THE INTERNATIONAL COMMISSION FOR THE CONSERVATION OF ATLANTIC TUNAS (ICCAT)

# TAGGING MANUAL FOR THE ATLANTIC-WIDE RESEARCH PROGRAMME ON BLUEFIN TUNA (GBYP)



## TAGGING MANUAL FOR THE ATLANTIC-WIDE RESEARCH PROGRAMME FOR BLUEFIN TUNA (ICCAT-GBYP)

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#### **1. INTRODUCTION**

There is currently a management plan in force for the eastern stock of the Atlantic bluefin tuna (ABFT), *Thunnus thynnus* (Linnaeus, 1758), with the objective of rebuilding the stock to the  $SSB_{F0.1}$  level with a probability of at least 60% by 2022. However the SCRS noted that, there are considerable data limitations in respect of the assessment; so that, when taking into considerations all the degrees of uncertainty, the SCRS analysis could change the estimated rebuilding probabilities. For these reasons new approaches are required to improve scientific advice.

In order to reduce the uncertainty about the stock dynamics of ABFT, improve the ability to estimate stock status and reference points and to provide robust management advice, the SCRS has indicated several priorities identified in the ICCAT Report 2008-2009 (II), 1: 224 and ICCAT Report 2008-2009 (II), 2: 223-224).

Under the GBYP research programme for ABFT, tagging is one of the activities to be conducted. The priorities of GBYP are:

1. Improve basic data collection through data-mining (including information from traps, observers, and VMS), developing methods to estimate sizes of fish caged, elaborating accurate CPUE indices for Mediterranean purse seine fleets, development of fisheries-independent information surveys and implementing a large scale well planned conventional and genetic tagging experiment;

2. Improve understanding of key biological and ecological processes through electronic tagging experiments to determine habitat and migration routes, broad scale biological sampling of live fish to be tagged and dead fish landed (e.g. gonads, liver, otoliths, spines, etc.), histological analyses to determine ABFT reproductive state and potential, and biological and genetics analyses to investigate mixing and population structure; ecological processes, including predator-prey relationships;

3. Improve assessment models and provision of scientific advice on stock status trough improved modelling of key biological processes (including growth and stock-recruitment), further developing stock assessment models including mixing between various areas, and developing and use of biologically realistic operating models for more rigorous management option testing.

Tagging studies have the potential to reduce key uncertainties about important population parameters, e.g. natural mortality, to provide fishery independent data for stock assessments and to reduce uncertainty about stock structure that may invalidate current assessment assumptions. The specific objectives of the tagging design in relation to conventional and PIT tagging are:

• Validation of the current stocks status definitions for populations of ABFT in the Atlantic and Mediterranean Sea. It is particularly important to consider possible sub-stocks units and their mixing or population biomass exchange in the Mediterranean Sea.

• Estimation of natural (M) and total mortality (Z) rates of ABFT populations by age or age-groups.

• Estimation of reporting rates for conventional tags, by major fishery and area, using the observer programs currently deployed in the Mediterranean Sea.

For the potential use of electronic tags are:

- Evaluate habitat utilization and movement patterns (spatio-temporal) of the spawning population within the Mediterranean Sea, with emphasis on: (i) vertical and horizontal distribution patterns of the spawning stock, to help calibrate the aerial surveys and estimate sighting probabilities; (ii) investigating how mature specimens use the spawning grounds (e.g., do ABFT visit the same spawning grounds every year to the exclusion of all others, or do they visit several spawning sites and, if so, over what periods); (iii) validation of the current stocks status definitions for populations of ABFT and estimation of mixing rates between management areas.
- Similar to previous, but for the Gulf of Mexico spawning grounds.

#### 1.1. Identification and physical description

#### Scientific name: Thunnus thynnus (Linnaeus, 1758)

ABFT (Fig. 1.1.1.) belongs to the *Scombridae* family. Long, fusiform and round body with small eyes considering its size, small conical teeth in a single row, short pectoral fins. It has two dorsal fins, the second of which (brown-brick red) is higher than the first (bluish/yellow). The finlets are yellow and the central caudal peduncle is black in adults and semi-transparent in juveniles. Dark blue in colour, tending to black on the dorsal side and in the upper area, silver lower sides with white and grey spots (which fade after death).



Fig. 1.1.1. Atlantic bluefin tuna © IEO

ABFT can reach a weight of over 600 kg, a length of over 3 m (Cort, 1990; Restrepo *et al.*, 2009) and live for more than thirty years (Neilson & Campana, 2007). It feeds on fishes, cephalopods and small crustaceans such as pelagic crabs (Fig. 1.1.2.) and krill (Ortiz de Zárate & Cort, 1986).



Fig 1.1.2. Atlantic bluefin tuna eating pelagic crabs © IEO

#### 1.2. Habitat

It is a fish with a highly evolved heat exchange system in its bloodstream and its internal temperature can be up to 21°C higher than the surrounding water (Carey & Teal, 1969). This is one of the reasons for its wide oceanic distribution.

ABFT can appear in the warm waters of the Bahamas of around 30°C (Rivas, 1954) and soon afterwards reappear in Norwegian waters (Mather III, 1962), where the water temperature is barely above 10°C. De Metrio *et al.* (2002) cite its presence near the Arctic circle (75°N) where temperatures of 5°C are recorded.

Recent aerial surveys in the western Mediterranean (Bonhommeau *et al.*, 2010; Sorell Barón, 2010) reveal that ABFT frequent surface waters in both the spawning and trophic seasons, and electronic tagging surveys also show that they often dive to great depths, sometimes to over 1000m (Teo *et al.*, 2007; Wilson & Block, 2009).

#### **1.3.** Geographical distribution and fishing areas

In the Atlantic Ocean, ABFT are found in waters between Labrador (Canada) and Brazil, including the Gulf of Mexico on the western side (Mather III *et al.*, 1995), and on the eastern side from Norway (Tiews, 1963) to Senegal (Ngom Sow & Ndaw, 2010) and Cape Verde (De Metrio *et al.*, 2002), including the Mediterranean and Black seas (Fromentin, 2006).

The evolution of ABFT fishing in the North Atlantic has passed through different phases over the last seven decades (ICCAT, 2010; Fig. 1.3.1.). The most outstanding of these was the development of Japanese longline in the 1960s and of purse seine in western fisheries; also the fall in fishing by the traps of the Strait of Gibraltar and the Mediterranean in the 1970s; in those years purse seine fishing began in the Mediterranean.



BFT-Figure 1. Geographic distribution of bluefin tuna catches per 5x5 degrees and per main gears.

Fig 1.3.1. Distribution of the Atlantic bluefin tuna fishing (ICCAT, 2010)

In the 1980s the purse seine fishery disappeared in the north of Europe (Nøttestad & Graham, 2004). From the 1990s purse seine fishing in the western and eastern Mediterranean (Libya and Turkey) increased along with the Japanese longline in the central and eastern Atlantic. In more recent years new hook fisheries have been established in the Mediterranean (De la Serna, 2004).

#### 1.4. The Atlantic population

For the purposes of resources management the North Atlantic population is split into two stocks: the Western and the Eastern, which includes that of the Mediterranean. There is mixing between the two with interannual variations (ICCAT, 2010). The separating line between the stocks (Fig. 1.4.1.) runs along the 45° W meridian of the northern hemisphere.



Fig 1.4.1. Dividing line of the Atlantic bluefin tuna stocks (45° W) © IEO

The separation of the eastern and western stocks is mainly based on the existence of two spawning areas, one in the Gulf of Mexico and the other in the Mediterranean Sea. Moreover, in the year in which this separation was adopted (1975) it was taken into account that most of the ABFT tagged were recovered in the same part of the ocean that they had been tagged in, and that there was no evidence of a spawning area in the central Atlantic (Mather III *et al.*, 1995).

Fromentin & Powers (2005) describe the ABFT population as a set of local populations, denominated metapopulations, occupying different habitats and having a certain degree of influence over one another.

Electronic tagging shows that ABFT crosses the dividing line of 45° N without any difficulty (Block *et al.*, 2005); nevertheless, when spawning they are faithful to their place of birth (spawning fidelity) (Fig. 1.4.2.).



Fig. 1.4.2. Daily positions of adult ABFT differentiated as western (in red) and eastern (in yellow). The ABFT populations share the same feeding areas but return to the spawning areas in the Gulf of Mexico (in red) and the Mediterranean (in yellow) (Block *et al.*, 2005; Rooker *et al.*, 2007).

De Metrio *et al.* (2002; 2004; 2005) and Fromentin (2010), from electronic tagging studies using pop-up tags, show the possible existence of resident populations in the Mediterranean since most of the fishes tagged remained on the side where they had been tagged (Fig. 1.4.3.).

Tudela *et al.*, (2010), by electronically tagging fishes during the trophic season (August and September 2008) in the western Mediterranean, obtained results which support the residence of ABFT in the interior of the Mediterranean, as migrations towards the eastern part of the Mediterranean do not take place.



Fig. 1.4.3. Pop-up positions of satellite tags applied to ABFT released in the Mediterranean Sea and eastern Atlantic in the period 1998-2006 (De Metrio *et al.*, 2002; 2004; 2005).Light blue circles: tags deployed in the eastern Mediterranean; red circles: tags deployed in the central Mediterranean; green circles: tags deployed in the western Mediterranean.

The results of conventional tagging in the Mediterranean (Arena & Li Greci, 1970; Godoy *et al.*, 2010) and the presence of ABFT larvae in the Levantine Sea (Karakulak *et al.*, 2004; Oray & Karakulak, 2005) support the hypothesis of the resident populations in the Mediterranean.

#### **1.5. Migrations**

Migrations depend on fish age and size, which are mainly related to reproduction and the search for food.

The migrations of adult fishes towards spawning areas in the Mediterranean and their return to the ocean for feeding have been known since the time of Aristotle (384 B.C. - 322 B.C.).

Migrations to spawning areas (Fig. 1.5.1.) get longer as the ABFT increase in size.



Fig 1.5.1. Genetic migrations © IEO

Trophic migrations of spawning fishes (Fig. 1.5.2.) begin when the reproductive period has come to an end. Many of these ABFT return to the Atlantic ocean on a trophic migration (Mather III *et al.*, 1995; Rodríguez-Roda, 1964; Medina *et al.*, 2010). The dispersion of schools after passing through the Strait of Gibraltar occurs in a north-south direction (De Metrio *et al.*, 2003) between June and December. Regarding the western stock, Mather III (1962) and Tiews (1963) were the first to publish evidence of transatlantic migrations of large spawning ABFT.



Fig 1.5.2. Trophic migrations © IEO

The migrations made by juvenile fish are generally shorter than those of the larger fish; nevertheless, transatlantic migrations have been reported since more than four decades ago, especially in certain years (Mather III *et al.*, 1967; Mather III & Jones, 1973; Aloncle, 1973; Cort, 1990). Rooker *et al.* (2006), in studies into the chemical composition of fish hard parts, show that these migrations take place in certain years in highly significant quantities.

# 2. ABFT FISHERIES AND SUPPLYING FISH FOR TAGGING IN THE ATLANTIC AND THE MEDITERRANEAN SEA

To carry out the present ICCAT tagging programme, a large number (several tens of thousands) of ABFT will be caught and tagged.

ABFT can be caught using various techniques, mainly purse seines, traps, live-bait boats, longline, troll line. However, some fishing methods are not suitable for tuna tagging, because the fish are no longer in good enough physical condition to survive after tagging. Therefore, catch procedures must be rapid and harmless for the fish; moreover, they need to be able to provide large quantities of tagged fish.

Tunas caught by longline, a passive gear, spend some time on the hook and become stressed by fighting against it before being hauled on board for tagging; this lowers their survival rates. Moreover, only a small number of fish are caught at one time. Both methods are unsuitable for large-scale tagging programmes.

Other fishing methods mentioned, namely live-bait boats, purse seines, and traps, appear to be suitable for the requirements of tagging. In particular, troll-line fishing has been used successfully for small-scale tagging of ABFT.

#### 2.1. Baitboat

Catching tunas by pole-and-line fishing is a rather ancient technique that has been and is used both in sport fisheries (angling) and in commercial fisheries. It consists of a hooked line attached to a pole that is made either of wood (including bamboo) or fibreglass. In modern times, this technique has been highly improved by equipping ad hoc medium-sized vessels, of up to around 40 meters, with several (10 to 20) fishermen handling poles almost all around the circumference of the boat. (Fig. 2.1.1.).



Fig. 2.1.1. Baitboat at the port of Tarife (Spain) (photo by M. Deflorio).

The boats carry live bait to entice the fish to aggregate around the boat by using bait and/or water spraying. Hooks at the line end are baited with live bait. When fishing starts, fishermen cast the hooks into the water and haul them back as soon as the fish bite, just a few seconds later. Tunas caught in this way are landed on the deck where they get free from the hook, due to the fact that the hook is barbless. In this way, several tons of tuna can be landed in just a few hours (FAO, 2006).

In order to catch tunas of over 10-15 kg, and up to 100 kg, fishermen still use one pole, fitted with a rope which is pulled through a pulley by another person. Baitboat fishing (Fig. 2.1.2.) is quite apt for large-scale tuna tagging programmes, where large numbers of tunas need to be caught within a short timeframe. Most importantly, pole-and-line caught tunas have proven to be in good condition for tagging purposes, since they are usually not stressed or injured.



Fig. 2.1.2. Baitboat fishing activities in the Strait of Gibraltar (photo by M. Deflorio).

When tagging tunas caught by live-bait boats, the hooked juvenile specimens are directly landed in the tagging cradle, whereas the larger ones are placed on tagging mattresses (Fig. 2.1.3.).





Fig. 2.1.3. Tuna specimen landed on a cradle (left) or on a mattress (right, photo by M. Deflorio).

#### 2.2. Tuna purse seine

Purse seines for catching ABFT are operated by large ad hoc vessels (up to 42 m long), known as purse seiners. They go after fish which have aggregated and are swimming comparatively close to the sea surface (i.e. in the mixing zone above the thermocline) both in high-sea waters and in coastal areas. Aggregated tuna resources up to a maximum depth of 300 m – but mostly at depths of 60-70 m – are targeted.

Tuna purse seiners (Fig. 2.2.1.) are usually equipped with high-tech instruments that also enable the captain to identify the size of the fish in the school detected by echosounder. This makes it possible to target the fishing at a particular size of fish to be tagged.



Fig. 2.2.1. Turkish tuna purse seine in the Levant Sea (photo by M. Deflorio).

Currently, purse seiners catch ABFT over 30 kg in weight, except in the Adriatic Sea, where smaller fish may be caught by the Croatian fleet.

The purse seine gear is made up of a large net which encircles the tuna school and is closed at the bottom to entrap the fish. The net measures 1500 to 2000 m in length and 120 to 250 m in depth. The mesh size of purse seines used in the Mediterranean Sea to catch ABFT is up to 200 mm in the body and the bottom part of the net, dropping to around 120 mm in the bunt. The top of the net is mounted on a floatline and the bottom on a steel chain (leadline) with steel rings, which allow the net to be "pursed".



When a school is detected, the vessel places itself on one side of the school; subsequently the skiff, a small high-powered boat, attached to one end of the purse seine, is released. The fishing vessel then encircles the school at maximum speed. Once the encirclement is finished (4-8 minutes), the end of the net attached to the skiff is transferred aboard the purse seiner and the two ends of the purse line cable are hauled in as quickly as possible in order to close the net at its bottom; this is called "pursing". In the case of large purse seines, these pursing operations may take around 15 to 20 minutes. The net is then pulled aboard the vessel. As a rule, this operation will take around one hour, provided there are no incidents. Subsequently, fish harvested from the purse seine are stored in the well, in brine, at  $0^{\circ}$ .

Recently, most of the fish are transferred to floating cages soon after the school capture (Fig. 2.2.2.) and the pens are transferred to a "tuna farm" for fattening activities (Ottolenghi et al., 2004).



Fig. 2.2.2. Fish transferring to a floating cage (photo by M. Deflorio).

For tagging purposes, at the end of pursing, the net is not fully drawn and is maintained at sea, so that the fish are left to dive in enough room, calculated according to the size of the school, so as to avoid injuries and reduce stress for the fish. In the case of schools made up of juvenile fish, up to 10-15 kg, they are caught one by one from the purse with a long handled scoop net and brought on board to be tagged. Soon after tagging, each fish is released into the sea keeping it away from the net. In the Mediterranean Sea, juvenile ABFT are generally sighted at sea and caught in spring and autumn. The highest concentrations of such fish generally occur in the Gulf of Lion and the Tyrrhenian Sea (Fromentin et al., 2003).

Adults ABFT, i.e. at least 5 years old and weighing an average of 35 kg (Santamaria et al., 2009), are too heavy to be brought on board by scoop net. Therefore, in order to be tagged with conventional tags, they are either:

a) individually seized and placed in a stretcher by a diver within the purse seine, brought to the side of the main vessel or of an auxiliary boat, tagged and then released away from the purse net; the fork length is estimated by comparison with a measuring pole (Fig. 2.2.3.); b) or transferred from the purse-seine net into a towing pen (or floating cage) attached to the purse seine; once in the pen and, in any case, once they have calmed down (which could take several hours), they can be caught by pole-and-line or another technique and then tagged and released.



Fig. 2.2.3. Atlantic bluefin tuna transfer on a stretcher for tagging purposes (photo by V. Papadopoulos).

Although purse seiners catch tunas in very large numbers, they are all caught at once. By the time the fish have been concentrated into the net, many of them may result already stressed, exhausted and injured, if not already dead. For this reason, only a fraction of purse-seined fish is suitable for tagging.

Individual tunas must therefore be checked carefully before tagging and those too distressed rejected in order to keep survival rates to an adequate level. Both reducing cramping of tunas in the purse seine, by keeping the net volume sufficiently wide, and transferring the large tunas into a pen until they calm down, may greatly help in achieving satisfactory survival rates.

#### **2.3. Traditional tuna traps**

The traditional tuna trap, known as *almadraba* in Spanish, *tonnara* in Italian and *madrague* in French, is a fishing technique for catching migrating tunas dating back thousands of years. It is a system of large nets that intercepts schools and small groups of tunas along their route near the coast and traps them (Fig. 2.3.1.).

The overall trap scheme consists of a long standing vertical net (actually a set of nets) erected perpendicular to the coast to intercept the fish and convey them into the actual trap, which is made up of a maze of pools or chambers.

The last of these (the so-called "death chamber") is fitted with a liftable floor which, when raised, clusters the fish into a restricted space so that they are easily caught and slaughtered. This simple maze works because the tuna are unable to see the exit from the central pool, and so remain inside.



Fig. 2.3.1. A traditional tuna trap. (http://www.photolib.noaa.gov/htmls/fish2059.htm)

The system of nets is connected to the shore, is anchored to the bottom and is kept perpendicular to the sea surface by a line of floats. The net mesh size is small enough not to let the tuna become entangled. The long intercepting net can be positioned so as to intercept tunas coming from either direction. For instance the Barbate (Spain) trap, close to the Straits of Gibraltar, is first positioned to intercept pre-spawning tunas entering the Mediterranean and, afterwards, is repositioned to intercept post-spawning tunas coming out of the Mediterranean (De Metrio et al., 2002; de la Serna et al., 2004). Other traps, such as the one in Carloforte (Sardinia, Italy), catch only migrating pre-spawning tunas (Corriero et al., 2003 and 2005).

In the last twenty years, traditional tuna traps have been used in Spain and Morocco, both on the Mediterranean and Atlantic coasts comparatively close to the Straits of Gibraltar, Tunisia and Italy. The traditional trap fishing season is adjusted to the tuna's biological cycle, in particular to their genetic migrations (Heinisch et al., 2008). The fishing season extends from spring to early autumn, according to the geographical location where the trap is set.

The traditional tuna trap has excellent potential for tagging adult ABFT. For the purposes of tagging, trapped tunas may be individually taken from the death chamber using either a scoop net (for smaller ones), pole-and-line (for small and medium-sized ones), or a stretcher handled by a diver. Hence, each fish to be tagged is brought on board a boat anchored alongside the trap.

#### 3. TAGS DESCRIPTION AND TAGS INSERTION METHODS

#### **3.1.** Conventional tagging

#### 3.1.1. Generalities

Conventional tagging is carried out by means of *conventional tags* also known as *dart tags* or *spaghetti tags* (Fig. 3.1.1.1.). These tags are simply designed, low cost and easy to insert into the fish. Conventional tags are typically used in large-scale tagging programmes, which is the present case with ABFT tagging.



Fig. 3.1.1.1. - Spaghetti tags with different types of dart.

Indeed, the need to deploy a large number of conventional tags originates from the need to obtain a sufficient number of returned tags in order both to validate the current stock status definitions for ABFT populations in the Atlantic and the Mediterranean and to assess their mortality parameters (natural and fishing-related). The use of a large number of conventional tags is also justified by the current low rates of tag returns. Since the aim of this type of tagging is to mark thousands of tunas, it derives that two requirements must be met:

- a) catching many individuals in a comparatively short time;
- b) tagging them rapidly and effectively.

A most important issue related to tuna tagging is the fish survival rate after tagging operations. Hence catching, handling, marking and releasing procedures must be as fast as possible, in order to reduce stress, and cause the least possible detriment to fish health.

#### 3.1.2. Tagging equipment

The equipment required for tagging should be available to the tagging teams several days before the start of tagging fieldwork. Hence, orders to acquire equipment must be placed well in advance of tagging operations.

Tagging personnel must be acquainted with all the individual pieces that make up the equipment before embarking, especially with tags and their applicators, the cradle fitting, and the recording forms to be filled out.

- Tags: each team should be provided with at least 10% more tags than needed for each tagging trip to allow for breakages and losses.
- Tag applicators: for rapid tagging, a sufficiently large number of tags (i.e. equal to the potential number of tuna that will be tagged) must be prepared in their applicators before each fieldtrip. Each tagging team will be provided with a number of applicators matching each daily tagging trip needs. These will be placed in a ready to use container.
- Tags holders: tag-loaded applicators must be suitably stored before embarking. Two storage methods have been used. The preferred container in the case of large-scale tagging is made up of a wooden block with holes drilled into it. The loaded applicators are placed into these holes in an upright position, with the sharp end facing up. Suggested measurements for one-hundred-applicator boxes are: 38 cm in length, 12 cm in width and 7-10 cm in height (Kearney and Gillett, 1982) or 36.5 x 12 x 7 (Itano, unpublished in Hallier, 2004). In each box, 4 rows of 25 holes are drilled to a depth of 1.5 cm. This depth is regarded as a good compromise between overall compactness and handling, i.e. how easy it is for the tagger to get hold of the applicators. The top should be painted white and the holes numbered sequentially. Boxes should be made of a wood that will not swell with water and should be provided with a cover. The number of applicator-loaded boxes to prepare should be calculated on the basis of the number of tags expected to be used on each trip. Care must be exerted when handling the loaded boxes since the applicator tips can wound both the taggers and the fish; moreover, fishing lines may become entangled in a set of applicators. In fact, during tagging operations, the box should be placed close to the cradle within reach of the tagger's hand. A safer way to store tag-loaded applicators is in plastic-canvas 'aprons', into which thin pockets are sewn to hold the applicators. These could be rolled up tightly when not in use (Anderson et al., 2004). The latter method is better for small-scale tagging.
- Cradle or tagging platform: these are the apparatuses where the fish is landed to be measured and tagged. A cradle consists of a working platform placed on top of a steel frame at a height which enables the tagger to work comfortably during large-scale tagging. The cradle frame is made from galvanised or stainless steel tubes in order to withstand the corrosiveness of sea water. Suggested measures are 160 cm in length, 108 cm in width and 95 cm in height (Kearney and Gillett, 1982). The cradle platform is made from tough but smooth vinyl material and is shaped like a large shallow spout, gradually sloping down towards one end.

The fish will be gently placed onto the platform and made slide down until the fish nose touches the platform stop. A drainage hole must be made at the lowest point of the platform. The tagging platform must incorporate a tape to measure fish length showing 0 at the end where the fish nose stops; otherwise centimetres should be marked on the platform surface with water resistant markers. In case of large-scale tagging, such as with live-bait boats, more than one cradle should be placed on board the vessel in order to take advantage of the fairly high catch rates.

- Tagging mattress: when dealing with tuna fish so big that use of the cradle is not advisable, a mattress filled with foam padding and covered with tough but smooth vinyl material can be used. Cm should be marked on the mattress surface with water resistant markers.
- Measuring tapes.
- Measuring boards.
- Plastic bucket to collect used applicators
- A piece of dark cloth (black, brown or red) to cover the tuna's eye to calm it down
- Cotton or rubber gloves for handling the fish, hat and protective glasses for protection from the hooks, boots and clothes suitable for the weather conditions on the fieldtrip.
- Recording forms (best if printed on plastic sheets suitable for writing) and tape recorder to record tagging and associated data.
- Waterproof boxes to store recording forms
- Writing tools: pencils, pens, pencil erasers, waterproof markers and alcohol to clean waterproof marks. Staplers.
- Bleach or and detergent to clean the applicators after use.
- Walkie-talkies to communicate between teams
- Binoculars
- Tuna fish identification sheets
- Spare parts

#### 3.1.3. Tag size

Larger implanted tags are potentially more visible than smaller ones to fishermen and others who will be handling the fish, but the larger the tag the greater the disturbance to the fish. Hence, as a general rule, small fish are tagged with small tags and large fish with large ones.

Small tunas have been successfully tagged with dart tags 10 cm in length and 1.5 mm in diameter; larger tunas have been tagged with 12.5 cm x 2 mm dart tags.

#### 3.1.4. Tagging team

A tagging team is usually made up of three people:

- The fish handler: he/she receives the fish from the fisherman, places it on the cradle, ensures that it reaches the right measuring position, measures it and calls out the measurement for the recorder (see below), holds the fish down while the tagger inserts the tag, and puts it back into the sea as soon as tagging operations have been completed.
- The tagger: he/she reads and calls out the fish length for the recorder, reads and calls out the tag number, inserts the tag into the fish.
- The recorder: a) during tagging operations, he/she fills out the recording form with the tagging data (sequential number, length, and tag number for each fish) and also records them on the tape recorder; b) before and after the tagging operations: he/she is responsible for recording and transcribing all the information required in the various recording forms; he/she must be familiar with all the recording forms and how to fill them out.

#### 3.1.5. Tag implantation

The tag must be implanted a couple of cm below the insertion of the second dorsal fin, so that its head, after perforating the skin and muscle, crosses the fish's sagittal plane through the second dorsal fin pterygiophores (i.e. the bones that support the fin rays) and its barb becomes firmly anchored through them.

Insertion in other locations on the body, namely below the first dorsal fin or directly into the muscle, must be avoided. The direction of tag implantation is from the back at an angle with the body of less than  $45^{\circ}$  in order both to minimize the drag due to water resistance during swimming and to ensure that the barb gets firmly anchored in the pterygiophores. Higher angles of implantation would affect the fish's swimming efficiency due to water resistance; lower implantation angles would risk inserting the tag head into the muscle alone.

Tag insertion is carried out with an applicator, a stainless steel tube with a sharp end. The applicator is slightly longer and slightly larger than the tag; the tag is placed inside the applicator so that its head is at the applicator sharp end and the barb is housed in an indentation at the other end of the applicator.

The tagger holds the tag-loaded applicator firmly in his hand and inserts it into the fish body with a brisk and calibrated movement of the hand. Soon afterwards, the tagger retrieves the applicator with a gentle and continuous backward movement of the hand and then checks whether the tag is correctly placed and ensures that the fish has not been badly damaged. It is most important that the tagger be experienced, so that he/she can work fast and correctly, otherwise either the tag will fall out or the fish will be harmed or both: in either case, the tagging would be a failure. Inexperienced taggers are strongly recommended to practice sufficiently on dead tuna fish of about the same size as those that are going to be tagged.

#### 3.1.6. Tagging procedure

Differences are expected according to the fishing methods used for catching the tunas and according to their size. In general, large-scale tagging involves capturing large amounts of tunas over a short period of time, whatever the catching method (live-bait boats, purse seiners, traps). Therefore it is advisable that more than one tagging team should carry out the tagging on each boat. The number of teams depends on the boat size and the space available.

Juvenile tuna are caught in large quantities by live-bait boats. In such cases, the correct procedure begins by identifying the best location on board the vessel for the tagging station: the cradle must be securely fastened to the boat so as to support the tagging work even in rough seas; the cradle tilt and height should be adjusted so as to satisfy the tagging team's needs and make their work as comfortable as possible; all the tagging material and other equipment should be placed in such a position as to be readily available.

The cradle position as well as those of the team members must be chosen in order to make handling the tuna as easy as possible (both when receiving them from the fishermen and when putting them back to sea), as well as disturbing the fishermen as little as possible.

The tagging team members must wear gloves when handling the fish; this protects both the fish and the team members' hands.

The tagging platform and vessel deck in its vicinity, the fish handler's and tagger's gloves, and any equipment that may come into contact with the fish body (e.g. the dark cloth to cover the eye, rulers, and so on) must be kept wet at all times so as not to damage the fish.

When fishing starts and tuna are caught, the fisherman hauls in the fish and passes it to the tagging team's fish handler, or else lands it directly on the tagging platform as gently as possible.

If barbless hooks are used, the caught fish drops by itself when the line is slackened. If this does not happen, the fish handler can remove the hook with a simple swift movement of the line. If the hook does not come off easily (whether it is barbed or not) because it is too deep and its removal may either take too much time or injure the fish, the fish must be rejected. Likewise all injured tuna with seemingly lower probabilities of survival once put back into the sea will be rejected. When the tuna handler receives the fish, he/she gently places it head-first onto the tagging platform of the cradle and slides it down until the fish reaches the platform stop (point 0).

The handler then reads and calls out the fish length (fork length), holding the fish firmly at all times until the tagger has inserted the tag, and puts it back into the sea. In the meanwhile, the tagging team's recorder must record all tagging data (fish size and tag number).

Speed when handling and tagging is crucial for the survival of tagged fish. Each tagging operation lasts just a few seconds overall if properly carried out. Tunas are best handled using both hands, one holding the caudal peduncle and the other sustaining the body, whereas holding the fish just by its tail may damage it. The tunas are always placed on the same side of their body when on the tagging platform, depending on the tagger's position and needs. The fish is put back into the water with its head pointing in the same direction as the boat.

#### 3.1.7. Hygiene

Hygiene is required since there is the potential for tunas to become infected at the site of tag insertion, which in turn would affect their chances of survival and the results of the tagging programme. Before each tagging trip, the tag applicators should be sharpened, thoroughly cleaned and sterilised by boiling in water for at least 15 minutes. Tags should be kept in their plastic wrappers until the day they are to be used when they can be inserted in the applicators; any unused tags which have been kept outside their wrappers should be thoroughly rinsed with fresh water before use. If applicators need to be used a second time on the same day, they must be washed carefully with washing powder and then rinsed thoroughly in several changes of fresh seawater before reusing.

The tagging team members should take care of their personal hygiene, washing their hands before loading the applicators with tags. During both loading and tagging operations, tag heads and applicator sharp end must never be touched with dirty hands or dirty gloves. Moreover, tag boxes or aprons must be kept away from possible sources of contamination.

Gloves, which are necessary for the tagger and the fish handler to protect their hands both from the applicators during tag insertion and from fish spines and hooks, must be made of easy-to-clean material. Their cleanliness is very important for hygiene purposes, both for the tagged fish and for the tagger's skin. Particular care must be exerted in removing fish mucus remains off cotton gloves; for this reason, rubber gloves are preferable.

#### 3.2. Archival tagging

#### 3.2.1. Generalities

In recent times, tag and release technologies have been developed and new electronic devices are used nowadays. Archival tags are small data loggers that record dates, times, swim depths, water temperatures, body temperatures and light levels. Light levels are used to calculate an approximate daily position of the tagged animal based on the time of dawn and dusk and the angle of the sun. Reliable estimates of latitude, however, usually require the use of sea-surface temperature, which can also be recorded by the tag and subsequently matched with relevant data sets obtained by satellite.

Archival tags can be attached externally or internally, and must be retrieved for their data to be downloaded. They are used most commonly on species that have a high likelihood of recapture, including fish, seabirds, sea turtles and marine mammals. Archival tags can record data every few seconds for up to 10 years, depending on the tag sampling frequency and battery life, and provide information about post-release fish mortality rates, oceanic movements and preferred water temperature, clarity and currents to provide new insights into some of the aspects of marine animals' biology.

Archival tags have been used to track return migrations of juvenile Atlantic bluefin tuna from the Bay of Biscay (Goñi et al., 2009) and of juveniles southern bluefin tuna from the Great Australian Bight to the Indian Ocean (Willis et al., 2009), the latter including details of their diving patterns and feeding events (marked by sharp drops in body temperature as food and cold water enter the stomach). Archival tags have similarly revealed pan-oceanic migrations of adult ABFT between spawning grounds in the Gulf of Mexico and the Mediterranean Sea, and feeding grounds off the US and European coasts, as far north as Iceland (Block, et al., 2001, 2005). In shelf seas, archival tags have traced the migrations of demersal fish, such as plaice and cod (Turner et al., 2002; Hunter et al., 2004a and 2004b; Svedäng et al., 2009), and revealed new information on behaviour, temperature, population distribution and the likely effects of climate change. Presently, the most used archival tags are the Mk9 tag (Fig. 3.2.1.1.) by Wildlife Computers and the LAT series tag (Fig. 3.2.1.2.) by Lotek. These tags are design to study also fish. Generally, the tag is suitable for both external attachment and internal implantation. The tag measures depth, temperature, and light-level. Optionally, for implantable applications, the light level and/or a second temperature sensor can be mounted on a sensor stalk.



Fig. 3.2.1.1. Mk9 archival tag (source: Wildlife Computers web site).



Fig. 3.2.1.2. LAT series archival tag (source: Lotek web site).

#### 3.2.2. Tagging equipment

- Archival tags.
- Conventional tags: fish must also be tagged with green conventional tags with a legend specifying the presence of an archival tag inside the fish and the reward.
- Conventional tagging material (e.g., applicators, cradle, mattress, recording forms), as specified under the conventional tagging section.
- Needle-holders.
- Scissors.
- Atraumatic needled sutures: the needle should have cutting edges, so that it can be held in the needle-holder. The size and curve of the needle will depend on the size of the fish (e.g. B Braun Silkam DS 30, size 0; Ethicon PDS II cp-1, size 0).
- Scalpel and disposable blades.
- Surgical gloves.
- 10% Povidone-iodine solution (e.g. Betadine).
- Rigid-framed net of knotless webbing: it can be useful for brailing small fish from the side of the vessel.
- Lift to take the fish onboard.
- Camera and/or videocamera.

#### 3.2.3. Tagging team

A tagging team is usually made up of four people:

• The fish handler, who receives the fish from the fisherman, places it on the mattress, measures its length and calls out the measurement for the recorder (see below), holds the fish down while the tagger inserts the tag and covers the fish eye with a piece of dark cloth if necessary, and takes care of putting it back into the sea as soon as tagging operations have been completed. In the case of very large fish that cannot be handled by just one person and are taken onboard by a lift, the fish handler will be supported by one or more fishermen.

- The tagger, who accomplishes the whole surgical operation to implant the tag inside the abdominal cavity (see paragraph 3.2.4), from cutting an incision in the abdominal wall to closing it.
- The tagger assistant, who takes care of all the surgical instrumentation, the archival tags and conventional tags and their applicators, and sterilize all pieces; he/she hands the surgical and tagging instruments and devices to the tagger upon his/her requests; he/she besides takes care of the hygiene of all devices that will get into contact with the fish to be tagged, e.g. mattress, parts of the deck close to the mattress, and so on.
- The recorder: a) before each tagging operation starts activates the archival tags; b) during tagging operations fills out the recording form with the tagging data (sequential number, length, and tag number for each fish); c) takes photographs and/or videos of the fish and tagging operation; d) before and after the tagging operations is responsible for recording and transcribing all the information required in the various recording forms; must be familiar with all the recording forms and how to fill them out.

#### 3.2.4. Tag implantation

Once the fish is hauled onboard and placed in the tagging cradle or on the tagging mattress, tags are implanted into the abdominal cavity of the fish. An incision about 2 cm long will be made with a sterile surgical scalpel blade in the abdominal wall about 1/3 the distance from the anus toward the base of the pelvic fins, and about 2 cm to the left of the centerline of the fish. Special care will be taken to cut through the dermis only and partially through the muscle, but not into the peritoneal cavity. A gloved finger will be inserted into the incision and forced through the muscle into the peritoneal cavity (Schaefer et al., 2007).

Next, a small amount of amoxicillin (15 mg of amoxicillin per kg of body weight) will be injected into the wound from a syringe without a needle (e.g. 0.1 ml of Betamox or Clamoxyl LA per kg). The tag, previously sterilized in 10% Povidone-iodine solution, will be inserted through the incision into the peritoneal cavity, with the stalk protruding outside. The incision will be closed with two surgeon's knots using a sterile needle and suture materials (Fig. 3.2.4.1.; Fig. 3.2.4.2.).



Fig. 3.2.4.1. Closing the incision where the archival tag has been placed (photo by G. Aranda).



Fig. 3.2.4.2. Surgeon's knot tying (source: U.S. Army Medical Department Center and School).

#### 3.2.5. Tagging procedure

ABFT to be tagged with archival tags will be caught with one of the fishing methods causing least stress for the fish (see chapter 2).

It is essential that the fish is not out of the water for more than 2-3 minutes. Therefore, all the material must be ready for use before the fishing operations begin and the archival tags activated. It is advisable to activate several tags in advance in order to have more than one ready; this will be useful in the case some tags do not work properly or one fish already tagged is rejected because deemed to have lowered survival chances.

The tagging team members in contact with the fish must wear sterile gloves; all the tags and surgical material must be sterilized between tagging operations.

Once the fish is hauled onboard, the fisherman will place it gently on the tagging mattress or, if the size of the fish allows it, it will be placed in the tagging cradle ventral side up and measured to the nearest cm. The eyes will be covered with a wet synthetic dark cloth as soon as possible to keep the fish calmed down. Hence, the archival tag is implanted as described above (3.2.4.).

The fish must be in excellent condition (not bleeding and with no apparent damage in eyes, gills, fins or skin).

Each fish will also be tagged with a conventional spaghetti tag.

Data on position, date, time, fork length, weight (if available), archival tag no., conventional tag no., duration of the whole operation and any other relevant information must be accurately recorded.

The fish is put back into the water with the head pointing in the same direction as the boat.

Refer also to conventional tagging procedure for further advice.

#### 3.2.5. Hygiene

In addition to the recommendations detailed for conventional tagging, all the tags and surgical material must be sterilized in 10 % Povidone-iodine solution.

#### 3.3 Pop-up tagging

#### 3.3.1. Generalities

In addition to the archival tags and as an evolution of them, a more refined type of tag and release technologies have been developed with the pop-up archival transmitting (PAT) satellite tags. Through GPS locating technology and release or 'pop-up' devices, satellite tags gather information about post-release mortality rates, oceanic movements and preferred water temperature, clarity and currents to provide new insights into some of the ocean planet's least understood pelagic fish.

PAT satellite tags are placed externally and are pre-set to detach from the fish body, rise to the surface and radio-transmit data summaries to the Argos satellite network. This network collects, processes and disseminates environmental data, and has a special channel dedicated to wildlife telemetry. PAT tags provide a means of collecting fishery-independent data, and have been deployed on animals such as tuna, marlin, sharks, swordfish, halibut, eels and sea turtles. Although satellite tags are obviously the more expensive option, they remain by far the best option for gathering relevant information on marine animals' biology.

Pop-up tags, from the tagging standpoint, consist of two parts: the active electronic apparatus on one hand, which is tethered to the implanting device. The latter is provided with a metallic or plastic dart to be inserted into the fish (Fig. 3.3.1.1.).



Fig. 3.3.1.1. Mk10-PAT tag equipped with a metallic dart (photo by M. Deflorio)

Much longer (5x) retention times and cleaner insertion sites (no ulceration or open wounds) using nylon darts over metal ones have been shown (Musyl, unpub. results). The tether is generally a monofilament. Because regular (i.e. nylon) monofilament hydrates and becomes brittle over time, fluorocarbon line (123 kg) is preferred for the tether. The length of the tether should be about 16-20 cm.

Probably one of the commonest ways for tags to become loose is through continual movement of the dart in the flesh, which inflames the surrounding tissue thereby providing a site of secondary infection. Over time, the surrounding tissue becomes necrotic and the dart simply rots out. To reduce or alleviate these vitiating forces, a swivel is placed halfway along the tether to reduce torque and precession.

This type of tag has already been used to tag ABFT (Block et al., 2001 and 2005; Lutcavage et al., 1999; De Metrio et al., 2002, 2004 and 2005) and have repeatedly proven to be a powerful tool to improve understanding of migration patterns in this fish. Nevertheless, some aspects of ABFT population structures and migrations (e.g. reproductive site fidelity) need to be further investigated.

Commercially available PAT tags vary according to their size, shape, assemblage, and, most important, performance (memory power, working time endurance, transmission power, service satellite technology, operating mode, depth resistance, and so on), as well as according to their price and the costs of satellite connection, data recovery and processing. PAT tags have also evolved since they became available on the market and are expected to continue to evolve.

Presently, the most used pop-up archival satellite tags are:

- the Mk10-PAT tag (Fig. 3.3.1.2.) and the MiniPAT tag (Fig. 3.3.1.3.) by Wildlife Computers;
- the PTT-100 tag (Fig. 3.3.1.4.) and the X-tag (Fig. 3.3.1.5.) by Microwave Telemetry:
- the PSAT tag series (Fig. 3.3.1.6.) by Lotek.

They are designed to track the large-scale movements and behaviour of fish. The tag is attached to the animal via a tether. A buoyant body and a corrodible pin allows the release of the tag from the fish so data can be transmitted to ARGOS satellite system.



Fig. 3.3.1.2. Mk10-PAT tag (source: Wildlife Computers web site)



Fig. 3.3.1.3. MiniPAT tag (source: Wildlife Computers web site)



Fig. 3.3.1.4. PTT-100 archival pop-up tag (photo by M. Deflorio)



Fig. 3.3.1.5. X-tag (source: Microwave Telemetry web site)



Fig. 3.3.1.6. PSAT tag series (source: Lotek web site)

#### 3.3.2. Tagging equipment

- Pop-up tags.
- Conventional tags: fish must also be tagged with green conventional tags with a legend specifying the presence of an archival tag inside the fish and the reward.
- Conventional tagging material (e.g., applicators, cradle, mattress, recording forms), as specified under the conventional tagging section.
- Surgical gloves.
- 10% Povidone-iodine solution (e.g. Betadine).
- Rigid-framed net of knotless webbing: it can be useful for brailing small fish from the side of the vessel.
- Lift to take the fish onboard.
- Camera and/or videocamera.

#### 3.3.3. Tagging team

Refer to archival tags tagging team (3.2.3.).

#### 3.3.4. Tag implantation and tagging procedure

Pop-up tags are rather expensive, so only a small number of tags are generally used in tagging programmes. Therefore, great care will be exerted in all steps of the tagging procedure. Otherwise the pop-up tagging procedure is very similar to the conventional tagging procedure. The technical requirements for each pop-up tag, which vary according to each brand and model, must be respected (e.g. temperature to keep the tag at before deployment, switching it from stand-by to on mode, checking whether it works, and so on). Before embarking, the taggers will check all the material needed for the operation, especially the tag. As a caution, it is assumed that the "fully" equipped tag (with tether and dart) will float (i.e. can tell "shed" from "dead"). Therefore, it is critical that scientists test this assumption before tag deployment.

The fish to be tagged must be at least 30-40 kg in weight (or about 120-140 cm), since smaller specimens would suffer because of the comparatively high drag caused by the tag. ABFT of such a size will be caught with one of the fishing methods causing least stress for the fish (see chapter 2).

Note that in the case of tagging with pop-up tags, there is no need to catch larger numbers of fish as in conventional and PIT tagging, but rather to ensure good fish condition. Indeed, whatever the fishing method, the physical condition of the fish to be tagged must be carefully checked before tagging.

Once the fish is taken on board, it is placed on a mattress (see paragraph 3.1.) to be tagged. The dart will be inserted using the applicator at the base of the second dorsal fin (Fig. 3.3.4.1., a), so that it becomes anchored in the pterygiophores (i.e. the bones that support the fin rays) (Fig. 3.3.4.1., c). In order to improve the tagging procedure, a large soaked mattress (or sponge) will be placed on the fish body to restrain it whilst it is being tagged instead of placing hands on the animal, which might cause abrasions and/or bruising to the epithelium.

In addition to the pop-up tag, a conventional tag should also be inserted into the fish, also at the base of the second dorsal fin. This "double-tagging" strategy would provide information on PAT tag shedding rates should a double-tagged specimen be re-captured.



Fig. 3.3.4.1. Insertion point of the anchor (i.e. spaghetti tag or dart of a pop-up tag) into the fish (illustration by S. Gelao; *c*: source from Kearney and Gillett, 1982)

If, after tagging has been carried out, it is found that the tagged fish is somehow injured or too stressed, the pop-up tag will be retrieved. The tag will also be removed from the fish when its implantation is not fully satisfactory, when insertion in a way that may threaten fish survival or cause the modification of its behaviour (e.g. by producing too much drag), or cause tag shedding. In either case, the tag will be extracted by cutting the fish's flesh around the dart, rather than by pulling it with force, so that it can be used with another fish.

The fish is immediately put back into the water, as soon as the tagging is completed.

#### 3.3.5. Hygiene

A broad-spectrum bactericide must be used in the pop-up tagging procedure.

The dart, tether and applicator tips should be liberally bathed in Betadine solution (a 10% solution of Povidone-iodine) immediately before insertion in order to lessen the risk of infections.

As for all other hygienic recommendations, refer to the conventional tagging chapter.

#### 3.4. PIT tagging

#### 3.4.1. Generalities

Passive integrated transponder (PIT) tags exploit radio frequency identification technology, which remotely identifies objects through the use of radio frequencies. Most of the tagging devices used in animals are passive.

A PIT tag consists of a small, glass-encapsulated electromagnetic coil and microchip with a unique alpha-numeric code. Other encapsulating media are also available in the PIT tag market (Fig. 3.4.1.1.).

The tag is inactive until energized by an electronic tag reader. The scanner sends a low-frequency signal to the microchip within the tag providing the power needed to send its unique code back to the scanner and positively identify the animal. Therefore, these tags can last many years, providing a long-term identification method.

PIT tags are relatively inexpensive, easy to implant, and are thought to have long retention times. However, tags implanted in the peritoneal cavity may also invoke tissue reactions that result in their encapsulation by connective tissue and migration away from the point of injection (Gheorghiua et al., 2010). In order to prevent such adverse events, especially where PIT tags are to be used in longer-term studies, as is the case for the ABFT, PIT tags should be implanted into the muscle tissue of the fish.

The main advantage of this type of tag is that they may provide quantitative estimates of conventional tagging reporting rates among different fisheries, which is the main uncertainty affecting the use of conventional tagging in estimating natural and fishing mortality values.



Fig. 3.4.1.1. Several models of PIT tags. (source: Biomark, Inc.)

#### 3.4.2. Tagging equipment

- PIT tags
- PIT tag applicators
- Conventional tags and conventional tagging material (e.g., applicators, cradle, recording forms), as specified under the conventional tagging section.
- Disinfecting material.

PIT tags implanted into fish require the use of a scanner to be used when potential tagbearing fish are caught. As in the choice of PIT tags, the read distance dictates the selection of the scanner, which should also be related to the tags to be used (as an example, FS2001F-ISO reader from Biomark provide an excellent read range, but is not compatible with food-safe HDX tags from Hallprint Pty Ltd.).

#### 3.4.3. Tag size

The size of PIT tags to be used is not expected to be a constraint in the case of the ABFT. Hence the size should be chosen so as to maximize the detection range.

As an example, 23 mm BIO23.B FDX tags from Biomark inc., and 22 mm HDX tags from Hallprint Pty Ltd., provide the maximum reading distance among the models available.

It must be noted that PIT tags are not easy to detect, and therefore they might be accidentally ingested by man. On this account, it may be interesting to consider the use of tags encapsulated in surgical plastic and food-grade resin (e.g., ENSID Technologies Ltd, Fig. 3.4.3.1.), rather than glass-encapsulated tags.



Fig. 3.4.3.1. Food-safe PIT tag (source: Hallprint Pty Ltd).

#### 3.4.4. Tagging team

A comparatively large number of fish will be tagged with PIT tags and, moreover, each tagging operation takes longer than conventional tagging. To be carried out properly, this type of tagging requires a three-person tagging team, similarly to conventional tagging: a fish handler, a tagger, a recorder. The latter will also be responsible for verifying whether the implanted PIT tag works properly; he will also help the tagger in disinfecting the tags and implanting instruments. The person in charge of fish handling will also help the tagger to calm down the fish in the cradle.

#### 3.4.5. Tag implantation

PIT tags are typically injected subcutaneously using a hypodermic syringe-like applicator (Fig. 3.4.5.1.).



Fig. 3.4.5.1. NJ Phillips Polymer PIT Tag Applicator (source: Hallprint Pty Ltd).

Implant location varies depending on the studied species. In the case of tunas, no information is available on tag migration within the fish body or tag shedding according to different tag insertion locations. However, because of both the limited detection range of this type of tag and the size of the fish to be tagged, PIT tags will be inserted on one side of the fish, just below the derma layers.

Taking into account the details of tagging procedures, the preferred fish side is the left one, and the place of insertion will be just in front of the distal extremity of the left pectoral fin, perpendicular to the fish's longitudinal axis, so that the longitudinal axis of the tag is oriented as far as possible in the same direction as the muscle myomers. In this way, the PIT tag will be placed in the central part of the fish body, which more or less corresponds to the point of maximum body convexity. This PIT tag location is also good for subsequent scanning procedures on caught fish.

#### 3.4.6. Tagging procedure

All the instruments are prepared before starting tagging activities, and needles must be disinfected opportunely.

The PIT tags, whose codes are previously recorded, are arranged in order in multicell distributors so they can be taken out sequentially, inserted into the needle and be ready for the implantation.

Load PIT tag syringe needles with PIT tags (one PIT tag per needle), load up at least enough needles with tags to get through a batch of fish.

Once the fish is hauled onboard, it is gently put onto a tagging cradle or tagging mattress on its right side, with the head facing the closed side of the cradle and measured.

The exposed eye will be covered with a wet synthetic dark cloth as soon as possible in order to sedate the fish. A right handed tagger will have the fish belly towards him and will handle the fish with his left hand and the syringe and needle with tag with his right hand.

The proper technique for tagging is to insert the needle at approximately a  $45^{\circ}$  degree angle with respect to the body surface, more or less perpendicular to the fish's longitudinal axis, so that the longitudinal axis of the tag is oriented as far as possible in the same direction as the muscle myomers, just in front of the distal extremity of the left pectoral fin (Fig. 3.4.6.1.).



Fig. 3.4.6.1. PIT implant location (tip of the pectoral fin) and particular of the position of the PIT within the muscle myomers. (photo by M. Deflorio; illustration by S. Gelao).

Once the tip of the needle has just broke the surface of the fish's skin, the needle should be flattened out almost parallel to the fish's body and inserted only far enough to insert the tag. In this way the tag will be inserted just below the derma layers.

The plunger on the syringe is then pushed forward to insert the tag. Immediately after tag insertion has occurred gently pull the needle out of the fish. Gently rub the insertion point with a finger to ensure the tag is completely inserted.

Each fish will also be tagged with a conventional spaghetti tag.

Data on position, date, time, fork length, weight (if available), PIT tag no., conventional tag no. and any other relevant information must be accurately recorded.

The recorder will then scan the fish surface by the portable reader/scanner device to check whether the tag works properly and will register the PIT tag number.

The whole of the operation area is then covered in a disinfecting solution and after the fish immediately returns free to the sea water, head first. The used needle is then placed in a container of alcohol for sterilization and disinfection purposes.

#### 3.4.7. Hygiene

In order to avoid infections and disease transfer, PIT tags and PIT tag injectors will be disinfected in a 70-80% ethyl-alcohol or 60-80% isopropyl-alcohol solution for a minimum of 10 minutes.

#### 4. ABFT TAGGING IN THE ICCAT PROGRAMME

ICCAT's eastern ABFT stock tagging programme expects to tag 36,000 individuals over a three-year period, from 2011 to 2013. Immature fish will make up the majority of the tagged fish. The programme will involve various types of tagging and ABFT individuals of different ages, over a three-year period, according to the plan summarized in Table 4.1.

Area	Methods to be decided	Age1	Age2	Age3	Total
Bay of Biscay	Bait Boat	500-600	800	600	2000
Gibraltar/Atlantic	Bait Boat/Trap	500-600	800	600	2000
Balearic Islands/Gulf of Lions	PS	500-600	800	600	2000
Western-Central Mediterranean	PS/Trap	800-900	1000	800-900	3000
Eastern Mediterranean	PS	800-900	1000	800-900	3000

Tab. 4.1. Numbers of ABFT to be tagged each year (2011-2013).

#### 4.1. Juvenile conventional tagging

ICCAT's current ABFT tagging programme requires a significant number of juvenile individuals (age: 1 to 3 years; Corriero *et al.*, 2005) to be tagged with conventional tags and released. The data gathered from juvenile tagging is expected to provide satisfactory estimates of natural mortality rates in ABFT during pre-adult life stages, a most important parameter for managing their stocks.

In all, the tagging programme requires the conventional tagging (i.e. with dart or spaghetti tags) of 12,000 ABFT specimens per year, from 2011 to 2013, in the Eastern Atlantic/Mediterranean area. The return of a significant number of tags is expected in order to estimate mortality rates among the Eastern stock. According to the programme, ABFT in the age range from 1 to 3 years - i.e. juveniles - will be tagged with conventional tags.

Based on current knowledge of Eastern Atlantic/Mediterranean ABFT fisheries, it appears that the best method for catching juvenile tunas, for tagging purposes, is by live-bait boats.

This method is especially suitable for tagging small tunas, since it is quite difficult to catch larger animals by pole-and-line, as well as to land them in the cradle without harming them. Tagging from live-bait boats is carried out mainly in the Bay of Biscay, where the highest catches of juveniles occur. Hence this is where tagging and release operations will be carried out.

Some 12,000 ABFT individuals in the approximately 4 to 15 kg weight range (Santamaria et al., 2009) will thus be tagged and released each year, from 2011 to 2013.

Juvenile ABFT are not subject to any genetic migrations, so they may be caught regardless of the spawning season. This concern apart, tagging operations will be carried out during the regular fishing season in the Bay of Biscay.

Tagging should be conducted in as many areas as possible. Spatial heterogeneity and incomplete mixing need to be considered within the tagging experiment. This can be addressed partly through tagging in different areas.

#### **5. LIST OF ACRONYMS**

ABFT	Atlantic Bluefin Tuna
CPUE	Capture Per Unit of Effort
F	Fishing mortality rate
FL	Fork length
GBYP	ICCAT Atlantic Wide Research Programme for Bluefin Tuna
HDX	Half-duplex system
ICCAT	International Commission for the Conservation of Atlantic Tunas
IOTC	Indian Ocean Tuna Commission
М	Natural mortality rate
PAT	Pop-up Archival Transmitting
PIT	Passive Integrated Transponder
PS	Purse Seine
РТТ	Pop-up Terminal Transmitter1
SCRS	Standing Committee on Research and Statistics
SSB	Spawning Stock Biomass (SSB $_{F0.1}$ is the equilibrium of SSB with fishing mortality rate F = 0.1)
VMS	Vessel Monitoring Systems
Z	Total mortality rate

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