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Research Article

Effect of Tributyltin on the Sex Ratio in Guppy *(Poecilia reticulata)*

İsmihan Karayücel, Olcay Kırıkoğlu, Seval Dernekbaşı

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ABSTRACT

In this study, the effect of tributyltin (TBT) on the sex ratio in guppy (*Poecilia reticulata*) during the labile period covering both embryonic and post hatching periods was investigated. The gravid females was fed an artificial diet containing TBT chloride at an environmental levels of 25, 50 and 150ng/g feed from 16th day after the first parturition until next parturition. The newly hatched larvae from untreated females were also fed by the same diet for 11 days. TBT caused various abnormalities like body shape and fin deformations in gravid females and no parturition was seen. The male ratio significantly increased to 70.74, 87.50 and 87.18 % in the experimental groups of guppy larvae fed by 25, 50 and 150ng TBT/g diet, respectively. Survival and growth of the larvae were negatively affected by TBT treatment. These results clearly showed the masculanization of guppy exposed to TBT for the first time.

Keywords: Guppy, endocrine disrupters, tributyltin, sex reversal

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INTRODUCTION

Endocrine disrupting chemicals (EDCs), a range of pollutants in the environment, are both natural and mostly man-made, found in many everyday products including plastic bottles, metals, detergents, flame retardants, additives or contaminants in food, toys, personal care products and pesticides that interfere with the body's endocrine system and produce adverse developmental, reproductive, neurological, and immune effects in both humans and wildlife and have received increasing interest in the possible health threat posed by them. Amongst them organotin compounds (OTs), with a worldwide annual production of 50,000 tons in 1996, particularly tributyltin (TBT) have been widely used for various industrial purposes such as production of PVC, textile, slime control in paper mills, disinfection of circulating industrial cooling water, agricultural fungicides, preservation of woods and rodent repellents. The use of TBT as biocides in antifouling boat paints is the most important contribution of organotin compounds and leads to the contamination of aquatic environment (Tian et al. 2015). Aquatic pollution resulting from its usage has been of great concerns due to its bioaccumulation potential, persistence in sediment up to several years and highly toxic effects on non-target organisms (Antizar-Ladislao 2008; Anastasiou et al. 2016). After the first negative effects were reported in several studies, the use of organotin-based antifoulings from paints was prohibited in the European Union since 2003 and imposed their complete removal from the ship hulls since 2008 (Anastasiou et al. 2016). Because of the relatively long half-life of OTs in sediments (0.9-5.2 years; Dowson et al. 1993), they are priority substances posing a serious threat to aquatic environment for a long time after their deposition (Anastasiou et al. 2016). TBT, currently a cause of great concern, is still found at high levels in water ecosystem and tissues of aquatic organisms in China (Jiang et al. 2001; Zhang et al. 2013), Japan (Harino et al. 2000), Great Britain (Harino et al. 2003), Taiwan (Shue et al. 2014), India (Garg et al. 2011), Croatia (Furdek et al. 2012), South Korea (Kim et al. 2014), Spain (Jiang et al. 2001), Italy, Tunisia and Portugual (Anastasiou et al. 2016).

After the demonstration of masculinization effect of TBT on approximately 260 gastropods species worldwide (Titley-O'Neil et al. 2011), studies of the effects of TBT especially on fish reproduction has also received particular attention. In fish, TBT disrupted steroidogenesis in zebrafish (*Danio rerio*) and brown trout (*Salmo trutta fario*) (McGinnis and Crivello 2011; Marca Pereira et al. 2014) and reproductive behaviour in male medaka (*Oryzias latipes*) (Nakayama et al. 2004) and male guppies (*Poecilia reticulata*) (Tian et al. 2015), decreased fertility and fecundity in Japanese whiting (*Sillago japonica*) (Shimasaki et al. 2006), inhibited gonad development in rockfish (*Sebastiscus marmoratus*) (Zhang et al. 2007; 2013) and mummichog (*Fundulus heteroclitus*) (Mochida et al. 2007). Although there are many studies dealing with the negative effects of TBT on fish reproduction, only three studies showed that TBT altered the sex ratio towards males at extremely low levels in Japanese flounder (*Paralichthys olivaceus*) (Shimasaki et al. 2003) and zebrafish (McAllister and Kime 2003; Santos et al. 2006). Interestingly, no studies are available dealing with the effects of TBT on sex differentiation in guppy.

In the present study, the guppy, native to fresh and brackish waters of north eastern South America and adjacent islands of the Caribbean and one of the famous tropical ornamental fish in the world was used as an experimental model. They are ovoviviparous fish with a short reproductive period, readily available, easily handled fish and therefore suitable for studying EDCs on the reproductive system since they have also strong visible secondary sex characteristics. They also tolerate comparatively wide ranges of temperature, salinity, and crowding (Tian et al. 2015). Therefore, the aim of this study was to investigate the effect of TBT on the sex ratio of guppy.

MATERIAL AND METHOD

Experimental Fish Stock and their Maintenance

The experiment was conducted at an indoor facility of the Faculty of Fisheries, University of Sinop. Experimental *P. reticulata* with an average weight of 0.50g were obtained from a commercial aquarium fish production facility. Fish were kept at 26±0.5°C (±SE) under a 14/10 h light/dark cycle. They were fed with a commercial diet (JPL Novo Bea Flakes with 45.2% protein and 8% fat) twice a day (at approximately 9:00 am and 3:00 pm) *ad libitum*. O_2 and pH were monitored weekly while the water temperature was checked daily during the study. Throughout the experiments, O_2 and pH were 5.57±0.03mgL-1 and 8.64±0.02, respectively (±SE). A total of 36 aquarium of 40cmX 20cmX 20cm were used for TBT treatment of gravid females and newly born fry at three different concentrations (25, 50 and 150 ng/g feed) with triplicates. Thermostatically controlled submerged heaters were used to maintain the water temperature at desired level. Aeration was provided to each aquarium through an air pump. All aquariums were cleaned weekly by siphoning the uneaten food and excreta and water were then topped up with clean aerated tap water at the desired temperature.

Preparation of Experimental Feed

TBT chloride (97% purity) was obtained from Merck. TBT incorporated in the diet at nominal concentrations of 25, 50 and 150ng/g feed. The selection of TBT doses was based on the previous studies (McAllister and Kime 2003; Shimasaki et al. 2003; Santos et al., 2006). These doses of TBT incorporated in the diet are also environmentally relevant (Santos et al. 2006). TBT dissolved in acetone was poured onto the food in a fume cupboard and mixed frequently by a spatula to ensure even distribution of the acetone/TBT solution. The food was left in a fume cupboard to dry, then stored in airtight containers at 4°C in a refrigerator. The control diet was prepared in a same manner without using acetone/TBT solution.

Determination of TBT Treatment Timing and Duration

Determination of TBT treatment timing and duration were based on the previous hormonal sex reversal studies indicate that the labile period for successful hormonal sex reversal covers both the embryonic i.e. treating the brooding female and post hatching periods (Takahashi 1975; Kavumpurath and Pandian 1993). Therefore, the gravid females were treated by the experimental diet from 16^{th} day after the previous parturition until the termination of gestation while the experimental diet was also given to the newly born fry from the untreated female for 11 days.

Experimental Design

TBT treatment to gravid female guppy; 9 gravid females which are distinguishable by their black sacs, denoting their pregnancy were chosen, maintained individually and fed with the control diet until the succeeding parturition. The resulting broods were served as control group (first brood). The females were reared for further 15 days and fed by control diet. From 16^{th} day after the first parturition, the females were fed with the experimental diets until next parturition.

TBT treatment to fry guppy; 9 gravid females were chosen, maintained individually and fed with the control diet until the succeeding parturition. After parturition, the females were moved individually to stock tanks and the resulting broods were kept in same unit and fed by the experimental diets at concentrations of 25, 50 and 150ng TBT/g feed for 11 days and the experimental groups were coded with T25 (for replicates T25-1, T25-2, T25-3), T50 (for replicates T50-1, T50-2 and T50-3) and T150 (for replicates T150-1, T150-2 and T150-3), respectively. Fish were reared by feeding with the control diet for further 49 day and sexing of the fish was done at 60^{th} day by external examination based on the presence of the male secondary sex characters using caudal, dorsal and anal fins as markers. The fish lacking these characters were classified as female. At the end of the experiment, the fry were also weighted nearest to 0.01 g to determine the effect of TBT treatments on the average weight of guppy. The second broods from the same females would be used as control group but unfortunately the females died.

Survival rate was calculated as: (Number of fish at the end of the treatment / Number of fish at the beginning of the treatment) x 100. Survival rates were calculated at 11^{th} day and 60^{th} day for experiment.

Statistical Analysis

Survival and average weight were expressed as the mean±SE. Survival (arcsine transformed) and average weight of groups were analysed for significant differences using analyses of variance (ANOVA) with a Tukey posthoc test. A P-value <0.05 was considered as significant. Sex ratios of the experimental groups were tested against one male: one female sex ratio using χ^2 test since the respective control groups could not be obtained because of the dead of the mothers. On the other hand, it should be considered that the sex ratios of progeny of the females from the same stock did not differ the expected sex ratio of 1:1.

Heterogeneity χ^2 tests were also used for the experimental groups to pool the data. Whenever more than 20% of the expected frequencies were less than five or any expected frequency was less than one, expected frequencies increased by combining adjacent categories into a single-pooled category (Armitage and Berry, 1994). Pooled sex ratios of the experimental groups were also tested against one male: one female sex ratio using χ^2 test. Minitab 17 was used for all statistical analyses.

RESULTS AND DISCUSSION

In the present study, we examined the effects of TBT on sex ratio of both gravid and larvae guppy by oral administration of TBT. The chosen concentarion of TBT (25, 50 and 150 ng TBT/g feed) were environmental levels according to the previous studies (McAllister and Kime 2003; Shimasaki et al. 2003; Santos et al. 2006).

Nine gravid females were planned to use in order to determine the effect of TBT on the sex ratios of broods by feeding with the diet including different concentration of TBT but females showed various abnormalities like body shape and fin deformations (Fig 1). No parturition was seen and therefore no broods were obtained. Only one female in T25 group gave dead broods and died after parturition. On the other hand, no deformation was observed after TBT application to the guppy fry in this study. Although there is no report on abnormality caused by TBT in any adult ovoviviparous fish species, similar malformations (skeletal and neuromuscular) were reported in medeka embryos exposed to TBT at ≥12.5μg/l during embryogenesis with concentration and time dependent manner (Bentivegna and Piatkowski 1998). Fent and Meirer (1992) reported that TBT exposure (concentrations ranging from 0.82 to 19.51 μg/l) led to mortality, behavioral, gross morphological and histopathological effects in minnows (*Phoxinus phoxinus*) eggs and yolk sac fry. Japanese medeka (*Oryzias latipes*) embryos exposed to TBT by nanoinjection at concentrations of 0.16, 0.80, 3.96, 19.2 and 82.1 ng/egg caused impairment of development like abnormal eye development, haemorrhage and deformity of tail (Hano et al. 2007). This study clearly showed that fish species that are livebearing and give birth to free swimming fry like guppy were much more susceptible to TBT than fry and TBT caused severe abnormal development in gravid guppies. Especially effect of TBT on malformations in female livebearing fish species should be studied more detailed.

Survival rates of the experimental groups of TBT treated guppy fry did not differ significantly on 11th and 60th day of the experiment (Table 1). On the other hand, significant differences were found in T25 and T150 groups between 11th and 60th day of the experiment showing that survival was reduced after 11th day of the experiment. Average weight of the experimental groups was significantly reduced by increasing level of TBT. Although this

Figure 1. Body shape and fin deformations in female guppy *(Poecilia reticulata)* after treatment with the diet containing TBT.

Table 1. Survival rates (%) and average weight (g) of guppy (*Poecilia reticulata*) fry fed by diet containing different concentration of TBT.

Experimental groups	Survival rate on 11th day	Survival rate on 60th day	Average weight on 60th day
T25	100±0.00[ax]	53.67±6.06[ay]	0.30±0.04[a]
T50	83.33±16.67[ax]	54.33±15.07[ax]	0.16±0.01[ab]
T150	100±0.00[ax]	48.66±5.70[ay]	0.15±0.05[b]

Different superscripts (a, b) within the same column denote significant differences.
Different superscripts (x, y) within the same row denote significant differences.

Table 2. Sex ratios of guppy (*Poecilia reticulata*) fry treated by diet containing different concentration of TBT at 60th day of the experiment

Experimental groups	Number of female (%)	Number of male (%)	P ($\chi 2$) Test (vs 1:1)	Average female (%)	Average male (%)	P ($\chi 2$) of pooled data of the replicates in the same experimental group (vs 1:1)
T25-1	8 (42.10)	11 (57.89)	0.491[NS]	29.26	70.74	0.008**
T25-2	1 (5.55)	17 (94.44)	0.000***			
T25-3	3 (75.00)	1 (25.00)	0.317 [NS]			
T50-1	3 (100.00)	0 (0.00)	0.083 [NS]	12.50	87.50	0.000***
T50-2	0 (0.00)	30 (100.00)	0.000***			
T50-3	3 (20.00)	12 (80.00)	0.020 **			
T150-1	2 (20.00)	8 (80.00)	0.058 [NS]	12.82	87.18	0.000***
T150-2	0 (0.00)	17 (100.00)	0.000***			
T150-3	3 (25.00)	9 (75.00)	0.083 [NS]			

*P<0.05, **P<0.01, ***P<0.001, NS: not significant (P>0.05)

study did not design for investigation of TBT effect on growth rate in guppy, our result still provide strong support for the fact that TBT do have an considerably suppression in growth of guppy fry. Similar results were also reported in rainbow trout (*Oncorhynchus mykiss*) (Seinen et al. 1981), silverside (*Menidia beryllina*) (Hall et. al. 1988) and Japanese flounder (*Paralichthys olivaceus*) (Shimasaki et al. 2003). Rainbow trout in the yolk sac fry stage exposed to tri-n-butyltin-chloride (TBTC) at concentrations of 0.2 and 1µg/l for 110 days showed a significant and a dose-related growth retardation resulting in a 44% decrease of the body weight in the 1 µg/l group at the end of the experimental period (Seinen et al. 1981). Reduced growth in larval inland silverside (*Menidia beryllina*) was reported by Hall et al. (1988). TBT concentrations of 93 and 490 ng/l significantly reduced growth by 20 to 22% in this species. In Japanese flounder fed an diet containing tributyltin oxide (TBTO) at concentration of 0.1 and 1.0 µg/g diet from 35 to 100 d after hatching showed suppressed growth (Shimasaki et al. 2003). This study was agreed with the previously mentioned researchers indicating that TBT may suppressed the feeding or metabolic rates which causing to a lower growth rate in TBT-treated groups than in the control and the inhibitory effect of triorganotin compounds on mitochondrial energy conservation.

Higher male ratios were obtained in the experimental groups (Table 2) showing male biased sex ratio. Significant deviations from one female: one male sex ratio were observed in some replicates of the experimental groups. No heterogeneity was observed with respect to sex ratios in replicates of the groups and data were pooled. Pooled sex ratios of the replicates in the same experimental group significantly differed from the balanced sex ratio of 1:1. In vertebrates, the genetic sex of the individual determined by the combination of male and female genetic factors at fertilization. However, sexual differentiation which refers to gonadal development after primary sex determination in fish is highly plastic, labile and susceptible to the presence of exogenous steroids. Even after primary sex determination in fish, sexual development can be triggered by external factors in contrast to the genotype. Treatments of embryos or larvae with exogenous androgens and estrogens lead to functional male and female phenotype and/ or vice versa and also called sex reversal which is a valuable tool in the elucidation of sex determining mechanisms and production of monosex populations in aquaculture industry (Pandian and Sheela 1995; Baroiller and D' Cotta 2001; Strüssmann and Nakamura 2002). The first report about the effect of TBT on sexual differention of fish was published by McAllister and Kime (2003). In that study, water exposure to zebrafish larvae from hatch to 70 days, to environmentally realistic level of 1 and 10 ng TBT/l resulted 80 and 90% male. 35 days post-hatched Japanese flounder larvae fed an artificial diet containing tributyltinoxide (TBTO) at concentrations of 0.1 and 1.0 μg/g diet from 35 to 100 days after hatching resulted male bias sex ratio (25.7% and 31.1% male, respectively) compared with the control (2.2%) (Shimasaki et al. 2003). Santos et al. (2006) demonstrated that fish exposed to TBT showed a bias sex ratio toward males (62.5% males in control tanks and 86% and 82% in TBT 25 and TBT 100 ng TBT/g, respectively) in zebrafish. Bias sex ratio towards male (62.5% males in control tanks and 86% and 82% in TBT 25 and 100 ng/g diet, respectively) was also reported in five days post-fertilization zebrafish larvae fed a diet containing TBT for a 4-month period (Santos et al. 2006). Our data for guppy are consistent with these results showing male biased sex ratio in the experimental groups (between 70.74-87.50%) with significantly differentiation from the balanced sex ratio of 1:1. Although the respective control groups could not be obtained, the sex ratios of progeny of the females from the same stock did not differ the expected sex ratio of 1:1. The result suggest that either the TBT treatment was lethal to genetic females or the TBT treatment caused to masculinization of genetic females. On the other hand aforementioned literatures support the latter hypothesis. Endocrine disruptors interfere with hormone-signaling by competitively binding to the androgen or estrogen receptor, thus activating the transcription of sex-hormone-dependent genes (e.g., xenoestrogens or xenoandrogens), binding but not activating the androgen or estrogen receptor (e.g., anti-androgens or anti-estrogens), modifying enzymatic pathways involved in biosynthesis of sex hormones and other unclear mechanisms (Liao et al. 2014). It is well known that E2 stimulates the hepatic production of yolk that is necessary for oocyte growth. In the ovary, E2 is produced by conversion of T catalyzed by the steroid synthesising enzymes, in particular the P450 AI. TBT is a well known P450 AI. The present and the earlier studies clearly showed increased levels of T and decreased levels of E2 in the ovaries of the fish exposed to TBT which could be related with an inhibation of P450 aromatase activity resulting male bias sex ratio.

CONCLUSION

This is the first report of TBT causing masculinization in guppy. In summary, the data presented here strongly suggest that TBT exposure to guppy resulted in abnormality in gravid females, biased sex ratio towards male and suppression of growth in guppy larvae.

Ethics Committee Approval: This study was conducted in accordance with ethics committee procedures of animal experiments.

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Research Article

Interactions Between Sea Turtles and Fishing Along the Aegean Coast of Turkey

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ABSTRACT

In this study, the interactions (encountering and bycatching) of the sea turtles with fisheries in the Aegean Sea were investigated.

Data were collected by face-to-face survey with a total of 404 fishermen in 98 fisheries cooperatives for 5 provinces (Çanakkale, Balıkesir, İzmir, Aydın and Muğla) between August 2016 and October 2017.

The interactions are more intense in places where fishing activities coincide with the living areas of these living beings. Although there is maximum (100%) interaction (visual contact) on the coast of Aydın (100%), the average annual encounter within these five provinces is quite high with 88%. While there is a significant difference in the rate of encounters with turtles had been found between the provinces ($p<0.05$), there is no significant difference in the rate of bycatching with fishing gears ($p>0.05$). Even though one of every four fishermen who participated in the survey had bycaught sea turtles at least once, the most of bycatching occurred in the fishing gears in the group of trammel/gill nets with 76%.

In areas where sea turtles are concentrated on the Aegean coast, applications should be carried out to prevent or reduce incidental catch of the fishing gears. Because the results show that these living beings are heading towards the Aegean north due to global warming.

Keywords: Aegean Sea, sea turtles, fishing gears

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INTRODUCTION

Although the Aegean Sea has an intended coastline with many bays, gulfs and islands, fishing grounds are limited due to steep, broken topography and a narrow continental shelf, and national waters of Turkey. Saroz, Çandarlı, Izmir, Sığacık, Kuşadası, Güllük and Gökova Bays as well as adjacent water of Gökçeada and Bozcaada are important fishery areas in the Aegean Sea and are rich in biodiversity similarly to the Mediterranean (Kınacıgil and İlkyaz, 1997). According to 2017 records, 30% (4027 fishing vessels) of Turkey's fishing fleet is located in the Aegean region (TUIK, 2018). 33% of the boats using the trammel/gill nets, as well 30% of the boats using both of longlines and handline are registered at the Aegean ports. In addition to the small-scale vessels, 6% of the trawlers and 17% of the purse seines are in the Aegean. This situation has lead to diversification of fishing gears for effective fishing. While fishing for large vessels is only carried out during the fishing season in non-banned areas, there is no such limitation for small boats. Small-scale fishermen usually make their living by fishing mostly on coastal areas.

The Bern Agreement which concerned the European Conservation of Natural Life and Natural Environment was signed by the European Economic Community and other member countries in 1982. The agreement was approved by the Council of Ministers dated 9 January 1984 and numbered 84/7601 and was published in the Federal Register dated 20 February 1984 and entered into force in Turkey. At the same time in the vernacular of the day according to Article 16 entitled "Fishing Forbidden Species" of the notification on the Arrangement of Commercial Fishing Regulations No. 4/1 of the notification No 2016/35 (Anonymous, 2016) which was enacted by published in the Federal Register dated August 13, 2016 and numbered 29800 and which was valid as of 01/09/2016, that is forbidden to catch, collect, keep on board, disembark, transport and sell species of *Caretta caretta* (Linnaeus, 1758), *Chelonia mydas* (Linnaeus, 1758) and *Dermochelys coricea* (Vandelli, 1761) in all our waters. Aegean fishermen use the name *C. caretta* for all sea turtles which they have seen. Only the fishermen in Muğla can distinguish the Nile turtle (*Trionyx triunguis*) from other turtle species because their morphological characteristics are obviously different.

The sea turtle population in the Eastern Mediterranean was seriously exploited as a target species in the first half of the 20th century. Nowadays, the most important threats to these turtle populations are anthropogenic effects in nest sites and interaction with fishing (Casale, 2008). Even though the dominant small-scale coastal fishing, traditional subsistence fishing and recreational fishing in coastal Turkey, there is no extensive study on bycatch of turtle. Despite of the dominant small-scale fishing, artisanal fishing and recreational fishing in coastal areas of Turkey, there is no extensive study on the bycatching of turtles.

In this study, the interactions (encountering, bycatching and saving) of sea turtles with the fisheries in the Aegean Sea were investigated. Within this framework, we have tried to reveal the situation of the bycatch of sea turtles with different fishing gears according to time and space and mentioned applications to reduce them.

MATERIAL AND METHODS

The study was carried out along the coasts of the Aegean Sea in Çanakkale, Balıkesir, İzmir, Aydın, and Muğla within 34 districts bound to these provinces and in 98 cooperatives areas between August 2016 and October 2017. The data were collected through a face-to-face survey of 404 fishermen in these cooperative areas. In this context, questions about general information about with used fishing gears, boat characteristics, fishing area, the average amount of catch and problems encountered, the conditions of interaction with sea turtles (encountering, bycatching and saving) and the reduction of the bycatching of sea turtles (propensity to use deterrents and the points of view of fishermen such as TED's, longline hooks, shark silhouettes, acoustic signals) were used. Legal research ethics committee approval permissions for the survey were obtained from the Aegean University Scientific Research and Publication Board and related Ministry. Every fisher interviewed was informed about the purpose of the work, the confidentiality of the collected data and their names would be preserved. The interviews were carried out after the fishers agreed to participate in the survey. Face-to-face interviews and survey were organized at ports, on fishermen's boats or in cooperative buildings. The survey was designed for the purpose of investigating the views of fishermen and their interactions with sea turtles, none of the questions have been made from multiple-choice or limited and they were open-ended to set fishermen free.

The total number of registered fishermen in the Ministry of Agriculture and Forestry has been accepted as the mainland along the coast of Aegean Sea (the shoreline from the Çanakkale border to the Antalya province border). In this case, the number of fishermen on the mainland is 5076.

The number of the survey to be made according to the sampling size account is calculated by the following formula *(Newbold, 1995).*

$$n = \frac{Nxt^2xpxq}{d^2(N-1) + t^2 * p * q}$$

N = Number of individuals in the population

n = Number of individuals to be sampled

p = frequency of observation of the event to be investigated (probability)

q = probability of not observation of the event to be investigated (1-p)

t = value in the t table at a certain degree of freedom and at the determined level of error

d = ± error

95% confidence level and 5% error margin were accepted in the calculation of the total sample size, and the probability of observation and non-observation of the event was taken as 50-50% due to heterogeneous construction. Accordingly, the number of fishermen to be sampled was calculated as 357, and random stratified sampling method was applied with the aim of providing a homogeneous distribution.

However, the survey data obtained during the field studies revealed that the current number of fishermen was 4569 on the Aegean Sea coast. In this case, it was calculated that the number of interviews required to be done by recalculating the sampling size account was 354 and the layer interval was 0.078. During the field studies, face-to-face interviews were made with 404 fishermen instead of 354 fishermen as a result of fishermen's approach to the subject and their willingness to participate in the survey.

Information on the target and catch composition was obtained from co-operative presidents and fishermen, and information on bycatch of sea turtles was taken only from fishermen. The obtained data were analyzed in MS-Excel and SPSS 22 programs. The chi-square test was used to compare the number of turtles according to the boat, season, area and fishing gears.

RESULTS AND DISCUSSION

It has been determined that current the number of registered fishermen is 4569 on the Aegean Sea coasts during fieldwork. In this case, it is calculated that the number of the survey to be made by recalculation of the sample volume is 354 and the interval of stratum is 0.078. A survey was conducted with 404 fishermen during the field studies. The number of the survey to be carried out according to the respectively were 60 for Çanakkale, 18 for Balikesir, 149 for İzmir, 26 for Aydın and 68 for Muğla, and the number of the survey was carried out the respectively 83 in Çanakkale, 26 in Balıkesir, 177 in İzmir, 32 in Aydın and 86 in Muğla.

All of **t**he fishers who carried out the survey were male individuals. The age distribution of 57% of fishermen were between 40 and 59 years. A large proportion (81%) is composed of elementary / secondary school graduates. In Behramkale, which is especially connected to the Ayvacık district of Çanakkale, the vast majority of the male individuals were directed to fisheries instead of continuing education-training, while the ladies were involved in education and training activities. 94% of fishermen are fishing with boats either by their own boat or obtained the right to use it by renting. The rest of the fishermen are boat workers. The number of fishermen who are members of the cooperatives in where is 85%. 80% of fishermen are fishing with less than 9 m of boats and 73% of them catch less than 10 kg per day. The distribution of fishing gear used by 400 of the 404 fishermen who participated in the survey is as in table 1.

Table 1. Fishing gears which used by fishermen and distributions

Fishing Gears	Number of Fishermen	Percentage of Fishermen (%)	Percentage of Fishing Gear Used (%)
Trammel/ Gill net	343	68.9	85.8
Longline	92	18.5	23.0
Trawl	6	1.2	1.5
Purse seine	9	1.8	2.3
Fishing line	33	6.6	8.3
Others	15	3.0	3.8
Total	498	100	125

The 252 fishermen use only a trammel/gill net, and 91 fishermen use a second fishing gear with a trammel/gill net. 13 fishermen use longline as the only fishing gear and 76 fishermen use thetrammel/gill net as a second fishing gear together with longline. For this reason, the number of fishing gear used can be increased to 498 and the rate to 125%.

89% of the fishermen who participated in the survey observed visual contact with sea turtles at least once during the catching operation and 274 fishermen had encountered sea turtles at least four times during the season. While all of the fishermen encounter sea turtles in Aydın, the least encounter with 82% is in Izmir (Figure 1). For this reason, there was a significant difference in the rates of encounters with turtles had been found between the provinces (P = 0.004). 77.3% of in the rates of encounters with turtles had been at most during the fishing operation in the summer. Followed by spring with 11,1%, autumn with 7,1% and winter with 4,5%. An encountering is observed absolutely within these five provinces in every seasons, at least of in the rates of encounter were in the winter and at most in the summer.

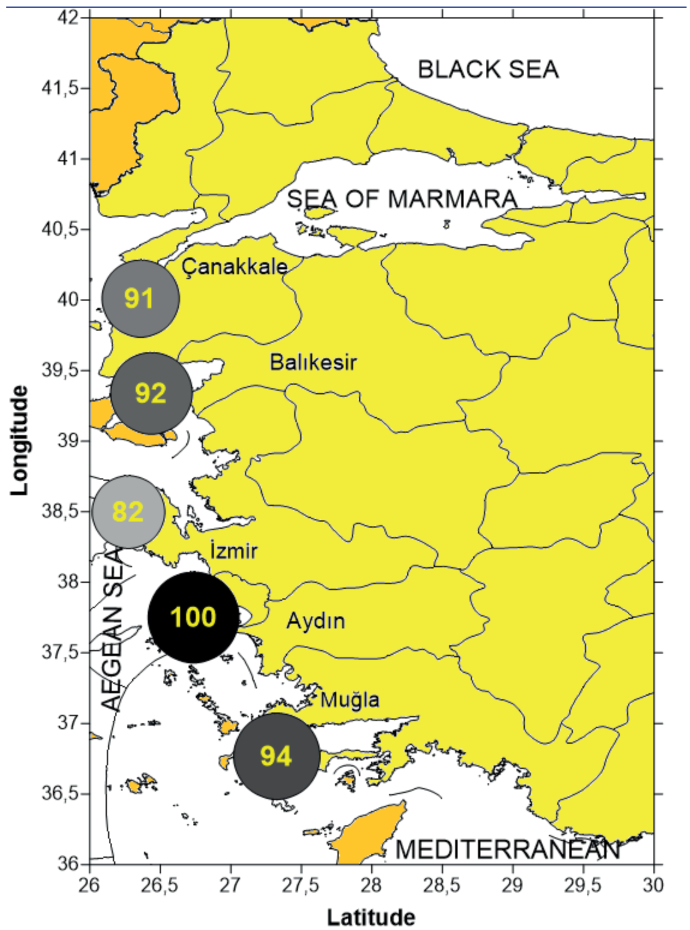

Figure 1. According to the provinces the rates of encounter with sea turtles.

While 299 (75%) of 399 fishermen who participated in the survey never bycaught sea turtle but 100 (25%) of them bycaught at least once. 46 of the 100 fishermen who said they caught sea turtles with fishing gear during fishing operations bycaught at least once, 40 of fishermen bycaught 2-4 times and 14 of fishermen bycaught more than 5 (Table 2). 18 of 79 fishermen in Çanakkale, 9 of 25 fishermen in Balıkesir, 35 of 177 fishermen in İzmir, 13 of 32 fishermen in Aydın and 25 of 86 fishermen in Muğla have bycaught sea turtle with their own fishing gears. Nevertheless, as incidental catching of turtles in the fishing gears there was no significant difference between the provinces (P = 0.054).

According to the number of vessels and fishing gear, the distribution of 25% of fishermen who bycaught sea turtle during the fishing operations is detailed in the table 2. According to this, trammel/gill net is the fishing gear that catches the most turtles, followed by longline, fishing line, purse seine, trawl and others. İzmir where most bycaught sea turtles come at the beginnig of the other provinces, the rate of catches is high in Balıkesir and Çanakkale.

The methods of save (release) of sea turtles is varied according to fishing gears (Table 3). Cutting the mesh in the trammel/gill nets and cutting the branchline of the longline are the most commonly used release methods. Six fishermen who make their living by trawl and 9 fishermen who make their living by purse seine stated that they had carried out the rescue process respectively by emptying the trawl bag or while collecting the nets, taking back from the edge of the net.

60 of the 100 fishermen who participated in the survey, saved a sea turtle in the marine environment without putting it in the boat, 40 of them rescued them on the boat. According to 99 of 100 fishermen who bycaught sea turtles even if not bycaught none of the turtles are injured, only a turtle saved from the trammel/gill net was injured in İzmir. None of the fishermen had any knowledge of how to intervene in the first phase. 98 of the 100 fishermen who bycaught the sea turtle said that the bycaught turtle's breathing was regular and quickly swam away after being rescued from the trammel/gill net, 2 fishermen did not notice the conditions of the turtles, for they did not pay attention to their breathing. A possible bycatching result 401 (99%). Fishermen had no knowledge of how to assist a sea turtle with irregular breathing, only a total of 3 fishermen said that they had enough knowledge about how they should intervene in a probable breathing problem in Küçükkuyu and Babakale and in the center of Çanakkale province.

322 (80%) of the fishermen were be unwilling to use Turtle Excluding Devices (TED), circle types of hooks for longline, dogfish silhouettes for trammel/gill net, remover devices that emit sound waves at certain frequencies according to non-target fishing composition, and in addition to this the newly developed fishing gears, equipments and methods for reducing the non-target fishing in the fishing gear of sea turtles. Only 81 (20%) fishermen willing to use these devices and methods since they are open to all technological developments for the sustainability of the ecosystem and the continuation of fishing.

Even though small-scale fisheries are dominant in the Aegean Sea, the non-target catching of sea turtles is not included in the evaluations. Although small-scale fishing (trammel/gill net, longline, fishing line, etc.) is not as effective as large-scale fishing (trawl, purse seine) in terms of production, it is in greater interaction because they use fishing areas together with sea turtles. The vast majority of the boats used in the Aegean Sea fishery are the

Table 2. Distribution of bycaught sea turtles according to provinces and fishing gears.

Number of bycatches	Provinces	Fishing Gears						
		Trammel/ Gill net	Longline	Fishing Line	Purse Seine	Trawl	Others	Total
at least once	Çanakkale	7	-	2	-	-	-	8
	Balıkesir	7	1	1	-	-	-	8
	İzmir	15	6	3	-	-	-	17
	Aydın	2	-	-	2	-	-	4
	Muğla	8	4	-	-	1	-	9
two to four	Çanakkale	5	-	2	-	-	-	5
	Balıkesir	1	-	-	-	-	-	1
	İzmir	12	2	-	2	-	-	15
	Aydın	5	1	-	1	-	-	6
	Muğla	6	7	2	1	2	1	13
more than five	Çanakkale	2	1	2	1	-	-	5
	Balıkesir	-	-	-	-	-	-	-
	İzmir	3	-	-	-	-	-	3
	Aydın	2	1	-	1	-	-	3
	Muğla	1	-	-	1	1	-	3

Table 3. The method and rate of rescue of sea turtles from fishing gears by fishermen.

Rescue Style	Number of Fishermen	Number of Fishermen (%)	Save (%)
Cutting the nets	354	71.5	88.1
Cutting the branch line of the longline	92	18.6	22.9
Cutting the fishing line	34	6.9	8.5
Emptying the trawl bag	6	1.2	1.5
Taking back from the edge of the net	9	1.8	2.2
Total	495*	100	123.1

**The total number seems to be high due to the fact that some fishermen also use two types of fishing gears.*

small-sized boats under 12 m. Because of the fact that the number of boats in small-scale fisheries is high and the lack of time closure increases the probability of interaction with sea turtles. However, most studies on the reduction of incidental catching of sea turtles and their fishing as well as the records kept are usually related to large-scale fisheries. In small-scale fishing (including traditional and subsistence fishing) and hand-line fishing, non-target catch is not generally taken in consideration (Peckham *et al.*, 2007). However, in some studies, small-scale fishing is also seen as an important risk factor for by-catching of sea turtles (Peckham *et al.*, 2007; Shigueto *et al.*, 2008).

Sea turtles have interacted intensely in areas where small-scale fishermen used the trammel/gill net, longline and fishing tackle sets in the Aegean waters, Since the trammel/gillnets used vary according to the target species, there are too many different types of trammel/gill net. The capture of sea turtles with the trammel/gill nets are varied according to the type of nets, the place where it is used and the fishing effort. While only one *C. caretta* was caught in the 131 operations with pelagic sword trammel/gill nets at the moonless night (Akyol *et al.*, 2012), drifting nets, which are a different type of trammel/gill nets, by-caught 5 *C. caretta* in 125 operations of albacore (*Thunnus alalunga*) fishing in the Aegean waters (Akyol and Ceyhan, 2012). Although there are many applications to reduce of incidental catching of sea turtles with trammel/gill nets, many tools and methods are still being tested to reduce of incidental catching. In Mexico where the tortoises are heavily distributed, in the Punta Abreojos region, emitting ultraviolet light LEDs (peak wavelength 396 nm) have been tested at 40 cm bottom of trammel/gill nets in order to prevent bycatching of *C.mydas,* and as a result, 209 *C. mydas* in the control net and 123 *C. mydas* in the experimental net were caught (Wang *et al.*, 2013). Another means of reducing the interaction of sea turtles with trammel/gill nets is the use of acoustic signal transmitter (pingers) (Kraus *et al.*, 1997). Experiments have shown that acoustic signal transmitters are initially effective for decreasing interactions of both dolphins and turtles with fishing gears.

Longline sets are the second fishing gear used widely in Aegean waters. Most of the fishermen using the trammel/gill nets use longline concurrently. Longlines can be set up in different depths and shapes depending on the target species. It throws depth varies from 10 m to 350 m in the Aegean Sea. Longline and sea turtles are in high interaction in areas of this depth. 28071 sea turtles were caught with longline in the Mediterranean Sea between 1990 and 2008 (Wallace *et al.*, 2010). Deaths of sea turtles which were caught with bottom longline are inevitable as they could not surface of the sea to breathe. The hook which is developed for catching of target species, and changed forages have been succeeded for decreasing of by-catching sea turtles. Yet J-type hooks have common usage, circle hooks have dropped the by-catch of sea turtle (FAO, 2010). Even the narrowest point of the circle hook is wider than the J-type hook. A decrease in mortality rates of *C. caretta* which are released from the circle hook have been observed (Foster *et al.*, 2012). Another factor affecting the interaction of turtles with the fishing gears are the feed used in longling fishing. The most preferred baits in fishing tunny and swordfish with pelagic longline are squid and mackerel. Sea turtles tend to eat the bait with small bites until it ends on the hook. Turtles swallow the hook together with the squid meat. Because it is more elastic than fish meat, and they cannot eat by biting squid meat in pieces (FAO, 2010). However, recently it has been established that the blue-painted squid meat drops bycatch of the sea turtles (Swimmer *et al.*, 2004). Flash light bars, dogfish silhouettes, radio frequency waves, chemicals, deterrent sounds also fall into the other ways of reducing the interaction of sea turtles with pelagic longline (Wiedenfeld *et al.*, 2015). However despite all this, it is not possible with today's technology to reduce the non-target catching to zero.

In the study, it was determined that sea turtles were caught in 67% of the trawl fishing boats. In trawls, success was achieved not only in the selectivity of size of the target species but also in the selectivity of the non-target species. The sea turtles caught in the trawl drowns, since they can not get out of the net to breath. When the data of trawling in the Mediterranean Sea between 1990 and 2008 was compiled, it was reported that 16041 sea turtles had been caught (Wallace *et al.*, 2010). The number of turtles caught by 11 trawlers during the 1999-2000 the open season between Mersin and Samandağ had been reported as 698 (Taşkavak *et al.*, 2006). In the same study, two of them were without TED and one of them was fishing by hanging a TED on the trawl net, while a total of 12 sea turtles were caught in TED-free net, 3 individuals of them were escaped through TED. Especially, since there is alot of sea turtle interaction in the shrimp trawls which take place in the bottom trawler, the strategies to reduce the interaction with some modifications (using TED) in these trawls started by the National Marine Fisheries Service (NMFS) 25 years ago in the Atlantic Ocean and in the Gulf of Mexico. It can be said that the TEDs are the most successful technological development in reducing the by-catching of turtles in trawls. It is possible to say that the TEDs are the most successful technological development in reducing the by-catching of turtles in trawls.

In the study, all purse-seine fishermen said that they caught sea turtles during the fishing operation. During the operation, turtles can coil in the network, and their pallets and crusts can be injured by pulling the net potently (NMFS, 2017). The turtle that is attached to a network, while being pulled by the net collecting roller both it can be damaged by falling from the top and it can be crushed while passing through a net reel (FAO, 2010). All of the purse-seine fisheries who participated in the survey, release turtle on the edge of the net without taking to the boat with aid of a assistant boat which is called skiff and ladle in the marine environment.

It is estimated that the annual number of by-catching of sea turtles in the world is 85000 (Wallace *et al.,* 2010) and in the Mediterranean is 44000 (Casale, 2011). Humber *et al.* (2014) estimated that the total number of sea turtles caught legally was 42000 per year, and that this number was 31268 only in Papua New Guinea, Nicaragua and Australia. In a survey conducted in Italian waters in 2014, it was estimated that bycaught turtles numbered 52340 individuals (Lucchetti *et al.*, 2017). According to the results obtained in this study, only 25% of the Aegean fishermen caught sea turtles at least once during the fishing operation between August 2016 and October 2017.

It is possible to state that while the sea turtles are thought to stop by the Aegean Sea for feeding purposes only, their living areas have headed for the north according to this study. The lack of any significant difference between the cities of bycatching supports the idea that sea turtles, who generally use the Mediterranean coasts for spawning and the Aegean Sea for feeding purposes, have headed north. This case can be explained by the way sea turtles head towards the Aegean coasts based on the warming of northern waters due to global warming.

For some fishermen, sea turtles are the living beings that come to the ensnared fish on the net, cause damage to nets and yield, and bring about time loss when they are caught. Sea turtles tear away the fish caught on the net by shivering the net, and leave a three-hole trace on the net by unripping with their flippers. However, fishermen claimed that the damage caused by sea turtles to their nets is less important than the damage by dolphins and seal attacks. These living beings are either released by splitting the fishing gear with powerful movements depending on the types and material of the fishing gear, or are saved from the fishing gear and put back in the marine environment depending on the attitude of the fishermen. The death of the sea turtle interacting with the fishing gear is inevitable when it cannot breathe from the surface, as it cannot pull through the gear or it goes unnoticed by the fisherman.

When fishermen encounter a wounded sea turtle, they reach out for the person or people, who will deal with the situation, barely. The intervention not only causes time loss, but can also make the fishermen feel guilty. For this reason, the fishermen leave these living beings in their environment without any intervention. The flow of data about turtles encountered or by-caught is missing due to the fact that there is no obligation to keep a logbook for boats under twelve meters. An appropriate registration method should be established to ensure reliable data flow associated with these protected species. Furthermore, incentives should be given to fishermen for the use of tools and methods that reduce by-catching instead of banning fishing activities in areas where turtles are abundant.

Considering the data obtained from the study areas, it can be said that sea turtles are commonly found along the Aegean coast. The reliability of the collected data is high, as it relies on that the fishermen have been fishing in this area for many years. While the first aim of the studies is the protection of sea turtles who live in the areas the study carried out, the protection of dolphins and seals, which fishermen encounter during fishing, is the second one. For a sustainable ecosystem, training packages containing information on the protection of these living beings can be prepared and active participation of fishermen can be ensured. In addition, more detailed studies are needed in the region because local fishermen have not encountered juvenile or hatchling individuals.

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Research Article

Effects of 17ß-estradiol Hormone on Growth and Sex Differentiation in the Mozambique Tilapia (*Oreochromis mossambicus*, Peters, 1852)

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ABSTRACT

In this study, the effects of 17-ß-estradiol hormone on the zootechnic performances and sex ratio of Mozambique Tilapia (*Oreochromis mossambicus*. Peters, 1852) fry were investigated in order to initially produce functional phenotypical females and then super males. To this end, different E2 concentrations (50, 100, 150 and 200 ppm) were tested over a period of 45 days in a closed recirculating system. The evaluations showed that the female ratio was significantly (p value= 0.04 and α=0.05) higher in all treatment groups ranging from 61.90% to 86.36% compared to the control group. Growth was significantly higher (F value=22.78 and p value=0.00) in the control group compared to the treatment ones.

Keywords: 17ß-estradiol hormone, Mozambique tilapia, Oreochromis mossambicus, sex difference

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INTRODUCTION

Mozambique tilapia (Oreochromis mossambicus. Peters, 1852) in mixed-sex cultures is known to reach sexual maturity long before reaching commercial size (Jiménez & Arredondo, 2000; Arboleda-Obregón, 2005). Therefore, in commercial farming of Mozambique tilapia, reproduction during grow-out is a major problem, leading to the overpopulation of ponds, and finally resulting in a wide range of fish sizes at harvest. As a solution to this problem, several techniques have been developed to produce larger and uniform fishes including all-male production (Mair et al., 1997; Tariq-Ezaz et al., 2004). Production of a monosex population in a tilapia culture through hormone sex reversal eliminates uncontrolled reproduction and allows the production of marketable-sized fish (Shreck, 1974).

The initial development of a monosex population requires the feminization of the XY genotypes during their sexually undifferentiated stage and the identification of the "neo-females" (females XY) through a progeny test (Mair et al., 1997). The feminization process is related to the fact that sex determination is under genetic control in Tilapias, but the ultimate differentiation of the gonads in fishes depends on endocrine signals, i.e. estrogens and androgens (Yamamoto, 1969; Arcand-Hoy and Benson, 1998; 1998; Uguz et al., 2003; Hayes, 2005). Indeed, the genetically prescribed sex in fishes (Atlantic cod Gadus morhua Nile tilapia Oreochromis niloticus, Honeycomb grouper Epinephelus merra) could easily be overridden by the applications of exogenous steroids if they are applied at the appropriate time and dose during early development (Shreck, 1974; Hunter and Donaldson, 1983; Uguz et al., 2003).

Even if successful feminization attempts have been conducted in the genus "Oreochromis" (Mair and Santiago, 1994; Rosenstein and Hulata, 1994; Vera-Cruz et al., 1996; Piferrer, 2001), more often they concern exclusively the Nile Tilapia (Oreochromis niloticus). There is very little information available about varying parameters such as hormone dose, treatment start time, duration of treatment and stocking density in Mozambique tilapia (Oreochromis mossambicus). Additionally, the global effects of estradiol-17ß (E2) on growth and sex-differentiation in this species have been studied in relation to the small number of publications related to this fish. Therefore, the present study was undertaken to determine the effects of E2 on the growth and the feminization of the gonads of sexually undifferentiated XY O. mossambicus fry. This is a preliminary step towards our ultimate objective of developing a breeding program to produce a male genetic population through supermale (YY) broodstock in EGE University in Western Turkey.

MATERIAL AND METHODS

Animal Culture Condition

This study was carried out in accordance with Guidelines for animal Care and Use of laboratory Animals over a period of five months (20 weeks). It took place in three phases and in three different breeding environments located in the experimental aquaculture unit of the Faculty of Aquaculture of EGE University in Turkey:

- First phase: Reproduction of broodstock maintained in the laboratory for four weeks in polyester tanks with a total volume of 200 Liters of water incorporated in a recirculation system at the ratio of one male to 3 females;
- Second phase: Sexual inversion of the larvae for 45 days in glass aquaria 30cm x 30cm x 30cm in size with a total volume of 27 lt;
- Third phase: Growth of fries for 12 weeks in a recirculating fresh water system at 28°C with UV filtration under a 14L: 10D photoperiod with a total volume of 80 L with artificial aeration and a daily water renewal rate of around 20%.

A total of 5 treatment groups (0, 50, 100, 150 and 200 ppm doses of 17ß-estradiol hormone) with 3 replications including 17 larvae per replication were settled. The experimental groups formed are presented in Table 1.

Feeding

In the current experiment, the fish were treated with a dose of 50, 100, 150 and 200 ppm 17ß-estradiol (E2) delivered within the feed. Hormones were given to the fry starting from the time they began exogenous feeding for a period of 45 days. The appropriate amount of hormone (diluted in 95% ethanol) was added to the trout starter feed (Çamlı yem, Turkey) using the spray method. The fish were fed ad libitum. Control fish were fed without the hormone treatment.

Routine Measurements and Weighings

The sampling method was used in the measurement and weighing of the experimental fish. A 1 mm precision measuring board and an electronic scale of 0.01 g sensitivity were used to assess the total length (L) and weight (W) of the fish at the beginning of this trial and after every month by sampling all living fish for each experimental group. Live fish and total height measurements were taken back to their environment. As it was planned that the female individuals should be used first for the observation of YY males, sexing was performed by observing the external shape of gonads by the naked eye according to Goto et al., (1999). At the end of the trial, 10 fish were chosen randomly and cut for gonad examination.

Parameters Studied in the Research

Gonadosomatic index (GSI)

Calculation of the gonadosomatic index of the groups was made using the following formula (Halver, 1989; Hepher, 1990). GSI = (Gonad weight (g) x 100 / Fish weight (g))

Daily Live Weight Gain (DLWG)

The growth rate of fish showing the daily live weight gain was determined according to the following formula (Wooten, 1990). DLWG g / day = (Wt - W0) / t where

Wt: fish weight at the end of the trial (g),
W0: Fish weight per experiment,
T: Trial period (days)

Specific Growth Rate (SGR)

Specific growth rate SGR. was calculated according to the following equation (Hoşsu et al., 2003; De Silva and Anderson, 1995):

SGR (% body wt.gain/day) =

$$\left[\frac{\text{Log}_n \text{ Final fish wt.} - \text{Log}_n \text{ initial fish wt.)}}{\text{Time interval}}\right] \times 100$$

Table 1. Experimental design of the trial

	17ß-estradiol hormone concentration (ppm)				
Trial duration	**Control group (0)**	**A group (50)**	**B group (100)**	**C group (150)**	**D group (200)**
30 days (28 °C)	K1 (17)	A1 (17)	B1 (17)	C1 (17)	D1 (17)
	K2 (17)	A2 (17)	B2 (17)	C2 (17)	D2 (17)
	K3 (17)	A3 (17)	B3 (17)	C3 (17)	D3 (17)
Number per treatment	51	51	51	51	51
Total number of larvae			255		

Condition Factor (K)
The weight-length relation of Fulton, which was used to determine the health of fish, was calculated using the following formula (Ricker, 1975). K = W.100 / L^3

Feed conversion rate
FCR = Amount of feed consumed (g) / Live weight gain (g) (Santinha et al., 1999).

Survival Rate (SR)
The survival rate of fish is calculated by the formula reported by Pechsiri and Yakupitiyage (2005). SR % = (Ns / Ni) x 100.
Ns: Number of fish at the end of the trial; Ni: Number of fish at the beginning of the trial.

Statistical Analysis

All results presented in this study are expressed as the mean ±SEM. All values were analyzed by the 1-way analysis of variance followed by Newman-Keuls multicomparison test to analyze the difference of means using SPSS 15.0 (SPSS, Chicago, IL) statistical software and (p<0.05) was considered statistically significant.

RESULTS AND DISCUSSION

Water Quality Parameters

Water quality parameters such as temperature and dissolved oxygen averages are given in table 2. Statistically no differences (p> 0.05) were observed between groups regarding the average temperature that ranged from 28.07±0.18 to 28.25±0.18 °C respectively in the Control and 50 ppm group. Inversely, statistical analysis showed significant differences related to the dissolved oxygen quantity between the experimental groups. However, all observed values (from 4.88±0.19 to 5.90±0.19) were above the recommended 3 ppm in the Tilapia rearing.

Female Ratio and Survival Rate

The sex ratios and survival rates obtained from the experimental groups at the end of the trial are given in Table 3.

The application of 17ß-estradiol to Oreochromis mossambicus fry during 4 weeks post-hatching was found to be significantly (P <0.05) successful for the sex-reversal in this species. The proportion of females was higher in hormone-fed fish groups than in control fish (P <0.05). The female proportion of the control group (K') was almost equal to the proportion of males (51.52%). A significantly high proportion of females were obtained in the 150 ppm and 200 ppm groups - 76.60 % and 86.36 % respectively. Our studies clearly showed that the female proportion is dose-dependent.

When we come to the survival rates, statistical analysis showed a significant difference between groups (P <0.05). Conversely, this parameter was higher in the treatment groups than in the control group (apart from the group of fish fed with 200 ppm hormonal feed). This was calculated as 80.39% and 64.71% respectively in the 50 and 100 ppm groups. There was no significant difference in fish mortality between 150 ppm and control groups (p> 0.05). The lowest survival rate was obtained in the 200 ppm group (43.14%) and allows us to suggest that high dose of 17ß-estradiol is fatal for O. mossambicus.

Growth Performances

The average height and weight values obtained from the measurements and weighings during the test are shown in figure 1. A strong correlation was observed between weight and length in all treatment groups. The ANOVA analysis revealed significant (p<0.05) differences regarding these two parameters.

The average weight of the Control group (25.87±1.02 gr) was significantly higher than those of the treatment groups (< 0.05) and

Table 2. Water parameters during the experiment

Average	A (50 ppm)	B (100 ppm)	C (150 ppm)	D (200 ppm)	K (Control)	F	p
Temperature (°C)	28.22±0.46 a	28.25±0.52 a	28.20±0.59 a	28.18±0.45 a	28.07±0.20 a	0.145	0.96
Diss. Oxygen (mg/g)	5.90±0.35 a	5.63±0.52 a	5.76±0.517 a	4.88±0.40 b	4.98±0.68 b	5.96	0.00

Tablo 3. Female proportion and survival rate in O. mossambicus treated with E2

Treatment groups	Female proportion (%)	Survival rate (%)
A (50 ppm)	63.41 ^{b}	80.39 ^{c}
B (100 ppm)	61.90 ^{b}	82.35 ^{c}
C (150 ppm)	76.60 ^{c}	68.63 ^{b}
D (200 ppm)	86.36 ^{d}	43.14 ^{a}
K (Control group)	51.52 ^{a}	64.71 ^{b}
P value (X^2 testi)	0.033	0.00

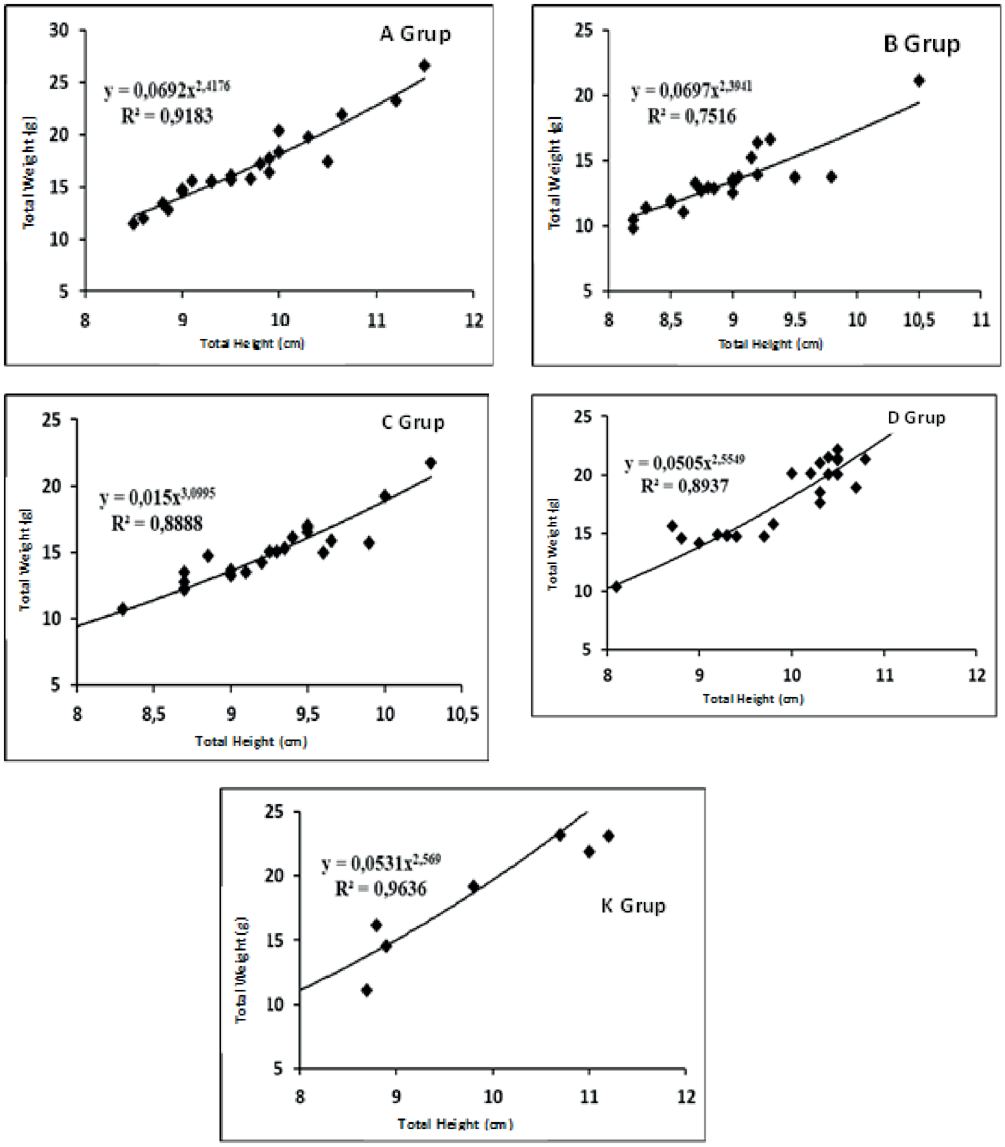

Figure 1. Weight and height relationship of treatment groups (n=7)

can be presented as follows: 13.44±1.02 gr for 100 ppm. 14.78±1.02 gr for 150 ppm. 16.65±1.02 gr for 50 ppm and finally 17.94±1.02 gr for 200 ppm groups. Therefore, it can be said that hormonal treatment with 17ß-estradiol has an inhibitory effect on growth in Mozambique tilapia. The trend in average total length remained the same with a significantly ($p<0.05$) higher value from the Control group (10.94±0.2 cm).

Table 4 shows the growth performances obtained in this experimentation. For all results, the corresponding values are significantly higher in the Control than in treatment groups ($p< 0.05$).

Feed Consumption

Figure 3 shows the variation of daily feed intake during the experimental period. Its analysis confirms the hegemony of the Control group over the others ($P< 0.05$). The highest daily feed intake was observed in this group. Feed consumption in the other experimental groups were relatively equal (Figure 2). Based on these results. it can be understood that hormonal treatment also diminishes feed intake (consumption) in O. mossambicus.

Meanwhile. there was no statistically significant difference ($P> 0.05$) between the groups in terms of condition factor (Figure 26). The condition factor values according to the groups are 1.80±0.04; 1.85±0.04; 1.88±0.04; 1.83±0.04 and 1.91±0.04 respectively for 50. 100. 150. 200 and 0 ppm (Control) groups.

The analysis of the FCR and FCE ratios revealed statistically significant differences between the values obtained in the different experimental groups ($p <0.01$). However, it was determined that the values of the fish fed on a diet containing estradiol were better than those of the control group. In contrast, the results between the hormone treated groups weren't different from each other ($p>0.05$). The lowest feed conversion ratio came from the

Table 4. Growth performances of O. mossambicus fed with feed containing different concentration of 17 ß-estradiol (90 days)

Average	50 ppm	100 ppm	150 ppm	200 ppm	Control	F	p
Live weight (g)	16.6±3.79 bc	13.44±2.28 c	14.68±2.75 bc	17.84±4.34 b	25.77±8.87 a	22.78	0.00
Total Len. (cm)	9.77±0.83 b	8.97±0.52 c	9.20±0.54 b	9.88±0.95 b	10.94±1.6 a	14.85	0.00
DLWG	0.18±0.04 bc	0.15±0.03 c	0.16±0.03 bc	0.20±0.05 b	0.29±0.1 a	22.78	0.00
SGR	5.66±0.30 bc	5.43±0.18 c	5.53±0.22 bc	5.73±0.31 b	6.09±0.46 a	17.26	0.00
K	1.80±0.31 a	1.85±0.15 a	1.88±0.12 a	1.83±0.18 a	1.91±0.21 a	1.09	0.38
Feed cons. (g)	7.80±0.06 c	5.99±0.73 d	7.13±0.93 c	8.38±1.58 b	18.32±3.36 a	14.4	0.00
FCR	0.50±0.12 b	0.46±0.06 b	0.50±0.09 b	0.56±0.19 b	0.83±0.41 a	12.01	0.00
FCE	2.12±0.48 a	2.23±0.31 a	2.07±0.33 a	1.94±0.54 a	1.43±0.51 b	11.79	0.00

Table 5. Evaluation results of the trial group (8 months) (Different letters indicate that the difference between the groups is important (p<0.05))

Treatment groups	A	B	C	D	Control	F Value	P Value
Body Weight (g)	57.5±10.5 a	47.4±17.8 a	52.5±19.0 a	57.6±16.2 a	58.8±16.2 a	0.75	0.56
Total Lenght (cm)	14.95±1.3 a	12.31±2.8 a	13.54±3.6 a	13.77±2.7 a	14.5±2.1 a	1.47	0.23
Condition Factor (F)	1.74±0.84 a	2.83±0.59 a	2.33±0.65 a	2.42±0.56 a	1.61±0.1 a	2.42	0.06
GSI	2.18±1.36 b	2.81±1.59 b	2.74±1.67 b	2.44±0.96 b	5.12±1.9a	3.15	0.02

fish fed on a 100 ppm estradiol diet (0.46 ± 0.04). As indicated above, the highest conversion rate was obtained from the control group (0.83 ± 0.04).

Histological Analysis
At the end of our experiment. after subjecting sex-reversed females to reproduction for 8 months, only a limited number of eggs and accompanying juvenile fishes were obtained. Subsequently, 10 individuals from each treatment group were sacrificed and subjected to gonadal examination and the GSI values excluded from the groups at the end of 8 months are presented in Table 5 together with the evaluation results.

The groups fed on the 17ß-estradiol diet did not differ from the control group (p>0.05) in terms of body weight, height and condition factor according to table 5 analysis (p>0.05). However, the analysis of GSI averages revealed a significant difference between the hormone fed fish and the control group fish (p<0.05). In addition, the histological analysis showed significant problems (in terms of numbers and severity) such as structural defects of the gonads (asymmetry, defective eggs) and deformities of some internal organs such as the air sac.

Water quality during the course of the experiment was within the recommended parameters for Tilapia species as suggested by Philippart and Ruwet, 1982; Azaza et al., 2008 and Soltan et al., 2013. No statistical difference was observed in average water temperatures between all treatment groups (p>0.05) which were all around 28 °C (Table 2). Dissolved oxygen (Table 2) stayed within acceptable limits as well (DO- 4 mg/L or higher) as per the recommendations of Azim and Little, 2008; Iwama et al., 1997 and Rappaport et al., 1976. The comparison of the mean weight values between experimental groups (Table 5) revealed statistically significant differences (P≤0.05) and generally the estradiol hormone-fed fish groups were found to have a similar average body weight (apart from the 200 ppm group) but lower than the fish in the control group. The same observations were reported by Johnstone et al. (1979), Hunter and Donaldson (1983), according to whom estradiol hormone induces lower growth performances. Piferrer (2001) and Alcántar-Vázquez et al., (2015) suggested that, unlike androgen, estrogen has no effect on growth in fish. The best (lowest) feed conversion ratio (FCR) was 0.46 ± 0.06 and was recorded in the 100 ppm estradiol-fed fish group while the control group showed the worst (highest) FCR - 0.83 ± 0.41. The FCR is considered as the best parameter for evaluating the acceptability of the feed and the final performance of the fish (Inayat and Salim, 2005). The FCR values obtained in our study were good and showed significant differences between the groups. All the fish fed on hormones were found to valorize feed better than those in the control group, although they consumed less. Our results are better than those of Malik et al. (2014) and Daudpota et al. (2016). Kalsoom et al. (2009), however, reported a higher FCR than ours in their study on Catla catla × Labeo rohita hybrid. The plausible reason for such a good FCR of the present study may be due to the combination of the availability of appro-

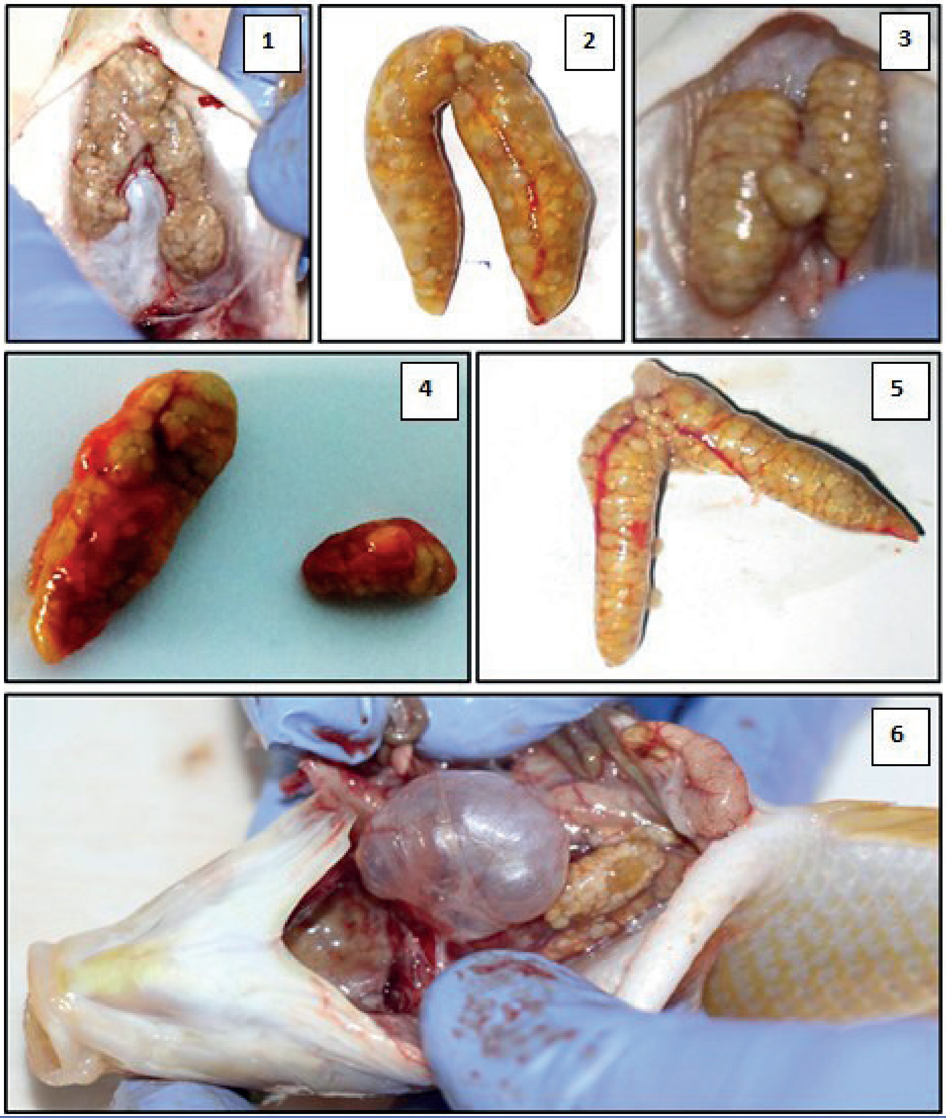

Figure 2. Deformations noticed during histological analysis: (1, 3, 4, 5: Asymmetric ovaries, 2: A female gonad full of losse and whitish eggs, 6: chick gonad and excessively blown airbladder

priate nutrient contents (protein rate of 50-55%) of the feed supplied, the optimized water quality and the great care provided in general. In the comparison of condition factor averages, it was seen that the K value of the estradiol-treated groups was slightly smaller than the control group even if the differences were not statistically significant (P>0.05). As recommended for freshwater fish by Bagenal and Tesch (1978), Hile (1936) and Martin (1949), these values were ideal and in the range of 2 to 4. Ayode (2011) stated that K values above 1 indicates the healthiness and the so-desired isometric growth of the fish for commercialization.

Sex ratios were female-oriented and ranged from 51.52% (control) to 86.36% (200 ppm) and exceeding 76.60% in the 150 ppm estradiol hormone-fed fish group. The 50 and 100 ppm groups showed sex ratios of 63.41 and 61.90% female respectively. These differences were statistically significant (P≤0.05). The later gonad analysis of the fish showed the presence of intersex individuals in the treatment group. The effect of estradiol concentration on sexual differentiation in O. mossambicus fish is clearly demonstrated in our experiment. These are in line with the results obtained by Alcántar-Vázquez et al. (2015) who reported in their work the use of O. niloticus fry of 10 mm total length on average since the size of the fish is very important for a successful sex reversal. The good sex reversal values observed in our study may be due to the initial length of the fry on the first feeding. As an example, VeraCruz et al. (1996) suggested that the initial length of fry for a successful feminization process of Tilapias should be less than 10 mm. In our study, the average initial length of Mozambique Tilapia pupils was 7 mm. Several works reported better results but with another estrogen hormones. Basavaraja et al. (1990) published a study in which 100% female individuals were obtained from O. mossambicus pups fed on a diet containing 50-100 ppm of diethylstilbestrol (DES). Similar results were reported in the Varadaj (1989) study. However, mortality also increased significantly with higher hormone doses, exactly like in the present study. Wang et al. (2008) used 17-ß-estradiol at different doses (50-200 ppm) for 60 days for the feminization of Coho salmon (Lepomys macrochirus) larvae fish. In the study conducted, larvae were fed with 17-ß-estradiol supplemented feeds that led to 100% female populations. However, regarding the fish of the control group, the hormone-fed ones were found to grow more slowly during the experimental period, like in this study. In addition, the success of this experiment can be explained by the results of Abucay and Mair (1997) who explained that unlike in flow-through systems where they can escape, sex manipulations are likely to be more successful in re-circulation systems in which metabolites, or filtrates are kept within the system.

The GSI averages in all experimental groups were weaker than in the control group. Similar situations were described by Hatikakoty and Biswas (2004) and Ganie et al., (2013) in O. mossambicus. They found that most of the ovaries of fish fed with the same hormone diet remained blocked in the early maturation stage. This suggests that increasing estradiol hormone dosage prevents gonadic maturation. The GSI value found in the control group was higher than that of the experimental groups and the result was that the individuals belonging to this group could successfully deliver eggs and larvae. It has also been suggested that the increased rate of deformity of gonad structures of fish is related to the increasing dose of estradiol. The same conclusions were reported on Nile Tilapia (O. niloticus) by Alcántar-Vázquez et al. (2015), on sea bass (Dicentrarchus labrax) by Chatain et al. (1999) and on Carassius auratus by Beardmore et al. (2001).

CONCLUSION

This study aimed to assess the effects of 17ß estradiol on the sex differentiation and growth of O. mossambicus. The findings showed that the female ratio was clearly and positively dose-dependent. In addition, as reported in the literature, poorer results were obtained in terms of feed consumption and growth parameters of all of the fish that were subjected to estradiol administration compared to the control group. In contrast, the survival rate (apart from in the 200 ppm group) and feed valorization were observed to be better in hormone treated fishes. These results suggest that the best temperature, dose and duration of application of 17ß estradiol to Mozambique tilapia (Oreochromis mossambicus) should be applied at 28 °C, 100-150 ppm and 30 days of application for successful feminization. Furthermore, it was noticed that feminization experiment using 17ß estradiol hormone in Oreochromis gender on Nile Tilapia, almost no research has been conducted on Mozambique one. That is why we strongly recommended continuing or even repeating the same research in order to establish better and more reliable standards for fish aquaculturists, fish farmers or anyone involved in this field who might be interested in this practice.

Conflict of Interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics Committee Approval: This study was carried out in accordance with animal welfare and the ethics of trial. All procedures were performed in accordance Law on Veterinary and Medical Activities and National Animal Welfare Act. Therefore ethical approval was not required.

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Research Article

Effects of Water Temperature on Sex Differentiation and Growth Parameters of the Mozambique Tilapia (*Oreochromis mossambicus*, Peters, 1852)

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ABSTRACT

In this study, the effects of water temperature on growth, survival rate, gonad development and sex ratios of Mozambique Tilapia (*Oreochromis mossambicus*) were studied by applying different temperature applications (28, 30, 32, 34 and 36°C) for a single period of 40 days. At the end of the study, in the control group (28°C), the males proportion was 47.62%. In fishes reared at 30°C, average male ratio was found as 73.68%. The highest male proportion was obtained in the 36°C temperature group (86.67%). Growth performances, feed utilization and gonad development were found to increase with elevating temperatures up to 34°C unlike the survival rate which has been found to fall with higher temperatures. In this study, sex differentiation rates differed significantly between all groups ($p<0.05$). However, the highest male rate obtained in group A and D. As hypothesized, results suggest that fish performed better at 30-32°C than 28°C or 34-36°C water temperature and the optimum temperature for a better expression of growth parameters in Mozambique Tilapia could be 30°C.

Keywords: Mozambique Tilapia (*Oreochromis mossambicus*), growth, survival, water temperature

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INTRODUCTION

In the last 30 years, the amount of global aquaculture has increased by 12 times, resulting in the highest growth rate in the food production sector close to 8% (Osvald and Mikolasek, 2016). Aquacultural production, which was estimated at 7.36 million tons in the 1980s, reached 16.48 million tons in the 90's and 54.8 million tons in 2005. The actual annual production turns around 73.8 million tons (FAO, 2016). A portion of this increase in global aquaculture production (around 12.31%) is provided by Tilapia culture (FAO, 2016). Tilapia is one of the earliest fish species cultivated in the world and as understood from the paintings in ancient Egyptian graves its cultivation dates back to 3000 years BC (Popma and Masser 1999). Several studies on the reproduction of a variety of fish species have shown that sex ratio is often influenced by the ambient temperature of the water (Patino et al., 1996; Blazquez et al., 1998; Yamamoto, 1999; Goto et al., 2000; Baroiller and D'Cotta, 2001; Azuma et al., 2004). This has been amply demonstrated in several species, including the Nile Tilapia. Unfortunately the Tilapia of Mozambique, which is very close to its cousin of the Nile, has not enjoyed the same ardor in scientific researches. The aim of this study is to investigate the effect of altered water temperature on Mozambique tilapias's (*Oreochromis mossambicus*) sex differentiation and growth parameters. In addition, it will also help to enrich the scientific literature on this subject so as to provide up-to-date sources to future researchers who will want to deepen research in this field.

MATERIALS AND METHODS

The experiment was carried out in the Fish Breeding Aquarium Unit of Ege University in Izmir (Turkey). The tilapia offspring used in the experiment were obtained naturally from the tilapia rootstocks in the laboratory of Ege University Aquaculture Faculty Aquaculture Department. The eggs were incubated at 28°C for 8 days at the root of the rootstock individual. During the incubation period of the eggs, water parameters (temperature, oxygen, and pH) were followed. In aquariums, cartridge water (10 microns) was used and the rested tank water was used. In order to keep the aquarium water clean, air-assisted mechanical filters have been used, and as a result of daily siphoning, fresh water has been introduced as water.

The desired amount of oxygen in aquariums is provided by the ventilation lines in the unit. The system uses a dual fluorescent system (950 lux) for a 14 hour dark period. In order to adjust the water temperatures in the aquarium, Eheim 100-300 W two different thermostatic heaters are used. In addition, Kern brand precision balance (0.01 g) for measuring fish weights, HI 9142 brand oxygen meter for oxygen measurements, Merck Pharo 100 Visible Water / Wastewater Analysis Spectrophotometer for measuring ph-nitrogen compounds and 2 mercury thermometers for temperature measurements were used.

The experiments were carried out in 15 glass aquariums, 30 * 30 * 30 cm in size and 18 liter in water volume. 255 new hatched *O. mossambicus* larvae were placed under 5 different experimental temperatures (28 (group K), 30 (group A), 32 (group B), 34 (group C) and 36°C (group D) with 3 replicates and then reared during 40 days. In the study, 1-1.5 mm sized extruder commercial trout start feed was used.

Estimation of Growth Parameters

Specific Growth Rate (SGR)
Specific growth rate SGR. was calculated according to the following equation (De Silva and Anderson, 1995):

SGR (% body wt.gain/day) =

$$\left[\frac{\text{Log}_n \text{ Final fish wt.} - \text{Log}_n \text{ initial fish wt.)}}{\text{Time interval}}\right] \times 100$$

Feed conversion rate
FCR = Amount of feed consumed (g) / Live weight gain (g) (Santinha et al., 1999).

Condition Factor (K)
The weight-length relation of Fulton, which was used to determine the health of fish, was calculated using the following formula (Ricker, 1975). $K = W.100 / L^3$

Gonadosomatic index (GSI)
Calculation of the gonadosomatic index of the groups was made by the following formula (Halver, 1989; Hepher, 1990). GSI = (Gonad weight (g) x 100 / Fish weight (g))

Survival Rate (SR)
The survival rate of fish is calculated by the formula reported by Pechsiri and Yakupitiyage (2005). SR % = (Ns / Ni) x 100.
Ns: Number of fish at the end of the trial; Ni: Number of fish at the beginning of the trial.

Histological Analyzes of Gonad and Sex rate (Masculinity rate)

For histological analysis, gonad samples taken from fish were fixed with 10% formaldehyde and stored. Tissue samples were fixed in 10% buffer formol solution for sectioning and kept until histological examination was performed. Blocked specimens were fixed to formol and 5 µm thick sections were taken from Leica 2125 Rotary microtome. Hematoxylin-Eosin staining was performed to examine the general structure (Luna, L.G. 1968, Bancroft et al., 1996; Presnell and Schreibman, 1997). The preparations were examined under different types of Olympus JX31 type phase contrast microscope and photographed with the Olympus E330 type microscope. In this way, sex rates of fish were determined.

Statistical Analyzes

The growth parameters (Live Weight (LW), Specific Growth Rate (SGR), Feed Conversion Ratio (FCR), Condition Factor (K) and Survival Rate (SR)) analyzed in this trial were obtained by One-way ANOVA test. The difference between groups was tested at a significance level of 5% ($p \leq 0.05$) with the Student-Newman-Keuhls multiple comparison test. For sex ratios, X2 test and Likelihood ratio test were applied. The results are given as Mean±Standard Deviation. All data were analyzed in SPSS 15.0 (SPSS, Chicago, IL) statistical package program.

RESULTS AND DISCUSSION

Water Aquality

The average and standard deviations values of water temperatures measured daily for each treatment throughout the study are given in Table 1. The lowest value was measured in the control group (28.06±0.23°C) and the highest water temperature was measured in D group (35.66±1.89°C). Even if differences between groups in terms of the amount of dissolved oxygen during the experiment were observed ($P \leq 0.05$), all values were found to be comprised in an acceptable range all along the study. The K (control) and A (30°C) groups showed the highest oxygen concentrations (5.05±0.74 ppm and 5.05±0.72 ppm respectively). The group with the lowest amount of oxygen was D group (4.40±0.75 ppm).

Growth Performances

The statistical significance levels of the growth parameters such as body weight (BW), total length (TL), condition factor (K), gonadosomatic index (GSI), specific growth rate (SGR), feed conversion ratio (FCR) are given in Table 1. Except for the condition factor, the differences between these parameters were found to be significant ($p \leq 0.05$). The groups with the highest body weight according to the last measurements were respectively A (30.497±1.98 g). B (27.762±1.81 g) and Control (23.780±1.60 g) groups. C and D groups reached 16.829±1.53g and 11.212±2.34g respectively and were found to be statistically lower than the previous groups ($p \leq 0.05$).

Table 1. Environmental parameters, growth performances, survival rate and masculinity rate of the experiment

Parameters	A (30°C)	B (32°C)	C (34°C)	D (36°C)	K (28°C)	F Value	P Value
Average water temperature (°C)	29.7±0.7 ^{d}	32.16±0.59 ^{c}	33.84±1.1 ^{b}	35.66±1.89 ^{a}	28.06±0.23 ^{e}	809.26	0.00
Average DOC (ppm)	5.05±0.7 ^{a}	4.64±0.66 ^{b}	4.48±0.74 ^{bc}	4.40±0.75 ^{c}	5.05±0.74 ^{a}	25.06	0.00
Weight (g)	29.9±11.8 ^{a}	31.23±10.3 ^{a}	17.76±5.03 ^{b}	9.42±4.12 ^{c}	25.74±7.82 ^{a}	20.84	0.00
Total Length (cm)	12±1.96 ^{a}	11.84±1.43 ^{a}	10.21±0.84 ^{b}	8.13±1.25 ^{c}	11.17±1.42 ^{a}	20.06	0.00
Gonodosomatic Index (GSI)	0.2±0.18 ^{a}	0.27±0.27 ^{a}	0.26±0.22 ^{a}	0.004±0.004 ^{b}	0.23±0.24 ^{a}	36.78	0.00
Specific Growth Rate (SGR)	6.3±0.66 ^{a}	6.37±0.50 ^{a}	5.85±0.37 ^{a}	5.00±0.85 ^{b}	6.05±0.50 ^{a}	15.06	0.00
Condition Factor (CF)	1.9±0.30 ^{a}	1.95±0.23 ^{a}	2.00±0.00 ^{a}	2.07±0.60 ^{a}	2.00±0.00 ^{a}	0.54	0.71
Food Conversation Rate (FCR)	0.45±0.3 ^{b}	0.44±0.37 ^{b}	0.66±0.30 ^{b}	1.90±1.26 ^{a}	0.56±0.27 ^{b}	6.33	0.00
Survival rate (%)	41.18 ^{b}	49.02 ^{b}	68.63 ^{a}	29.41 ^{c}	62.75 ^{b}	-	0.034
Masculinity rate (%)	73.68 ^{b}	50.00 ^{c}	42.86 ^{c}	86.67 ^{a}	47.62 ^{c}	-	0.034

For length measurement, average values of 11.678±0.36 cm and 11.218±0.33 cm were obtained respectively from A and B groups. However, the fish in group D showed the lowest length (7.99±0.43 cm). A strong relationship has been noticed between the two parameters as shown in figure 3.

The highest SGR was determined in group B (6.37±0.50) followed by group A (6.33±0.66) and the lowest in the group D (5.00±0.85). K did not differ statistically between groups (p≤0.05). The highest K-value was found in group D (2.07±0.60) and the lowest in group A (1.90±0.30).

The GSI differences were found statistically significant among the treatment groups and D group (p≤0.05). Accordingly, the highest GSI was obtained from group B (0.27±0.27) and the weakest from group D (0.004±0.004).

Temperature was found to influence significantly feed consumption in *O. mossambicus* (p≤0.05). Feed consumption was statistically similar in the groups C (16.50±2.50 g). D (17.93±5.18 g) and Control (18.3±3.48 g). But fishes kept at 30°C (20.33±2.11 g) and 32°C (25.42±6.14 g) showed a higher feed intake.

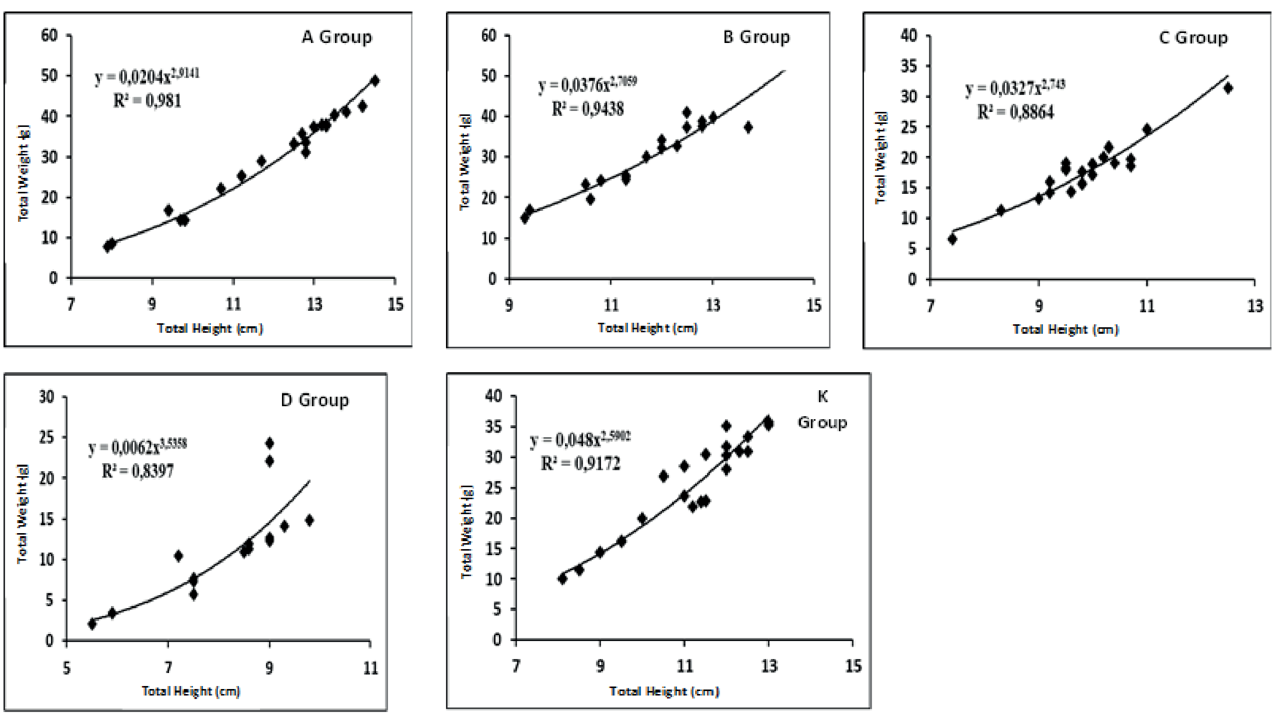

Figure 3. Length (Height)-weight relationship of the experimental groups (n = 51)

Table 2. Average egg diameter between experimental groups

Groups	Average egg diameter (µm)	F value	p value
A (30°C)	170.390±107.58'b (104.300-236.481)	14.017	0.00
B (32°C)	261.154±113.93'b (195.063-327.245)		
C (34°C)	457.517±137.15 a (391.427-523.608)		
K (28°C)	240.309±113.24'b (174.218-306.399)		

In terms of FCR, fish belonging to group B had the lowest average value of 0.44±0.37. Then followed A Group (0.45±0.28). Control group fishes were observed to have better FCR (0.56±0.27) than those grown at 34°C (0.66±0.30).

Histological Analized for Gonad

Ovarium

Egg sizes calculated by using Gonadal-Squash method are presented in Table 2.

The difference between groups was statistically significant ($p \leq 0.05$). Individuals reared under 34°C were found to have larger egg diameters than the others (457.52±137.15 µm). After that, group B follows (261.15±113.93 µm). Control and group A had egg diameters of 240.31±113.24 µm and 170.39±107.58 µm respectively.

At the end of the experiment (90 days after eclosion), there were no mature eggs in all treatment groups. The totality of the eggs recorded were at an intermediate stage of development (previtellogenic and vitellogenic stages) in general. Unfortunately, no egg cells were seen in D group because of its excessive immaturity (Figure 1).

Testes

In Figure 2 representative testis images by treatment are given below.

The analysis of these different images allows us to make the following explanations:

- The gonad from the control group is in the process of maturation. Although there are some black dots that prove the existence of spermatozoa, it can be said that the gonad is generally full of primitive reproductive cells;
- Sperm cloud is common in the gonads of fish grown under 30 and 32°C; these clouds are a sign that they are full of spermatozoa. They also accumulate so much as to form a real warehouse;
- In those kept below 34°C, sperm clouds are less noticeable than in the previous group. But they tend to accumulate. Here, too, it can be said that the gonad is on the maturation stage;
- The gonads of fish grown at 36°C are almost completely immature. There is no sperm cloud. It is full of spermatogonies confirming that the gonad remains immature.

Survival Rate (SR)

The difference between the groups for SR was statistically significant (Table 1), and the effect of temperature on this topic was significant ($p \leq 0.05$). The highest survival rate was obtained at 34°C (68.63%). The lowest one was seen in the 36°C group (29.41%). The differences between the groups A, B and control groups were not significant ($P > 0.05$).

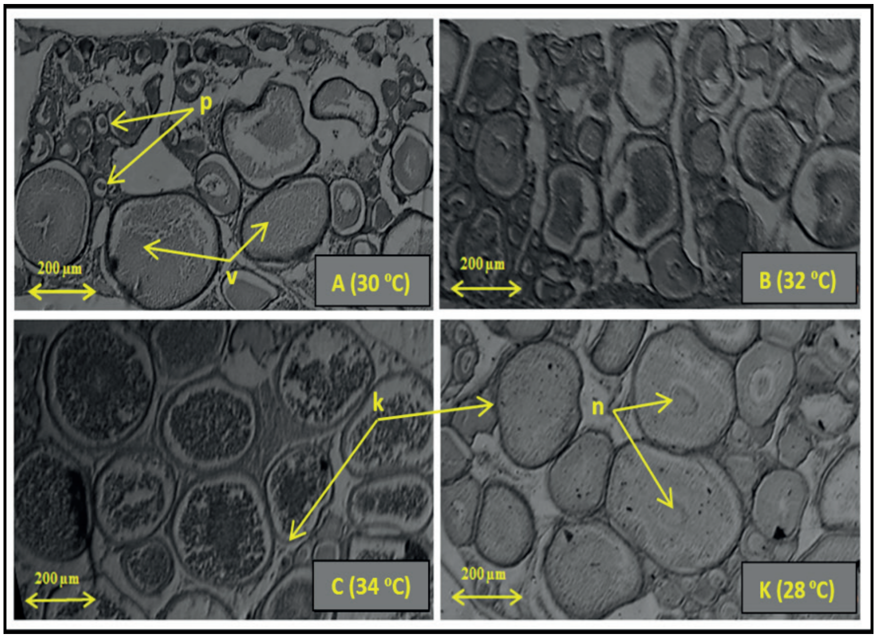

Figure 1. Female gonad sections by temperature (X 10, HE) (n: nucleus, p: previtelogenic oocytes, v: vitellin globules, k: korion)

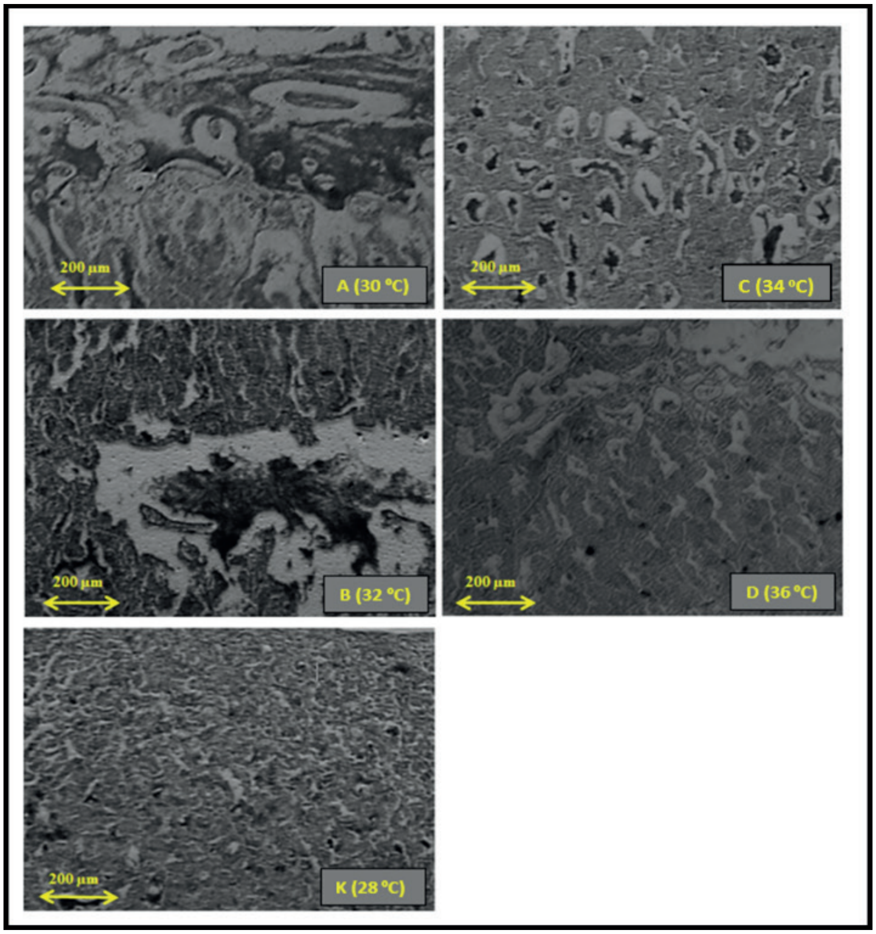

Figure 2. *O. mossambicus gonad* (testes) sections showing sperm cloud and spermatogenic cells in different development stages

Sex Ratios

The sex ratios obtained from the groups at the end of the trial were given in Table 1. The differences between groups were statistically significant (p≤0.05). The highest masculinity rate was obtained from the groups reared under 36 and 30°C (86.67% and 73.68%, respectively). No intersex individuals were observed after the examination of the gonad. The lowest male ratio was recorded as 42.86% in the C group (34°C). A balanced sex-ration was obtained in control (28°C) and B group (32°C).

The results of the present study show that survival rate and sex ratio, gonad development, mean weights, lengths and growth rates of juvenile *O. mossambicus* were significantly influenced by temperature. The highest masculinity rate was obtained from fishes reared under 30 and 36°C respectively (73.68% and 86.67%). No intersex gonad has been found. Our results are similar those published by Baroiller et al. (1995), Tessema et al. (2006); Azaza et al., (2008) and Soltan et al. (2013) who investigated the effects of temperature on Nile Tilapia (*Oreochromis niloticus*) of which juvenile fries were exposed to different temperatures ranged from 22 to 36°C. At the end of their studies, the highest male ratio was obtained with individuals reared at 36°C and ranged between 80 and 84 %. The conversion rate to the male character in tilapias may not always result in the success of the transformation activity. In some cases, the resistance of the two characters to the difficult conditions may affect this. That is, if a gender is more resilient than the other in a difficult condition, the gender ratio cannot be evaluated only with classical information. For example, in some scientific studies, males are more resistant to temperature than females (Altun et al., 2006: El Said, 2013). In addition, differences in gender ratio are caused by parental influence (Baroiller, 1996). In addition, these differences may result from the heterogeneity of the population, that is, the mixture of two or more strains. First of all, we did not practice an adaptation phase before exposing fishes to different temperatures. In the work conducted by Çetinkol (2012), fish were kept at 27°C for 1 week before being exposed to three different temperatures (24, 28, 32°C). Many scientific researches stated that sudden temperature changes can be damaging for the offspring (Sultan et al., 2009; Altun et al., 2006; Wang and Tsai, 2000). According to these researchers, exposing fish to masculinizing temperatures put their survival chances at risk. Secondly, Wang and Tsai, 2000; Borges et al., 2005 demonstarted that Tilapine species begin to struggle considerably at temperatures of 34°C. They emphasized that in *Oreochromis mossambicus*, deformity and cannibalism ratios rose to significant levels with increasing temperature from 32 to 35°C. Concerning the gonad development, exposure of new-hatched Mozambique Tilapia fries to 36°C resulted in insignificant ovary size, reduced testicular size (immaturation) and in weaker growth performances as well (Table 1 and 2). Our findings are in line with those obtained by Byerly (2003) who examined the effects of elevated sub-lethal temperatures on the development of gonadal germ cells as well as somatic growth of channel catfish fries Ictalurus punctatus. For that 2 days-old channel catfish fries were exposed to different temperatures ranging from 27 (control) to 36°C for a period of four weeks. The results indicated that exposure to 34°C reduced gonad size with a slight decrease in overall body weight while the one to 36°C resulted in a significant reduction in oocyte number and ovarian/testicular area.

In mammals, heat-dependent germ cell loss has been observed only in males (Ito et al, 2003). But for fishes germ cell loss occur also in females indicating that they are also heat sensitive (Strussmann et al., 1998, Fat-Hallah. 2000. Ito et al, 2003). Exposure to elevated temperature has been reported by many researchers (Scott et al, 1979; Pinart et al, 1999; Fat-Hallah, 2000) to cause an inhibition of testicular development. Temperature influence the physiological processes (Brett and Groves, 1979) and the growth in many fish species (Jobling 1981; Elliott, 1982; Jonassen et al., 1999). The specific growth rate, feed rate, average live weight gain, feed conversion rate and condition factor obtained at the end of our study are shown in Table 2. The analyzes of results help to notice that fish groups reared at temperature comprised within 28 and 32°C showed better performances than the other groups. The highest performances was seen in B group (32°C), while the fish kept at 36°C showed the weakest ones. This is in accordance with findings from Dikel (2009) who stressed that every fish species is known to have a specific temperature optimum to survive and grow. Within this thermal tolerance range, maximal growth is obtained at optimal temperature. With increasing temperature, the uptake of nutrients also increases to maximum and then decreases when approaching the upper tolerance level (Warren and Davis, 1967; Dikel, 2009). At elevated temperatures, feed intake becomes more vigorously and the digestive processes of the fishes are then accelerated (Cossins and Bowler, 1987). According to Heap and Thorpe (1987), the 0-group malpigmented and normally pigmented turbot (*Scophthalmus maximus.* L) And turbot-brill hybrids, *S. maximus x S. rhombns* (L) developed faster at elevated temperatures not only because of much improved appetite but also because of an increase in food conversion efficiency (FCR). Similar results have been reported by Cai and Curtis (1990) in which the growth rate and food consumption, expected the assimilation efficiency in triploid grass carp (*Ctenopharyngodon idella*) increased with environmental temperature. The results of the present study are in line with these findings. But they differ slightly from those obtained by Al-Asgah and Ali (1997), who reported that the total weight gain and specific growth rate of Nile Tilapia differed significantly ($p<0.05$) at different water temperatures and increased with higher temperature up to 29°C, after which no significant increase was observed. They then suggest that the optimum temperature for *Oreochromis niloticus* lies somewhere near 29°C. We think that the variability in the reported results may be by the species differences.

In our study, the values for the feed conversion ratio (FCR) decreased with the increase in water temperature up to 32°C indicating a better utilization of feed per unit live weight gain. It corresponds to the results of Al-Asgah and Ali (1997). According to Dikel (2006)'s study, Tilapia's health has been reported to be seriously threatened by lowering the water temperature below 12-13°C. Under normal conditions, Tilapia, which has optimum growth around 27-28°C, loses its growth potential as it goes under this temperature. However, as the temperature is increased above 30-32°C, a significant decrease in growth rate is observed. Similar results were obtained from Fattah et al. (2008).

CONCLUSION

Our studies demonstrated that growth performance of juvenile Mozambique Tilapia (Oreochromis mossambicus) was affected by water temperatures. Exposure of fries to 36°C had a marked inhibitory effect on both gonadal and somatic development. Accordingly, the 36°C exposure caused several physical and physiological abnormalities in the exposed fish. It is then clear that an exposure temperature of 36°C is deleterious to Mozambique Tilapia. These observations help us to suggest shortening of the exposure time to 1-2 weeks maximum in order to minimize the effects on health and growth of this fish for in case of its large scale production.

Since the studies conducted on this species are limited, the data obtained are considered to be important. Same experiments should be performed in another rearing environment such as concrete ponds, with different feeds in order to better understand the environmental influences on the reproduction of this fish because it is inevitable that continuing studies on Mozambique Tilapia under the above assessments will lead to more information on this species, which is important for aqua culturists.

Conflict of Interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

Ethics Committee Approval: This study was carried out in accordance with animal welfare and the ethics of trial. All procedures were performed in accordance Law on Veterinary and Medical Activities and National Animal Welfare Act. Therefore ethical approval was not required.

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Research Article

Zooplankton Biodiversity in Reservoirs of Different Geographical Regions of Turkey: Composition and Distribution Related with Some Environmental Conditions

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ABSTRACT

The zooplankton fauna and the relationship with their environmental variables were investigated on the epilimnion layer of the seven reservoirs (Demirdöven, Devegeçidi, Menzelet, Sır, Ömerli, Porsuk, Tahtalı) of different regions (Marmara, Aegean, Mediterranean, Central Anatolia, Eastern Anatolia, Southeastern Anatolia) throughout the summer months (2015) in Turkey. According to the trophic conditions reservoirs varied between oligo- and eutrophic status. Chlorophyll*a* concentration, measured for the estimation of primary production, was determined considerably high along the investigation period. A total of 62 zooplankton species were identified with the contribution of 44 rotifers, nine cladocerans and nine copepods. Except one reservoir, all of the others were dominated by rotifera group, and also in each study site dominant taxa were changed at species level. On the other hand common dominant taxa for all reservoirs was the rotifer *Polyarthra vulgaris* Carlin, 1943 with 95 % frequency. In terms of zooplankton species, most of the studied reservoirs showed less than 50% similarities, due to their different limnological conditions and different geographic locations. Reservoirs in high trophic conditions in the present study were represented by low species diversity. Water quality in the reservoirs with respect to biological data were determined as ß-mesosaprobic. The using limnological and biological indices to determine water quality were consistent. Densities of main zooplankton groups, and also frequent species of the reservoirs correlated with epilimnion layer depths and total phoshorus concentrations significantly. On the other hand rotifera variation was affected mainly by physical variables (pH, temperature, dissolved oxygen concentration, conductivity), and crustacean variations were related with total phoshorus. The comperative assessment between limnological variables and zooplankton community in this reservoirs was studied for the first time.

Keywords: Turkey, trophic state, limnological conditions, rotifera, biological indices

INTRODUCTION

Rapid population growth and development of the industry cause the need for freshwater to increase in Turkey as well as around the world. Therefore, many of reservoirs were built for drinking water supply, irrigation, flood control, and energy generation in Turkey since the 1930s. But, nowadays as a result of the urbanization and industrialization, reservoirs faced with the eutrophication hazard. Also, persistence of the eutrophic conditions causes loss of biodiversity, and may destroy the balance of the food chain from the bottom to the top (Brito et al., 2011). For this reason, limnological and biological variables should be investigated and followed for assessment, and obtained data should be used to improve the water conditions of reservoirs.

Biotic and abiotic factors of the reservoirs might alter the zooplankton species diversity, density, biomass, and spatio-temporal distribution. Life cycles of zooplankton are between days to weeks (Brock et al., 2005). Due to feeding and reproduction form alterations among the groups (Hutchinson, 1967), they show varied reactions to the abiotic conditions. Also, zooplankton groups, especially rotifers, respond quickly to these alterations, and consequently they are known as biologic indicators to estimate the water quality in the freshwater ecosystems (Sladecek, 1983; Herzig, 1987; Saksena, 1987; Hanazato, 2001; Pereira et al, 2002). Quick response of rotifers to the alterations in their environment results from their small-sizes, permeable integument (Arora and Mehra, 2003), rapid reproductive rates, and also ability to generate dense populations (Pace, 1986). Because of the rotifer density variation informs about eutrophication, they are used as trophic state indicators (Chen et al., 2012).

This present investigation was designed to determine the zooplankton community structure of seven reservoirs (Demirdöven, Devegeçidi, Menzelet, Sır, Ömerli, Porsuk, Tahtalı) from different river basins of Turkey and analyzed the relationship between zooplankton assemblages and various limnological variables for the first time.

MATERIALS AND METHODS

In the present study, a total of seven reservoirs (Devegeçidi, Demirdöven, Menzelet, Sır, Ömerli, Porsuk, Tahtalı) from different six regions from Turkey were investigated (Figure 1). General features of each reservoir was given in Table 1.

The study was carried out in the summer months (June, July, August) in 2015, simultaneously, in each reservoir. Samples were

Figure 1. Sampling locations. (I: Demirdöven, II: Devegeçidi, III: Menzelet, IV: Sır, V: Ömerli, VI: Porsuk, VII: Tahtalı)

Table 1. General features of studied reservoirs.

Dam Lake	Demirdöven	Devegeçidi	Menzelet	Sır	Ömerli	Porsuk	Tahtalı
Site	**I**	**II**	**III**	**IV**	**V**	**VI**	**VII**
Coordinates	40° 02′ 22.56″ N 41° 44′ 11.4″ E	38° 03′ 24.67″ N 39° 59′ 09.55″ E	37° 42′ 10.66″ N 36° 53′ 45.18″ E	37° 35′ 40.37″ N 36° 45′ 29.06″ E	41° 05′ 09.37″ N 29° 24′ 33.92″ E	39° 38′ 07.17″ N 30° 13′ 10.34″ E	37° 42′ 10.66″ N 36° 53′ 45.18″ E
Geographical Region	Eastern Anatolia	Southeastern Anatolia	Mediterranean	Mediterranean	Marmara	Central Anatolia	Aegean
Locality	Erzurum	Diyarbakır	Kahramanmaraş	Kahramanmaraş	İstanbul	Eskişehir	İzmir
Basin	Aras	Dicle-Fırat	Ceyhan	Ceyhan	Marmara	Sakarya	Küçük Menderes
River	Tımar creek	Devegeçidi creek	Ceyhan river	Ceyhan river	Riva stream	Porsuk creek	Tahtalı creek
Building date	1986-1996	2009-2010	1980-1989	1987-1991	1968-1973	1966-1972	1986-1999
Building purpose	Irrigation	Irrigation	Energy-Flood control	Energy	Drinking water	Irrigation, Flood control, Drinking water	Irrigation, Drinking water
Altitude	1788 m	747 m	621 m	1281 m	83 m	887 m	52 m
Volume	34,25 hm^3	202,32 hm^3	1.950 hm^3	1.120 hm^3	386,5 hm^3	431 hm^3	306,65 hm^3
Catchment area	1.45 km^2	32.14 km^2	42 km^2	47.50 km^2	23.10 km^2	23.40 km^2	23.52 km^2
Max. Depth/ Epilimnion depth (ave±stdev)	30 m/ 2 m±1.5	17 m/ 2 m±2.3	30 m/ 9 m±2.3	30 m/ 14 m± 4.2	20 m/ 6 m± 1.5	35 m/ 8 m±1.2	20 m/ 10 m±1.7

collected from the surface to the bottom of the epilimnion layer at the deepest point of the reservoirs (Table 1).

Some physicochemical variables [water temperature (T), dissolved oxygen concentration (DO), pH, electrical conductivity (EC)] were measured using by multiparameter (YSI 6820) with one meter intervals along the epilimnion layer, and water transparency was determined using by Secchi disk, *in situ*. The water samples for nutrient analysis [total nitrogen (TN), total phosphorus (TP)] and also Chlorophyll*a* (Chl*a*) concentration (as the primary production=phytoplankton biomass) were taken with 5 L Van-Dorn bottle from the surface, middle and bottom at the epilimnion layer, and they were mixed to obtain a composite sample. Nutrient analysis were performed according to APHA, AWWA,WEF (1989), and Chl*a* concentration was determined according to Nusch (1980). All variables were measured in triplicate.

Zooplankton was sampled with a closing net (55 μm mesh size, 0.6 m diameter opening, 1 m length) vertically from to the end of the epilimnion layer to the surface, and fixed with 4% formaldehyde solution. Zooplankton species were determined using a binocular microscope. The identification of the zooplankton species was performed according to the relevant taxonomic keys (Dussart, 1969; Koste, 1978; Margaritora, 1983). Enumeration, measurement, and also biomass calculations of the zooplankton species were performed according to EPA Standard Operating Procedure LG403 (U.S. Environmental Protection Agency, 2010). The body length of 20 randomly chosen individuals were measured via by camera attachment to use for the biomass calculation.

Trophic status of the reservoirs were determined according to Carlson's Trophic State Index (TSI) (Carlson, 1977). Species richness (*R*) of zooplankton was given as the total number of species. Shannon-Wiener diversity index (*H'*) (Shannon and Weaner, 1949), and Pielou evenness (*J*) (Pielou, 1966) indices for zooplankton were computed monthly. Saprobic index (S) was calculated according to the formula developed by Pantle and Buck (1955), and individual valence of each rotifer species were determined with reference to Sladecek (1983). To determine the Quotient of Community similarity degree for each pair of sites by using the zooplankton species Sørensen Similarity Index (QS) (Sørensen, 1948) was performed, and also a cluster analysis depending on determined species were used to compare the zooplankton compositions between studied sites (Bray and Curtis, 1957). Because of the data showed not normal distribution considering to the Shapiro-Wilk test, non-parametric tests (Spearman, Kruskal-Wallis) were selected. Non-parametric Kruskal-Wallis test was performed to determine differences of physicochemical variables and Chl*a* concentration, between study sites. To determine the correlation between biological (zooplankton and Chl*a*) and environmental data Spearman's rho was used. To investigate correlations between biological (zooplankton abundance and Chl*a* concentration) and physicochemical variables (Table 2) linear model of Redundancy Analysis (RDA) was employed via CANOCO 4.5 computer program (ter Braak and Šmilauer 2002). Monte Carlo permutation test (999 unrestricted permutations) was used to test the importance of correlation between biotic and abiotic variables.

RESULTS AND DISCUSSION

Different geographic regions and morphometric features (Table 1) of the investigated reservoirs were concluded in variations of main physical and chemical variables, with some exceptions. Water temperature, conductivity, nutrients, transparency and epilimnion layer depth showed significant differences between studied reservoirs (Table 1). Water temperature showed regional differences in summer months, and also conductivity variation showed similar patterns with water temperature. Nutrient concentrations were considerably high in every site (Table 1). Transparency of the sites were low, generally, due to summer months high primary production as given Chl*a* concentration in each reservoir. Epilimnion layer were differed based on the vertical water temperature variations of each reservoir (Table 1). Trophic state index (Carlson, 1977) of the studied reservoirs were ranged from 38 to 62 (Table 2), that means they changed between oligotrophic and eutrophic conditions. Components of CTSI [TSI (SD), TSI (CHL), TSI (TP)] were correlated each other significanlty. The negative correlation between transparency (Secchi depth) and Chl*a* (r=-0.606, p=0.004, N=21) was the indicator, that transparency of the reservoirs not depends on the phytoplankton abundance only.

Table 2. Summer variation of some physcicochemical variables and Chl*a* concentration (mean±stdev)

Site	T (°C)	DO (mg L^{-1})	pH	EC (mS cm^{-1})	TN(mg L^{-1})	TP (μg L^{-1})	Trans. (m)	Epilimniyon (m)	Chl*a* (μg L^{-1})	CTSI
I	17.3±3.6	7.3±0.7	8.0±0.6	80.1±17.4	1.4±0.1	29.7±5.7	1.0±0.1	2.0±1.5	6.9±1.6	57 (eutrophic)
II	27.1±1.6	6.9±2.1	7.9±0.7	323±9.0	2.3±0.7	28.7±7.4	2.0±0.4	2.0±2.3	9.3±6.5	54 (eutrophic)
III	25.4±1.8	8.7±0.6	8.7±0.8	323±14.8	1.7±0.2	7.2±4.7	4.0±1.4	9.0±2.3	2.7±1.5	38 (oligotrophic)
IV	24.2±0.9	6.7±2.7	8.0±0.7	464.4±84.7	1.8±0.3	12.5±5.8	2.0±1.2	14.0±4.2	10.1±5.8	48 (mesotrophic)
V	26.2±2.4	8.6±2.0	8.5±0.3	320.4±11.6	1.8±0.2	15.0±4.9	2.0±0.13	6.0±1.5	11.1±6.5	49 (mesotrophic)
VI	23.0±2.0	9.3±4.3	8.8±0.2	457±20.1	3.1±1.5	108.7±108.2	1.0±0.4	8.0±1.2	11.2±11.6	62 (eutrophic)
VII	26.9±1.5	7.9±1.8	8.5±0.2	381±13.5	1.9±0.2	11.1±1.9	4.0±1.0	10.0±1.7	4.5±2.8	42(mesotrophic)
Kruskal-Wallis (by sites) df=6; N=21										
χ2	13.749	5.818	7.948	17.541	12.658	17.075	14.684	17.557	11.833	
P	0.033	0.444	0.242	0.007	0.049	0.009	0.023	0.005	0.066	

Table 3. Summer zooplankton variation of the studied reservoirs.

		I			II			III			IV			V			VI			VII	
Copepoda	**J**	**J**	**A**	**J**	**J**	**A**	**J**	**J**	**A**	**J**	**J**	**A**	**J**	**J**	**A**	**J**	**J**	**A**	**J**	**J**	**A**
Acanthodiaptomus denticornis (Wierzejski, 1887)								*		+		+				*	*	*		+	
Acanthocyclops venustus Normann & Scott, 1906																+	+	+			
Acanthocyclops viridis (Jurine, 1820)										+	+					+	+				
Cyclops abyssorum Sars, 1863	+		+																		
Cyclops vicinus (Sars, 1863)				+																	
Eudiaptomus vulgaris (Schmeil, 1896)	+	+	+																		
Metacyclops stammeri Kiefer, 1938	+						+	*		*						*	+				
Thermocyclops crassus (Fischer, 1853)													+	*							
Thermocyclops dybowskii (Lande, 1890)										+	+	+									
Cladocera																					
Alona quadrangularis (Müller, 1776)																			*		
Bosmina longirostris (Müller, 1785)				*	*	+	+			+	+	*	+	+	+	+	+	+	+	+	*
Ceriodaphnia puchella Sars, 1862																			*	+	+
Ceriodaphnia quadrangula (Müller, 1785)							+	*	*	+	+	+				+					
Daphnia cucullata Sars, 1862	+	+	+	+			+			+	*					+	+		*		
Diaphanosoma brachyurum (Lievin, 1848)			*	+		+	*	+	+		+	+		+	+		+	+		*	+
Disparalona rostrata (Koch, 1841)	*																				
Leptodora kindtii (Focke, 1844)				+																	
Moina micrura Kurz, 1874				+		*															
Rotifera																					
Adineta vaga (Davis, 1873)																					+
Anuraeopsis fissa (Gosse, 1851)													+	+	+						
Ascomorpha coelata De Beuchamp, 1932						+															
Ascomorpha ecaudis Perty, 1850																	+	+	+	+	+
Ascomorpha ovalis (Bergendahl, 1892)				*											+						
Ascomorpha saltans Bartsch,1870								*	+	+	+	+									
Asplanchna priodonta Gosse, 1850		*	+		+	+	+	+	+	+	+	+	+	+	+	+	*	+			
Asplanchna sieboldi (Leydig, 1854)														+	+						
Brachionus angularis Gosse, 1851				*	+	+								+				+		*	
Brachionus calyciflorus Pallas, 1766						*					*			+				*			
Brachionus caudatus (Barrois & Daday, 1894)					+	+															
Brachionus diversicornis (Daday, 1883)					*	+									*						
Brachionus falcatus Zacharias, 1898					+	+															+
Brachionus diversicornis (Daday, 1883)					*	+									*						
Brachionus urceolaris Müller, 1773								*													+
Cephalodella gibba (Ehrenberg, 1830)					*																
Colurella colurus (Ehrenberg, 1830)												*									
Conochilus dossuarius Hudson, 1885																					+
Conochilus unicornis Rousselet, 1892		+				+	+				+	+			+						
Epiphanes macrourus (Barrois & Daday, 1894)					+	+													+		
Filinia limnetica (Zacharis, 1893)														+	+						
Filinia longiseta (Ehrenberg, 1834)					+	+															
Filinia opoliensis (Zacharias, 1898)					+																
Filinia terminalis (Plate, 1886)					+	+									*						
Hexarthra intermedia (Wiszniewski, 1929)					+	+															
Hexarthra mira (Hudson, 1871)														*	+			+	*		+
Kelicottia longispina (Kellicott, 1879)	+	+	+				*			*											
Keratella cochlearis (Gosse, 1851)				+	+	+	+	*		+	+	+	+	+	+	+	+	+			*
Keratella quadrata (Müller, 1786)	+	+	*							*			+			+	+	+			
Keratella tropica (Apstein, 1907)						*															
Keratella valga (Ehrenberg, 1834)											+	+									
Lecane lunaris (Ehrenberg, 1832)													*				+				

Table 3. Summer zooplankton variation of the studied reservoirs. (continued)

	I			II			III			IV			V			VI			VII		
Rotifera	J	J	A	J	J	A	J	J	A	J	J	A	J	J	A	J	J	A	J	J	A
Polyarthra dolichoptera Idelson, 1925				+	+	+								+	+	+	+	+			
Polyarthra vulgaris Carlin, 1943	+	*	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Pompholyx complanata Gosse,1851				+	+																
Pompholyx sulcata Hudson, 1885													+	+	+	+	+	+			
Rotaria rotatoria (Pallas, 1776)															+						
Synchaeta oblonga Ehrenberg, 1832				*	*	+	+	+	+	+	+	+	+	+		*	*	+			
Synchaeta pectinata Ehrenberg, 1832																			+	+	
Synchaeta stylata Wierzejsk, 1893															+						
Trichocerca capucina (Wierzejski & Zacharias, 1893)									+		+			+	+			+			
Trichocerca cylindirica (Imhof, 1891)					+	+								+	+						
Trichocerca pusilla (Jennings, 1903)										+	+	+	+	+	+		*	+	+		
Trichocerca ruttneri Donner, 1953						+								+	+						
Trichocerca similis (Wierzejski, 1893)						+	+	+	+			+	+	+	+				+	+	+

*: only once

Table 4. Sørensen's similarity coefficient index.

Site	I	II	III	IV	V	VI	VII
I	1	0.18	0.48	0.31	0.24	0.34	0.09
II		1	0.46	0.37	0.41	0.39	0.30
III			1	0.80	0.47	0.61	0.30
IV				1	0.48	0.59	0.39
V					1	0.58	0.31
VI						1	0.35
VII							1

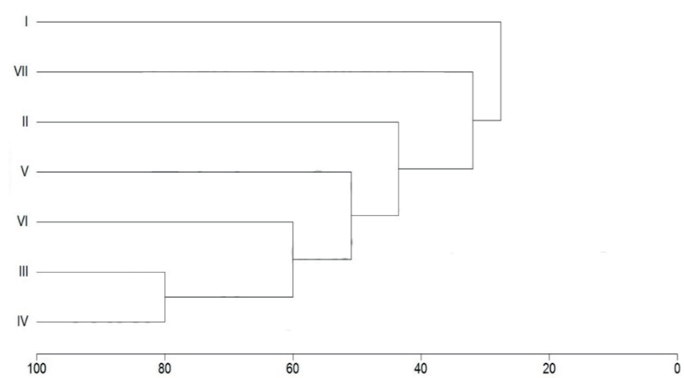

Figure 2. Dendrogram based on differences in zooplankton diversity between the studied reservoirs.

Also the positive significant correlation between TSICHL and TSITP (r=0.492, p=0.023, N=21) showed that in the studied reservoirs phosphorus may be limiting factor (Gołdyn et al., 2003). The water quality and ecological conditions of the studied reservoirs affected by their intended use (drinking water, energy, flood control and irrigation). Due to some of the studied reservoirs are drinking water resources, meso- and eutrophic quality of the waters may resulted as a drinking water quality problem (Palmstrom et al., 1988; Smith et al., 2002; Davies et al., 2004).

A total of 63 zooplankton taxa were identified (Table 3). Rotifers were dominant as numerically with 44 species. Crustaceans were represented by nine cladocerans and nine copepods throughout the sampling period (Table 3). The similarity of seven reservoirs from different regions was shown in Table 4. In terms of zooplankton species, site III and site IV showed highest incidence of similarity (0.80) (Table 4), due to their location in the same region (Table 1). On the other hand, according to the Sørensen's index, based on the common species of the sites (Table 3), zooplankton taxa of site IV were more similar with site III, IV and V (0.61, 0.59, 0.58, respectively) (Table 4). Also, similar results were verified by cluster analysis (Figure 2). Similarities or dissimilarities between the reservoirs were shaped by different abiotic conditions of the sites in different regions (Figure 2).

Species richness (R), as the total number of zooplankton species, changed between 9 (site I) - 26 (site II) species (Table 5). Shannon diversity index (*H'*) was determined between 1.59 (site III)- 2.29 (site V), and Pielou Evenness index (*J*) was ranged from 0.55 to 0.75 (Table 5). According to the description of Mason (1983) trophic state of aquatic environments evaluated between 1-3, therefore in terms of diversity index studied sites found as meso- and eutrophic status. The diversity results of the studied site showed similarity with TSI (Carlson, 1977). In the present study, species diversity of the sites, with high trophic conditions, found in low val-

Table 5. Index results of the studied reservoirs.

Site	Species richness (*R*)	Diversity index (*H'*)	Evenness index (*J*)	Saprobic index (*QS*)
I	9	1.66	0.75	1.52 (oligosaprobic)
II	26	2.16	0.66	1.64 (ß-mesosaprobic)
III	13	1.59	0.62	1.67 (ß-mesosaprobic)
IV	17	1.95	0.69	1.57 (ß-mesosaprobic)
V	25	2.29	0.71	1.63 (ß-mesosaprobic)
VI	20	1.65	0.55	1.58 (ß-mesosaprobic)
VII	15	1.68	0.62	1.59 (ß-mesosaprobic)

ues (Reed, 1978). Meso- and eutrophic reservoirs (Table 2) in the present investigation represented by low species diversity and on the contrary they had high densities than other sites (Figure 3A) (Reed, 1978). The high abundances of limited number of zooplankton species in the eutrophic sites caused unbalanced environments (*J*, Table 5). The saprobic index of the studied reservoirs varied from 1.52 (site I) to 1.67 (site III) and these values attributed to the ß-mesosaprobic, except site I (Table 5). Saprobic values showed the high organic matter decomposition levels in the reservoirs (Nandini et al., 2016). Most of the identified rotifer species in the study (24 of total 44 rotifer species) are known as eutrophication indicators due to their saprobic degrees (beta-mesosaprobic and alpha-mesosaprobic) (Sladecek, 1983).

In freshwater ecosystems rotifers dominate the zooplankton fauna (Saksena, 1987), and are also used as the biological indicators to determine the trophic status of the environment (Sladecek, 1983; Saksena, 1987). In this way, not only qualitative features but also quantitative characteristics (density) of the rotifers are important to evaluate the water quality, that high rotifer abundances expresses eutrophication (Sendacz, 1984). Most of the studied reservoirs, except site I where the copepods represented 48.2% of total zooplankton abundance, were dominated by rotifers in terms of relative abundance (ind/L) (Figure 3A).

Total zooplankton abundance (ind/L) varied between 132±21 ind/L (site VII) and 3258±718 ind/L (site II) (Table 6). Statistically total zooplankton and total rotifer abundance showed differences between study sites (Kruskal-Wallis; $H_{(6,N=21)}$=15.706, p=0.015; $H_{(6,N=21)}$=14.874, p=0.021, respectively), on the contrary no significant monthly differences were found between density of main zooplankton groups and total zooplankton (Kruskal-Wallis; H, df:2, P>0.05, N=21).

Seven of the identified taxa during the study showed ≥ 50 frequency (% F), they were *Polyarthra vulgaris* (Carlin) (95% F), nauplii (76% F), *Asplanchna priodonta Gosse* (67% F), *Keratella cochlearis* Gosse (62% F), *Bosmina longirostris* (Müller) (57% F), *Diaphanosoma brachyurum* (Lievin) (52% F), and *Trichocerca similis* (Wierzejski) (52% F), respectively. On the other hand, dominant taxa varied temper to reservoirs (Table 6). According to the frequency and dominance results rotifer *P. vulgaris* determined as the common dominant species all of the sites.

Main zooplankton groups [(copepoda: r=0.591, p=0.010, N=21);

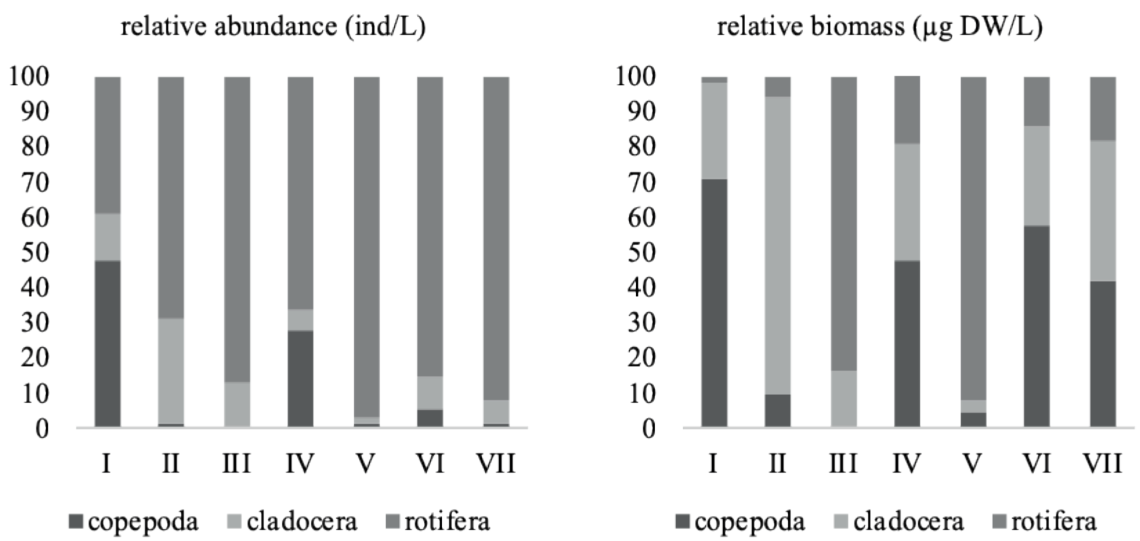

Figure 3. A- Relative abundance (ind/L) and B- Relative biomass (µg DW/L) variation of main zooplankton groups.

Table 6. Length and weight variations of dominant zooplankton taxa (N% ≥10 of total zooplankton abundance) of the reservoirs.

				Length (μm)		Weight (μg DW)		Total zooplankton abundance	Total zooplankton biomass
Site	N		Relative abundance (%N) in related reservoir	min-max	ave±stdev	min-max	ave±stdev	(ind/L) total±stdev in related reservoir	(μg DW/L) total±stdev in related reservoir
I	37	Nauplii**	29	132.9-261.7	190.5±38.6	0.1-0.5	0.3±0.1	339±76	723±56
	29	*K.quadrata*	19	109.0-147.4	131.9±8.1	0.03-0.1	0.1±0.01		
	24	*P.vulgaris*/**	16	108.0-178.4	153.5±18.8	0.04-0.2	0.1±0.04		
	47	*D.cucullata*	13	553.7-1519.1	885.7±235.4	0.8-16.9	4.1±3.6		
	25	*E.vulgaris*	11	901.7-157.7	1138.6±189.7	6.1-23.0	10.9±4.3		
II	60	*D.cucullata*	29	414.0-964.7	629.6±149.3	0.4-4.4	1.4±1.0	3258±718	1625±876
	71	*P.dolichoptera*	17.2	51.7-124.0	88.5±20.5	0.004-0.1	0.02±0.002		
	39	*P.vulgaris**	15.5	87.4-181.1	126.9±25.9	0.02-0.2	0.06±0.04		
	51	*K.cochlearis***	14.6	65.8-118.7	81.2±11.9	0.001-0.003	0.001±0.0001		
III	88	*P.vulgaris**	47.5	78.7-163.4	109.6±16.1	0.02-0.13	0.04±0.02	144±40	147±59
	93	*S.oblonga*	18.8	73.6-214.5	154.6±30.6	0.004-0.1	0.04±0.02		
	67	*T.similis***	11.4	114.3-166.5	139.2±9.9	0.08-0.24	0.14±0.03		
	77	*D.brachyurum***	10.1	347.1-988.8	620.4±130.5	0.4-3.7	1.4±0.6		
IV	58	*P.vulgaris*/**	36.8	86.2-173.1	139.5±25	0.02-0.16	0.09±0.04	163±14	50±9
	60	Nauplii**	22.9	90.3-313.7	139.9±36.5	0.1-0.6	0.1±0.01		
V	71	*K.cochlearis***	22.1	68.5-132.9	95.1±19.6	0.001-0.005	0.002±0.001	1828±410	172±75
	29	*P.dolichoptera*	21.4	59.0-89.2	70.8±7.2	0.01-0.02	0.01±0.004		
	33	*P.vulgaris*/**	12.5	63.6-162.7	105.5±22.2	0.01-0.1	0.04±0.03		
	26	*T.cylindirica*	11.6	108.6-261.9	212.2±33.3	0.1-0.9	0.5±0.2		
VI	98	*K.cochlearis***	39.97	66.6-122.4	90.4±13.9	0.0006-0.004	0.002±0.0007	1407±243	199±64
	31	*P.sulcata*	25.53	102.3-186.9	139.8±23.7	0.03-0.2	0.09±0.04		
	52	*P.vulgaris*/**	10.74	68.6-114.6	93.2±11.0	0.005-0.02	0.01±0.004		
VII	69	*P.vulgaris*/**	41.68	60.6-147.0	93±20.5	0.007-0.1	0.03±0.02	132±21	24±9
	54	*A.ecaudis*	24.58	65.0-147.0	90.3±19.7	0.003-0.1	0.02±0.002		
	40	*T.similis***	10.61	129.1-162.7	139.7±8.4	0.11-0.22	0.14±0.03		

*common species for all sites; ** frequent species ≥50%F

(cladocera: R=0.604, p=0.005, N=21); (rotifera: r=0.495, p=0.023, N=21)], and also total zooplankton (r=0.590, p =0.005, N=21) densities were correlated posivitely TP concentration. Moreover total zooplankton abundance showed positive significant correlation with Chl*a* concentration (r=0.520, p =0.016, N=21).

The density of copepoda (r=-0.485, p =0.010, N=21), cladocera (r=-0.481, p=0.032, N=21), rotifera (r=-0.441, p =0.046, N=21), and also total zooplankton abundance (r=-0.588, p =0.005, N=21) correlated negatively with increasing epilimnion layer depth. The common dominant species of the study sites *P.vulgaris* density correlated negatively (r=0.445, p=0.009, N=21) with epilimnion layer depth like total rotifer abundance. Phytoplankton growth rates may decrease through the depletion of phosphorus stock levels in the epilimnion, that may resulted as the lack of food for zooplankton (Arhonditsis et al., 2004). This case was supported by the negative correlation between Chl*a* concentration as phytoplankton biomass, and epilimnion layer depth (r=-0.304, p=0.018, N=21).

Total zooplankton biomass (μg DW/L) ranged between 24±9 μg DW/L (site VII) and 1625±876 μg DW/L (site II). Cladoceran and total zooplankton biomasses between sites showed significant differences (Kruskal-Wallis; $H_{(6,N=21)}$=7.584, p=0.004; $H_{(6,N=21)}$=10.009 p=0.006, respectively). Average individual length (μm) and weight (μg DW) values of the dominant species were given in Table 6. The highest biomass in site II related with the dominance of big-sized cladoceran *D. cucullata* in June (943 ind/L). Relative biomass dominance of the main zooplankton groups varied for each site, that although rotifers were the predominant group along the study period, because of their small sized bodies they could not have a high contribution to the total zooplankton biomass, generally (Figure 3B).

Zooplankton fauna of Devegeçidi (site II) (Bekleyen, 2001; Bekleyen, 2006), Porsuk (site VI) (Apaydın Yağcı et al., 2013), and Tahtalı (site VII) (Özdemir Mis et al., 2009) reservoirs were investigated, previously. Zooplankton fauna of site II, VI and VII showed similarities with the previous studies, but the identified taxa in the pres-

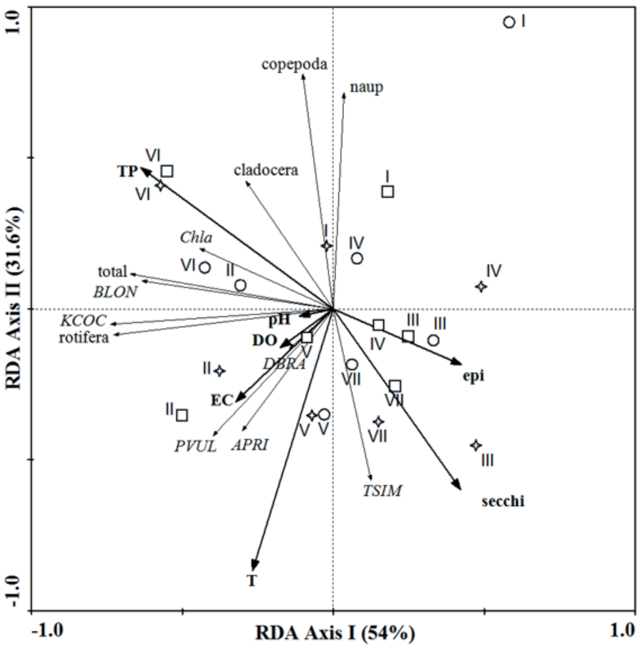

Figure 4. RDA ordination plot. (Roman numerals (I-VII) explained the reservoirs codes; PVUL: *P. vulgaris*, APRI: *A. priodonta*, KCOC: *K. cochlearis*, BLON: *B. longirostris*, DBRA: *D. brachyurum*, TSIM: *T. similis*, naup: nauplii; circle: June, square: July; star: August).

ent study were fewer than the former. This is probably related to the sampling procedures and times, and also may be affected by the trophic and environmental conditions changing in time. Because of the lack of available data on the trophic conditions of the previous studied sites, it is not possible to compare before and after. Zooplankton variation of the other reservoirs, and the relationship between zooplankton fauna and their environmental conditions in all reservoirs were investigated for the first time in the present study.

In the redundancy analysis (RDA) Chl*a*, total zooplankton, main zooplankton groups, and most frequent taxa abundances were used as biological data (Figure 4). Furthermore, after the control of environmental variables with regard to the Variance Inflation Factor (VIF<10) (ter Braak and Šmilauer 1998), and a stepwise forward selection (FS), total nitrogen (TN) (VIF>10) was removed from the data set, thereby eight limnological variables were used in the multiple comparison (Figure 4). According to the RDA analysis first two axes explained 85.6% of total variation. First axis was in a relation with largely epilimnion layer depth (0.3225) and pH (-0.0850), whereas second axis correlated with TP (0.3916) and pH (-0.0202), mainly. The sampling times of each reservoir were grouped in itself, generally (Figure 4). The reservoirs (site I, II, IV) identified as eutrophic according to the TSI, showed significanly correlation with TP, whereas oligo- and mesotrophic sites related mainly water temperature, epilimnion layer depth and transparency (Secchi disk depth) (Figure 4). Eutrophic conditions were related with high nutrients, that eutrophic sites represented by high zooplankton abundance (Table 6). Relatively higher transparency, compared to the other sites, in the oligotrophic site III resulted in low zooplankton abundance. The negative correlation between total zooplankton abundance (both at group and species level) and epilimnion layer depth may seen in RDA graph (Figure 4). Because TP is the nutritional source for the primary producers, total zooplankton density (both at group and species level) was related with TP indirectly and also significantly, as seen on the multiple comparison (Figure 4). An other evidence, that support this relevance, was the positive correlation between Chl*a* and TP concentration (r=0.505, p=0.020, N=21). According to the ordination plot rotifer taxa were related with pH, DO, EC, and T, mainly (Figure 4), because they are not selective on food quality, and they are also consumers of detritus and bacteria (Ruttner-Kolisko, 1974; Conde-Porcuna et al., 2002). The common dominant species of the reservoirs *P. vulgaris*, and most frequent species of the study showed similar behaviors as in the groups they belonging to.

High rotifer abundance (Almeida et al. 2009; Lodi et al., 2011; Špoljar, 2013) in eutrophic waters attributed to the avaliable food (phytoplankton, bacteria, and especially detritus) for rotifers and also their short generation times (Sed'a and Devetter, 2000; Nogueira, 2001, Gazonato Neto et al., 2014, Haberman and Haldna, 2014). When all data and statistical results of the present study are evaluated, the dominance of rotifers in both density and diversity are related with trophic status of the studied reservoirs. The siginifant correlation between total rotifer abundance and total phosphorus (TP) (r= 0.495, p=0.023, N=21) (Stemberger, 1995) of investigated sites is the evidence that trophic conditions of the water affected their composition and dynamics relatively (Arora and Mehra, 2003). An other probability, that may clarify the dominance of rotifers, was the pH values of the studied reservoirs. Rotifer species of eutrophic waters prefers pH≥7 (Berzins and Pejler, 1987) similar in the present study. pH levels shows increasing in nutrient-rich waters with dense photosynthetic activity resulting from algal growth, accordingly most of the rotifer species may find optimum conditions for their development. Also in the studied reservoirs cyanobacterial blooms occured in the summer months (Köker et al., 2017). It is known that these blooms provide an opportunity to reconstitute the zooplankton population from big-bodied to the small sized species (Gilbert, 1996). This information was supported by the results of the present study. The small-sized evasive rotifer *P. vulgaris* was determined as the common dominant and most frequent (95% F) species in the reservoirs during the study period.

In conclusion, both the laboratory data and the statistical results verified each other. Physicochemical and biological data showed that water bodies are under pollution pressure. The statistical results of the present study stated the possibility the usefullness of the zooplankton groups, especially rotifers, as a good ecological indicators (Montagud et al., 2019). Also, when considering their intended use, especially as drinking water, trophic conditions and biological patterns of the reservoirs must be controlled and followed for safe use.

Conflict of Interests: The authors declare that for this article they have no actual, potential or perceived conflict of interests.

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