SEEDLING PRODUCTION OF ATLANTIC BLUEFIN TUNA (ABFT) *Thunnus thynnus*. THE SELFDOTT PROJECT.

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Introduction

The Atlantic Bluefin tuna (ABFT), *Thunnus thynnus*, L. 1758, is a migratory scombrid which has been feeding the human populations, especially in the Mediterranean area for centuries. In the last 15 years, the wild populations is being overexploited mainly due to the appearance of the fattening activity, addressed to the Japanese market, and consisting in the massive capture of individuals in the spawning areas, feeding them with very oily pelagic raw fish, and slaughtering several months later (FAO, 2008). To avoid the collapse of the wild populations, the International Council for Conservation of Atlantic Tunas (ICCAT) has implemented maximum allowed captures (quota) and this quota is being reduced year by year (ICCAT, 2008), which put in danger the fattening activity in the foreground, and maybe the

fishery itself in the middle distance.

One way to alleviate the pressure on the wild fishery of the Atlantic bluefin tuna and aid in its conservation is the domestication of the ABFT and the development of a self-sustained industry which the first step is to close the life cycle in captivity, as it is already done in the Pacific bluefin tuna, *Thunnus orientalis* (Sawada et al., 2005). This aquaculture industry will propagate the ABFT in captive conditions, rear the larvae and produce fingerlings for further grow-out on suitable, scientifically formulated and environmentally performing feed, as it is done successfully in the EU for species such as the Atlantic salmon (*Salmo salar*), European seabass (*Dicentrarchus labrax*) and gilthead seabream (*Sparus aurata*).

The SELFDOTT project (From capture based to self-sustained aquaculture and domestication of bluefin tuna, *Thunnus thynnus*), funded by the 7ºFP of the European Commission is implementing the knowledge already obtained on the artificial control of reproduction of the ABFT to obtain viable eggs. These eggs have been used to study embryonic and larval development to produce fingerlings for further rearing, while at the same time develop a commercial, pelleted feed for the grow out of ABFT, thus reducing or eliminating the practice of raw fish importation and feeding by the fattening industry.

In the area of reproduction, the objectives of SELFDOTT are to (a) enhance the existing knowledge on the reproductive biology (onset of puberty, gametogenesis, and gamete quality) and artificial spawning-induction of ABFT; (b) optimize microsatellite markers to genotype ABFT and their progeny, with applications to broodstock management and fisheries populations; (c) obtain fertilized and viable eggs, and ship them to larval rearing facilities for the production of juveniles; (d) develop appropriate broodstock diets for the support of reproductive maturation and gamete quality; and (e) study the behaviour of long-term reared wild broodstock and establish monitoring procedures in order to develop the knowledge for a self-sustained aquaculture of the ABFT.

Material and Methods

For the spawning induction activities of 2009, two ABFT broodstocks were available each in Spain and Malta. The broodstocks used in Spain were composed of 60 ABFT placed in two floating cages located at El Gorguel (Cartagena, Spain) (25 m diameter x 20 m depth). Cage R1 contained 35 ABFT with an estimated mean body weight of 100 kg caught in the Balearic Sea in June 2007 and kept in captivity for 3 years. Cage R2 contained 30 ABFT with an estimated body weight of 80 kg, also caught in the Balearic Sea in June 2008 and kept in captivity for 2 years. This last broodstock was donated by the Spanish Fisheries General Secretary to the IEO from a quota excess in February 2009. Both cages were fitted with a 2 cm mesh net to restrict the entry of opportunistic small pelagic fish that can eat the eggs being released. The broodstocks in both cages were fed to satiety once a day with raw fish

consisting mainly of mackerel (*Scomber scombrus*) and Spanish mackerel (*S. japonicus*). On 26th and 27th June 2009 an egg collector was placed in cage R1. The system consists of a curtain that is 6 m high. The curtain surrounds the entire perimeter of the cage and hangs from the surface down into the water. Eleven cones protrude outwards from the curtain and each cone has a cylindrical collector at the end of it, where the BFT eggs are collected. To avoid the entering of opportunistic fish into the collector, a "predator mesh" was placed at the entrance of the cones. The entire system is made with a polyethylene 500 μm mesh.

Two similar ABFT broodstocks were also available for induce spawning in Malta. Due to the failure in inducing spawning in the very protected, but shallow waters of Marsaxlokk Bay in 2008, the original broodstock maintained inside Marsaxlokk Bay in 2008 was moved to another site, approximately 2 km off the coast of Marsaxlokk Bay. In addition to this cage, a new broodstock (2009 Stock) was selected in another cage in March 2009. These two broodstocks were reared in separate cages next to each other. Egg collection devices were fabricated for the two spawning cages. Egg collection was to be done manually from the surface of the water after spawning. A PVC curtain that went down to a depth of 3 m below the surface was installed for both cages around the perimeter of the cage. This was done to contain any floating viable eggs that would then be collected by plankton nets from the surface.

Another captive broodstock (maintained in Calabria-Italy by the company MareNostro) belongs to a regional research consortium (ALLOTUNA) funded by European Union Structural Funds through the region of Puglia (Italy), was available. The two research consortia have some common partners, and have signed a collaboration agreement and coordinate their activities closely, including the transfer of viable eggs.

On the 26th and 27th June, 15 captive-reared BFT from cage R1 in Spain were administered an implant loaded with gonadotropin releasing hormone agonist (GnRHa) at a dose of 6 mg GnRHa/fish according the technique developed in the former EU funded project REPRODOTT (Mylonas et al., 2007). Cage R2 was not implanted, waiting for the results from cage R1.

Spawning induction in Malta was carried out on the 27th June, 2009, when 15 of 32 BFT were implanted with GnRHa implants. The implantation procedure took approximately one and a half hours and all 15 fish were implanted without any problems. The 2008 broodstock group of 30 fish was not induced at this stage. After induction, both cages were checked for any floating eggs twice a day, at sunrise and just before sunset.

In 2010, the egg collector was placed in both broodstock cages (R1 and R2) in Cartagena, on 17th June. No GnRHa implants were used.

In Malta the broodstock, outside the bay is made of 40 fish caught in south Malta in June 2008 and 2009. Inside the Bay were 30 fish, caught in south of Malta in 2009. Spawning induction in Malta was carried out in 15 ABFT in the cage outside the Bay on 5th June, 2010,

and 15 ABFT too in cage inside the Bay on 24th June. A second implantation was carried out on 25th June in the cage outside the Bay when 10 ABFT were implanted (4 of which had been previously implanted in early June).

Results and Discussion

In 2009, the Spanish broodstock begun spawning on 29th June, three days after hormonal treatment, and spawned daily for a period of 17 days with a total of 140 million eggs. Daily fecundity ranged between 285000 to 34 million eggs. Fertilization success was almost 100%, as probably only fertilized eggs floated to the surface and were collected. Although the GnRHa implants could not be improved further in terms of their duration of hormone release, the resulting daily spawning for a period of at least 17 days after treatment achieved is considered quite adequate for this fish.

No eggs were collected from the broodstock maintained in Malta in 2009, even though chasing and spawning behavior were observed after the second implantation. Based on gonadal data obtained from another broodstock maintained in the same site, as well as from fish randomly sacrificed from this stock after the hormonal therapy, it seems that the hormonal treatment might have been given too late in the season, as most of the fish where in advanced apoptosis. Another complication was introduced into the equation due to the hydraulic conditions of the site caused by very strong water currents at the depth that the fish may have spawned, resulting in the dispersal of any eggs released prior to their entering the area that could be captured by the employed egg collector.

In 2009, husbandry tasks were satisfactorily supplied in ABFT viable eggs (IEO- 346 000, MRRA- 450 000, IFREMER- 540 000 and HCMR- 386 000) from Cartagena (Spain: 1 486 000) and Calabria (ALLOTUNA-Italy: 836 000). The ABFT egg shipment results were notably improved and successful but still critical points have to be solved in terms of egg temperature pre-packing conditioning, transport boxes insulation related to transport ambient temperature and duration.

For ABFT larval rearing, several experiments have been implemented using the 3 targeted methods: Mesocosm (HCMR, IEO), pseudo green water (IEO, HCMR and MRRA) and clear water (IFREMER, IEO). The rearing attempts have been conducted until 17 (IEO) and 60 dph (HCMR) in mesocosm, 24 (MRRA), 60 (HCMR) and 73 dph (IEO) in pseudo green method, and 10 (IEO) and 20 dph (IFREMER) in clear water method. In mesocosm, swimbladder inflation rate was 50 % (HCMR), while ranging between 50 (HCMR) and 58 % (IEO) in pseudo green method, and between 1.2 (IFREMER) and 5 % (IEO) in clear water method. The presence or the addition of microalgae seemed to give a significant advantage to mesocosm and pseudo green rearing methods during the early development stages in terms of growth and survival rates compared to the clear water rearing method. The ABFT growth

during larval rearing (30 dph) in pseudo green method is shown in Fig. 1.

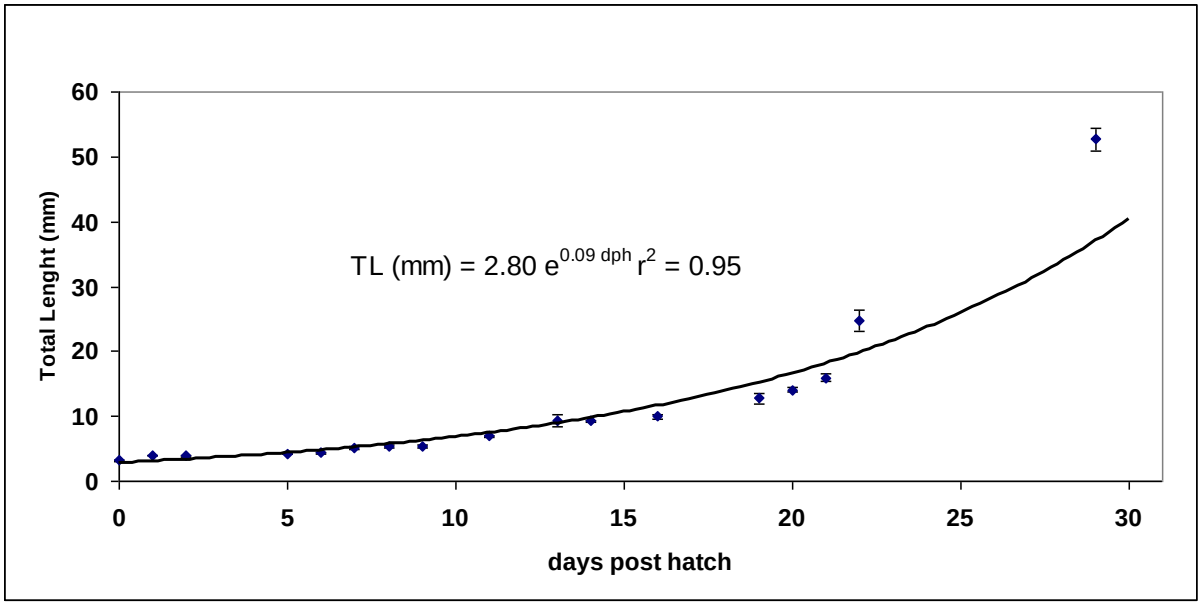

Fig. 1 ABFT growth during larval rearing (30 dph) in pseudo green method (error bars is the standard deviation of the mean)

The feeding sequence of yolk sac larvae after Artemia naupli strongly influenced both survival and growth, highlighting the critical importance to satisfy the energetic and nutritional needs at that stage. A few ABFT individuals ranging between 8 and 14 cm total length were produced. The ABFT growth performed after weaning is showed in Fig. 2. Light intensity, lighting regime, hydrodynamic, surface cleaning, feed sequence and nutritional quality have been identified as the main factors to be fine tuned mainly during the larval rearing stage. The best survival in 2009 (73 dph) was observed in Cartagena with a weight of 30 g and 14 cm of total length. In Fig. 3, there appear the ABFT development stages at 0, 34 and 73 dph.

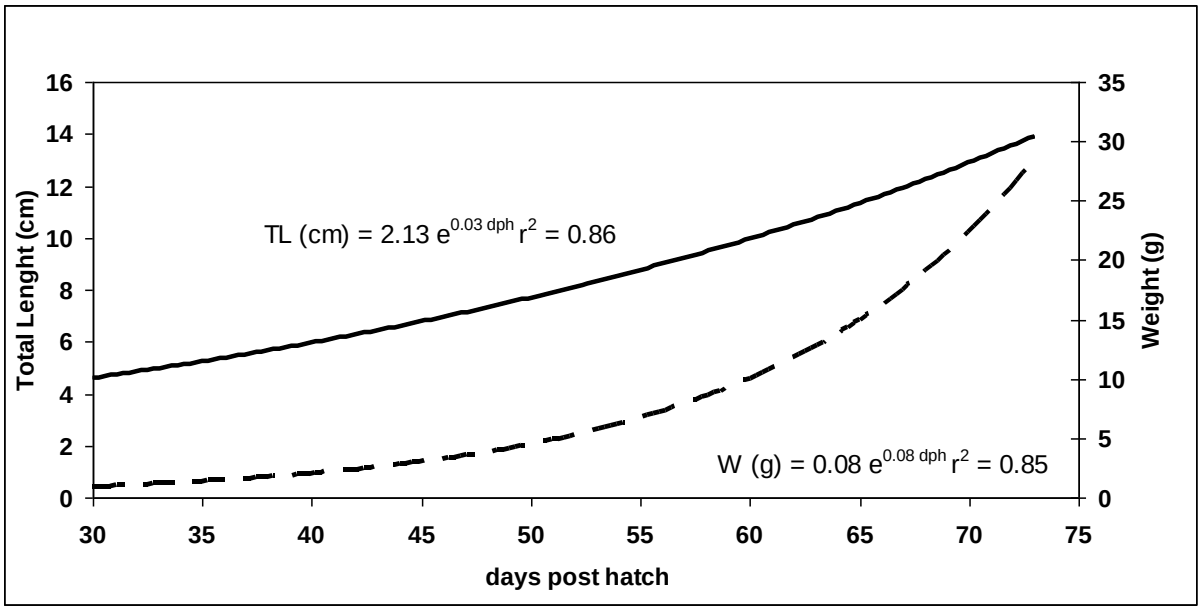

Fig. 2 ABFT growth (total length and body weight) performed after weaning (larval rearing was performed in pseudo-green method)

In 2010, spawnings were obtained in both sites (Spain and Malta). In Cartagena (Spain) ABFT eggs started to be observed in both cages from 18th June, at around 20ºC, just the day after the egg collectors were placed. So it is not possible to assure that spawnings were not take place before. These spontaneous spawnings continued during one month up to the water temperatures were above 26ºC. A total of 60 million eggs were collected. The first analysis did not show hatching rate differences between eggs obtained from spontaneous and hormonal induced spawnings. Nevertheless, it must be taking into account that only the floating eggs were collected from the cages.

In Malta around 280 000 buoyant eggs were collected over 10 spawns between 5th June and 7th July from the cage placed outside the Bay. The surface sea water temperature fluctuated between 20.4ºC–23.9ºC. Once again the cage inside the Bay failed to produce eggs.

As it was done in 2009, several egg batches have been transported in June-July 2010 from Cartagena (Spain) and Calabria (Italy-ALLOTUNA project) to the larval rearing laboratories involved in the project (IFREMER, MRRA and IOLR-NCM) in order to carry out the larval rearing trials planned in the SELFDOTT project. Larval rearing trials in Malta were carried out with the local spawns. The results of all these trials are not yet available. Updated information could be obtained in the SELFDOTT web site: www.selfdott.org.

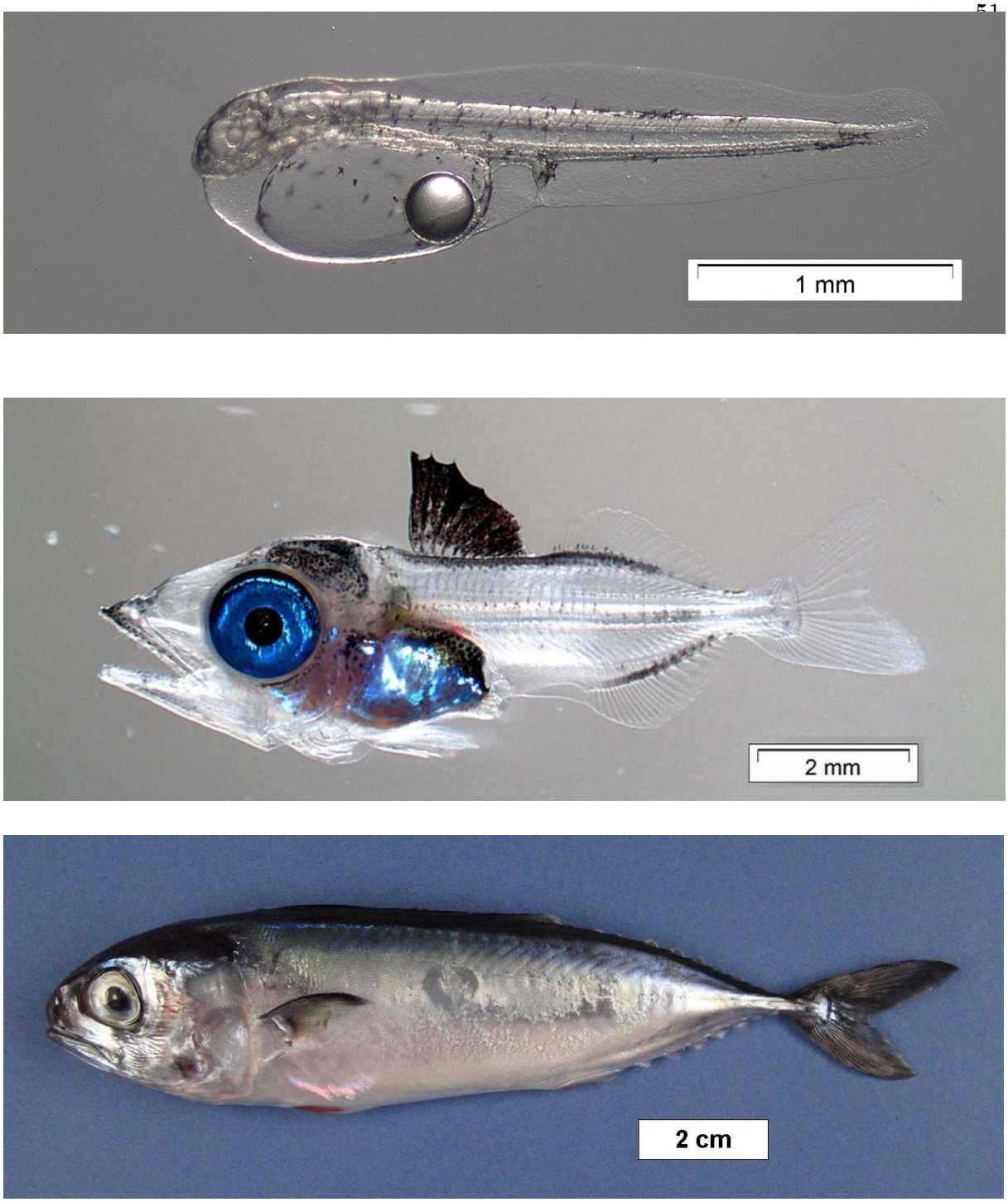

Fig. 3 ABFT development stages related to age, at 0, 18 and 73 dph.

Conclusion

Massive spawnings with or without hormone induction are available from captivity adapted broodstock in the spawning period. The complete mortality of the reared ABFT juveniles beyond two months of life seems to be related mainly to nutritional deficiencies and collision against the tank walls. Therefore, the main efforts should be given in these areas to produce juveniles which could be reared up to commercial size.

References

FAO (2008) Capture based aquaculture. Global overview, (ed. by A. Lovatelli and P.F. Holthus), *FAO Fish.Tech.Pap*., **508,** 314 p.

ICCAT (2008) Recommendaton amending the recommendation by ICCAT to establish and multiannual recovery plan for bluefin tuna in the eastern Atlantic and Mediterranean. *International Committee for the Conservation of Atlantic Tuna*, 27 p.

Mylonas, C.C., Bridges, C.R., Gordin, H., Belmonte Ríos, A., García, A., Gándara, F. de la., Fauvel, C., Suquet, M., Medina, A., Papadaki, M., Heinisch, G., De Metrio G., , Corriero, A., Vassallo-Agius, R., Guzmán, J.M., Mañanos, E. and Y. Zohar (2007) Preparation and administration of gonadotropin-releasing hormone agonist (GnRHa) implants for the artificial control of reproductive maturation in captive-reared Atlantic bluefin tuna (*Thunnus thynnus thynnus*). *Rev.in Fish. Sci.*, **15**: 183-210.

Sawada, Y., T. Okada, S. Miyashita, O. Murata and H. Kumai (2005) Completion of the Pacific bluefin tuna *Thunnus orientalis* (Temminck et Schlegel) life cycle. *Aquacult. Res.*, **36**, 413-421.

Please note: This research has been carried out with financial support from the Commission of the European Communities, specific RTD programme of Framework Programme 7, SELFDOTT. The Community is not liable for any use that may be made of the information contained therein, nor does it necessarily reflect its views and in no way anticipates the Commission's future policy in this area.