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Atlantic Bluefin Tuna (*Thunnus Thynnus*) Farming and Fattening in the Mediterranean Sea

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The Atlantic bluefin tuna (Thunnus thynnus) is one of the tunas with the highest commercial value and it is supporting the capture-based tuna aquaculture industry in the Mediterranean Sea. This is a seasonal activity and it involves the capture of fish from the wild and their rearing in sea cages for periods ranging between 3 months to 2 years. Short-term rearing is done mainly to: (a) achieve a greater body fat percentage and (b) obtain a better price by not flooding the market in the brief fishing period. Due to the increasing fear of a collapse of the fishery, the International Commission for the Conservation of Atlantic Tunas currently reduced the total allowable catches for 2010 to 13,500 mtn from 32,000 mtn previously. Therefore, there is great interest in establishing a proper and sustainable tuna aquaculture industry. This necessitates the development of specific technologies for tuna aquaculture that will not rely on captured individuals from the wild, as it is practiced today. This article reviews the methods used for the farming and fattening of the species in the Mediterranean Sea, and the current status of the efforts at controlling reproduction in captivity.

Keywords *Thunnus*, Atlantic bluefin tuna, aquaculture, reproduction

1. *INTRODUCTION*

The Atlantic (or Northern) bluefin tuna (*Thunnus thynnus*) is one of the tuna species with the highest commercial interest for fisheries, and the one supporting the capture-based tuna aquaculture—farming and fattening—in the Mediterranean Sea (Ottolenghi, 2008). Based on their separate spawning areas, as well as somewhat different life history traits, the Western Atlantic and the Eastern Atlantic/Mediterranean Sea stocks are considered as different by the International Commission for the Conservation of Atlantic Tunas (ICCAT), the international body responsible for the management of and allocation of fishing quotas (Total Allowable Catches [TACs]) for the Atlantic bluefin tuna (Schaefer, 2001; Block et al., 2005; ICCAT, 2005; Rooker et al., 2007). As of 2004, the Eastern Atlantic/Mediterranean Sea stock accounts for 95% of the global catch (FAO, 2006). Due to the increasing fear for a collapse of the fishery and the intensification of the pressure from non-governmental organizations (NGOs), ICCAT currently reduced the TACs for 2010 to 13,500 mtn—from 32,000 mtn three years ago—and restricted the purse seine fishing period to only one month (ICCAT, 2009). The ICCAT also left open the possibility to decide on a suspension of all fisheries for Eastern Atlantic and Mediterranean Sea bluefin tuna in 2011 in the case of a serious threat of fishery collapse, which would be detected with a subsequent stock assessment.

Atlantic bluefin tuna farming and fattening in the Mediterranean Sea is a seasonal activity and it involves the capture of fish from the wild and their rearing in sea cages for periods ranging between 3 months to 2 years. Short-term rearing is done to achieve: (a) a greater fat percentage in the muscle, which is desirable by the sushi and sashimi markets in Japan (Figure 1), (b) a better price by not flooding the market in the brief fishing period of June–July, and (c) a certain weight increase over the

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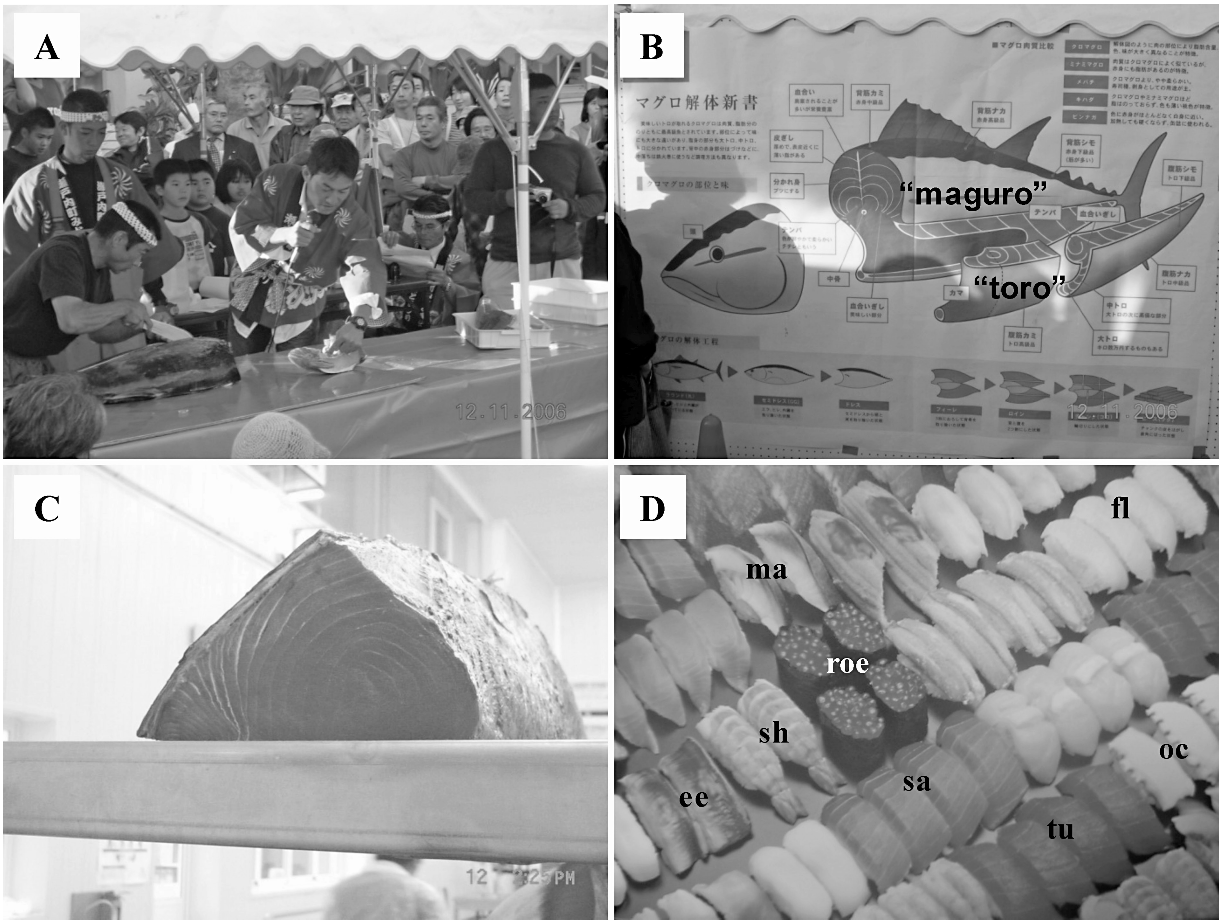

Figure 1 Sushi and sashimi preparation of bluefin tuna. (A) A specialist bluefin tuna butcher in Japan cutting a fresh Pacific bluefin tuna. The cut piece of flesh on the bench indicated by the announcer is the priced "toro," the flank part of the tuna that is almost 50% fat and is the most expensive part of the fish. (B) A schematic presentation of the butchering of bluefin tuna, showing the various sections, including the "toro" and the "maguro," the latter being the typical bluefin tuna piece known to sushi/sashimi connoisseurs. (C) A piece of farmed Atlantic bluefin tuna "maguro" at a Spanish processing facility (Gines Mendez España, Mazarron). (D) Sushi from a variety of fishes, including "maguro" (tu), shrimp (sh), salmon roe (roe), salmon (sa), octopus (oc), freshwater eel (ee), mackerel (ma), and flounder (fl). (Figure is available in color online)

captured fish. According to ICCAT, the Atlantic bluefin tuna rearing operations are classified as "fattening" if rearing is done for a short period (3–7 months) using mature fish (>30 Kg in body weight), or "farming" if rearing is done for a longer period of time (up to 2 years) and involves juvenile fish (8–30 Kg in body weight) (ICCAT, 2008a). In the latter case, rearing is done to increase body weight and reach the minimum size used in the Japanese market. This article provides a review of the farming and fattening operations of the Atlantic bluefin tuna in the Mediterranean Sea and the current status of the efforts at controlling reproduction in captivity.

2. *FISHERIES*

Commercial fishing of Atlantic bluefin tuna is done using hand trolling, baitboat, longlines, set traps (Almadrabas and Tonnaras), and purse seines (ICCAT, 2008b). In the Eastern Atlantic and Mediterranean Sea, the major fishing gear for Atlantic bluefin tuna up to the 1970s has traditionally been the set traps. Later on, purse seines became the most prevalent fishing method and in the past few years they accounted for 70–86% of reported catches, since this method captures the fish alive and supplies the tuna fattening industry (Ottolenghi, 2008). Hand trawling and longlines are allowed to operate throughout the year, whereas set traps are operating during the reproductive migration period (April–September). Purse seines are restricted during the reproductive season for fish >130 cm fork length (>30 Kg body weight), which are the reproductively mature fish supporting the tuna fattening industry throughout the Mediterranean Sea, with the exception of Croatia. Croatian tuna farmers are the only ones allowed to harvest fish that are smaller than the above limit (but >8 Kg).

During their spawning migrations in the Mediterranean Sea, Atlantic bluefin tuna form large schools, which can be detected by their swimming and feeding activities on the surface. Schools may be homogeneous or heterogeneous regarding age and body sizes (Ottolenghi, 2008). In addition, young tuna schools are often associated with smaller tunids, such as skipjack (*Katsuwonis pelamis*) or bonito (*Sarda sarda*). The purse seiners used for the Atlantic bluefin tuna in the Mediterranean Sea are highly efficient, modern fishing vessels equipped with fish-finding sonars and in the past have been supported by spotter airplanes (now prohibited by ICCAT). Once a tuna school is located, a long (>1 Km) and deep (200 m) seine is deployed from the mother ship by an accessory boat, which encircles the fish. Along the bottom of the net there are metal rings connected with a cable, which once tensioned from the mother ship closes the bottom of the seine creating a "purse" where the fish are trapped. In the past, the fish would have been slaughtered on the spot and transferred to processing-freezing ships for transportation to the markets. Currently, 99% of the catch of the purse seine fleet is sold to fattening operations throughout the Mediterranean Sea. The remaining 1% consists of individuals that die accidentally during the purse-seining activities or during transport to the fattening sites, and these fish are sold directly to the market. This development has led to an important stock assessment problem, since fish are not immediately harvested and there is no effective method to determine the fish biomass or size- and age-structure of the captured population. As a result, it has become exceedingly difficult to obtain the necessary data for appropriate stock assessment analysis to be undertaken (FAO, 2005; ICCAT, 2008b). Furthermore, as the growth in body weight attained in culture cannot be ascertained, the calculation of annual fishery catches based on the reported biomass of tuna harvested by the fattening industry is not considered reliable.

Due to the apparent overfishing of the Atlantic bluefin tuna stocks in the Mediterranean Sea in the last decade and the reduction in the stock biomass (ICCAT, 2008b), the ICCAT recommendation 08 from 2008 (ICCAT, 2008a) established a multiannual recovery plan for Atlantic bluefin tuna in the Eastern Atlantic and Mediterranean and fixed the TACs for the member states at 22,000 mtn for 2009 and 18,500 mtn for 2011, a significant reduction from the 32,000 mtn TACs that existed throughout the previous decades. The same recommendation allowed purse seine fishing activities in the Eastern Atlantic and Mediterranean only in the period between April 15 and June 15, and confirmed the prohibition of the use of airplanes or helicopters for helping to search for Atlantic bluefin tuna schools. With increasing fear of a fishery collapse and mounting pressure from NGOs, with recommendation 09 of 2009 (ICCAT, 2009) the ICAAT further reduced the TACs for 2010 to 13,500 mtn, while the purse seine fishing was restricted to a brief 1-month period from May 15 to June 15. The same recommendation established the possibility to decide on a suspension of all fisheries for Eastern Atlantic and Mediterranean Sea bluefin tuna in 2011 in the case of a serious threat of fishery collapse, which would be detected with a subsequent stock assessment. As the farming and fattening operations in the Mediterranean depend completely on access to the wild stocks of Atlantic bluefin tuna, these recent developments affected significantly the farming and fattening industry, which has been downsized in the last 3 years and is faced with the possibility of a complete shut-down, if a suspension is implemented on the fishery of the Atlantic bluefin tuna in the Mediterranean Sea.

3. *FARMING AND FATTENING OPERATIONS*

As mentioned earlier, Atlantic bluefin tuna farming and fattening in the Mediterranean Sea is a seasonal activity and it involves the capture of fish from the wild and their rearing in sea cages for periods ranging between 3 months to 2 years. The vast majority of fish are migrating mature spawners—body weight between 40 and 400 Kg, minimum allowable size 30 Kg in body weight and 130 cm in fork length—that are captured during their reproductive migration and most often when they are in their spawning areas. At this time, the fish have spent a large amount of energy for building their gonads (Mourente et al., 2002), and their muscle fat content is reduced greatly, which is not desirable by the sushi and sashimi market. Rearing of mature spawners is classified as "fattening" (ICCAT, 2008a) and its main objective is to achieve a greater fat percentage in the muscle, which is done by feeding the tuna with a diet of small pelagic fish with a high fat content (Belmonte et al., 2007). A secondary objective is to supply the market for a longer period of time, thus obtaining a better price by not flooding the market during the brief fishing period. Inadvertently, a certain amount of growth—estimated at between 20–40% depending on initial size—may also be achieved during the rearing period, which is also profitable to the farmers.

Another sector of the Atlantic bluefin tuna production in the Mediterranean Sea is classified as "farming" (ICCAT, 2008a), which is allowed only for Croatia because this was historically the first one established (in the early 1990s) and is the smallest activity in terms of fish biomass. In this sector, the Atlantic bluefin tuna captured are immature (8–20 Kg in body weight and < 130 cm in fork length). These juvenile tunas are reared in captivity for up to 2 years, reaching a harvest size between 30 and 50 Kg (Ticina et al., 2007). At this size they do not obtain the highest price in the Japanese market, which favors fish 150–250 Kg in body weight, but maintaining them longer increases the risk and is not practiced.

Recently, a 232 Kg wild bluefin tuna (probably a Pacific specimen) was auctioned at a price of $175,000 at Tokyo's Tsukiji market in January 2010 (approximately 547 euro Kg^{-1}), a record price for the new millennium (BBC News, news.bbc.co.uk/2/hi/asia-pacific/8440758.stm). However, the average price of farmed Atlantic bluefin tuna in Japan is much less, at around 36 euro Kg^{-1}, but when the market demand is high and tuna meat is of high quality the price can occasionally reach 120 euro Kg^{-1} (Aguado et al., 2004). Due to these very high prices, the farming and fattening of Atlantic bluefin tuna has experienced an amazingly rapid expansion since its start in the beginning of the 1990s. At present, 68 fattening companies of Atlantic bluefin tuna are officially recognized by ICCAT, and these companies are spread out throughout the Mediterranean Sea (http://www.iccat.int/ffb.asp). Today, the countries

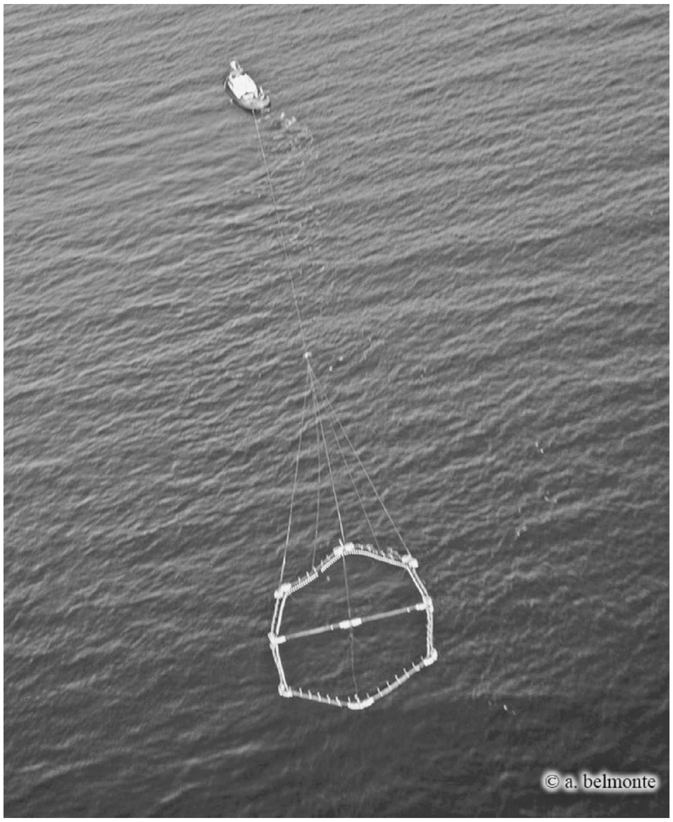

Figure 2 A tug boat hauling an Atlantic bluefin tuna transport cage from the fishing area to the rearing site. The cage is hexagonal (22-m sides) and 20–22 m in depth.

involved in Atlantic bluefin tuna fattening are Cyprus, Greece, Italy, Malta, Spain, Tunisia, and Turkey, while farming takes place only in Croatia. The highest volumes of production in recent years are coming from Malta, Tunisia, Croatia, Italy, and Turkey.

3.1. Farming and Fattening Systems

After capture by the fishing fleet, the Atlantic bluefin tuna are transferred into a transport cage (30 to 50 m in diameter, 20 m deep), which is placed near the purse seine and it is connected with the purse seine by sewing the two nets together. An opening is created and the fish are herded from the purse seine into the towing cage. The transport cage may be circular (50 m in diameter) or hexagonal (22 m sides). The transport cage is then towed to the prospective fattening site by a tug-boat at a very low speed (1–1.5 knots), in order to avoid collapsing of the net and reducing the available space for the fish (Figure 2). Because of the low transportation speed, the towing trip can take several weeks, which adds tremendously to the cost of production ($\sim$2,000–3000 € day^{-1}) and increases the danger of stressing and damaging the fish. In Spain, fish mortality during transport from the fishing grounds to the rearing facilities has decreased from 21% in 1995 to 4% in 2000, due to technical improvements in husbandry (Norita, 2003). In Turkey, transport mortalities have been reported to be 10% (Oray and Karakulak, 2003). The above has resulted in the establishment of fattening operations in countries that are located as near as possible to the areas of capture, such as Malta, Italy, Croatia, Tunisia, Turkey, and Cyprus.

To avoid losses and speed up the fattening process, during this long towing period, the fish are fed using a variety of frozen fish (see later). Upon arrival to the fattening site, the transport cage is placed next to the rearing cage and the fish are transferred inside it in a similar way used earlier for their transfer from the purse seine into the transport cage. The rearing cages may be circular of 50–120 m (50–60 m in Croatia) in diameter and made of high density polyethylene tubes (HDPE), but also can be rectangular (50 $\times$ 100 m) (Figure 3) (Belmonte and de la Gándara, 2008; Hattour, 2005). The depth of the cages is 20–35 m (18–21 m in Croatia) at the side of the net and the mesh size used is 25 cm. As both the transport from the fishing grounds and the transfer to their definitive rearing cages is stressful to the fish, it is usually necessary to allow a period of 10–15 days of calming the fish down, before starting feeding at full ration (Hattour, 2005). According to Farwell (2003), the stocking density of Atlantic tuna during the rearing phase ranges between 5 and 6.2 Kg m^{-3}, but most operations report lower values in the range of 2–4 (1–2 in Croatia) Kg m^{-3}.

In Croatia, where juvenile Atlantic bluefin tuna are used for farming, the mortality has been reported to be about 15–20% per fattening season (Katavic et al., 2003), while during the adaptation period in the rest of the Mediterranean (first 2 months after capture), the stress mortality is 60% of the total mortality ratio (Norita, 2003). However, from 1995 to 2000 the mortality of Atlantic bluefin tuna reared at the Tuna Farm of Mediterráneo S.L. (Spain), was reduced from 15.8 to 3.7%. Such levels of mortality can be considered normal for the industry in recent years. However, accidental events of natural catastrophes may result in higher mortalities, or even complete loss of a stock.

3.2. Feeding and Growth

Since the primary objective of Atlantic bluefin tuna fattening and farming is to obtain a substantial increase in muscle fat content, the majority of the feed fish are fat-rich, low-cost species (Belmonte et al., 2007), including sardinella (*Sardinella aurita*), pilchard (*Sardina pilchardus*), herring (*Clupea harengus*), mackerel (*Scomber scombrus*), horse mackerel (*Trachurus* sp.), chub mackerel (*Scomber japonicus*), bogue (*Boops boops*), and some cephalopods (Vita et al., 2004). This is a major issue regarding the sustainability of Atlantic bluefin tuna fattening operations, since the natural populations of these small pelagic species are also affected by the increased fishing pressure. Furthermore, the use of raw fishes, often imported from distant seas, entails the risk of importing diseases that may affect not only the Atlantic bluefin tuna themselves, but also the populations of wild fish in the vicinity of the rearing cages. In this regard, it is worth mentioning the occurrence of mass mortality

Figure 3 Atlantic bluefin tuna circular cages (A) and rectangular cages (B) off the coast of Murcia, Spain.

in natural populations of Australian sardine, which may have been caused by a disease agent carried by the feed-fish fed to Southern bluefin tuna (WWF, 2005).

During the rearing phase, the fish are fed to satiation with frozen fish once a day, though some authors reported that fish may be fed 1–3 times a day (Ottolenghi, 2008) or even 6 times a day (Katavic et al., 2003). However, this seems to be very difficult to manage for such large operations. Some operations offer a daily ration of approximately 2–10% of the estimated Atlantic bluefin tuna biomass, depending on water temperature and fish size composition of the reared population (Farwell, 2003; Ottolenghi et al., 2004, 2008). The feeding ration and the feed-fish species composition varies among different countries, as well as between operations in the same country, with species composition depending on availability and cost of shipment. Feeding may be done manually by throwing the feed-fish from the service boat into the rearing cage using shovels (Figure 4A). Another method is to place the fish in a metal cage in the center of the rearing cage and to have a diver controlling the number of fish falling into the rearing cage, depending on the feeding activity of the tuna. Yet another method is to put a frozen block of feed-fish in a floating cage made of steel mesh in the middle of the tuna cage. As fish thaw they fall through the mesh and are consumed by the tuna swimming below. This method was used first in Australia in the farming of Southern bluefin tuna. Finally, feeding systems have also been constructed, utilizing a tube running from the service boat to the center of the cage, along which the feed is pumped mixed with water (Figure 4B).

During feeding, tuna behave in a hierarchical manner with the larger specimens eating first while the smaller fish wait at

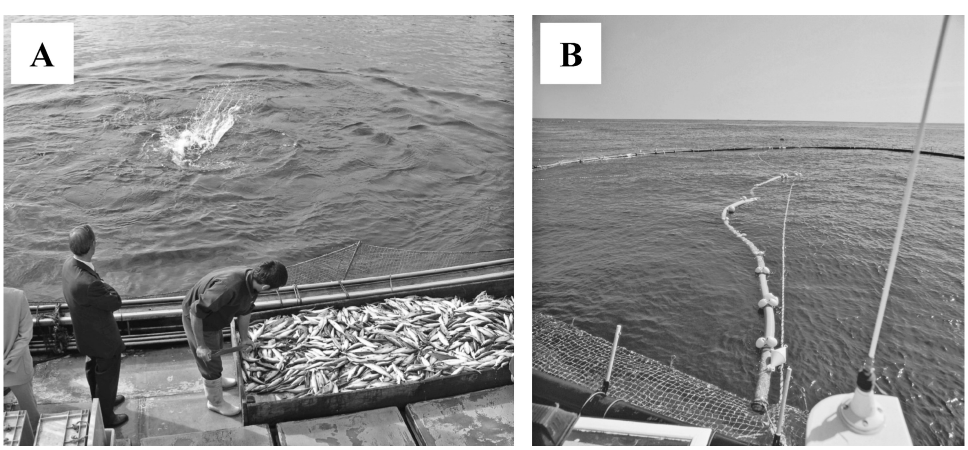

Figure 4 Feeding farmed Atlantic bluefin tuna with raw fish. (A) Manual method and (B) feeding system using a water pump and a hose.

the bottom of the cage until the former begin to lose interest in the feed. At this time, they move away from the surface where the feed is placed and the smaller fish swim up and start feeding. In most countries, a scuba diver is placed in the cage during feeding to monitor the activity of the fish (Ottolenghi, 2008), in order to avoid overfeeding. Wastage from feeding and accumulation of uneaten feed from the bottom has been identified as one of the main negative environmental impacts of aquaculture (Karakassis et al., 2000). In the early days of Atlantic bluefin tuna farming and fattening, when not much was known about their behavior, little attention was paid to how the feed was discharged into the cages, leading to both a high degree of wastage and significant negative effects on the sea bottom.

Without any accurate initial length and/or weight measurements of the Atlantic bluefin tuna captured in the wild and transferred into the cages for fattening, the growth and feed conversion ratios (FCR) achieved during the rearing are nothing but estimates. The estimated FCR, which are estimated on a wet feed/wet tuna biomass, are usually high and range between 10–20:1 (Mourente et al., 2002; Farwell, 2003; Katavic et al., 2003). For large specimens it may be even beyond 40:1, whereas for smaller juvenile fish it may be as low as 10:1, and it is higher at winter temperatures (Mourente et al., 2002). These values are not surprising, if one considers that Atlantic bluefin tuna are not really "grown," but are "fattened," as they are fed heavily and for the purpose of increasing their body fat content in a short period of time of a few months (Aguado-Giménez and García-García, 2005). After 8 months of fattening, Atlantic bluefin tuna exhibited a weight increase of 40–50% in smaller fish and 10–30% in the larger ones (Norita, 2003), whereas Percin and Konyalioglu (2008) reported an overall 25–35% increase in body weight at the end of the fattening period.

It has been estimated that during the reproductive season, Atlantic bluefin tuna breeders lose as much as 50% of their body fat, reflecting utilization for gonadal development as well as metabolism for providing energy for swimming during migration (Mourente et al., 2002). Since one the major objectives of tuna fattening operations is increasing the fat content of the fish, it is customary for some operations to evaluate the proximate composition of the muscle from the farmed fish at the end of the rearing period. Such measurements have indicated that the muscle fat content can be around 17%, depending on the type and quality of feeder fish used, the location of the fattening operation, and the water temperature during the fattening operation (Belmonte, unpublished data).

3.3. Health Management

Health management is not an aspect that is taken into great consideration in the fattening and farming operations of Atlantic bluefin tuna, since major disease and health problems have not been encountered yet, except for some limited situations of catastrophic losses due to environmental factors, such as water turbidity due to rainfall run-off, a feed-related mortality due to the lipid component of the feed fish (Roberts and Agius, 2008) and a mass mortality due to a bacterial outbreak (Mladineo et al., 2006). There are various reasons for the lack of major disease problems. First of all, due to the high oxygen demand of Atlantic bluefin tuna, farming and fattening is done at relatively exposed coastal areas, with strong currents, and the fish are stocked at very low stocking densities (2–4 Kg m^{-3}) compared to that of other fishes reared in typical Mediterranean aquaculture facilities (10–20 Kg m^{-3}). Also, the fish that are stocked in the cages are not young fry, but mature adults (for fattening) or advanced juveniles (for farming), and already have a well-developed immune system. It has also been proposed that the partial endothermy of Atlantic bluefin tuna conveys some enhanced immunocompetency to the fish (Mladineo et al., 2008). Another important reason is that the rearing cycle of the fattening operations is limited to 6–8 months, with a substantial fallowing period during spring and early summer. This latter explanation is not valid for the farming operations in Croatia, where fish are reared for periods of at least two years and the sites are always stocked with fish, as the production cycles have a significant overlap. Information on the diseases and health problems of Pacific and Southern bluefin is much more available, as the industry for these species has been in existence for a longer period of time (Munday et al., 2003; Di Maio and Mladineo, 2008; Hayward et al., 2008; Johnston et al., 2008).

3.4. Harvesting

When Atlantic bluefin tuna are consumed raw, as sushi and sashimi, the quality requirements for their flesh are much stricter than when the fish are to be consumed cooked. For example, the accumulation of lactic acid from the anaerobic metabolism of the muscle during struggle on a hook or when the fish are seined and moved on deck to die of suffocation—as practiced for Atlantic bluefin tuna that are caught by the long-line and the Almadraba/Tonnara fisheries, respectively—can be tasted in fish consumed raw, but not when cooked. Tuna with a high concentration of lactic acid in their flesh are well recognized in the Japanese wholesale market (referred to as "yake"), based on the appearance—color and texture—of the flesh and either obtain a much lower price or are not considered appropriate for sushi or sashimi. When Atlantic bluefin tuna fight before death, they produce a lot of lactic acid, which may be exaggerated when the fish are not allowed to swim (movement restriction by intense crowding or by lifting the nets to the water surface), as they cannot obtain adequate oxygen. Furthermore, due to their ability to conserve metabolic heat produced by the muscles (Graham and Dickson, 2001), their body temperature can increase tremendously during fighting (reaching 28–32°C at the core), resulting in severe degradation of the flesh for the sushi and sashimi market. Since > 90% of the farmed Atlantic bluefin tuna in the Mediterranean Sea are destined for this market, the underlying criterion for the slaughtering methods is the maintenance of optimal flesh quality, and this inadvertently means that death must occur as quickly as possible, without prior stressing or exhausting the fish.

There are three major methods employed for killing Atlantic bluefin tuna reared in fattening operations. These include, in order of prevalence: (a) shooting on the head underwater using a power-head ("lupara"), (b) shooting on the head from above the water, and (c) hitting on the head with a metal spike or a core. The first two methods are used for tuna that are larger than 80 Kg, whereas the third method is only used for smaller individuals. The method of electrocuting using an electric harpoon, which was evaluated in the early 2000s, is no longer employed (Soto et al., 2006).

Shooting underwater is the most commonly employed method for killing farmed Atlantic bluefin tuna. With this method, the tuna are killed one-by-one by means of an underwater shot on the head using a lupara loaded on a jab-stick or spear gun (Figure 5C and insert). Depending on the size of the rearing cage, the number of fish inside and the number of fish that need to be killed, a mild reduction in the available volume may be done by lifting one of the sides of the cage. Alternatively, a seine also called "culling net" (Figure 5A) may be used to isolate the portion of the population that will be killed in one day, which can be 200–300 fish, depending on body weight. However, the crowding achieved is nowhere near what is required for the other two killing methods (see below). The fish are allowed plenty of space to swim around as usual, and are killed one-by-one by a shot on the head. Alternatively, a group of fish from the population of the rearing cage may be herded into a smaller "slaughtering cage" (10 × 10 m surface area) that is either connected to the rearing cage with a "window" or is a part of the culling net (Figure 5B). The isolated fish are shot with the power-head one-by-one (Figure 5D). An underwater shot with the power-head rarely misses the head, but ideally it should hit the brain each time, resulting in immediate death of the fish and cessation of any voluntary movement. Once a fish is killed, it is captured by another diver and lifted on deck using a mechanical crane (Figure 5E). Then, the fish is bled by slicing its lateral arteries, its brain destroyed with a metal core—in case the shot to the head did not already destroy the brain—and a metal wire is pushed through the hind brain and spinal cord to stop any involuntary muscle movement (Figure 5F). In some operations, immediately after landing the shot fish on board the service boat, the head is cut off using a chain saw; therefore, a metal core is not necessary to create an opening for the metal wire used to destroy the spinal cord. The advantage of the lupara method is that (a) it allows for a certain degree of selection of individual fish to be killed and (b) it is the best method from the point of view of flesh quality, as well as animal welfare, as it results in instantaneous death of fish that are normally swimming or under mild or moderate crowding conditions in a slaughtering cage or culling net. The disadvantage of this method is that it is time consuming and inefficient in killing a large number of fish in a day, and it is not suitable for small fish or when a large number of fish must be killed and processed.

Shooting on the head from above the water is the second most commonly employed method, as it allows killing a large number of fish in a short period of time. A group of 20–30 fish are separated from the main population, by herding them into one side of the rearing cage using a seine. Alternatively, a group of fish from the population of the rearing cage may be herded into a smaller slaughtering cage—as for the previous method—that is connected to the rearing cage. The isolated fish are then brought quickly (∼ 1 min) to the surface in order to restrict their movement, as the bottom of the cage is lifted rapidly using hydraulic winches. At this time, trained marksmen standing on a service boat or platform next to the cage shoot the fish on the head using a shot gun loaded with single-bullet cartridges (same as the ones used for the lupara, Figure 5C insert). Usually a single shot is enough to cause immediate death, if hit directly on the brain, but occasionally a second shot may be required. Once all of the fish in the particular group are killed, the tuna are hoisted on deck of the service boat using a mechanical crane. There, the fish are processed as described earlier for the lupara method, in terms of bleeding and destroying the spinal cord. The process is repeated multiple times, until the working day is over or the required number of killed fish is achieved. The advantage of this method is that the tuna are killed within a very short period of time (1–5 min) after the exposure to severe stressful conditions (i.e., crowding and movement restriction), therefore, flesh is maintained in optimal conditions. Furthermore, this is a very efficient killing method as it allows processing a large number of tuna in a day. The disadvantage is that this method is only applicable to large fish (> 80 Kg), as it is more difficult to aim at a larger number of smaller fish in a crowded area, reducing the accuracy and efficiency of the method.

The method used for smaller fish (< 80 Kg) involves a manually applied coring or spiking of the brain—referred to as "Ike Jime" or "Shinkei Nuki" in Japanese. This is the method employed in Croatia almost exclusively, since harvested fish are usually < 50 Kg, and in other farms in the Mediterranean Sea where often a number of small fish are present in the population of larger individuals. In Croatia, the fish to be killed are crowded close to the surface by lifting the bottom of the cage or using a seine to gather them into one site of the rearing cage. Fish are either captured by divers by grasping them from the opercula and are gaffed (a "gaff" is a stick with a hook or a barbed spear) by two people from a service boat and landed on deck using a slide. When they are moved on deck the lateral arteries are severed to bleed the fish and a core is pushed through their brain case destroying the brain. Then, a metal wire is pushed through the hind brain and spinal cord. In the rest of the Mediterranean where large Atlantic bluefin tuna are farmed, this method is used for any small fish that are present in the population. Divers herd the already crowded small tuna into the nets of the slaughtering cage or culling net, capture them and turn them upside down—which tends to immobilize them— and hoist them on deck of the service boat using a mechanical crane. Then the fish are processed as described above.

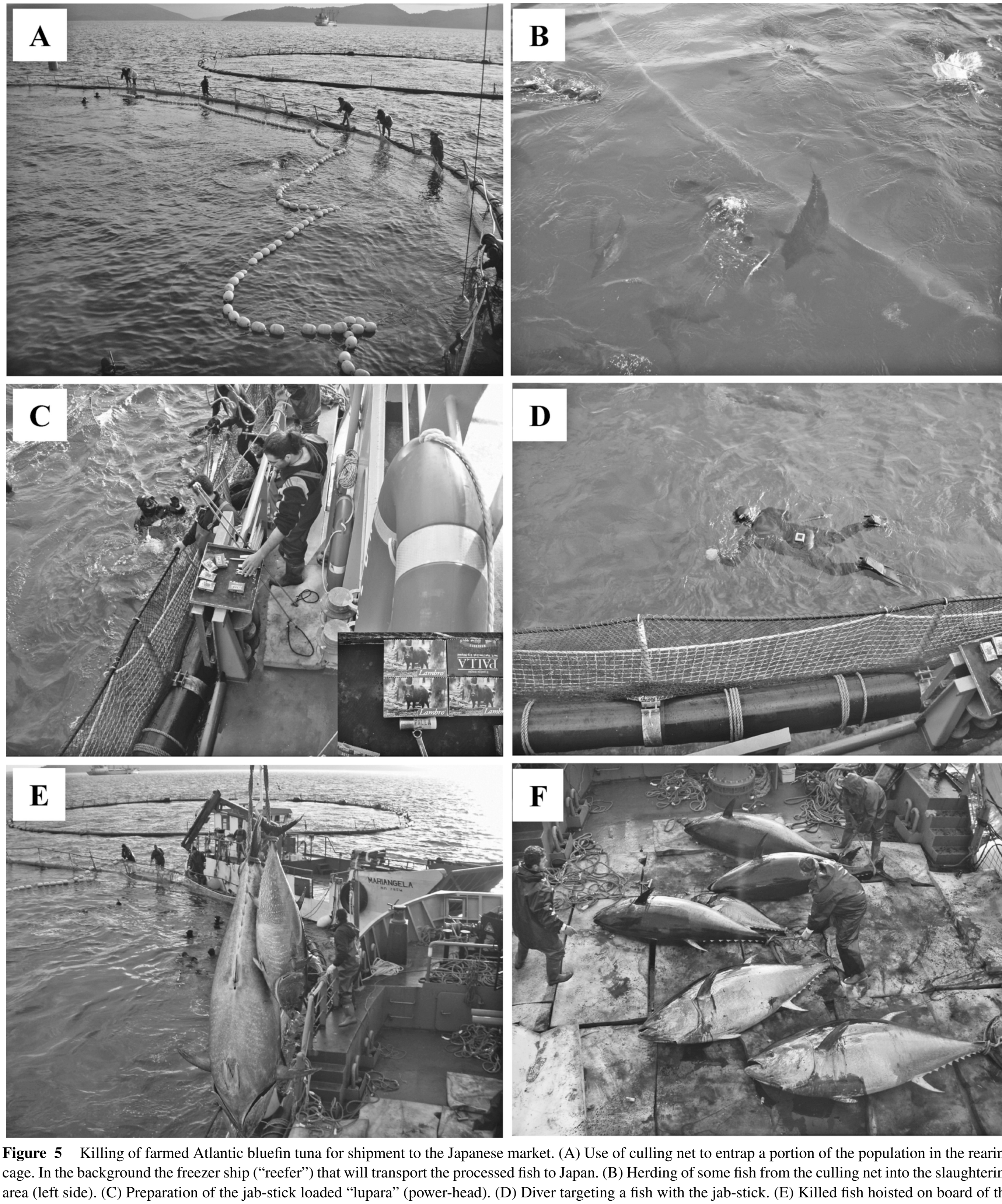

Figure 5 Killing of farmed Atlantic bluefin tuna for shipment to the Japanese market. (A) Use of culling net to entrap a portion of the population in the rearing cage. In the background the freezer ship (“reefer”) that will transport the processed fish to Japan. (B) Herding of some fish from the culling net into the slaughtering area (left side). (C) Preparation of the jab-stick loaded “lupara” (power-head). (D) Diver targeting a fish with the jab-stick. (E) Killed fish hoisted on board of the service boat. (F) Cored and bled fish ready for transfer to the Japanese reefer.

As mentioned earlier, less stressed fish that are harvested quickly and processed properly achieve better prices, confirming the importance of killing tuna correctly, not only for fish welfare reasons (Ugolini et al., 2005). Pre-slaughter and killing procedures might be enhanced in a way that fish excitement or stress conditions are minimized, not only to ensure proper fish welfare, but also high-quality tuna flesh for the sashimi market (Buentello et al., 2008). Reports do exist on the effects of stress induced by killing on the flesh quality of bluefin tuna and its shelf life (Lowe et al., 1998; Takii et al., 2005; Soto et al., 2006; Messina and Santulli, 2008). In a comparative study between the two main killing methods of farmed Atlantic bluefin tuna—the lupara (underwater after moderate crowding) and shot-gun (above water, after severe crowding to the surface), it was found that the shot-gun method resulted in significant changes in various blood indicators of primary and secondary stress, suggesting that fish killed by a shot-gun after severe crowding on the water surface are significantly more stressed (Messina and Santulli, 2007, 2008). This was accompanied by higher depletion of muscle glycogen and a reduction in pH, which causes a reduction in shelf life of the processed flesh. In addition, the flesh of these fish had a higher production of total volatile basic nitrogen and malondialdehyde, both markers of nitrogen and lipid degradation. Both parameters are known to respond to severe stress (Poli et al., 2005). Therefore, similar to the observation in other farmed fishes (Pottinger, 2001; Poli et al., 2005), the data obtained in farmed Atlantic bluefin tuna suggest that the killing method, through the applied stress and increase in anaerobic and anaerobic activity, can affect negatively flesh quality.

3.5. *Markets*

Atlantic bluefin tuna from the Mediterranean can reach the Japanese market in two forms: frozen or fresh. The prices for these two products are very different—but so are the costs of shipping to Japan—and are around 25–43 € for fresh and 12–21 € for frozen tuna (Table 1). Production figures (in mtn) for the last four years were 21,000 in 2003, 24,000 in 2005, 21,800 in 2006, and 22,800 in 2007.

In 1996, the first year in which fattening of adult Atlantic bluefin tuna began in Spain, all fish were exported fresh and were shipped to Japan by air. At that time, the supply was scarce in relation to the enormous demand in Japan. This meant that prices were high, the mean price for fresh product during 1996 being 43 € Kg^{-1}. Although this price seems very high, especially compared to other marine farmed fishes, it included air transport (30% of the sales price), customs tariffs in Japan, auctioneers' commissions, transport within Japan, etc., all of which were charged to the producer in the Mediterranean (Belmonte and de la Gándara, 2008). In subsequent years, there was a rapid increase in the number of Atlantic bluefin tuna farms in the Mediterranean Sea, which resulted in significant increases in supply and a corresponding drop in prices. As a result, from 2002 and onward the vast majority of the production of Atlantic bluefin tuna farms began to be exported in frozen form rather than fresh, since this meant lower costs. Frozen fish are sold whole, "free on board," which means that once the tuna has left the fattening facilities and is loaded onto the freezer vessel (called "reefers"), all the costs corresponding to freezing, transport, processing, etc. are borne by the buyer. Although the sale price is much lower now and for 2008 ranged between 12 and 17 € Kg^{-1} (depending on individual fish size: 12 € for 30–60 Kg fish, 14 € for 60–120 Kg fish, and 18 € for fish > 120 Kg) the business is more profitable for the producer.

Table 1 Annual distribution of fresh vs. frozen product of farmed/fattened Atlantic bluefin tuna to the Japanese market, along with average market prices

Year	Frozen Product (%)	Price (Frozen) €	Price (Fresh) €
1996	0		43
1997	5	n/a	40
1998	3	n/a	38
1999	0		43
2000	35	n/a	38
2001	70	21	32
2002	70	23	30
2003	70	13	20
2004	90	13	21
2005	30	14	29
2006	80	14	29
2007	80	13	26
2008	85	16	19
2009	85	12	20

n/a = Not available.

The industry and market changes that took place due to the evolution of supply and demand during the years after 1996 were accompanied by the devaluation of the Japanese yen (¥) against the Euro, which made imports more expensive for Japanese consumers, and led to a shift in the balance of imports and exports in Japan in favor of the latter. This situation produced a change in consumer eating habits, buying less expensive substitutes to the bluefin tuna, such as the wild-caught yellowfin tuna (*Thunnus albacares*) or bigeye tuna (*Thunnus obesus*). Since there is an abundant supply of these two species, caught in the Pacific Ocean by the approximately 400 fishing vessels of the Chinese and Taiwanese fleets, these tunas can be supplied to the market at highly competitive prices.

Production costs in Spanish operations range from 8 to 9 € Kg^{-1}, including the purchase price for wild tuna paid to the fishing boats, the cost of towing to the anchored facilities, feed, labor, auxiliary craft, etc. (Belmonte and de la Gándara, 2008). In the 2006–2007 season, the sale price of farmed Atlantic bluefin tuna for freezing varied between 14.50 € Kg^{-1} for fish weighing more than 120 Kg and 12.40 € Kg^{-1} for smaller fish (Belmonte and de la Gándara, 2008).

3.6. *Fishing and Aquaculture Regulations*

With the intention of better managing this fishery and protecting the wild stock from over-fishing, during the last decade, the ICCAT adopted a series of measures to control the fishery in the Mediterranean Sea. The first measures included: (a) an increase in the minimum catch size from 10 to 30 Kg, (b) a reduction in the number of permissible fishing days from 11 to 6 months, (c) the presence of observers at cage facilities, and (d) the prohibition of at-sea transshipment (Commission, 2007). One of the measures taken with regard to the fishing period was to suspend fishing by purse seining between July 1 and December 31. Atlantic bluefin tuna aggregate for reproduction in the spawning areas along the Mediterranean, when the water temperature increases to 24°C. As the water temperature increases earlier in the Eastern compared to the Western Mediterranean Sea, it means that fishing boats off the coasts of Turkey, Egypt, Libya, and Malta start their fishing season much earlier than in the spawning areas around the Balearic Islands in Spain (thus giving them more days in which to fish). The further reduction in the fishing season between April 15 and June 15 imposed by ICCAT in 2008 (ICCAT, 2007, 2008b) and the current reduction to a single month between May 15 and June 15 imposed by ICCAT in 2009 (ICCAT, 2009) has resulted in a serious negative impact on fattening operations in the Western Mediterranean Sea (i.e., Spain), where facilities are now receiving insufficient stocks for cost-effective production and some companies have been forced to cease operations as of 2010. As a result, more and more the Atlantic bluefin tuna fishing and fattening industries are moving towards the Eastern Mediterranean Sea. In this area, monitoring of the wild catches is more problematic, since this fishery zone is less stringently regulated and there is no reliable information as to what is being caught there, unlike the case of the Balearic Islands, where there has been a much stricter control exercised by Spain, whose boats are fishing in the area.

Recently, the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) at the fifteenth meeting of the Conference of the Parties that was held in Doha (Qatar) on March 3–25, 2010 discussed the proposal by Monaco to amend Appendix I to include the Atlantic bluefin tuna (http://www.cites.org/eng/cop/15/prop/E-15-Prop-19.pdf). Appendix I of CITES includes species that are threatened with extinction, for which, therefore, international trade is prohibited. The proposal was not adopted by the conference due to solid opposition from nations such as Japan, Korea, Libya, and Turkey, and the Atlantic bluefin tuna fattening and farming industry has survived for the next few years, albeit with significant reductions in allowable catches from previous years.

4. *TOWARDS A SUSTAINABLE AQUACULTURE: CONTROL OF REPRODUCTION IN CAPTIVITY*

Development of any industrial aquaculture operation requires an absolute control of the production cycle of the particular fish in question. The sustainability of the fattening and farming of Atlantic bluefin tuna in the Mediterranean Sea is questionable, at least at the magnitude that has been operating in the last decade, therefore, development of a proper aquaculture industry for the Atlantic bluefin tuna is seen by many as the only way to both satiate the great demand for sushi and conserve the wild stocks of this magnificent and historic fish. One of the prerequisites for domestication and the establishment of a sustainable aquaculture industry is the capacity to control reproductive processes of fish in captivity, and to acquire high quality seed (i.e., eggs and sperm) for grow-out of the marketable product (Mylonas et al., 2010). This allows (a) the reliable production of seed, (b) the manipulation of reproductive season through photothermal manipulations, in order to extend the period of seed production, and (c) the improvement of desirable traits through selective breeding.

In the wild, sexual maturity in Atlantic bluefin tuna is achieved at 3–5 years of age in the Eastern Atlantic–Mediterranean Sea stock (Abascal et al., 2004; Corriero et al., 2005). The median size at sexual maturity of females is 103.6 cm in fork length, whereas 100% of the fish are mature above 135 cm (Corriero et al., 2005), which corresponds to a body weight of 30–35 Kg. Spawning of Atlantic bluefin tuna in the Mediterranean Sea takes place from May to July (Corriero et al., 2003; Karakulak et al., 2004), and the major spawning areas have been identified around the Balearic Islands (Spain), south of Malta, the South Tyrrhenian Sea (Italy), and the Levantine Sea between Turkey and Cyprus (Nishida et al., 1997; Medina et al., 2002; Corriero et al., 2003; Karakulak et al., 2004; Heinisch et al., 2008). Atlantic bluefin tuna have an asynchronous ovarian development (Corriero et al., 2003, 2007) and spawn multiple times during the reproductive season. The spawning frequency has been estimated to be 1.2 days (i.e., each female spawns every 1.2 days, on average), producing a relative batch fecundity of $\sim$ 98,200 eggs Kg^{-1} body weight (Medina et al., 2002). Given an average spawning season of 3 weeks per female, it is expected that annual fecundity for a 100 Kg female can reach 172 million eggs. Spawning takes place at the water surface in the evening hours at water temperatures usually $> 23°C$ and the pelagophil eggs hatch in about 28 hr (Mylonas et al., 2007). In a recent trial on the potential of mature fish caught by purse-seine in the spawning grounds to continue spawning while being towed to the fattening sites, it was demonstrated that the vast majority of spawning took place between 03:00 and 05:00, with some spawns occurring also during the daytime (Gordoa et al., 2009). Spawning behavior (i.e., chasing) and subsequent release of fertilized eggs has been observed recently in captivity, occurring also in the very early hours of the day (F. de la Gandara and A. Belmonte, unpublished data).

Reproduction in captivity of the bluefin tuna was first accomplished in Japan with the Pacific bluefin tuna (Kumai, 1998; Lioka et al., 2000; Miyashita et al., 2000; Masuma et al., 2003; Sawada et al., 2005; Masuma et al., 2006). Fish are maintained in large cages or enclosures and are allowed to spawn naturally. In June of 2002, artificially bred Pacific bluefin tuna broodstock—i.e., the F1 of wild breeders—produced 1 million eggs for the first time at Kinki University. This closure of the production cycle was a significant step forward and paved the way for the future of full-scale aquaculture of this species. Currently, a limited quantity of fully farm-raised Pacific bluefin tuna is being sent to the Japanese market on a regular basis.

In captivity, almost all fishes exhibit reproductive dysfunctions (Zohar and Mylonas, 2001). In females, failure to undergo oocyte maturation, ovulation, and spawning is the most common dysfunction. In males, the problem is less serious and it includes the reduction in sperm quantity or quality in males. It is thought that these dysfunctions are the result of captivity-induced stress (Sumpter et al., 1994; Pankhurst and Van Der Kraak, 1997), the lack of the appropriate spawning conditions (Zohar, 1989a, 1989b; Battaglene and Selosse, 1996), and/or the lack of some essential dietary components in the diet (Watanabe and Vassallo-Agius, 2003). Reproductive dysfunctions often weaken as consecutive generations of broodstock are being produced from cultured parents, as fish are inadvertently selected for characteristics adaptive to the culture environment. Development of an aquaculture industry for the Atlantic bluefin tuna, as for any other species established so far, necessitates a study of its reproductive biology and establishment of methods for the control of maturation and spawning in captivity (Bromage and Roberts, 1995). These methods may be useful not only during the early establishment of the industry, but they can prove to be important management tools in order to ensure efficient spawning and a consistent supply of gametes for the future (Mylonas et al., 2010).

Studies have recently been undertaken to develop methods for the control of reproductive maturation in captive-reared Atlantic bluefin tuna (Corriero et al., 2007; Mylonas et al., 2007; Corriero et al., 2009). Mature migrating Atlantic bluefin tuna (5–12 years old) were captured in the Mediterranean Sea and reared in floating cages. During the natural spawning period (June–July) of two consecutive years, fish were implanted underwater with a controlled-release delivery system (Figure 6A) loaded with gonadotropin-releasing hormone agonist (GnRHa), in order to induce maturation, ovulation/spermiation, and spawning (Mylonas et al., 2007). There were no differences between the histological appearance of the testes of GnRHa-treated and Control males (Figure 6B), and almost all of them contained intra-testicular spermatozoa (Corriero et al., 2007). However, the proportion of spermiating Control males ($n = 17$) was only 12% compared to 26% for the GnRHa-treated males ($n = 19$). Also, there were no differences between Controls and GnRHa-treated fish in sperm concentration, initial spermatozoa motility, or duration of forward motility, which ranged between $29.02 - 48.54 \times 10^{10}$ spermatozoa ml^{-1}, 58–63% and 9–8 min , respectively. In females, GnRHa implantation induced

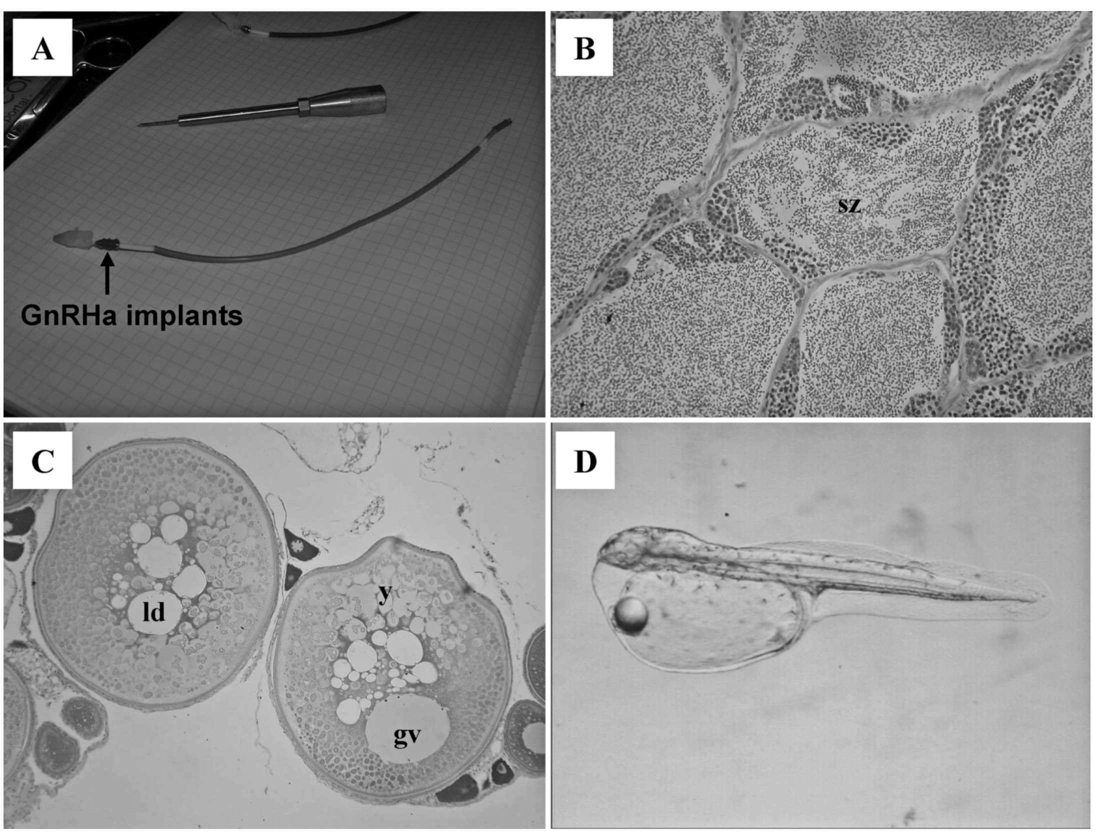

Figure 6 Induction of maturation and spawning of Atlantic bluefin tuna. (A) Implants (arrows) containing gonadotropin-releasing hormone agonist (GnRHa), assembled with an arrow head and visual tag for underwater administration to captive broodstock. (B) Histological section of a testis from an untreated captive male. (C) Histological section of the ovary from a GnRHa-implanted female, showing oocytes in the process of maturation, with a migrating germinal vesicle (g) and coalescing lipid droplets (ld) and yolk globules (y). (D) Atlantic bluefin tuna pre-larvae at the day of hatching (from Mylonas et al., 2007).

oocyte maturation 2–8 days after treatment in 63% of the fish (Figure 6C), and 88% had post-ovulatory follicles in their ovaries, compared to 0 and 21%, respectively, for Control females. In addition, two females in 2005 were found to be ovulated at the time of sacrifice and their eggs were inseminated in vitro with sperm from spermiating males, producing viable embryos and larvae (Figure 6D). Also, fertilized eggs were collected from the cages demonstrating that it is possible to induce maturation, ovulation/spermiation, and spawning in captive-reared Atlantic bluefin tuna using a GnRHa-based therapy.

In another experiment in Italy, a captive stock of Atlantic bluefin tuna was induced to spawn using the same GnRHa therapy, which resulted in spawning of 20 million eggs over four consecutive days (De Metrio et al., 2010). The estimated fertilization rate was 80% for the first two days and 30% for the next two. Larval rearing was undertaken in a commercial hatchery and resulted in the first ever production of aquacultured Atlantic bluefin tuna fry up to the size of 8 cm in total length at 60 days post hatching. More recently in 2009, another broodstock of captive-reared fish maintained in Spain (SELFDOTT, EU 7th FP Programme, http://sites.google.com/site/selfdottpublic/) was induced to spawn using the same GnRHa implants, resulting in daily spawning for 17 days, producing a total of 170 million fertilized eggs (F. de le Gandara and A. Belmonte, unpublished data). The eggs were sent to various research hatcheries around the Mediterranean Sea, and larval rearing trials produced a small number of fingerlings, demonstrating the feasibility of the aquaculture production of Atlantic bluefin tuna. The broodstock used in Italy by De Metrio et al. (2010) spawned reliably after treatment with GnRHa implants for three consecutive years (2008–2010), whereas the broodstock used in Spain spawned spontaneously in 2010, without the use of any hormones, producing eggs for a period of at least 2 weeks (F. de le Gandara and A. Belmonte, unpublished data).

These findings pave the way for a new era in Atlantic bluefin tuna production—a true aquaculture—where this species will be propagated in captive conditions, through rearing of the larvae and grow-out of fingerlings on scientifically formulated feeds, as is done successfully for species, such as the Atlantic salmon (*Salmo salar*), European sea bass (*Dicentrarchus labrax*), and gilthead sea bream (*Sparus aurata*).

5. *THE FUTURE OF ATLANTIC BLUEFIN TUNA FARMING AND FATTENING*

There are various reasons for the popularity of farming the bluefin tuna (all three species): the high growth of the species (30 Kg in 3 years), its high market value, and a dress out percentage (percentage of usable flesh) of 80%, in comparison to 50% for European sea bass or gilthead sea bream. Therefore, there is great interest to establish a sustainable farming industry. This, in turn, necessitates the development of specific technologies for tuna aquaculture that will not rely on capture of seed from the wild, as it is practiced almost exclusively today, with the exception of the Pacific bluefin tuna in Japan (Sawada et al., 2005). As the natural bluefin tuna populations that currently supply the raw material for the industry are severely over-fished, the recommendations of the GFCM/ICCAT working group on sustainable practices for bluefin tuna farming in the Mediterranean state that, “In the long term, the potential sustainability of bluefin tuna farming is linked also to the research advances in the successful ‘domestication’ of the species.”

It is also worth mentioning that at the present, bluefin tuna farming is based on the high prices fetched by this product on the Japanese market, and that any crisis in the latter would have a major knock-on effect on the sector. However, once Atlantic bluefin tuna can be produced entirely in captivity and fed efficient commercial diets, then rearing of fish for 3 years to 20–30 Kg body weight may be the standard of the Mediterranean tuna aquaculture (not fattening any more!) industry, and can create a new product (i.e., fresh tuna steak) supplying world markets. This product may be profitable at much lower prices than the ones expected now for sushi/sashimi quality flesh. Finally, it is also expected that the development of a self-sustained aquaculture industry of the Atlantic bluefin tuna will help in alleviating the pressure on the wild fishery and aid in the conservation and recovery of this magnificent fish.

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