Contract Amendment Nr. 1
To the Short- Term Contractor BFT Growth in Farms Study (ICCAT GBYP 05/2020-d) of the

ATLANTIC-WIDE RESEARCH PROGRAMME FOR BLUEFIN TUNA (GBYP Phase 10)

Final Report (Deliverable 5)

Zadar, July 26th 2021

Contractor

Pelagos net farma d.o.o., tuna farm company

This project is co-funded by the European Union

Background

Following intensive fattening operations in several Mediterranean countries in the late 1990s a very strong impact on BFT fisheries and its management was made. With the purse seine captured fish being transferred to fish farms, apparently, catches were frequently under-reported, and the size composition of fish entering fish farms remains a challenge. The appropriateness of a non-invasive stereoscopic video monitoring with respect to the size estimates over the time of farming/fattening continue to be discussed. In spite of some available data on bluefin tuna size structure at stocking, the size information at harvesting or from markets do not always match predicted growth factors.

By the beginning of 2000s a real farming was conceived in Croatia. This was then followed by a sudden increase in demands for bluefin tuna juveniles, the majority of which are obtained by purse seine fishing. Juvenile fish of ages 2-3 years are not landed but transferred to towing cages, then placed into the grow-out cages where they are raised up to three years.

At the 21st Meeting of the Commission, the SCRS was requested to provide an update on the potential growth rates of bluefin tuna in farming/fattening facilities, with the aim of improving the consistency of the growth rates derived from eBCD s, as stipulated in paragraph 28 of Rec. 18-02. Namely, it is recognized that the growth rates derived from the eBCDs are not coherent with the SCRS tables and growth rates published in 2009 by the SCRS.

Therefore the SCRS has asked CPCs to undertake studies as to consider the difference among geographic area (including Atlantic and Mediterranean), and the different farming/fattening strategies in providing an update on the potential growth rates of bluefin tuna. In performing this task, the SCRS should invite independent scientists who have appropriate expertise to review the analysis. Relevant CPCs should ensure that the scientists for the studies can have access to and, as required by the protocol, assistance to carry out the trials. Consequently, the Atlantic-Wide Research Programme for Bluefin Tuna (GBYP) was committed to undertake scientific studies in selected farms to identify growth rates including in weight and size gains

of recognizable individual fish during the farming/fattening period along the eastern Atlantic and Mediterranean.

Given the particular practice of the Croatian tuna farming along the central eastern Adriatic capturing the juvenile fraction of the Bluefin tuna by purse seine fishing, PELAGOS NET FARMA D.O.O. was contracted to carry out the first phase of the GBYP growth in farms study in one of the identified case studies (contract ICCAT GBYP 09/2019-d), specifically in Croatian waters. This study was adapted to the extent possible to the normal farming procedures and farming strategy of farm selected, that is representing general bluefin tuna farming practice in Croatia where caging may last from one and half up to three years. An experienced scientific team supported contractor to performe required tasks, with particular references to seasonal measurements of growth rate of caged fish by means of steroscopic camera system (SCS), as well as the collection, elaboration, and analysis of a data on initial length distribution, data from intermediate sampling and data on size and weight at the end of farming period by SCS and direct measurement during harvesting operations with particular focus on tagged fish.

Objectives

Overall objective is to obtain reliable data to estimate growth rate of farmed bluefin tuna under given range of conditions and situations including size structure, growth period, environmental and zoo technical factors. Specific objective of this document is to integrate scientific information provided by four (4) reports already submitted.

Activities, Material and Methods

Juvenile Bluefin tuna were collected in 2019 from 6 individual purse seine catches during regular fishing season in the Adriatic (Table 1). At rearing site fish were distributed into two experimental cages, one of this containing 1506 fish (cage HRV011001, group EBCD HR19900165-G-LT01) and 1688 (cage HRV011005, group EBCD HR19900216) respectively (Figure 1).

Catch date	Fishing vessel name	Location (latitude-longitude)	Estimated number of fish	Estimated biomass (kg)
Jun 4th 2019	TACOMA	*42°53'70" 14°46'10"*	214	1880
Jun 30th 2019	KALI	*43°2'3" 15°7'7"*	870	8700
Jun 30th 2019	TACOMA	*43°3'68" 15°4'3"*	1150	11500
Jul 1st 2019	PELAGOS II	*43°1'3" 15°7'36"*	332	6580
Jul 2nd 2019	PELAGOS II	*43°2'38" 15°7'31"*	165	3160
Jul 2nd 2019	TULJAN DVA	*43°2'34" 15°8'22"*	463	8986

Table 1.

The experimental trial was performed at the commercial tuna farm Balabra located in the central eastern Adriatic which capacity is 1.200 tonnes in 14 cages (ICCAT number ATEU1HRV00011). The farm is located about 9 Nm from company's headquarters in the port of Zadar-Gazenica (Figure 1, 2 and 3).

Figure 1. Position of two experiment cages #1 and #5 within Pelagos-net farm near Balabra island

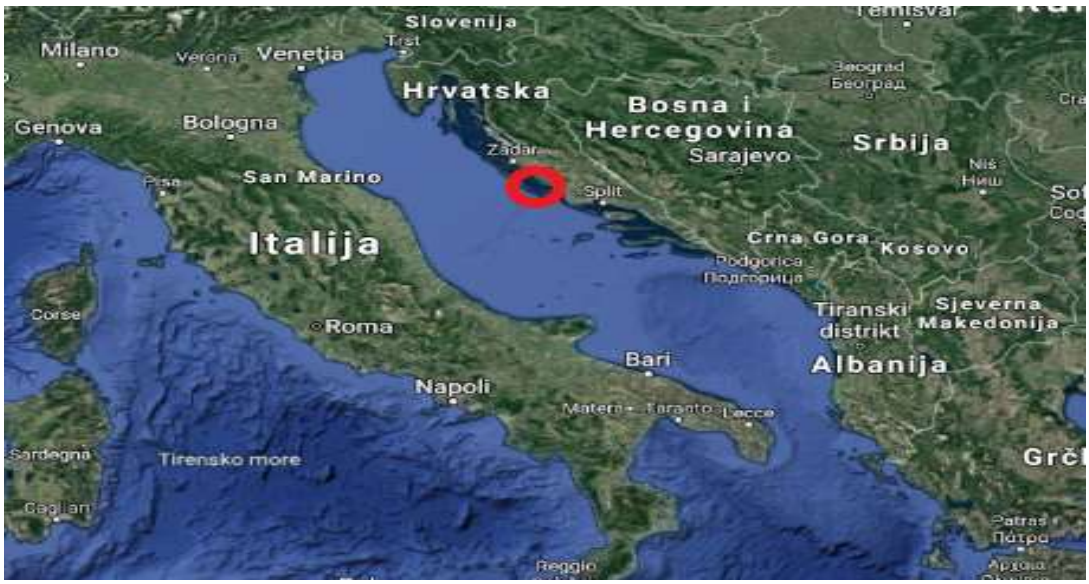


Figure 2. Surrounding area of Pelagos-net farm located in the East Mediterranean Sea

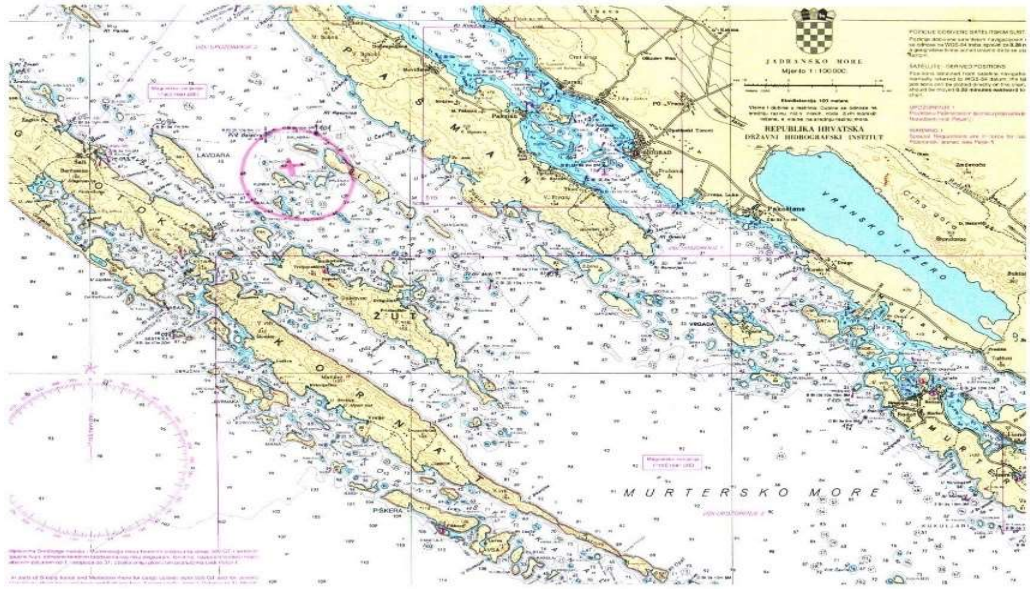


Figure 3. Surrounding area of Pelagos-net farm located in the central Eastern Adriatic

Based on standardized age-length key for the East Atlantic and Mediterranean (Rodriguez-Marin at al., 2016) majority of fish belong to age 2+, and far less to age 3+. Captured fish estimated in total quantity of 3.194 pcs and 40.806 kg, average weight 13 kg were kept alive, transferred to towing cages.

The initial size structure of the fish in the holding cage obtained by SCS is presented in Figure 3.

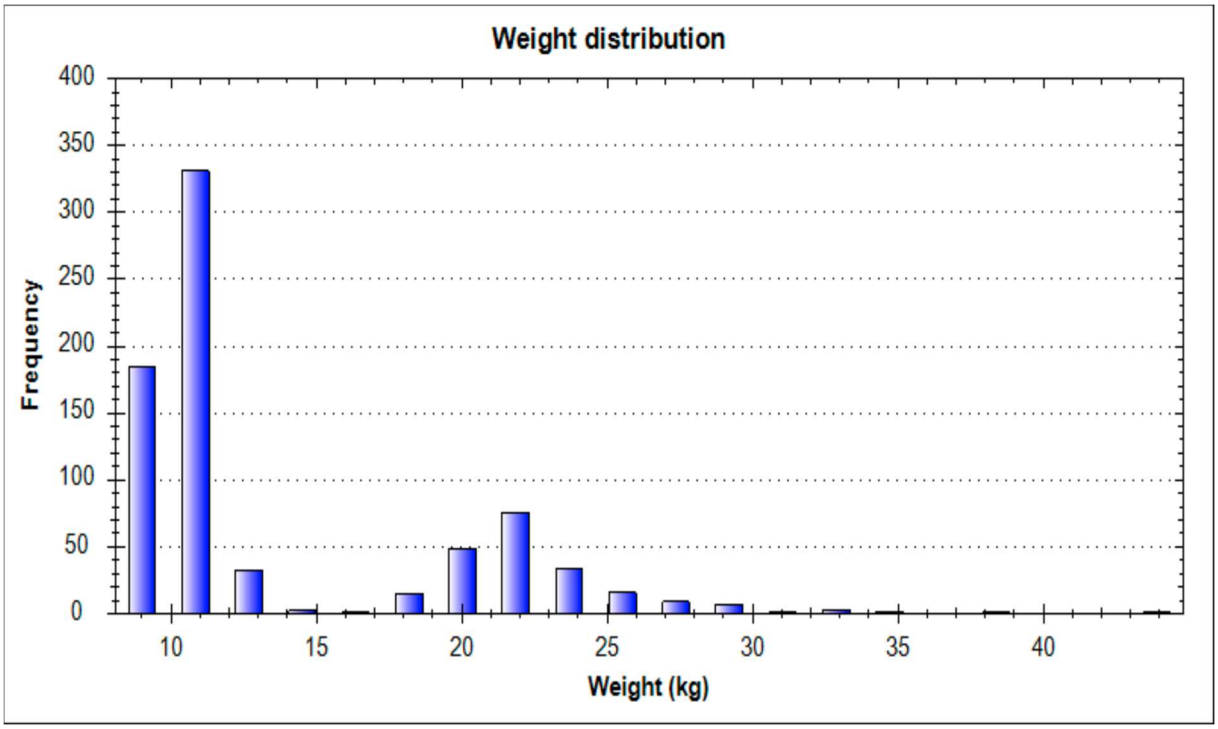
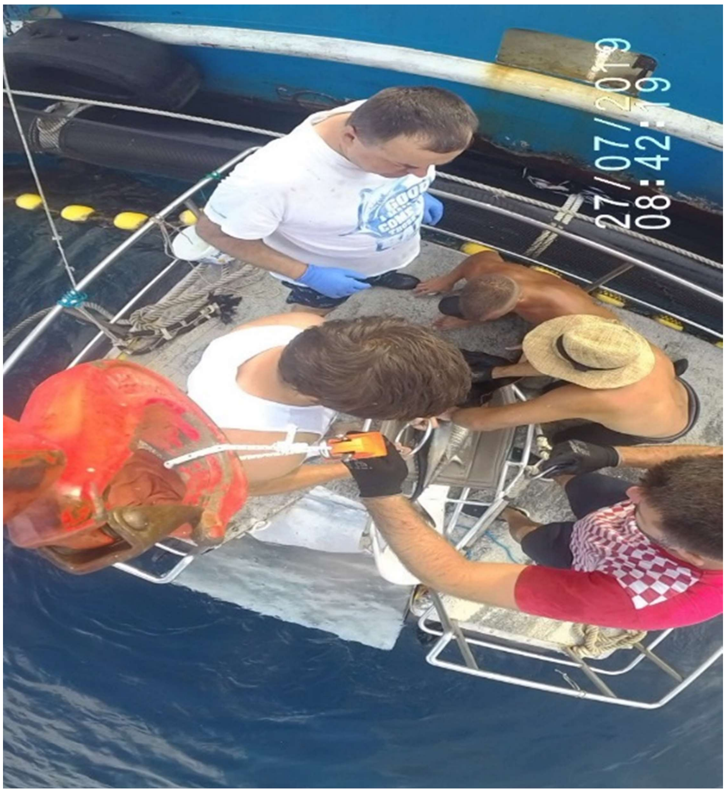


Figure 4. Weight distribution of fish at stocking, estimated by Stereo camera video system and converted from fork length (FL cm) to round weight (RWT kg).

To get insight into growth rate over fixed growing period, internal tags were inserted directly to the surface of the fish aiming not to affect the animal's behaviour, or cause injury by rubbing or tearing the tissue where it is inserted. The basic information, such as when and where it was tagged and the size at releases into holding system was recorded as to match up the release information with the recapture ones, and finally to learn how much captive tuna grew during that time period.

All tagged fish were measured in SFL (nearest cm) and weight (RWT in kg) (Figure 5a-5d).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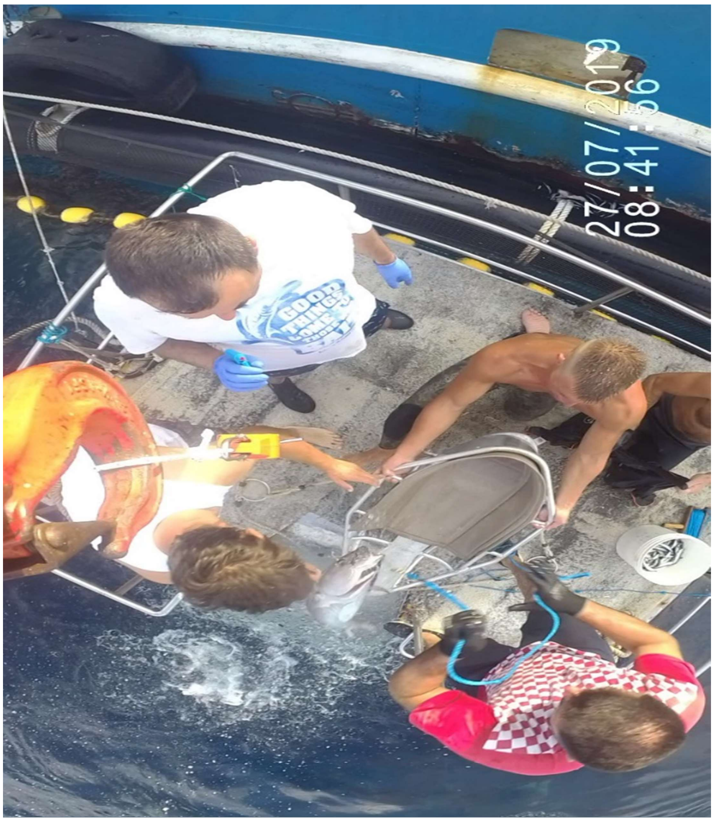
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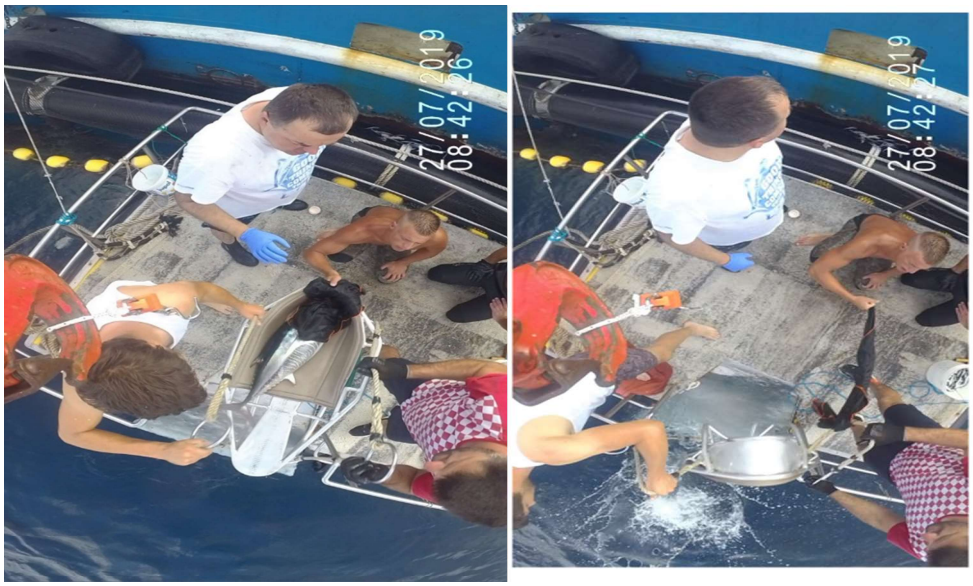


Figure 5a, 5b, 5c, and 5d. Fishing, tagging, measuring in length (FL cm) and round weight (RWT kg), and releasing back into a rearing cage

External tagging trial on July 12th 2019.

In total, 12 bluefin tuna juveniles were sampled from cage HRV011005, tagged with external clips combined with the cutting of the second dorsal fin. In the first week following tagging two fish died, both were examined and processed by the scientists involved.

Internal PIT tagging: from July 26th to July 31st 2019.

During five days, total of 206 bluefin tuna juveniles were hooked and marked by inserting PIT tag into a muscle on the top of the head. Age of tagged fish were estimated 2+ years (approx. 160 ind.) and 3+ years (>40 individuals).

Following PIT tagging the oxytetracycline was administered at the prescribed dose (Figure 6a-6d). The time needed to complete tagging operation was recorded.

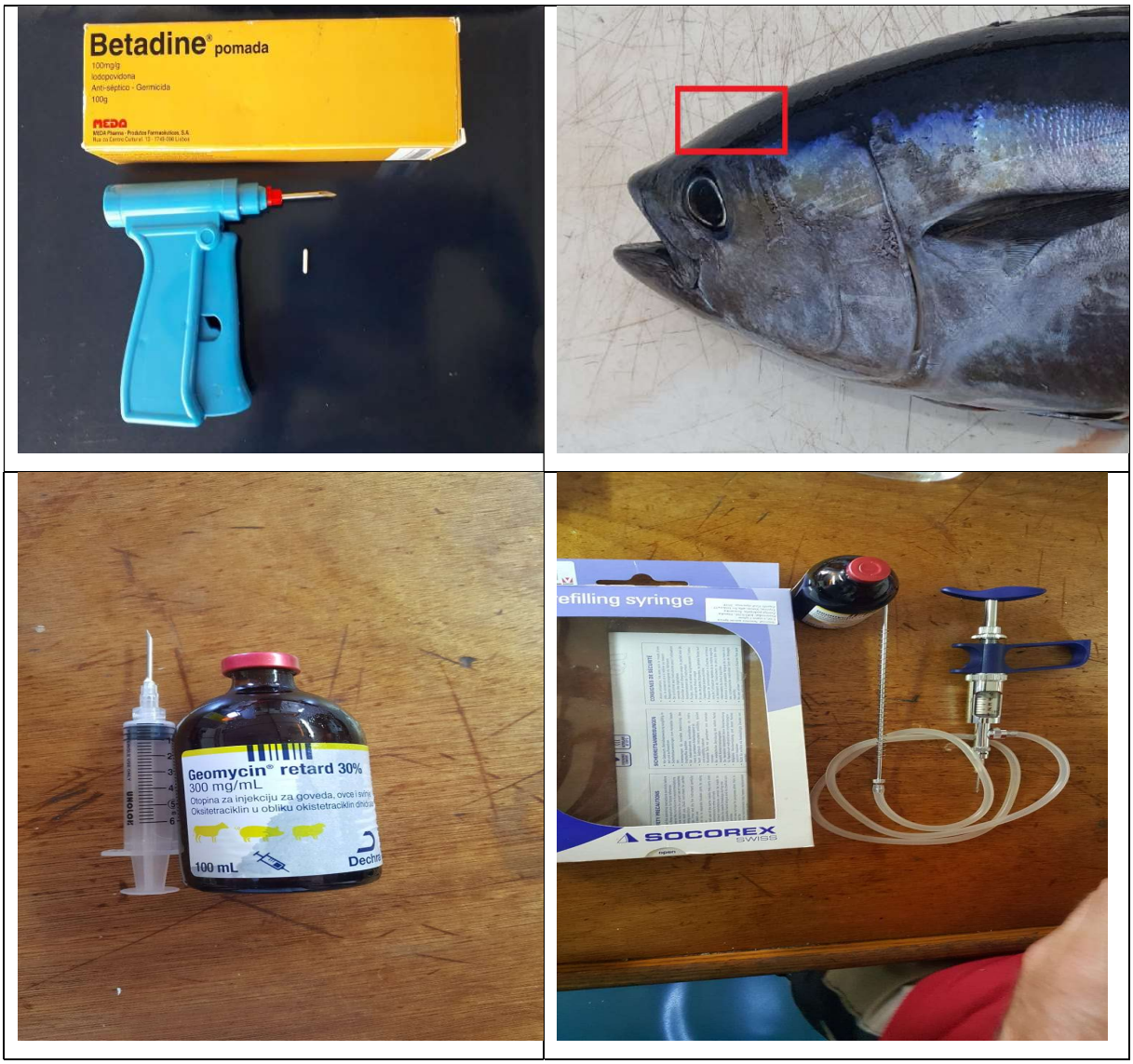


Figure 6a, 6b, 6c, and 6d. Place to insert the PIT tag by an applicator, and iodine based antiseptic - geramycin cream to prevent infection (upper). Oxytetracicline applied to each tagged fish in a prescribed dose (down)

After the tagging was done, the divers reported that tagged fish separated itself from the respective school and seemed quite dizzy. However, there was only a total of two morts in the cage HRV011005, which were examined and processed by the scientists engaged. Eventually, ten days after tagging, on July 31st, the isolated tagged fish joined the rest of the school showing an usual behaviour.

The tuna were fed to satiety – AD LIBITUM in quantity presented in Figure 7. The fish were fed daily when possible, usualy early in the morning with defrost pelagic fish (sardines, herring, anchovy, mackerel).

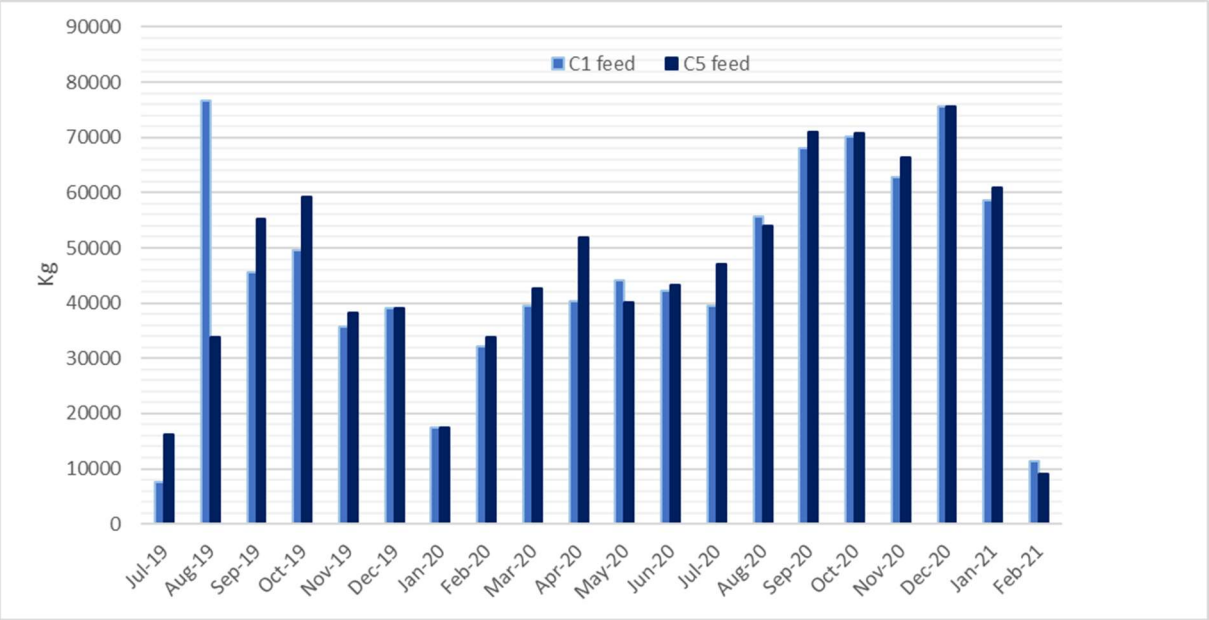


Figure 7. The quantity (kg) of feed distributed in two experimental cages by months during breeding period.

Temperature (T°C) and oxygen saturation is monitored (Figure 8), as well as daily feeding activities, including quantity of food given.

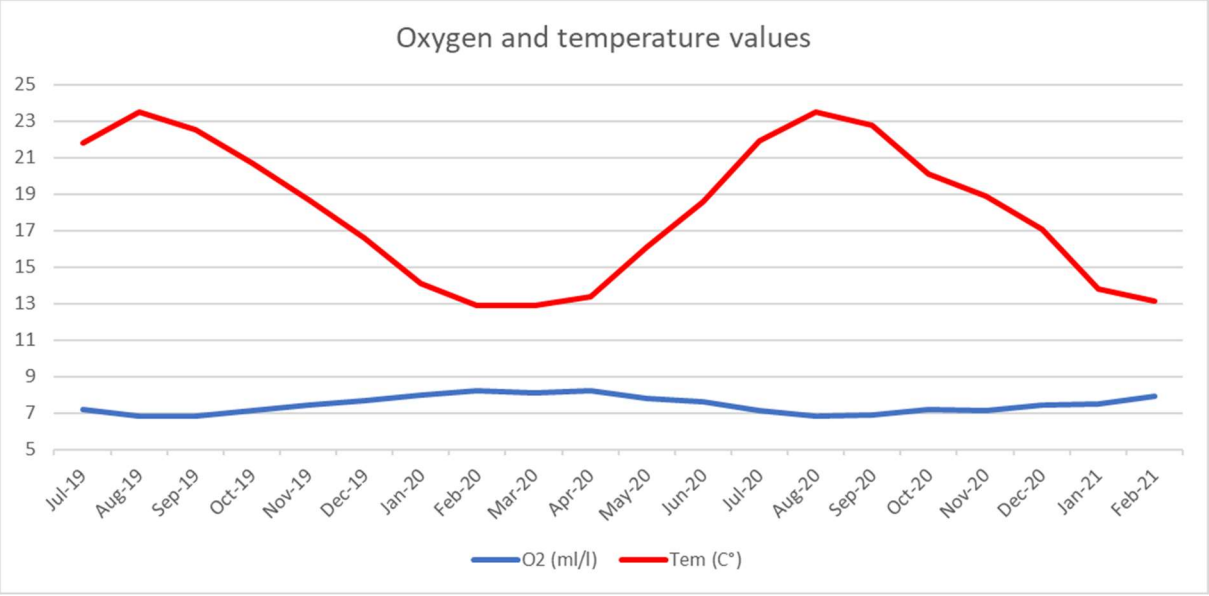


Figure 8. Oxygen (ml/l) and temperature (T°C) at the farm during the experiment

The length of fish was recorded seasonally by stereoscopic camera system in two cages and converted into weight. It was assumed that 30 minutes of recording should be enough to atract most of the fish to move around submersed underwater camera, and to get a representative sample of the bluefin tuna that have been stocked. Three replicates were conducted at each cage. By 30 minutes recording it is expected to cover at least 20% of rearing population by readable images, that should be satisfactory for biomass estimate in grow out cages. To stimulate the fish passing through the field of view of the cameras, bit was distributed over the surface of the cages. Differences among replicates were tested by nested ANOVA (StatSoft).

Figure 9. Stereoscopic camera system AQ-1 AM 100

Recorded imagery is stored directly onto the hard drive of the computer. To avoid errors, filmed BFT were only measured when the bodies are straight or close to be straight. Conversion of SFL obtained by underwater SCS to round weight (RWT) was made by means of L-W relationship integrated into camera software:

$$RW = (2.3139*10^{-5}) *FL^{2.9840}$$

May sampling

On May 2020 samples of 20 specimens from each cage (HRV011005 and HRV011001) with tagged BFT (40 specimens total) were fished and analyzed for the biometric parameters, with particular references to gonad maturity status. Among 40 specimens sampled from both

experimental cages only one tagged fish was detected. After detecting the PIT tag the tagged fish were examined for signs of tissue reaction to the tag.

Sampling at harvesting

Fish were sampled at regular harvesting, killed and individually measured for SFL with measuring callipers (MC) and subsequently weighted (±0.1 kg) against which the SC size data estimates could be compared. A length frequency histogram at harvesting show tagged fish were almost equally distributed over all size classes in experimental cages (Figure 10 and 11).

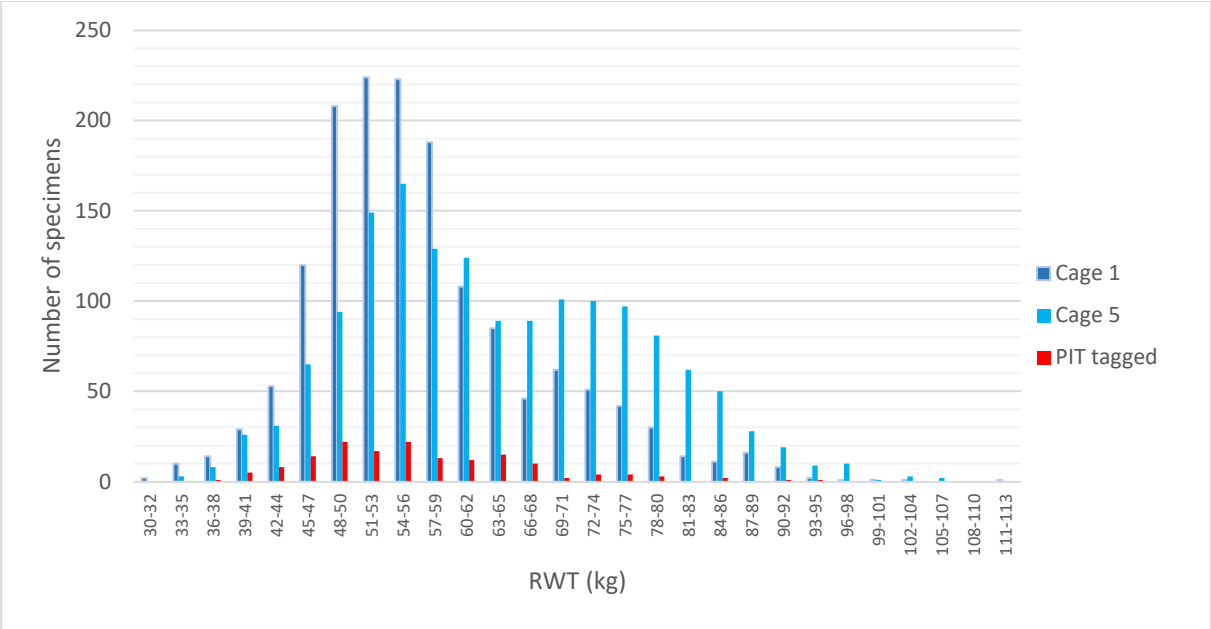


Figure 10. Size distribution of tagged bluefin tuna in cage 1 and cage 5 at harvesting

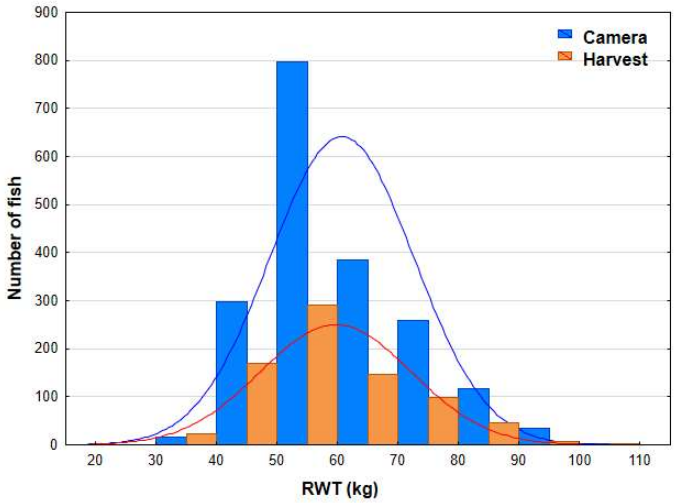


Figure 11. Stereoscopic camera system (SC) sampling design that includes three replicates for two cages at the farm. Differences among replicates were tested by nested ANOVA. No significant differences were recorded among replicates for each cage.

After detecting the PIT tag fish were weighted and measured in size, first spine of dorsal fin and otholits were sampled, and examined for sign of tissue reaction to the tag.

Results and Discussion

Fin cliping as an external tag showed no good retainability. Out of 12 tagged fish only one was detected at the harvesting.

Sumarized results of PIT taging operation are presented in Figure 12. Registred mortality was 1% and 22% of tagged fish were not recovered.

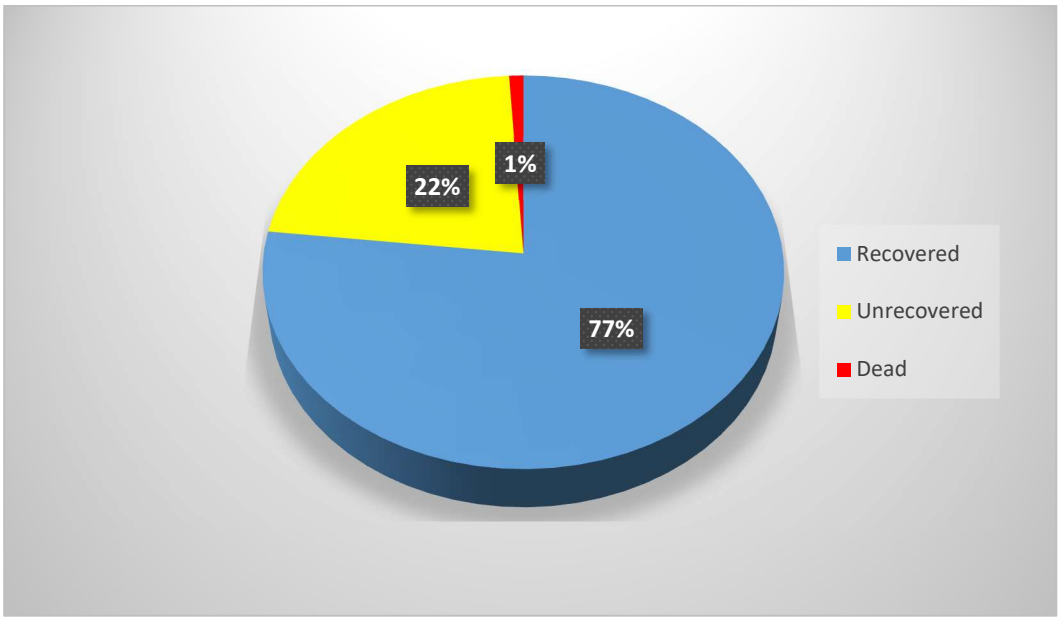


Figure 12. Sumarized results related to recoveredd, unrecovered and dead bluefin tuna juveniles tagged at s Net Farm (Adriatic) after 19 months of caging

From the total tagged 206 fish, 158 were harvested, while 46 were not recovered (Figure 13) . It is worthy to notice that only 2 fish died, both during tagging operation.

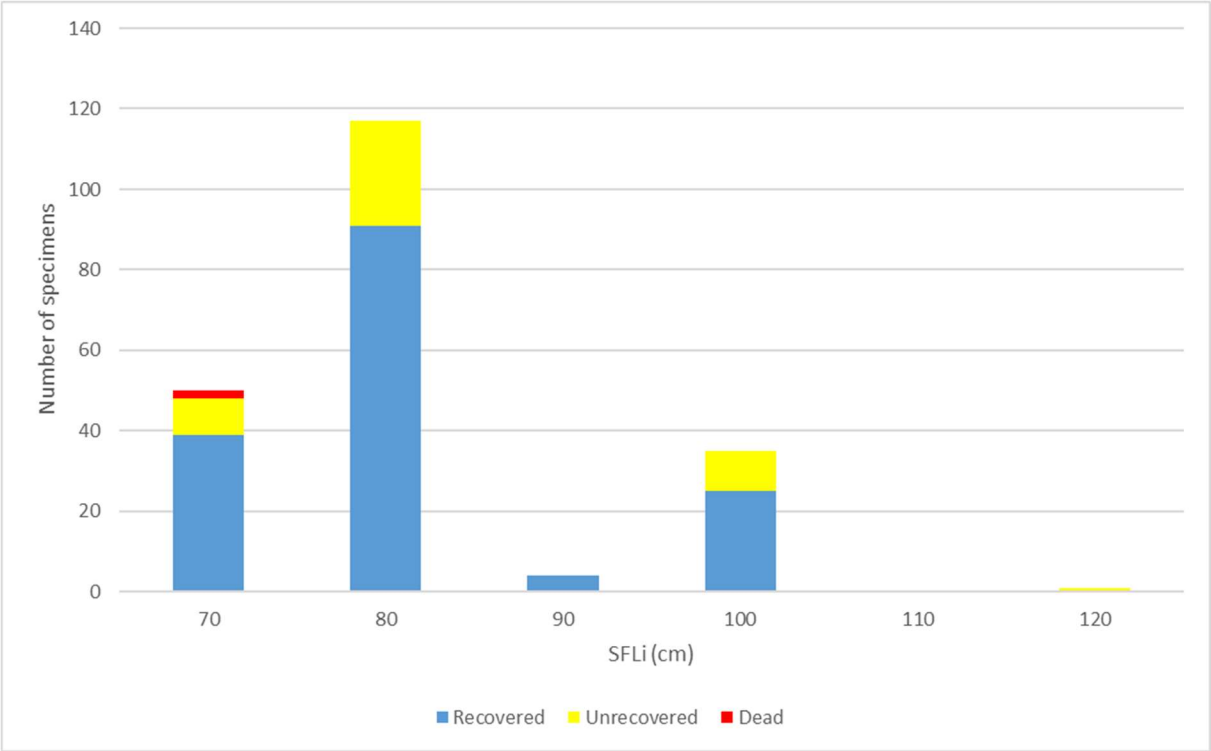


Figure 13. Distribution of harvested, unrecovered and dead tagged bluefin tuna from different initial lenght classes.

The proportion of the unrecovered fish could be result of a detector failure during harvesting operation as well as failure of readers being forced with harvesting routine procedures . Further factor, at least theoretically, could be loss of the tag. However, we believe that the loss of tag , even possible, is less likely. Following surgical examination, we have concluded that after a year and half in the host the tag was not encapsulated with surrounding tissue. Because of complete healing of wound site, and no visible signs of inflammation or rejection, it suggests that an encapsulated tag appears to be biologically inert and/or BFT did not recognize it as foreign body.

Figure 14. Sampled fish having PIT tag inserted in the muscle head. No visible inflammations were observed.

The epidermal pigmentation of wound sites appeared normal enabling to locate injection site visually. Complete healing of the wound had occured at sampling 9 months after insertion of PIT tag (May sampling). The glass-encapsulated tag appears to be biologicaly inert with no sign of inflamation of wound area tissue (Figure 14).

The presence of the tag did not apear to adversly affect the growth or condition (L-W relationship) of the tagged fish (Figure 15).

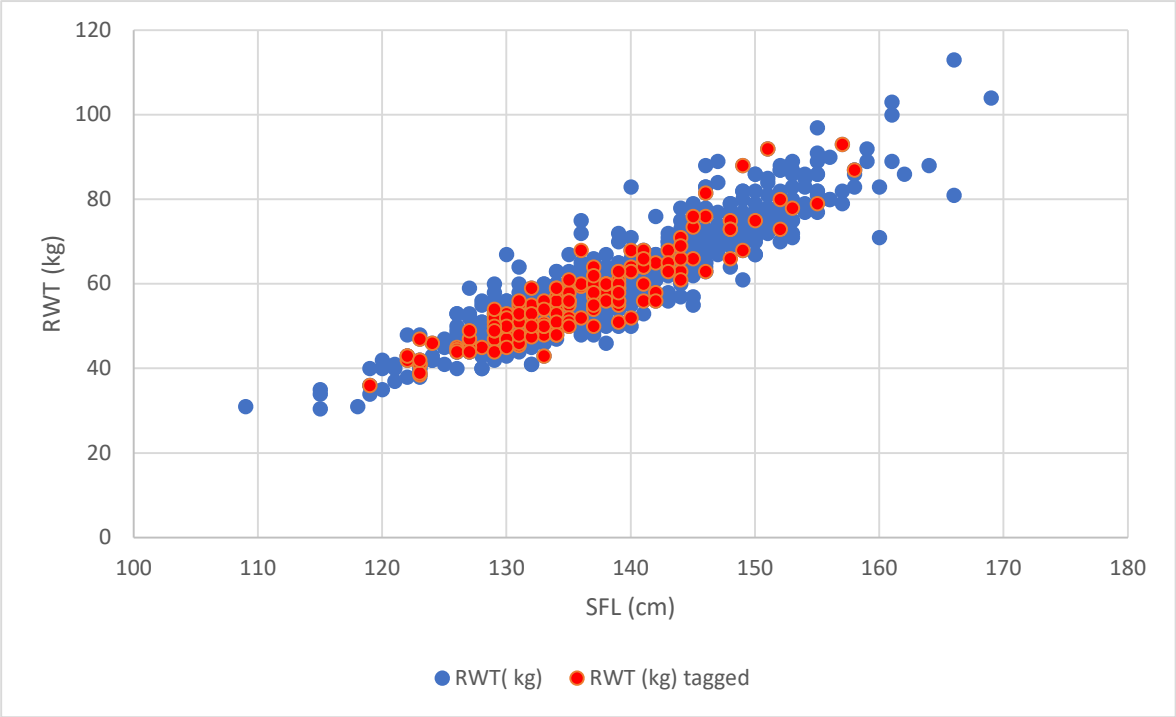


Figure 15. LW distribution of tagged (n=156) and non tagged (n=938) bluefin tuna from experimental cages #1 and #5.

An average body weight increase (BWI) over entire rearing period was around 500 percent. No significant difference in BWI was found between cages when the non-parametric Mann-Whitney U test was applied (Figure 16).

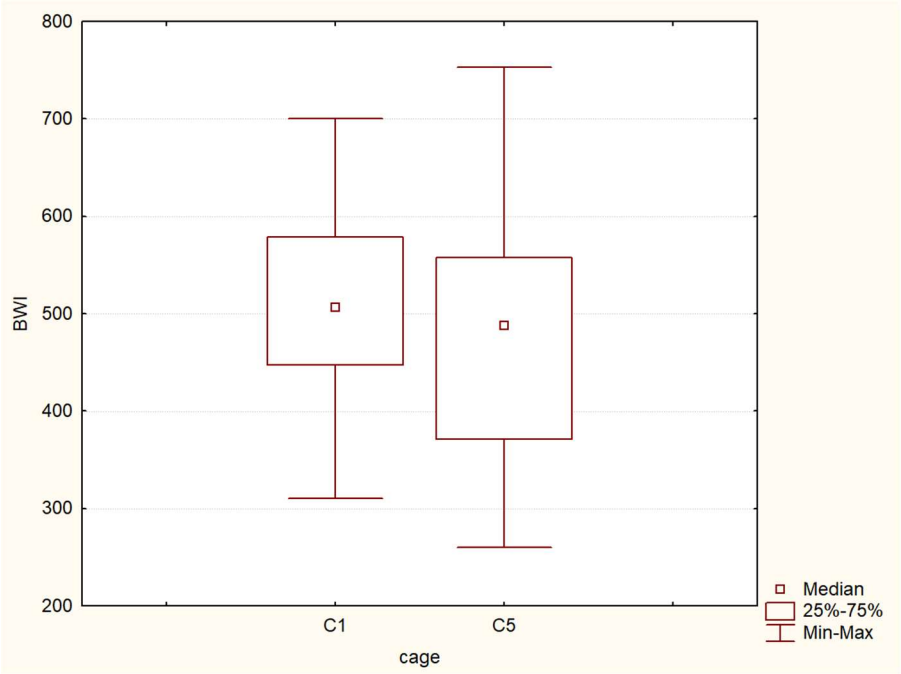


Figure 16. No significant difference in BWI was found between cages when the nonparametric Mann-Whitney U test was applied.

In relation to initial size and/or age group BWI of tagged fish age 2+years (n=127) was in average 525.1 percent (SD=81.2), while age group 3+ years (n=29) was 334.4 (SD =54.4) (Figure 16). This is in line with Rodrigues-Roda (1964) who studied stages of maturity of eastern atlantic bluefin tuna at different size and came to conclusion that the growth is rapid precisely up to the lenght of 115 cm, and after this growth rate slow down. Maturation greatly affect the growth rate. It is presumed that most of 3+ years fish could spawn and consequentely contributed to decrease in food consumption from May to July (Figure 17).

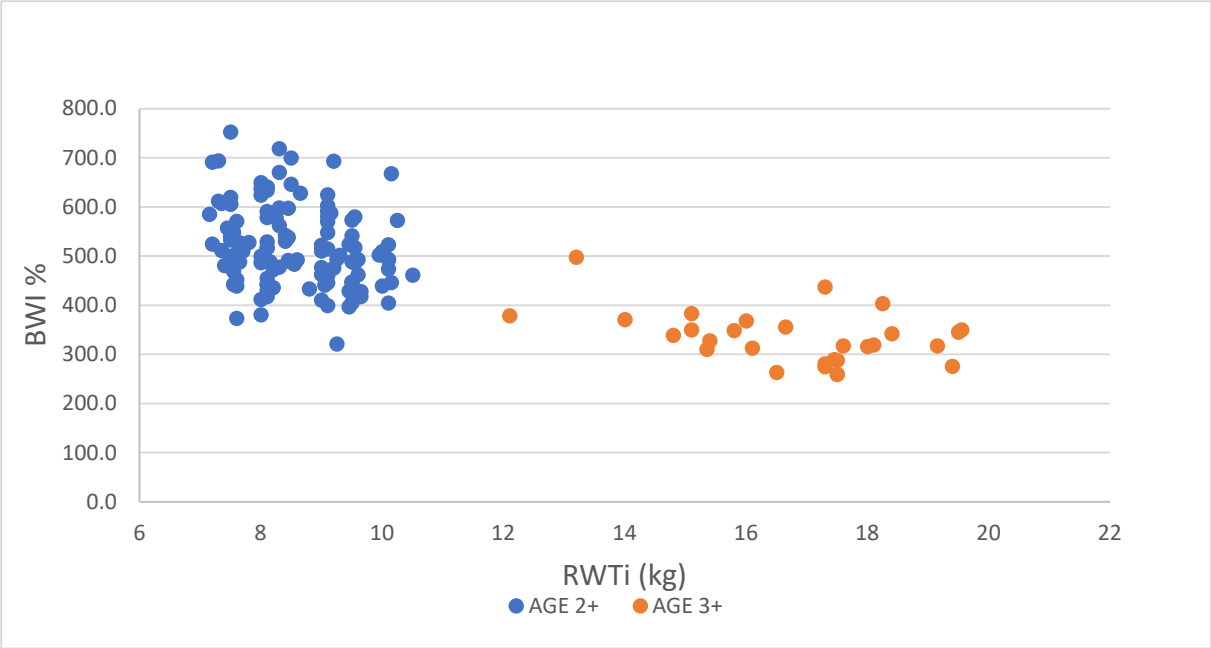


Figure 17. Body weight increase (BWI) in relation to initial weight (RWTi) corresponding to initial round weigt (RWTi) and estimated age class of tagged bluefin tuna respectively in two experimental cages

These differences are significant (Figure 18), and may be explained by relatively high variability in size and/or age frequencies related to sexual activity of juvenile and adolescent bluefin tuna.

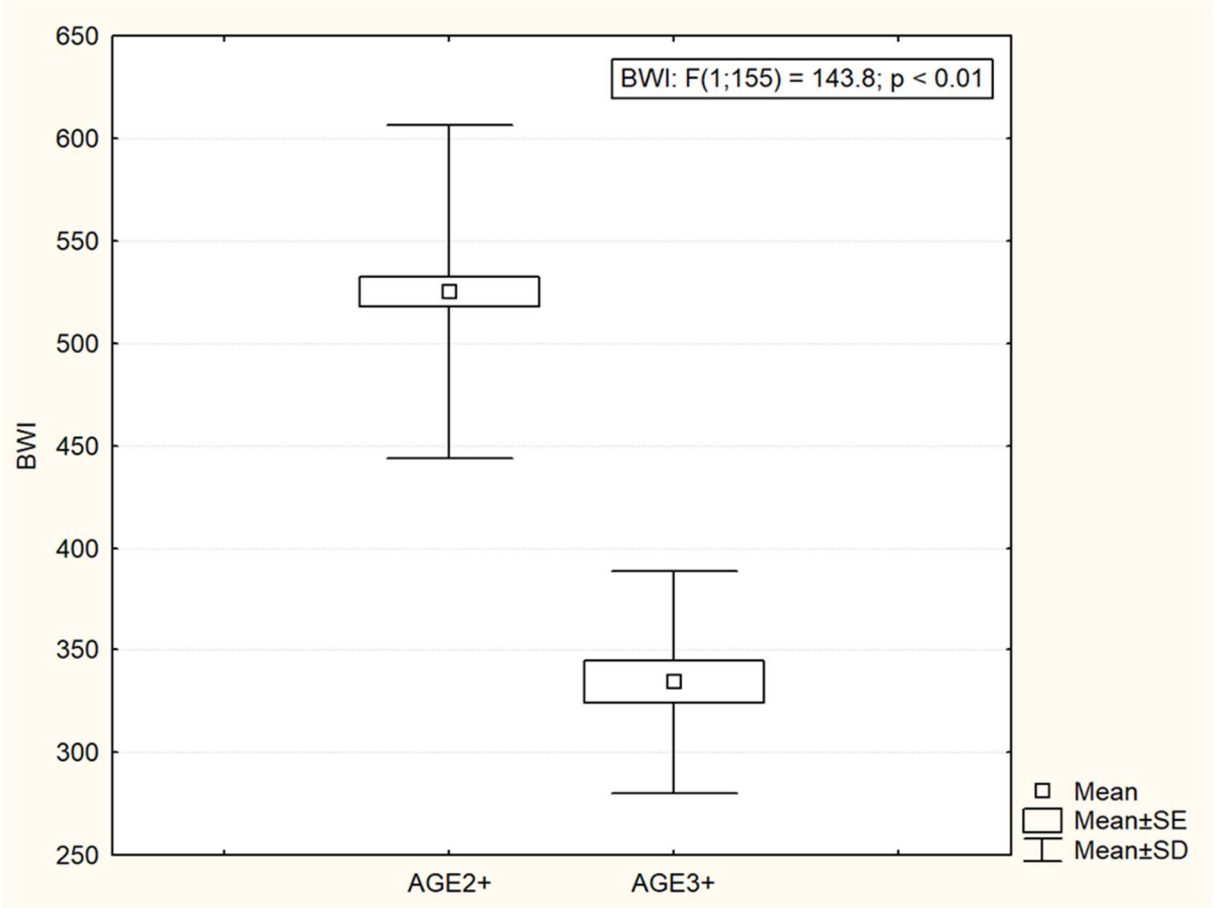


Figure 18. Body weight increase (BWI) of tagged tuna at age 2+ and 3+ directly measured at harvesting. ANOVA indicated significantly different body weight increase between ages of tagged tuna.

In May sampling we have found that larger fish have already initiated gametogenesis. From GSI it is evident that advanced age group, both females and males (estimated initial age 3+ years) are capable of generating mature gonads during the current spawning season. (Figure 19).

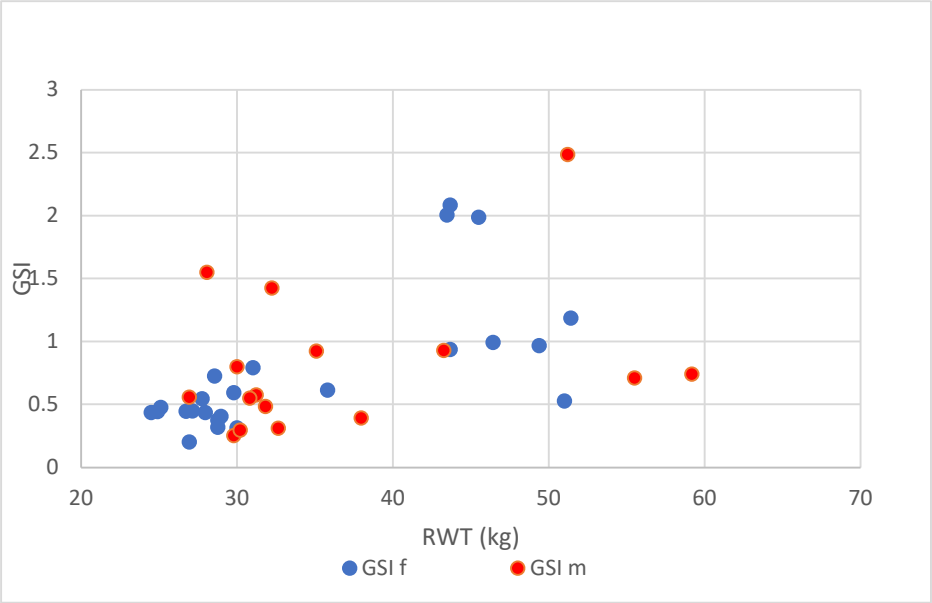


Figure 19. Gonadosomatic index (GSI) of blue fin tuna after 10 months of farming, sampling in May

Consistent with Corriero et al. (2005) gonad maturation could be completed at GSI > 0.5. The presence of vitelogenic oocytes (Figure 20) are reliable indicators of sexual activity (Corriero et al. 2020). From the data presented in Figure 17 and Figure 18 it is evident that maturing fish during their fourth year of life (3+) show a lower mean body weight increase compared to younger age class fish (2+).

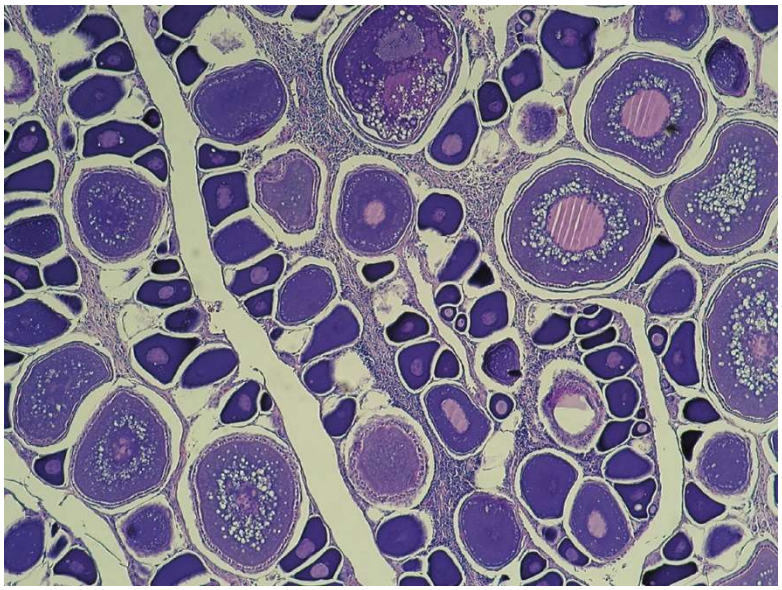
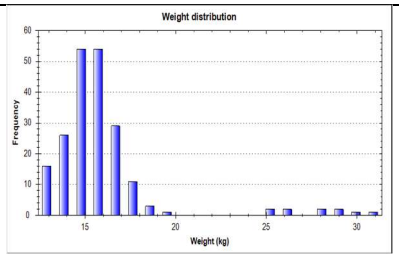
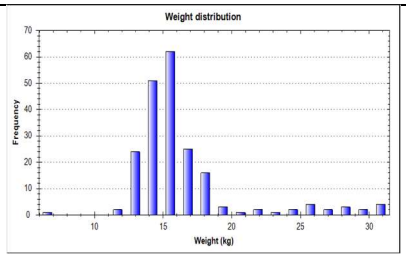
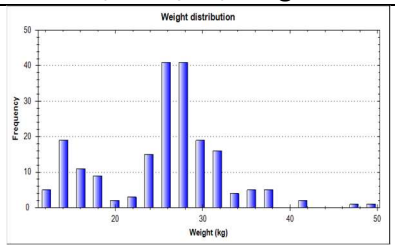
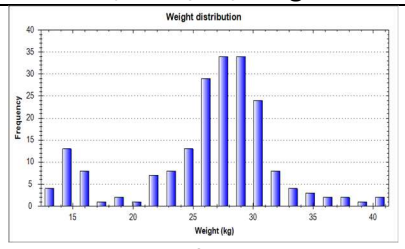
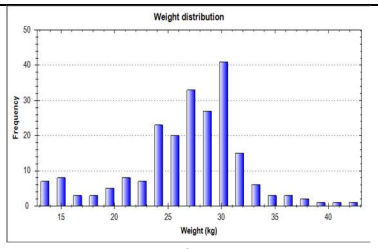
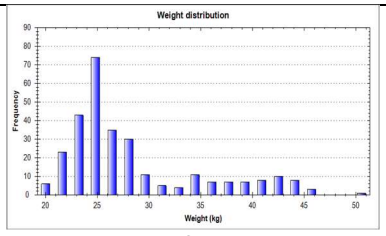
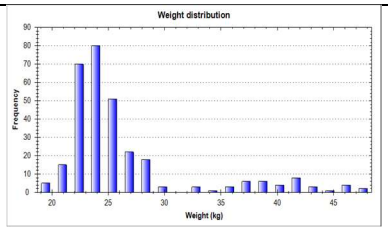
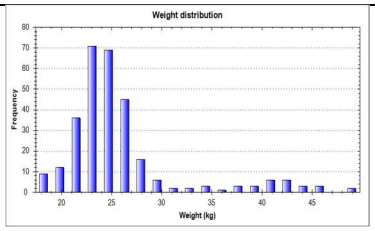
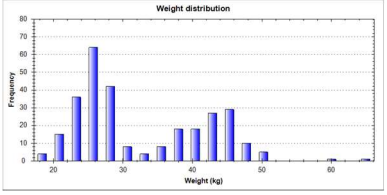
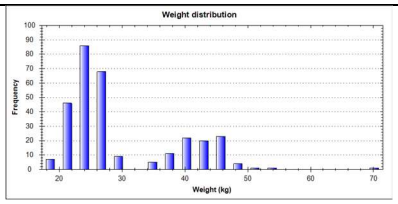


Figure 20. Histology of bluefin tuna ovary sampled in May showing advanced gonadal development

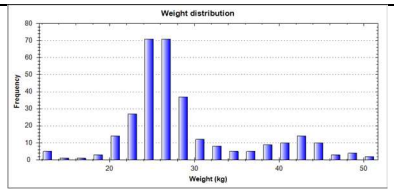
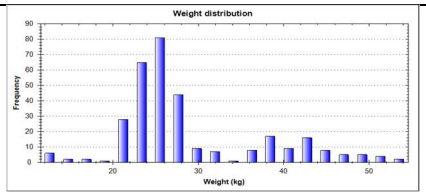
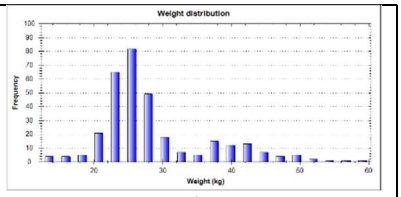
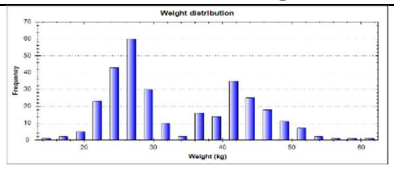
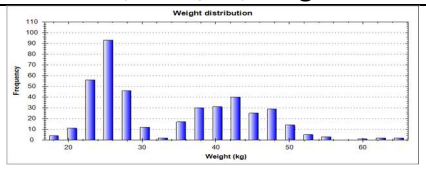
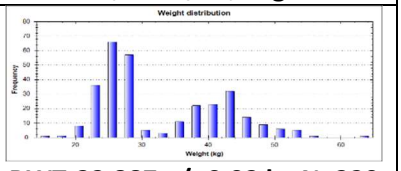
27./28. August 2019 AQ-1 weight reports C1 and C5

C1			
	RWT-15,876 +/- 3,03 kg N=204	**RWT-16,360 +/- 3,98 kg N=205**	
C5			
	RWT-25,550 +/- 6,87 kg N=199	**RWT-26,309 +/- 5,63 kg N=200**	**RWT-26,770 +/-5,37 kg N=217**

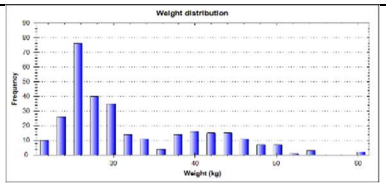
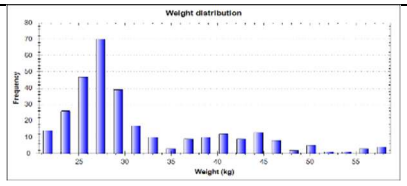
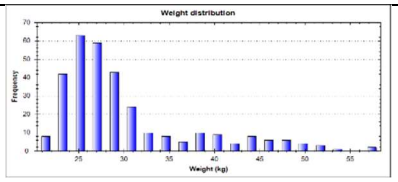
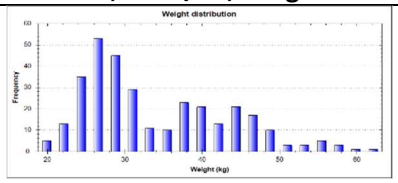
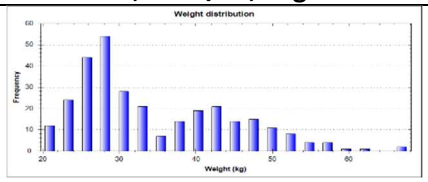
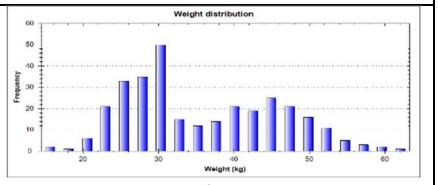
27./28. December 2019 AQ-1 weight reports C1 and C5

C1			
	RWT-28,293 +/- 6,61 kg N=293	**RWT-26,295 +/- 5,96 kg N=305**	**RWT-25,766 +/- 5,86 kg N=298**
C5			
	RWT-32,587 +/- 9,3 kg N=290	**RWT-29,641 +/- 8,95 kg N=304**	

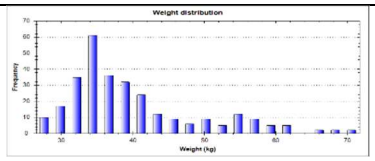
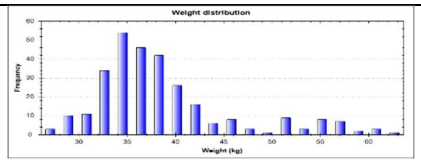
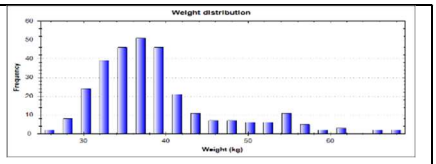
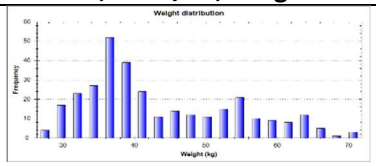
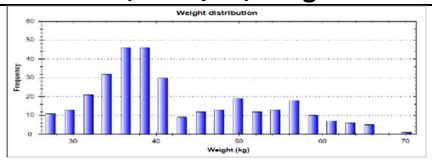
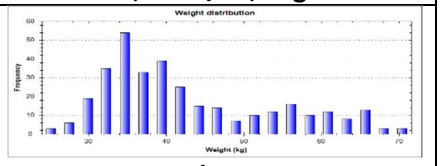
9./10. April 2020 weight reports C1 and C5

C1	 RWT-28,768 +/- 9,1 kg N=360	 RWT-28,802 +/- 8,49 kg N=362	 RWT-28,561 +/- 7,5 kg N=377
C5	 RWT-33,515 +/- 9,56 kg N=307	 RWT-33,862 +/- 10,35 kg N=319	 RWT-32,887 +/- 9,93 kg N=320

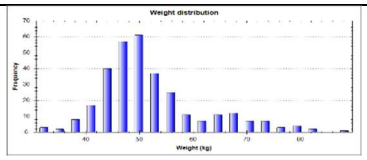
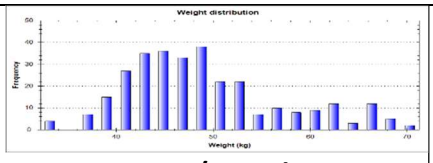
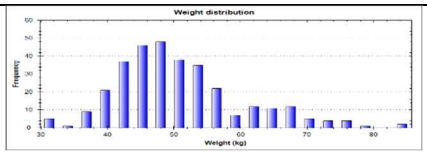
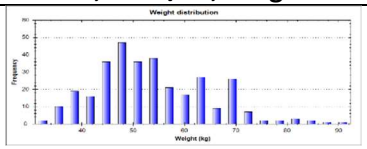
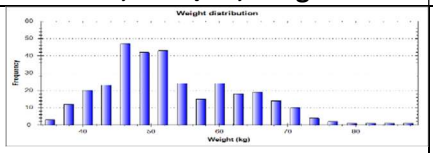
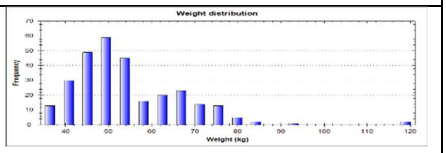
18./19. May 2020 weight reports C1 and C5

C1	 RWT-32,147 +/- 8,49 kg N=307	 RWT-31,381 +/- 8,3 kg N=303	 RWT-30,028 +/- 7,4 kg N=315
C5	 RWT-33,883 +/- 9,16 kg N=322	 RWT-34,388 +/- 9,92 kg N=304	 RWT-35,694 +/- 9,96 kg N=313

11. August 2020 weight reports C1 and C5

C1	 RWT-39,878 +/- 9,16 kg N=293	 RWT-38,628 +/- 7,16 kg N=293	 RWT-38,884 +/- 7,9 kg N=299
C5	 RWT-43,425 +/- 10,5 kg N=318	 RWT-42,528 +/- 9,78 kg N=324	 RWT-42,847 +/- 11,03 kg N=337

11./12. November 2020 weight reports C1 and C5

C1	 **RWT-52,075 +/- 9,65 kg N=315**	 **RWT-49,049 +/- 8,02 kg N=307**	 **RWT-50,714 +/- 9,53 kg N=320**
C5	 **RWT-53,668 +/- 10,94 kg N=322**	 **RWT-53,254 +/- 9,77 kg N=324**	 **RWT-54,286 +/- 12,41 kg N=292**

5. February 2021 weight reports C1 and C5

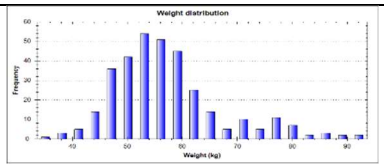
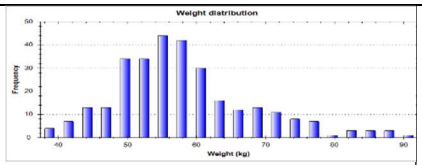
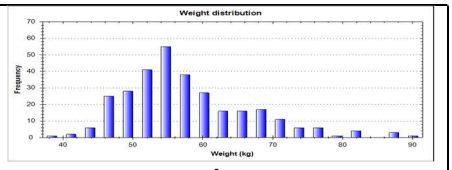
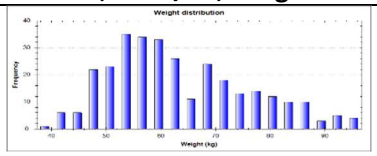
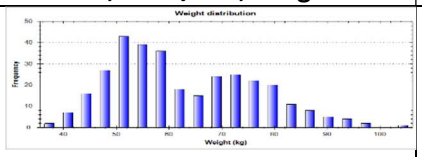
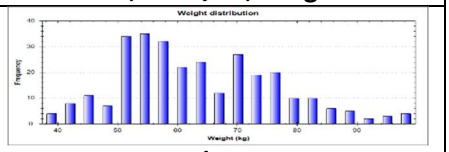
C1	 **RWT-57,059 +/- 9,96 kg N=337**	 **RWT-58,001 +/- 10,01 kg N=327**	 **RWT-57,676 +/- 9,01 kg N=304**
C5	 **RWT-63,280 +/- 12,62 kg N=310**	 **RWT-62,865 +/- 13,4 kg N=325**	 **RWT-63,635 +/- 12,93 kg N=295**

Figure 21. Round weight (RWT) of bluefin tuna in two experimental cages (C1 and C5) calculated from straight fork length /SFL) obtained from stereoscopic camera system throughout farming cycle

No significant difference in mean weight (RWT) calculated from length recorded by SC at different 30-minutes time intervals (**Figure 22**).

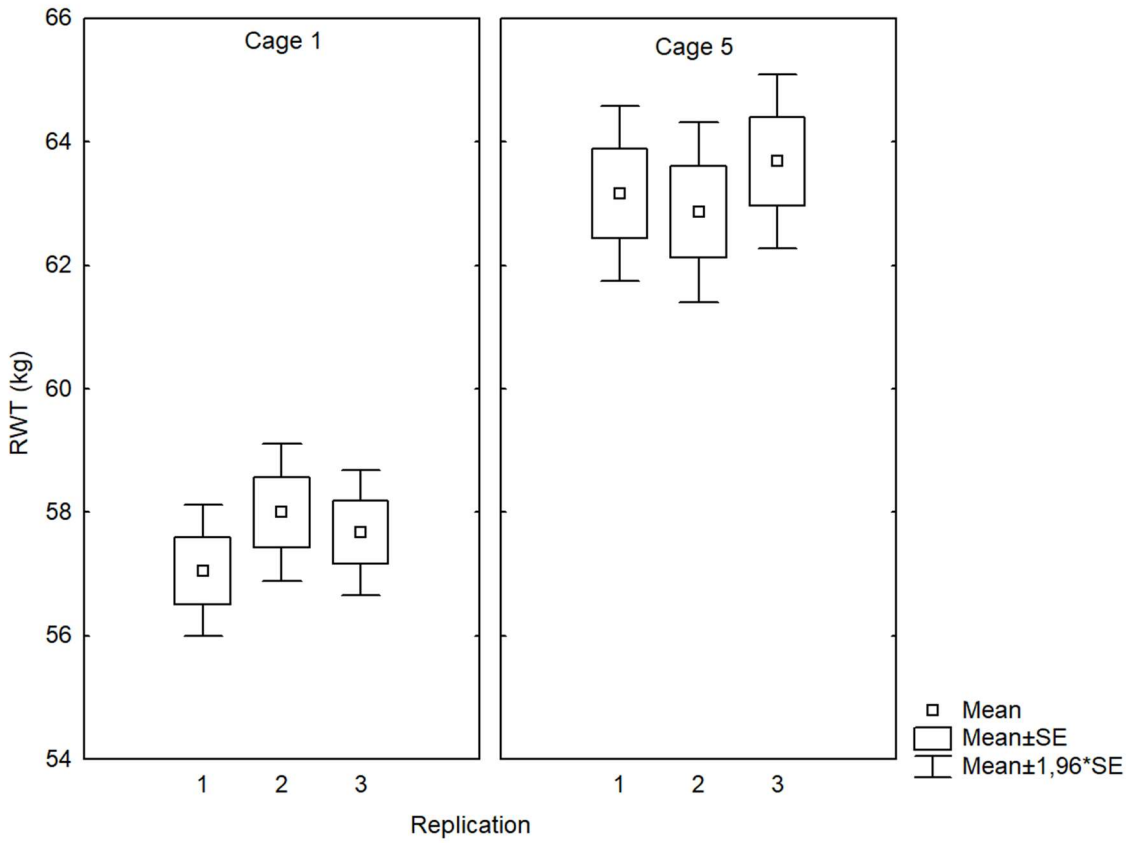


Figure 22 . Stereoscopic camera system (SC) sampling design that includes three replicates in calculating round weight (RWT) for two cages at harvesting. Differences among replicates were tested by nested ANOVA. n.s. indicate no significant difference among replicates.

The difference in RWT relationships by cages estimated by SC recordings and real measurement of killed fish at harvesting was plotted as shown in **Figure 23.** Some difference in mean weight of fish estimated from SC readings and directly measured can be explained by relatively high variability in size frequencies (**Figure 10**) and by SC reader who is aiming to catch up 20% of the fish reared in cage. Other factors that may affect differences could be the lack of normal distribution in tested variables, and unbalanced number of data obtained by stereoscopic camera system and directly measured fish at harvesting.

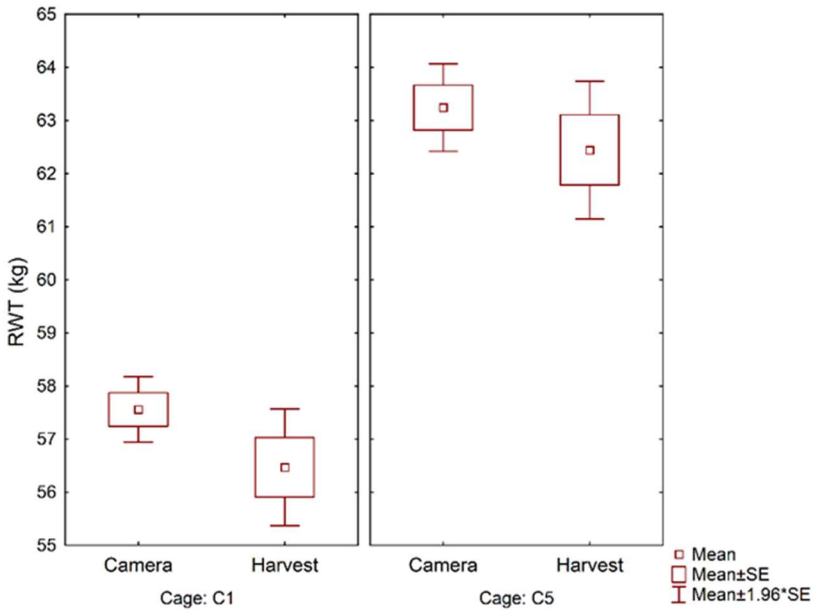


Figure 23. Round weight (RWT in kg) in cage C1 and C5 estimated by SC and directly measured at harvesting.

Conclusions and Recommendations

- A study to identify growth rates including in weight and size gains of individual bluefin tuna juveniles (> 8 kg) during the 19 months of caging in the Adriatic (Croatia) have been successfully carried out from 2019-2021. PIT tagged fish were almost equally distributed over all size classes in experimental cages.
- During 19 months of farming bluefin tuna juveniles reached the overall harvested weight between 58 and 64 kg. A mean value of body weight increase of 500% over tested period did not differ between two experimental cages.
- Body weight increase of maturing fish is greatly retarded during the spawning season compared to immature bluefin tuna. The fish having initial weight from 8-10 kg (estimated age 2+ years) increased body weight 525%, while for the fish of 14-20 kg (estimated age 3+) body weight increase was 334%.
- Registered mortality of tagged fish over the entire farming period was neglectable (1%). Unrecovered portion (22%) could be addressed to combination of several factors, such as detector failure and failure of readers being forced with harvesting routine procedures.

- The retention of external clips attached to the anal fin combined with cutting part of second dorsal fin was very low (1 against 12 tagged fish).
- Encapsulated tag appears to be biologically inert with no sign of inflammation of wound area tissue. There was no effect on the length/weight relationship of the tagged fish.
- Underwater video recordings can be easily used on a routine basis to obtain reliable data on size frequency distribution of BFT reared in grow out cage. Using the appropriate L-W equation, obtained length information from footages can be easily converted into quite precise fish biomass as to adjust feeding regime with other zootechnician measures (i.e.stocking density), and thus improve farm management as whole.

Acknowledgements

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