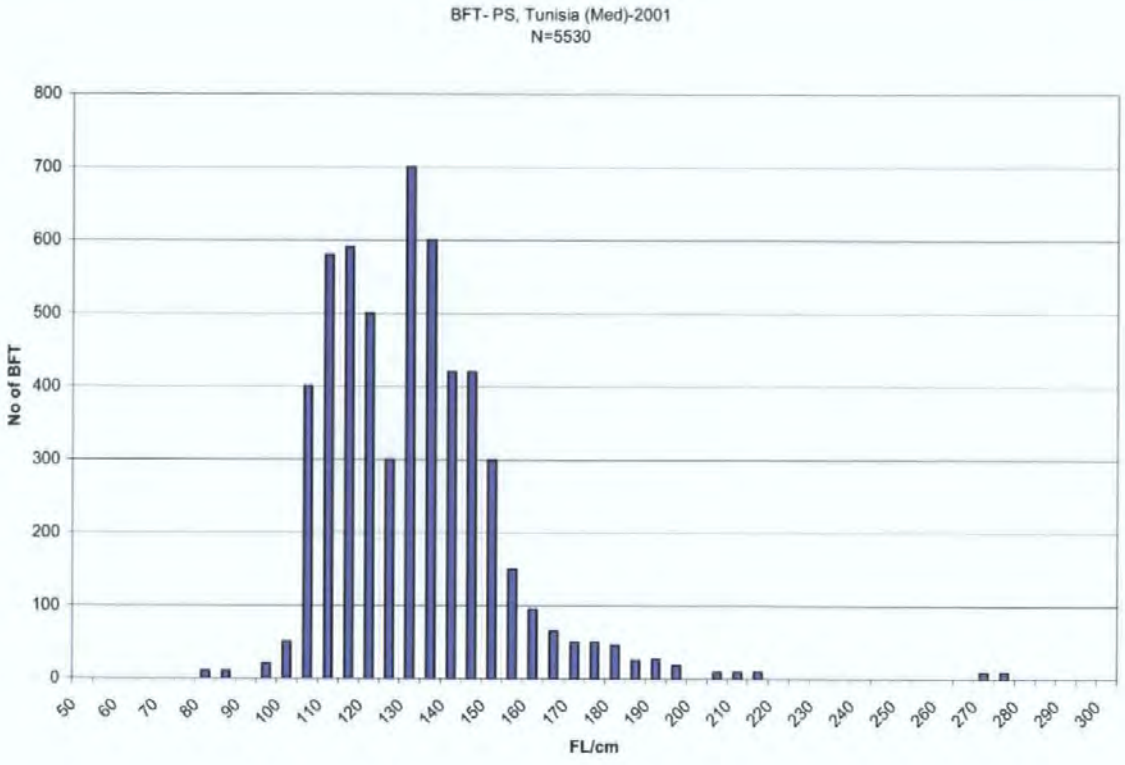


Fig. 2.13 Length distributions of bluefin tuna landed by Tunisian purseiners

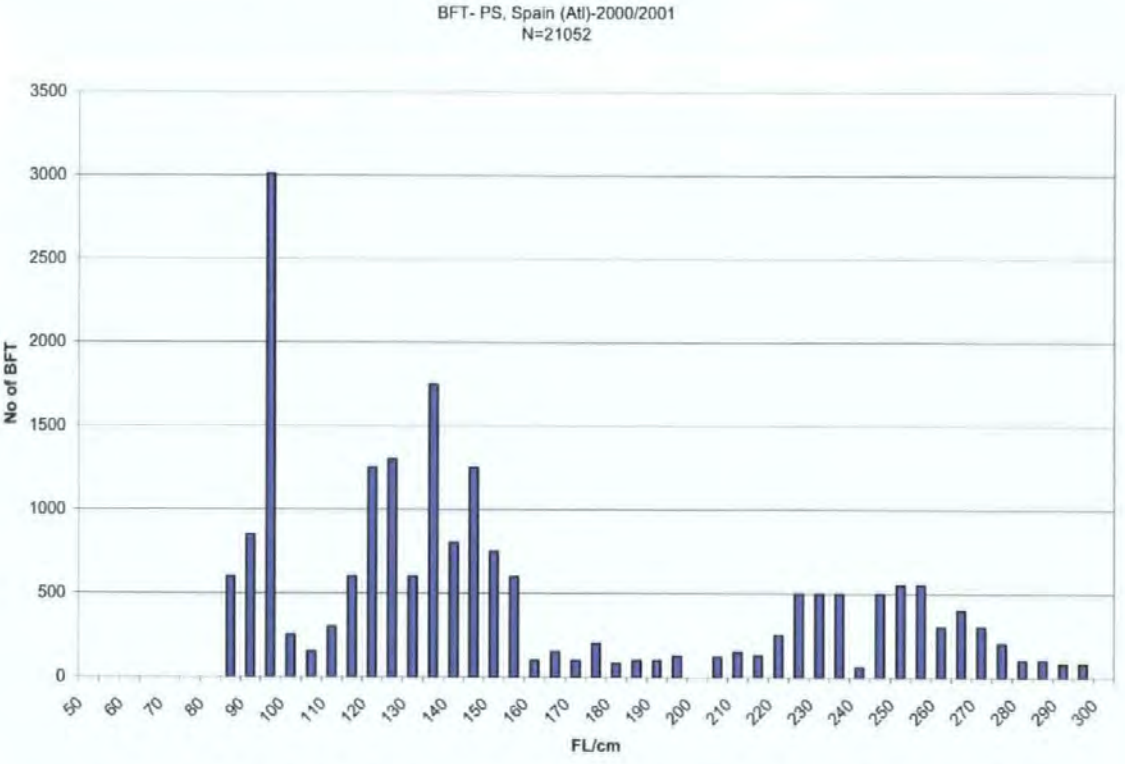


Fig. 2.14 Length distributions of bluefin tuna landed by Spanish purseiners

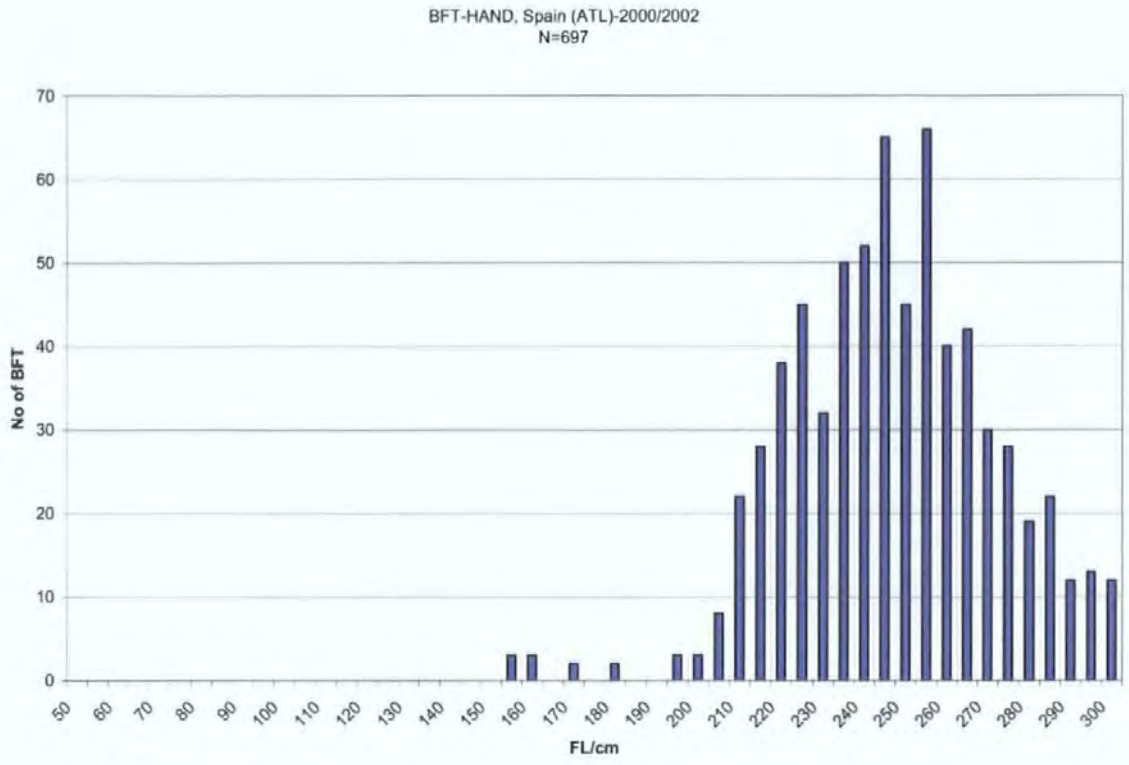


Fig. 2.15 Length distributions of bluefin tuna landed by Spanish hand lines

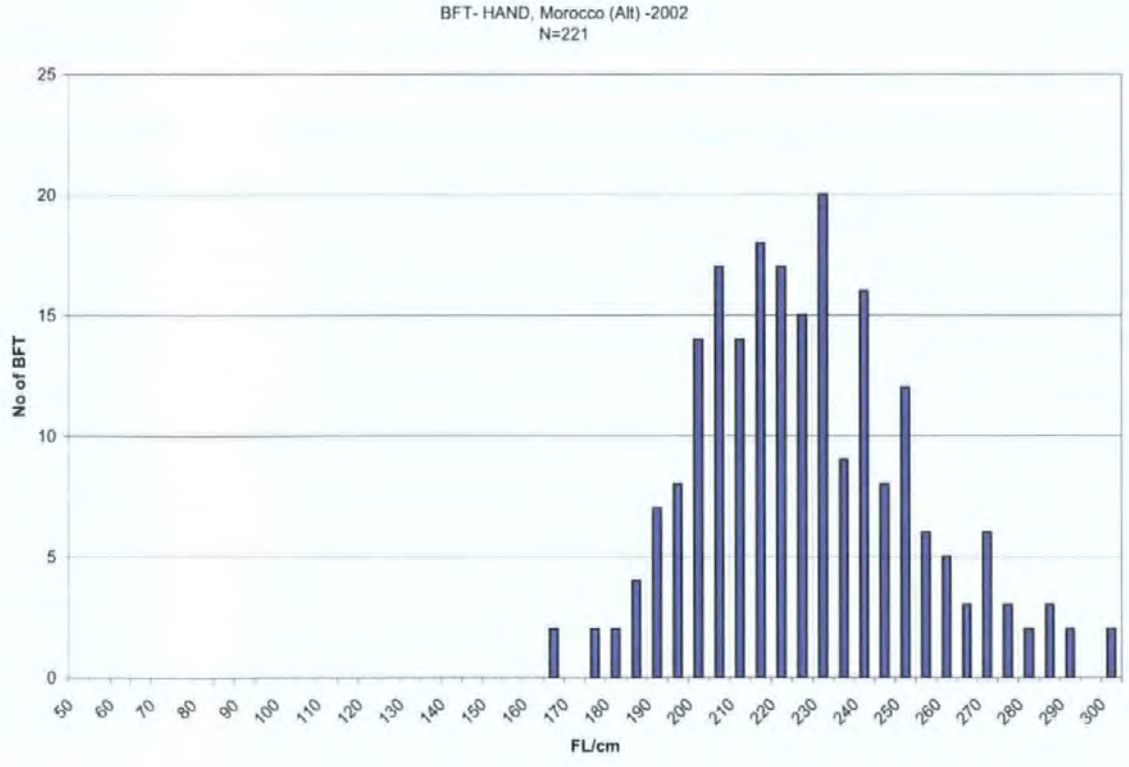


Fig. 2.16 Length distributions of bluefin tuna landed by Moroccan hand lines

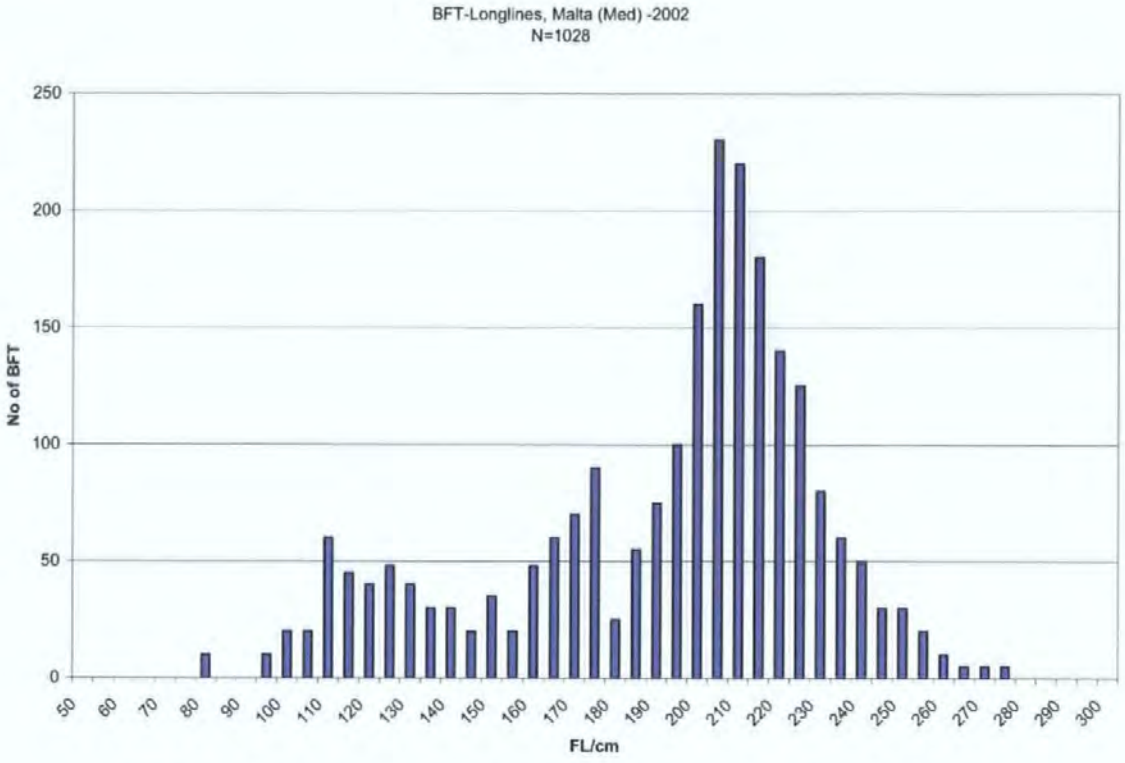


Fig. 2.17 Length distributions of bluefin tuna landed by Maltese long lines

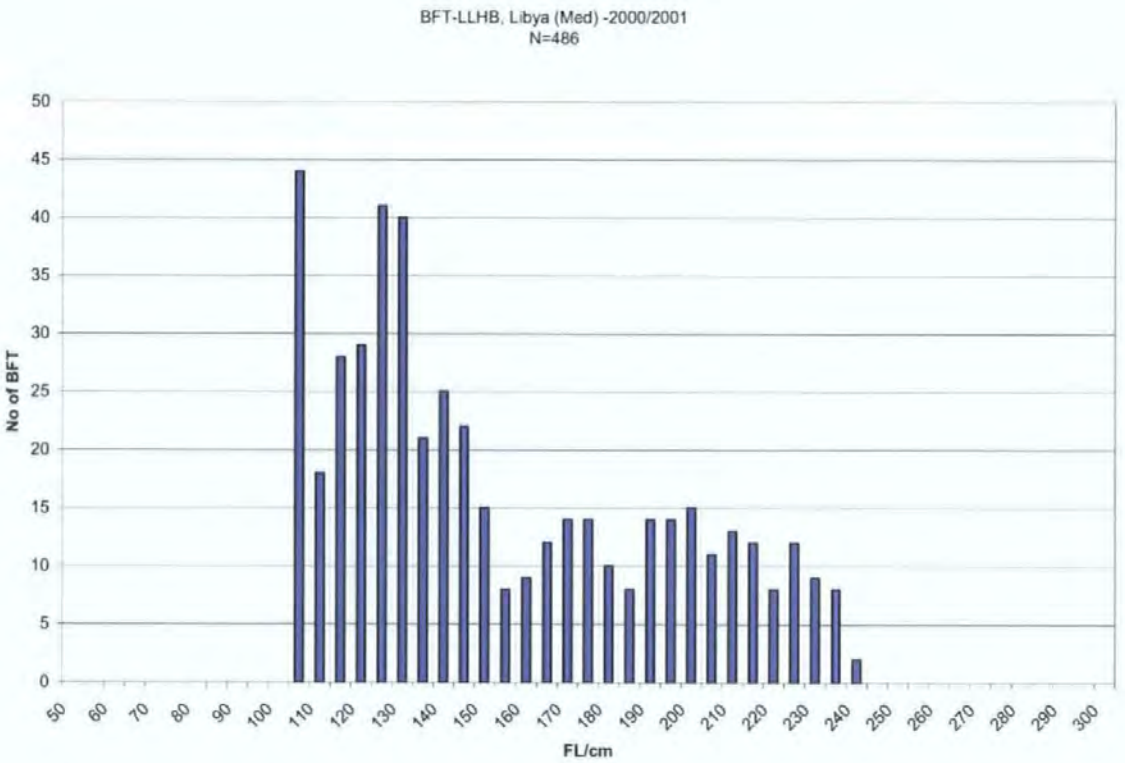


Fig. 2.18 Length distributions of bluefin tuna caught by Libyan longlines

Table 2.3 gives the mean fork length per country per gear together with the standard deviation from the mean.

Country	Gear	Number of samples	Mean FL/ cm	Standard Deviation
Morocco	Handline	65	235	5.6
Tunisia	Trap	118	209	14.5
Tunisia	Purse seine	200	125	12.1
Spain	Handline	5 035	240	6.2
Spain	Purse seine	7 341	180	13.2
Malta	Longline	141	210	5.1
Libya	Trap	153	160	11.9

Table 2.3 Mean FL and standard deviation per country per gear

The statistical test ANOVA was carried out to determine whether any differences are present between the results obtained using different gears in different countries. At the 95% confidence level, a value of 0.2550 was obtained. This indicates that a significant difference is present between the mean fork length calculated for the bluefin tuna targeted by different gears.

The individual length and weight data collected from bluefin tuna samples caught around the Maltese Islands during the year 2000 are given in Table 2 of the Appendix.

All the data collected during the duration of the study with regards to length-weight relationships is summarised in Table 2.4 and Fig. 2.19.

Country	A	b	R^2	Gear	Number of samples
Morocco	1.01×10^{-2}	1.7854	0.794	HAND	65
Tunisia	15.3×10^{-5}	2.6381	0.854	TRAP	118
Tunisia	4.00×10^{-4}	2.4295	0.987	PS	200
Spain	3.74×10^{-5}	2.8589	0.879	BB	5 035
Spain	3.62×10^{-5}	2.8673	0.799	PS	7 341
Malta	2.60×10^{-3}	2.0775	0.771	LL	141
Libya	2.00×10^{-4}	2.9957	0.878	TRAP	153

Table 2.4 Length-weight relationships for bluefin tuna caught by several gears and fishing areas during the year 2000 (PS= purse seine, BB= bait boat, LL= longline)

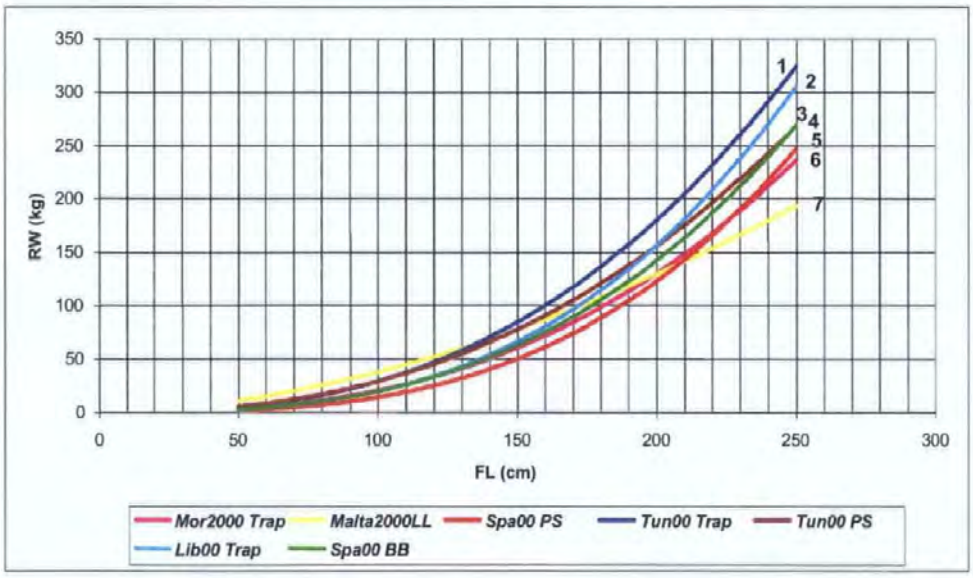


Fig. 2.19 Comparison of bluefin tuna length-weight relationships in different regions of the Mediterranean utilising different gears

An ANOVA test was carried out to study the differences between these relationships. At the 95 % confidence level, a value of 0.1324 was obtained indicating that a significant difference exists between the relationships.

Fig. 2.20 and Table 2.5 compare data obtained from this study with previous studies.

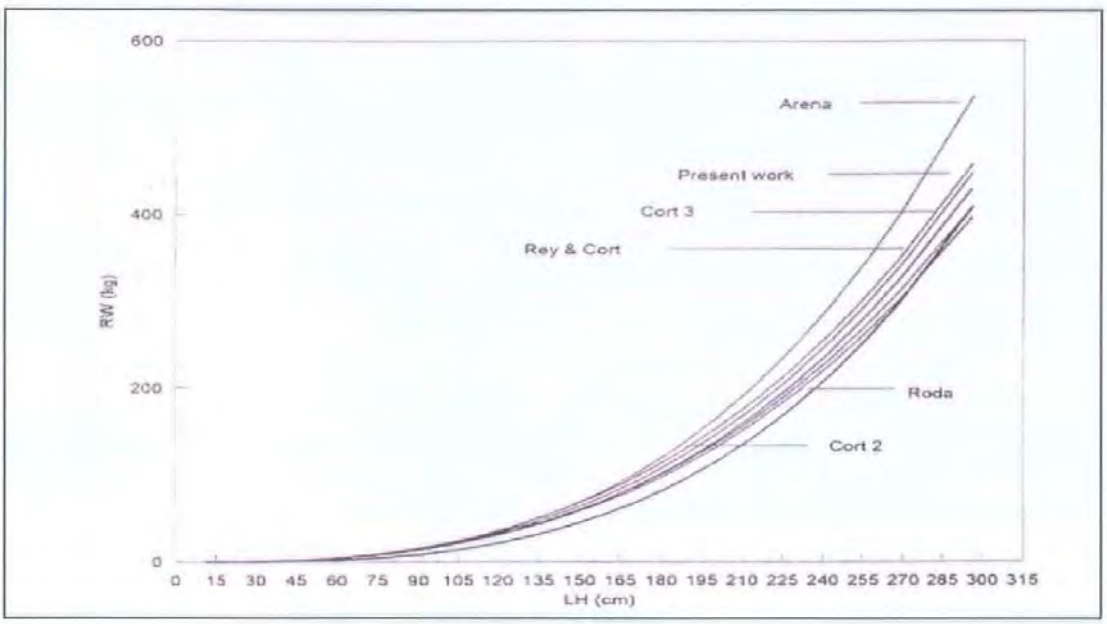


Fig.2.20 Comparison of the results of this study with previous studies (FAO-COPEMED Annual Report, 2002)

An ANOVA test was carried out to study the differences between these relationships. At the 95 % confidence level, a value of 0.0114 was obtained indicating that no significant differences exist between the relationships.

	A	B
Present work – Mediterranean	5.608×10^{-5}	2.797
Rey & Cort, East Atlantic (not published) (1990)	2.950×10^{-5}	2.899
Parrack & Phares, West Atlantic (1979)	2.860×10^{-5}	2.820

Table 2.5 Values for growth curve Round Weight = a Fork Length b

An ANOVA test was carried out to study the differences between these relationships. At the 95 % confidence level, a value of 0.4124 was obtained indicating that a significant difference exists between these three relationships.

2.5 Discussion

From the analysis of the data collected from the Maltese Islands, it is evident from Fig. 2.8 that there were no significant changes in the distribution of sizes over the whole study period of 5 years (1999 – 2003). In fact, the mean length remained of the same magnitude, i.e. between 193 and 219 cm fork length. At the 95% confidence level, no significant difference was found between the mean fork length calculated for the different years. In fact a value of 0.0221 was obtained.

The peak in Fig 2.5 is not as distinct as in the rest of the figures since during the year 2001 only a small number of bluefin tunas were sampled due to the reasons explained above. From Fig. 2.3 – 2.8, one can observe that the bluefin tunas caught are rather large. In Malta bluefin tuna is only caught using surface longlines which target tuna during its spawning period. At this time of their life cycle, large tunas tend to swim in the upper region of the water column to release their eggs in the surface waters and therefore are more easily caught by the hooks of the drifting surface longlines.

Size distribution was also studied in different regions of the Mediterranean for bluefin tuna using different gears. At the 95% confidence level, a value of 0.2550 was obtained indicating that significant differences were present between the mean fork lengths of tuna targeted by different gears.

Traps target bluefin tuna before their spawning season. As such the size distributions of bluefin tuna caught by traps are more widely distributed. In fact the standard deviation for the fork lengths of tuna targeted by traps ranged between 11.9 and 14.5. The mean fork lengths determined for this gear were 209 cm and 160 cm.

Purse seines entrap whole shoals of fish. As a result, even in this case the size distribution of bluefin tuna is very widespread and the standard deviations of the mean fork length ranged between 12.1 and 13.2.

In the case of handlines, only larger specimens are caught since bluefin tuna would be present in the upper region of the water column during its spawning season which in this case coincides with the fishing season. The mean fork lengths determined were 235 cm and 240 cm and the standard deviation ranged only between 5.6 and 6.2.

These differences in the size distributions could be mainly attributed to the differential selectivity of the fishing gear, the different spatial – temporal stratum, the condition factors and the number of sampled specimens.

Table 2.3 summarises the length – weight relationships determined for each region for each main gear. Although the relationships obtained are very close, differences exist.

These can be attributed to factors such as the hydrographical characteristics of the particular fishing area and period, the differential catchability due to the prevailing environmental factors and the differences in the fishing strategy adopted amongst vessels.

Results obtained were also compared with previous studies carried out in the Mediterranean and the length – weight relationships agree well with each other since at the

95% confidence level, no statistical difference was found between the R^2 values of the different length - weight relationships calculated.

On the other hand when the length - weight relationship obtained in this study for the Mediterranean region was compared with the reported length – weight relationships determined for the east and west Atlantic (Table 2.4), significant differences were observed. This could be a factor indicating that the Mediterranean stock has got a different length – weight relationship and could therefore be considered as an independent stock residing in the Mediterranean during its whole life cycle. More studies in the same line of this experiment need to be carried out in the east and west Atlantic since the only reference material available is rather old.

2.6 Summary

1. No significant changes were observed in the size distributions of bluefin tuna caught around the Maltese Islands during the period 1999 - 2003.

2. Significant differences were observed in the distribution of sizes of bluefin tuna caught by the different gears. A recommendation should be made to the SCRS of ICCAT to utilise these separate relationships when determining population sizes from data collected from different gears during stock assessments since these would give more reliable results.

3. A significant difference was observed between the length – weight relationship of the Mediterranean when compared to the east and west Atlantic length – weight relationships. This could be an indication that the Mediterranean population has got its own distinct characteristics and thus needs to be considered as a different sub population.

Chapter 3

AGE DETERMINATION

3.1 Introduction

Knowledge on individual growth is essential for assessment purposes. Age – length keys allow the transformation of length distributions into age distributions which are in turn essential in the determination of the population demographic structure as well as its exploitation rates. Different methods have been used in the studies of age determination, including otolith studies, studies of the vertebrae, etc. In the case of bluefin tuna, the spines are the most commonly used skeletal parts for age determination. The first ray of the first dorsal fin is normally used.

This experiment was sponsored by project FAO-COPEMED 'Large Pelagics Programme'. The author attended a training course in Fuengirola, Spain in order to learn the methodology applied for spine extraction and reading. The course was delivered by Ms. Cristina Rodriguez from the Spanish Oceanographic Institute in Santander. The necessary equipment was given to the Malta Centre for Fisheries Sciences by the project FAO-COPEMED and all the collection of dorsal spines and preparation of slides was carried out in Malta by the author. The slides were then read in the Spanish Oceanographic Institute in Santander, first by the author and then corroborated by Ms. Rodriguez in order to ensure validity of results and standard procedures. The results obtained were presented and published by the author during the annual ICCAT Scientific Committee for Research and Statistics meeting in 2000.

3.2 Literature review

The dorsal fin spines are the most commonly used skeletal parts for age determination of bluefin tuna. The first ray of the first dorsal fin is normally used. However many other

studies have been carried out using other bony structures like vertebrae or otoliths (Prince *et al.*, 1985). The otoliths are difficult to find since they are quite small compared with the size of the head and moreover the head of tunas is very fleshy and bloody. This means that if one can accurately pinpoint where the cut must be done, the probability of finding them is enhanced. Tuna otoliths are rather dense and ridged, so it is necessary to prepare sections about 0.34 mm thick.

Both vertebrae and spines present difficulties to age interpretation of large bluefin tuna. Age reading from vertebrae differs depending on whether the whole vertebra is used or only a horizontal section (Prince *et al.*, 1985). Many techniques have been developed in order to improve the visualisation of the bands or rings. One of the most common is staining with alizarin red (Berry *et al.*, 1977). Reading from spines is complicated by the re-absorption of the nucleus from age 3. Nevertheless, both structures have proved to be suitable for direct ageing and spines have been used to establish the Mediterranean bluefin tuna growth parameters (Cort, 1991; Mather *et al.*, 1995).

The advantage of using the dorsal spine is that the extraction and sampling preparation for reading is more straight forward compared to otoliths or vertebrae. Also the latter involves the purchase of fish whilst in the first case the damage done to the fish is nearly negligible and the market price is not affected.

Compean Jimenez & Bard (1980, 1983) first started reading fin rays for the purpose of age reading in bluefin tuna caught from the Atlantic. Rey & Cort (1984) worked out a length-age key by reading dorsal rays of Atlantic bluefin tuna. As regards the Mediterranean Sea, Tserpes & Tsimenides (1995) & De Metrio & Megalonfonou (1989) studied growth of other large pelagics by reading transverse sections of rays from the anal fin.

Many of these techniques provide good results in ageing younger fish but age estimation of adult bluefin is more complicated. Reading interpretation of bluefin tuna spines in adult fish is rather difficult since most of the first rings would have been reabsorbed. One must then back calculate body size at age based on the relationship that exists between the growth of the spine and the fish. The diameter of the first complete ring is measured and it is used to determine the corresponding number of years. Then the subsequent rings are added on, finally ending up with the estimated age of the bluefin tuna (Cort, 1990).

No previous studies have been made about the population dynamics of bluefin tuna caught in the Mediterranean (ICCAT, 1997). Age-length keys allow the transformation of length distributions into age distributions. Knowledge on individual growth is essential for assessment purposes by means of analytical models. These are essential in order to know the population demographic structure as well as its exploitation rates thus leading to more sustainable management.

3.3 Materials and Methods

A total of 72 bluefin tuna spines (42 males, 30 females) were collected around the Maltese Islands during May and June 1999 (Fig.3.1), ranging in length from 106 cm to 290 cm. From the individual weights of the bluefin tuna, the individual lengths were calculated using the length - weight conversion for bluefin tuna for the Mediterranean (ICCAT Field Manual, 1990).

Fig. 3.1 Map indicating area around the Maltese Islands from where bluefin tuna was caught (Farrugia, A. et al., 2000)

The method of extraction, preparation and sectioning of spines used was the one described by Compean-Jimenez & Bard (1980).

The first dorsal fin of each bluefin tuna to be studied was spread open (Fig. 3.2) and the membrane between the two first dorsal rays was cut. Then the first dorsal ray was bent forward until the ligaments were broken. The ray was then turned round to the right and to the left alternatively until it came out. Each ray was kept in a container labelled with the bluefin tuna number. The spiny rays were kept at 15°C until laboratory analysis was carried out.

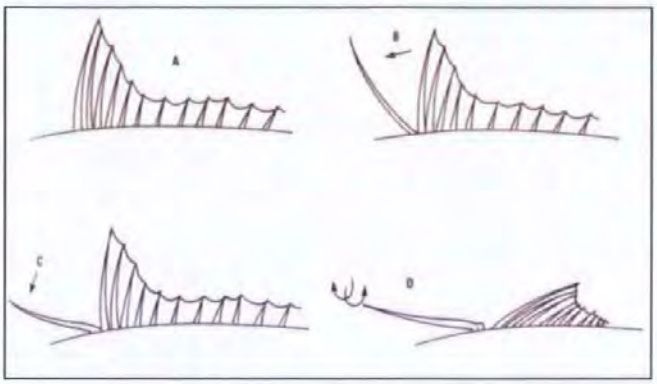


Fig 3.2 Technique for the extraction of the first dorsal ray (Farrugia, A. et al., 2000)

Before starting the analysis, any remaining connective tissue was removed. An effort was made to start the sectioning in the same position. The cuts were made using a low rotating diamond saw. The lubricant used during cutting was a mixture of 25% special oil, 25% liquid soap and 50% water. Three serial cross-sections about 0.7 mm thick were obtained from each spine at the point near condyle base (Fig. 3.3) using a low speed saw. Later, the sections were mounted on slides covered with a highly transparent resin that was useful both to fix the samplings and to clarify the possible bands or annuli.

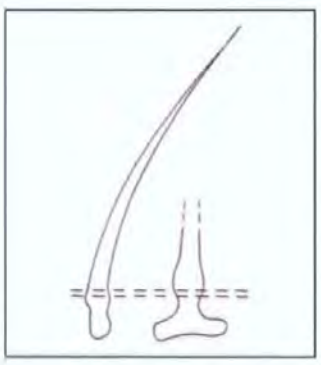


Fig. 3.3 Region along dorsal ray from where section has to be taken (Farrugia, A. et al., 2000)

The measuring and reading of the spinal sections was carried out with a profile projector using a zoom of 10. A binocular lens was also used together with a micrometer to determine ring diameter.

Interpretation of growth bands was based on the recognition of narrow translucent and wider opaque zones, assumed to represent slow and fast growth, respectively. Therefore, the number of translucent zones (rings), interpreted as annual events, was counted in order to assign an estimated age to the fish. As the nucleus of the spine is reabsorbed and the first rings begin to disappear from age 3, the mean diameter of the first rings of younger specimens was used to date the first visible ring of older specimens (Rodríguez-Marín, 2004). Two readings of each spine were taken independently. When there was disagreement between counts of translucent bands, spines were read for a third time.

Most of the samples belonged to large fish older than 4 years old, which means that it was impossible to find all the rings since normally the nucleus or centre of the spine would have been reabsorbed and consequently the first rings have disappeared. For this reason, the table prepared by Cort (1990) which provides the parameters (mean, standard deviation, and confidence interval) of the ring diameters for ages 1 to 7 years old was used. Based on these parameters, the first visible ring was identified and assigned its respective age according to the table. Then all the successive rings were counted and measures of their respective diameters (mm) taken when possible.

3.4 Results

The study of 72 spines allowed the build up of a preliminary age-length key including larger bluefin tuna (Table 3.1). The parameters obtained from measuring the diameter of the corresponding rings are given in Table 3.2. Bluefin tuna spines were collected between the months of May and June. Most of them presented the last visible ring near the border (Fig. 3.5a) indicating that the bluefin tuna had been targeted during a period of fast growth.

The bluefin tuna longline fishery was mostly composed of large fish ranging from 86 to 275 cm (Fig. 3.4). The mean fork length of the whole sample of bluefin tuna was 198 cm with a standard deviation of 5.6. The demographic composition of the catch made in 1999 is presented in Table 3.3 using the before mentioned age-length key. Individuals from some length classes are missing since it was not possible to assign and age due to the low number of spines in some length groups.

In Malta, bluefin tuna are caught during their spawning season and as such large tunas are caught. Therefore for the larger length groups there were enough samples but not for the smaller ones. In general most fish belong to the 8 to 10 years old age group. Fig. 3.5 are photomicrographs of some of the dorsal spine sections indicating clearly the presence of the annual rings, the vascularised nucleus and the regions of slow and fast growth.

Although sex data has been recorded, analysis of the spinal sections by sex has not been possible to perform due to the wide range of lengths and the low number of samples in each length class. The base data can still be used in the future when more samples will be collected in order to prepare a different age length key for male and female bluefin tuna.

Length/Age	4	5	6	7	8	9	10	11	12	13	14	15	N
106-110	1.00												1
111-115													0
116-120			1.00										1
121-125													0
126-130		1.00											1
131-135													0
136-140		1.00											1
141-145													0
146-150				1.00									1
151-155			1.00										1
156-160			1.00										1
161-165													0
166-170			1.00										1
171-175													0
176-180													0
181-185						1.00							1
186-190					1.00								1
191-195						1.00							1
196-200					1.00								3
201-205								1.00					2
206-210							1.00						3
211-215					0.20			0.60		0.20			5
216-220							0.50			0.50			4
221-225					0.25		0.38		0.13	0.25			8
226-230						0.20	0.40	0.40					5
231-235								0.50	0.25	0.25			4
236-240						0.44		0.33	0.11	0.11			9
241-245								0.67		0.33			3
246-250									1.00				2
251-255								0.25		0.50	0.25		4
256-260								0.33	0.33			0.33	3
261-265									0.50		0.50		2
266-270										1.00			1
271-275								1.00					1
276-280								1.00					1
281-285													0
286-290												1.00	1
N	1	2	4	1	7	7	10	18	7	11	2	2	72

Table 3.1 A preliminary age-length key prepared for large bluefin tuna (The fractions represent the distribution of bluefin tuna present at each age class)

Age	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Mean	2.23	3.43	5.14	6.30	7.48	8.59	9.58	10.44	11.26	12.09	12.68	13.58	14.36	15.10	16.41
s.d.	0.21	0.25	0.45	0.32	0.30	0.29	0.29	0.37	0.46	0.52	0.74	0.82	0.88	1.10	
Sy	2.18	3.38	5.03	6.25	7.46	8.57	9.56	10.40	11.20	12.00	12.50	13.27	13.93	13.72	
Sx	2.28	3.48	5.24	6.34	7.51	8.62	9.61	10.48	11.33	12.18	12.86	13.89	14.80	16.48	

Table 3.2 Values and parameters (Mean ring diameter of dorsal spinal section, s.d.= standard deviation, Sy = Inferior limit of confidence interval, Sx = superior limit of confidence interval) obtained from measuring

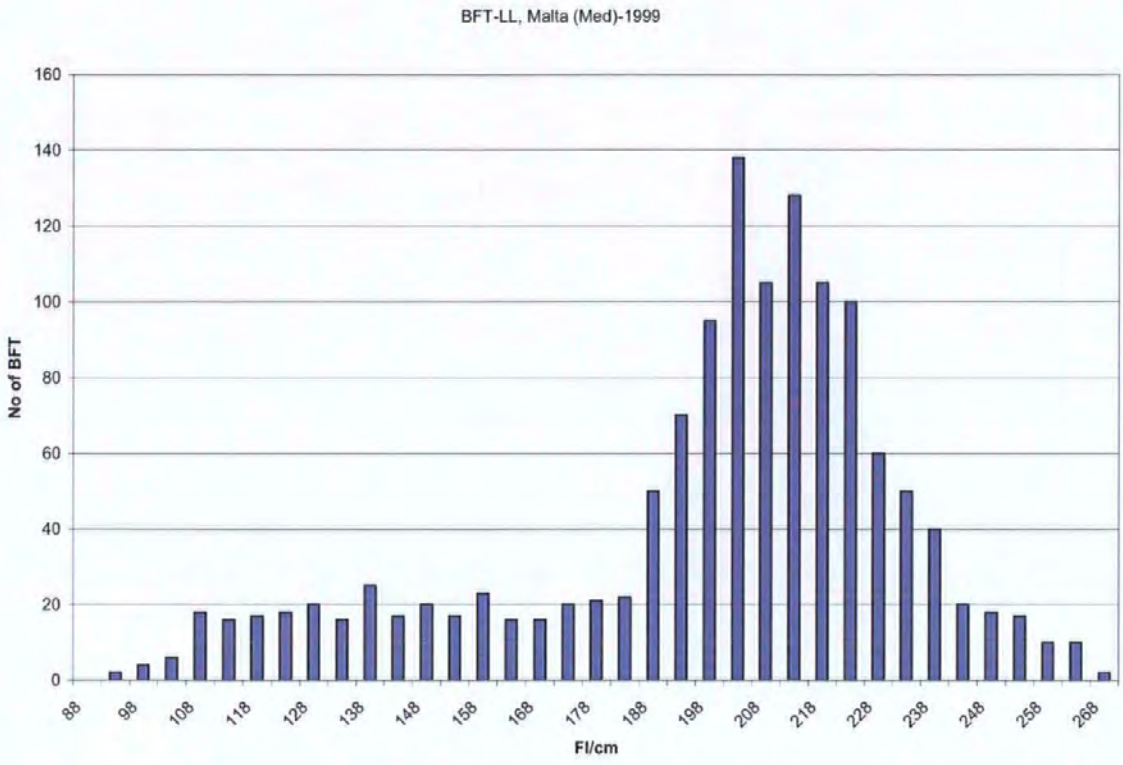
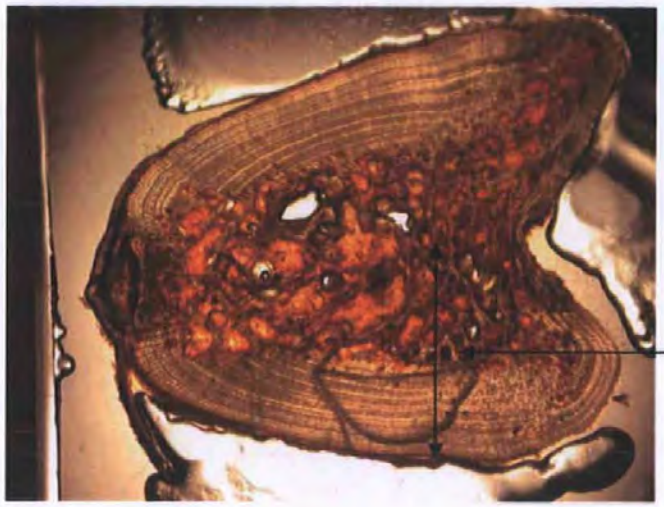
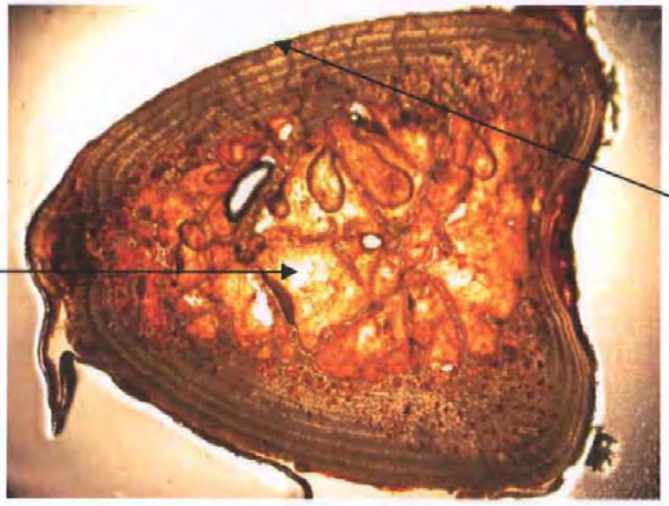


Fig. 3.4 Length-frequency distribution of bluefin tuna caught by longline fishery around the Maltese Islands in 1999

Age	4	5	6	7	8	9	10	11	12	13	14	15
N° fish	12	31	80	17	242	159	213	216	34	102	3	2
%	1.1	2.8	7.2	1.5	21.8	14.4	19.2	19.4	3.0	9.2	0.2	0.2

Table 3.3 Demographic composition of bluefin tuna catch for 1999 in Maltese Islands obtained after extrapolating the results to the whole bluefin tuna catch obtained during 1999





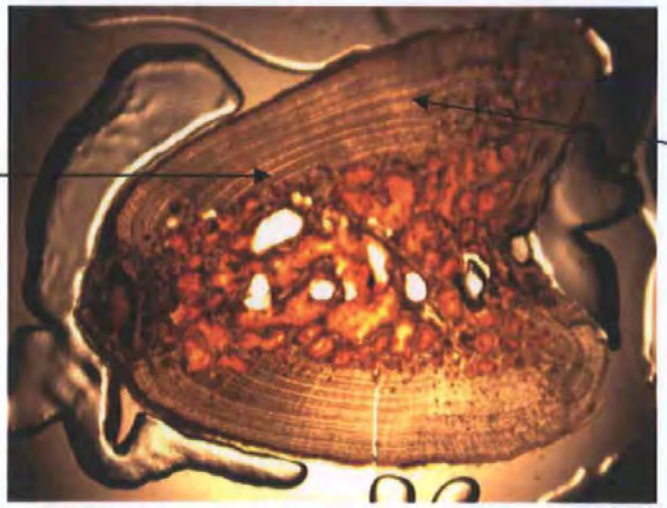


Fig. 3.5 Bluefin tuna dorsal spine sections (annuli clearly visible)

3.5 Discussion

Values obtained in Table 3.1 are in good agreement with those obtained by Rey *et al.*, (1984) and Cort (1990). The latter studies had only been carried out on young tuna (up to 8 years of age).

The readings of these dorsal spines allowed the creation of a preliminary age-length key for larger bluefin tuna up to the age of 15 years. The number of collected spines is rather small and as such the collection and reading of more spines will allow the creation of a more reliable age-length key.

As most of the bluefin tuna dorsal spines were collected during the Maltese bluefin tuna season, i.e. between May and July, the last visible ring was found very close to the edge and therefore was not taken into account in ageing. Regrettably, some of the length classes were not satisfactorily represented since these length groups were less abundant. As such, collection and reading of spines should continue. But, in general, the age-length key is very representative of the length distribution of the bluefin tuna landed by longlines and provides an important stage for future studies.

Differences in age and growth between males and females usually exist as has already been described by other authors for the western stock (Butler *et al.*, 1977; Hurley *et al.*, 1981). The collection of new data will allow the construction of age length keys taking into account sex.

These age – length keys will provide essential information in all aspects of studies carried out on bluefin tuna. In this case, this age – length key will be used in the determination of age at first maturity as will be explained in the next chapters.

3.6 Summary

1. Age determination by a range of methods has proved elusive especially in older fish.

2. This age - length key will allow the determination of the age of bluefin tuna up to the age of 16 years. This key will provide essential information in all aspects of studies carried out on bluefin tuna. In this study, this will be used to determine the age at first maturity in the experiments of reproduction.

3. As yet no age - length key has been determined for large bluefin tuna caught in the Atlantic. This study will serve as an important step in deepening research in this field.

4. Further studies will allow age determination for bluefin tuna caught in the Atlantic. This could then be compared with the age-length key of the Mediterranean. If significant differences are found, this would further confirm the presence of a permanent residing bluefin tuna stock in the Mediterranean Sea.

Chapter 4

ASSESSING THE REPRODUCTIVE STATUS OF BLUEFIN TUNA IN THE MEDITERRANEAN

4.1 Introduction

The presence of potential spawning areas in a particular region could be an indication for the completion of a full life cycle. The proposed methodology of this study was to determine the gonadosomatic index of female bluefin tuna and then study it in relation with the qualitative stage of the gonads. This can be based on histological studies in which the development of the ovary cycle, the presence or absence of atresia and the presence of postovulatory follicles can be determined. This would help to further confirm the presence of an independent resident stock within the Mediterranean.

Although no specific studies have been carried out on the age at first sexual maturity of bluefin tuna, there is some evidence of the presence of a difference in the attainment of the first sexual maturity between the western and the eastern Atlantic populations. The aim of this part of the experiment was to identify the size and age of first sexual maturity for female bluefin tuna caught in the Mediterranean and to determine whether it differs from that of the Atlantic stocks.

FAO-COPEMED 'Large Pelagics Project' sponsored the first part of the experiment. The author collected all the female bluefin tuna samples for the first part of the experiment. Two other observers, one in Italy and one in Spain helped in the collection of samples for the second part of the experiment.

The author attended courses with regards to reproductive studies in Malaga, Spain. All courses were delivered by Dr. David Macias, University of Malaga, Spain. Histological samples were all prepared in Malta but then reading was done in Spain both by the author

and by Dr. David Macias in order to ensure validity of results. A paper has been prepared and has been accepted by the Journal of Applied Ichtyology for publishing.

4.2 Literature review

The Western Mediterranean and the Adriatic Sea are considered to be spawning areas for bluefin tuna (Piccinetti, 1980). The highest concentrations of bluefin tuna larvae are found in the south of Italy, to the north of Sicily and around the Balearic Islands (Dicenta, 1975, Piccinetti, 1995). The spawning season of bluefin tuna extends from June to July in areas with a surface temperature in the region of 24 °C (Arena, 1979).

A popular method used for the study of the sexual maturity is the calculation of the gonadosomatic index (West, 1990; De Martini *et al.*, 2000). This method, which is both fast and cheap, expresses the developmental stage of the gonads in terms of gonad weight to fork length ratio (Wootton, 1990). Unfortunately, the gonadosomatic index alone, although able of providing useful proxies of reproductive activity, is not a reliable parameter to distinguish between mature inactive and immature fish (West, 1990; De Martini *et al.*, 2000). Thus its use can introduce a consistent bias in the determination of the size at sexual maturity.

An obsolete methodology for the calculation of the sexual maturity is represented by the use of macroscopic arbitrary scales (Nikolsky, 1963; Holden & Raitt, 1974; Rodríguez-Roda 1964, 1967). This kind of classification, in addition to the same limitations of the gonadosomatic index, depends on the subjective interpretation of the observer. Thus, the histological analysis of ovaries represents the only reliable, although more expensive, method for the assessment of sexual maturity in fish (West, 1990).

Western Atlantic bluefin tuna sometimes mature as early as age 6 and are considered fully mature by age 8, at a weight of 135 kg (Baglin, 1982; NRC, 1994). This corresponds to 190 cm fork length when applying the weight-length conversion factor provided by ICCAT (Field Manual, 1990) whereas Rodriguez-Roda (1967) found that Mediterranean bluefin tuna seem to mature as early as age 3, at a weight of 15 kg and be fully mature by age 5.

Reports from the Scientific Committee for Research and Statistics (ICCAT) have assumed that bluefin tuna first spawn successfully at age 8 in the Atlantic compared to age 5 in the Mediterranean. However, according to de la Serna *et al.* (1999), age at first maturity in the Mediterranean occurs before the estimated ages. This may be due to the different environmental conditions. Some scientists even attribute this different behaviour to over fishing.

Taking into consideration that identifying the age at first maturity of highly exploitable fish species like bluefin tuna is critical for the effective management (Huppell & Sullivan, 2000, SCRS, 2001) extensive investigation to study reproductive studies and age at first maturity was proposed. Also if different ages for the Mediterranean and the Atlantic could be fully proven, this could lead to the conclusion that the Mediterranean population is a different sub population with its own characteristic features.

During the last 5 years, the Standing Committee for Research and Statistics (SCRS) of ICCAT has strongly recommended more research to further enhance the reproductive studies of bluefin tuna since these are essential for the sustainable management of populations.

4.3 Materials and Methods

4.3.1 Reproductive Studies

Sex determination of bluefin tuna can only be done by directly examining the internal organs. Thus, information on sex could only be obtained from carcasses that have not yet been eviscerated.

The gonads of bluefin tuna can be found in the ventral part of the body cavity and in sexually mature fish; both male and female gonads are often over 30 cm long. Distinguishing between male and female tuna is relatively easy. Male gonads have a relatively uneven appearance and irregular shape, with many nodules present on the external surface. Cross sections of male gonads have a characteristic rectangular shape and when sexually ripe, milt is easily seen. In contrast females generally have a smooth external appearance and in cross section, the gonad is oval in shape and occasionally has a hole (or lumen) in the middle.

Five Maltese fishermen were asked to retain bluefin tuna on board as a whole fish i.e. without removing the gills and guts. Upon arrival to the main landing port in Malta, the author went to do the observations and collect the necessary samples. First identification of sex was carried out and then the female gonads that were identified were weighed on board using a small electronic scale.

This measurement was then used to calculate the Gonadosomatic Index (according to Kume & Joseph, 1969) of bluefin tuna. The Gonadosomatic Index is a numerical value

which gives an indication of the maturity stage of the organism. It can be calculated as follows:

$$\textbf{Gonadosomatic Index} = \frac{\textbf{Weight of Gonads in g}}{\textbf{(Fork Length of Fish in cm)}^3} \times 10^4$$

From the value obtained, one can get an idea of the maturity stage of the fish. It also gives an indication of the type of eggs present at different size classes.

Samples were also collected from all the 48 female gonads observed in order to study the absolute fecundity of the species by means of histological studies. In the case of the gonads, the samples taken were a cross section throughout the whole thickness of the ovary because it has been demonstrated that the size and the maturity of oocytes vary radially along the distance to the ovary centre in several teleostean fish species (Emerson *et al.*, 1990).

In each case, small samples of tissue were cut using a chemically cleaned scalpel as soon as the fish was gutted. The histological processing entails the risk of variation in the volume of tissues as well as their deformation. In the case of gonads, these variations usually increase as the follicular development progresses. In order to avoid these as much as possible, each sample was kept in a small bottle labelled with the tag number and full of phosphate buffered formalin. In order to preserve the tissues well it was made sure that the tissues for preservation did not exceed a volume of 5 cm^3, that they were cut as soon as possible and that the volume of the phosphate buffered formalin in each sample bottle was always three times as much as the volume of the tissue.

In the laboratory the phosphate buffered formalin in each sample bottle was replaced with a fresh solution. Afterwards the sample bottles were kept in a cool place until the histological analysis could be performed.

Before starting the embedding in wax, it was important to take the tissue sample out of the sample bottle and dry the excess fixative solution. Then a sample of around 0.5 cm thickness was cut. This was then washed in phosphate buffered formalin for three times (20 minutes each), dehydrated in a series of ethanol solutions of increasing concentration, n-butylic alcohol and embedded in paraffin wax using an automatic tissue processor.

After the excess n-butyl alcohol was drained, the samples were submerged in liquid wax at 60 °C for 2 hours. The samples were then transferred to another container full of liquid wax and left there for 2 hours for embedding.

5 μm thick sections were cut using a microtome. Before cutting, each paraffin block was carved in a truncated pyramid form so that in the sectioning the order of the same series could be easily recognisable.

During cutting the preferred thickness was in the range of 5 and 10 micrometres since this is the thickness of a mature oocyte. For each sample, the maximum number of sections that fit the glass was taken. At least, two separated samples with a minimum distance equal to the diameter of an oocyte of the greatest size were cut.

The samples were then stretched in a bath with distilled water at 40 °C. Once stretched, the sections were gathered with the glass properly labelled and that would have been previously treated with an adherent solution (poly-l-lysine, 0.02% in distilled water). Sections were then dried for a minimum of 24 hours in an oven at 37 °C.

The procedure shown below was used to dewax and stain the samples with Mallory's Trichrome stain.

Xylene	10 minutes
Xylene	10 minutes
Xylene	10 minutes
Alcohol, 100%	5 minutes
Alcohol, 100%	5 minutes
Alcohol, 96%	5 minutes
Alcohol, 96%	5 minutes
Alcohol, 70%	5 minutes
Alcohol, 50%	5 minutes
Rinse in distilled water	5 minutes
Sublimate corrosive	20 minutes
Distilled water,	just rinse
Fuschin acid	1 minute
Distilled water,	just rinse
Phosphomobilic acid	75 seconds
Distilled water,	just rinse
Liquid Mallory	75 seconds
Distilled water,	just rinse

The sections were then dehydrated after the stain by immersing in 96 % ethanol for 5 minutes and by immersing in absolute ethanol 100 % for another 5 minutes.

The stain was then fixed with eucalyptol for 15 minutes and clarified with xylene for 10 minutes. Slides were mounted with DPX. After keeping for 24 hours at 37 °C for the polymerisation of the mounting media, the sections were ready for microscopic examination.

All slides were carefully observed under the microscope and then photographs were taken using the camera attached to the microscope. Photographs were taken both under low and high power.

To estimate the reproductive condition of bluefin tuna, two different histological classifications systems were used, one for estimating sexual maturity and the other for assessing the activity stage of mature females. Each ovary was histologically classified according to both systems (Hunter & Goldberg, 1980).

A female is considered sexually mature when it has the capability of reproduction in a determinate spawning season. Histological signs of sexual maturity are the presence in the ovary of yolked oocytes, hydrated oocytes or post ovulatory follicles. The immature females that have not yet reached sexual maturity are unable to reproduce in a determinate season.

Four different stages of sexual activity have been considered:

- **Inactive females**: no yolked oocytes and no atresiac structures present
- **Prespawning females**: these females show signs of an imminent spawning like hydrated oocytes or oocytes present in the nuclear migration phase. In this case, no

postovulatory follicles or extended atresia are present. Oocytes densely stored in the ovary.

- **Spawning females**: histological analysis indicate signs of past spawning (post ovulatory follicles) and enough vitellogenic oocytes to complete more spawning.
- **Post spawning females**: these females show signs of past spawning (post ovulatory follicles) but not enough vitellogenic oocytes to complete more spawning. In this case extended atresia would be present and also the oocytes are not densely packed.

4.3.2 Age at first maturity

Bluefin tuna ovary and spine samples were collected from 501 bluefin tuna during the bluefin tuna fishing season i.e. between May and September 1999-2003 in several locations in the eastern, central and western Mediterranean (Fig. 4.1). For each fish, the fork length (FL) was measured to the nearest centimetre as described in Chapter 2 and date and place of capture were recorded.

Fig. 4.1 Locations throughout the Mediterranean from where bluefin tuna gonads and spines were collected for the determination of age at first maturity

Ovary slices were fixed in 10% buffered formalin and histological analysis was carried out as described above in section 4.3.1. Oocyte atresic stages were classified according to Hunter and Macewicz (1985). On the basis of the classification scheme used, the distinction between immature and mature inactive fish was based on the presence of atresia of vitellogenic follicles, sign of past reproductive activity. As previously reported (Corriero *et al.*, 2003), no sign of atresia can be observed in bluefin tuna captured some months after the reproductive season. Therefore, for the present study the only samples used were those collected during a temporal window (May-September). This allowed a clear distinction between mature and immature specimens.

The body length at median sexual maturity (L_{50}) was estimated using nonlinear regression. A derivative-free maximum likelihood method (Proc NLIN; SAS, 1989) was used to fit percentage maturity by 5 cm length class to the two-parameter logistic model,

$$P_x = 100/(1+\exp^{(a-bFL)})$$

Where P = percentage mature at length x; and

$L_{50} = (-a/b)$.

4.4 Results and Discussion

4.4.1 Reproductive Studies

From the analysis of the data of Table 4.1, the female bluefin tuna caught around the Maltese Islands, were present during their spawning season and all of them were adults. The mean gonadosomatic index for the whole sample was 4.75 with a standard deviation

of 0.82. Such a high average for the gonadosomatic index indicates the presence of mature spawning females. When high values of gonadosomatic indices are present within a stock, the maturity of the stock is assured to be fully recruited and will therefore constitute the annual potential fecundity for the whole population.

Gonad Weight (kg)	GSI
1.75	0.8
1.75	2.55
1.75	1.06
1.8	1.48
2.5	1.14
3.6	3.62
4.75	4.92
4.75	4.46
1.2	1.62
1.5	1.23
4.5	2.16
7	5.34
6	7.5
5.5	4.83
11	8.37
10	7.61
11	5.41
3.7	2.29
2.2	3.21
1.8	3.66
1.7	3.85
0.7	0.5
1.3	5.78
1.7	6.33

Gonad Weight (kg)	GSI
2	5.8
2.2	5.08
0.85	5.73
1.1	5.77
1.3	6.01
1.2	5.72
1	5.12
0.63	4.61
1.45	5.52
0.95	4.86
1.4	5.21
1.1	5.12
1.7	5.24
2.2	4.99
0.55	4.02
0.68	3.57
5	4.39
6	3.66
13.5	12.68
3.45	4
3	19.22
5	2.75
5	3.85
1.4	5.42

Table 4.1 Gonad weight and calculated Gonadosomatic Index for female bluefin tuna observed

Histological studies carried out on bluefin tunas caught by the Maltese longliners further confirm that all the bluefin tuna females studied were mature. These females contained a large number of yolked oocytes, nuclear migration stage oocytes or postovulatory follicles. All these factors suggest an imminent or recent spawning period.

Regarding the activity stages, the studied bluefin tuna ovaries could be classified in three different stages (Fig. 4.2, 4.3 and 4.4):

12 ovaries (or 25 % of the whole sample) were in the prespawning stage;
34 ovaries (or 70 % of the whole sample) were in the spawning period and had enough vitellogenic oocytes to complete several more spawnings in the same season;
whilst the remaining 2 ovaries (or 5 % of the whole sample) showed completely spent ovaries.

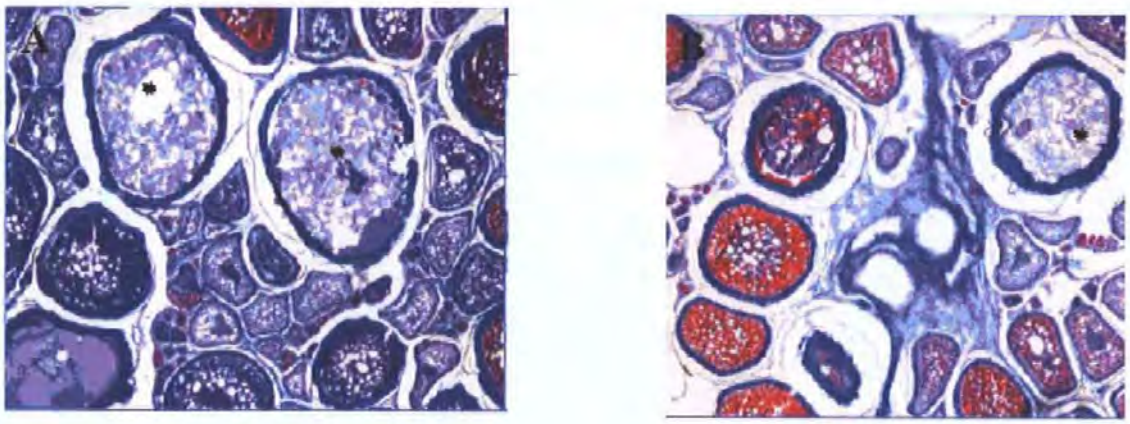


Fig. 4.2 A and B Microphotographs of gonads of bluefin tuna caught in Malta. Mature spawning BFT female showing hydrated oocytes (Microphotographs taken at the Histological Laboratory of IEO Malaga, Spain

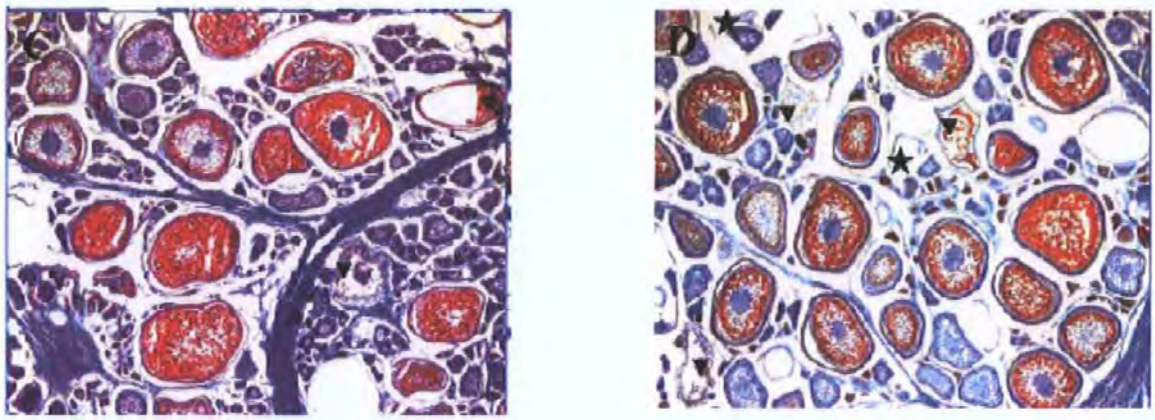


Fig. 4.3 C and D Microphotographs of gonads of bluefin tuna caught in Malta) Mature female showing atresia and postovulatory follicles. (Microphotographs taken at the Histological Laboratory of IEO Malaga, Spain

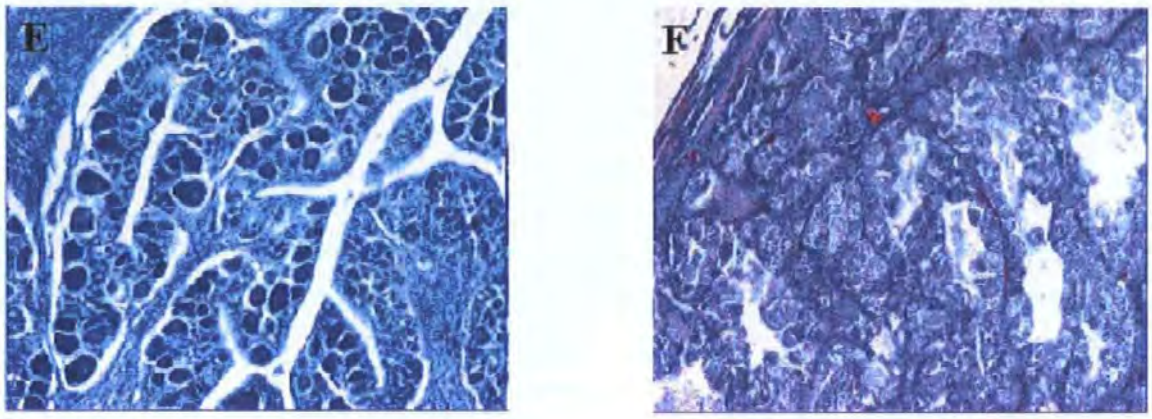


Fig. 4.4 Microphotographs of gonads of bluefin tuna caught in Malta. E Immature female F Mature male (Microphotographs taken at the Histological Laboratory of IEO Malaga, Spain)

The above results were compared to similar works carried out in the Mediterranean. Hattour, A. (2002) carried out histological analysis of bluefin tuna ovaries coming from Tunisian purse seiners. These had been caught during the months of April and May, 2001. Results showed that in this case the bluefin tunas were present in a prespawning stage and that they were going to spawn imminently, possibly in the central Mediterranean.

The histological analysis of bluefin tuna ovaries caught by Libyan fishermen during the months of May and June, 2000 showed that all the females were mature with an imminent or recent spawning period. These results indicate that these tunas were spawning in a

nearby area, possibly in the central Mediterranean during the period between late May and mid June (Tawil, M. *et al.*, 2001).

All the above results strongly indicate the presence of a spawning area very close to the Maltese Islands, i.e. in the central Mediterranean during the period between late May and the beginning of June. The presence of sexually mature female bluefin tuna and the presence of a spawning area in the Mediterranean lead to the conclusion that the Mediterranean bluefin tuna is capable of completing its life cycle within the Mediterranean and that a permanent residing stock could be present.

4.4.2 Age at First Maturity

On the basis of the ovary classification scheme used, 57 individuals (11.3% of the specimens analysed) were immature and 444 (88.7%) were mature (Table 4.2, Fig 4.5).

Size class (FL in cm)	Size of sample	% Mature
< 95	16	0
95 – 99	2	0
100 – 104	68	60
105 – 109	21	43
110 – 114	21	90
115 – 119	21	81
120 – 124	27	77
125 – 129	30	90
130 – 134	37	89
135 – 139	40	100
≥ 140	266	100

Table 4.2 Percentage and frequency of mature and immature bluefin tuna female specimens by size class

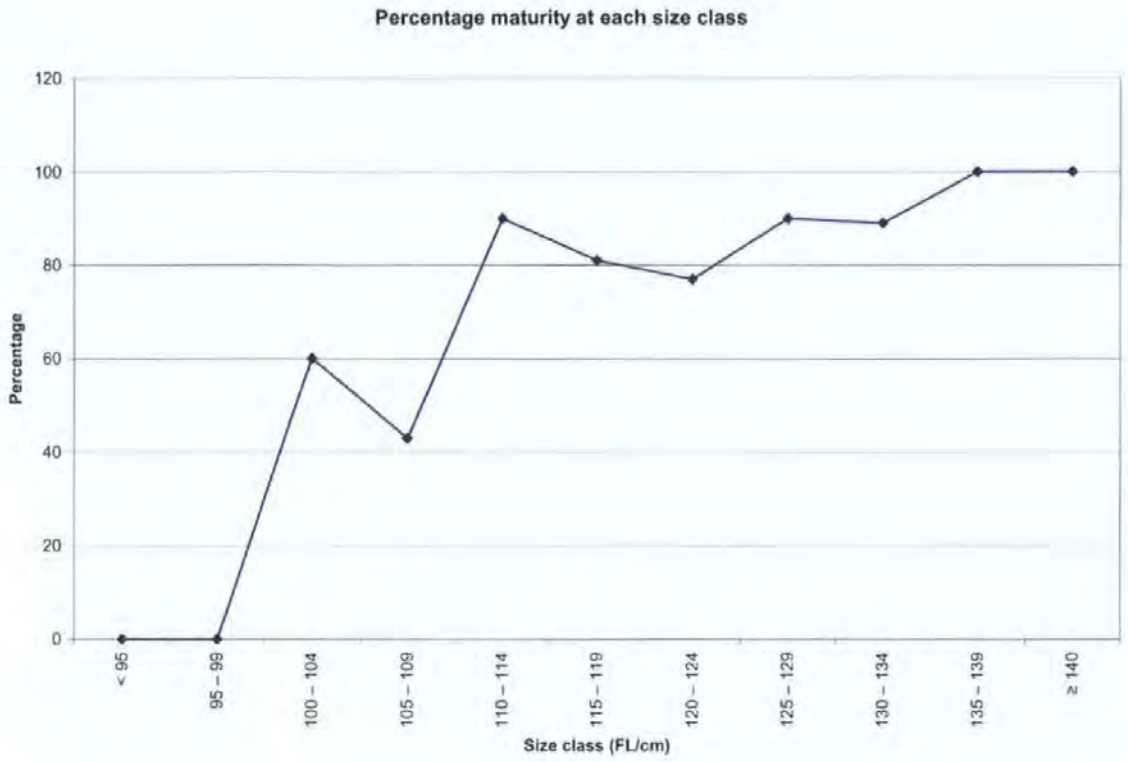


Fig 4.5 Variation of percentage maturity with size class

The ovaries of the immature fish were characterized by previtellogenic or early vitellogenic oocytes and absence of atresic vitellogenic follicles.

On the basis of the histological pattern, the mature fish were additionally subdivided as follows: 269 (54% of the total sample) were active non-spawning since they showed late vitellogenic oocytes but no sign of recent or imminent spawning; 39 8% of all the fish sampled were active spawning as they showed post-vitellogenic oocytes and/or postovulatory follicles; 7% of all the fish analysed were inactive because they had vitellogenic follicles but displayed also major vitellogenic atresia or they had previtellogenic or early vitellogenic oocytes plus late stages of atresia. No mature fish was found below 100 cm. All the fish of over 135 cm were found to be mature.

All the spines analysed showed the complete formation of the ring corresponding to the previous year of their life. Among the 20 fish included in the 100-104 cm size class, 16 belonged to the age group 3 and 4 to the age group 4. The 40 fish contained in the 135-139 cm size class belonged to the age group 4 (4 individuals) and 5 (36 specimens).

In this section, the size of first sexual maturity for the Mediterranean bluefin tuna using a method based on the statistic elaboration of data coming from the histological analysis (De Martini *et al.*, 2000) was determined.

On the basis of the macroscopic evaluation of the ovary maturity stage, Rodriguez-Roda (1967) estimated that 50% of the female bluefin tuna of the Mediterranean stock are reproductively active at the size of 97.5 cm (fork length), while 100% maturity is reached between 115 and 120 cm. Tawil *et al.* (2001) in a preliminary approach to the study of sexual maturity based on the histological analysis of the ovaries of 21 bluefin tuna, reported mature specimens above 115 cm (fork length). In a stereological study on bluefin tuna fecundity, Medina *et al.* (2002) reported that the smallest mature female sampled in the Balearic waters was 116 cm FL. During a histological description of the ovarian cycle, mature females over 110 cm FL were found (Corriero *et al.*, 2003). Since none of the cited investigations have been carried out with the methodology needed for the determination of the first sexual maturity, the information provided are nevertheless useful for stock management and tend to support the evidence of the author's experiment.

The present study, performed on a consistent number of specimens with the required methodology for this type of research, indicates that 50% of the Mediterranean bluefin tuna reaches the first sexual maturity at 104 cm (fork length).

The analysis of the spines indicates that all the specimens had completed the formation of the ring corresponding to their previous year of life. This finding is in agreement with Cort (1991) and Megalofonou and De Metrio (2000) who reported that ring completion occurs during April and May for bluefin tuna caught in the Mediterranean. This data indicates that 100% maturity is reached at the age of 4-5 years.

In the case of the Western Atlantic, no study reports the size of 50 % sexual maturity. The only data available indicates that maturation starts at the age of 6 and 100 % maturity is reached by the age of 8 years at a fork length of 190 cm (NRC, 1994).

4.5 Summary

1. The time of spawning and location in the Mediterranean appear established but methods of determining sexual maturity need to be refined.

2. Adult bluefin tuna targeted in the Mediterranean during the months of May and June are sexually mature and present during their spawning period.

3. The presence of spawning fish within the Mediterranean indicates that the bluefin tuna can complete its life span in the Mediterranean without having to migrate into different waters in order to complete its life cycle.

4. A similar study should be extended to the male bluefin tuna to determine whether they follow the same reproductive process.

5. Mediterranean bluefin tuna start to mature at age 4-5, having reached a fork length of 100 cm.

6. At age 5-6 and having a fork length of 135 cm all Mediterranean bluefin tuna females are sexually mature.

7. It can be concluded that for the Mediterranean, female bluefin tuna reach first sexual maturity at a markedly lower age and size than the western Atlantic population.

8. The difference found between Western Atlantic and the Mediterranean provides further evidence for the existence of a separate Mediterranean population.

Chapter 5

THE DEVELOPMENT OF ELECTRONIC TAGGING EXPERIMENTATION OF BLUEFIN TUNA IN THE MEDITERRANEAN

5.1 Introduction

The argument for the presence of a resident stock in the Mediterranean can be strengthened through the use of pop off satellite tags which are employed to study the large-scale movements of large pelagics which frequent the surface.

This tagging section aims to identify and describe the migrations and movements of bluefin tuna within the Mediterranean and also if present between the Mediterranean and the Atlantic Ocean.

This part of the project was carried out in collaboration with FAO-COPEMED Large Pelagics Project and Prof. Gregorio de Metrio (University of Bari, Italy). The author was in charge of all the organisation of work involved. The bluefin tunas were made available for this research by AJD Tuna Ltd. and Melita Tuna Ltd. Tags were provided by the University of Bari. The tagging was carried out by Prof. Gregorio de Metrio because of the very high costs of equipment involved and due to the critical situation of the bluefin tuna once on board the fishing vessel no errors could be risked. Decoding of data was all carried out in the University of Bari.

5.2 Literature review

To understand better the life history of bluefin tuna and develop competent management strategies, temporal and spatial movement patterns must be identified (Gulland, 1983). Data on bluefin tuna dispersal patterns have been difficult to obtain because of the limited resolution of analytical tools available for studying pelagic fish. To date, tag and recapture programs using conventional tags (fisheries dependent indicators of movement) have been

the method of choice for describing the distribution of Atlantic bluefin tuna (Lehodey *et al.*, 1997).

Conventional tags and released data indicate that trans Atlantic movements of all size classes of Atlantic bluefin tuna occur (Brunemeister, 1980; Cort *et al.*, 1993; Mather *et al.*, 1995). Recently microprocessor-based, data storage tags (archival tags) have been developed for monitoring the geoposition (based on ambient light levels), thermal physiology and diving behaviour of large pelagic vertebrates (Block *et al.*, 1998; Delong *et al.*, 1992; Metcalfe *et al.*, 1997).

Although the data intensity of archival tags is high (2 megabytes), their major limitation is the need to recapture the animal to access the data. This requires the deployment of large numbers of tags in species with high exploitation rates. In addition, the multinational nature of most oceanic fisheries complicates the coordination of archival tag recoveries. Archival tags have been deployed recently on Atlantic bluefin tuna (Block *et al.*, 1998) but significant numbers of returns take years to retrieve. Block *et al.*, (2005) analyzed the data over a nine-year period and discovered that bluefin tuna have a complex migratory life-cycle that varies depending on the season, as well as the age and body size of the fish.

Satellite tags (conventionally towed or attached) have been employed to study the large-scale movements and physiology of marine mammals, birds and sea turtles (Jouventin *et al.*, 1990; Kooyman *et al.*, 1996; Mc Connell *et al.*, 1992; Papi *et al.*, 1995; and Renaurd *et al.*, 1994). These tags have been deployed successfully to basking sharks (Preide, 1984) but are only applicable for the largest pelagic fishes that frequent the surface. The new pop off methodology will broaden the scope of satellite tag utility to most large pelagic organisms.

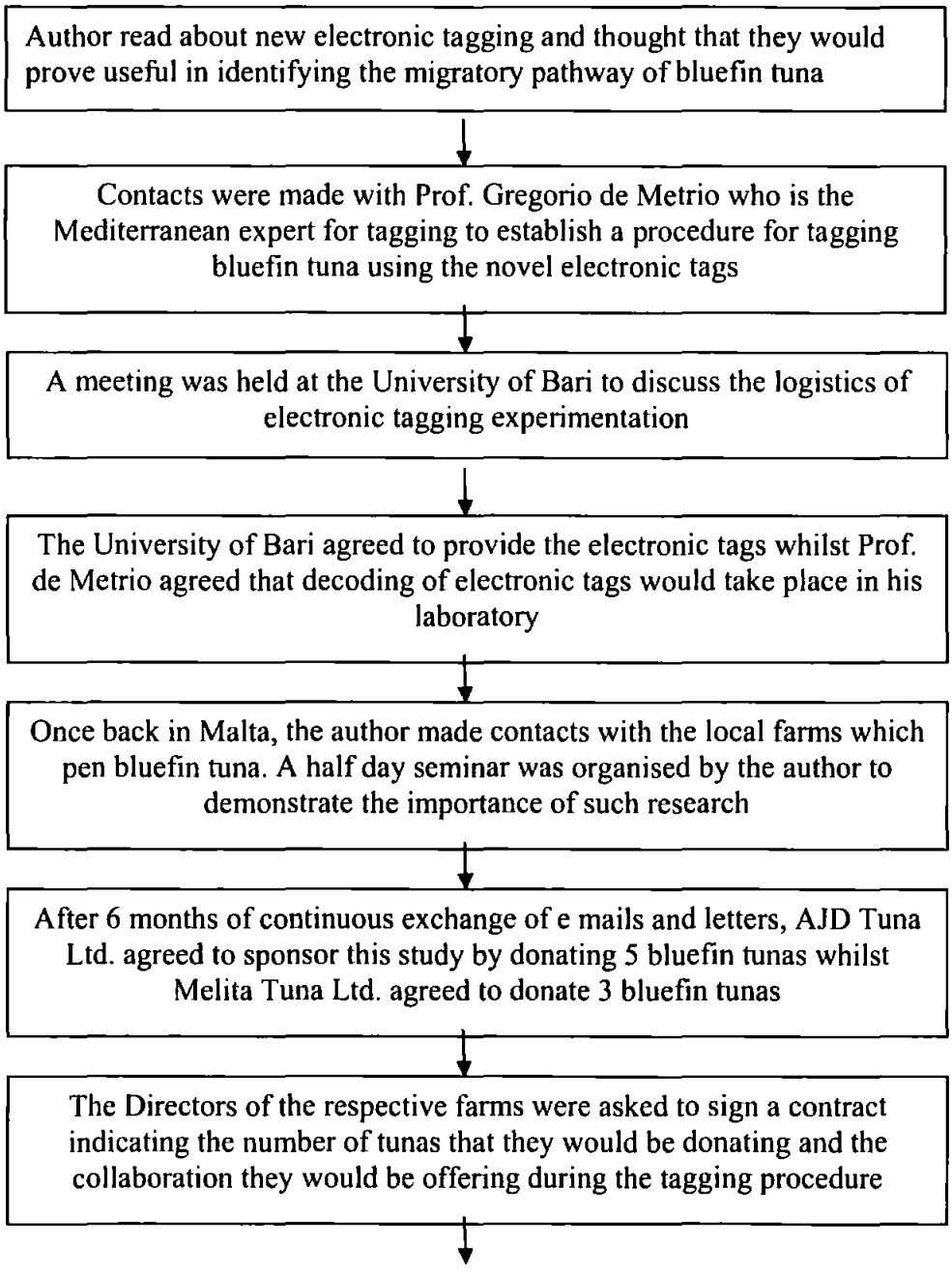
Cort & Liorzou (1995) revised tagging data available since the initiation of tagging activities until 1993 and came up with various conclusions including the fact that trans-Atlantic migrations of bluefin tuna are possible and occur on a frequent basis. They also reported recapturing a bluefin tuna which had been tagged in the Atlantic. Turner (1999) continued studying the frequency of trans-Atlantic migrations and determined the percentage of tagged tuna which crossed the Atlantic.

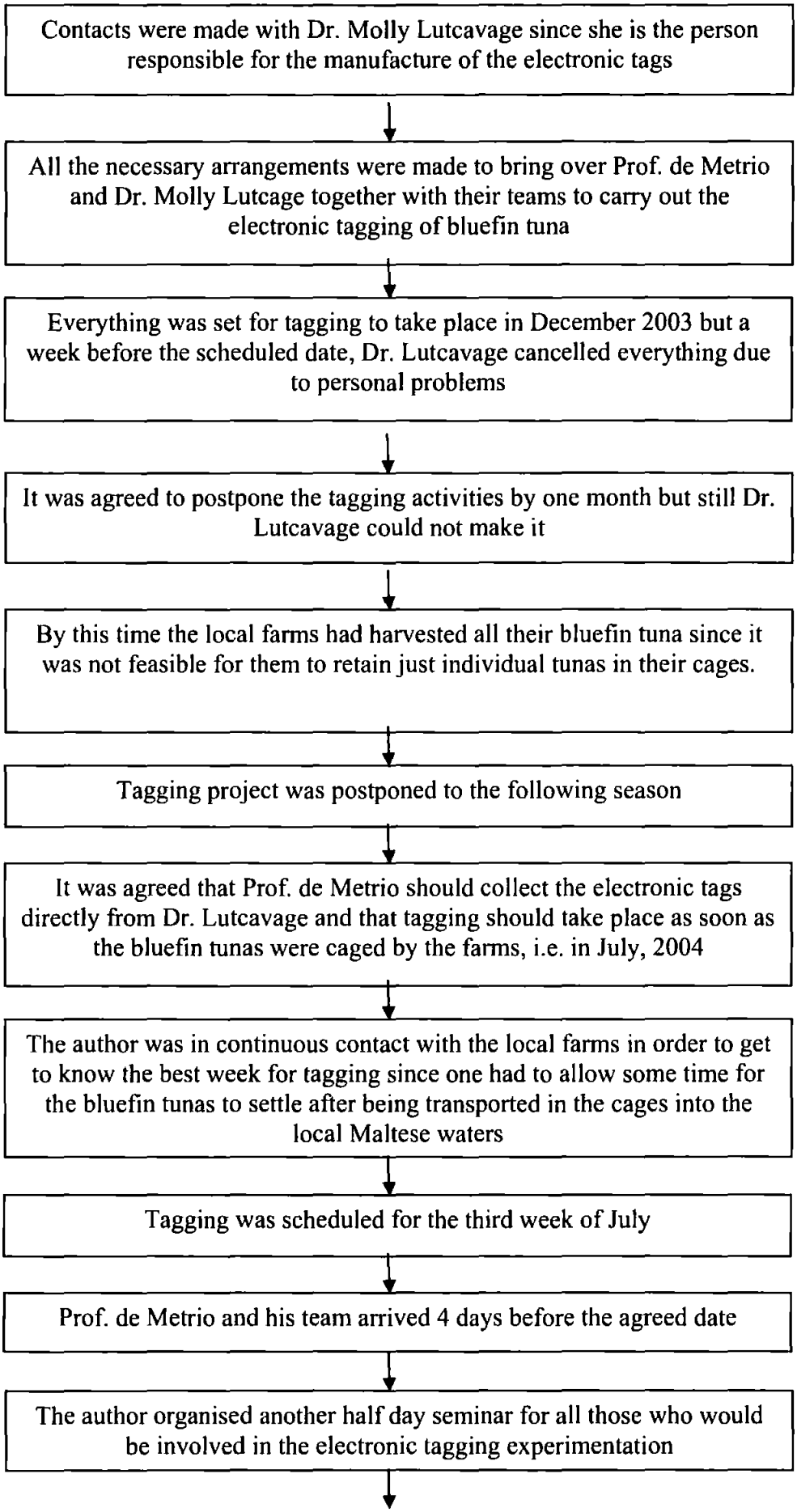
The successful deployment in recent years of implantable and pop up satellite archival tags has rapidly enabled researchers to examine the movements of large pelagic fishes (Metcalfe & Arnold, 1997; Block *et al.*, 1998, Lutcavage *et al.*, 1999; Kitigawa *et al.*, 2000; Marcinek *et al.*, 2002; Block *et al* 2001; Gunn & Block 2002; Boustany *et al.*, 2002; Seitz *et al.*, 2002). These new techniques are providing the major advances that will be necessary to understand the distribution of oceanic organisms in relation to their changing physical and biological environments.

The development of electronic tags began to give a different picture of movements of bluefin tuna marked off the east coast of the United States and in the Gulf of Mexico than had been possible by means of conventional tags (Block *et al.*, 1998, 2000; Lutcavage *et al.*, 1999, 2000). Additional work with electronic tags by European scientists (De Metrio *et al.*, 2000) began to provide some answers to the other half of the distribution and movement problem. At this point in time, the SCRS is beginning to examine other hypothesis on movement and mixing of fish between the east and the west and between the Atlantic and the Mediterranean, including consideration of reassessing the present stock division boundary.

5.3 Materials and Methods

The most crucial part of this experiment involved the development of an effective procedure for the electronic tagging of large pelagics. Such a procedure means getting everything and everybody ready for the tagging process. Planning and managing such an expensive project involved a large contribution from the part of the author and took almost two years to become a reality. Fig 5.1 gives an overview of the operational research involved.




Contacts were made with Dr. Molly Lutcavage since she is the person responsible for the manufacture of the electronic tags
All the necessary arrangements were made to bring over Prof. de Metrio and Dr. Molly Lutcage together with their teams to carry out the electronic tagging of bluefin tuna
Everything was set for tagging to take place in December 2003 but a week before the scheduled date, Dr. Lutcavage cancelled everything due to personal problems
It was agreed to postpone the tagging activities by one month but still Dr. Lutcavage could not make it
By this time the local farms had harvested all their bluefin tuna since it was not feasible for them to retain just individual tunas in their cages.
Tagging project was postponed to the following season
It was agreed that Prof. de Metrio should collect the electronic tags directly from Dr. Lutcavage and that tagging should take place as soon as the bluefin tunas were caged by the farms, i.e. in July, 2004
The author was in continuous contact with the local farms in order to get to know the best week for tagging since one had to allow some time for the bluefin tunas to settle after being transported in the cages into the local Maltese waters
Tagging was scheduled for the third week of July
Prof. de Metrio and his team arrived 4 days before the agreed date
The author organised another half day seminar for all those who would be involved in the electronic tagging experimentation

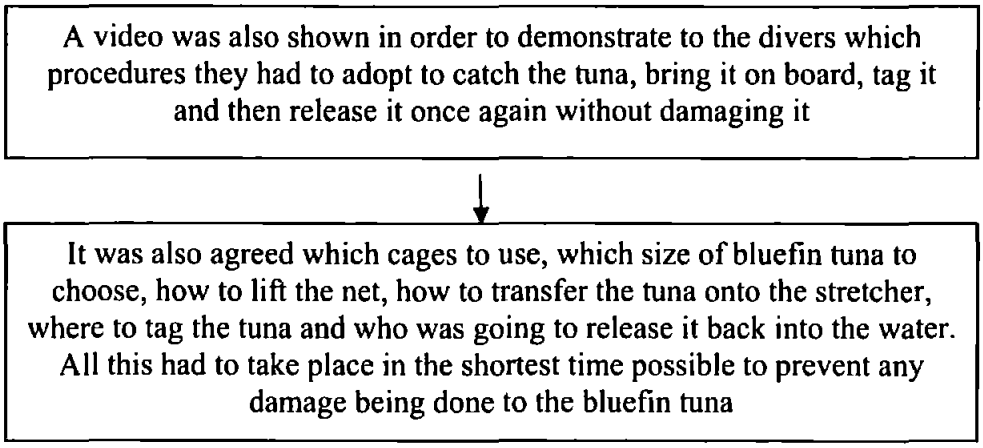


Fig 5.1 Overview of the operational research of the tagging project

A total of eight bluefin tunas were tagged around the Maltese Islands using PAT tags (Wildlife Computers INC, Redmond, WA, USA).

Each satellite tag attached externally to a fish is released at a pre-programmed time because of a corrosive linkage. It then floats to the surface and transmits continuously to ARGOS satellites. The tag provides a fisheries-independent measure of the straight-line distance travelled from the point of tagging. The lithium battery, microprocessor and 0.150 W satellite transmitter are all packaged in a carbon fibre tube. A streamlined float constructed of syntactic foam and a microballoon resin composite are secured to the trailing end of the tube. Each torpedo-shaped tag (Fig. 5.2) weighs between 65 and 71 grams in air. The centre of buoyancy and mass are such that the tag floats with the antenna extending upward upon reaching the surface. While attached the antenna extends parallel to the fish and the syntactic foam float provides sufficient lift to keep the tag off the body at low speeds.

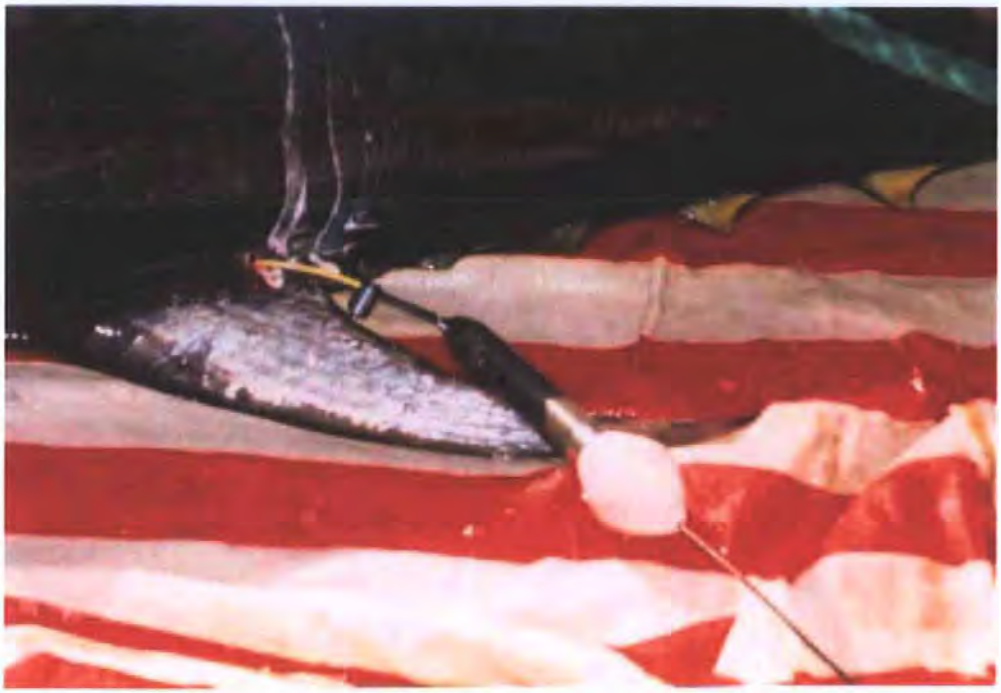


Fig. 5.2 Actual structure of the archival pop up tag used for tagging bluefin tuna

Tagging was carried out on farmed bluefin tuna (Fig. 5.3) caught by purse seine nets. This is the only gear which allows bluefin tuna to remain alive. All other gears including longlines lead to the death of the bluefin tuna and as such could not be used for tagging purposes since the tuna has to be still alive. Tuna caught by hooks remains alive for a maximum of 3 hours (Carabott, 1999, *pers. comm.*). If the hook is removed and the tuna is released again, the tuna would eventually die due to injuries which prevent it from eating.

The tags were programmed to detach themselves from the tuna and float to the sea surface after intervals of 120 and 300 days. A selection of the archived data could then be obtained through the Argos satellite system including the pop up position of the tag.

After intensive planning of the actual procedures to be carried out by the whole team involving more than 15 persons, the first tagging activity was conducted off the North East coast of Malta (35:58:40 N and 14:25:57 E) on the 20th July, 2004. The second tagging

activity was conducted off the South-East coast of Malta (35:50:72 N and 14:35:05 E) the day after. Details of the tagging activities are summarized in Table 5.1.

The bluefin tunas were caught individually by restricting the area of the net and lifting it (Fig. 5.4, Fig. 5.5 and Fig. 5.6). The individual tunas were pulled onto a prepared stretcher (wet vinyl covered pad) on the deck of a small fishing vessel. Once on deck, a saltwater hose was placed in the mouth of the bluefin tuna to irrigate the gills and the eyes were kept covered with a sea water soaked blindfold (Fig 5.7).

The curved body length was measured so that later on the body mass could be estimated. The fish could not be weighed directly since on board the weight is measured by hooking the tuna to the scales and this procedure would have killed the tuna.

Tagging was carried out on board using a hand-held harpoon. The pop off satellite tag was attached to the bluefin tuna by using a titanium dart machine. The dart was inserted 10 cm deep at the base of the second dorsal fin, where it was anchored through the bony projections and connective tissues radiating ventrally from the fin. Each tag was connected to its anchor by a 25 cm long monofilament leader attached through the eye loop at the front end of the tag. The eye loop was fixed in place by a thin, stainless steel wire that was exposed to sea water externally and connected internally to a battery. At the programmed time, the microprocessor activated the battery which then passed a low voltage across the wire promoting corrosion and release.

Each bluefin tuna was kept on deck for an average time of 2 minutes. Experiments carried out on captive tunas held in the Pacific indicated that because the tuna body narrows after the second dorsal fin, tags placed here have minimal contact with the body and therefore do no disturb with the normal swimming patterns (Block *et al.*, 1998).

ARGOS	Deployment	Deployment location			Programmed		Expected	BFT data		Tool used	Type of
IDs	Date	(Coordinates as Minute Decimal)	Lat. (N)	Long. (E)	Time		pop-up date	FL (cm)	W* (kg)	to tag	Dart
	(dd/mm/yy)	Area			(days)		(dd/mm/yy)				
7386	20/07/04	Off the north-eastern coast of Malta	35:58:40	14:25:57	120	Days	17/11/04	150	59	hand-held harpoon	Titanium
41804	20/07/04	Off the north-eastern coast of Malta	35:58:40	14:25:57	120	Days	17/11/04	145	53	"	"
41800	20/07/04	Off the north-eastern coast of Malta	35:58:40	14:25:57	150	Days	17/12/04	147	55	"	"
7457	20/07/04	Off the north-eastern coast of Malta	35:58:40	14:25:57	150	Days	17/12/04	172	86	"	"
41797	20/07/04	Off the north-eastern coast of Malta	35:58:40	14:25:57	180	Days	16/01/05	152	61	"	"
52617	21/07/04	Off the south-eastern coast of Malta	35:50:72	14:35:05	300	Days	16/05/05	220	172	"	"
41805	21/07/04	Off the south-eastern coast of Malta	35:50:72	14:35:05	300	Days	16/05/05	232	200	"	"
41799	21/07/04	Off the south-eastern coast of Malta	35:50:72	14:35:05	300	Days	16/05/05	229	193	"	"
* The weight of the fish was derived from the length – weight relationship suggested by José Louis Cort (1990) for the summer season.											

Table 5.1 Details of the tagging activities carried out in Malta, July 2004

Fig. 5.3 Bluefin tuna fattening cage from which bluefin tuna for tagging were captured

Fig. 5.4 Encircling the bluefin tuna in order to capture a single bluefin tuna and take it on board

Fig. 5.5 Directing the bluefin tuna onto the stretcher in order to be taken on board for tagging

Fig. 5.6 Lifting the net in order to make the area for bluefin tuna smaller and thus making capturing of bluefin tuna easier

Fig. 5.7 Lifting the bluefin tuna using a stretcher to take it on board

5.4 Results

Table 5.2 shows the results obtained from the tags that were detected by satellite after surfacing at sea. From the total of 8 tags released, 7 resurfaced. None were acquired on the expected pop up date but premature tag shedding took place. The tags surfaced after intervals of between 11 to 98 days.

The positions at which the various tags were first detected by the Argos satellite system indicated that almost all tags surfaced close to their release positions, however pop ups were observed throughout the Mediterranean, starting from close to the Maltese Islands (the point of release), up to Lampedusa, off the coast of Lebanon, off the coast of Cyprus, close to Linosa Island and the extreme eastern of the Gulf of Sirte (Fig. 5.8).

Home	ARGOS	Tag	ICCAT tag	Deploy ment	Programme d	Expected	Pop Up	Time of tag	Pop-up location (Coordinates as Minute Decimal)		
IDs	IDs	Ids	IDs	date (dd/mm /yy)	time (days/month s)	pop-up date	Date	on fish (days)	Area	Lat	Long
1	7386	04P01 97	05376	20/7/04	120 days	17/11/04	29/9/04	71	North-west off Malta	3642.00 N	1324.00 E
2	41804	04P02 13	05361	20/7/04	120 days	17/11/04	17/9/04	59	Close to Lamped usa Island	3530.00 N	1300.00 E
3	41800	04P02 09	05379	20/7/04	150 days	17/12/04	12/10/0 4	84	off the coast of Lebano n	3212.00 N	3412.00 E
4	7457	04P01 98	05377	20/7/04	150 days	17/12/04	26/10/0 4	98	off northea stern coast of Cyprus	3554.00 N	3448.00 E
5	41797	04P02 06	05374	20/7/04	180 days	16/1/05	16/8/04	27	Close to Linosa Island	3660.00 N	1248.00 E
6	52617	04P02 17	05369	21/7/04	300 days	16/5/05	10/9/04	51	Extreme eastern of the Gulf of Sirte	3124.00 N	1918.00 E
7	41805	04P02 14	05378	21/7/04	300 days	16/5/05					
8	41799	04P02 08	05373	21/7/04	300 days	16/5/05	1/8/04	11	Close to Linosa Island	3600.00 N	1242.00 E

Table 5.2 Table summarising tag returns

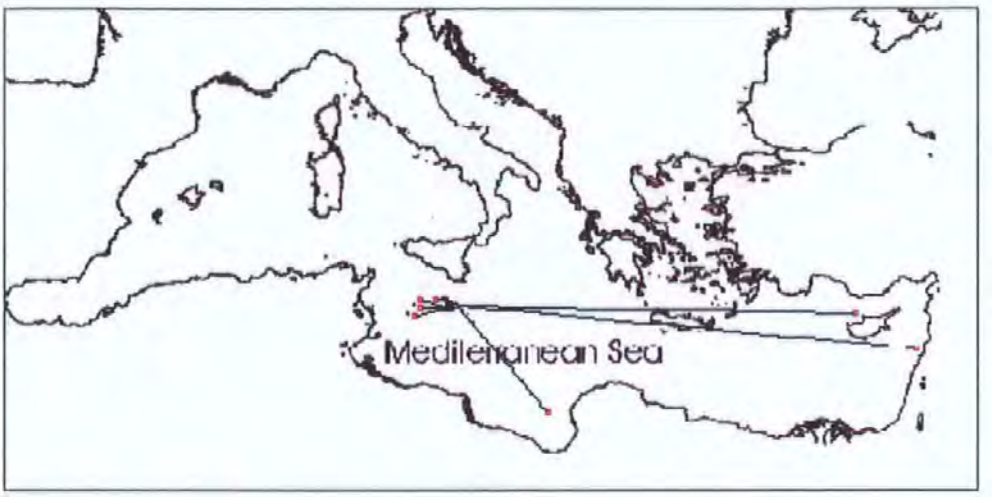


Fig 5.8 Pop up locations of tags attached to bluefin tuna

To date, only three sets of archived data have been processed. These were received from tags 5, 6 and 8, which were detected within 11 to 51 days after their deployment. The depth profiles showed that only one fish went deeper than 1000 m, when the safe release mechanism started the tag detachment. All the three bluefin tuna showed extensive vertical movements for the first few days after release.

5.5 Discussion

A recent review of the scientific bases for the management of Atlantic bluefin tuna recommends that the two stock hypotheses be rigorously tested especially now that there is an increasing evidence of a resident Mediterranean stock (ICCAT 1998). The report emphasised the need to quantify the extent of bluefin tuna movement within the Mediterranean and if present between the Mediterranean and the Atlantic regions and the effects these movements might have on the choice of management strategies. Determination of both the extent of mixing of mature bluefin tuna and the fidelity to a specific spawning ground is critical for future bluefin tuna management. The pop-off

satellite technology has the potential to improve the identification of discrete biological stocks, which is paramount to the implementation of effective management.

The results of the tagging activities carried out indicate that all the tagged bluefin tuna remained in the Mediterranean and none migrated into the Atlantic. In fact pop ups were observed throughout the Mediterranean, starting close to the Maltese Islands (the point of release), up to Lampedusa, off the coast of Lebanon, off the coast of Cyprus, close to Linosa Island and to the extreme eastern of the Gulf of Sirte. These results may further confirm that not all the bluefin tuna migrate back into the Atlantic but that some remain in the Mediterranean throughout their whole life cycle. However, the timescales observed are very short in comparison to the life span of the bluefin tuna.

A major shortcoming of this study is that the number of tagged bluefin tuna was very small, only 8 bluefin tunas being tagged. The cost of materials was the main limiting factor. Each pop off satellite tag costs in excess of 1000 Euros whilst each alive bluefin tuna has a potential market value of more than 10 Euros per kilogram. The costs of this experiment were more than 20 000 Euros without taking into account other overheads incurred like for example the cost of bringing the experts to the tagging site and paying the divers which helped during the activity. In order to make the results significant, this type of tagging activity has to continue both in the Atlantic and in the Mediterranean.

A further shortcoming was that the pop ups took place before the set dates. This fact was reported to the Wildlife Computers INC, USA so that if possible it will be prevented in future tagging activities. If pop ups could be programmed to take place after longer periods they could be used to provide more conclusive evidence of where the bluefin tuna

completes its life cycle. One has to remember that the complete life cycle of bluefin tuna can take more than 15 years.

5.6 Summary

1. Advances in tagging technology will assist in defining migration patterns.

2. The results obtained so far lend support to the theory of a separate residing stock in the Mediterranean. When further tagging activities will be carried out in the future, they will provide the best evidence of the separate bluefin tuna stocks which are present in the Mediterranean and in the Atlantic.

3. Early release of tags may have compromised the quality of the anticipated evidence.

Chapter 6

INNOVATIVE APPROACHES IN STOCK IDENTIFICATION

6.1 Introduction

In the first part of the experiment, the stock specificity and stability of chemical signatures in the otoliths of bluefin tuna will be analysed. Otolith chemistry of juveniles from eastern and western Atlantic regions will be quantified to determine the discriminatory power of otolith chemistry for stock identification.

This research was carried out in collaboration with Dr. Jay Rooker (Texas A & M University at Galveston, USA). Organization of work and collection of samples was all carried out by the author in Malta. Extraction of the otoliths was done under the supervision of Dr. Jay Rooker who visited Malta for this purpose. Otoliths were then transferred to the University of Galveston, USA where chemical analysis was carried out. The main aim of this part of the experiment was to compare the otolith chemistry of Atlantic and Mediterranean bluefin tuna. At the moment a paper is being prepared with the results obtained at the end of the experiment.

Recently, several recent population genetics studies, using both nuclear (Pujolar, 2001) and mitochondrial (Vinas, 2001) molecular markers, could not reject the hypothesis of a single genetic population of the Mediterranean bluefin tuna population. However the opportunity of having samples from new localities not studied previously could corroborate the single population supposition in the Mediterranean (Vinas *et al.*, 2002).

This second part of the experiment was carried out in collaboration with FAO-COPEMED Large Pelagics Project and in collaboration with Dr. Carles Pla (University of Girona, Spain). Three courses were attended in Fuengirola, Spain. Samples in Malta were collected by the author whilst the other samples were collected by observers who attended

the same courses and used the same sampling methodology. Genetic samples were then processed and studied by the author in the University of Girona, Spain under the supervision of Dr. Carles Pla. Several papers have been presented by the author during the annual ICCAT Scientific Committee for Research and Statistics (1999, 2000, 2001 and 2002).

6.2 Literature review

The premise of otolith chemistry is that certain elements are incorporated into otoliths in proportion to their concentrations in the environment and thus these elemental fingerprints can be used to distinguish individuals from different environments or regions. It also provides age and growth information and contains a lot of information about the organism's habitat.

Otolith chemistry is increasingly used as a technique to differentiate stocks, and interest in its application as a recorder of time and environmental conditions has increased substantially in the past decade (Campana, 1999; Thresher, 1999; Secor & Rooker, 2000; Campana & Thorrold, 2001). Otoliths precipitate as the fish grows and elements from the individual's surroundings are integrated into the aragonite-protein matrix. As otoliths are metabolically inert, resorption or remobilisation of newly deposited elements during ontogeny is negligible. Consequently, the chemical composition of otoliths may serve as natural tags or chemical signatures that reflect differences in the chemical composition of the individual's habitat.

Recent work suggests that otolith chemistry can be used to identify natal origin and assess the relative contribution of different nursery areas to mixed adult stocks (Thresher, 1999;

Thorrold *et al.*, 1998, 2001). Moreover the approach has been used recently to assess stock specificity of tunas and findings suggest that otolith elemental analysis has promise for assessing the population connectivity of pelagic stocks (Rooker *et al.*, 2001b). As such chemical analysis of the otoliths may prove an important tool in the identification of stocks.

In the Atlantic Ocean, the existence of two putative stocks is generally assumed (ICCAT, 1997). This division is based on the knowledge of two assumed separated spawning grounds, one in the Mediterranean Sea and one in the western Atlantic. Recent genetic studies which have been carried out with bluefin tuna, try to confirm the possible stock differentiation between the western Atlantic and eastern Atlantic – Mediterranean stock (Pujolar & Pla, 2000; Alvarado Bremer *et al.*, 1999).

Concerning the Mediterranean Sea, a series of genetic studies were carried out in recent years (Pla *et al.*, 1995; Pla *et al.*, 1998; Pujolar & Pla, 2000). These studies using both protein electrophoresis and mitochondrial DNA sequencing could not reject the hypothesis of a single genetic population of Mediterranean bluefin tuna.

De la Serna *et al.*, (2000) reported data on a bluefin tuna recaptured in a trap located in the Spanish south - Atlantic coast, close to the Strait of Gibraltar. The recaptured tuna was tagged in the Western Atlantic. Furthermore, this document reported for the first time the preliminary genetic study of 15 bluefin tuna belonging to the same shoal as the recaptured one. The results from the genetic analysis of the mitochondrial DNA show a distributional pattern similar to the one found for several places in the eastern Atlantic.

It has been recommended by ICCAT to go deeply into genetic analysis of tuna captured during migration in order to get to know better the migratory pathways of the bluefin tuna and enhance the knowledge of the genetic population structure of the Mediterranean Sea. This could shed more light on the identification of Mediterranean stocks.

6.3 Materials and Methods

6.3.1 Chemical Analysis of Bluefin Tuna Otoliths

The most crucial part of this experiment involved finding a sponsor and then developing a method for the extraction and analysis of bluefin tuna otoliths. All the planning and management of this experiment was carried out by the author as detailed below in Fig. 6.1.

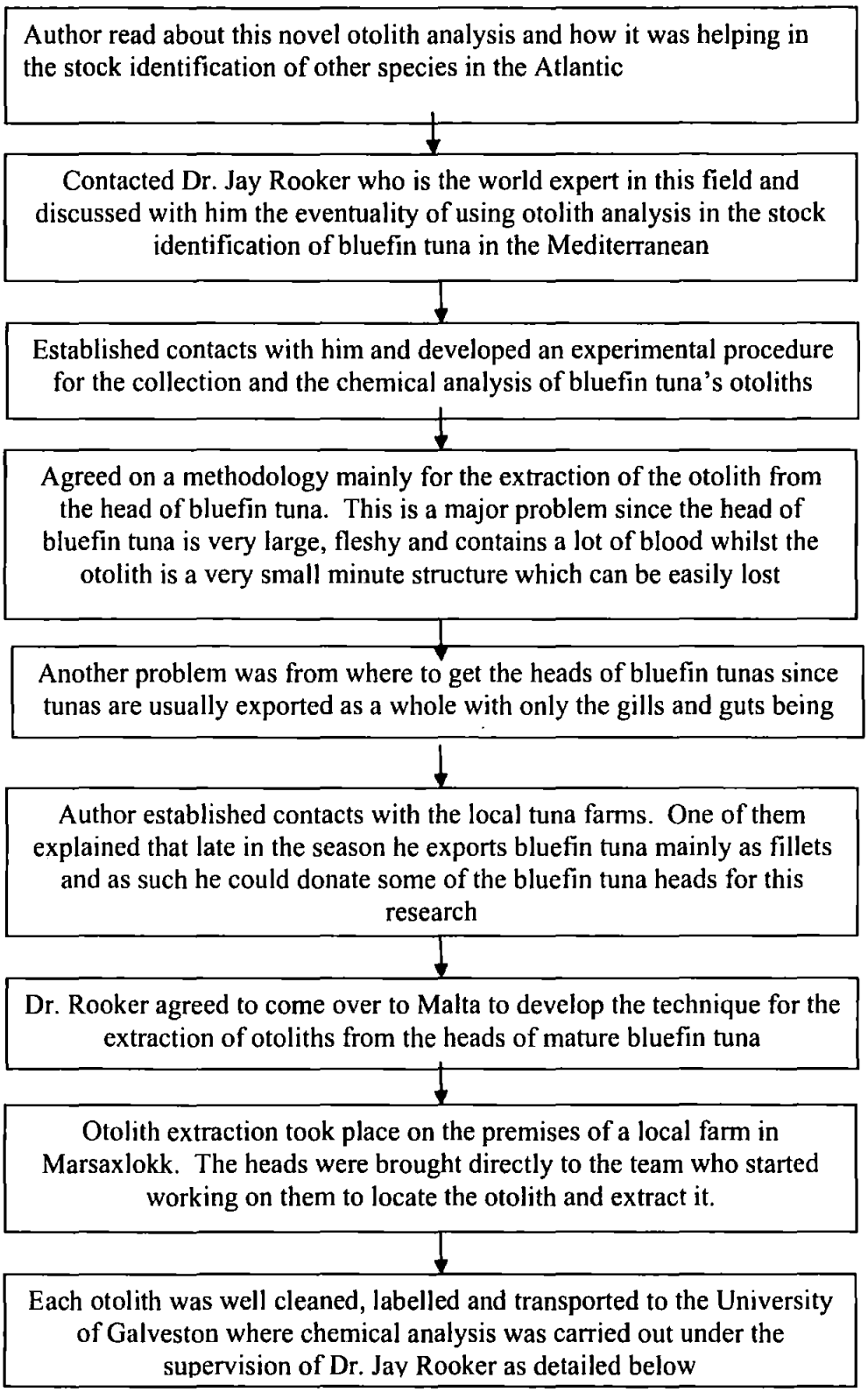


Fig 6.1 Operational management involved in project

Samples were collected during the harvesting period of farmed bluefin tuna caught and fattened around the Maltese Islands (September, 2003). The samples could not be collected from wild caught tuna directly since in order to extract the otolith the whole head is needed. Wild caught tuna are usually gilled and gutted out at sea and then after dressing they are exported directly to Asian markets with the head still on. Therefore there were no heads from wild caught tunas available for research purposes.

An agreement was made with one of the tuna farm owners in Malta. This arrangement involved that the farm owner would donate the heads of the filleted tunas for otolith extraction. Table 6.1 gives the main characteristics of the bluefin tunas from which the otoliths were extracted. The mean fork length was 170 cm whilst the mean round weight was 109 kg. Selection of single otoliths (i.e. right or left sagittae) for elemental analysis was based on random assignment. Otoliths were extracted from bluefin tunas harvested on the 20th September, 2003 off the south-eastern coast of Malta (Lat. 35:50:72, Long. 14:35:05).

Fork Length (cm)	Round weight (kg)	Fork Length (cm)	Round weight (kg)
145	55	152	63
140	53	218	171
147	58	233	198
165	82	229	193
159	61	148	58
220	178	144	54
232	195	147	53
215	182	172	86
150	57	152	61
145	52	217	172
147	50	232	199
168	86	227	191

Table 6.1 Characteristics of the bluefin tuna from which otoliths were extracted

Otoliths were stored in dry well sealed sampling bottles. These were clearly labelled and sent to the University of Galveston, Texas for chemical analysis.

Before elemental analysis, otoliths were carefully cleaned from surface contaminants. All reagents used were ultra-pure grade and all implements and containers were cleaned with 1% nitric acid and rinsed with 18 megohm doubly deionised water. The collected otoliths were first soaked in doubly deionised water to hydrate biological residue adhering to the surface of the sample and then any residue was removed using fine tipped forceps.

The otoliths were then soaked in 3 % hydrogen peroxide for 5 minutes to dissolve the remaining biological residue and immersed for 5 minutes in 1 % nitric acid to remove surface contamination. Otoliths were then flooded with doubly deionised water for 5 minutes to remove the acid. Finally, otoliths were dried under a Class 100 laminar-flow hood and stored in plastic vials. Otolith mass was reduced by approximately 4% as a result of the decontamination procedure.

In preparation for instrumental analysis, every otolith was weighed to the nearest 0.01mg. Internal standards were added to all solutions to compensate for possible instrumental drift.

Elemental concentrations were determined using a Perkin Elmer ELAN 5000 quadrupole inductively coupled plasma mass spectrometer (ICPMS) (Perkin Elmer, Inc., Shelton, CN, USA). Levels of lithium, magnesium, manganese and barium were determined using external calibration standards. Levels of calcium and strontium were quantified after 100 fold dilution using external standards without matrix matching. Samples were analysed at random to avoid possible sequence effects.

Procedural blanks and two certified reference materials (CRMs) were concurrently digested and analysed following the same procedures. Limits of detection (LODs) were calculated based on three readings and standard deviation of the mean procedural blank and converted to a dry weight basis. The limits of detection were lithium 0.01, magnesium 0.19, manganese 0.06, barium 0.01, strontium 0.90 and calcium 0.46 (values expressed as $\mu g\ g^{-1}$ dry weight, calcium in percentage).

Multivariate analysis of variance (MANOVA) was then carried out by the author to test for spatial and temporal differences in otolith chemistry. Nursery ground and year were used as fixed factors in separate MANOVA models. Pillai trace (V) was chosen as the test

statistics as it is the most robust to violations of homogeneity of covariance (Wilkinson *et al.*, 1996). Univariate tests for each element were analysed using analysis of covariance (ANCOVA) and a preliminary model (interaction regression) was used to determine if slopes of regression lines (homogeneity of slopes assumption) differed. The main significance test of ANCOVA (homogeneity of y intercepts) was performed for all elements because the assumption of parallel slopes was met. Tukeys's HSD test was used to find *a posteriori* differences (a = 0.05) among sample means. Linear discriminant function analysis (LDFA) was used to classify juveniles from different nurseries and/or year classes.

Small differences in otolith weights and fish lengths occurred among sites and years and thus the relationships between elemental concentration and otolith weight prior to performing LDFA were examined. The effect of the size (otolith weight used as a proxy for fish size) to ensure that differences in fish size among samples did not confound any site specific differences in otolith chemistry was removed. Concentrations were weight - detrended by subtraction of the common within-group linear slope from the observed concentration (Rooker *et al.*, 2001a).

The relative importance of individual elements in discriminating across spatial and temporal scales was assessed using the F test. Elements with large F values were removed. Correlation of elements used in the discriminant function model was evaluated using the Tolerance statistic. Such estimates range from 0 to 1 and a small value indicates that a variable is highly correlated with one or more of the other variables (Wilkinson *et al.*, 1996).

Prior to statistical testing, residuals were examined for normality and homogeneity among factor levels. Within group distribution and variance were examined and an outlier was

removed in one case (high manganese value) to meet parametric assumptions. The results obtained from the analysis of the otoliths (Fig. 6.2) collected in the Mediterranean were compared with published results of otoliths (Rooker, 2001) of bluefin tuna collected from nursery areas in the west Atlantic. The comparison was made with bluefin tuna of the same size class, i.e. in the range of 50 – 200 kg.

6.3.2 Genetic Studies

As stated previously, bluefin tuna targeted by longliners are usually gilled and gutted out at sea and therefore the collection of samples had to be done on board in order to be able to immediately collect the samples for genetic analysis and preserve them accordingly.

Small samples of liver, heart and muscle were cut using a clean scalpel as soon as the bluefin tuna was brought on board and gutted. In order to preserve the tissues well, it was made sure that the tissues for preservation never exceeded a volume of 5 cm^3, that they were cut as soon as possible and that the volume of the 90% alcohol in each sample bottle was always three times as much as the volume of the tissue.

Samples of bluefin tuna were collected for mitochondrial DNA sequence analysis during the bluefin tuna season of the year 2000. These were collected by the author in three Mediterranean locations; Libya (22), Tunisia (23) and Malta (12) (Fig. 6.2, Table 6.2).

Fig. 6.2 Main locations from where samples for genetic analysis were collected

Location	No of samples	Date	Average FL/cm
Libya	22	June 2000	206.1
Malta	12	July 2000	137.3
Tunisia	23	June 2001	230.3

Table 6.2 Description of samples collected for study

In the laboratory, the alcohol solution in each sample bottle was replaced with a fresh solution. Afterwards the sample bottles were kept in a cool place until the genetic analysis could be performed at the University of Girona, Spain.

The laboratory techniques applied are as outlined in Vinas *et al.*, (2001), with very minor modifications.

For the bluefin tuna samples obtained from near the Maltese Islands, a combination of primers was used in order to obtain the complete mitochondrial DNA control region sequence: L15998-PRO (Alvarado Bremer *et al.*, 1995) which is complementary to the

$tRNA^{pro}$ flanking D-loop fragment. This was used in combination with the FST (Pla *et al.*, 1995), corresponding to the $tRNA^{phc}$ gene adjacent to the 3 ´ control region end.

The full length (863 bp) of the bluefin tuna mitonchondrial control region was edited by eye with Programs SEQ ED. (version 1.3) and XESEE (Cabot & Bekenbach, 1989) and aligned using *Thunnus thynnus* (GenBank accession number X82653) sequences as reference. The total of sequence variation was assessed estimating nucleotide diversity (Nei, 1987) and haplotypic diversity (Nei & Tajima, 1981).

Gene phylogenia was reconstructed using the Neighbour-Joining (NJ) algorithm (Saitou & Nei, 1987) on a matrix of Kimura two parameter distance model (Kimura, 1980). A bootstrap test (Felsenstein, 1985) of 1000 replicates was carried out to check the strength of each branch of the tree. All these calculations were performed using the PHYLIP package (Felsenstein, 1993).

For all the samples obtained from the different regions of the Mediterranean Sea, the loop sequence of each individual was obtained following the laboratory outlines described in Vinas (2001). The combination of primers used was: L15998-PRO primer which was complementary to the $tRNA^{pro}$ flanking D-loop fragment with CSBDH (Alvarado Bremer *et al.*, 1995) corresponding to the control sequence block (CSB) of the mitochondrial D loop region. Sequences were read in ABI prism 310 Genetic analyzer available in the Laboratory of Ichthyology Genetics at the University of Girona, Spain.

Sequences were edited by eye with the programs SEQ ED. (version 1.3) and Bioedit (version 5.0.0; Hall, 1999) and aligned using *Thunnus thynnus* sequences as reference (Genbank accession number X82653). Sequences variation was assessed estimating nucleotide diversity (Nei, 1987) and haplotypic diversity (h; Nei & Tajima, 1981) using the

Arlequin package (version 2.0; Schneider, 1997). Gene phylogenia was reconstructed using the Neighbor-Joining (NJ) algorithm (Saitou & Nei, 1987) on a matrix of gamma Tamura Nei (alpha = 0.27) (Tamura, K. & Nei, M. 1993, Wakely, J. 1993). A bootstrap test (Felsenstein, 1985) of 1000 replicates was carried out to check the strength of each branch of the tree. All the phylogenetic calculations were performed using the MEGA package (version 2.1; Kumar *et al.*, 2001).

The extent of the population subdivision using an analysis of molecular variance was also analysed (AMOVA, Excoffier *et al.*, 1992), available in the software package Arlequin. The significance level of the population subdivision was determined by a 3000 fold non parametric permutation procedure also implemented in the Arlequin software.

6.4 Results and Discussion

6.4.1 Chemical Analysis of Bluefin Tuna Otoliths

Multivariate analysis of variance indicated that otolith chemistry of bluefin tuna (*Thunnus thynnus*) collected in the Mediterranean Sea (near the Maltese Islands) and western Atlantic nurseries differed significantly (Pillai's trace = 3.55, $P < 0.01$). Univariate contrasts indicated that concentrations of only one element, lithium, differed significantly (ANCOVA, $P < 0.05$) between nursery areas. The concentration of lithium was higher for *Thunnus thynnus* collected in the Mediterranean than in the western Atlantic (Fig 6.4).

Discriminant analysis, based on concentrations of all six elements, indicated that 71% of these individuals were apparently correctly assigned to their native nursery area (Mediterranean Sea 67%, western Atlantic 75%). One record of otolith manganese from

the central Mediterranean Sea was identified as an outlier and classification success was improved to 85% by removing this case from the discriminant model. The F value of lithium was markedly higher than other elements (10.3). Values of two other elements; manganese and barium were moderately high (1.8 and 5.1 respectively), suggesting that these elements may also be useful for discriminating Mediterranean and western Atlantic juveniles. Correlation of elements in the discriminant model was moderate (Tolerance = 0.3 – 0.5).

Age 5 and age 6 bluefin tuna collected from the same region of the Mediterranean Sea were compared and distinct differences in otolith chemistry were observed. Results of MANOVA showed that elemental signatures of age 5 and age 6 *Thunnus thynnus* were significantly different (Pillai's trace = 23.62, $P < 0.001$). Univariate tests indicated that otolith concentrations of lithium, magnesium, strontium and barium were significantly different between age classes in the Mediterranean Sea (ANCOVA, $P < 0.05$). Concentrations of lithium and magnesium were approximately twofold greater in age 5 bluefin tuna while strontium and barium were markedly higher in age 6 individuals.

Fig 6.3 A bluefin tuna otolith

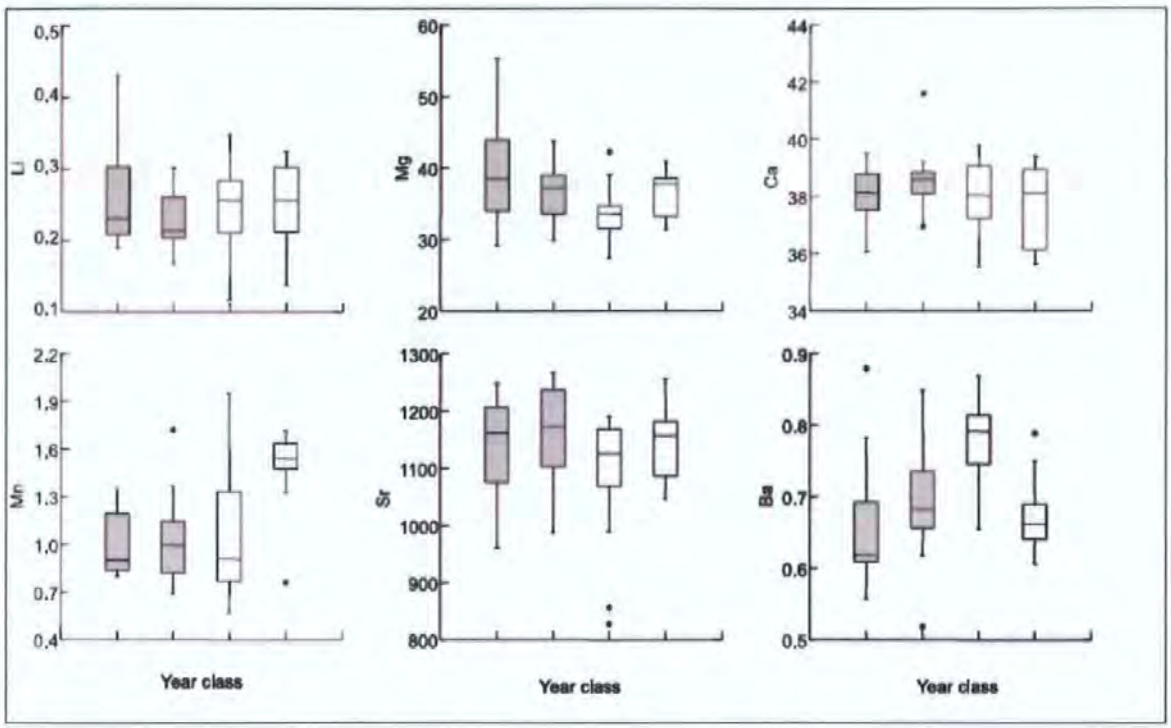


Fig 6.4 Box plots of elemental concentrations of otoliths of bluefin tuna collected from the central Mediterranean. Concentrations given in parts per million with the exception of calcium

6.4.2 Genetic Studies

The complete mitochondrial DNA control region sequence was obtained for all the samples collected from the Maltese Islands. Every sequence was unique with a haplotypic diversity of h = 1 and a nucleotide diversity of Π = 0.00335 for the entire sample. Although the high degree of observed DNA variation, these figures were very similar to the results already obtained in Spain with a total Mediterranean haplotypic diversity of h = 0.096 and Π= 0.035 (Pla *et al.*, 1998).

The tree topology clustered the sequences in three divergent clades. Only the third clade showed 100% bootstrap values. These three clades were also previously observed in the Mediterranean (Pla *et al.*, 1998) with a high sequence divergence of the third clade. The clade I comprised eight sequences (72%), the clade II presented two unique sequences (18%) and the remaining belonged to the third clade (9%).

Although the sample size was rather small, a preliminary analysis of the heterogeneity of the clade distribution could be done using as a reference the frequencies already observed. The results of these analysis showed homogeneity of the clade distribution (Fig. 6.5 and 6.6) between the samples from the rest of the Mediterranean and the Maltese samples (P = 0.3440 +/- 0.0150), suggesting a great homogeneity between all the bluefin tuna present in the Mediterranean Sea.

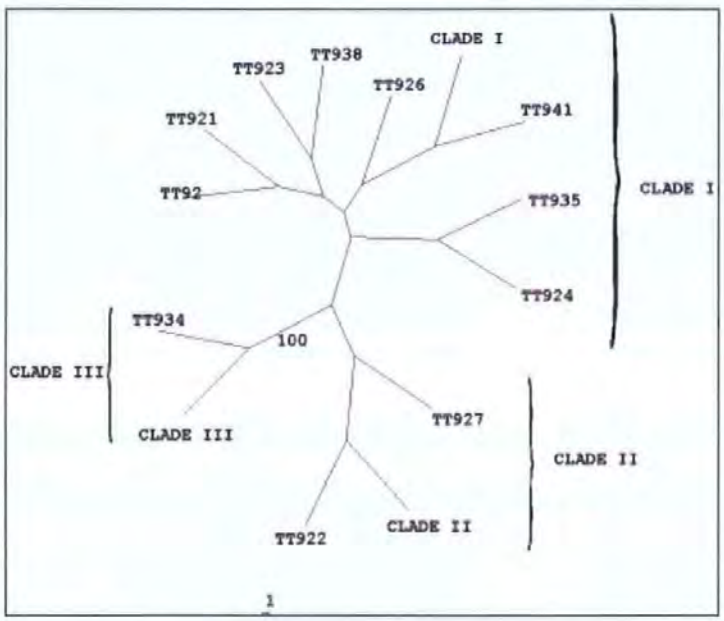


Fig. 6.5 Neighbor-Joining tree using Kimura two-parameters distance of the 12 distinct sequences. Numbers in the internal branches showed bootstrap values higher than 75% after 1000 replicates. Clade I, II and III sequences are used as references for their own clades

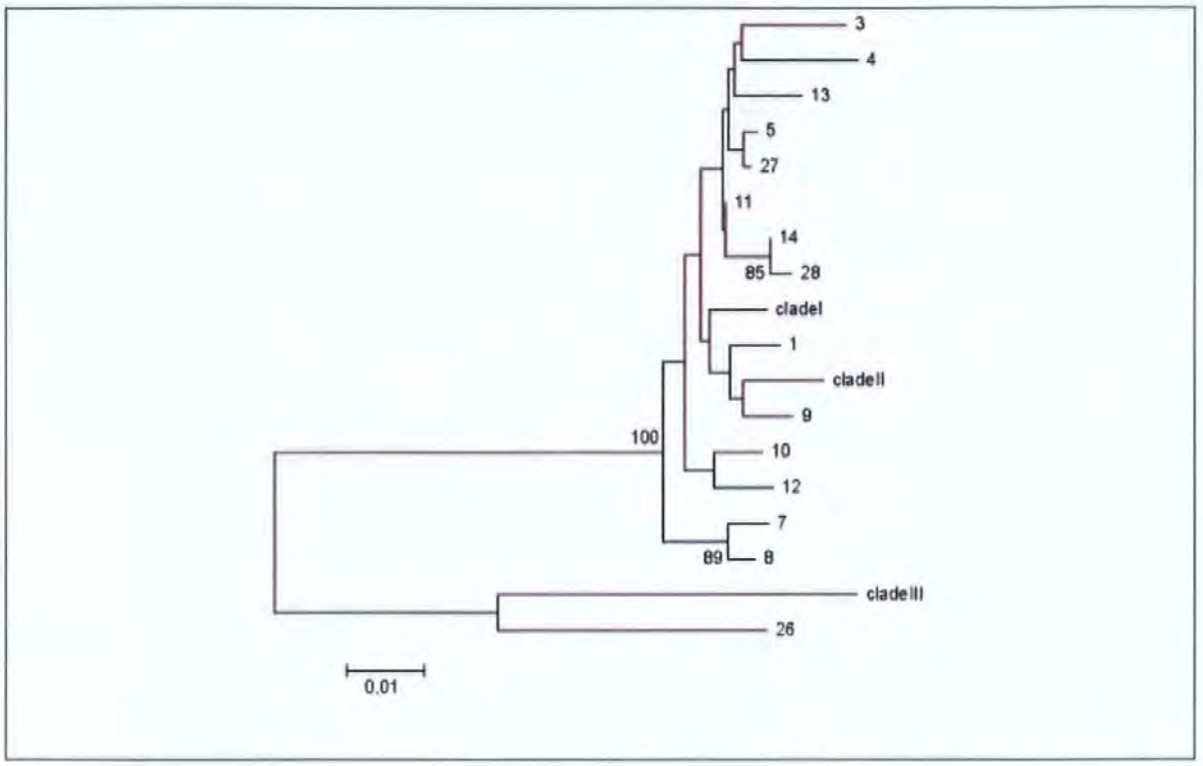


Fig 6.6 Phylogenetic tree based upon the Neighbour-joining distance of the distinct sequences. The numbers on the branches show bootstrap values exceeding 50%

The 373 base pair sequences of the 5′ mitochondrial control region of each individual were obtained for samples collected from the other parts of the Mediterranean. According to Vinas (2001), this section corresponds to the first domain of this mitochondrial region. The comparison of the 81 sequences revealed 48 haplotypes (Table 6.3), with frequencies 3 is to one. The haplotypic diversity for the overall samples was estimated to h= 0.993+/-0.003. Haplotypic diversity of each sample was very similar to the rest of the entire sample and also with the previously calculated for 269 Mediterranean bluefin tunas (h=0.9999+/-0.001, Vinas, 2001). Among the 48 distinct haplotypes, 42 variables sites were found, with 20 singletons and 22 parsimonious. Thus, the level of nucleotide diversity for the entire sample was pie=0.042+/-0.007. This is very similar with the previous results already obtained for the whole Mediterranean (pie=0.044+/-0.021).

Location	No of samples	No of haplotypes	Molecular diversity indices	
			H	Π*
Libya	22	19	0.985 +/- 0.018	0.020 +/-0.011
Malta	12	9	0.954 +/- 0.046	0.016 +/-0.009
Tunisia	23	20	0.985 +/- 0.009	0.015 +/-0.008
Total set	57	48	0.993 +/-0.003	0.020 +/-0.010

- Nucleotide diversity calculated after removal of introgression type haplotypes

Table 6.3 Samples size, number of haplotypes and molecular diversity indices for each sample and for the entire data set

The tree topology clustered the sequences in two highly divergent clades named clade I and clade II (Fig. 6.7), supported by 100% bootstrap values. The clade II was highly divergent from the clade I. The clade II was approximately 5% of the individuals (4 of 81) which was not significantly different (P = 0.602 +/- 1.55) from the frequency (6%) previously found by Vinas (2001) for this clade in the Mediterranean.

For the AMOVA analysis it was decided to remove the clade II sequences from the analysis. The highly divergence of this clade probably could have inflated artificially the nucleotide diversity of the samples. The overall comparison among the four samples resulted in the rejection of the homogeneity, with a slight differentiation (sigma = 0.013) but with a significant probability (P less than 0.001). However the pair wise comparison of the samples (Taula 3) revealed the genetic differentiation of the samples collected from the Malta location. Moreover, when 269 sequences from 6 different Mediterranean locations were included in the analysis, the AMOVA analysis also indicated significant differences only for the Malta location.

Since this was the first time that the genetic differentiation within the Mediterranean was carried out and considering the small sample size (12) of Malta, this heterogeneity was probably caused by the small sample size. Clearly a more exhaustive analysis could be done by increasing the size of future samplings.

When the Malta location was not included in the analysis of the molecular variance, none of the remaining locations differed significantly (Φ_{st} = 0.005; P = 0.097). Similarly the remaining samples (Libya and Tunisia) showed no genetic differentiation when they were compared to the pooled Mediterranean locations (Φ_{st} = 0.002; P = 0.149).

Thus, these results do not reject the hypothesis of homogeneity in the Mediterranean.

	Libya	**Malta**	**Tunisia**	**Mediterranean**
Libya	-	0.029	0.009	0.001
Malta	0.003*	-	0.029	0.026
Tunisia	0.042	0.003*	-	-0.002
Mediterranean	0.465	0.0034*	0.027	-

- significant probabilities after Bonferroni correction

Table 6.4 Results AMOVA pairwise comparison among the three different samples and the previous data from the Mediterranean. Values above diagonal shows the Φ_{st} and values below its probability.

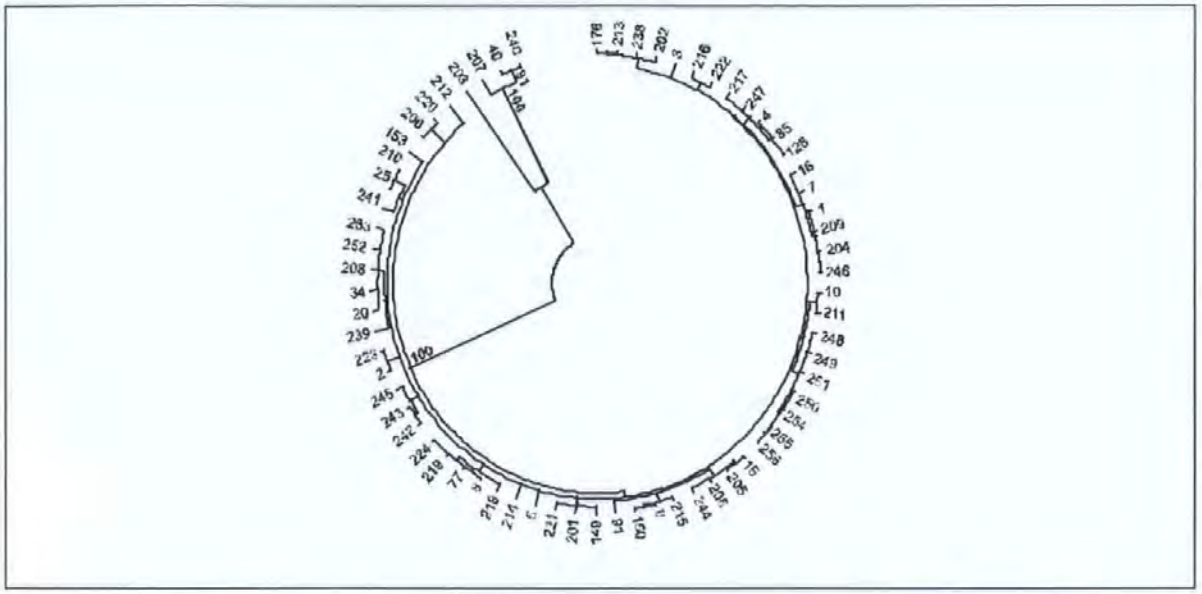


Fig 6.7 Neighbor-joining tree with Tamura-Nei distance (α = 0.27) of the bluefin tuna haplotypes. Values in the branches.

The genetic studies have demonstrated the low genetic differentiation with the Libyan and Tunisian locations. Thus, these locations are genetically closely related to each other and also to the rest of the Mediterranean. On the other hand, the genetic differentiation of the Maltese sample was probably due to the small size effect but clearly more analysis involving comparisons between individuals targeted during the same year would be desirable to corroborate these findings.

6.5 Summary

1. The chemical analysis of bluefin tuna otoliths appears to give good evidence of the origins and spatial history of bluefin tuna.

2. The study of the otoliths has demonstrated that the chemical signatures in the otoliths of bluefin tuna from the Mediterranean and the Atlantic nurseries are relatively distinct and even show some degree of temporal persistence.

3. The indications from the otolith samples examined, in comparison with similar samples from the east and west Atlantic, are that a confidence level of more than 75% is attainable

4. Data is critically needed to estimate exact origins of bluefin tuna populations and findings from future otolith based assessments will most likely play a central role in providing a means of identifying source and relative contributions of different nursery grounds in the Mediterranean Sea and the Atlantic Ocean.

5. Utilizing DNA 'fingerprinting' and related techniques will enable identification of distinct units of bluefin tuna to be identified.

6. The results obtained in the genetic studies show that the samples analyzed are part of the general distribution of bluefin tuna in the Mediterranean Sea. In this sense, this data could be used to identify the genetic homogeneity of the bluefin tuna distribution in the Mediterranean Sea and the hypothesis that this is a separate stock in all senses.

Chapter 7

EXPLOITATION TECHNIQUES FOR BLUEFIN TUNA

7.1 Introduction

The first part of this chapter aims to analyze the old and new exploitation techniques for the bluefin tuna in the Mediterranean in order to determine trends in landings, presence of illegal, unreported and unregulated catches and to conclude whether the bluefin tuna population in the Mediterranean is being overexploited or not. The second part of this chapter analysis the difficulties arising from the new exploitation of bluefin tuna, i.e. tuna farming.

7.2 Exploitation of Bluefin Tuna

It is well known that Japan is the main market for tuna from world fisheries. It is also the underlying force driving the current development of the tuna fishing sector in the Mediterranean. Farmed tuna from the Mediterranean has a higher oil content than its Australian equivalent and is more appreciated in Japan because the oil gives the flesh a more reddish colour which makes it more attractive. Mediterranean farmed tuna (Fig. 7.1) is either exported fresh by air cargo or, more usually deep-frozen by means of cargo vessels.

Fig. 7.1 Mediterranean farmed bluefin tuna

Imports of farmed tuna from the Mediterranean increased dramatically in Japan since 1997, to the extent that 80% of Mediterranean tuna imported in 2002 was of farming origin (Table 7.1).

Japanese Imports (metric tons)		
Country	**Farmed**	**Total**
Croatia	3, 150	3,491
Cyprus	No farmed tuna	6
France	No farmed tuna	97
Greece	No farmed tuna	397
Italy	1,640	2,602
Libya	No farmed tuna	216
Malta	2,351	2,637
Spain	6,006	6,925
Tunisia	No farmed tuna	358
Turkey	1,405	1,405
Total	14,553	18,135

Table 7.1 Total bluefin tuna imports to Japan from tuna farming by Mediterranean countries during the year 2002 (Miyake, P. estimated from BTSD data)

The impacts of the increasing farming products sent to the Japanese market have been significant. Until the early 1990s, the Japanese tuna *sashimi* market was extremely specialised into two major categories, one for very expensive, high quality prime products and another for popular price and quality. Only fat bluefin and southern bluefin in pre-spawning condition entered into the high quality market. These were served at top class Sushi restaurants or Japanese restaurants. All the remaining tunas were sold at much reduced prices and served at the public restaurants and supermarkets.

Previously bluefin tuna and small southern bluefin tuna had only been accepted at the less quality market. Due to the new trends of exploitation, these are now fattened by farming. This group of tunas started to constitute a middle quality category in the market, filling the

gap between the previous two extreme categories. The price for the middle quality tuna is still much less than the top quality meat but considerably better than lean red meat tunas.

These fish (fattened farmed tuna) provide the public with fatty meat called 'toro' which only rich people could have eaten before. These farmed tuna are now sold even in the supermarkets and used in the popular and inexpensive *sushi* bars such as rotating sushi bars (sushi being carried on conveyors which customers pick up). In a way, it brought 'toro' taste to the public and enriched the Japanese people's food habit. On the other hand, it dragged down the price of high quality meat tuna, as well as pushing down the lower quality meat tuna.

All indications in late 2003 pointed to the saturation of the Japanese market due to the overproduction of farmed tuna from the Mediterranean. As a result, in 2003, prices fetched by Mediterranean farmed tuna in the Japanese market fell down due to an over supply crises (WWF, 2004). This fact uncovered the paradox that the strong and extremely rapid development in the production of farmed tuna in the Mediterranean during the last years had been made following a purely short-term perspective, seeking immediate huge profits, without taking into consideration the economical sustainability of the business vis-à-vis international markets and the sustainability of the bluefin tuna fishery.

7.2.1 Changes in the fishing industry

Tuna farming has brought a revolutionary change to the Mediterranean fishing industry. It has created a lot of new jobs for farming, the tuna fish price went up for fishermen, and changed the operational procedure completely (including fishing area and season as well as net lifting procedures). The fish caught by purse seiners used to be sold at the local markets for canning and just a little was used for fresh fish consumption. Therefore the price was

quite low, less than a dollar per kilogram. Now there is a whole new field of farming and there is an increasing demand for purse seine caught fish with a much higher price paid, more than 9 dollars per kilogram.

The demands from the tuna farming industry have created increasing fishing pressure on some small pelagic stocks targeting for feeding farmed bluefin tuna. Some of these fisheries affect stocks already in decline, such as the anchovy. Conversion rates reported for farms in Italy, Spain and Turkey range from 10 kg to 25 kg of baitfish consumed to produce only 1 kg of tuna. This low conversion efficiency from feed to tuna meat also makes tuna farming a wasteful practice, entailing a high ecological footprint. In fact, the demand for bait fish, such as sardine, mackerel, squid and anchovy (Fig. 7.2) has increased, as 3 to 5% of the mass of the fish farmed are required daily for feeding. In the Italian market, the prices for frozen mackerel for bait have increased by about 75% since 1998, frozen squids by 40 to 110 %, depending on the size and quality, and sardines by 80%.

Fig. 7.2 Baitfish used for feeding farmed bluefin tuna

7.2.2 Changes in fishing gear

When targeting bluefin tuna for farming, the type of gear to be used is very important. In fact it must take into account the stress that occurs during fishing and should provide tunas that are able to adapt easily and rapidly to captivity. Tunas should have suffered minimum physiological stress for better survivability.

The fishing technique most physiologically suited to target bluefin tuna for farming is the purse seine (Doumenge, 1999). It is almost the only gear used for farming besides the small amount caught by traps.

Purse seiners and traps target bluefin tuna mainly during their spawning migratory pathway in the Mediterranean when they are travelling in shoals. Since the development of tuna farming in the Mediterranean, a change in gear has been observed throughout the Mediterranean. In fact the amount of bluefin tuna targeted by drift nets has completely disappeared since the ban of drift net fishing in the Mediterranean by the European Commission in 1998. Also the landings of longlines have dropped drastically since the bluefin tunas landed by this gear are already dead and therefore cannot be used for farming.

Purse seining is a modern fishing technique developed in the 1950's. It involves shooting a large net off the stern of a fishing vessel, with a bottom weighted line and a top float line that extends the net vertically in the water. A second smaller vessel (skiff) pulls one end of the net from the purse seiner as both vessels encircle the shoal of fish from opposite directions until finally reconnecting the skiff end of the net with the purse seine vessel.

The purse seiner then draws the purse line closed, creating a purse to entrap the shoal of tuna. Purse seining is a very efficient system that can be defined as an industrial fisheries tool, is species selective and in the Mediterranean Sea does not entail high bycatches of cetaceans (Tudela, 2002b). It is almost the only system that allows the transfer of live fish to the fattening cages and is therefore an essential component for industrial tuna farming.

The high tech purse seine activity is now being privileged in front all the other fishing gears in the Mediterranean, given the interest of the industry to secure live captures to fill the cages. This, in turn, has resulted in an increasing vertical integration of the business through either formal agreements between tuna fishing ship owners and farmers or, directly, through the direct involvement of the farming industry in fishing operations (like the case of Murcian producers who own some units of the Spanish and French purse seine

fleet). Another direct consequence has been the rapid increase in purse seining fishing capacity.

Fuelled by the high demand from Mediterranean farms, the French and Spanish tuna purse seine fleets underwent an intense modernisation during the last few years (including vessel replacement by more highly efficient units) by utilising funds from the European Union (Financial Instrument for Fisheries Guidance – FIFG). This has resulted in a net increase in their fishing capacity. In other countries, projects for the building of new purse seine fleets have been made public like Algeria and Greece.

The case of Turkey is especially paradigmatic, in the sense that tuna farming production is currently booming linked to domestic purse seine catches, in spite of not having been allocated any quota from ICCAT for bluefin tuna for the whole period 2003 –2006 (WWF; 2004)

The amount of bluefin tuna caged for farming in the Mediterranean in 2002, estimated from industry data (Miyake, 2003) amounts to a total of 14, 620 metric tons. If compared to purse seine production in 2002 (15, 830 metric tons, ICCAT, 2003), it is clear that that year virtually all purse seine catches in the Mediterranean were caged into tuna farms. This suggests that any further sharp increase in farmed production in the Mediterranean with respect to 2002 figures would surely originate from illegal over quota catches, given the current limit set by ICCAT at 32, 000 metric tons per year for 2003 – 2006, that represents a theoretical freeze in fishing effort.

Nevertheless, by departing from this 'saturation' point reached in 2002, information available for 2003 indicates a clearly alarming trend. The report of the second meeting of the *Ad hoc* GFCM/ICCAT Working Group on Tuna Farming in the Mediterranean, held in

Turkey in December 2003, reports that according to data from the Japanese industry, about 21, 000 metric tons of bluefin tuna were introduced in Mediterranean cages in 2003. Whereas it is not clear if this estimate also includes inputs into unreported farms, it agrees with other well informed sources that pointed to a farming capacity in Mediterranean farms in 2003 amounting to 25, 000 metric tons (WWF, 2004). Given that this live tuna was almost exclusively supplied by purse seiners (except a marginal amount by traps), it is worth highlighting that Mediterranean purse seine catches higher than 21, 000 metric tons have only historically been met with total captures of 40, 000 metric tons or more. Clearly, this level of purse seine catch is not compatible with a total annual quota of 32, 000 metric tons for the year 2003 – 2006, as it is currently in force.

7.2.3 Changes in fishing areas

Nowadays spotter planes are used to spot the shoals of tuna. Fishermen are then informed about the location of the shoals and they move to these areas where shoals of bluefin tuna (reaching more than 600 individuals) would be present. Most would be caught since purse seines and traps are very efficient gears. The areas where purse seiners move are mainly the spawning areas of the Mediterranean. In fact the high yields of the purse seiners also show the high vulnerability of the spawning bluefin tuna to this method. Artisanal surface longline catches have decreased considerably from these areas apparently as a result of the tunas being taken from the shoals by the purse seine nets.

Consequently the establishment of closed purse seine areas should be considered for particular areas of the Mediterranean which are the main spawning areas with the main aim of protecting bluefin tuna during their spawning season. Even small areas would contribute to a significant protection for spawning and recruitment in the Mediterranean

Sea and would therefore contribute to the sustainability of tuna farming in the Mediterranean.

7.3 Is the bluefin tuna population being overexploited?

Bluefin tuna (*Thunnus thynnus*) is a widely distributed species throughout temperate waters in the Atlantic and Mediterranean Sea. Due to its highly migratory character, several fisheries target bluefin tuna by means of different gears. Fisheries are mainly seasonal and directed to different fractions of the bluefin tuna population.

Thus, reproductive tuna are caught during genetic migration to known spawning areas, i.e. the Balearic Islands and the Adriatic Sea. In Morocco bluefin tuna is targeted by traps located in the Strait of Gibraltar and close to northern African coasts. Once in the spawning grounds, bluefin tuna is caught by longliners and purse seiners. Before and after the spawning season, younger bluefin tuna is caught by purse seine, bait boat and trolling gears targeting them in their feeding grounds. Furthermore, bluefin tuna is also caught by bottom hand line, in very limited areas, for longer periods.

For the last years, bluefin tuna feeding and fattening activities (confining fish within large floating marine netcages) has experienced a great development. Wild bluefin tuna, mostly provided by purse seine vessels and to a lesser extent by traps, are towed to several aquaculture units located throughout the Mediterranean Sea. From the study carried out, the decrease in the number of traps and the subsequent in the number of purse seiners throughout the Mediterranean Sea is quite evident.

Under and mis-reporting is the main source of uncertainty related to catch data. Though most of the countries fishing for bluefin tuna are now ICCAT members (i.e. they have to

report annual catches), ICCAT official statistics are unfortunately highly misleading. This was clearly demonstrated in 1998 when several countries asked for a revision of the official ICCAT statistics for the 1991 – 1997 period. This request was actually motivated by the implementation of a Total Allowable Catch in 1996; the quota of each country being proportional to its historical catches. Not so surprisingly, this revision led to a 20 to 25 % increase in total catches (ICCAT, 2000).

The second problem is related to the fishing of bluefin tuna less than 1 year of age. This activity is still important in most coastal Mediterranean countries, but it is not reported since the size limit of landings of bluefin tuna is fixed at 6.4 kg (with a tolerance of 15 % for fish between 3.2 and 6.4 kg). This widespread under reporting which results from both industrial and small-scale fisheries, is impossible to evaluate accurately and strongly biases the assessment and management procedures.

To address unreported catches ICCAT compares the catch data reported by the different countries (Task I data) with the import figures to Japan (biannual Bluefin Tuna Statistical Document, BFSD). A comparison of 1999 figures amounted to an estimated 3, 242 metric tons of unreported catch in the Mediterranean by Spain, Croatia, France, Italy, Portugal and Morocco. This was about 10% of the quota set for that year and almost certainly still below the actual catch (WWF, 2004).

Changes in gear technology and tactics mainly affect the purse seine fleet, which at the moment is the largest one in the Mediterranean Sea. In comparison to 1970, a standard purse seine was in 2000 twice as long and four times more powerful in horse power. Though such a change can easily be modelled, it is more difficult to model objectively the recent increasing use of powerful positioning and prospecting equipment, such as bird

radar, sounder, sonar and aircraft as well as new storage equipment, such as carrier vessels with deep freezing storage and pool systems (Liorzou, 1999).

Because large tunas have a considerable value on the Japanese market, the purse seine fleet that used to target small fish until the 1980s, now targets both small and big fish. Subsequently, purse seiners strongly expanded their fishing area in the Mediterranean Sea.

In 2001, the Algerian Fisheries Firm Union Peche, announced that they had signed an agreement with the Spanish-Portugese ship building company Navalfoz. According to the agreement, the fleet of 20 tuna purse seiners with a deck length of 30 metres and 1 measuring 47 metres would be built. This example illustrates the powerful economic interest behind tuna farming activities that are fuelling the development of the sector in the Mediterranean. The economic investments associated with this operation amounted to 20 million $. It is also noteworthy that this development was agreed despite the fact that Algeria did not become a Contracting Party to ICCAT until February 2001 and lacked any quota for bluefin tuna allocated by ICCAT until 2003. With an aim to get a better quota from ICCAT, Algeria supplied ICCAT with revised figures on national catches of bluefin tuna for the last years. The new figures showed a peak in landings in 1993 and 1994, surprisingly the same years that ICCAT uses as the reference point for quota allocation among the different states. Algeria was finally given a quota of 1,500 metric tons, a volume which does not justify the building of the enormous purse seine fishing capacity announced. Such changes in both selectivity and fishing area are known to bias the effort estimate. This case illustrates the powerful economic interests behind tuna farming activities that are fuelling the development of the sector in the Mediterranean Sea.

Turkey has also seen an increase in purse seine fishing for bluefin tuna, going from 28 purse seine units in 2002 to 50 purse seine units targeting bluefin tuna in 2003. Bluefin

tuna catch in 2002 amounted to 2,300 metric tons, 1,400 metric tons of which were transferred into farms. It is important to note that Turkey has only become a Contracting Party of ICCAT since August 2003 and that there is no quota for bluefin tuna allocated from ICCAT to this country for the whole period 2003 – 2006. In this context, all Turkish production is arising from 'illegal' fishing.

Another source of uncertainty relates to the cooperation and competition between fishing vessels. Purse seiners tend to work in teams of about 5 boats. As soon as a boat catches a shoal, it shares its catch with its partners but also with boats of other teams if the latter arrives at the fishing location before the former has finished surrounding the shoal with its seine. The teams change from year to year and there is also strong competition between them. These changes, which affect fishing effort positively and negatively, are highly difficult to quantify without detailed information provided by observers on board who must be present continuously throughout the whole bluefin tuna fishing season.

Improving standard stock assessment procedure would involve reducing these subjective uncertainties, and it therefore appears necessary to consider the perverse effects related to the implementation of a Total Allowable Catch on the Mediterranean bluefin tuna stock. There is no doubt that this management measure has increased the level of mis- and under-reporting and decreased the quantity and quality of information related to the fishing effort. The official statistics have become less trustworthy and their deterioration will probably continue without the implementation of efficient controls of the present rules.

Regarding the last stock assessment made by the ICCAT scientists in 2002, the low quality of the input information available led the Scientific Committee for Research and Statistics to conclude that it was not possible to make definitive management recommendations based on the results obtained, since they were not considered to be reliable enough. In

essence, this was the ultimate explicit recognition by bluefin tuna specialists of the impossibility to carry out a rational management of the stock under current conditions. Consequently the standard stock assessment procedure based on official statistics is now inoperative.

7.4 Difficulties arising from tuna farming

This new practice in the Mediterranean, i.e. tuna farming, is threatening one of our most valuable resources. The bluefin tuna is already under considerable pressure and has been declining for years. Now tuna farming has opened up a new section on the Japanese market, which has further increased the demand for bluefin tuna and made the situation of wild stocks even more perilous. Tuna farming has given rise to various problems in the sector as will be discussed below.

7.4.1 Increasing effort

As the increase in price and demands for the purse seine caught bluefin tuna for the tuna farming, the fishing efforts have been also concentrating on this type of tuna. This has an undesirable impact on bluefin tuna stocks. Firstly, more effort is being exerted for smaller fish and possible consecutive increasing proportion of smaller fish in the total catches would result in lower yield per recruits. This indicates a sign of over fishing. Secondly the east Atlantic population (including the Mediterranean population) had already attained the level of a maximum sustainable yield when farming started to increase (ICCAT, 2002).

Therefore the increasing effort and consequent catches should have further reduced the stock size, according to the analysis of the ICCAT Scientific Committee for Research and

Statistics. The Commission adopted a catch restriction policy since 1995 but implementation of quota system is getting harder and harder and non compliance seems to be increasing, as the demands for bluefin tuna in the market continue increasing. During the 13th Special Meeting of ICCAT held in Bilbao in 2002, ICCAT adopted an unsustainable annual quota of 32, 000 metric tons for the years 2003 – 2006, 23% higher than the maximum level scientifically determined.

7.4.2 Possibility of tuna 'laundering'

The current management system based on quotas is largely non operational since there are no effective mechanisms in place ensuring a monitoring of overall catches in real time. This means that it is not possible to stop the whole fishery when the total annual quota has been met because neither is ICCAT receiving continuous updated information on catches during the fishing season nor is it empowered with the political mechanisms member states require to do so. Tuna farming is increasing this problem and total catches on the stock on a national basis are reported to the management authority – ICCAT- *a posteriori*, the following year or even two years later.

Another serious problem is the increasing uncertainties in statistics. Unfortunately, the ICCAT Bluefin Tuna Statistical Document (BFSD) is required for only dead fresh or frozen tuna products, and the international trade of live tuna is not recorded in these documents. In other words, the live tuna could be imported from any country without any documentation.

Some countries, such as Malta, require BFSD to be completed, even if the fish are alive, and issue re-export BFSD when the products are shipped out of the country. Some other countries require fishermen to report catches and register the sales of live fish, even though

BFSD is not required. On the other hand, trade between countries of the European Union are not considered as foreign trade and hence there is no need of such actions regardless the condition of fish (live or dead). Such a lack of documents continues to mask transfers between fleets and farms from France, Spain, Italy, Malta and Cyprus.

Once they are transferred into the pen, the identities of origin of the fish are lost. When these tuna are lifted, harvested and exported to other countries, a BFSD has to go with it and the authority of the country where farming took place sign such a document. In other words, the countries where the bluefin tuna were caught are not necessarily signing the documents. For example, bluefin tuna farmed in Malta but caught by a Libyan vessel will be issued with a re-export certificate from Malta and not by the catching country. Consequently, the quantities of bluefin tuna exports from one country can exceed their real (reported) catches by a significant quantity. The origins of these fish are lost. Only exception is the case where the country request BFSD even when the fish are imported live (e.g. Malta). Thus, if one country caught over their quota and exported it as live fish, to find out such over usage of the quota would be difficult.

Finally the transfer of European farms, mainly Spanish, toward North African countries (even if both countries are equally bound to ICCAT obligations) to avoid control for their activities, is already taking place. This has led some to speak about 'IUU farms' spreading around the Mediterranean (mimicking the concept of IUU – illegal fishing). WWF (2004) reported that Libya and Tunisia did not report their farming activities to the GFCM/ICCAT Working Group on Tuna Farming last year, after having been formally required to do so.

7.4.3 Body weight increase of farmed tuna

As the fat content increases, tunas are expected to increase their body weight during the farming period. Tuna farmers expect at least 25% increase in body weight during the few months farming. Therefore, in order to estimate the original live weight of fish at the beginning of farming, the weight of lifting at the end of farming has to be converted by applying a factor of increase.

This weight increase is having strong implications for the control of quota since there is room for countries/farms to argue high weight gains in their farms to hidden excess catches. At this regard, it is noteworthy the broad range of weight gain percentage reported for farms in Italy, Spain and Turkey (from 10% to 50%).

The ICCAT scientific requirements are to report 'catches' in weight. Therefore in principle, even if the fish are captured and sold alive to the fish farmers of other countries, the catches in weight (at the time of the capture) must be reported by the flag states. Actually most of the countries are implementing reporting of all the captures. If they are reported correctly, then the landing (lifting) data of farmed fish should not be added to the bluefin catches. However, if the implementation of such a rule is not really effective and they are not reported, the landing weight less growth during farming must be estimated and added to the reported catches. On the other hand, if there is a large mortality during the farming, landing can be even less than the weight of the fish entered into the cage when farming started.

Also, in countries such as Croatia, increasing amounts of fish are now being kept over a year in cages. This means that only a part of the catches of one year is landed in that year and the rest would be landed in the following years. Therefore, the landing (or harvest

from the farming) has no relation with the catches. These elements all add up to the increasing uncertainties of the bluefin tuna catch statistics.

7.4.4 Conversion factors from products to the round weight of bluefin tuna

Generally, the farmed bluefin tuna products are exported to the Japanese market and reported in the weight of products (such as gilled and gutted, dressed, loins, etc.) The SCRS established conversion factors from products weight to live weight for bluefin tuna. However, those are based on fish captured from the wild. Therefore, it is apparent that the conversion would be different for farmed tunas. Further investigations should start in this particular area.

7.4.5 Difficulties in sampling

Bluefin tuna size data used to come from size measurements of the catches made by various gears such as purse seine, trap, etc. However, since farming activities of bluefin tuna started, such sampling from the catches is getting more and more difficult, because live tunas are moved from purse seines or traps directly to the cages. This is leading to inadequate sampling by various countries. As such no reliable stock assessments can be carried out.

7.4.6 Effects on the environment

Dense farming may cause water pollution in two aspects, one from the leftover of bait and cultured fish and another from processing. In Malta stringent Environmental Impact Assessments are carried out prior to the start up of the operation. These data are then used as the benchmark. Water and sediment analysis are carried out on a regular basis together

with benthic surveys in order to follow continuously the effects on the environment. In order to keep control of pollutants, Spain is limiting the number of cages which could be operated in a certain area. However, as the operations continue to expand all over the Mediterranean, this problem will remain. This issue needs to be looked into further in order to try and find methods which are environmentally friendly.

The effects on human sanitation of the chemicals and/or medicines possibly used with baits need to be evaluated. In most of the places, the use of the chemicals and medicines (e.g. hormones, antibiotics) for farming is prohibited by the law. However, the implementations are not well studied. These should also be studied and monitored with much care.

7.4.7 Future of tuna farming

The farmed bluefin tuna industry is expected to continue increasing in the coming years due to further technical developments and the market demand for its products. The long-term sustainability of the industry will depend on several factors:

- The supply of food fish (small pelagics)
- The development of formulated feeds
- Improvements in feed formulation to ensure meat quality
- The continued availability of seed material
- The expansion of marketing activities away from reliance on Japanese markets
- Improved harvesting techniques
- Improved offshore technologies for culture systems

Environmental and ethical concerns will continue to affect the functioning and image of the industry. Regulations are needed to create and control the traceability of products as well as quality and environmental issues, e.g. by tagging all bluefin tuna which are transferred into cages.

The prospect of achieving the captive breeding bluefin tuna and being able to manage the complete life cycle could represent a base from which the industry could further expand. This issue alone would remove ecological concerns and guarantee a more sustainable future for the sector. On the 7th July, 2005, members of the European Union funded research team REPRO-DOTT (Reproduction and Domestication of *Thunnus thynnus*), announced the first successful hormonal induction of a captive breed stock of the bluefin tuna to obtain eggs and sperm. In vitro fertilisation was carried out successfully and viable larvae were produced. This is a very important achievement in controlling reproduction of the species as it proves that it is able to mature in captivity and produce viable gametes for successful fertilization. This might be the first step at controlling the whole life cycle of the fish in captivity and save the bluefin tuna from extinction.

Chapter 8

DISCUSSION, MANAGEMENT STRATEGIES AND RECOMMENDATIONS

8.1 Introduction

This chapter aims to analyze the results obtained in the experiments described in the previous chapters in order to determine whether the Mediterranean population is a separate stock or not. The management strategies that should be followed together with the recommendations for the future are given at the end of the chapter aiming to ensure the future sustainability of the species.

8.2 Stock Identification

In the experiments discussed in the previous chapters, differences were observed between the biometric relationships determined by this study and the biometric relationships available for the Atlantic. Although more experiments in line with the experiments carried out in the Mediterranean need to be carried out in the Atlantic, the results obtained indicate that the Mediterranean stock has its own distinct characteristics and therefore needs to be managed as a separate stock.

The age - length key determined by this study will allow the determination of the age of Mediterranean bluefin tuna up to the age of 16 years. This key will provide essential information in all aspects of studies carried out on bluefin tuna. As yet no age - length key (till the age of 16 years) has been determined for bluefin tuna caught in the Atlantic. Further studies will allow age determination for all bluefin tuna caught in the Atlantic and this could then be compared with the age - length key of the Mediterranean. If significant differences are found, this would lead to the hypothesis of the presence of a permanent residing bluefin tuna stock in the Mediterranean Sea.

Maturity studies carried out on bluefin tunas caught by the Maltese longline fishery in the Mediterranean indicate that most of the bluefin tuna females studied were mature. These females contained a lot of yolked oocytes, nuclear migration stage oocytes or postovulatory follicles that suggest an imminent or recent spawning period. The presence of spawning areas within the Mediterranean shed light on the fact that the bluefin tuna can complete its life cycle in the Mediterranean without having to migrate into different waters in order to spawn.

The present study, performed on a consistent number of female specimens indicates that 50% of the Mediterranean bluefin tuna reaches the first sexual maturity at 104 cm (fork length). The analysis of the spines indicates that all the specimens had completed the formation of the ring corresponding to their last year of life. This finding is in agreement with Cort (1991) and Megalofonou and De Metrio (2000) who reported that ring completion occurs during April and May for bluefin tuna caught in the Mediterranean. This data indicates that the estimated age of most of the specimens is 50 % sexual maturity at 3 years while 100% maturity is reached at 5 years. In the case of the Western Atlantic, no study reports the size of 50 % sexual maturity. The only data available indicates that maturation starts at the age of 6 and 100 % maturity is reached by the age of 8 years at a fork length of 190 cm (NRC, 1994). It can be concluded that for the Mediterranean, female bluefin tuna reach first sexual maturity at a markedly lower age and size than the Atlantic population.

The pop-off satellite technology has the potential to improve the identification of discrete biological stocks, which is paramount to the implementation of effective management. Till now no concrete conclusions could be drawn since the number of pop up tags attached was small. However the results obtained from this preliminary study indicate that bluefin tuna

present in the Mediterranean remain in the Mediterranean and that not all migrate back to the Atlantic as was previously thought and assumed.

The study of the otoliths has demonstrated that the chemical signatures in the otoliths of bluefin tuna from the Mediterranean and the Atlantic nurseries are relatively distinct and even show some degree of temporal persistence. Data are critically needed to estimate exact origins of bluefin tuna populations and findings from future otolith based assessments will most likely play a central role in providing a means of identifying source and relative contributions of different nursery grounds in the Mediterranean Sea and the Atlantic ocean.

The genetic studies have demonstrated the low genetic differentiation with the Libyan and Tunisian locations. Thus, these locations are genetically closely related to each other and also to the rest of the Mediterranean. On the other hand, the genetic differentiation of the Maltese sample was probably due to the small size effect but clearly more analysis involving comparisons between individuals targeted during the same year would be desirable to corroborate these findings. In this sense, this data could be used to confirm the genetic homogeneity of the bluefin tuna distribution in the Mediterranean Sea and the hypothesis that this is a separate stock in all senses.

In addition to the above research areas, evidence for stock identification was also sought from work being done by others. The determination of the accumulation of methyl mercury in the tissues of bluefin tuna is also a good way of identifying the stock since it can be used together with the structural and chemical characteristics of the otoliths as an indicator of the physiological and habitat characteristics of the species.

Among marine organisms, bluefin tuna tends to accumulate relatively large amounts of mercury because they are high in the food chain and because they have a long life cycle. In most species, mercury content increases with size (Davenport, S. 1995). Given the commercial importance and the high demand for bluefin tuna on the food market, it was important to determine the presence and quantities of some heavy metal pollutants in the dorsal muscle of these fish. Mercury levels in bluefin tuna targeted in the Mediterranean were found to be higher than the levels allowed by the Italian legislation (Storelli *et al.*, 1993).

Mercury levels were determined in 34 samples of bluefin tuna caught from the Libyan coast and the concentration of mercury from the samples analysed ranged between 0.2 to 1.54 ppm. The average concentration was 0.52 ppm (Hassan, T. E., 1987). In a study carried out by Storelli *et al.*, (2002) total mercury concentration in bluefin tuna ranged from 0.84 from 0.16 to 2.59 mg/kg wet weight (mean 1.18 mg/kg)

When bluefin tuna samples were analysed, methyl mercury was the predominant form present in the muscle comprising 57.4 – 94.7 % of the total mercury content (Cappon & Smith, 1982).

In a study carried out by Morales *et al.*, (1991), the mercury levels in the flesh of bluefin tuna ranged widely. In juvenile fish, they were high and significant, showing the dependence of mercury level on fish size. The amount of mercury increased significantly in the older fish but was poorly correlated with weight. The elemental composition of fish otoliths was shown to reflect pollution, geographical effects and temperature of the environment in which the fish had been living.

In the case of the Atlantic, the only reference material that could be found was the routine analysis carried by the U.S Food and Drug Administration - Centre for Food Safety and Applied Nutrition. During the Food and Drug Administration Programme (1990 – 2003), the levels of methylmercury in tuna ranged between 0.02 and 1.06 ppm. These data could not be directly compared with data for the Mediterranean since the species of tuna was not specified.

8.3 Management strategies

Over the past decades, the central Mediterranean has seen changes in the fleets fishing in this area. Prior to 1994, the central area of the Mediterranean was fished predominantly by industrial longliners of both coastal and non coastal states. Lately the industrial longlining effort started decreasing whilst the purse seine fleet started to increase significantly as described in the first chapter. This was mainly due to the development of the tuna farming or penning development. Also more purse seining fleets were observed to shift their effort to the central Mediterranean.

Prior to the development of tuna penning, the artisanal longlining fleet, the industrial longlining fleet and purse seining fleet never reported any gear conflict. Conflicts have now been reported pertaining more to the physical characteristics attached to the tuna penning operation. In fact, in 2002 there were serious frictions between local Maltese tuna fishermen (small to medium scale longliners) and the tuna penning industry and its associated purse seine fleet. Disputes involving Italian (from the Adriatic) and Spanish fishermen, on one hand and Maltese longliners, on the other, required the presence of the Armed Forces. This situation, overcrowding and the high technology arising from the physical characteristics of the penning operations have greatly affected artisanal longline

fisheries in the Mediterranean. The livelihood of artisanal fishermen has been grossly affected since it disrupts their fishing operation by causing physical damage to their longlines which result in a decrease in landings and profits.

Members of ICCAT, Cooperating Parties and observers of ICCAT have adopted ICCAT's recommendations to decrease fishing effort after 1994. This measure was aimed at reducing landings throughout the Mediterranean basin. That is to say that the 25% decrease in allowable catches has been counterbalanced by a shift that has seen effort in this area increase by a greater percentage. The increase in efforts towards this area has been generally by the purse seining fleets which target bluefin tuna during its spawning season and aim at catching tuna and keeping it alive.

Purse seining exerts a more efficient fishing effort than longlining due to the technological sophistication of this fishing operation. This means that the pressure on bluefin tuna fisheries in the Mediterranean has increased considerably over the last years. The decline in abundance of resources and the risk of their exhaustion has been announced by international organisations such as the conference on the International Trade of Endangered Species (CITES). There are various approaches to sustainability, ecological, biological, economical and social. An ecological equilibrium and socio-economic equilibrium greatly depend on each other and sustainability can only be guaranteed if they are in harmony. It is vital for future fisheries management to follow the precautionary approach by keeping a *status quo* in the absence of sufficient information in order to assure that the existing equilibrium is not upset.

The control of fishing effort in the Mediterranean is highly regarded as a major tool for the sustainability of Mediterranean fisheries by regional bodies such as the General Fisheries Commission for the Mediterranean and ICCAT.

Until now the total allowable catches of bluefin tuna yearly amount to around 32, 000 metric tons in the Mediterranean Sea and just 3,000 metric tons in the Atlantic Ocean. But since the hypothesis was that trans-Atlantic migration exists, the population of Atlantic bluefin tuna was managed by ICCAT and decisions were equally taken by all those concerned with fishing for bluefin tuna both in the Mediterranean Sea and the Atlantic Ocean.

This means that the countries fishing in the Atlantic were fully involved in the decisions to be taken regarding fishing in the Mediterranean Sea. This cannot be considered to be the best solution any more. It is right to have a single managing body like ICCAT but since there are further studies suggesting that the Mediterranean stock is a separate one, more power in the decision making procedures has to be given to the Mediterranean coastal countries which fish in the Mediterranean Sea over the countries which fish only in the Atlantic Ocean. Then ICCAT should be the managing body overall.

If more data support the evidence of a separate Mediterranean stock, then one should consider the management of the Mediterranean bluefin tuna population to be done by a Mediterranean regional body like GFCM (General Fisheries Commission for the Mediterranean). This should be the regulatory body for the bluefin tuna in the Mediterranean and then discussions should be held between GFCM and ICCAT so that similar decisions are taken with regards to the bluefin tuna population in the Atlantic Ocean and in the Mediterranean Sea.

Tuna farming is considered as capture based aquaculture since it depends on the wild stocks for supplying the fish. This is having serious implications on the management of fish of the wild stock. Bluefin tuna is just being kept for a few months in cages to be sold

out season and fetch higher prices. In fact it is mainly kept in cages to enhance the quality of the meat and enhance the lipid content. But now the most important factor is not that it has to be supplied fresh but that it must have an ideal lipid concentration in its meat. The trend is just to enhance its lipid content, harvest it and deep freeze it and then wait until the best market opportunities are available.

Initially tuna farming showed huge profits and this fact attracted many business people into this new type of industry. The problem is that they invested so heavily that they have created pressure to develop purse seine fleets with new technology with an increasing fishing pressure. All these have lead to a huge over capacity.

A strict and immediate moratorium on the development of new tuna farms in the Mediterranean should be considered. One needs to go beyond the simple creation of a positive list of nationally authorised farms as recently adopted by ICCAT since this does not provide for any kind of limitation in numbers.

A true tuna aquaculture independent of capture fisheries would involve controlling all the stages of the life cycle of bluefin tuna. Therefore now the only remaining solution seems to be that tuna farming must find a way in order to avoid interfering with the normal spawning. But on the other hand it should help to enhance it. This can only be done either by fishing after natural spawning has already occurred or by setting up closed fishing areas. This could be achieved by shifting the bluefin tuna fishing season from May – July as it is at present to the end of June – mid August. Another option could be the setting up of closed spawning areas.

One could also suggest the collection of eggs from the bluefin tuna held in captivity, growing them in artificial nurseries and then releasing them back into the wild in order to re-establish the natural wild population.

8.4 Recommendations

1. Biometric relationships in line with those carried out in Chapter 2 will have to be carried out in the Atlantic in order to fully confirm differences between the west Atlantic, the east Atlantic and the Mediterranean.

2. As suggested in Chapter 3, the collection of new data will allow the construction of age - length keys (from age 1 to age 16 or more), also taking into account sex, for the different Atlantic and Mediterranean populations. These keys are of fundamental importance in all aspects of bluefin tuna being studied.

3. Further pop up tagging will need to be carried out throughout the Mediterranean and Atlantic to make the results obtained in Chapter 5 more significant. Since the analysis was carried throughout the Mediterranean but on a small scale, more analysis is needed to fully confirm the hypothesis.

4. Continuation of the experiments detailed in Chapter 6 to measure otolith chemistry, methylmercury and genetic analysis could be carried out on bluefin tuna in order to determine their origin and further confirm the presence of a residing Mediterranean population.

5. Further collection of otoliths and muscle samples from the Mediterranean and Atlantic tunas can be used to suggest age structure, critical life history periods, growth

environments stock structure, food web position and migration history. This will provide the data needed to further confirm if a permanent residing Mediterranean population exists or not.

6. It is vital for future fisheries management to follow the precautionary approach by keeping a *status quo* in the absence of sufficient information in order to assure that the equilibrium is not upset.

7. The control of fishing effort in the Mediterranean is highly regarded as a major tool for the sustainability of Mediterranean fisheries by regional bodies such as the General Fisheries Commission for the Mediterranean and ICCAT. In this respect, a possible measure for the management of bluefin tuna stocks in the Mediterranean could be:

i. curtailing the shifting of fleets in order to allow the historical fishing patterns to exploit their respective areas ensuring the relative stability of fishing effort distribution.
ii. creating a specific management regime to control the fishing effort on the different types of fishing operations taking place in any one area to avoid conflicts between all stake holders.
iii. establishing closed seasons so as to allow the juveniles to grow and the adult bluefin tuna to spawn.
iv. establishing a direct reporting system to ICCAT through the installation of electronic logbooks on all industrial fishing vessels targeting bluefin tuna in the Mediterranean sea so as to have direct control of the quota.

8. One could also suggest trying the collection of eggs from the bluefin tuna held in captivity, growing them in artificial nurseries and then releasing them back into the wild in order to re-establish the natural wild population. The rapid growth of tuna farming is unsustainable and harming already depleted Mediterranean wild tuna stocks. The fast development of new tuna farms in the Mediterranean should be halted until the implications of this activity on the environment, the tuna stock and the fish stocks used as baits are properly addressed at the appropriate international and national levels. Initiatives like limiting the fraction of tuna quota susceptible of being farmed or establishing minimum farming sizes for tuna would be the kind of measures which would lead to a more sustainable Mediterranean bluefin tuna population.

9. New management measures have to take into account the new developments in the bluefin tuna fishing industry and should aim for the sustainable exploitation of the species.

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