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| | EUROPEAN COMMISSION<br>RESEARCH AND INNOVATION DG | Final Report |

**Project No:** 311904

**Project Acronym:** TRANSDOTT

**Project Full Name:** TRANSLATION OF DOMESTICATION OF THUNNUS THYNNUS INTO AN INNOVATIVE COMMERCIAL APPLICATION

# Final Report

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**Project coordinator name:**
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HEINRICH-HEINE-UNIVERSITAET DUESSELDORF

Final Report

# PROJECT FINAL REPORT

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# Final Report

**Please note that the contents of the Final Report can be found in the attachment.**

# 4.1 Final publishable summary report

## Executive Summary

Due to declining stocks and increased fishing pressure there are serious concerns that the present fisheries and fattening industry for Bluefin Tuna (Thunnus thynnus) is not sustainable and that every effort should be made to develop BFT aquaculture. The TRANSDOTT project proposal was aimed to further advance the technologies developed in SELFDOTT and implement them within the industry. The major objective of this project was to translate/ transfer the state of the art technologies to the industry which has the experience in up-scaling and implementing it into a working protocol. TRANSDOTT represents a “top-down” approach from one Enterprise, three SME´s and three non SME´s to build on the scientific results obtained from previous projects.
Starting in April 2012, based on an already established broodstock in Malta thirty BFT were assigned to a broodstock cage fitted with a PVC egg collector. The PVC egg collector was made deeper for improved egg collection, but the BFT broodstock biomass was not as large as expected and the egg collector was not effective due to strong currents in the cage area that ripped the egg collector material in 2012. For the following years, more fish were added to the broodstock on a yearly basis and the spawning biomass was 2,380kg in 2013 and 4,800kg in 2014. The broodstock were fed an improved diet throughout the project, from the beginning of April to enhance spawning quality. The sea temperatures were also monitored using data loggers at different depths of the broodstock cage. All fish introduced into the broodstock cage were tagged and biopsied after the spawning period or in a separate cage before transfer. The egg collection method was reassessed following the failures of 2012 through MRRA/MAR’s collaboration with Korean researchers and the use of a ‘trawl-net’ type of egg collector that was installed outside the cage so as to collect the eggs by use of the currents. The fish spawned naturally in both 2013 and 2014 however a drop in SST in 2013 stopped spawning that was once again started by GnRHa induction. In 2013 egg collection started on the 16th June and over 10 million viable BFT eggs were collected. In 2014, spawning started on the 8th June and over 40 million viable BFT eggs were collected, with a maximum of 6.2 million eggs collected in one day. This was positive proof of the work done on the current studies in previous years and the set-up of the egg collector. Egg parental monitoring through DNA analysis was carried out for samples taken from Malta and Croatia in 2012 and from eggs collected in Malta in 2013 and 2014. With this system it was clearly observed that a number of females and males were participating in spawning events.
Eggs obtained in all years were distributed to partners and interested institutes or companies that were willing to collaborate with TRANSDOTT by sharing results. In 2012 it was only possible to make 7 shipments of eggs from Malta that were obtained from another tuna farm in the north and 1 shipment from Croatia. Following the problems of 2012, all larval rearing partners in Israel, Malta and Spain received BFT eggs on more than one occasion depending on hatchery space availability and spawning quantities during 2013 and 2014. A total of 8.6 million eggs were sent to the partners in 2013 (16 shipments) and 26 million eggs in 2014 (28 shipments). BFT eggs were also sent to companies outside the consortium for research purposes in Germany and Spain. This proved that Malta is the ideal location for the production and distribution of BFT eggs throughout the Mediterranean and beyond, due to very favorable logistics and short travelling distances on land.
A major problem experienced by all partners was the contamination of eggs by alien species such as Sarda sarda (Atlantic bonito) and Auxis rochei (Bullet tuna) among others. Some of these fish grew faster and cannibalized BFT larvae, causing major problems. All partners removed the faster growing alien species from the larval rearing tanks by fishing them out individually. In 2014 studies were carried out to try and separate the alien species at the egg stages through floatation rates.
In 2012, due to the lack of biological material, a few studies mainly focused on larval rearing conditions for BFT and on live prey enrichments with DHA during the critical rotifer feeding stage for improved eye development, prey acquisition and early growth took place. Trials combining DHA with 400 mg taurine / l grew significantly better and survived longer. Moreover, the 400 mg/l taurine supplementation treatment markedly reduced the presence of calcium oxalate urinary crystals in the urinary duct. In 2013 IOLR showed that dietary taurine (4.4 mg taurine / g DW) significantly

improved survival and tank biomass in 14 dph BFT larvae. FB reported that the use of copepods as a first food significantly reduced bottom-mortality by 5 dph and until 27 dph with the subsequent feeding of yolk sac larvae (16 dph) reduced cannibalism between 11 to 30 dph. Trials in 2013 and 2014 tried to reduce the dependence of BFT larvae on live sea bream yolk-sac larvae, however there is no significantly viable replacement.FB produced juveniles in all years through their innovative method of feeding copepods plus yolk-sac larvae and from the 2014 season fish of 2 to 2.5 kg have survived. The IEO in Spain received BFT eggs from TRANSDOTT in 2014 and they produced around 5,000 juveniles that were transferred to sea cages for grow-out by similar methods. MFF produced around 10,000 larvae at 22 dph however a lack of yolk-sac larvae forced over-feeding and water quality deterioration which led to the loss of all larvae by 30dph. European SME´s should be further encouraged by the positive results of TRANSDOTT to continue with the work already started and bring this technology into full commercial large-scale production. International interest has grown and Europe can continue to be at the forefront of this development.

**Summary description of project context and objectives**

Project Context and Main Objectives

Since the early 1990's a “capture-based" aquaculture industry has been developed in the Mediterranean Sea (Ottolenghi et al., 2004), which involves the capture of migrating wild BFT to their spawning grounds by purse-seiners, towing them to floating cages where they are then fattened for periods ranging from 2 months to 2 years (Miyake et al., 2003; Directorate General for Fisheries, 2004; Ottolenghi et al., 2004; FAO, 2005). The Atlantic bluefin tuna (BFT) is the largest tuna species. It has an elongated fusiform body, being more robust at the front. Its maximum length can exceed 4 m and its official maximum weight is 726 kg, but weights up to 900 kg have been reported in various fisheries of the West Atlantic and Mediterranean Sea (Mather et al. 1995). This practice became so widespread and common place in the Mediterranean Sea that some called it "the gold rush of the Mediterranean"(Fish Farming International, 2005). The fishing pressure on the BFT Mediterranean populations has threatened the well being, future health and numbers of the eastern ABFT, so much so, that a proposal was brought to the CITES meeting in Doha in 2010, to ban international trading of this species, an act that was finally voted against. However, the issue of the survival of the BFT keeps consistently appearing in the news media during the last decade with articles that appeared in Time magazine (24 July, 2006) and in TV documentary features (EuroNews Futuris Documentary 21st September, 2010). In the Mediterranean Sea, the species of greatest value is the Atlantic bluefin tuna, Thunnus thynnus (BFT) (Fromentin & Powers, 2005; Chow et al., 2006), a very close relative of the Pacific BFT (T. orientalis) and the Southern BFT (T. maccoyii) (Collette et al., 2001; Nelson, 2006). All major tuna species, which land together over 6 million tons annually, are considered fully or over exploited and their fishing campaigns are managed by international commissions, assigned to the different fishing areas of the world oceans. These commissions collect data, have scientific advisory committees which recommend to the commissions on the fishing quota for the BFT (and other tunas). Fuelled by the increasing demand for this unique fish by the sashimi-sushi market in Japan, Europe and the United States (Catarci, 2004), the expansion of the fattening industry is considered to be threatening the wild stock, which is now considered to be overfished (Fromentin & Powers, 2005). The International Commission for the Conservation of Atlantic Tunas (ICCAT) the international organization responsible for the conservation of tunas in the Atlantic Ocean and its adjacent seas, is implementing a 4-year gradual reduction in the Total Allowable Catch (TAC) for Eastern Atlantic and Mediterranean BFT (ICCAT, 2007), in an effort to address the increasing scientific (Fromentin & Powers, 2005), public (Bregazzi, 2006) and NGO concern (Atrt, 2006) over the status of the wild stocks and the threat of extinction of the species due to overfishing. With the new fishing regulations and much smaller quotas for the ABFT the total landings of the three bluefin tuna species accounts for no more than one percent of the total landings of the other members of the Thunnid subfamily / tribe. (ICCAT,2011).
Recently due to the success of the rebuilding programme and new stock assessments it has been decided to increase the quota for Eastern and Mediterranean Bluefin tuna by just under 20% a year over the next three years (14-04 BFT Recommendation by ICCAT amending the recommendation 13-07 by ICCAT to establish a multi-annual recovery plan for Bluefin tuna in the eastern Atlantic and Mediterranean).
One way to alleviate the pressure on the wild fishery of the BFT and aid in its conservation would be the domestication of the BFT and the development of a self-sustained industry, which will propagate

this species 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 sea bass (Dicentrarchus labrax) and gilthead sea bream (Sparus aurata). Therefore, there is a great interest in developing captive Atlantic BFT broodstocks and larval rearing methods to support the sustainable development of BFT aquaculture. Studying the reproductive biology and larval rearing of this species in captivity would also result in a better understanding of its life history, which is necessary for management of the wild stocks. The idea of domesticating the ABFT was translated in the late 1990's and early 2000 into a RTD long term program. It started in a major scientific and professional meeting in Cartagena, Spain, called "The first Conference for the Domestication of Thunnus thynnus, (DOTT) the bluefin tuna (BFT) were
conducted: REPRODOTT (Q5RS 2002-01355), in which the feasibility of spawning the BFT in captivity was proven possible, ending in collecting fertilized eggs in the broodstock cage and hatching them in 2005 (Mylonas et al 2008). A large volume of basic knowledge and practical know-how was developed during this project (Corriero et al, 2007a, 2007b, Heinisch et al, 2008). The REPRODOTT project paved the way for the following project, the SELFDOTT (KBBE-2007-1-2- 09); "From capture based to SELF-sustained aquaculture and Domestication Of bluefin tuna, Thunnus Thynnus" which started in 2008 and will be terminated at the end of November 2011 (after a one year of extension of the project without additional funding). Some of the results of the SELFDOTT have not been published yet, however many publications dealing with the different aspects of the project are in preparation as well as the final report, which is due in the end of January 2012. There were three major objectives that were all successfully achieved during the SELFDOTT project:

1. Improving controlled spawning of captive BFT and collecting the resulting fertilized eggs.
2. Developing techniques for mass rearing of the BFT larvae and production of juveniles.
3. Developing artificial diets for weaning BFT larvae from live food, juveniles feed and grow-out feed.

Further to the above goals, spawning (even without induction) was attained regularly during the spawning seasons (over 3 years), eggs were collected in the millions and sent to the different hatcheries to be studied and for larval rearing and juvenile production. Each year from 2008 to 2011, results were improving, and a large volume of new knowledge, both basic and practical was accrued . In the summer of 2011, for the first time, a few hundred BFT juveniles, produced within the framework of the SELFDOTT project, were stocked in a rearing cage in the open sea near Cartagena, Spain. These were reported to have reached an average body weight of 1kg by October 2011. At the same time, artificial feeds were used for rearing experiments, based on Japanese techniques developed over the last few years at Kinki University, Japan (Biswas et al, 2006, Sawada, 2011). Diets developed within the framework of SELFDOTT project supported the weaning stages (day 24 post hatching), juvenile growth as well as grow-out diets (SELFDOTT final report, 2012). Improvements to the diets developed so far will continue and there is a need to design feeds for earlier stages (day 15 post hatching), so that the current protocol of feeding large numbers of newly hatched larvae of other marine fish species such as sea bream may be substituted by feeding artificial diets, with the aim of reducing such a costly and cumbersome procedure of producing this substitute larval food.
On the very encouraging progress of the SELFDOTT project, the TRANSDOTT project (call identifier: KBBE-2012.1.4-02) proposal is aimed to further advance the technologies developed in SELFDOTT and in Japan and implement them within the industry. This will maintain the European momentum in this field and enable Europe to become a world leader in sustainable BFT aquaculture. TRANSDOTT is the acronym chosen for the title of this proposed project and stands for: "TRANSLATION OF DOMESTICATION OF THUNNUS THYNNUS INTO AN INNOVATIVE COMMERCIAL APPLICATION".
The major objective of this project is to translate / transfer the state of
the art technologies to the industry which has the experience in up-scaling and implementing it into a working protocols. The main partners in the TRANSDOTT Project Proposal are SME’s that have a background in the hatchery industry including the rearing of BFT eggs and larvae. All these SME’s are interested in developing BFT aquaculture due to the fast growth rates and high market value of this species. The scientific and technological aspects of the project will be guided by three partners from the research and development field in mariculture but with vast experience in BFT projects

through their major contributions in DOTT, REPRODOTT AND SELFDOTT. In this way, the TRANSDOTT Project can be a valid continuation of the previous DOTT projects so that a sustainable BFT aquaculture industry may be developed.

The project is divided into five Work-Packages (WP);

WP 1. Co-ordination and Planning
WP 2. Improvement in broodstock management and gamete production.
WP 3 Further development of egg, larval rearing and feeding strategies.
WP 4 Improving fingerling production and testing, transportation to cages and on-growing techniques.
WP 5. Dissemination, Exploitation, Commercialization.

The campaign of domesticating the BFT in the Mediterranean Sea, which started in the beginning of the 2000's has reached the point of moving from the "Research and development" stage to the translation of results into commercial processes. TRANSDOTT is designed to further advance the level of the BFT domestication technology through the major input by industry and backed by the scientific partners.

**Description of main S & T results/foregrounds**

Description of the Main S & T Results/Foregrounds

WP2.1 Broodstock Management and Handling (MFF, UDUS, MRRA/MAR, IOLR/NCM)

Following the egg collection attempts of Year 1 (2012), in preparation for the spawning period of Year 2 (2013), another 15 fish with an average BW of 70kg were added to the spawning biomass following the loss of 11 fish during 2012, particularly during and in the aftermath of the spawning period. Moreover, the 2012 fish were smaller than predicted and more fish were required. These Atlantic Blue-fin tuna were kept in a 28m diameter cage with a 10m deep net from January 2013. For the following year, more fish were added and the 2014 spawning stock consisted of 34 fish from the 2013 group with 23 fish of 60kg average BW added in January 2014. The spawning biomass in 2013 was estimated at 2,380kg and in 2014, following the addition of more fish, it was estimated at 4,800kg. The spawning cage was moored at approximately 1 km from the shore at the MFF site at Xrobb L-G#a#in off Delimara in Malta, throughout the year. The geographical coordinates are N 35°50'80" E 14°35'60". All added fish were caught the previous year from Maltese waters.

Feeding management for 2013 and 2014 was similar to 2012 and the broodstock were fed on sardines and mackerel until April, after which they were fed a nutritionally enhanced diet with 50% squid throughout the three months prior to the spawning period. From 2013, the mesh of the net used to hold the broodstock was increased to 150mm (tuna size net) from 22mm (sea bream sized net) used in the previous year, in order to avoid current disturbances during egg collection. All individual tagging of the broodstock was done during the latter part of 2013 in October after the spawning period, combined with muscle sampling for sex determination and microsatellite analysis for each fish in the spawning cages.

All throughout the year in 2013 and 2014, the broodstock were fed to near satiation, six times per week. As observed in previous years, feed consumption of the broodstock BFT increased during the latter half of April and in May, but then became constant and started to decrease at the end of May. A decrease in feed intake during the spawning period is an indication that the fish are maturing and this has been shown to happen in previous years in BFT in the Mediterranean (SELFDOTT Final Report, 2012). The diet was enhanced with squid as this has proven beneficial in marine broodstock nutrition (Watanabe and Vassallo-Agius, 2003).

It is known to support reproductive maturation and gamete quality in BFT as shown during the EU funded SELFDOTT project when fish fed the experimental diet containing 50% squid showed a more advanced gonadal development, with females having oocytes of a higher diameter than those fed the control diet. Moreover, the improved broodstock diet was associated with a lower occurrence of atresia of vitellogenic oocytes and a higher vitellogenin receptor gene expression. Sperm characteristics, namely sperm release, sperm concentration, sperm motility, duration of movement

and sperm velocity were all improved by the experimental diet (SELFDOTT Final Report, 2012). This allowed the Deliverable 2.4 to be achieved and submitted to the Commission Portal.

Tagging and Muscle biopsies of individual broodstock:
A new underwater tagging and biopsy system has been developed. This can be used with a laser sighted spear-gun for use under water. It was used in a similar form in October 2013 and again for the new fish added to the broodstock in January 2014. This allowed the individual genetic characterization and the determination of the parental origin of the eggs and larvae produced by the broodstock. Before fish were added in January 2014, they were kept temporarily in a separate cage and tagged before being added to the spawning cage. The samples that were analyzed are muscle samples from the Atlantic blue-fin tuna (ABFT) broodstock in Malta taken using the biopsy tool as outlined in Deliverable 2.2
They are in total 47 samples which were sampled on two separate occasions and two different cages. One sampling took place in October 2013 and the second one in January 2014. The total genomic DNA extraction was carried out using the peqGOLD Tissue DNA kit (Peqlab) and for the genetic analysis of the broodstock the Fragment Analysis method was used. The 94 broodstock samples were genotyped with a multiplex of 11 microsatellite markers out of the 16 initial microsatellite markers that were ordered. The 5 microsatellite markers that were left out gave no reproducible results by the first genotyping. Moreover, the concentrations of the three microsatellite markers (TET03211, TET03137 and TEF01274) were increased. The second amplification of the samples (94) worked much better than the first one we obtained better results by analyzing them with the STRand analysis software v2.4.59 (Melissa Locke, Eric Baack & Rob Toonen, 2000), that was used for the genotypic/scoring of our microsatellite data. By leaving out 5 of the loci, we amplified finally 11 microsatellite loci instead of 16, which is an efficient number of microsatellite loci for the respective number of samples. In this way, we achieved reproducible results for the tuna broodstock that show us the allele sizes and the genotypes for every DNA sample via the STRand analysis software v2.4.59 (http://www.vgl.ucdavis.edu/informatics/strand.php/) . These results allowed Deliverables 2.2 and 2.3 to be attained and also milestone MS2.
A TRANSDOTT Bluefin Tuna (Thunnus Thynnus) Broodstock DNA Fingerprint Table was provided as part of Deliverable 2.5 and submitted to the Commission.
Together with this deliverable the results of the sex test using the “Hot Blot” technique and Parentage studies were combined. From comparisons of the staining of the ZRP antibody it was concluded that 15 females and 11 males were present. Obviously an arbitrary standard is used to judge whether a particular sample is a male of female and in this case we do not have absolute values therefore the numbers should have +/- error margin of at least 10%. The test also tends to over-estimate the number of females.
Further sampling was carried out on additional brood stock added to the old brood stock cage at the end of the 2013 season. The results indicated 7 females and 9 males. It could be expected that the sex ration of these additional fish was skewed due to the fact that they came from the production cage after harvesting and these tend to be smaller fish which are usually males. Again an overestimation of the number of females can be predicted. On the whole the use of the muscle biopsy is more accurate when ZRP and VTG concentrations in the fish plasma are high. Therefore sampling just after the spawning season should be recommended. The difference between results from October sampling and January sampling are significant in terms of signal strength.
Since the spawning performance could be affected by minimal disturbance it was best to avoid fish losses, UDUS, MRRA/MAR and MFF decided that the planned handling trials on BFT involving the use of different anesthetics and novel delivery systems for underwater injection should be abandoned.

WP 2.2 Behaviour and Monitoring (UDUS, MFF, MRRA/MAR)

Temperature

Six two-channel temperature and relative light level data loggers (HOBO Pendant® Temperature/Light Data Logger 64K) were attached to the broodstock net; three at the surface (1m below sea level) and three at the bottom of the net (10m deep). All data loggers were programmed to monitor both parameters every 10 minutes and were downloaded on a weekly basis prior to spawning and analyzed via appropriate HOBOware Software so that a clear indication of the water

temperatures was observed. Temperature and light intensity were measured throughout each season with HOBO data loggers. Temperature rose sharply in 2013 year from around the 13th of June with a rapid rise from 20 °C on the 13th of June to over 24°C by the 20th of June. Thereafter there was a decrease in temperature back down to 23 °C on the 3rd of July. As temperature was followed further for 10 days it rose to over 25°C. In 2014 temperatures in general were slow to rise and at the beginning of June they even dropped back to 17°C briefly. Beginning on the 7th of June a strong rise was seen with 24°C being reached for the first time on the 13th of June. This was preceded by a sharp increase of 4°C in temperature over 4 days. From the 13.06 onwards variations in temperature were correlated with daily peaks in solar radiation which were over 24°C on the 18th a cold front moved in and SST was depressed to 20.5°C. .Again temperatures began to rise again and ranged between 22.5°C to 24.5°C into the beginning of July. Temperatures continued to increase and for the period from the 7th July onwards temperatures were above 24°C rising to 27°C in August.

Currents

After mounting the sensor outside of the cage it was found that current speed was relatively low at approximately 10cm/s . Small increases at the surface at night were probably caused by wind. On the concomitant direction measurements at low current speeds <10cm/sec the current direction oscillated frequently. During the night 30cm/s speeds were reached and flow was relatively uniform in direction 150-250°. When the transponder was placed inside the cage the patterns of both speed and current direction were completely different. In terms of current speed maximum values were around 15 cm/sec and at a depth were the tuna were swimming measureable current speed differences could be seen. Only higher current speed values were seen at the surface.

Current direction was highly mixed inside the cage 6probably due to the egg collecting curtain placed inside. When surface mounted the temperature of the senor was around 26° C but when placed on the bottom of the cage large vertical movements up to 4-5m were recorded accompanied by 4°C changes in temperature Each movement of the cage bottom which has a finer mesh than the cage sides was accompanied by temperature changes..

FACET: It would appear that outside the cage current speeds of up to 50cm/sec can be found at the surface but in general they are in agreement with the “Hand measurements shown in the table above. Inside the cage due to the egg-collectors values were slightly higher overall 5- 10 cm/sec but non-directional. These current velocities had a negative effect on the ability to collect eggs in season 1 and could be partly responsible for the inability to collect eggs in 2012.
Similar studies were carried out in 2013 using the same equipment again in configurations with bottom mounted inside the cage and also top mounted looking downwards. From the recordings it was found that the average current speeds lower in the cage ranged between 0 to 0.25 m/s. From the current direction it could be seen that within the cage itself there are very mixed currents from all directions. At the bottom of the cage however on the hours around midnight a strong directional current going North can be seen. It is clear that the wind effects the current direction particularly on the surface as can be seen by the red coloration.
Since the sensor is attached to the cage bottom then the movements of the cage itself can be followed in this recording. It can be clearly seen that sensor rolls are correlated with the bottom currents as visualized in the direction results. No change in current speed at these times can be discerned. The surface mounted sensor did not give any further information only to confirm that currents up to 15-20 cm/sec could be observed in the bottom of the cage. One could speculate that these may be caused by swimming of the fish themselves and may form a vortex giving multi directional currents in the center of the cage.A marked difference in speed of the current as one progresses from the bottom to the top of the cage was observed indicating that when eggs are released they will be exposed to 10 -15 cm/ sec currents.
Data collection by data loggers attached to BFT. Description of BFT broodstock behaviour and vertical movements in response to various husbandry conditions

Although Data loggers as shown below were attached to the broodstock in Malta none were recovered.These types of operations had been successful previously but the broodstock cage design had been altered in 2014 such that no supporting rail was used making identification of floating object more difficult after their release and also large waves could swamp the surface and wash out any floating objects.

The report above on monitoring forms the basis for Deliverable 2.4 which was submitted to the Commission

WP 2.3 Induction (UDUS, MFF, MRRA/MAR, IOLR/NCM)

The data loggers (data shown above) and sea water temperatures were monitored and observed during May and June so as to determine the timing of the spawning induction. Previous spawning during EU funded DOTT projects have indicated that once the BFT are induced, they produce a more consistent number of eggs as the spawning of different females is coordinated. Once the broodstock get more acclimatized to their captive surroundings, they will then be able to spawn naturally without induction, but for the first few years this natural spawning is not as reliably consistent as an induced spawning. Since the broodstock was young and used for the first time this year for the TRANSDOTT project, ten of the thirty BFT were implanted on the 15th June when the average sea water temperature was around 22.7oC at the bottom of the cage and the surface average seawater temperature was 23.5oC. This is in accordance with the sea water temperature profile being consistently above 22oC.

WP 2.4 Egg Collection (MFF, MRRA/MAR)

The collaboration of two other Maltese tuna farms with Korean research teams was the main link that helped TRANSDOTT scientists to collect eggs successfully in Year 2 (2013) and during the extended period in 2014. In fact, the team from the Korea Marine Fish Hatchery Association, led by Mr Wan-Kyu Park contributed a Korean prototype “trawl net” egg collector.

For Year 2 of the project, it was decided by the MFF and MRRA/MAR team, in conjunction with UDUS and IOLR scientists that the traditional egg collector curtain was to be used in conjunction with the Korean “trawl net” egg collector during the spawning season. Firstly, the traditional egg collector was reinforced for attachment to the cage circumference and for a much better and durable resistance to currents and wind. This was installed in place on the 25th May however it was ripped again by the 19th May when Force 7 – 8 winds struck the area. The whole PVC egg collector was dismantled and taken for further reinforcements.

Consequently, as the spawning period window approached, plans to install the Korean egg collector with the traditional PVC egg collector were made and on the 13th June, everything was set up, albeit with the PVC egg collector mainly in use as a current guide to help the currents flow into the underwater egg collector net. Following studies during 2012, and after consultation with local fishermen and MFF divers, the Korean egg collector was installed outside the cage facing towards the prevailing current direction. The top end of the wide opening that was around 6m wide was attached to the cage PVC frame and the bottom end was 4m below and attached to the rope pillars of the net. The trawl net tapered towards the cod end 15m away where a 2m long egg collection bag would collect any accumulated eggs that are swept in by the currents. At the entry to this bag there was a larger mesh net that collected debris in the form of seaweed, jelly fish and larger materials. After the installation of the egg collectors on the 13th June, 2013, egg collection was attempted on the 14th and 15th June, however no eggs or a negligible amount of eggs were collected respectively.

The scientific team on location decided to wait for the surface sea temperature (SST) to rise before induction as it was still around 21°C and it was assumed that SST around 24°C would be a good period to induce. However, against expectations, fertilized eggs were first collected on the 16th June, 2013 when the SST was 21.5°C – this corresponded with the SST in Spain (around 21.0°C ) when natural spawning and egg collection was successful in previous years (SELFDOTT Final REPORT, 2012). For the EU funded DOTT project broodstock in Malta, this was a breakthrough and the first substantial amount of BFT eggs collected naturally from the MFF cage site, indicating an end to the various problems with egg collection that were encountered on this site over the years (SELFDOTT Final Report, 2012). Egg collection quantities increased to over 1.5 million eggs per day until the 24th June, 2013 during this first phase of the spawning period. At this stage the SST was hovering around 23.7°C. During the next couple of days, the SST dropped to below 23°C and spawning ceased on the 27th June, 2013. No eggs were collected even after the SST rose once again above 23°C.

It was decided to induce the broodstock with GnRHa implants and 10 fish were induced on the 1st

July, 2013 as the SST was predicted to rise over the next days. Spawning resumed on the 4th July, 2013 as the SST rose above 23°C, and opened the second phase of this 2013 BFT spawning period in Malta. Here it should be noted that the commercial farm to the north of the island did not induce their broodstock which had incidentally also stopped spawning with the drop in SST; and in fact their fish did not produce any more eggs; clearly indicating the positive effect of the GnRHa implants towards spawning stimulation. In this case, the drop in SST halted spawning and the implants were enough to re-stimulate spawning.

During this second phase of the spawning period, SST was around 23.8°C on average and a maximum of over 2.5 million eggs were collected on 6th July, 2013. This was the peak of the spawning season for 2013, and when the SST rose over 24°C after the 10th July, the numbers of eggs collected diminished and the last few eggs were collected on the 14th July, 2013.

Since the type of egg collector was new for 2013, egg collecting procedures were altered accordingly and standardized during the spawning season in 2013. Eggs were collected at sunrise at around 5:00am, from a rubber dinghy that then transports the eggs back to the adjacent working barge. For collection, the egg collection mesh bag is lifted out of the water into a bucket with around 5L of sea water. The mesh was opened and the content was released inside the bucket. When there was a large number of eggs, the seawater in the bucket was topped up to aorund 10L or the eggs were aplit into 2 buckets. Following this procedure, the debris net was emptied and rinsed before setting up the egg collector bag once again for the next day. Once the eggs were taken on board the workbarge, a pragmatic assessment was done and oxygen was added gently to the seawater for the next 40 minutes until the eggs were transported back to the egg packing facility.
Once the eggs reach the packing facility, the eggs are placed into a larger 100L round container where the debris that passed through the debris net out at sea in removed by the use of a coarse hand net (mesh size 1.1 – 1.3mm). At this stage, larger eggs belonging to foreign species, sea weed, and foreign species fish larvae and jellyfish medusa were removed. The eggs were left to settle at the surface of this container before being transferred to 2L measuring cylinders for egg volume measurements.

Previously, induction was thought to be optimal when the sea surface temperature was above 24oC; however spontaneous spawning in BFT broodstock in Spain showed that these fish are able to spawn spontaneously in sea water temperatures between 21 and 27oC (SELFDOTT Final Report, 2012). The implants were prepared by UDUS using the latest underwater speargun technology and implants.

As a result of no egg collection from the broodstock cage at the MFF site following the first induction (due to various reasons highlighted in the sections below), another induction was carried out on ten fish on the 23rd June when the sea surface water temperature was 25.7oC. Of the ten implanted BFT, 8 were not induced during the first induction procedure on the 15th June whilst two of the fish were induced for a second time.

When induction took place, the diver estimated the BFT body weight and this data revealed that the BFT were relatively small, averaging 41.5kg and 37.5kg for the first and second implantations, respectively. This is a relatively small average BW compared to previous BFT used for broodstock, but the decreased feed intake as the spawning period approached and the fact that the median size at sexual maturity of wild females corresponds to a body weight of 30 – 35 kg (Corriero et al., 2005) are strong indications that the BFT broodstock in this experiment should have spawned. However the reduced spawning biomass, coupled with the broken egg collector curtain and other egg collection problems did not yield any BFT eggs from this broodstock cage. The eggs were then left to settle inside the measuring cylinders and their volumes were carefully measured and added u. In the meantime, a sample was taken for examination under the microscope so that the fertilization rates were calculated. Another sample was taken for egg diameter and hatching rate measurements in the laboratory while yet another sample is frozen for parentage analysis at UDUS in Germany (see later). After the BFT eggs were confirmed to have good quality and the volumes were measured, the eggs chosen for use in experiments in Malta or for shipment to the partners were washed with sterile sea water (sand filtered, 10m, 1m and UV filtered) and prepared for packaging for transportation. As they were put aside, they were kept in a net with flowing seawater and oxygenated.

In the extension of the project, during the 2014 spawning season (Year 3), the same system for egg

collection was used, however the PVC egg collector curtains were not used as it was thought that they may disturb the sea water current flows entering the trawl-net egg collector. The spawning biomass had also increased by this time and the egg collector trawl net was installed on the cage during the first week of June when the SST was still 19.0°C. As reported earlier during Year 2 of this project and as previously reported in the SELFDOTT Project from the Spanish team (SELFDOTT Final Report, 2012) eggs were first collected naturally and without induction when the SST was 20.8°C (around 21.0°C). Eggs were collected thereafter over 35 days from 8th June, 2014 until the 12th July, 2014.The spawning SST's varied between 20.8C° and 24.2°C throughout the spawning period, with an average fertilization rate of 99.8% among buoyant eggs. The largest volume of eggs collected in one day was 6,220 ml; equivalent to over 6.2 million BFT eggs. In 2014, the egg collection data indicated that the best temperatures for egg collection were between 22.0°C and 24.0°C. Egg collection volumes decreased significantly from the 5th July onwards when the SST reached over 24.5°C. Following this decrease in egg collection volumes, 13 fish were implanted with GnRHa implants on the 7th July but this was to no avail as the spawning season was over at this stage.

During this final year, a total of over 40 million BFT eggs were collected throughout the spawning season, however, although the egg collection system is very efficient, further improvements are required for the future, especially with the need to eliminate alien species that are less than 1% but a great liability when larval rearing takes place. Suggestions to improve the system include closing the cod end of the egg collector only during the BFT spawning time window between midnight and 3am so that only BFT eggs are collected, however the ultimate egg collection will be when a land based facility will be constructed in Europe for the provision of BFT eggs during the spawning season, and further down the line for the supply of out of season eggs.

These results form part of the completion of Milestone MS 3.

WP 2.5 Egg Incubation and Shipment (MFF MRRA/MAR)

Viable fertilized eggs were collected from the MFF cage site as described in WP 2.4 above. The main challenge in BFT egg transportation was for the longer transit times experienced with the Israel shipments, when there was a risk of hatching of the eggs. For this reason it was extremely important to have the logistics planned out from the cargo handling company.

After egg volume measurement described above, the eggs were then transferred to a known volume of well oxygenated sterile sea water from where they were equally distributed by simple proportion according to the number of cardboard boxes required for shipment. The boxes were lined with polystyrene and contained a plastic transparent flexible cubitainer that was topped up to contain 12L of sterile sea water after the eggs are added. The temperature of the sea water was kept at around 22°C so as to ensure that the egg don't hatch during the shipment when the travel time was over 24 hours.

When the ambient spawning temperatures were above 22°C, the sea water was cooled before packing. A total of 150,000 blue-fin tuna eggs were packed per 12L volume of sterile sea water into a 20L volume cubitainer. The air layer was then injected with pure oxygen so as to maintain optimal oxygenation conditions throughout the shipment. The oxygen level of the sea water inside the box was at 30ppm when the eggs were stocked. Before injecting the cubitainer with pure oxygen, a data logger was placed inside the sea water so as to record the sea water temperature throughout transportation. Two 250ml ice packs were placed inside the box so as to maintain temperatures from increasing during transport in the hot summer months. Once everything was prepared, the boxes were closed and prepared for transportation to the local airport. Transportation labels were attached to all boxes indicating consignee and shipper addresses and contact phone numbers. The cargo handler who prepares all flight schedules and bookings was the best way to determine the most appropriate route to the destination country. In all a total of 8.5 million BFT eggs were shipped in 2013 and 26 million BFT eggs were shipped to hatcheries for experimentation during 2014.

Maternity pattern from the BFT eggs for the spawning days in 2014, Malta
The DNA extraction from the eggs of each of the 21 spawning days were amplified against the Dloop region primers in order to define the potential number of mothers that spawned this period.The

PCR products were sent for sequencing at Macrogen, Netherlands. The sequences were aligned using the BioEdit programme with the ClustalW Alignment method. On only 5 days did single mothers spawn. There are 14 mothers that spawned on more than one day during the spawning period. In Season 2014 it seems clear that early on in the season in the middle of June more females participated in a spawning event. Later on only single mother were seen. A spawning pause of 2 days was seen between spawning events for individual mothers. Handling trials on BFT using anesthetics and delivery systems were not carried due to lack of suitable fish and the closure of the project on the 30th Sept. 2014. Fish which are treated with anaesthetics cannot be resold and therefore would have represented an added cost to MFF who had already kept the brood stock before the beginning of the project and also for a further 6 months. The brood stock has also been maintained beyond the end of the project to help in the transition of TRANSDOTT to a commercial enterprise therefore Deliverable 2.6 was partially fore filled and submitted but milestone MS4 was achieved.
During the 2014 season great efforts were made to utilize the supply of egg provided by the MFF broodstock and the facilities of MRRA for basic science work carried out by scientists from UDUS, IOLR/NCM, MARC, and MFF. These involved the identification of Alien species and egg flotation rates.

Alien Species
These experiments were carried out in collaboration with MFF. The adult bluefin tuna in are kept in sea-cages where they spawn. These sea-cages are an open system. So the eggs of the foreign species can flow into the egg-collector brought by the currents. Different fish species may have an overlapping spawning time with Bluefin Tuna. The foreign species which commence spawning with Thunnus thynnus in June is Trachinus draco (greater weever) and on the other hand they may start earlier to spawn for examples Sarda sarda (atlantic bonito). Open-cage egg collecting systems do not collect only tuna eggs but foreign species as well. Three different methods for the identification of foreign eggs was used. The first one was using morphological characteristics. Using this method three different kinds of eggs could be identified. The first ones were T. thynnus eggs). An important point is, that we do not know whether this eggs are only tuna eggs. They have a spherical shape and an average diameter of 1mm. In addition the chorion is smooth and unornamented and inside the eggs there is a clearly visible oil droplet. Secondly the small eggs, they are visually smaller than the T. thynnus eggs and have a clear chorion. The last type of egg contains an ornamented chorion. They are the largest and oil droplets not exist. Furthermore there is a difference in the hatching time, whereas the T. thynnus eggs and the small eggs need around 34 hours to hatch, the ornamented eggs need more than 72 hours. To summarise the main points of the morphological characteristics, we can say, that we had three kinds of eggs. The differences between them are first the size and second the structure of the chorion.
The second method used was the Proteome-analysis with the gel electrophoresis. It is one of the methods to identify the foreign species. With this you can check proteins of the eggs and the larvae as well. The results of this method gave a distinction between muscle and eggs of the relevant species.The conclusion of this method is, that we had three different kinds of samples with three different results, this means we can't compare BFT Eggs with BFT and also foreign species muscle samples. So we only can say there is a different, if we compare eggs with eggs, but we can't get a clear identify of the foreign species.
With the last method, the DNA–analysis you can make a clear identification. The DNA-analysis included three steps. It starts with the DNA extraction of the samples. A polymerase chain reaction with the d-loop (displacement loop) of the T. Thynnus or the CoxI (Cyclooxygenase) of the Scombridae amplifies this DNA. The last step is the sequencing. The four identified species are only preliminary results. The first two individuals are Argyrosomus japanicus (Japanese Meagre) and the the Sarda sarda (Atlantico bonito). Also Scomber colias (Atlantico chub mackerel) and the Trachinus draco (Greater weever) could be identified as foreign species.

Conclusion and recommendations
It is difficult to identify the foreign eggs and larvae rapidly using morphological methodology and molecular techniques are expensive and time consuming but yield reproducible results. The main threats are that the foreign species are more resistant to environmental influences and develop faster. Furthermore a high mortality causes a shift in tuna/foreign species ratio, so there are more foreign species at the end of incubation during larval rearing. To solve this problem, it is important to improve the egg-collection and the egg-collector as well. Moreover it can help to think about the opening time of the egg-collector which should be keyed to the spawning times of the tuna which are

thought to be during darkness between 02:00 and 05:00 at night.

Determination of egg rising rates and their Influence on Egg collection
Using a specially constructed flotation chamber (this method is originally in Coombs 1981; modified by Tanaka 1990) direct measurements were made on freshly collected eggs to ascertain their rising rates.The mean results from 11 egg batched shown in the table above indicated a mean rising speed of 2.7cm/secs. If these are then used together with the current measurement data an estimate of the influence on egg distribution when collecting in open sea cages can be determined. From calculations made it was concluded that eggs which were spawned at a depth of 10m from the surface would reach the surface after approximately 60 minutes if no currents were present. However in Malta around the broodstock cages currents of >10 cm/sec are often observed meaning that eggs would reach the surface over 350m from the cage.

WP3 Development of egg, larval rearing and feeding strategies

WP 3.1. Improvement of Embryo's First Critical Days

Effect of delayed hatching time for BFT eggs during prolonged transportation on larval quality
Most work on this was carried out during 2013 and 2014 after the failed egg collection attempt in Malta in 2012. The eggs sent from MFF to IOLR and ARDAG (both in Eilat, Israel) took between 25-29 h from collection at sea to arrival in Eilat during the 2013 and 2014 seasons, which were the longest transit times among all the partners. Nevertheless, the hatching percent of total eggs and only live eggs (80.6%, 87.0, respectively) as well as larval survival to 1 dph (79.5%) at the IOLR in 2014 of the project were very good and similar to hatching rates calculated at MFF where the spawning broodstock is off-shore. On the other hand, these values were surprisingly higher than ARDAG and considerably greater than FB. The hatching and survival values of IOLR were calculated by placing a live egg in each of 12 (5 ml) wells/plastic plate where three plastic plates were stocked / cubitainer. The plates were covered and placed in temperature controlled incubators until hatching where the newly hatched larvae were counted. The next day the larvae were recounted to calculate survival to 1 dph. This approach was similar to that of ARDAG except that their plates were covered and floated on the tank's water surface. P5. FB received their eggs after 28 h, acclimated them to hatchery conditions (20-24 #C), removed dead eggs and hatched larvae and then later estimated the number of hatched YSL in several samples. However, there may be a number of sources of error in estimating the number of eggs by volume as well as the dead eggs resulting from transport. Temperature fluctuation may have been greater during transit as well. Taken together, the results suggest that it is feasible to ship eggs for up to 30 h without excessive egg mortality at hatching provided that temperature shock is minimized in route. For the future, a standardized protocol for calculating percent hatching should be adopted.

Protocol to introduce transported eggs to hatchery conditions in terms of the rate of pH, oxygen and temperature acclimation

Both IOLR and ARDAG practiced very similar protocols when receiving eggs from Malta which were in transit from 25-29 h. Upon opening the cubitainer, which was placed floating in one of the tanks (temperature about 23 #C) of the experimental system, any odor emanating from the bag was noted and the dissolved oxygen (DO), temperature and pH were measured and recorded. In all cases the DO was well above 200% and the temperature ranged from 21-24 #C. The cubitainer was left to float in the tank until temperature equilibrium was reached. Frequently, the pH ranged from 6.5-7.1 (from released CO2 acidifying the water). This was raised to about 7.7 through dripping 1 N NaOH together with aeration for mixing, a process that took about 15 min. This is relatively rapid for this process but to extend the period significantly might mean the eggs would hatch before acclimation and stocking in the experimental system (about 30 eggs / l). After pH normalization, the eggs were then placed into a 15 l bucket with aeration and samples taken, using a 10 ml pipette, to count live, dead and fertilized eggs as well as recording their developmental stage. The hatching and survival values at IOLR were calculated by placing a live egg in each of 12 (5 ml) wells/plastic plate where three plastic plates were stocked / cubitainer (36 replicates). The plates were covered and placed in 22 #C controlled incubators until hatching where the newly hatched larvae were counted. At the end of the next day the remaining live larvae were counted to calculate survival at 1 dph.
Similarly FB measured temperature, oxygen and pH upon opening the cubitainers. The eggs were

transferred to a 40 L bucket where hatched larvae and alien species larvae were removed. Incubator water was slowly added, raising the temperature 1 #C/h to acclimate the eggs to hatchery tank conditions. When tank and cubitainers parameters were similar, the eggs were transferred to the incubators.

WP 3.2 Classic Larval Rearing

Protocols to enrich the live food with DHA and taurine based on BFT larval survival, growth and weaning success

The taurine enrichment treatments resulted in three significantly ($P<0.05$) different rotifer taurine levels (1.1, 4.4, 6.4 mg/g DW) while there was a significant ($P<0.05$) rotifer taurine dose response in larval whole body taurine levels (4, 10.4, 15.9 mg/g DW).
There was a significant ($P<0.05$) dietary taurine effect on larval survival at the end of the 15 dph experiment where fish fed the moderate taurine diet (4.4 mg taurine/g DW rotifer) represented 55.1% of the remaining larvae (755 fish) while the control (1.1 mg taurine/g DW rotifer) and high (6.4 mg taurine/g DW rotifer) taurine treatments represented 17.1 (235) and 27.8% (381), respectively, of remaining fish. Higher survival can frequently mean smaller fish. Although the moderate taurine diet (4.4 mg taurine/g DW) demonstrated an increase in DW over the control and high taurine diet (1.1 and 6.4 mg taurine/g DW, respectively), it was not significant ($P>0.05$). However, if the tank biomass (DW x no. of surviving individuals) is calculated, the moderate taurine (4.4 mg taurine/g DW) fish show a significantly higher tank biomass compared to the other treatments.
In order to understand the mechanism explaining the survival and biomass promoting effect of taurine, the fatty acid profiles of larvae fed the different rotifer treatments were examined. As taurine is the main precursor for bile salt synthesis in fish, increased levels of this nutrient might improve the emulsification and absorption of dietary lipids in the lumen of the digestive tract resulting in higher levels of the essential n-3 long chain polyunsaturated fatty acids; DHA and EPA. However, no difference in (a) DHA, (b) EPA or their (c) DHA/EPA ratio was observed in BFT larvae from the different treatments suggesting that bile salt synthesis was not a factor affecting improved survival.
Taurine plays a role in protecting the outer membranes of photoreceptors from photo-damage, which may impact on larva's hunting efficiency and prey acquisition. Consequently, we studied if a correlation existed between dietary taurine and taurine accumulation in different sections of the retinal photoreceptors (rods and cones) using an immunohistochemistry technique and in the outer and inner membranes of the photoreceptors examined there was a significant ($P<0.05$) dietary taurine dose-dependent response on taurine accumulation. However, despite a significant increased accumulation of taurine in these retinal sections, as a function of taurine in the diet, no change in larval rotifer consumption (mastaxes/larva) rate was found, meaning that the taurine-driven enhanced survival and tank biomass was not due to improved vision and prey capture. The physiological mechanism linking larval performance with dietary taurine may be more associated with muscle development or another pathway that was not investigated in this study.
In summary, these studies recommend enriching rotifers with (1) a high DHA enrichment preparation (32%) and adding moderate taurine levels (400 mg/l) to the enrichment medium in order to produce rotifers having 4.4 mg taurine/g DW (resulting in 10.4 mg taurine/g DW larva) which will markedly improve survival and tank biomass during early (2-14 dph) BFT larval culture.

The effect of rearing salinity on larval prey consumption, growth, survival and incidence of urinary crystals

There was a significant ($P<0.05$) increase in prey consumption with larval age in both salinity treatments reaching over 40 rotifers/larva/90 min by 12 dph. Moreover, the prey consumption rates between the treatments did not differ significantly ($P>0.05$) from each other (apart from one instance at 6 dph) suggesting that salinity did not affect hunting and ingestion success. Similarly, larval length increased significantly ($P<0.05$) with larval age in both salinity treatments but here too there were no differences ($P>0.05$) in growth (length) between salinity level treatments.
On the other hand, fish reared in 40 ‰ survived remarkably better ($P=0.014$) than larvae grown in 30 ‰ seawater. In fact, the lower salinity was ultimately lethal to almost all BFT larvae. Nevertheless, fish reared at 40 ‰ had increasingly larger ($P<0.05$) urinary calculi with age while fish at the lower salinity exhibited no calculi (apart from a small one at 12 dph).
Despite the relatively large salinity difference BFT larvae from the control 40 ‰ and treatment 30 ‰

ingested prey and grew very similarly. On the other hand, fish reared at 30 ‰ showed much poorer survival, indicating the factor increasing mortality was more associated with osmoregulatory challenge rather than the incidence of calculi in the urinary tract. Virtually no fish in the 30 ‰ demonstrated the presence of urinary calculi while fish in the 40 ‰ exhibited a much high incidence, which grew with larval age. Future studies should test a narrow salinity range less than the ambient 40 ‰ (35-36 ‰) to verify if both mortality and the presence of urinary calculi can be markedly reduced.

Evaluation of various weaning diets on reducing the dependence on feeding BFT larvae yolk-sac pre-larvae

Due to a lack of sea bream yolk-sac larvae at the Malta hatchery, it was imperative that MFF test various weaning diet alternatives, which are regularly used to rear larvae of other fish species in aquaculture. The alternatives to yolk-sac larvae that have been tested at MAR are as follows:

• Frozen bream eggs
• Frozen meagre larvae
• Frozen mysis
• Adult artemia
• Mackerel flakes
• Live wild sardine larvae/fry
• Dry feed

In the absence of yolk-sac larvae, the above feeds were offered to the BFT on a daily basis from 15 days post hatching at intervals of 60-90 minutes. The BFT demonstrated a positive initial response to all the alternatives to yolk-sac larvae, in terms of feeding behaviour, but would only taste and reject the alternative feeds. On the occasions that yolk-sac larvae were available and offered to the BFT larvae, there was an immediate feeding response. The only rearing tanks that had BFT survivors older than 40 days post hatch were in tanks that had received some level of yolk-sac larvae. These were the only BFT larvae that were successfully weaned onto dry feed.

BFT eggs were transferred to the hatchery facilities of FB and the larvae were reared in 25 m3 tanks in a RAS system using seawater (37 ‰) at a temperature of 23-24 #C. Exogenous feeding larvae (2 dph) were fed rotifers (Brachionus plicatilis) previously enriched with Ori-Green (Skretting, Stavanger, Norway) and exposed to a light/dark photoperiod of 16/8. Mesocosm reared copepods (mixture of Acartia sp.-95% and Trigriopus sp.-5%) were fed to the fish larvae from 3-18 dph. Rotifers and copepods were supplied twice a day to maintain a minimum density of 10 prey / ml in the rearing tanks. The weaning onto a commercial diet (Skretting Tuna Starter; 300–500 of particle diameter) started at 16 dph. Microalgae (Tetraselmis chuii) were also provided from first feeding up to 15 dph. The larvae were sampled (anesthetized with ethyl-4-aminobenzoate) at 0, 1, 2, 3, 4, 6, 11, 17, 18, 25 27, and 28 dph. Total length (TL), yolk and oil volume and mouth size were determined as well as histological analysis of the organogenesis of the digestive system, visual system and other structures (Dr. Manuel Yúfera et al. in Aquaculture 426-427 (2014), 126-137: “Organogenesis of digestive system, visual system and other structures in Atlantic Bluefin tuna (Thunnus thynnus) larvae reared with copepods in mesocosm system”). In summary, the study showed that the digestive system, sensory and visual structures, thyroid gland, swim bladder, and the kidney and heart differentiate very early during the larval ontogeny of this species, and was fully functional by 17–18 dph under FB rearing conditions. The results show that at day 16 dph tuna larvae had dry feed in the stomach, but they are still dependent on YSL to successfully transit this developmental phase and will not survive without YSL supplementation to their diet.

The period of weaning at ARDAG facility began when the feeding of rotifers was no longer sufficient. During this period, predation or cannibalism became more and more frequent and mortality rate increased dramatically even though the stomach contained dozens of rotifers. In addition, the larvae showed very little interest in Artemia during this period. The feeding of dry Skretting weaning diet was not successful under the conditions at ARDAG and limited success was achieved with Skretting 800-1500 mm diet when hydrated using the “water spray” technique. Larvae that survived were weaned successfully by 30 dph. In addition the following alternatives were tested: Artemia (nauplii and adults), dried blood worms and gilthead sea bream eggs. During these tests, a significant response was observed only when the BFT larvae were offered gilthead sea bream YSL and post-larvae. Overall, ARDAG's experience indicated that between 15 to 30 dph, the weaning diet was insufficient and an additional food source is required to increase the survival rate of the BFT larvae. This may be related to the nutritional value or attractiveness of the weaning feed compared with live feed that also triggers the predation instinct of the larvae or it may be due to inadequate rearing conditions for BFT larvae.

WP 3.3 Alternative Feeds for BFT Larvae; Copepods (FB, MRRA/MAR)

Description of co-feeding of BFT larvae with rotifers and copepods

Tuna larvae hatch at 24-36 hours after spawn. The first two or three days the larvae fed on endogenous reservoir (yolk sack), the mouth and anus are still closed and the eyes are in development. The first food supplied to the tuna larvae consists of rotifers and the smallest stages of copepod nauplii (40-70 microns). At 9 dph the copepod smallest stage size is mixed with the second stage size. At 12 dph the rotifers feeding is stopped and the supply of sea bream YSL begins. At day 22 dph only copepod instar sizes 1 and 2, and sea bream YSL are fed. On day 25 dph the weaning phase begins. Copepod large sizes and sea bream YSL are still supplied for 7 days. On 32 dph tunas are weaned to dry food and no more live food is given.

Description of early weaning of BFT larvae at YSL feeding stage

For tuna larvae to develop completely there is a dependence on the availability of YSL. The feeding of gilthead sea bream YSL, which is widely grown in European aquaculture, has been shown to be effective food in BFT larviculture. In addition to stimulating normal feeding behaviour, the feeding of YSL appears to reduce cannibalism as well. On the other hand, the supply of YSL necessary to feed BFT post larvae is massive. In order for a commercial hatchery to produce thousands of tuna fingerlings, around 500.000 sea bream YSL is necessary to feed one thousand tuna larvae/ day until 25 dph. This means, there must be a production of at least 2 to 4 million sea bream YSL to feed 4 batches of 1.000 tuna larvae at the same time.
In order to reduce the dependence of YSL, FB performed in 2014 an early weaning trial. The organogenesis of the digestive tract in fish from this trial was described in a recent paper published by Yufera et al. (2014) concluding that 17 dph larvae have the capacity to be weaned (described earlier in this report). FB installed 4 automatic feeders in one tuna culture where no sea bream YSL were supplied. At 16 dph tuna larvae had dry food in the stomach indicating that weaning can be achieved at this developmental stage but at present the larvae still need to be fed YSL to pass the weaning stage with adequate survival rates. Further work must be performed in order to achieve early weaning with tuna larvae culture.

WP 3.4 Mesocosm Larval Rearing (MFF, IOLR/NCM, MRRA/MAR, PAN)

Evaluation of co-feeding rotifers and selected mesocosm species (copepods or ciliates) versus rotifers and mesocosm water, in terms of BFT larval growth, development and survival

The experimental design consisted of eight 1.5 m3 l conical cylindrical tanks fed by ambient (40 ‰) seawater that was sand (10 µm) and activated charcoal-filtered as well as UV-treated. The tanks were exposed to a light intensity of 500 lux and a light: dark photoperiod of 16:8. The tanks were stocked with 20 BFT eggs/l and were “greened” with the micro-algae Nannochloropsis oculata (500x103 cells/ml) when the larvae began feeding at 2 dph. This allowed the testing of the control treatment (10 rotifers/ml) and co-feeding treatment (7 rotifers/ml + 30 ciliates/ml) in replicates of 4 tanks/treatment until 14 dph. The combination of rotifers and ciliates in the co-feeding treatment was calculated to equal the prey biomass of the rotifer control and was fed to the larvae from 2-6 dph. After this, these fish were fed the control only- rotifer treatment. However, overall survival in this study was poor and insufficient numbers of larvae could be sampled for dry weight at the end of 14 dph. Nevertheless, the results presented here indicate a significant growth advantage (larval length) and a tendency for improved survival when co-feeding rotifers and a Mesocosm prey during first feeding.
An early significant ($P<0.05$) effect of co-feeding rotifers and ciliates on prey acquisition was shown in 3 and 4 dph larvae. However after this point, there is no significant ($P>0.05$) difference in rotifer feeding between treatments at any age. In fact, there is a tendency for the control fish to consume more rotifers than larvae in the co-feeding treatment. Despite the fact the mouth gape of BFT larvae can easily accommodate rotifers of this size, the data suggests that consuming the smaller ciliates at first feeding provided some nutrient and/or energy advantage to the larvae to successfully hunt more rotifers. However, by 5 dph the fish may have changed their size preference to the larger prey. Consuming more rotifers in the co-feeding treatment may have given these larvae a growth

advantage that was significantly (P<0.05) expressed later on in development. Fish co-fed rotifers and ciliates from 2-6 dph were markedly (P<0.05) longer at 2 and 3 dph. Interestingly, these fish grew better (P<0.05) than the control larvae at 12 and 14 dph as well. Noteworthy that this was considerably after the co-feeding had stopped and the only-rotifer feeding had begun. Although there was a trend of better survival in the co-feeding treatment, it was not significant (P>0.05). On the other hand, the overall survival of fish in this study was very poor resulting in high variability among tanks and reducing the possibility of statistical significance in this parameter.
Overall, this study suggests a growth and possibly survival advantage in the co-feeding of rotifers and ciliates during the first days of feeding. It also underscores the importance of early prey consumption on larval performance later on in development. However, whether the smaller size of ciliates compared to rotifers facilitated consumption at the start of exogenous feeding and/or that the critical nutrients in ciliates promoted growth and survival still must be elucidated in future studies.

Description of improvements in classic and Mesocosm larval rearing BFT

Mesocosm
Atlantic Bluefin Tuna (T. thynnus) eggs were placed in a two bigger volume of water (200,000 L) to simulate “natural hatching and rearing conditions” at MFF facility in Malta. An aeration system was built up for each tank separately and 2.5 million tuna eggs were placed in a starting volume of 50,000 L of natural seawater. The water was coloured green with concentrated Chlorella. After 24 hours around 95% of the eggs hatched. And the water level was gradually increased over several days until maximum volume was reached. The BFT larvae were fed with Rotifers and other ciliates (E. vannus) and later on with enriched brine shrimps (A. salina). The rotifers which were put as food for the Tuna larvae in the Mesocosm tanks was also fed with several kinds of algae (Chlorella spec., Nannochloropsis gaditana, Tetraselmis cordiformis, Isocrysis galbana).Techniques have been developed to allow greater control over water flow, temperature, salinity and aeration. nDue to the size of the tanks used in Malta (200m3), it has proven very difficult to clean the bottom, which is critical for maintaining high water quality. Different methods of siphoning were tested, however an effective method is still to be developed. Further improvements to water quality will be made using higher flow rates and by adding oxygen to the water as soon as the dry feed is offered. Furthermore, the use of a robot siphon is suggested. Apart from rotifer proliferation in the Mesocosm BFT larval rearing tank, another rotifer stock was cultured adjacent to the Mesocosm tank so that a back-up rotifer supply was available and rotifers were added when required. The addition of nutrients to promote the growth of live algae (Isochrysis and Tetraselmis) has become more controlled, by monitoring the levels of key nutrients in the water.
A major problem in the BFT larval rearing from eggs collected in sea cages has been the presence of alien species that are collected with the BFT eggs. There is a now a greater understanding of what needs to be done in order limit the number of invasive species being added to the Mesocosm tanks. This includes separation of larger sized eggs by mesh net plus procedures that have been developed in order to catch/remove any invasive species that may be found in the Mesocosm tanks.
These large volume trials proved to be very effective, in fact around 10,000 BFT larvae survived at 24dph even though the larvae were only fed on traces of sea bream yolk-sac larvae due to lack of availability. This lack of yolk-sac larvae caused major problems as the larvae were not feeding well and the numbers dropped to around 500 by 30dph, when the water quality parameters could not be maintained due to overfeeding and insufficient water exchange. This emphasises the need for a provision of live yolk-sac larvae for feeding the BFT juveniles until they can be weaned onto the dry feed.

Rearing Protocol:
• Improved survival has been demonstrated by using a longer initial photoperiod, 18-24hours, which is then reduced to 12 hours at 8 dph
• Improved survival has been demonstrated by using a lower initial temperature, 23.5-24oC, which was then increased to 24-24.5oC from 10 dph
• It was beneficial to feed the larvae with rotifers at a density of 15/ml or higher, and at a rate of approximately 450 rotifers per L per BFT larvae. In addition, following on from the work at IOLR, all rotifers were enriched with 400 ppm taurine.
• The larvae of many species of marine finfish are reared at salinity lower to that of the ambient, resulting in better survival and swim bladder inflation. When BFT larvae were reared at lower salinities it was found to have a negative effect on survival.

Invasive Species:
• There is a now a greater understanding of what needs to be done in order limit the number of invasive species being added to the Mesocosm tanks. Procedures have also been developed in order to catch/remove any invasive species that may be found in the classic larvae rearing tanks.

Ocean Acidification Effects on Bluefin Tuna Larvae Thunnus thynnus

Within the remit of the EU FP7 TRANSDOTT project and the German BIOACID project it was possible to combine techniques established to investigate the influence of Ocean Acidification (OA) on eggs and larval stages of marine fish with broodstock management of a top Oceanic Predator, the Bluefin Tuna, Thunnus thynnus.
Over the past few years it has become viable to hold broodstock of the ABFT in captivity and induce them to spawn thus providing fertilised eggs in large number for aquaculture. Since it is not possible to hold fully adult BFT in incubation conditions as yet the possible effect on early life stages was investigated using both control and simulated "Oceans of the Future" levels of $CO_2$ of 390 µatm and 1200 µatm. Freshly fertilised BFT eggs were hatched at stocking densities between 15,000 – 20,000 eggs per 80L flow-through tanks which were fed from either an incubation header tank (IKS Isostar pH-Stat) set to pH 7.5 (Treatment) or directly from the main seawater supply pH 8.1 (Control).Temperature and pH were monitored in both test and control tanks with an IKS system. Hatching rates were determined 34 hours after spawning. Eight replicate tanks were used for both control and treatment incubations and sub sampling took place every third day to monitor survival and growth rates of larvae (Days Post Hatching, DPH). An air lift was used to provide circulation within the tanks. On day three post hatching, Nannochloropsis was added together with enriched rotifers. On day seven post hatching larvae were checked for calcinosis, a deposition of calcium phosphate in the renal tract that ended up killing them. The experiment was repeated seven times. It started with eggs from the 06.08.2014 to eggs from the 07.12.2014. All results you can see here are preliminary results from batch 5 (eggs from the 06.29.2014). There are no significant differences in these results. Especially growth rates and survival rates were similar in control and treatment tanks. The hatching rates and the occurrence of calcinosis were minimal increased in the presence of high $CO_2$.

WP4.1 Fingerling Production (ARDAG, MFF, FB, IOLR/NCM, MRRA/MAR, PAN, SARC)

All hatcheries within the framework of the TRANSDOTT project were able to stock BFT eggs during the spawning season of year 2 (2013) and in the extended period during year 3 (2014).

In Year 2, BFT larvae trials in Malta did not yield any fingerlings due to the very small size of the hatchery, plus the presence of alien species that preyed upon the BFT larvae as discussed earlier in WP3. In the case of ARDAG, there was firstly a significant improvement in egg collection and shipping parameters during 2013 which contributed to a larger quantity and better quality of eggs available for larval rearing. During this second year, ARDAG produced a total of 40 fingerlings that were weaned to dry feed supplied by Skretting by 40 DAH. The mortality rate of the juveniles increased dramatically at 20 DAH due to cannibalism, malnutrition (unsuccessful weaning), and tank collisions. At 50 DAH, the 18 remaining juveniles were fed with 1500 µm dry feed as well as with 0.5 g gilthead sea bream (S. aurata) fingerlings. The last surviving fingerling weighed 84 g at 85 DAH.
In the case of FB, there were 1,120 weaned BFT juveniles at 30 DAH of which 800 were kept for feeding trials and 100 were transferred to the large outdoor tanks in Year 2 of the project. These produced 533 fingerlings at 10g. These BFT showed phenomenal growth to 2kg in four months in outdoor tanks. The main challenges during the weaning and grow-out of the juveniles was due to alien species during the earlier stages and collisions with the sides of the tanks after weaning. Survival was always shown to be better when the juveniles were introduced into larger tanks.

In Malta during 2014 (Year 3), there were 500 surviving BFT at 30 DAH in a large 200m3 rearing tank, however due to a lack of yolk-sac larvae for feed, the BFT were fed the Skretting dry diets mixed with moist pellets and some chopped sardines which led to a water quality degeneration with no survivors. At the FB, higher flows and better bottom cleaning, accompanied with a more efficient

yolk-sac larvae feeding protocol will yield thousands of BFT juveniles from this system. For ARDAG in Israel, there were lower survival rates and fewer BFT juveniles at 30 DAH than in Year 2 due to high mortality during the first ten DAH and insufficient number of yolk-sac larvae for feed. These juveniles were fed mainly on 500-800 µm followed by 1250-1500 µm dry feed by Skretting. In the case of Malta and ARDAG, while several fingerlings were produced during Year 2 and 3 of the project, the numbers of surviving juveniles was considered too low to attempt to transport the BFT fingerlings to the sea cages.

In FB in Spain, 1.8 million BFT larvae were seeded into larval rearing tanks in 2014, as opposed to the 810,000 BFT larvae seeded in Year 2. From these BFT larvae, there were very promising survival rates during the copepod feeding stages. However in 2014, as opposed to Year 2, there were problems with sea bream yolk-sac larvae supply from a nearby sea bream hatchery. For this reason, the yolk-sac larvae were fed to only one batch of BFT juveniles resulting in good survival in this batch. Despite the Yolk-sac larvae problems, the results were nevertheless very promising with 550 surviving BFT juveniles at 10g. The weaning diets produced by SARC were based on previous successful work carried out during the SELFDOTT Project (Selfdott Final Report, 2012) and FB reported a very good feeding performance on these diets. Growth was not sufficient in Malta and Israel so the conditions fior weaning were not optimum and so the juvenile BFT did not respond well to these diets.

Overall, the results over Year 2 and the extension year yielded a lot of data that will be used for BFT juvenile production by marine hatcheries in the coming years. Following the production of BFT larvae, the yolk-sac larvae feeding stage is still essential between 12 DAH and 32 DAH, so it is strongly advised that hatcheries that aim to produce BFT juveniles should have an adequate supply of sea bream or other similar marine species yolk-sac larvae as a transition diet until the BFT are weaned onto the dry feed.

The IEO facilities in Mazzaron, Spain also recieved BFT eggs from the Malta broodstock, and their method with the combination feeidng of rotifers and copepods, followed by sea bream yolk-sac larvae was successful and over 5,000 BFT juveniles were transferred to the cages. Another hatchery, Kilic in Turkey, also produced thousands of juveniles for ongrowing trials, following feeding of rotifers and a very high amount of sea bream yolksac larvae.

WP 4.2 Fingerling Transportation (MRRA/MAR, MFF, UDUS, IOLR/NCM, ARDAG, FB, PAN)

During Year 2 in FB, Spain, from 1,000 weaned BFT juveniles, around 100 individuals were transferred to a large circular tank (3,000m3) for ongrowing without any mortality. These fish reached 2 kg in 4 months so the growth was phenomenal. In the following year, from 1,300 weaned BFT, two batches were transferred to large outdoor tanks (100 at 41DAH and 123 BFT at 35 DAH, respectively) whereas another 105 individuals were transported to cages 15 hours drive away from the FB hatchery at 45 DAH.

The batches that were reared in the large tanks were fed on the SARC diet and FB moist diet. Both groups showed very good feeding behaviour and growth. The group that was transferred to the cages following a 15 hour drive had a 20% survival. FB used two different types of methods to transfer tuna fingerlings.
1. The first method was using transparent plastic bags where 1-5 tuna fingerlings were collected to be transferred.
2. The second method was using a bucket with an internal foam layer that covered the internal surface in order to avoid collisions with the walls.
There was no difference reported in terms of survival for both methods that resulted in a 100% survival when transferring from the weaning tank to the larger tanks or to the transportation tank. The “plastic bag technique” is becoming the more popular method for transfer of tuna fingerlings to tanks; however the success of the second method indicates that other options can be available and should be investigated in future studies. The transportation tank used by the IEO facilities in Mazzaron, Spain was circular, and white with internal black stripes. These stripes help the BFT juveniles to distinguish the perimeter of the tank and with this system, collisions are significantly reduced. In fact, the BFT spawning tank that has been constructed in Spain is striped to aid the BFT broodstock to identify the sides of the tank easily. In the case of ARDAG, a total of 21 fingerlings

were transferred from IOLR\NCM hatchery to ARDAG (29, 45, and 58 DAH) and a total of 20 fingerlings were transferred from the larval rearing tanks to larger tanks in ARDAG (43 and 63 DAH). In both cases, the fingerlings were handled and carried in transparent plastic bags to minimize handling damage and avoid wall impact. The survival rate employing this method of transfer was 80%. The low number of surviving fingerlings did not allow testing the effects of anesthetics and stocking density on survival. Due to the lack of surviving fingerlings for experimentation in Malta, no fingerlings were transferred to larger tanks or to cages at sea for on-growing.

WP 4.3 Fingerling to Grow-out (MFF, PAN, IOLR/NCM, ARDAG, FB, PAN, SARC)

Among the main partners of the TRANSDOTT consortium, only FB managed to transfer juveniles for ongrowing. However, IEO in Mazzaron, Spain also transferred juveniles to cages for ongrowing. Another partner, Kilic from Turkey who shared their results with the TRANSDOTT consortium during the Final Meeting in Düsseldorf, Germany produced thousands of BFT juveniles for ongrowing.

Previous studies have shown that the biggest cause of mortality in juvenile BFT during the grow-out phases is due to tank side-wall collisions or net-cage collisions (SELFDOTT Final Report, 2012). Although in ARDAG grow-out trials were not conducted in sea cages due to the insufficient number of fingerlings, the remaining fingerlings were kept in tanks at the hatchery for the grow-out phase. To lower the incidents of tank collisions during Year 3, a 100-200 lux "night light" was kept on for the duration of the trial. In addition, the grow-out tanks were lined with plastic sheets, at a safe distance from the walls, to absorb the impact in the occurrence of a collision. These modifications may have contributed to the fact that fewer incidents of collision related mortality were observed in Year 3 in comparison to Year 2. Some other methods of prevention have been successful, for example as mentioned earlier, by painting stripes on the sides of the rearing tanks or net cages or even by placing a very fine mesh net that “protects” the juveniles from hitting the sides. It has been noticed, among the larval rearing partners in Malta, Israel, Spain and even Turkey that it is best to transfer the fingerlings to the cages as early as possible after their complete weaning onto the dry feed.

In the case of FB in Spain, the BFT grew to over 2.5kg by 160 DAH. Furthermore, improvements in the number of weaned juveniles produced over the 3 years of the TRANSDOTT Project indicate that from Year 1 to Year 3 the number of BFT juveniles increased from around 200 weaned BFT in 2012 to around 1,000 in 2013 and 1,300 in 2014. At the time of writing there are still survivors from the 2014 spawning in the outdoor tanks in Spain with a weight of 2-2.5 kg. The survival from seeded larvae decreased from 2013 to 2014 due to problems encountered in sea bream yolk-sac larvae supply, indicating the importance of this step in the production of BFT juveniles.

Throughout the years during the TRANSDOTT Project, the improvements in BFT juvenile production techniques were phenomenal, following up from the advances that were already gained during the previous SELFDOTT project (SELFDOTT Final Report, 2012). During the SELFDOTT Project, BFT juvenile production on a larger scale was only carried out at IEO in Mazzaron in Spain where huge numbers of BFT eggs were produced. The TRANSDOTT Project, with BFT eggs produced in Malta, had a much wider distribution in Malta, Spain and Israel – with interest shown but non-partners for BFT eggs and collaboration. In fact, high numbers of juvenile BFT were produced for on growing in 2 locations in Spain (FB and IEO) and in Turkey whereas Malta and Israel also contributed to an increase in the numbers of weaned BFT.

Results from all partners indicate that the future for BFT aquaculture is very promising with MFF in Malta and IEO in Spain committing to independent BFT egg production for the coming years, plus the construction of a Land Based Facility for BFT broodstock in Spain. Larval production techniques, especially with the development of techniques for rearing copepods for larval rearing, and with the feeding of marine yolk-sac larvae prior to dry feed introduction seem to be very important steps in the production of BFT juveniles.

Results Comparison with TRANSDOTT Projections in Description of Work Table 1:

SELFDOTT=Broodstock maintenance and egg production: Two broodstocks have been used in Spain and Malta with differing success rates for egg production. Consistency and no disturbance of

the broodstock during the season as well as enhanced diets to improve GSI values. Seawater temperature monitoring carried out and used to assist in decision making for spawning season determination.
TRANSDOTT =Broodstock will be limited to Malta but with contingency plans and MOU with another tuna farm in Malta for egg collection (see later). Supplemented diets started early without disturbance to the fish as from 3 months prior to spawning. Sex determination and DNA finger-printing within the first year which was not carried out in SELFDOTT. Environmental monitoring as in SELFDOTT with lower sensor number and geared to commercial time frame and costs with 3 point monitoring but with window of induction opportunity determination.
COMMNENT: All successfully carried out and implemented.
SELFDOTT= Broodstock induction was carried out twice in Spain (afterwards spontaneous spawning occurred twice) In Malta induction was carried out four times with standard implants provided by HCMR.
TRANSDOTT = Broodstock induction in Malta using an improved delivery system using only titanium carriers and laser sighting system with a pneumatic adjustable pressure spear gun. New "user friendly" GnRHa implants supplied by UDUS at lower cost and higher user flexibility with optional numbers of implants during the project.
COMMENT: All successfully carried out and implemented.
SELFDOTT= High budget for the design of a specialized egg collector which proved not to be functional. Reverting to the conventional egg collector used in Japan or in Malta for amberjack egg collection resulted in egg collection in Spain and Malta.
TRANSDOTT = Two egg collectors used previously in Malta will be fused together to create a deeper egg collector that should give better results. In addition, new cage movement sensors (UDUS) will be used to detect current direction and thus counter egg loss by concentrating collecting resources.
Egg collectors changed to "Korean Plankton" net design outside the cage. Use of Current sensors and rising rates of eggs have helped in increasing efficiency of collection.
SELFDOTT= Critical points for improvement of larval survival were identified and partly overcome in SELFDOTT. The first few days after hatching are vital and tank hydrodynamics have been identified to increase survival.
TRANSDOTT = Further work on aeration and hydrodynamics will be done to reliably improve the survival at this critical stage.
COMMENT: Some work carried out and protocols derived.
SELFDOTT= Trials in Malta showed that feeding copepods along with rotifers indicated larval survival improvement.
TRANSDOTT = Larger scale experiments with co-feeding of rotifers with copepods will be carried out during TRANSDOTT larval rearing (FB).
COMMENT: Highly successful feeding with copepods at all stages by Futuna Blue and this has led to better survival rates also in other commercial experiments.
SELFDOTT= Feeding 15 DPH BFT larvae on yolk-sac larvae showed significant improvements on the survival of BFT larvae.
TRANSDOTT= TRANSDOTT will feed yolk-sac larvae but will also aim to substitute the yolk-sac larvae with early weaning diets.
COMMENT: Yolk-sac larvae appear to be the key to improve survival rates and have the added advantage of preparing the larvae for the transition to dry feeding.
SELFDOTT= Transfer to cages and the subsequent days resulted in many mortalities during SELFDOTT due to net collisions at night. Some trials were done with lights on cages during night-time.
TRANSDOTT= Experiments will be carried out to investigate ways in preventing collisions after transport to cages. More work will be done on cages and tanks by the different partners during night-time.
COMMENT: Due to low number of fingerlings produced only partial improvements were made here.
SELFDOTT= Some efforts were done on anaesthetics and BFT handling during SELFDOTT; these trials resulted in the need to develop anaesthetic delivery systems for these large fish.
TRANSDOTT= During TRANSDOTT, trials will be carried out to produce effective delivery systems so that handling of large BFT may be improved without losses.
COMMENT: These experiments were not carried out due to the lack of fish resources but trial handling techniques have been designed.

SELFDOTT= A successful grow-out diet was formulated through the work of IEO , SARC and Fuentes which lead to 5Kg + fish surviving until March 2012.
TRANSDOTT=These successful diet compilations will be applied to the grow-out in Israel, Malta, Spain and Italy using a higher number of fingerlings. Effective weaning and grow-out diets or their alternatives will be provided by SARC
COMMENT: Dry diets were improved on and also the use of moist feeds.

## Potential impact and main dissemination activities and exploitation results

Potential Impacts

Tunas constitute the most valuable fishery worldwide, and most tuna stocks are considered fully exploited, over-exploited or depleted (FAO, 2002). In the Mediterranean Sea, the species of greatest value is the Atlantic Blue-fin tuna (BFT). Fuelled by the increasing demand for this unique fish, a “capture-based" aquaculture industry has developed in the Mediterranean Sea (Ottolenghi et al., 2004). This activity involves the capture of migrating wild fish and their fattening in floating cages for periods of, usually, a few months, before they are slaughtered and sent fresh or frozen to the sushi and sashimi markets in Japan, Europe and the United States (Catarci, 2004). In 2007, the ICCAT began implementing a 4-year gradual reduction in the total allowable catches TACs for Eastern Atlantic and Mediterranean BFT in an effort to address the increasing scientific, public and NGO concern over the status of the wild stocks and the threat of extinction of the species due to overfishing.

The development of a self-sustained industry that will propagate this species in captive conditions, rear the larvae and produce fingerlings for further grow-out on is considered as one of the ways to alleviate the pressure on the wild fishery of the BFT and aid in its conservation - BFT domestication. The funding of the TRANSDOTT project was of the utmost importance for Europe to maintain continuity, research potential, resources and qualified man-power for the future in a similar manner as it has been done in Japan over the last 30 years bringing them to the forefront of this field.

Based on the success in controlling reproduction in captive BFT which was achieved in a previous “DOTT” projects TRANSDOTT has applied this technology to succeed in being one of the few resources available within the EU for viable BFT eggs and this has led to increasing interest from other hatcheries and fish farms throughout the European Union. BFT eggs are a sought after commodity and have been exported with success to countries as far away as USA and Korea by European producers for use in RAS for research studies. Malta has shown itself to be an ideal “hub” for the development of a Tuna propagation unit, with a naturally strategic location as well as good internal and external logistic capabilities and natural spawning conditions.

The control of reproduction and the improvement of gamete quality is a major prerequisite for the development of self-sustained aquaculture of any species. TRANSDOTT made use of induced and natural spawning to propagate a species that is one of the most valuable fishery resources of the Mediterranean Sea, relating to gametogenesis in captivity and the quality of the produced gametes. Such control is imperative for the reliable production of eggs and juveniles, necessary for the BFT industry to switch from a capture-based fattening activity with heavy impact on the wild populations of BFT, to a self-sustained aquaculture industry, producing a unique product under totally controlled conditions and of high nutrient value for both the EU consumer and the export market. A factor which has a high socio-economic importance for the region.

In the case of Pacific BFT, spontaneous reproduction has been observed in captive fish by Japanese researchers for twenty years, but in a very irregular and unreliable way. As a result, a self-sustained aquaculture industry for the PBFT still does not exist, although the University of Kinki in Japan have made great advances, and the necessity of wild fish capture for fattening still exists. The Japanese government has for these reasons just begun to build a land-based PBFT breeding facility for gamete generation and has banned the catching of juvenile tuna.

The basis of the current EU approach to increasing the production of biological resources (i.e., the Knowledge Based Bio-Economy) is the advancement of knowledge and application of innovation for (a) the sustainable production and management of these resources, (b) the increased competitiveness

of the agriculture and aquaculture sectors, (c) the safeguarding of consumer health and (d) the reduction in the environmental impact of farming activities. TRANSDOTT has begun to address some of these aspects by bringing innovative new technology to the forefront. The development and optimization of a flexible, reliable microsatellite and mitochondrial DNA genotyping system has been a useful tool for general use in BFT aquaculture to manage the broodstock (e.g., recognition of the number of spawning females, to recognize broodstock fish with higher reproductive success, to estimate genetic relatedness of individuals in order to avoid inbreeding, to estimate genetic variability of the broodstock whose loss will limit the potential for genetic gain from artificial selection, to support selective breeding programs etc). TRANSDOTT will contribute to providing both sustainability and traceability of the products from BFT aquaculture, goals set by certification agencies such as Marine Stewardship Council or Best Aquaculture Practices or the EU for "Farm to Fork" traceability requirements. These tools are now available under the work carried out by TRANSDOTT.

TRANSDOTT has used better broodstock nutrition to produce higher fecundity and better-quality gametes, which in turn translates to healthier, stronger viable larvae with lower mortalities during the larval rearing period . The success of the copepod enrichment of the live feed period has generated greater potential for larval rearing and improved survival rates. A key impact has been the increase in larval numbers and the production of fingerlings in sufficient quantities under the right conditions for the dry food diets to be applied.

One of the very important limitations of undertaking any type of research in the biology and culture of BFT, is the absence of any BFT research facility in the EU or Mediterranean area so far. Such a land-based facility for BFT will provide infrastructure, personnel and fish. Plans are underway to initiate a land-based facility in Spain but there have been a number of set-backs and production of eggs is envisaged probably in one to two years' time. Therefore, for any type of BFT aquaculture research to be implemented, a commercial partner has to provide (a) its own concession, which almost always has a limited allowance for cages and fish biomass, (b) service boats and personnel, which may be needed for other commercial activities, (c) insurance and watchman services, in addition to the ones required for the commercial operations. In addition, the cost of feeding BFT is significant plus the fact that each fish is worth upwards of a few thousands of Euros. TRANSDOTT has therefore been a collaboration link between SMEs and academia with a "top-down " approach. Since 75% of the TRANSDOTT funding has gone to the SME´s then the main impact is designed to be at the commercial level, translating research findings into scaling up to commercial production.

There has been significant growth of the Bluefin Tuna farming industry over the past twenty years. Fisherman in Australia introduced a ranching concept to Southern Bluefin Tuna (SBT) in the early 1990s and subsequently achieved great economic success. Atlantic and Pacific Bluefin Tuna ranching has emerged during the last decade in Mediterranean countries, such as Spain, Italy, Turkey, Malta, Cyprus, Croatia, Greece and Tunisia, as well as in Mexico and Japan. Development is starting in several other countries including the United States. In all cases, the market potential is very strong. Although Japan is still the major consumer of tuna, demand from developed economies is steadily increasing. Tuna capture quotas exist in all regions and act as a constraint on industry growth. Scarcity has increased prices, and the sashimi and sushi restaurants have presented tuna as a refined eating product. After observing the farming practices of the Australian SBT fishermen, the ABFT industry realized that by retaining the captured fish and implementing the same "ranching" philosophy, it could significantly increase profitability. In Australia, ranching is the process whereby wild juvenile Tuna (between 10-20kg) are captured at sea and then transferred to cages, where they are fattened until they reach market size of c. 40kg. This process of takes approximately three to four months and allows the farmers to catch a higher number of small fish (still satisfying their quota limits), fatten them to market size in sea cages and then sell a larger biomass to the market. The advantage is that the fishermen maximize the value in their fishing quotas and obtain a better price for their produce as the farmed product has a higher fat content.

The official quota for ABFT in Europe had been reduced from over 32,000t in 2001 to 18,500t in 2011. Unofficially, the quantity caught used to be estimated to be closer to 60,000 tons p.a. at its peak, but dropped significantly in recent years. The recent decision by ICCAT ("International Commission for the Conservation of the Atlantic Tuna") to increase fishing quotas in the Eastern Atlantic Ocean and the Mediterranean by 60 percent in the next three years could increase supplies

but may not be sustainable in the long term. It is clear that ranching will continue and TRANSDOTT may have a major impact on the source of fish for ranching. Two different products could be envisaged; on the one hand 2 - 5g fingerlings (35 days) which are then grown-out by individual farms or a larger product the 1,5 kg juveniles which can be grown within 130 days. These juveniles will replace the current stock the ABFT ranchers are unable to obtain from the wild today due to depletion and restriction in quota´s.

It is envisaged that from the results of the present project a large number of European hatcheries could embrace the technology needed for the production of BFT fingerlings. It will not be necessary for each to have their own Broodstock as sufficient "seed" can be generated by three to four centres based within the Mediterranean basin. Transport of eggs is a tried and tested protocol and as long as hatching is avoided during transportation, large distances can be covered.

The Dissemination activities of the project have been based around the Web Site (www.TRANSDOTT.eu) which has published most of the details of the meetings etc. The work has been supported by numerous press releases and information provided to the industry "Ticker Services" which appear on a daily basis. Articles have also appeared in Aquaculture Newspapers and also successfully in the National papers. The publication of the article " Million Dollar Babys" in "Die Zeit" in Germany has certainly done a great deal to make the project well known. One of the juvenile producing companies in the project, Futuna Blue have been extremely active in the production of Video clips: (http://www.youtube.com/watch ?v=qudAJaTEHvg) for You-Tube for the general public. Moreover, an international documentary film was produced by the German TV companies Arte /ZDF. The TRANSDOTT project was part of the EU sponsored exhibition Science Cities which was a Europe-wide evening of Science spread throughout the community for the general public of all ages. This was extremely well attended and a video clip is included in the report (Science City)
http://www.youtube.com/watch?v=LGVdoaMUc_g&feature=youtu.be

The coordination meetings and the final workshop meeting in Düsseldorf were made available to visiting scientists and the final workshop "Tuna - Future Strategies for Propagation, Sustainability and Restocking" that was attended by an international audience with review lecturers form Japan , Australia and Europe on all of these topics. Video clips of individual lectures have been made available on the internet at http://mediathek.hhu.de/user/TRANSDOTT with over 2000 hits within the first two months.. Presentations were made at a number of Aquaculture conferences and publications are now in preparation. Consideration has been given to both IPR and Patents by all the partners and a strategy evolved which is covered by the consortia agreement to protect and develop these assets.

Further areas of exploitation of the results have been listed in the reports but all three SME´s have registered their interest in continuing with Tuna propagation. The Broodstock will be maintained by MFF at least for 2015 and FB and ARDAG have both indicated their willingness and interest in further exploitation and commercialization. The spin-off firm form UDUS, TUNATECH is actively involved in setting up a Tuna propagation unit within the Mediterranean with support from investors and there are a number of other investor groups who are hoping to begin with tuna propagation in the near future. Extra hatchery space will be generated from some of these projects and at the same time "downtime space" especially in European hatcheries can be used in June and July for ABFT propagation. TRANSDOTT has successfully completed most of the deliverables and tasks which it has undertaken. Our knowledge base has increased once more and a number of SME´s should now be in a position to exploit this to the full, proving that TRANSDOTT was indeed instrumental in translating FP project results into innovative commercial applications. The European Aquaculture Industry is at the forefront of this new revolution in Atlantic Bluefin Tuna propagation and together with the European Commission should capitalize on this long-term investment made through the "DOTT" projects.

**Address of project public website and relevant contact details**

Public Website:
www.TRANSDOTT.eu

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## 4.2 Use and dissemination of foreground

## Section A (public)

## Publications

| LIST OF SCIENTIFIC PUBLICATIONS, STARTING WITH THE MOST IMPORTANT ONES | | | | | | | | | | |
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| No. | Title / DOI | Main author | Title of the periodical or the series | Number, date or frequency | Publisher | Place of publication | Date of publication | Relevant pages | Is open access provided to this publication ? | Type |

| LIST OF DISSEMINATION ACTIVITIES | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| No. | Type of activities | Main Leader | Title | Date | Place | Type of audience | Size of audience | Countries addressed |
| 1 | Articles published in the popular press | HEINRICH-HEINE-UNIVERSITAET DUESSELDORF | Es darf nicht immer Sushi sein | 11/08/2012 | FAZ | Medias | 60000 | Germany |
| 2 | TV clips | Futuna Blue España SL | www.youtube.com/watch?v=1YvieI8DyM8 | 20/08/2012 | YOUTUBE | Medias | | WWW |
| 3 | Organisation of Conference | HEINRICH-HEINE-UNIVERSITAET DUESSELDORF | EUROPEAN CONTRIBUTIONS TO SUSTAINABLE ABFT AQUACULTURE - PAST, PRESENT AND FUTURE. BROODSTOCK MANAG | 22/11/2012 | Kushimoto Japan | Scientific community (higher education, Research) - Industry - Policy makers | 200 | Kinki Univ Globoa COE Meeting Japan, USA, Australia, Europe |
| 4 | Organisation of Conference | ISRAEL OCEANOGRAPHIC AND LIMNOLOGICAL RESEARCH LIMITED | THE COMBINED EFFECT OF DHA AND TAURINE IMPROVES RETINAL DEVELOPMENT, PREY INGESTION AND GROWTH IN FI | 21/02/2013 | World Aquaculture Society Meeting Nashville, Tennessee February 21-25, 2013 | Scientific community (higher education, Research) - Policy makers - Medias | | World Aquaculture Society Meeting |
| 5 | TV clips | HEINRICH-HEINE-UNIVERSITAET DUESSELDORF | Rote Thun | 23/05/2013 | http://www.arte.tv/guide/de/047013-000/der-rote-thun | Scientific community (higher education, Research) - Policy makers - Medias | | Germany and France |
| 6 | Articles published in the popular press | HEINRICH-HEINE-UNIVERSITAET DUESSELDORF | Year’s first Med bluefin eggs shipped to Spain, Israel/ Undercurent News | 18/06/2013 | http://www.undercurrentnews.com/2013/06/18/years-first-med-bluefin-eggs-shipped-to-spain-israel/ | Scientific community (higher education, Research) - Industry - Civil society - Policy makers - Medias | | Worldwide |
| 7 | Articles published in the popular press | HEINRICH-HEINE-UNIVERSITAET DU | Million Dollar Babys | 12/09/2013 | Die Zeit ( German Newspaper) | Medias | 100000 | Germany |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | ESSELDORF | | | | | | |
| 8 | Articles published in the popular press | Ministry for Sustainable Development, the Environment and Climate Change | Special Net Enhances Tuna egg Collection | 30/09/2013 | Hatchery International | Medias | 10000 | Global |
| 9 | Videos | HEINRICH-HEINE-UNIVERSITAET DUESSELDORF | Sciencity Exhibition TRANSDOTT | 27/11/2013 | http://www.youtube.com/watch?v=LGVdoaMUc_g&feature=youtu.be | Civil society - Medias | 16000 | Germany |
| 10 | Videos | Futuna Blue España SL | Reportaje a Futuna Blue España en Tierra y Mar | 07/01/2014 | http://www.youtube.com/watch?v=qudAJaTEHvg | Medias | | Spain |
| 11 | Posters | HEINRICH-HEINE-UNIVERSITAET DUESSELDORF | TRANSDOTT - TRANSLATION OF DOMESTICATION OF THYNNUS THUNNUS INTO A INNOVATIVE COMMERCIAL APPLICATION - ADVANCES IN 2013: | 11/06/2014 | World Aquaculture meeting | Scientific community (higher education, Research) | 1000 | Global |
| 12 | Oral presentation to a scientific event | ISRAEL OCEANOGRAPHIC AND LIMNOLOGICAL RESEARCH LIMITED | THE COMBINED EFFECT OF DHA AND TAURINE IMPROVES RETINAL DEVELOPMENT, PREY INGESTION AND GROWTH IN FIRST FEEDING LARVAE OF ATLANTIC BLUE FIN TUNA (Thunnus thynnus)(ABFT) | 11/06/2014 | WorldAquacultue meeting Brisbane Australia | Scientific community (higher education, Research) | 100 | Global |
| 13 | Organisation of Workshops | HEINRICH-HEINE-UNIVERSITAET DUESSELDORF | Future Strategies for Propagation, Sustainability and Restocking | 15/09/2014 | Haus Der Universität Düssewldorf, Germany | Scientific community (higher education, Research) - Industry - Policy makers - Medias | 120 | Global |
| 14 | Videos | HEINRICH-HEINE-UNIVE | TRANSDOTT WORKSHOP P | 10/11/2014 | http://mediathek.hhu.de/user/T | Scientific community (higher educat | 100 | World Wide |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | RSITAET DU ESSELDORF | RESENTATIONS-TUNA PROPAG ATION | | RANSDOTT | ion, Research) - Ind ustry - Civil society - Policy makers - Medias | | |
| 15 | Oral presentation to a wider public | HEINRICH-H EINE-UNIVE RSITAET DU ESSELDORF | Million Dollar Babie s ? successfull start to breed Bluefin T una (Thunnus th ynnus) in aquacultur e systems in Spain ? also in Germany? | 11/11/2014 | Eurotier Forum Aquakultur Han nover Exhibition | Scientific comm unity (higher educat ion, Research) - Ind ustry - Policy makers - Medias | 100 | Global |
| 16 | Oral presentation to a wider public | Futuna Blue España SL | “H2020 en el sector de alimentos del mar . El mejor camino de innovar en Europa” "Transdott, a good example of coll aborative project" | 17/12/2014 | Ctaqua, El Puerto d e Santa María, Spain | Scientific comm unity (higher educat ion, Research) - Ind ustry - Civil society - Policy makers - Medias | 100 | European |

## Section B (Confidential or public: confidential information marked clearly)

| LIST OF APPLICATIONS FOR PATENTS, TRADEMARKS, REGISTERED DESIGNS,UTILITY MODELS, ETC. | | | | | |
|---|---|---|---|---|---|
| Type of IP Rights | Confidential | Foreseen embargo date dd/mm/yyyy | Application reference(s) (e.g. EP123456) | Subject or title of application | Applicant(s) (as on the application) |

| OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Type of Exploitable Foreground | Description of Exploitable Foreground | Confidential | Foreseen embargo date dd/mm/yyyy | Exploitable product(s) or measure(s) | Sector(s) of application | Timetable for commercial use or any other use | Patents or other IPR exploitation (licences) | Owner and Other Beneficiary(s) involved |
| Commercial exploitation of R&D results | Protocol for Egg Transport and Packing | Yes | | Tuna egg tranportation | Aquaculture | Within the next 5 years | Not applied for | TRANSDOTT Consortium |
| Commercial exploitation of R&D results | Egg hatching and live feed protocol for fingerling production | Yes | | Information for prospective purchasers of Tuna eggs | Aquaculture | Witin the next 5 years | Not applied for | TRANSDOTT Consortia |
| Commercial exploitation of R&D results | Biopsy sampling of Broodstock with new Tools developed for Sampling, Tagging and Implanting | Yes | | Production of custom made Tools together with Analysis services | Aquaculture Broodstock | 5 years | Patent application being processed | UDUS owner Beneficiaries TRANSDOTT Consortia |
| Commercial exploitation of R&D results | Egg Collecting Methodology Techniques and Guidelines | Yes | | Cage Egg collection | Aquaculture | 5 years | Not applied for | MARC + TRANSDOTT Consortia |
| Commercial exploitation of R&D results | Production of yolk-sac larvae for weaning from live food to dry food | Yes | | Fingerling production | Aquaculture | 5 Years | Not applied for | TRANSDOTT Consortia |

| ADDITIONAL TEMPLATE B2: OVERVIEW TABLE WITH EXPLOITABLE FOREGROUND | |
|---|---|
| Description of Exploitable Foreground | Explain of the Exploitable Foreground |
| Protocol for Egg Transport and Packing | Description of treatment and cleaning of the eggs before shipment. Standized protocols for numbers of eggs, shipping conditions and containers to be used and initiation and measurements to made by recipients when eggs are received. Prospective purchase of eggs from commercial tuna propagation unit. IPR covered in Consortial Agreement. Future Research on temperature control. |
| Egg hatching and live feed protocol for fingerling production | Development of protools outlining hatching conditions most suitable to give maximum survival in terns of Temperature, Water Movement, Aeration and Tank size. Followed by feeding sequence of both live food organisms and dry diets .Prospective purchase of eggs from commercial tuna propagation unit. IPR covered in Consortial Agreement. Impact of copepods as live food essential for future success. Research into microdiets and alternative fish meal substitues needed. |
| Biopsy sampling of Broodstock with new Tools developed for S | New tools have been developed which allow three processes to be carried out simultaneously. 1) A muscle biopsy sample can be taken under water by a diver using a modified harpoon system 2) At the same time selected broodstock can be implanted with an EVAC pellet conatining GnRHa a sysnthectic ann |

| | |
|---|---|
| ampling, Tagging and Implanting | laog to induce spawning. 3) These fish are alos atgged visually and electronically at the same time with a dart with a colour coded sequence and also a mic ro-PIT tag. IPR covered in Consortial Agreement and patent apllication pending. Commercial hatcheries exploitation of broodstock properties for genetic tr aits such as faster growth. |
| Egg Collecting Methodology Tec hniques and Guide lines | Together with Colleagues from Korea an new type of net has been designed by MRRA and has proved to be more effective for open weater egg collection. The commercial production of these types of nets and the material used are key areas for exploitation. The layout and guidelines for the use of such nets are highly dependent on local logistics and conditions. IPR with Korean and MARC devlopment. Demand in future open ocean egg propagation units. |
| Production of yolk-sac larvae for weaning from live food to dry food | The production of yolk-sac larvae food at specific times in the life cycle of the fingerling rearing is a key parameter to the success of fingerling production. Will be essentila for commercila production of large quantities of fingerlings.IPR covered in Consortial Agreement. Research required on generation techn ology, synchronisation or storage of material. |

## 4.3 Report on societal implications

## B. Ethics

| | |
|---|---|
| **1. Did your project undergo an Ethics Review (and/or Screening)?** | No |
| **If Yes: have you described the progress of compliance with the relevant Ethics Review/Screening Requirements in the frame of the periodic/final reports?** | |

**2. Please indicate whether your project involved any of the following issues :**

**RESEARCH ON HUMANS**

| | |
|---|---|
| **Did the project involve children?** | No |
| **Did the project involve patients?** | No |
| **Did the project involve persons not able to consent?** | No |
| **Did the project involve adult healthy volunteers?** | No |
| **Did the project involve Human genetic material?** | No |
| **Did the project involve Human biological samples?** | No |
| **Did the project involve Human data collection?** | No |

**RESEARCH ON HUMAN EMBRYO/FOETUS**

| | |
|---|---|
| **Did the project involve Human Embryos?** | No |
| **Did the project involve Human Foetal Tissue / Cells?** | No |
| **Did the project involve Human Embryonic Stem Cells (hESCs)?** | No |
| **Did the project on human Embryonic Stem Cells involve cells in culture?** | No |
| **Did the project on human Embryonic Stem Cells involve the derivation of cells from Embryos?** | No |

**PRIVACY**

| | |
|---|---|
| **Did the project involve processing of genetic information or personal data (eg. health, sexual lifestyle, ethnicity, political opinion, religious or philosophical conviction)?** | No |
| **Did the project involve tracking the location or observation of people?** | No |

**RESEARCH ON ANIMALS**

| | |
|---|---|
| **Did the project involve research on animals?** | Yes |
| **Were those animals transgenic small laboratory animals?** | No |
| **Were those animals transgenic farm animals?** | No |
| **Were those animals cloned farm animals?** | No |
| **Were those animals non-human primates?** | No |

**RESEARCH INVOLVING DEVELOPING COUNTRIES**

| | |
|---|---|
| **Did the project involve the use of local resources (genetic, animal, plant etc)?** | No |
| **Was the project of benefit to local community (capacity building, access to healthcare, education etc)?** | No |

**DUAL USE**

| | |
|---|---|
| **Research having direct military use** | No |
| **Research having potential for terrorist abuse** | No |

# C. Workforce Statistics

**3. Workforce statistics for the project: Please indicate in the table below the number of people who worked on the project (on a headcount basis).**

| Type of Position | Number of Women | Number of Men |
|---|---|---|
| Scientific Coordinator | 0 | 5 |
| Work package leaders | 0 | 5 |
| Experienced researchers (i.e. PhD holders) | 1 | 8 |
| PhD student | 0 | 0 |
| Other | 8 | 18 |

| | |
|---|---|
| **4. How many additional researchers (in companies and universities) were recruited specifically for this project?** | 5 |
| **Of which, indicate the number of men:** | 5 |

## D. Gender Aspects

| | |
|---|---|
| **5. Did you carry out specific Gender Equality Actions under the project ?** | No |

| **6. Which of the following actions did you carry out and how effective were they?** | |
|---|---|
| **Design and implement an equal opportunity policy** | Not Applicable |
| **Set targets to achieve a gender balance in the workforce** | Not Applicable |
| **Organise conferences and workshops on gender** | Not Applicable |
| **Actions to improve work-life balance** | Not Applicable |
| **Other:** | |

| | |
|---|---|
| **7. Was there a gender dimension associated with the research content - i.e. wherever people were the focus of the research as, for example, consumers, users, patients or in trials, was the issue of gender considered and addressed?** | No |
| **If yes, please specify:** | |

## E. Synergies with Science Education

| | |
|---|---|
| **8. Did your project involve working with students and/or school pupils (e.g. open days, participation in science festivals and events, prizes/competitions or joint projects)?** | Yes |
| **If yes, please specify:** | EU Science City Düsseldorf Nov.2013 |

| | |
|---|---|
| **9. Did the project generate any science education material (e.g. kits, websites, explanatory booklets, DVDs)?** | Yes |

## F. Interdisciplinarity

| **10. Which disciplines (see list below) are involved in your project?** | |
|---|---|
| **Main discipline:** | 4.1 Agriculture, forestry, fisheries and allied sciences (agronomy, animal husbandry, fisheries, forestry, horticulture, other allied subjects) |
| **Associated discipline:** | 1.5 Biological sciences (biology, botany, bacteriology, microbiology, zoology, entomology, genetics, biochemistry, biophysics, other allied sciences, excluding clinical and veterinary sciences) |
| **Associated discipline:** | |

## G. Engaging with Civil society and policy makers

| | |
|---|---|
| **11a. Did your project engage with societal actors beyond the research community? (if 'No', go to Question 14)** | Yes |
| **11b. If yes, did you engage with citizens (citizens' panels / juries) or organised civil society (NGOs, patients' groups etc.)?** | Yes, in communicating /disseminating / using the results of the project |
| **11c. In doing so, did your project involve actors whose role is mainly to organise the dialogue with citizens and organised civil society (e.g. professional mediator; communication company, science museums)?** | No |
| **12. Did you engage with government / public bodies or policy makers (including international organisations)** | Yes, in communicating /disseminating / using the results of the project |
| **13a. Will the project generate outputs (expertise or scientific advice) which could be used by policy makers?** | Yes - as a primary objective (please indicate areas below multiple answers possible) |
| **13b. If Yes, in which fields?** | |
| **Agriculture** | Yes |
| **Audiovisual and Media** | No |
| **Budget** | No |
| **Competition** | No |
| **Consumers** | No |
| **Culture** | No |
| **Customs** | No |
| **Development Economic and Monetary Affairs** | No |
| **Education, Training, Youth** | No |
| **Employment and Social Affairs** | No |
| **Energy** | No |
| **Enlargement** | No |
| **Enterprise** | Yes |
| **Environment** | No |
| **External Relations** | No |
| **External Trade** | No |
| **Fisheries and Maritime Affairs** | Yes |
| **Food Safety** | Yes |
| **Foreign and Security Policy** | No |
| **Fraud** | No |

| | |
|---|---|
| **Humanitarian aid** | No |
| **Human rightsd** | No |
| **Information Society** | No |
| **Institutional affairs** | No |
| **Internal Market** | No |
| **Justice, freedom and security** | No |
| **Public Health** | No |
| **Regional Policy** | Yes |
| **Research and Innovation** | Yes |
| **Space** | No |
| **Taxation** | No |
| **Transport** | No |
| **13c. If Yes, at which level?** | International level |

## H. Use and dissemination

| | |
|---|---|
| **14. How many Articles were published/accepted for publication in peer-reviewed journals?** | 0 |
| **To how many of these is open access provided?** | 0 |
| **How many of these are published in open access journals?** | 0 |
| **How many of these are published in open repositories?** | 0 |
| **To how many of these is open access not provided?** | 0 |
| **Please check all applicable reasons for not providing open access:** | |
| **publisher's licensing agreement would not permit publishing in a repository** | No |
| **no suitable repository available** | No |
| **no suitable open access journal available** | No |
| **no funds available to publish in an open access journal** | No |
| **lack of time and resources** | No |
| **lack of information on open access** | No |
| **If other - please specify** | |
| **15. How many new patent applications ('priority filings') have been made? ("Technologically unique": multiple applications for the same invention in different jurisdictions should be counted as just one application of grant).** | 0 |

| 16. Indicate how many of the following Intellectual Property Rights were applied for (give number in each box). | |
|---|---|
| **Trademark** | 0 |
| **Registered design** | 0 |
| **Other** | 0 |

| | |
|---|---|
| **17. How many spin-off companies were created / are planned as a direct result of the project?** | 3 |
| **Indicate the approximate number of additional jobs in these companies:** | 50 |

| | |
|---|---|
| **18. Please indicate whether your project has a potential impact on employment, in comparison with the situation before your project:** | Increase in employment, In small and medium-sized enterprises |

| | |
|---|---|
| **19. For your project partnership please estimate the employment effect resulting directly from your participation in Full Time Equivalent (FTE = one person working fulltime for a year) jobs:** | 10 |

# I. Media and Communication to the general public

| | |
|---|---|
| **20. As part of the project, were any of the beneficiaries professionals in communication or media relations?** | No |

| | |
|---|---|
| **21. As part of the project, have any beneficiaries received professional media / communication training / advice to improve communication with the general public?** | Yes |

| 22. Which of the following have been used to communicate information about your project to the general public, or have resulted from your project? | |
|---|---|
| **Press Release** | Yes |
| **Media briefing** | Yes |
| **TV coverage / report** | Yes |
| **Radio coverage / report** | Yes |
| **Brochures /posters / flyers** | Yes |
| **DVD /Film /Multimedia** | Yes |
| **Coverage in specialist press** | Yes |
| **Coverage in general (non-specialist) press** | No |
| **Coverage in national press** | Yes |
| **Coverage in international press** | Yes |
| **Website for the general public / internet** | Yes |

| | |
|---|---|
| **Event targeting general public (festival, conference, exhibition, science café)** | Yes |

| **23. In which languages are the information products for the general public produced?** | |
|---|---|
| **Language of the coordinator** | Yes |
| **Other language(s)** | Yes |
| **English** | Yes |

| | |
|---|---|
| **Attachments** | TRANSDOTT Futuna Blue 2012-2014.mp4, ScienceCity TRANSDOTT Film.mp4, APPENDIX B Abstracts Workshop Düsseldorf.pdf, APPENDIX A IPR Patent Survey Form.pdf, ADDITIONAL LOGO s etc.docx, Malta Egg Collection and Shipping 2013.mp4, Futuna Blue Tuna Fingerlings TRANSDOTT 30 August 2013.m4v, Feeding Adult Tuna.mp4, TRANSDOTT Underwater Induction.mp4 |
| **Grant Agreement number:** | 311904 |
| **Project acronym:** | TRANSDOTT |
| **Project title:** | TRANSLATION OF DOMESTICATION OF THUNNUS THYNNUS INTO AN INNOVATIVE COMMERCIAL APPLICATION |
| **Funding Scheme:** | FP7-CP-TP |
| **Project starting date:** | 01/04/2012 |
| **Project end date:** | 30/09/2014 |
| **Name of the scientific representative of the project's coordinator and organisation:** | Prof. Christopher Bridges HEINRICH-HEINE-UNIVERSITAET DUESSELDORF |
| **Name** | |
| **Date** | 14/01/2015 |

This declaration was visaed electronically by Christopher BRIDGES (ECAS user name nbridgch) on 14/01/2015