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Aquaculture

journal homepage: www.elsevier.com/locate/aqua-online

Age structure and growth of bluefin tuna (*Thunnus thynnus*, L.) in the capture-based aquaculture in the Mediterranean Sea



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ARTICLE INFO

Article history:

Received 7 May 2013

Received in revised form 18 December 2013

Accepted 19 December 2013

Available online 3 January 2014

Keywords:

Bluefin tuna

Age

Growth

Caudal vertebrae

Capture-based aquaculture

Mediterranean Sea

ABSTRACT

The principal objective of this study was to estimate the age and growth, length–weight relationships and condition factors of the Mediterranean bluefin tuna (*Thunnus thynnus*) in a capture-based aquaculture farm and to reveal possible differences among farmed (reared in sea cages for 18 months), fattened (reared in sea cages for 6–7 months) and wild specimens. Moreover, to determine the age composition of the fish destined for fattening and farming operations with the current lack of demographic information makes the bluefin tuna stock assessment extremely difficult. For this purpose, a total number of 2096 specimens (ranging from 102 to 295 cm in fork length and from 33 to 540 kg in round weight) were sampled, from the Greek Bluefin Tuna Farm (GR 01/2004), in the Ionian Sea (38° 26′ 0.07″ N, 21° 1′ 48.85″ E), during the five year period 2007–2011. In addition, length and weight literature data from wild specimens captured in the central Mediterranean Sea were analyzed. The results revealed high percentage difference in weight between wild and reared in sea cages bluefin tuna of the same fork length, which reached a maximum of 43.9% in large specimens. The condition factor of the reared in sea cages bluefin tuna ranged from 1.24 to 3.16 with a mean value of 2.04 ± 0.19 . The mean condition factor of the farmed specimens (1.92 ± 0.17) was lower than that of the fattened specimens (2.08 ± 0.15). This difference was consistent both in younger and older specimens in the sample. Estimated ages that were obtained using the caudal vertebrae of 619 reared in sea cages specimens ranged from 5 to 18 years. It was observed that the age of fish has a significant impact on the condition factor. The comparison among the mean condition factor values of each age class revealed that older fish (10–20 years old) present higher values than younger ones (5–9 years old). With a view to estimating the precision of the aging method, the Average Percent Error (APE) and the Coefficient of Variation (CV) were calculated (APE = 1.89% and CV = 2.46%). The von Bertalanffy growth model was fitted to mean lengths at estimated ages and the growth parameters were determined ($L_{\infty} = 360.3$ cm, $k = 0.083$ yr⁻¹, $t_0 = -0.942$ yr).

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1. Introduction

The Atlantic bluefin tuna (*Thunnus thynnus* L.) is one of the tuna species with the higher economic interest, which sustains important recreational and commercial fisheries as well as the capture-based tuna aquaculture industry. The International Commission for the Conservation of Atlantic Tunas (ICCAT), based mainly on the separate spawning areas, one in the Gulf of Mexico and one in the Mediterranean Sea, and some different life history characteristics, recognizes two management units: the west and east Atlantic stock, the latter including the Mediterranean Sea. In the last few years, the increase in fishing pressure in step with the intensity of farming activity has resulted in a dramatic biomass reduction of the Atlantic bluefin tuna stocks. Different population analyses, showed a general increase in fishing mortality for large fish and a decline (40%) in spawning stock biomass (ICCAT, 2008, 2013), while the IUCN (World Conservation Union) assessment added bluefin tuna to the Red List as an endangered species.

In the Mediterranean Sea the rapidly expanding bluefin tuna farming industry, is a capture-based aquaculture as its activity is entirely based on the stocking of wild-caught specimens (Mylonas et al., 2010; Ottolenghi et al., 2004). Almost 99% of the purse seine fleet catches are sold to capture-based fattening farms in eleven countries throughout the Mediterranean Sea. The total tuna production derived from the farms is difficult to calculate as the initial cage stocking information (i.e. biomass and fish size), is only a rough estimate and any weight gain is generally kept confidential by the farmers (Ottolenghi, 2008). The total official production registered in 2003 was approximately 19,000 tons and 22,000 tons in 2004 (FAO/GFCM/ICCAT, 2005) while according to recent statistics, the annual bluefin tuna production after reaching a peak in 2006 declined continuously (ICCAT, 2013).

Although the ICCAT reduced catch quotas for the eastern Atlantic and Mediterranean bluefin tuna, these were nearly double of what scientists recommended (FAO/GFCM/ICCAT, 2005). Besides, the data of total imports of processed bluefin tuna reported by Japan to ICCAT for 2007 revealed that the catches were significantly higher than the total allowable catch (TAC) for that year (ICCAT, 2008), raising serious concern for the survival of this resource. According to MacKenzie et al.

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(2009), even if a near-complete ban on all bluefin tuna fishing in the NE Atlantic and Mediterranean were implemented and enforced from 2008 to 2022, the population would probably fall to record lows in the next few years, unless environmental conditions promote exceptionally high recruitment. Due to the increasing fear for the collapse of the fishery and the intensification of the pressure from non-governmental organizations, ICCAT reduced the TACs to 12,900 tons for the years 2011 and 2012 while the purse seine fishing period was restricted to 1 month, from May 15 to June 15 (ICCAT, 2012). Finally, for the years 2013 and 2014, the TACs were established in 13,500 tons per year (ISSF, 2013).

Data on size and age of fish are very important for fisheries management, as they form the basis for the calculation of growth and mortality rates. Several scientists, using various calcified structures such as vertebrae, spines and otoliths have studied, for many years, age and growth of the wild bluefin tuna in the Atlantic Ocean (Berry et al., 1977; Compeán-Jiménez and Bard, 1983; Cort, 1991; Farber and Lee, 1981; Lee et al., 1983; Prince et al., 1985) and the Mediterranean Sea (Farrugio, 1980; Megalofonou, 2006; Megalofonou and De Metro, 2000; Rodríguez-Marin et al., 2005; Santamaria et al., 2009; Sella, 1929). However, given that the bluefin tuna farming practice is a relatively new activity – started in 1985 in the Mediterranean Sea (FAO/GFCM/ICCAT, 2005) – there is a complete lack of aging studies while only a few estimate the length–weight relationships, condition factors and growth performances of this species under farming conditions (Aguado-Giménez and García-García, 2005; Galaz, 2012; Katavić et al., 2002; Percin and Akyol, 2010; Tičina et al., 2007; Tzoumas et al., 2009).

The principal objective of this study was to estimate the age and growth, length–weight relationships and condition factors of the Mediterranean bluefin tuna in a capture-based aquaculture farm and to reveal possible differences in growth between farmed (reared for 18 months), fattened (reared for 6–7 months) and wild specimens, using the existing literature data. Moreover, to determine the age composition of the fish used for the fattening and farming operations with the current lack of demographic information makes the bluefin tuna stock assessment extremely difficult.

2. Materials and methods

2.1. Sampling

During the five year period 2007–2011, a total of 2096 bluefin tuna were sampled at harvesting from the Greek Bluefin Tuna farm, in the Ionian Sea, in the central Mediterranean (Fig. 1). Fork length (FL, $n = 2096$), round weight (RW, $n = 2041$) after bleeding, as well as date and place of sampling were recorded for each specimen. Length measurements (the straight line from the end of the upper jaw to the posterior of the shortest caudal ray) were taken to the nearest centimeter (cm) and weight to the nearest kilogram (kg). For the purpose of age estimations, the caudal vertebrae were collected from a total of 619 specimens, of which 242 were recorded as fattened (reared for 6–7 months) and 134 as farmed (reared for 18 months). Vertebrae were preserved dry in plastic bags and refrigerated until processing and examination. The wild bluefin tuna data sets that were analyzed for comparison purposes were obtained from the study of Santamaria et al. (2009). The fish were caught by the Italian fishing fleet in several areas of the central Mediterranean Sea, during the spring and summer months mainly, from 1998 to 2005. The sea surface temperature in the area throughout the year ranges from 13.7 to 26.4 °C (<https://www.seatemperature.org>, October 2013).

2.2. Farming conditions

The Greek Bluefin Tuna farm (GR 01/2004), has a capacity of 1000 metric tons per year. It is located at a distance of around 6.44 km off the coast, at the Echinades Islands (38° 26' 0.07" N & 21° 1' 48.85" E), and uses 50 m diameter HDPE cages with nets 20 m deep at the side and ~29 m deep at the bottom which are moored in water of a total depth of 45–65 m. In this area, the water temperature ranges at 14.7–27.4 °C, 14.2–26.4 °C and 14.1–25.9 °C at depths of 2 m, 10 m and 25 m, respectively (Tzoumas et al., 2009). All reared in sea cages bluefin tuna were caught by purse seine fleets operating at the fishing grounds between Malta and Libya in the central Mediterranean, and



Fig. 1. Map indicating the Greek Bluefin Tuna farm position (small red circle) in the Ionian Sea and the bluefin tuna fishing grounds (big blue circles) in the eastern and central Mediterranean Sea.

near Cyprus in the eastern Mediterranean, during the months of May and June. The fish sampled had been reared in sea cages for 6–7 or 18 months from their arrival at the farm, in late June to beginning of August, and until harvesting, in late December of the year of catch to mid February of the following year. During summer, mean sea surface temperatures range from 22.8 °C in June to 25.6 °C in August while during winter, from 16.9 °C in December to 15.1 °C in February (<https://www.seatemperature.org>, October 2013). According to Tzoumas et al. (2009) bluefin tuna were fed with mackerel (*Scomber scombrus*), herring (*Clupea harengus*) and sardine (*Sardina pilchardus*) in a considerable proportion twice a day, 6 days in a week. At the beginning of the rearing period, the amount of total fresh food per total weight of bluefin tuna in each cage was 5–6% per day while at the end of the rearing period about 2%.

2.3. Aging procedure using vertebrae

The 35th and 36th caudal vertebrae were used to estimate the age of fish. To obtain these vertebrae, a transversal cut was made at the caudal area between the 4th and the 5th finlet. The 35th vertebra was the first in the sectioned part and was segregated together with the 36th vertebra from the rest of the caudal vertebrae. After they were cleaned and peeled, they were left to dry for at least two months.

For each sample, the largest radius of the vertebral cone was calculated by measuring the largest diameter of the vertebral cone to the nearest mm with a plastic caliper. As both vertebrae were available in most of the samples, the anterior surface of the 35th was used preferentially for measurement and interpretation. However, both anterior and posterior surfaces were used when annual growth zones were difficult to interpret. Age was estimated by counting the annual growth zones observed on the inner surface of the cones of whole vertebrae (Fig. 2A). One annulus was interpreted as one ridge and one groove (Berry et al., 1977). In some samples multiple narrow within-year ridges and grooves or lines may be formed (Fig. 2B). These samples may be difficult to read and the annuli must be interpreted by studying jointly all the features and structures mentioned above (Berry et al., 1977). Three readings of each vertebra ($n = 619$) were made independently by the principal reader (VR1), at weekly intervals. Vertebrae were rejected from the analysis when the three readings provided different annuli-counts. When two of the readings agreed, and the third differed by one annulus only, the age was derived from the two similar readings. In a total of 70 fish (11.3%), vertebra readings were made by two other readers from the same laboratory, one experienced (VR2) and one less experienced reader (VR3). All readers had received training in VR1's counting criteria prior to the exercise. Vertebrae were never read simultaneously and the readers never had prior access to information on size of fish or date of capture while they were counting growth zones.

2.4. Precision of age estimates

The precision or the reproducibility of repeated counts was calculated using the index of Average Percent Error (APE) (Beamish and Fournier, 1981) as well as the Coefficient of Variation (CV) (Chang, 1982). Specifically, these indexes determine if there are systematic differences in age estimations between the readings of one or more readers. Usually, the APE and CV produce similar values; however the latter is statistically more rigorous and also is more flexible (Campana et al., 1995).

2.5. Estimation of growth parameters

The von Bertalanffy growth model was used to describe length-at-age of reared in sea cages bluefin tuna. The von Bertalanffy parameters were estimated according to the equation: $L_t = L_\infty[1 - e^{-k(t - t_0)}]$, where L_t is the fish fork length at age t in cm, L_∞ is the asymptotic length

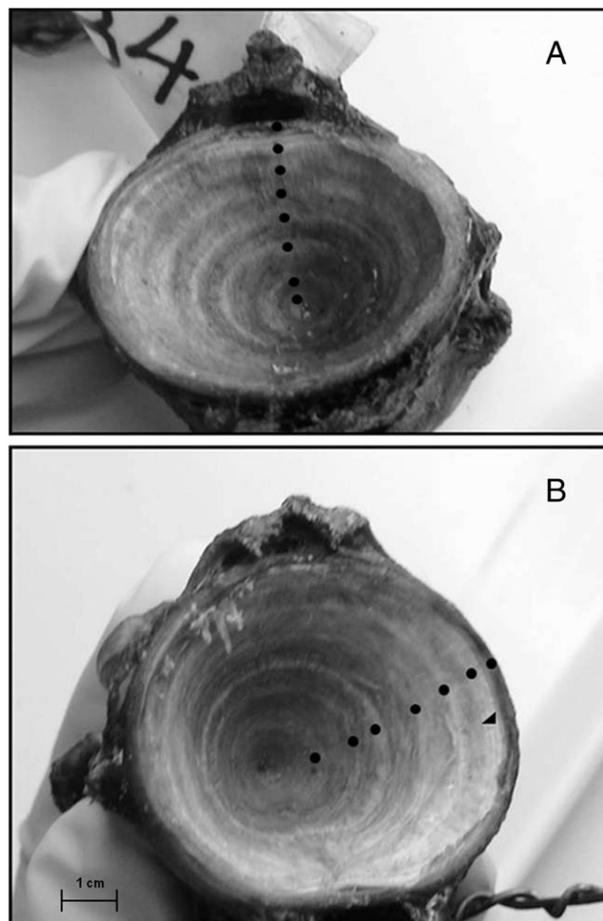


Fig. 2. (A) Vertebra of a reared bluefin tuna estimated 8 years old. (B) Vertebra of a reared bluefin tuna estimated 7 years old. The circles present the annual growth bands (ridges), while the triangle presents fine lines on the middle or slightly towards the distal side of the ridges.

in cm, k is the growth coefficient in yr^{-1} and t_0 is the theoretical age in yr when the fish has zero length (von Bertalanffy, 1938). Because of the lack of small fish in our sample, the mean sizes at age 1, 2, 3 and 4 were taken from a previous study on the age estimation of bluefin tuna, which has used the same skeletal structure (Farrugio, 1980). The growth performance index phi-prime (Φ') was used to compare bluefin tuna growth rate in the present study with those estimated in previous ones. This index was calculated using the equation: $\Phi' = \ln k + 2 \ln L_\infty$ (Sparre, 1987).

2.6. Length–weight relationships and condition factors

The length–weight relationships were estimated using the equation $W = a \times FL^b$, where W is the round weight in kg, FL is the fork length in cm and a, b are constants determined by the method of least squares. To estimate the percent differences in weight with respect to length between the wild and reared in sea cages bluefin tuna the Weight difference Index was used:

$$IWFL = 100 \times \left(1 - a_1 a_2^{-1} FL^{b_1 - b_2}\right)$$

where IW_{FL} is the weight difference index for a given FL class, a_1, b_1, a_2 and b_2 are the constants of the length–weight relationships of wild (subscript = 1) and reared (subscript = 2) samples, respectively. If $IW_{FL} > 0$, then the weight in reared bluefin tuna is greater than in wild

ones, whereas if $IW_{FL} < 0$ then wild bluefin tuna are heavier than the reared ones.

The Fulton's Condition factor (K) was calculated using the equation $K = 10^5 \times W/FL^3$, where W is the round weight in kg and FL the fork length in cm (Froese, 2006). To compare the condition factors of the reared specimens with those of the wild ones of the same area, the Fulton's Condition factor for the wild bluefin tuna was estimated using the length and weight data from the study of Santamaria et al. (2009).

2.7. Statistical analysis

Descriptive statistics were obtained for each measured or calculated parameter of the sampled bluefin tuna while histograms or frequency polygons were used to present graphically fork length, round weight and age distributions. To compare the mean values among different groups one-way ANOVA was used after the data were tested for normality and homogeneity of variances. When the variables were not normally distributed, the non-parametric Kolmogorov-Smirnov and Kruskal-Wallis tests were used, to determine possible statistical differences among the medians for two or multiple samples, respectively. Moreover, to identify statistical differences between the fattened and farmed samples of bluefin tuna the above tests were used regarding FL, RW and K data. Linear and multiplicative regression

models were used to determine length-length and length-weight relationships, correspondingly. The values of the constant b (slope) were compared with Student's t-test while hypothesis tests were used to detect any significant differences on the relationships. The level of significance in all cases was considered at $P \leq 0.05$. All statistical analyses and graphs were done using Statgraphics Centurion XV and Excel (Microsoft Corporation).

3. Results

3.1. Size distributions

The fork lengths of 2096 reared bluefin tuna ranged from 102 to 295 cm with a mean value of $198.7 \text{ cm} \pm 40.3$ while their round weights ranged from 33 to 540 kg with a mean value of $177.5 \text{ kg} \pm 102.0$. The length frequency distribution (Fig. 3A) presented its first peak at 150 cm, its second at 170 cm and its third at 235 cm. The weight frequency distribution presented its most significant peaks at 80 kg, 120 kg and 260 kg (Fig. 3B). The lack of young specimens with round weight less than 33 kg is due to the fact that the minimal allowed weight of reared in sea cages bluefin tuna is 30 kg.

Significant differences were revealed comparing the medians of fork length (Kruskal-Wallis test: $t = 133.7$; $P < 0.05$) and round

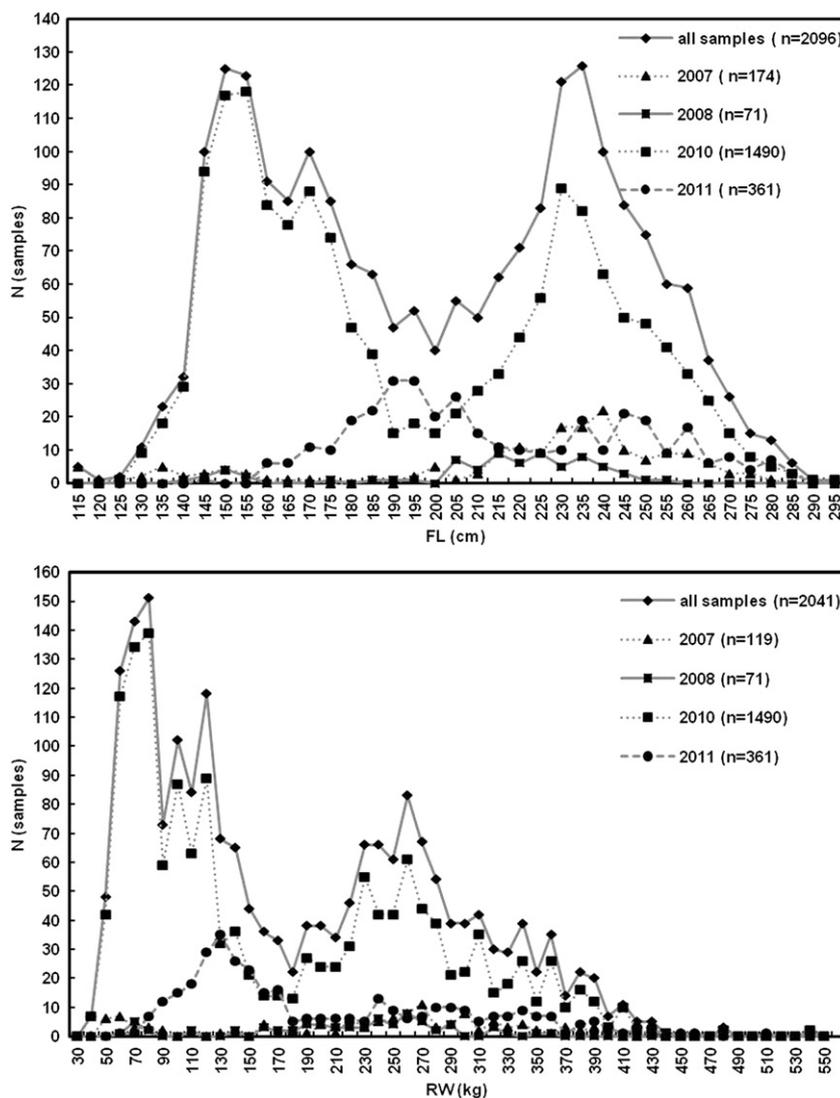


Fig. 3. Frequency distributions of fork length (FL) and round weight (RW) of reared bluefin tuna in the Greek Bluefin Tuna farm for the period 2007–2011 (all samples) and by year.

weight (Kruskal–Wallis test: $t = 96.1$; $P < 0.05$) in the years of sampling. The mean round weights, fork lengths and distributions of the specimens into the three size class categories, school (FL measure less than 130 cm), medium (FL measure 130 to less than 200 cm) and giant (FL measure 200 cm or greater) by year and area of capture are shown in Table 1. It was observed that the samples from the fishing grounds between Malta and Libya presented larger fish than those from fishing grounds near Cyprus. Besides, a decline of 22% for the giant specimens was evident from 2007 to 2011.

3.2. Age and growth estimation

A significant and positive linear relationship ($R = 0.953$) was found between vertebral cone radius and fork length (Fig. 4). The equation obtained was the following:

$$FL = 60.796 + 5.4218R (R^2 = 0.909; n = 619; P < 0.05).$$

Age estimations from vertebrae were obtained from a total of 619 specimens, ranging from 135 to 295 cm in fork length and from 43 to 540 kg in round weight. All vertebrae were considered readable. Estimated ages ranged from 5 to 18 years. The obtained size–age key is presented in Table 2. The age group 10 was the most abundant (14.2%), while the age groups 5 (0.8%), 16 (1.0%), 17 (0.6%) and 18 (0.2%) presented the lower number of samples. No specimens younger than 5 years old were observed, and fish that had already completed their fifth year of life were quite rare. For fattened specimens ($N = 242$), the estimated ages ranged from 5 to 17 years, with age 12 as the most abundant age class. Estimated ages of farmed specimens ($N = 134$) ranged from 6 to 18 years, with age 8 as the most abundant age class (Fig. 5).

The precision of the aging method was estimated using the three readings ($n = 619$) of the principal reader, VR1. The mean values of the Average Percent Error and Coefficient of Variation were 1.89% and 2.46%, respectively. In addition, no significant bias was detected between the three readers, VR1, VR2 and VR3 (ANOVA; $n = 70$; $P > 0.05$). The comparatively high APE and CV between VR1 versus VR3 and VR2 versus VR3 demonstrate the difficulty that less experienced readers have in using bluefin tuna vertebrae to estimate age (Table 3).

The von Bertalanffy equation for the theoretical growth of bluefin tuna in length was $L_t = 360.3[1 - e^{-(0.083)(t + 0.942)}]$, with correlation coefficient $R^2 = 0.997$. Between fattened and farmed specimens, differences in the mean size at age were only minor and not consistent for all age groups (ANOVA; $P > 0.05$). Therefore, they were not further considered for growth analysis. The growth curve based on the full data set is given in Fig. 6. The index phi-prime (Φ'), calculated using the L_∞ and k values, was 9.285.

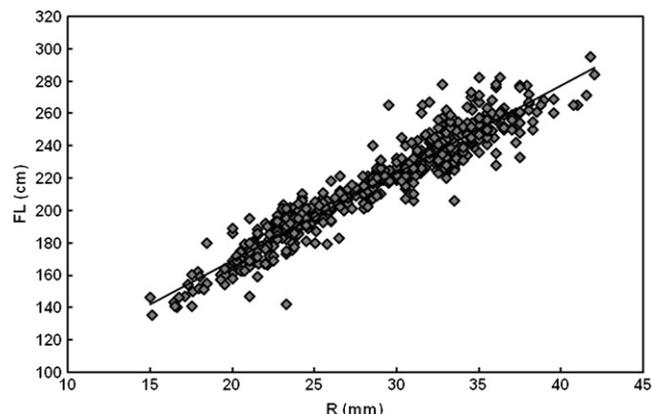


Fig. 4. Relationship between fork length (FL) and vertebral cone radius (R) of reared bluefin tuna ($n = 619$).

3.3. Length–weight relationships and condition factors

The regression parameters and statistics of length–weight relationships are given in Table 4. All regressions were highly significant (ANOVA; $P < 0.0001$). The slopes of the regression lines were significantly different from 3 (all samples: $t = 67.8$; $n = 2041$; $P < 0.05$; fattened: $t = 78.4$; $n = 253$; $P < 0.05$; farmed: $t = -80.3$; $n = 195$; $P < 0.05$), indicating positive allometric growth for the fattened bluefin tuna and negative allometric growth for the farmed ones. The Student's t -test for comparison of the slopes showed that there were significant differences ($t = 113.4$; $P < 0.05$) between fattened and farmed bluefin tuna.

The condition factor K of 2041 bluefin tuna ranged from 1.24 to 3.16 with a mean value of 2.04 ± 0.19 . For each sampling period the K values are given in Fig. 7A. A reduction of the mean condition factor was observed from 2007 to 2011 (Fig. 7A). In addition, a significant decrease of the K values was noticed from December to February (Kruskal–Wallis test: $t = 388.7$; $P < 0.05$). The higher values of K were in December (mean 2.17 ± 0.21) while the lower in February (mean 1.95 ± 0.19). A positive and significant linear relationship was found between K and FL ($K = 1.99 + 0.0002FL$; $R^2 = 0.21$; $n = 2041$; $P < 0.05$), however, the correlation coefficient ($R = 0.458$) indicated a weak relationship between the variables. The higher values of K were presented in large fish and the lower values in small fish. Comparing the K values among the estimated age groups significant differences were found (Kruskal–Wallis test: $t = 143.7$; $P < 0.05$). Mean K values ranged from 1.89 to 2.11 and it was obvious (Fig. 7B) that older fish (10–18 years old) present higher K values than younger ones (5–9 years old). Finally, significant differences were observed between the K values in farmed and fattened specimens, that were also confirmed both in the youngest (age ≤ 9 yrs) and the oldest fish (age ≥ 10 yrs) of the sample. The mean condition factor of the farmed specimens was

Table 1

Mean values and standard deviations (SD) of round weights (RW) and fork lengths (FL) of reared in sea cages bluefin tuna, as well as frequency distributions into the three size class categories (school, medium and giant) by year and area of catch.

Year/area	N	Mean RW \pm SD	Mean FL \pm SD	% frequency		
				School ^a <130 cm	Medium ^a 130 to <200 cm	Giant ^a ≥ 200 cm
2007	174	232.5 \pm 102.0	218.3 \pm 41.4	5.2	15.5	79.3
2008	71	211.9 \pm 72.7	210.6 \pm 28.6	0.0	18.3	81.7
2010	1490	166.4 \pm 102.3	192.3 \pm 40.8	0.5	56.1	43.4
2011	361	198.3 \pm 95.5	212.9 \pm 32.0	0.0	42.7	57.3
All	2096	177.5 \pm 102.0	198.7 \pm 40.3	0.8	49.1	50.1
Cyprus	404	84.4 \pm 49.7	159.2 \pm 22.2	0.0	94.1	5.9
Malta	1692	200.5 \pm 98.5	208.1 \pm 37.9	1.0	38.4	60.6

^a School (FL measure less than 130 cm), medium (FL measure 130 to less than 200 cm) and giant (FL measure 200 cm or greater).

Table 2
Age-length key by 10 cm of reared in sea cages bluefin tuna sampled from a Greek farm during the years 2007–2011.

FL (cm)	Age (years)														Total N
	5	6	7	8	9	10	11	12	13	14	15	16	17	18	
130–139	1														1
140–149	2	4	3												9
150–159	2	6	3												11
160–169		9	12	2											23
170–179		1	19	7	1										28
180–189		1	11	36	5										53
190–199			4	22	32	6									64
200–209			1	5	24	20	4								54
210–219				2	3	24	16	2	1						48
220–229					5	22	22	24	4						77
230–239					2	16	19	30	20	4					91
240–249						5	14	18	17	2					56
250–259					1		2	12	20	13	9		1		58
260–269							3	4	3	10	9	2			31
270–279										1	5	3	2		11
280–289										1		1	1		3
290–299														1	1
Total N	5	21	53	74	73	88	71	86	66	46	25	6	4	1	619
%	0.8	3.4	8.6	12.0	11.8	14.2	11.5	13.9	10.7	7.4	4.0	1.0	0.6	0.2	100%
Mean FL	146.8	158.8	173.6	187.9	201.1	216.8	227.7	236.2	243.2	252.4	261.5	270.7	273.0	295.0	217.5
SD ^a	9.0	10.0	13.3	9.0	13.1	11.7	13.0	12.0	10.9	10.8	9.0	8.9	12.2	0.0	39.0
SE ^b	4.0	2.2	1.8	1.1	1.5	1.2	1.6	1.3	1.4	1.6	1.7	3.6	6.1	0.0	0.1

^a Standard deviation.

^b Standard error.

lower (1.92 ± 0.17) than the one of the fattened specimens (2.08 ± 0.15) (Fig. 7C). However, both the fattened and the farmed specimens were heavier and presented higher K values than the wild ones of the same fork length and age (Fig. 8A–B).

The index IW_{FL} revealed high percentage difference between the weight of reared in sea cages bluefin tuna and the wild ones (Fig. 9). In particular, comparing our data with the data set of wild specimens (Santamaria et al., 2009) caught in areas near the sea farm the weight differences range from 16.4% for the FL class of 50 cm to 43.9% for the FL class of 300 cm.

4. Discussion

Very few studies till now have estimated the age and growth of wild bluefin tuna in the eastern Mediterranean Sea using hard parts (Megalofonou, 2006; Megalofonou and De Metrio, 2000; Santamaria et al., 2009; Sella, 1929) and this is the first one to estimate age and growth of reared in sea cages specimens using the vertebra method.

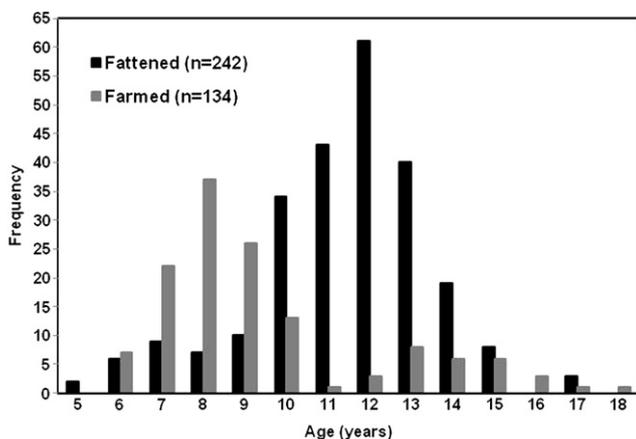


Fig. 5. Age frequency distributions of fattened and farmed bluefin tuna (n = 376) in the Greek Bluefin Tuna farm.

Vertebrae were chosen by various scientists in the past for aging wild bluefin tuna because annual growth bands are well distinct on the inner surface of the vertebral cones. Nevertheless, the method presents difficulties in the sampling since vertebra extraction does interfere with the market value of the fish. In addition, the method is time consuming in vertebra cleaning, separation and drying out processes. In contrast to the above, our sampling of reared in sea cages bluefin tuna during harvesting and processing at the deck of factory ships makes it possible to obtain the caudal vertebrae without interfering with the market value of the fish. Moreover, in our samples, annual growth bands were clear and well distinct, as in wild specimens. This could be attributed to the fact that both fattened and farmed bluefin tuna are kept in sea cages only for a short period of few months (6–18 months) and therefore almost all annual growth bands on their vertebrae are formed during their lifetime before captivity. The main problem associated with the aging method used in this study was the difficulty in distinguish the crowded growth bands at the outer margin of vertebrae in large specimens. At the age of 10 and onwards the ridges became less pronounced and the grooves were very narrow. However, similar problems were also noted by several authors in aging giant wild bluefin tuna using vertebrae (Berry et al., 1977; Farber and Lee, 1981; Lee et al., 1983; Prince et al., 1985; Rodriguez-Marin et al., 2005). Comparisons of age estimates from different hard parts in wild bluefin tuna revealed that there is a tendency to estimate fewer years in vertebrae than in other calcified structures in specimens older than 9 to 10 years old (Rodriguez-Marin et al., 2007). Otolith sections for example give more accurate age estimates than scales, vertebrae and spines in fish over 4

Table 3
Measures of precision, APE and CV, comparing pairs of age estimations of a sample of 70 reared in sea cages bluefin tuna.

Measures of precision	VR1 ^a vs VR2 ^b	VR1 vs VR3 ^c	VR2 vs VR3
Average percent error (APE)	2.57%	5.43%	6.83%
Coefficient of variation (CV)	3.64%	7.68%	9.66%

^a Principal reader.
^b Experienced reader.
^c Less experienced reader.

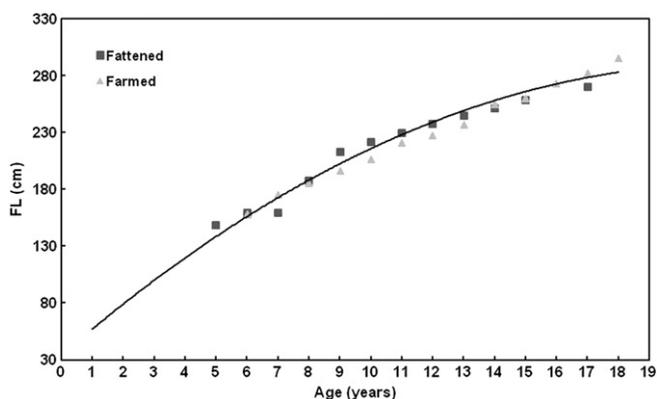


Fig. 6. Von Bertalanffy growth curve for reared in sea cages bluefin tuna from the Mediterranean Sea and mean fork lengths at age for fattened and farmed specimens.

or 5 years old (Berry et al., 1977; Farber and Lee, 1981; Lee et al., 1983) however, otolith extraction and preparation is very difficult, time consuming and interferes with the market value of the fish. On the other hand, spines give accurate results in young bluefin tuna, up to 3 years old, however, in older fish the central area of the spine begins to reabsorb and consequently the bands disappear (Cort, 1991; Rodriguez-Marin et al., 2005). Considering the differences observed between the age estimations using various hard parts as well as the advantages and disadvantages that have been described for each method, it would be recommended in a future study to estimate the age of the reared bluefin tuna using different calcified structures (otoliths, spines, vertebrae) from the same fish.

A basic assumption in growth studies using calcified structures is that fish size and the size of the calcified structure are closely related throughout the entire life cycle, which means that the growth zone or band on the calcified structure can be related to a time scale (Casselman, 1989). Our results showed a significant relationship between vertebral cone radius and fork length even in giant bluefin tuna. According to age estimations, bluefin tuna were from 5 to 18 years old during harvesting. Testing the precision of age estimation showed that the values of precision indexes were acceptable and kept up with the values of the existing bibliography. Lee et al. (1983) using vertebrae, obtained an average percent error ranging from 0.3% to 6.3% and a Coefficient of Variation ranging from 0.4% to 7.2%. According to Campana et al. (1995), the allowable limit of APE for large pelagic fish is 10%. The mean lengths at age of the reared in sea cages bluefin tuna presented intermediate values in accordance with other studies of wild fish from the Mediterranean Sea and the Atlantic Ocean (Table 5). Therefore, it was assumed that the growth in length of the reared fish is not much faster than the growth in length of the wild ones to differentiate considerably the length at age data. By contrast, the great differences observed in weight between wild and reared fish of the same length lead us to conclude that the reared in sea cages bluefin tuna grow mostly in weight than in length. The von Bertalanffy parameters L_{∞} and k are reversely connected and are influenced by the range of the samples as well as by the data distribution. Underestimation of age leads to higher asymptotic length and lower growth factor, while overestimation of age leads to the opposite result. Compared with previously published estimates of

Table 4
Regression parameters and statistics of the length–weight relationships in reared in sea cages bluefin tuna.

	n	a	b	R ²	P value
All	2041	2×10^{-5}	3.027	0.977	$P < 0.0001$
Fattened	253	1×10^{-5}	3.069	0.967	$P < 0.0001$
Farmed	195	3×10^{-5}	2.908	0.952	$P < 0.0001$

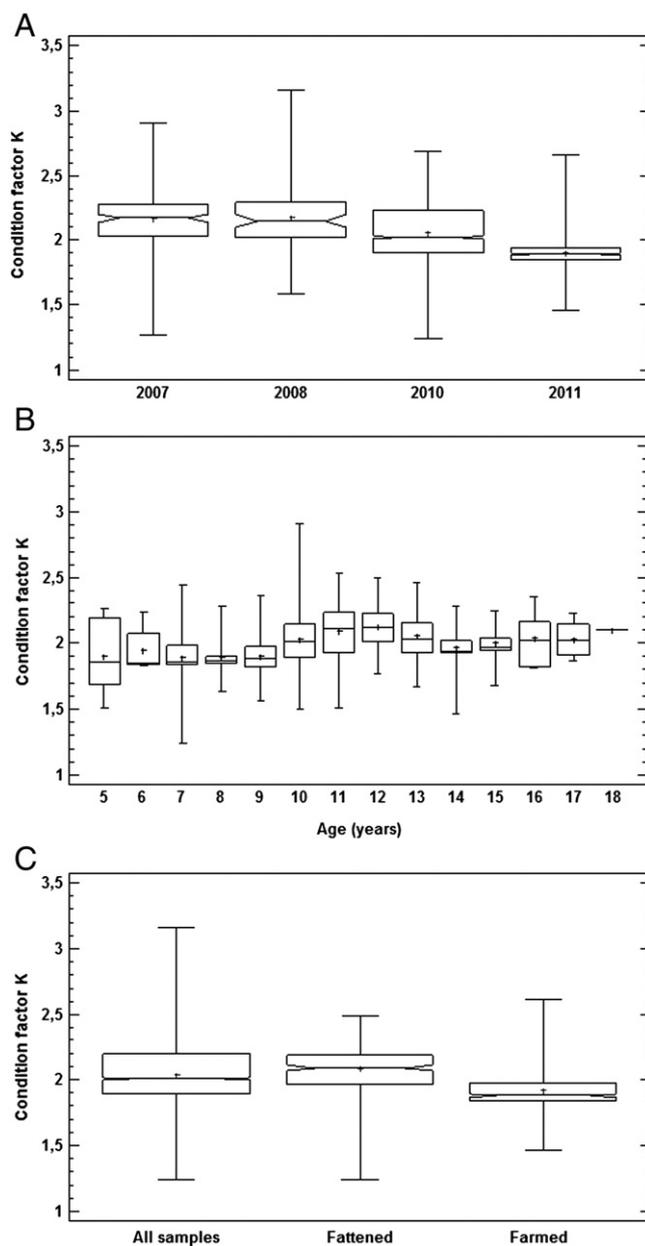


Fig. 7. Box-and-whisker plot of (A) condition factor K by year for reared bluefin tuna; (B) condition factor K by age group for reared bluefin tuna; (C) condition factor K for fattened and farmed bluefin tuna. The box itself represents 50% of all cases, and extends from the 25th to the 75th quartiles. The line inside the box shows the median. Points beyond the whiskers (outliers) are drawn individually.

growth parameters for bluefin tuna from the Mediterranean and Atlantic areas, some differences are obvious: L_{∞} presented intermediate value and k was in the high range. However, the calculated values of ϕ' were fairly similar (Table 5).

Due to the fact that there is no efficient method to establish the fish biomass moving into the towing or farm cages it is rather difficult to determine the size and age composition of the fish. Our results provided significant data not only on the length and weight distribution of the farmed bluefin tuna in Greece but also on age compositions. The medium size and giant bluefin tuna presented equal percentages, in the whole sample however size distributions along the years change significantly with giant tuna diminishing considerably from 2007 to 2011. The fish sampled in the period 2007 and 2008 were heavier than in 2010 and 2011. Due to this reduction in size many of the fish of 2010 and 2011 had to be farmed for 18 months in order to gain weight and

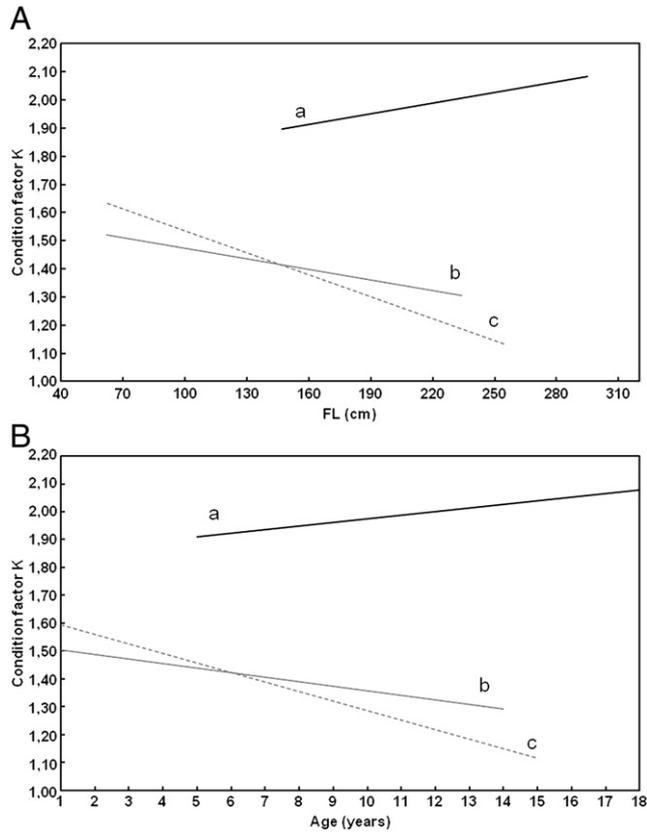


Fig. 8. (A) Trend lines of condition factor K with regard to fork length FL in wild and reared bluefin tuna, in the central Mediterranean Sea. (B) Trend lines of condition factor K with regard to age in wild and reared bluefin tuna, in the Mediterranean Sea (a: reared; b: wild females; c: wild males).

be more profitable. It is a fact that the Mediterranean farmers selectively harvest only the large fish after the traditional 5–7 month fattening period and carry-over the less than 100 kg fish to the next year for further farming for periods of 1.5–2.5 years and sell their fish at a larger size (Tzoumas et al., 2009).

Age compositions revealed that the fattened bluefin tuna consist mainly of specimens of 12 years old while the farmed bluefin tuna of 8 years old. Considering the period of captivity, the age composition of fish at the time of capture can be also estimated. We deduced that at the time of capture the fattened specimens were mostly 11 years old (25.2%), whereas the farmed ones were mostly 6 years

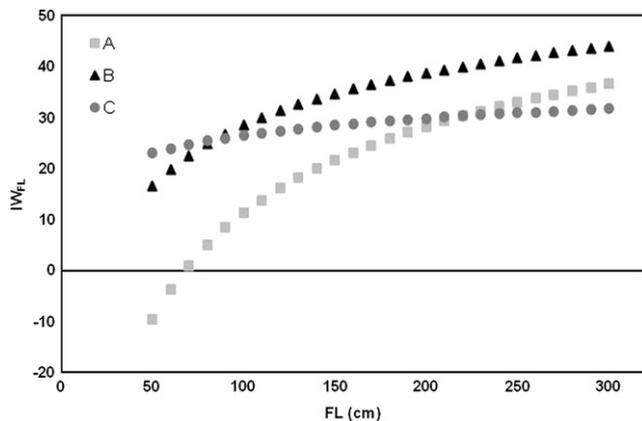


Fig. 9. Weight difference indexes IW_{FL} derived from the reared in sea cages bluefin tuna of the present study and the wild bluefin tuna examined in other studies (A: Aguado-Giménez and García-García, 2005; B: Santamaria et al., 2009; C: Sinovič et al., 2004).

old (27.6%). Since the bluefin tuna mature at ages 3–5 years old (Abascal et al., 2004; Corriero et al., 2005), we assumed that all fish aged 6 years and older had spawned at least once, with proportion 98–99%. The proportion of spawners, 8 years and older fish, was 84–91%. However, it was noticed that from 2007 to 2011 the abundance of fish greater than 9 years old declined by almost 30%. The fact that smaller and smaller fish were stocked in aquaculture farms in recent years is an indication of bluefin tuna overfishing.

Our results showed that there are statistically significant differences between wild and reared in sea cages bluefin tuna regarding to condition factor and growth in weight. When the FL–RW relationship and condition factor (K) of wild and reared in sea cages bluefin tuna were compared, the reared bluefin tuna were significantly heavier (Fig. 10), and the values of their condition factor higher (Fig. 8A–B). In wild bluefin tuna, the older specimens showed lower K values, probably because of the possible reproductive/migratory phase of the fish. In reared bluefin tuna, K values increased significantly with FL and age, in contrast to those of wild ones. Similar results were also presented in previous studies from farms in the western and eastern Mediterranean (Aguado-Giménez and García-García, 2005; Galaz, 2012; Percin and Akyol, 2010). The small specimens due to their high metabolic rate may devote most of the energy input to maintain their standard requirements (Brill, 1987) and consequently they never reach the overweight level observed in larger specimens during winter.

It is well known, that the condition factor of fish could be influenced not only by the age, sex, and stage of maturation, but also by the season, water temperature, and amount and type of food consumed. The findings of Galaz (2012) showed that the water temperature influences inversely proportional the condition factor of bluefin tuna. The highest values of K were found during winter, particularly in February (2.12) while the lowest were found in July (1.81). In our study, K values were high during winter but a slight decrease in mean values took place from December (2.17) to February (1.90). This could be probably explained by the reduction of food quantity supplied at the end of rearing period.

Unexpected differences were also found between fattened and farmed specimens, with farmed specimens that were reared for a period of 18 months having lower condition factors than the fattened ones that were reared for 6–7 months only. The differences were consistent even when fattened and farmed specimens of the same age or size group were compared. At the moment it is difficult to explain these differences because there are several factors, such as quantity and quality of food supplied, density and homogeneity of fish size in cages that could affect the growth of reared bluefin tuna. Nevertheless, low values of K in farmed bluefin tuna have been also found in other studies. Percin and Akyol (2010), who studied the morphometry and growth of bluefin tuna in a Turkish farm in the Aegean sea, noticed that despite the 18-month feeding period of fish with fork lengths ranging between 113 and 286 cm (171 ± 1.4 cm) their condition factor K was relatively low and ranged from 1.30 to 1.55 in males and from 1.29 to 1.51 in females.

The differences observed in weight (IW_{FL}) between the reared in sea cages and wild bluefin tuna (Fig. 9), revealed a significant increase of weight in the reared specimens. According to our estimations the highest value of weight gain was 43.94%. However, the weight gain is underestimated, due to the fact that we compare the weights of wild and reared fish having the same length and thus we don't consider the weight gain from the relative length growth of reared fish, which is more representative in juvenile and young bluefin tuna. Tičina et al. (2007) showed that juvenile bluefin tuna stocked at an average weight of 6.4 kg are able to increase their initial biomass by more than 340% within 511 days of farming. Katavić et al. (2002) had shown earlier that juvenile bluefin stocked at an average weight of 12 kg are able to increase their weight by 375% in 540 days of fattening to an average weight of 45 kg.

Table 5

Comparison of growth parameters, phi-prime (Φ') indexes and mean fork lengths at age of bluefin tuna estimated by different authors using the vertebra aging method.

Authors Date	Sella (1929)	Mather and Shuck (1960)	Rodriguez-Roda (1964)	Farrugio (1980)	Farber and Lee (1981)	Olafsdottir and Ingimundardottir (2003)	Present study 2013
Area	Mediterranean	West Atlantic	East Atlantic	Mediterranean	West Atlantic	East Atlantic	Mediterranean
L_{∞}	420.6	371.0	344.0	351.2	401.0	308.4	360.3
K	0.057	0.069	0.090	0.080	0.080	0.085	0.083
t_0	-1.806	-1.373	-0.970	-1.087	-0.920	-3.342	-0.942
Φ'	9.223	9.159	9.273	9.197	9.462	8.999	9.285
Age	Mean fork lengths (cm)						
1	64.0	57.0	55.3	54.0	44.8		
2	81.5	77.0	79.0	76.8	67.6		
3	97.5	95.0	116.2	97.9	91.6	134.0	
4	118.0	114.0	130.1	117.4	116.2	128.0	
5	136.0	133.0	146.9	135.4	138.8	160.4	146.8
6	153.0	149.0	165.1	152.0	157.9	171.1	158.8
7	169.0	163.0	178.1	167.3	176.3	184.0	173.6
8	182.0	177.0	192.9	181.4	189.7	199.5	187.9
9	195.0	190.0	206.5	194.5	200.8	205.3	201.1
10	206.0	201.0	220.3	206.5	217.2	208.8	216.8
11	216.0		221.5	217.6		219.8	227.7
12	227.0		244.0	227.9		216.1	236.2
13	239.0		246.0	237.4		221.2	243.2
14	254.0			246.1		236.3	252.4
15						241.7	261.5
16						247.3	270.7
17						265.0	273.0
18							295.0

Growth rates and condition factors obtained in this study probably are not representative for the entire bluefin tuna farming industry in the Mediterranean Sea; however, they provide very important indications on the growth performances of the bluefin tuna under given farming conditions. Summing up, we believe that the present results will contribute to the proper management of bluefin tuna. The pattern of exploitation developed for bluefin tuna needs to be based on accurate growth information because the stock is considered fully exploited.

5. Conclusions

The main conclusions of the present study are the following:

- The size distributions of reared in sea cages bluefin tuna change significantly along the years with giant tuna being considerably diminished

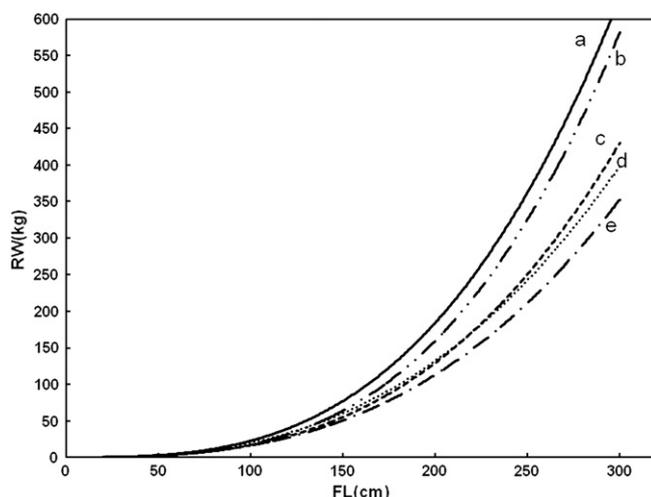


Fig. 10. Length (FL) – weight (RW) relationships of wild and reared in sea cages bluefin tuna in the Mediterranean Sea (a: present study; b: Aguado-Giménez and García-García, 2005 (fattened); c: Sinovčić et al., 2004; d: Aguado-Giménez and García-García, 2005 (wild); e: Santamaria et al., 2009).

from 2007 to 2011. Moreover, the abundance of older fish, greater than 9 years old, declined by 30%. The decline of abundance of the larger and older specimens in the capture-based aquaculture probably indicates the severe decline of the bluefin tuna stocks.

- There are significant differences both in length-weight relationships and condition factors between wild and reared in sea cages bluefin tuna. The mean condition factor of the farmed specimens (reared for 18 months) was lower than the one of the fattened specimens (reared for 6–7 months) and the differences are consistent even when fattened and farmed specimens of the same age or size group were compared.
- In wild bluefin tuna, the larger specimens show lower condition factors, probably because of their reproductive/migratory phase or the high water temperatures during their catch in summer (22.8–25.6 °C). It is known that the water temperature influences inversely proportional the condition factor.
- In reared in sea cages bluefin tuna, condition factor values were high during winter, but a slight decrease in mean values took place from December to February probably because of the reduction of food quantity supplied at the end of rearing period.
- In reared in sea cages bluefin tuna, condition factor increases significantly with FL and age. The smaller specimens due to their high metabolic rate may devote most of the energy input to maintain their standard requirements and consequently they never reach the over-weight level observed in larger specimens during winter.
- The growth in length of the reared in sea cages bluefin tuna is not much faster than the growth in length of the wild ones to differentiate considerably the length at age data and growth curves.
- The estimated ages and von Bertalanffy growth parameters for the reared in sea cages bluefin tuna during harvesting will help to estimate the age composition at the time of capture and to improve the stock assessment, which is the basis for an effective management of the stock.

Acknowledgments

This study is part of a PhD thesis that is conducted at the Faculty of Biology, National and Kapodistrian University of Athens. We would

like to express our most sincere thanks to Mr. A. Tzoumas, general director of Bluefin Tuna Hellas, for the cooperation during the samplings and the valuable help during the bluefin tuna measurements and Mrs. V. Kousteni for the readings of a number of vertebrae as a less experienced reader. We are also indebted to the reviewers for their suggestions to improve the manuscript. Moreover, Mr. H. Mori as well as the Greek and Japanese commercial fishermen are gratefully acknowledged for their assistance on board.

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