

**SHORT-TERM CONTRACT FOR BFT GROWTH IN FARMS
STUDY (ICCAT-GBYP 03/2020) OF THE ATLANTIC-WIDE
RESEARCH PROGRAMME FOR BLUEFIN TUNA
(ICCAT GBYP Phase 10)**

BETWEEN

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

AND

**TUNIPEX, S.A. EMPRESA DE PESCA DE TUNÍDEOS
(PORTUGAL)**

DELIVERABLE #5:

Final Report

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FINAL REPORT OF ACTIVITIES

Executive summary

ICCAT requested an update on the potential growth rates of bluefin tuna in farming/fattening facilities, with the aim of improving coherence within the growth rates derived from eBCD, as stipulated in paragraph 28 of Rec. 18-02.

Given the particular situation of the Portuguese tuna traps located along the South coast capturing the adult fraction of the bluefin tuna exiting the Mediterranean after the reproduction season, a contract was established between ICCAT and Tunipex (with IPMA as scientific sub-contractor) to fulfill the required work in Portuguese traps in the eastern Atlantic Ocean.

The Tunipex tuna trap, where the tagging operations took place, is located about two and a half nautical miles from the coast of the Algarve, between about 20-60m depth. The central location of the trap is at: Lat= 37.01332 (North); Long= -7.71035 (West).

Between the 3rd and 17th of August 2020, 107 (one hundred and seven) adult bluefin tunas were individually weighted, measured, double tagged and returned to the cage for fattening. All fish were harvested as a batch at the end of the season. However, this particular season there were delays associated with the Covid pandemic, and as such harvesting only occurred in December 2020.

Data regarding initial and final weight and length, feeding amounts, stereoscopic camera measurements and environmental parameters were collected and reported.

The overall weight increase for the harvested fish had a mean of 40.3% (varying between 12% and 74%), for fish that were fattened between 116 and 132 days between tagging and harvesting.

The growth data in this study were collected and reported successfully according to the ICCAT contract. At the end of the report, we also provide some additional recommendations, specifically in terms of onboard tagging operations, that may be considered for adjusting the tagging strategy on future phases of the ICCAT/GBYP tagging and growth in farms projects.

Background

During the 21st Special Meeting of the Commission, the SCRS was asked to provide an update on the potential growth rates of bluefin tuna in farming/fattening facilities, with the aim of improving coherence within the growth rates derived from eBCD, as stipulated in paragraph 28 of Rec. 18-02. Consequently, GBYP was committed to carry out a broad study on this topic, involving ad hoc experiments in selected farms along the eastern Atlantic and Mediterranean. Such broad study was planned within GBYP Phase 8, and included several preparatory tasks, such as elaboration and distribution of a detailed questionnaire submitted to all the operative BFT farms, and meetings with farm owners, local authorities and scientists in the five areas where the study was to be developed. The implementation of the study started in GBYP Phase 9, involving tagging experiments to determine individual growth trajectories, intensive monitoring of representative cages, including the record of relevant environmental variables and food provided to caged fishes and seasonal measurements of their growth by means of stereo-cameras measurements, as well as the elaboration and analysis of a database including data on initial length distributions from stereo-cameras and data on final sizes and weight at the end of farming period obtained during harvesting operations.

In this sense, and given the particular situation of the Portuguese tuna traps located along the South coast capturing the adult fraction of the Bluefin tuna exiting the Mediterranean after the reproduction season, a contract was established between ICCAT and Tunipex (with IPMA as scientific sub-contractor) to fulfill the required work in Portuguese traps in the eastern Atlantic Ocean.

The work developed within the frame of this contract is a continuation of the 2019 study with methodological improvements in order to increase the number of fish effectively weighed to provide a more robust analysis.

Objectives

The objective of this document is to integrate the scientific info already provided in Deliverables 2, 3 and 4 (update of work and draft final report) of the Project ICCAT/GBYP - Phase 10 - Short-term contract for BFT growth in farms study (ICCAT-GBYP 03/2020) of the Atlantic-wide Research Programme for Bluefin tuna in a single scientific Final Report. This Final Report, as requested in the signed contract, includes:

- a) a full description of the initial conditions of the monitored cages (origin of the caged fishes, date and area of capture, characteristics of the monitored cage, initial number and biomass of caged fishes, etc.);

- b) a detailed description of all the methodologies and protocols applied for monitoring environmental variables, biological sampling of dead fishes and taking measurements of live fishes - both directly and through stereoscopic cameras-, as well as tagging operations (how protocols have been applied, any departure from the protocol, difficulties encountered, etc.);
- c) files containing videos and raw data from stereoscopic camera measurements of tagged fishes carried out after the first official stereoscopic camera measurements at caging of the whole catch;
- d) detailed tables and graphs including:
- length and weight of any fish dead in the monitored cages due to causes other than harvesting operations, as well relevant data on biological samples from these fishes, if any,
 - weekly records of environmental parameters (T-Temperature, S-Salinity, DO2-Dissolved Oxygen) in the monitored cages,
 - daily quantities and types of food given to the trial cage/s,
 - length and weight of tagged fishes at tagging and at harvesting, as well as information of deployed tags and biological samples taken from these fishes,
 - length and weight of each fish harvested from the monitored cages (specifying date of harvesting),
- e) an Executive Summary of the final report.

Full description of the work carried out

Methodology

Tagging for the Growth study in the Tuna trap owned by company Tunipex and located off the South coast of the Algarve province (Portugal) started on the 3rd of August and was completed on the 5th of August with 85 fish tagged (5 fish in excess of the 80 contracted).

In order to compensate for the high mortality that occurred within one week after the tagging event, 22 additional fish were tagged on the 17th of August, therefore making a total of 107 fish tagged for this contract.

Fish were held in a temporary cage (trap cage) before being tagged and transferred to the monitoring and farm cage (officially named by ICCAT as Farm Cage PRT902). Both cages have the same dimensions: LxWxH 120m x 55m x 30m (H is limited by the sea floor, so it is the same as water depth).

Fish to be tagged were isolated on a knot-less net to minimize damage to the fish before hauling onboard on a stretcher with a digital scale and weighted (see Lino et al., 2019).

The stretchers used were individually identified (to subtract the weight of the stretcher to the measured weight) and had a mesh along the middle of the whole stretcher length to guarantee that all water was purged before weighting. Furthermore, fish weight was registered immediately before hauling back into the water (not at hauling on board) to ensure sufficient time for the water to be purged to the maximum extent possible.

All tagged fish were individually weighted, measured with a tape for SFL (Straight Fork Length) and double tagged with conventional tags provided by ICCAT (Annex 1).

In order to try to solve the high proportion of unrecovered tagged fish in the 2019 study, which might have been partially caused by tag shedding (Lino et al, 2019), the double tagging protocol was modified. The applicator was transformed to insert both tags in parallel, at the same distance to the dorsal fin, at an angle of 45 degrees of the fish body surface (Figure 1).



Figure 1 - Tag applicator handle modified for the double tagging used in 2020

In addition, the tags used in the 2020 study were of the double barbel type, inserted through the second dorsal fin. These replaced the single barbel tags that were used in the 2019 study, that were inserted deep in the muscle (Figure 2).

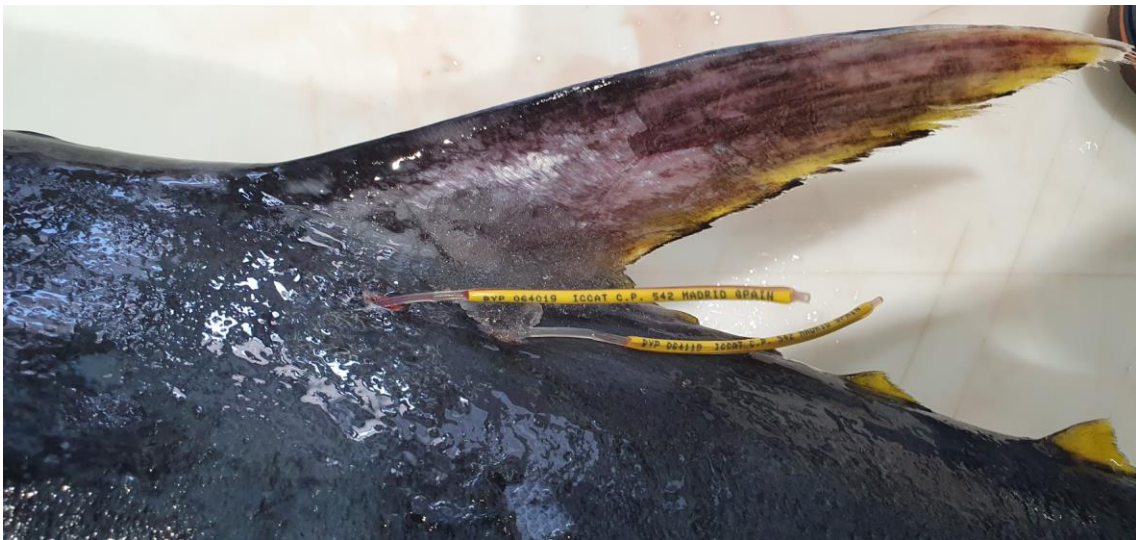


Figure 2 - Tags inserted through the muscle and the second dorsal fin at a 45 degree angle

All tagged fish were individually monitored with a stereoscopic-camera system (Annex 2) immediately after tagging, noting that it was only possible to obtain length measurements from 58 out of 107 fish (54%).

Differences between the two weights obtained (weighted on board vs calculated from length by the stereoscopic-camera) were analyzed using a Two sample t-test (Welch). Fulton's condition factor (K) at tagging for each individual was calculated using the following equation (Nash, Valencia and Geffen, 2006):

$$K = 100*(W/L^3)$$

where W is the Weight in grams and L is the Length in cm

All fish were moved from the trap cage to the farm cage PRT902 and the amount and type of food provided to the fishes in the monitoring cage was recorded on a daily basis (Annex 3). Weights and lengths and date of harvesting for all non-tagged fish farmed together with the tagged fish are included in Annex 4.

Surface and bottom temperature, air temperature, current direction and speed, wind direction and speed, water visibility (m), wave height and direction and cloud coverage were recorded on a daily basis, most of the days during the morning and afternoon (Annex 5).

Deliberate harvesting of tagged fish was scheduled to start in late October/early November but due to COVID limitations the freezer vessel only allowed the harvesting to occur between the 11th and the 14th of December 2020.

All harvested fish (both tagged and others not tagged) were sacrificed underwater with a "lupara" and individually weighted onboard the freezer vessel on a floor weight scale and measured with a tape (SFL). No biological samples were collected from this study due to the COVID limitations on the number of scientific staff onboard the freezer vessel.

A description of the number of tagged fish that died (i.e. not deliberately harvested), and those that were harvested but that couldn't be identified individually because of tags

shedding, is provided. The length and weight distribution at tagging event and at harvesting is also provided, as well as an analysis of the weight increase during caging.

All analysis was carried out using R version 3.6.3 (R Core Team, 2020)

Results and Discussion

An analysis of the length and weight measured on board vs the length estimated and the weight calculated by the stereo camera AM100 showed that for the 58 fish for which it was possible to obtain a measurement with the camera, there was no statistical difference between the SFL measurements (Two sample t-test (Welch), p-value = 0.16).

However, the weights obtained from the AM100 estimates and those directly measured with a scale were statistically different (Two sample t-test (Welch), p-value = 0.04). There was an overestimation of the total weight by the stereo camera compared to the on board weighting by more than 10% (723kg more in a total of 6920kg).

After the final round of harvesting in cage PRT902 where fish were concentrated, from the total tagged 107 fish, 63 tagged fish were harvested while 7 were not recovered and 37 were found dead after tagging (Table 1 and Figure 3). This represented a mortality of nearly 35% (which is a substantial improvement over the 42% from the 2019 study).

Table 1 - Number of fish Harvested, Dead and Unrecovered per 10cm size class

Classe_SFL	Harvested	Dead	Unrecovered	Total
140	1	1		2
150	1	1		2
160	2	1	1	4
170	6	4	1	11
180	8	5		13
190	10	9	2	21
200	13	8	1	22
210	17	4		21
220	3	0	1	4
230	1	1		2
240	1	3		4
250	0	0		0
260	0	0	1	1
Total Result	63	37	7	107

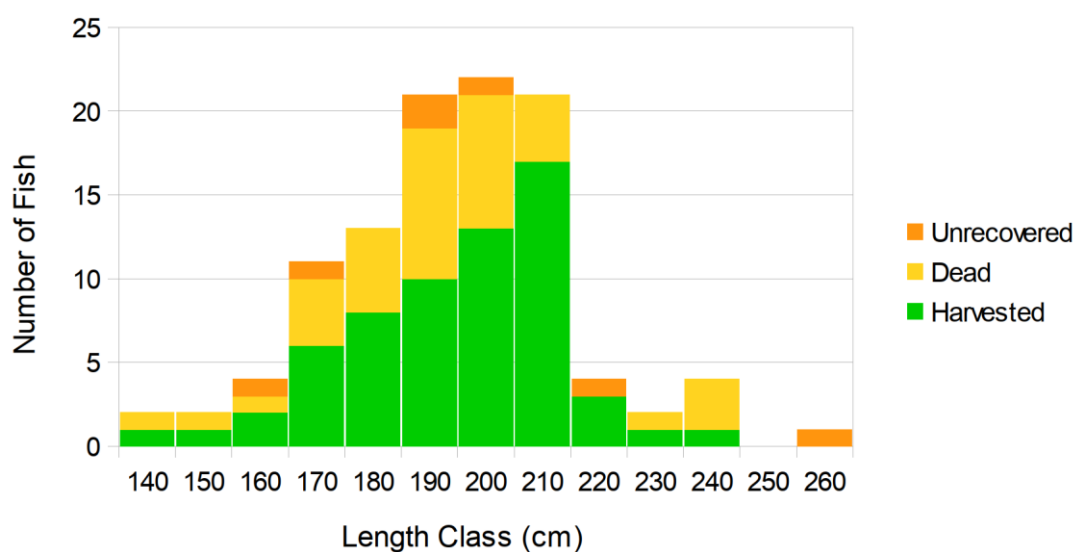


Figure 3 - Number of fish tagged per 10cm size class with Number of Fish Harvested (Green), Dead (Yellow) and Unrecovered (Orange)

Even if mortality was reduced by improved handling procedures it is still quite a high proportion of the tagged fish and something that needs to be taken into account for future studies.

Considering that we are dealing with very big and sensitive animals, the applied methodology to catch the tunas and immobilize them into the stretchers still represents a high stress factor and impossible to avoid to achieve the aim of this study (to have a measured value of the initial weight).

However, a study carried out in Maltese farms where fish were hooked and pulled to the stretcher had zero mortality out of 3 fish that were successfully tagged (Rouyer et al, 2019). This could indicate that the high mortalities in Portugal could be attributable to the bad initial condition of the fish and/or the high stress induced by the specific methodology used for capturing the fish. Using the hooking method in could be a solution to mortality to be tested.

Nevertheless, it is important to have actual values for this particular methodology. It is worthy to notice that most of the mortality occurred within the first two weeks after tagging (Figure 4).

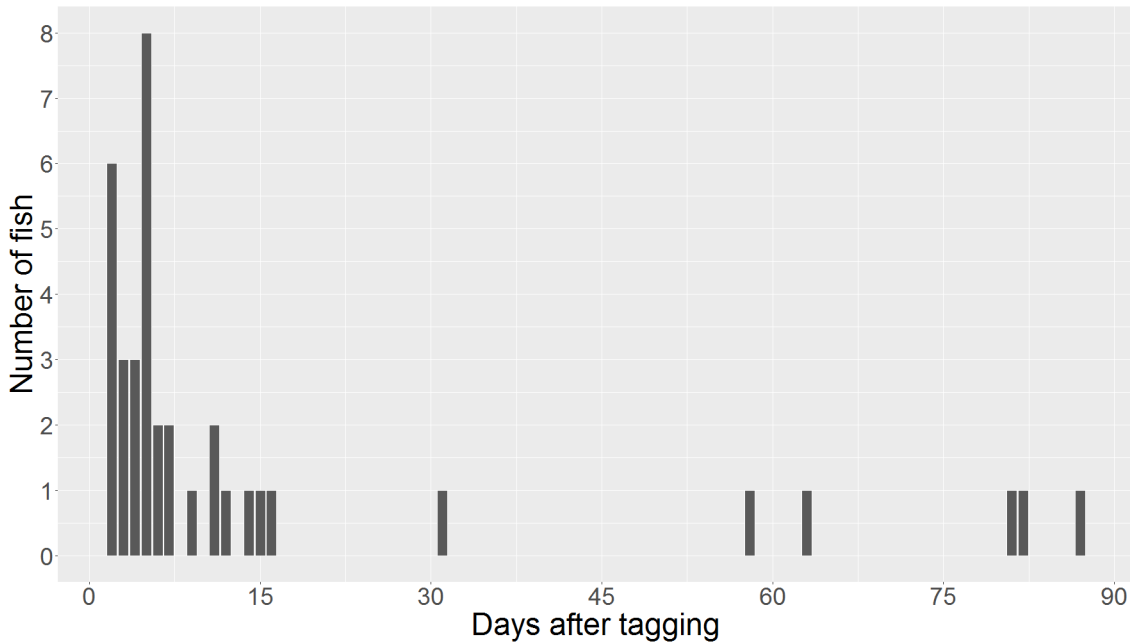


Figure 4 - Number of fish dead per day after the tagging event

Regarding the unrecovered fish, the number of fish that could not be recovered at the end of the harvest was 7 out of 107 (6.5%) which is a substantial improvement over the 2019 study (20.2%). The unrecovered fish could be the result of a combination of factors:

- Loss of both tags
- Release of excessive fish ordered by Portuguese authorities on the 6th August since the majority of unrecovered tunas belonged to the first tagging days, between August 3rd and 5th. Although all the efforts were made to avoid the release of tagged fish it is very difficult to separate tagged from non-tagged inside the same cage.
- The passage of Barbara storm in the Algarve coast between 19th and 20th October which caused the loss of 79 fishes. Some of these fishes were found in a

much degraded condition and so it was very difficult to confirm if the tags were lost.

- Tags were not noted by the scientific observers during harvesting since fish were harvested at a rate of 350 fish per day and tags were colonized by algae.

In any case it is a very low proportion of the total tagged fish.

Although this study did not perform any method comparison, the reduction in unrecovered fish could be a result of the modification of the tagging procedure. It is worth noting that of the 100 fish recovered only 3 had a single tag, which means that 90.7% retained the two tags.

The analysis of the weight data of the fish dead prior to intentional harvesting (Figure 5) suggests that weight increase does not start immediately (as had already been observed in the 2019 study). The overall weight increase of the harvested BFT varied between 12.0% and 74.0% (mean = 40.3%, SD = 13.6%), noting that all fish were harvested between 116 and 132 days after tagging.

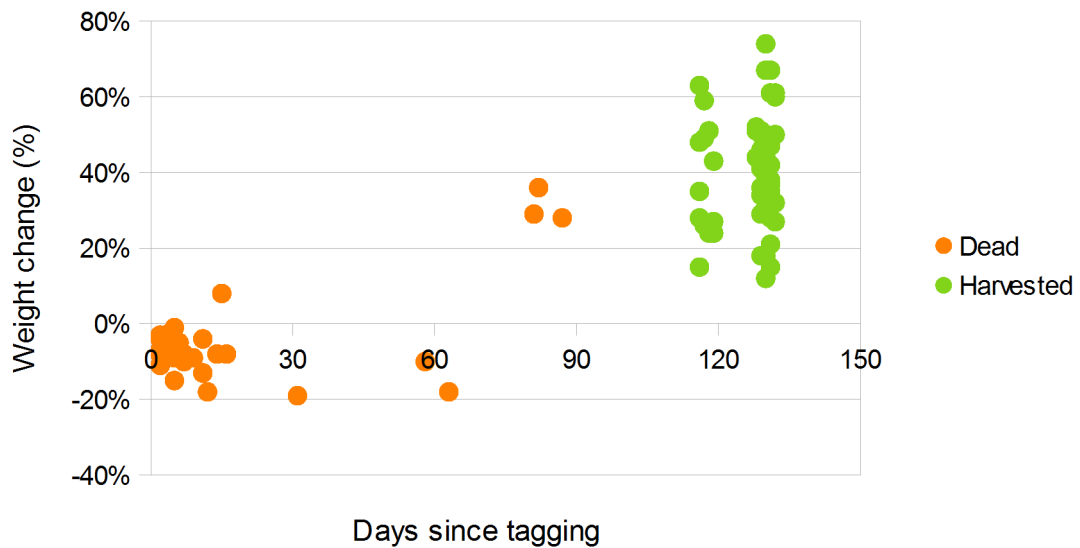


Figure 5 - Weight change as a proportion of initial weight with time after tagging.

According to the Tunipex staff it is normal that captured fish do not feed for 3 to 4 days after capture, so the initial weight decrease is normal. This could also be an effect of water/blood loss after harvesting which would be more relevant initially, before somatic weight growth is measurable. In this study a different situation was observed where 3 fish actually survived to between 31 and 63 days after tagging and their final weight was lower than the initial. These extremely thin fish that apparently did not feed but survived are relevant for the analysis of future tagging studies.

Pooling the data for all dead and harvested fish from this study and the previous 2019 results shows (Figure 6) an evident pattern of growth over time.

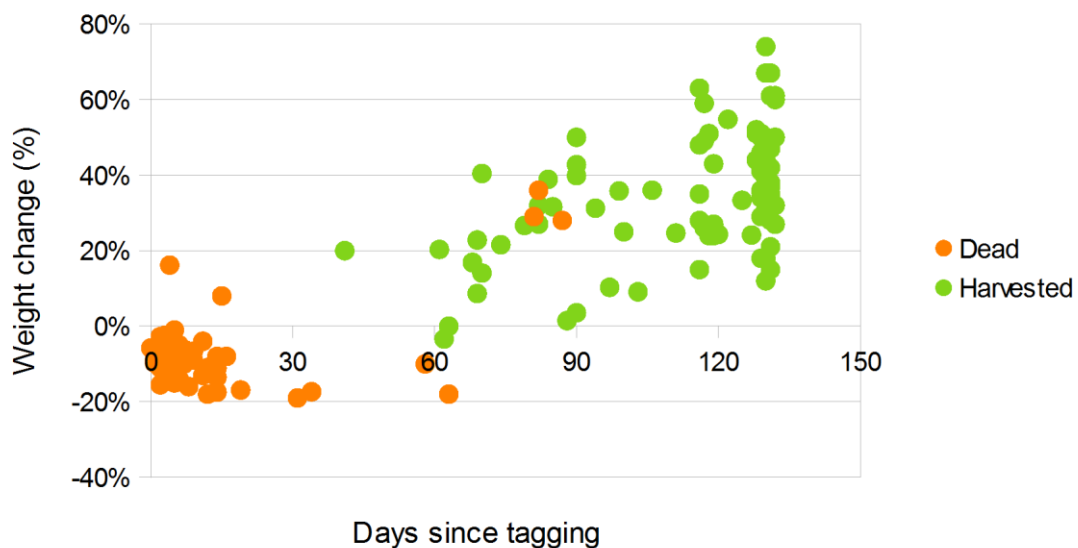


Figure 6 - Weight change as a proportion of initial weight with time after tagging. Pooled data from the 2019 and 2020 studies.

The pooled weight increase values show a trend of increasing weight gain over time but with a high range of individual variability. The maximum value observed (74% increase) is not an isolated value (outlier) but an extreme case of weight gain starting from an initial low condition. In this particular case a 204cm SFL fish that weighted 112kg (K=1,32) and that recovered to 195kg (K=1,86) after 130 days.

Plotting the weight change as a proportion of initial weight with the initial Fulton's K for the 2019 and 2020 pooled data of Harvested fish (Figure 7) seems to show a tendency for a decrease with increasing K.

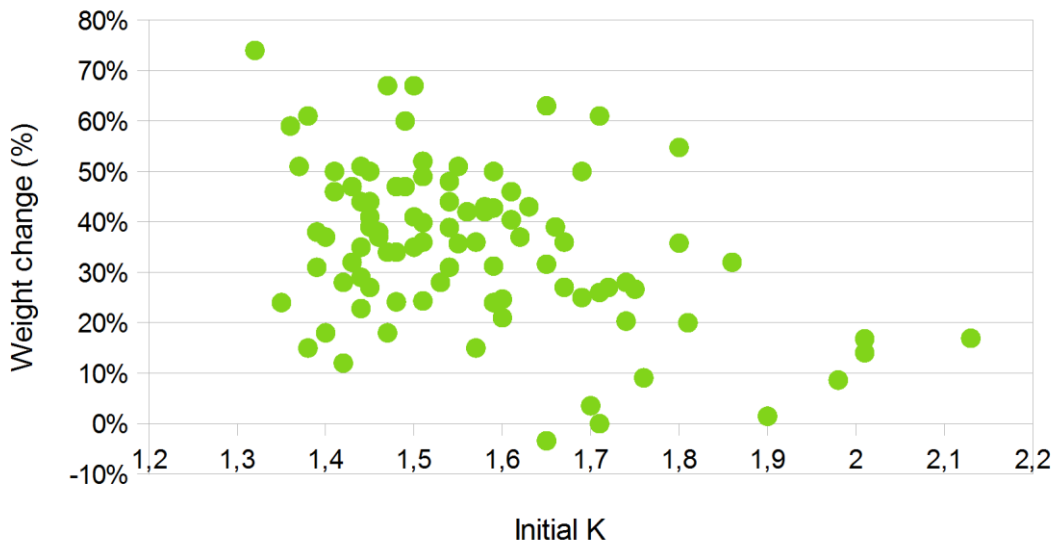


Figure 7 - Weight change as a proportion of initial weight with initial Fulton's K. Pooled data from 2019 and 2020 studies.

In fact plotting the Weight change in function of the initial length (Figure 8), seems to show a trend of decreasing weight gain with size, but also a reduced weight gain in smaller sized fish. Given the low number of fish these results allow only to speculate if this could be a result of competition for food inside the cages when small and larger fish are mixed.

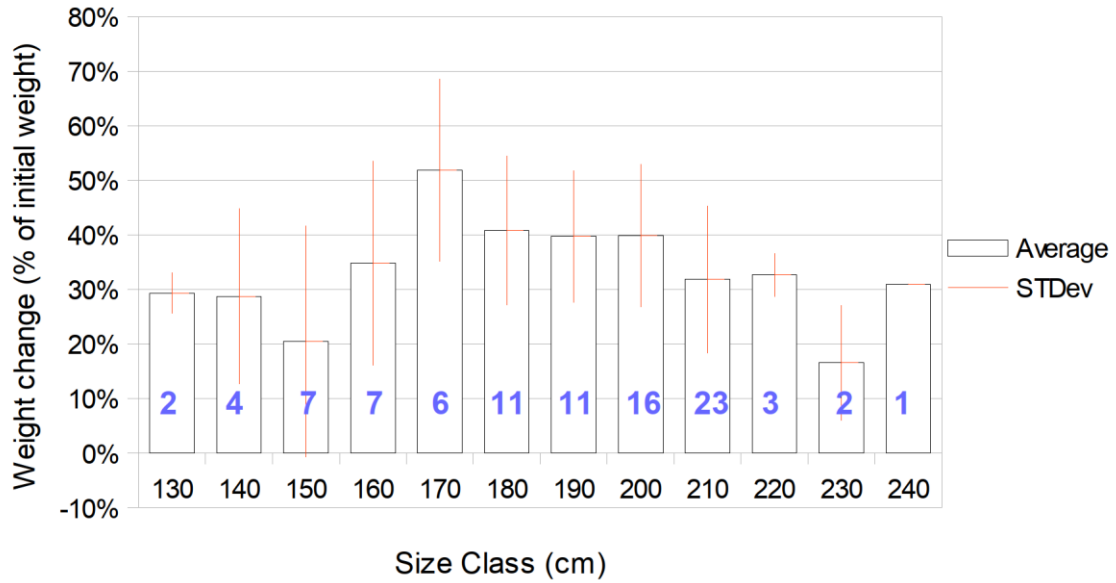


Figure 8 - Weight change as a function of initial size. Bars represent the average and the number in blue the number of fish in each class.

Nevertheless, the increase in weight for the tagged fish was within the expected values since their weight at harvesting is within the interval of untagged fish (Figure 9).

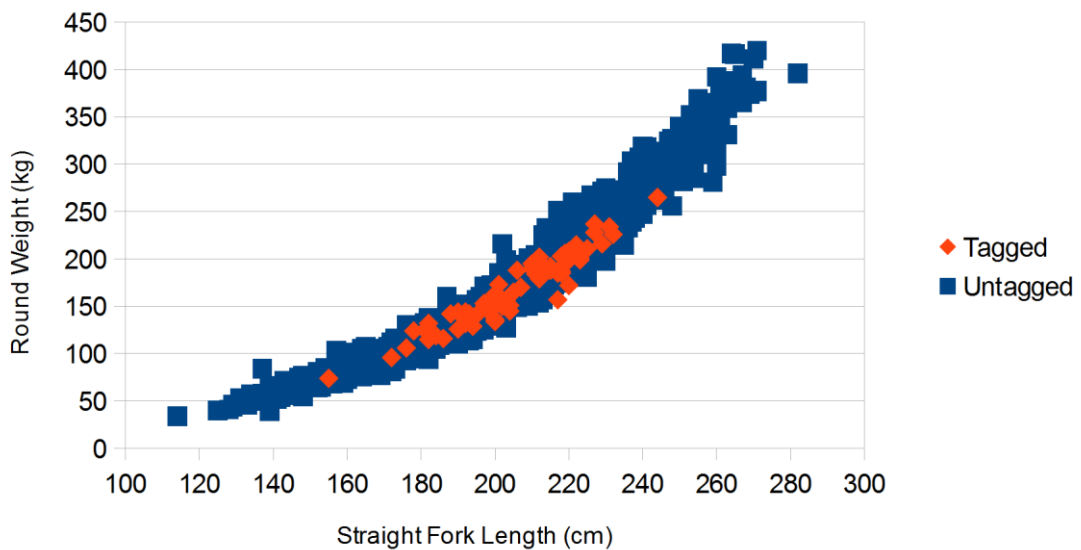


Figure 9 - Straight Fork Length vs Round Weight of all fish in the monitored cage

An ANCOVA test showed that the factor Tag is not affecting the relation between length and weight of harvested fish ($p\text{-value} > 0.95$) which means that tagging and handling the fish did not significantly affect the weight gain of fish.

Conclusions and Recommendations

- The overall weight increase for the harvested fish varied between 12% and 74%, with a mean of 40.3%. Those fish were fattened in the Tunipex BFT farm between 116 and 132 days between tagging and harvesting.
- The mortality of tagged fish over this project was relatively high but lower than on the 2019 study (35% vs 42%).
- The results of the comparison between direct measurements and values derived from the stereo camera are further evidence of the inadequacy of the L-W equation used in the AM100 for the low condition tunas after spawning which constitute the majority of the catch in this area.
- Tag retention was also extremely high during the project (90.7% of fish retained both tags, 2.8% lost one tag). We note that the conventional tags used showed again very high algae growth after just some weeks after tagging. This could explain that tags were not detected during harvesting thus accounting for the 7 unrecovered fish (or it could be due to loss of both tags). We would recommend using tags with some anti-fouling to prevent algae growth in the future.
- Switching from single barbel tags inserted in the muscle to double barbel tags inserted through the dorsal increased substantially the number of recovered fish. The operational change from multiple harvest events over time to a single final event could also have contributed to the increased recovery. However, the sampling design does not allow testing which factor was more relevant.
- Tagging and handling does not seem to have affected weight gain significantly since at harvesting tagged fish weight was not significantly different from non-tagged fish of the same length.

- We suggest to carry out further studies to investigate if mortality was induced by the handling stress or by the low fish condition,
- Another line of research should investigate if suboptimal growth of smaller fish occurs due to competition with larger fish, allowing for optimization of fattened fish

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