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A study on oocyte development of the Atlantic bluefin tuna (*Thunnus thynnus*, Linnaeus 1758) under farming conditions

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Abstract: Body length and weight measurements and histological samples were collected from slaughtered Atlantic bluefin tuna (*Thunnus thynnus*) that were fattening under farming conditions for the determination of oocyte development in February 2015 (n = 10), May 2015 (n = 1), June 2015 (n = 1), July 2015 (n = 9), and July 2016 (n = 3) during harvest and soon after newly dead. The developmental phases of oocytes were evaluated based on their diameters, fork body length and weight of fish, surface water temperature, and species' reproduction season. According to the histological results, the oocytes were at the beginning of the regeneration and maturation phases in February; in the preovulation phase in May, advanced vitellogenic oocyte with yolk globules and atretic vitellogenic oocytes in June, and previtellogenic oocytes in July 2015. In July 2016, the oocytes were previtellogenic, vitellogenic, and advanced vitellogenic. Individuals were between 119 and 280 cm in fork length, weighed between 30 and 455 kg, and showed natural oocyte development at 16.7–22 °C surface water temperature under cage farming facility conditions during the species' natural spawning season of May, June, and July.

Key words: Atlantic bluefin tuna, gonadal development, oocyte, histology

1. Introduction

The Atlantic bluefin tuna (*Thunnus thynnus*, Linnaeus, 1758) is one of 32 species and subspecies in the family *Scombridae* (subfamily *Scombrinae*) [1]. It is distributed in the Atlantic Ocean and its marginal seas [2]. There are two major populations which are distributed in the Eastern and Western Atlantic [3]. The Eastern population, which migrates to the Mediterranean Sea, spreads from Norway to the Canary Islands and from the southern coast of South Africa to the Mediterranean Sea [2]. While a number of different spawning behaviors have been reported for various species of the tuna family, the Atlantic bluefin tuna is characterized by spawning migration behavior [3]. It has been reported that the species spawns in May and the beginning of June in the southern part of the Mediterranean Sea [4] and between June and August in the northern and middle parts of the Mediterranean Sea [2].

The volume of Atlantic bluefin tuna fishing in the Eastern Atlantic and Mediterranean Sea was more than 50,000 t in 1996 [2]. However, after International Commission for the Conservation of Atlantic Tunas' (ICCAT) total allowable catch quota regulation, this value has drastically decreased especially in recent years. The latest announcement for 2019 was 32,240 t and for 2020 was 36,000 t [5]. An alternative method which supports the Atlantic bluefin tuna stock is fattening operations [2]. In many Mediterranean countries (Croatia, Italy, Malta, Spain, Tunisia, Turkey, Libya, Morocco) fish are caught during reproduction migration (May–June) and are transferred to fish cages where they are fattened for 3 or 4 months (from July to October or from March to July). After being harvested, they are sold to the markets in Far East countries [2,6,7]. Since 2002, the Atlantic bluefin tuna fattening operations have been carried out by six commercial companies in cages in the Aegean Sea and the Mediterranean Sea in Turkey [8,9].

Since the 2000s, when fattening operations have become common [7], various studies were conducted in the Mediterranean Sea on the subjects of the reproductive biology and oocyte development of the Atlantic bluefin tuna [1,4,10–12].

Another important method for supporting and controlling of the wild fish stocks is cultivation of this species in artificial environments. However, lots of difficulties have been reported regarding culture especially in the early

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periods [13,14]. One of the problems is mass mortality during first feeding when live food size, type, and quality are very important [14]. Moreover, Southern bluefin tuna (*Thunnus maccoyii*) has a bottleneck which is high larval mortality especially within 10 days posthatching like other cultured *Thunnus* species [15]. Another important issue is collisions. Tuna has higher oxygen demands than other teleostei. The juveniles have some morphological changes to enhance swimming ability, which may cause mortality due to collisions [16]. Other mortality reasons are absence of swim bladder inflation, sinking of larvae during the night, and incorrect prey capture when starting exogenous feeding [13]. Besides these issues, the mechanism of oocyte development is not still completely understood [11]. First spontaneous spawning was recorded in 1979 at Kinki University (Japan) with a 5-years-old captivated bluefin tuna. After that, few successful attempts have been recorded since 1987 [17]. Some studies demonstrated that a broodstock could be spawned with hormone application after one-year acclimation-captivity period [18]. Also in some studies, after one-year captivity some untreated broodstock could show good fecundity like the hormone-treated fish [10,17]. Therefore, observations on oocyte development of this species can be considered a valuable area of study.

This study reports on the oocyte development of the Atlantic bluefin tuna fattening in farming facilities by investigating the relationship between oocyte diameters and fish lengths, fish weights, and surface water temperatures.

2. Material and methods

2.1. Study area and farm

The samples were taken from the fattening facilities of a fish farm (Akua-Group Aquaculture Inc.), located in the offshore waters of Gerence Bay (Çeşme, İzmir), Aegean Sea. Net cages were high-density polyethylene (HDPE), 50 m in diameter, 30 m in depth; with mesh opening size of 8.5 mm. The stock density of fish was 2–3 kg/m^3, with a 2000–2500 fish/cage ratio. The average surface water temperature was 13 °C for February 2015, 16.7 °C for May 2015, 22 °C for June 2015, 25.5 °C for July 2015, and 21.9–22 °C for July 2016. Temperatures were recorded during fish feeding in the morning hours.

2.2. Measurements and sampling

After fork length and total weight measurements, oocyte tissue samples were taken with gonads from slaughtered fish by a scalpel blade during necropsy for histological examinations. The length (216 ± (SD) 48 cm, min = 119, max=280) and weight (234 ± (SD) 143 kg, min = 30, max = 455) values are given in Tables 1 and 2. The samples of ovary and testes were collected during the harvest in February 2015 (n = 10). For macroscopic observation, only ovarian tissues were taken from the newly dead fish in May 2015 (n = 1), in June 2015 (n = 1), in July 2015 (n = 9), and in July 2016 (n = 3). Briefly, samples were collected randomly during the harvesting process (n = 10) and during necropsy from newly dead fish (n = 14). Macroscopic images of female gonad (ovary) (Figure 1A) and male gonad (testicles) (Figure 1B) are given in Figure 1. Biometric measurements of the females were compared using Kruskal–Wallis analysis.

2.3. Histological evaluation

The oocyte samples were fixed in 10% formalin immediately after necropsy. After tissue processing, samples were embedded in paraffin blocks and cut with a rotary microtome (Leica RT 2125 RTS) to 5–6 µm and stained using the Hematoxylin-Eosin staining method at Aquaculture and Fisheries Engineering Department Laboratories, Faculty of Agriculture, Aydın Adnan Menderes University [19]. After staining, the preparations were examined under a light microscope (Novex B series BTS and Olympus CX31) with Image Focus Ver. 3 program and microphotos were taken using CMEX DC5000 and Olympus DP20 microscope cameras. Diameters of oocytes were measured in pixels, subsequently converted to micrometers by using dpi. In total, 300 oocytes per ovarium were measured. The developmental phases and diameters of the oocytes were classified according to the methods used in a prior study [12]. Only oocytes were evaluated histologically.

3. Results

Lengths and weights of the samplings were found different among the months ($P<0.05$). After studying the first sampling in February 2015, it was clear that the reproduction organs (gonad and testis) could be recognized macroscopically before histological examination, due to the fact that the same results were obtained through macroscopic and histological examinations. Based on these results, female–male ratio was 7:3 (70%:30%) (n = 10). Fish lengths and weights, fish sex, and histologically evaluated oocyte development statuses are given in Tables 1 and 2.

The histological examination of the oocyte samples taken from the individuals (n = 7) in February 2015 showed that oocytes (39–348 µm in diameter) were in the beginning of the regeneration (previtellogenic, lipid oocytes) and maturation phases (previtellogenic, vitellogenic oocytes).

In May 2015 (n = 1), oocytes were in the preovulation phase (Figure 2A). In June 2015 (n = 1), oocytes were observed in the ovulation and postovulation phase (Figure 2B). In the same sample, there were atretic vitellogenic oocytes, which is considered to be a sign of postovulation phase.

Table 1. Fish length (cm), weight (kg), fish sex, and histologically evaluated oocyte status-1, (2015) RP: regeneration phase; PP: preovulation phase; OP: ovulation phase; STP: stationary phase.

Month	No.	Fork length	Total weight	Sex	Oocyte status
February	1	250	335	Male	-
February	2	280	455	Female	RP, PP
February	3	255	351	Female	RP, PP
February	4	263	369	Male	-
February	5	262	389	Female	RP, PP
February	6	251	322	Female	RP, PP
February	7	272	407	Female	RP, PP
February	8	257	334	Male	-
February	9	230	277	Female	RP, PP
February	10	224	247	Female	RP, PP
May	11	195	156	Female	PP, OP
June	12	178	100	Female	OP
July	13	161	66	Female	STP
July	14	-	-	Female	STP
July	15	-	-	Female	STP
July	16	-	-	Female	STP
July	17	-	-	Female	STP
July	18	185	116	Female	STP
July	19	119	30	Female	STP
July	20	-	-	Female	STP
July	21	199	135	Female	STP

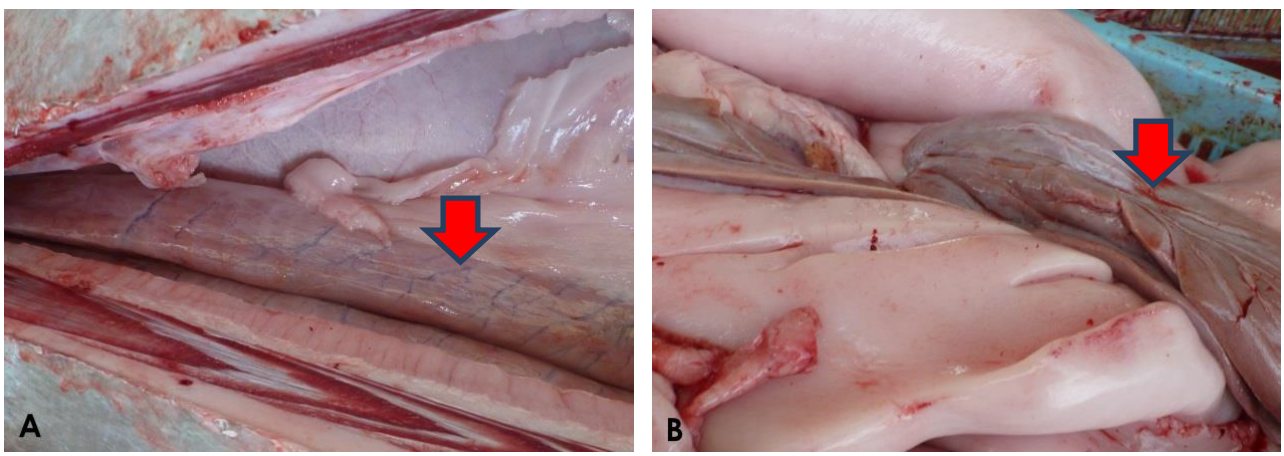

Figure 1. Macroscopic images of female gonad (ovary) (A) and male gonad (testicles) (B), arrows: reproductive organs (ovary and testicle) (Original)

In July 2015 (n = 9), oocytes were in the stationary phase (previtellogenic oocytes). In July 2016 (n = 3), oocytes were in the beginning of maturation, ovulation, postovulation, and regeneration phases, simultaneously. Previtellogenic (Figures 3A and 3B), vitellogenic, and advanced vitellogenic oocytes (Figure 3B) were recorded during this stage of the study.

Table 2. Fish length (cm), weight (kg), fish sex, and histologically evaluated oocyte status-2, (2016) RP: regeneration phase; PP: preovulation phase; OP: ovulation phase, PSP: the postovulation phase

Month	No.	Fork length	Total weight	Sex	Oocyte status
July	22	185	116	Female	OP, RP, PP, PSP
July	23	168	82	Female	RP, PP, PSP
July	24	173	78	Female	RP, PP, PSP

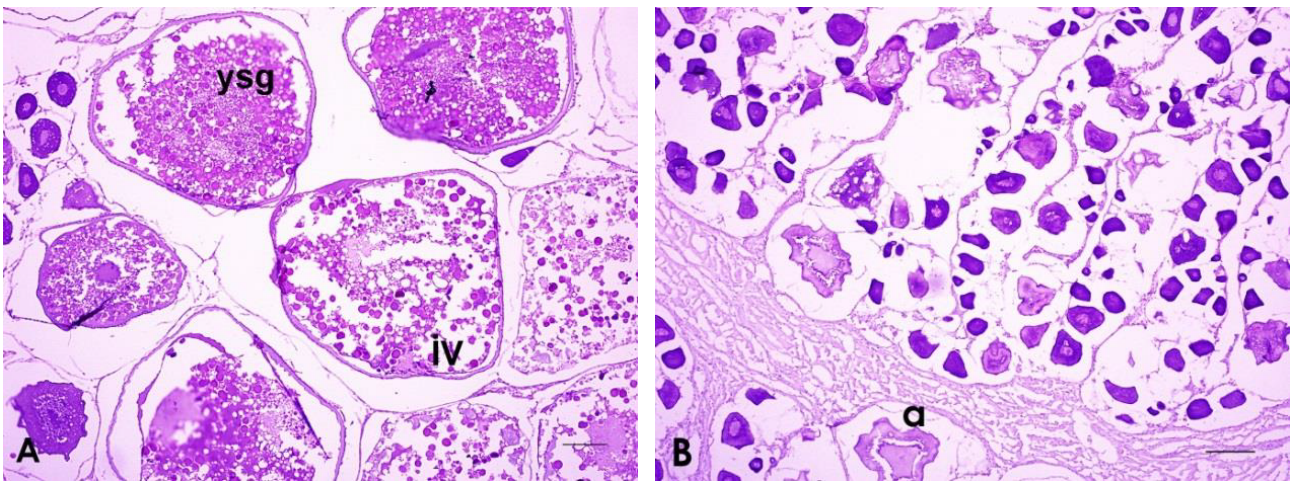

Figure 2. A) Oocytes with a diameter of 93–604 µm (preovulation phase), ysg: oocyte yolk globules, iv: advanced vitellogenic phase and oocytes. Scale bar: 100 µm, (15 kg; May 2015), B) 34–164 µm (ovulation and postovulation phase), a: atretic vitellogenic oocyte. Scale bar: 100 µm, 100 kg (June 2015)

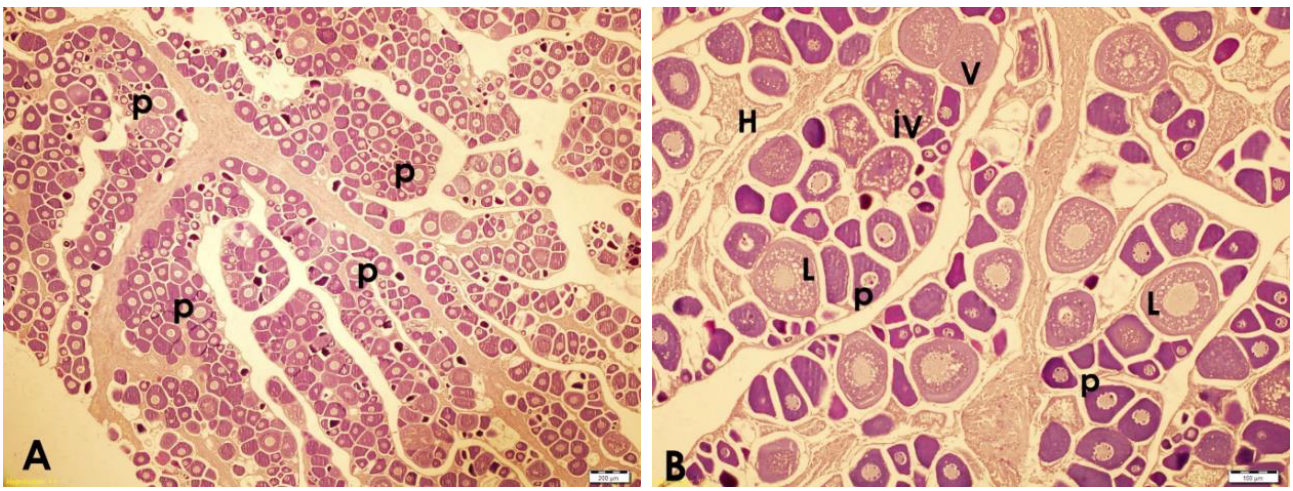

Figure 3. A) Oocytes with a diameter of 20–220 µm (beginning of maturation), B) 50–550 µm in diameter, (ovulation, postovulation, and regeneration phase) p: perinucleolus oocyte, v: vitellogenic oocyte, iv: advanced vitellogenic oocyte, H: hydrated oocyte, L: lipid oocyte. Scale bar: 200 and 100 µm, (78–116 kg; July 2016).

4. Discussion

Reproductive characteristics and female/male ratio in farming facilities are important subjects in terms of stock control and the efficient organization of fisheries operations. There have been various morphological, histological, and endocrinological studies in this context regarding Atlantic bluefin tuna. One of the most commonly used methods in female-male discrimination

Table 3. Classification of location, sample time, fork length, and oocyte status in different *Thunnus* species (LSF: lipid stage follicles, VF: vitellogenic stage follicles, POF: postovulatory follicles, AYS: advanced yolked stage, MNS: migratory nucleus stage, HS: hydrated stage, RP: regeneration phase, PP: preovulation phase, OP: ovulation phase, STP: stationary phase, PSP: the postovulation phase).

Species	Location	Sample time	Fork length (cm)	Oocyte status	Reference
Atlantic bluefin tuna	Gulf Mexico/ western Mediterrenean	February to July (n = 147) / mid-June to mid-July (n = 40)	172–326 cm/ 120–240 cm	LSF: April > June in Mexico, VF: similar in two location, highest POF in April, lowest values were in June	[26]
Pasific bluefin tuna, *Thunnus orientalis*	Japan	Late May, early August	Mean FL was 139.6 (±20.5) in 2011, 151, 6 (±16.8) in 2012	Spawning class 70.2% in 2011, 93.8% in 2012 with AYS, MNS, HS, POF	[27]
Southern bluefin tuna, *Thunnus maccoyii*	South Australia	2014	118–131 cm total length	50 µm in diameter oocytes were unyolked oocyte, up to 130 µm were early yolked oocyte	[28]
Atlantic bluefin tuna	Gulf of Mexico	May–October 2008–2010	134–292 cm	134–185 cm were immature, ≥185 cm were mature, ≥134 cm were perinuclear and lipid stage with vitellogenic oocyte	[29]
Atlantic bluefin tuna	İzmir, Turkey	February, May, June, July (2015), July (2016)	119–280 cm	RP, PP in February, PP, OP in May, OP in June, STP in July (2015), OP, RP, PP, PSP in July 2016	[Present study]

is the examination of sex steroids by taking blood samples during the spawning season [20]. For example, in European sea bass (Dicentrarchus labrax), one of the most cultured fish in Turkey, macroscopic (gonadal color and shape) or microscopic (histologically germ cells) methods can be used to determine the sex ratios. In addition, other methods of stripping procedures and cannulation in reproductive season and gonad squash mount technique can be used with juvenile fish [21]. In yellowfin tuna, sex determinations can be made by measuring 11-ketotestosterone in mucus extracted from fin clips [22].

According to a study on another most cultured species gilthead sea bream (*Sparus aurata*) as protandric hermaphrodite from Tunisia (Gulf of Gabes), male: female ratio was 1.18:1, 50% of the males with 18.75 ± 0.19 cm total length transformed to females [23].

Necropsy and histological sampling methods used in the present study are also quite common [12,16,24]. In a previous study, the female: male ratio in the stock was recorded to be almost at 1:1 ratio, with 41.9% male individuals in the stock of yellowfin tuna (*Thunnus albacares*) [20]. In our study fish were sampled randomly and female: male ratio was recorded to be 7:3 (n = 10, 70%:30%) in February 2015. However, this unexpected outcome probably resulted from the limited amount of sampling.

Surface water temperatures in the Aegean Sea where our samplings were carried out were recorded between 16.7 °C and 22 °C, for May and June 2015, respectively. This difference might be explained by the fact that our samples were collected at a more northern region. According to a study run in a fattening farm [25] with higher water surface temperatures at a location slightly southern of the region in the present study stated that reproduction season started in May for Atlantic bluefin tuna. However, in the present study the oocyte development was observed in late May, June, and July. This difference can be explained by the fact that temperature starts to increase earlier and causes earlier spawning in the southern part of the Mediterranean Sea.

In fattening farms, most of the fish show final oocyte maturation problems without hormone application. In a previous study, some fish had undergone hormone applications which resulted in oocytes with migratory nucleus or hydrated oocytes (preovulation and ovulation phase); however, oocyte maturation was not observed in the control group [10]. In another study, oocyte formation and advanced vitellogenic oocyte diameter of caged fish without hormone application were similar to wild fish, but gonadal size and final oocyte maturity were poor in the female individuals in the control group [4]. In our study, releasing oocytes from fish without hormone application appeared to be possible.

According to a study [20], active female ovaries contain postvitellogenic oocytes and atretic vitellogenic oocytes in minor amounts. Furthermore, a study [9]

showed that active oocytes collected from wild fish in the Mediterranean Sea between May and early June had been in the postovulation phase and included hydrate oocyte and postvitellogenic oocytes. These findings are similar to our own observations carried out in May and June 2015 and July 2016. These results suggest that all fish we studied were active in terms of reproduction during these months and that oocyte status is an important datum. Some of the studies which explained oocyte status in *Thunnus* species are mentioned in Table 3.

According to the results of the present study, breeding without hormone application is possible for Atlantic bluefin tuna at least for the first oocyte development (activity in late May, June, and July in the Aegean Sea). Regular monitoring of both oocyte release and embryonic development of fertilized oocytes in caged individuals might be beneficial in understanding the reproduction of this fish under farming conditions.

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