

The Effect of Ploidy on Growth and Feeding Pattern of Diploid and Triploid Turbot *Scophthalmus maximus* Under Communal Rearing Condition

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Abstract

Triploidy induction is a useful tool to produce sterile fish that prevents interbreeding of farmed fish with wild counterparts. In this study, growth performance and feed conversion rates of 12-months old diploid and triploid turbot that reared both communally and separately for 16 months at constant water temperature determined. At the end of the experiment, diploid, triploid, and communally reared groups reached an average weight of 672.4±21.0 g, 659.2±25.3 g and 660.5±12.0 g, respectively. Any significant differences were not observed in the weight of separately reared diploid and triploid turbot groups. In contrast, triploid fish in the communal group were significantly heavier than diploids at the end of the rearing period. The feed conversion rate of the ploidy group did not show any significant difference between groups. The highest mean specific growth rate was observed in the triploids in communal groups, whereas the diploids in communal groups had the lowest mean specific growth rate. The weight and length gain rates of the groups were similar. Diploid turbot growth appeared to be negatively affected by communal ploidy rearing. The results of the present study indicate that triploid turbot farm escapees may be advantageous in terms of growth in nature.

Introduction

Turbot *Scophthalmus maximus*, also known as, *Psetta maxima*, is a marine demersal sinistral form of flatfish. From the Black Sea to the Baltic Sea, it exists in a wide geographic range. Turbot subjects to intensive fishing pressure because of its high commercial value (Nikolov et al., 2015, Prado et al., 2018a; Firidin et al., 2020). According to a recent report, turbot's catches drastically decreased from 11548 tonnes in 1994 to 5843 tonnes in 2018 (FAO, 2020a). Such reduction due to overfishing, the result of eutrophication and changes in the coastal ecosystem. Aquaculture practices and stock enhancement programs are vital to meet the high market demand, (Aydin et al., 2020). Artificial spawning and seed production techniques are widely applied in Europe (Ruyet, 2002; Lugert et al., 2019), Turkey (Aydin

et al., 2020; Polat et al., 2021), China (Cang et al., 2018), and Korea (Han et al., 2018). Moreover, turbot aquaculture farms have mainly been established in Europe and China during the last decades reaching a total production of 59615 tonnes (FAO, 2020b). On the other hand, countries such as Turkey (Zengin et al., 2007), Russia (Maslova, 2002), Denmark (Støttrup, 2002), Belgium (Delbare and Clerck, 2000), Spain and Norway (Coughlan et al., 1998) have released turbot juveniles to enhance depleted turbot stock as well as take precautionary measures to encourage the amount of spawning stock biomass. Despite the benefit of providing an alternative source of protein, there is public concern regarding hatchery-reared fish moving to the wild environment (either escapee from the farm or intentional releases for stock enhancement) for the sustainability of natural resources (Prado et al., 2018b).

Although triploidy induction is a common method used in plants, early trials of triploidy induction for fish date back to only the 1940s (Gjedrem, 2005). In general, when the fish reach sexual maturity, a low growth rate, deterioration in meat quality, and mortality are observed. However, sexual maturity is not expected in triploid fish, so superior growth, high meat quality and low mortality are expected. (Thorgaard, 1986; Dunham, 1990; Hussain et al., 1995). To prevent these undesirable traits and to improve yield, triploid induction extensively utilizes in freshwater and anadromous fishes, marine fishes, and shellfish (Piferrer et al., 2009), as well as turbot. Moreover, to benefit from different fields, transgenesis has recently been applied to several fish species, such as Atlantic salmon, taking place in markets around the USA (Cebeci et al., 2020). However, transgenic fish still poses questions about the potentially deleterious impact of escaping or released transgenic fish on natural habitats. Therefore, sterile triploid fish also recommend eliminating the risk of transgenesis fish escaped from farm to natural habitat (Piferrer et al., 2009). On the other hand, the impact of triploidy is species-specific and different results have reported in many fish species (Arai, 2001; Felip, 2001).

The performance of triploid fish in their natural environment has mainly documented in terms of reproductive interactions between released/escapees triploid and diploid wild relatives in different fish species (Piferrer et al., 2009). On the other hand, the growth and feeding behaviour of triploid fish reared in communal condition is rare (Mori et al., 2006). Aquaculture performance of triploid barfin flounder was studied under the communal rearing conditions and Benfey (1999) explained that behavioural interactions between triploids and diploid controls are confounding.

Triploid induction in turbot can be obtained by cold shock application just after fertilization (Piferrer et al., 2000; 2003). There are many reports on triploid turbot biology such as early cleavage patterns of eggs (Aydin and Okumuş 2017), growth and gonadal development (Cal et al., 2006), sex-dependent synaptic behaviour (Cuñado et al., 2002), flesh quality (Hernández-Urcera et al., 2016; Ayala, et al., 2020), digestive enzyme activity (Domingues et al., 2019), haematology (Cal et al., 2005), immunology (Budiño et al., 2006), and body morphology (Hernández-Urcera et al., 2012). In literature, several studies have indicated that triploid turbot exhibits similar growth and feeding responses compared to their diploid counterparts (Piferrer et al., 2003; Cal et al. 2006; Domingues et al., 2019). The comparison of growth and feed utilization of triploid and diploid fish reared in communal is noteworthy to assess the behavioural effect of diploid or triploid fish on their counterparts and to figure out the possible effect of either released or escapees cultured triploid turbot on the wild environment. However, there have been no investigations on the effects of growth and feed utilization of triploid turbot under the circumstantiate of communal rearing with its diploid partners. Therefore,

this study aimed to determine the growth and feed usage of the triploid and diploid turbots on the circumstantiality of mutual rearing. For this purpose, individually tagged diploid and triploid turbots, reared both separately and in the same place for 15 months, were studied.

Materials and Methods

Fish material and Triploidy Application

Turbot broodstock used in the experiment were obtained from Marine Fish Hatchery in Central Fisheries Research Institute, Trabzon, Turkey. Eggs were fertilized in seawater by mixing with stripped milt from two different males for one female. After 20 min of fertilization for triploidy, fertilized eggs were exposed to cold water just below 0°C for 6.5 min as recommended by Piferrer et al. (2000) and Aydin and Okumuş (2017). Fertilized eggs were incubated at ~14°C for 5 days and larval rearing was carried out according to Çiftçi et al. (2002). Eggs, larva, and juveniles from each ploidy were kept separately during all rearing period.

Ploidy Assessment

When the experimental fish reached 6 months old, 120 control (diploid) and blood samples from 240 cold shock treated (triploid) juveniles were taken for ploidy evaluation. Ploidy levels were determined by measuring the long axis of erythrocytes (40 erythrocytes per fish) from dried smears of their blood under a light microscope (Piferrer et al., 2000). The long axis of erythrocytes was $11.4 \pm 0.02 \mu\text{m}$ in diploids, and $14.2 \pm 0.16 \mu\text{m}$ ($P < 0.001$) in triploids, and the triploid rate was calculated as 99.2%.

Rearing Conditions and Experimental Design

Turbots were reared in a flow-through system at the Marine Hatchery in Central Fisheries Research Institute in Trabzon, Turkey. Seawater with a 17.9 ± 0.03 ‰ salinity was pumped from a depth of 40 m, and the water exchange rate was 20 times a day for all experimental tanks. The seawater used in the hatchery was pre-treated with pressurized sand filters and a UV sterilization system. Pre-treated seawater was then pumped to all tanks from one reservoir with heating systems to maintain the desired temperature at all times. The water was aerated with two air stones at a moderate rate in each tank. Temperatures were measured twice a day; dissolved oxygen (DO), pH and salinity values were measured once a week. During the entire grow-out period, the water temperature was kept at $8.0 \pm 1.14^\circ\text{C}$, pH was 6.98–8.08, the salinity was 17.8–18.2 and DO was higher than 80%.

Three replicates were formed in a total of three groups: only diploid (150 fish), only triploid (150 fish), and diploid (75 fish) and triploid (75 fish) turbot grown

communally. For the first period of the experiment (5 months), 12 months post-hatching turbot were stocked into nine indoor, round-cornered square water tanks (500 L). For the rest of the experimental period (10 months), nine indoor raceway tanks (2000 L), each stocked with 50 fish, were used. All fish were individually marked with PIT tags (TX1400L, Biomark) and weighed individually.

Feed Consumption and Growth

Turbot diet containing 52% crude protein, 10% crude lipid, 1% crude fibre and 13% crude ash having gross energy of 19.8 kJ/g (Kiliç Yem Corporation, Turkey) was used in the experiments. Experimental fish were fed by hand with extruded turbot diet once a day to apparent satiation (*ad libitum*) (Aydin et al., 2011). The feed weight consumed by the fish in each tank was measured. Uneaten pellets were collected by siphoning 45 min after each feeding to be included in calculations to identify the real feed intake and FCR. The fish were starved for 24 h before each measurement. Initial stocking density was determined as 10 kgm² and did not exceed common standards. Growth parameters were recorded monthly. Growth parameter indices; specific growth rate (SGR), length and weight gain ratio (LGR and WGR), feed conversion rate (FCR), were calculated according to Altinok et al. (2020) based on the following formulae;

$$\text{SGR} = 100 \times (\ln(\text{final weight}) - \ln(\text{initial weight})) / \text{day} \quad (1)$$

$$\text{LGR} (\%) = 100 \times (\text{final length} - \text{initial length}) / \text{initial length} \quad (2)$$

$$\text{WGR} (\%) = 100 \times (\text{final weight} - \text{initial weight}) / \text{initial weight} \quad (3)$$

$$\text{FCR} = \text{feed intake} / \text{weight gain} \quad (4)$$

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA), and if significant differences were found, means were compared by the Tukey's test ($P < 0.05$) after confirmation of normality and homogeneity of variance (Zar, 1999). Statistical analyses were performed with Statistica 7 (Stat Soft. Inc. Tulsa, OK, USA).

Results

The initial, periodic, and final average weight of each fish group are presented in Figure 1. As presented in Figure 1, the initial mean weights of the fish groups were found to be similar ($P > 0.05$). Diploid, triploid, and communal reared turbot groups were grown at the proper constant water temperature (at 18°C) for 16 months and had an average weight of 672.4±21.0 g, 659.2±25.3 g and 660.5±12.0 g, respectively (Figure 1). When both ploidies reared together, the weight of diploid turbot was found to be 604±28.5 g, whereas their counterpart triploid turbot was 716±39.3 g. Turbot weight at the end of the experimental period was not significantly affected by ploidy when reared separately, but when reared communally, they were significantly affected by ploidy ($P < 0.05$) (Figure 1).

Growth parameter indices of FCR, SGR, WGR, and LGR are presented in Table 1. The feed conversion rate (FCR) of the diploid groups was slightly higher than that of the other groups; however, no statistically significant difference was found. The growth parameters SGR, WGR, and LGR were statistically different between the experimental groups ($P < 0.05$) (Table 1). The highest mean SGR was found in the triploid communal groups (0.51±0.01), while the lowest SGR was observed in

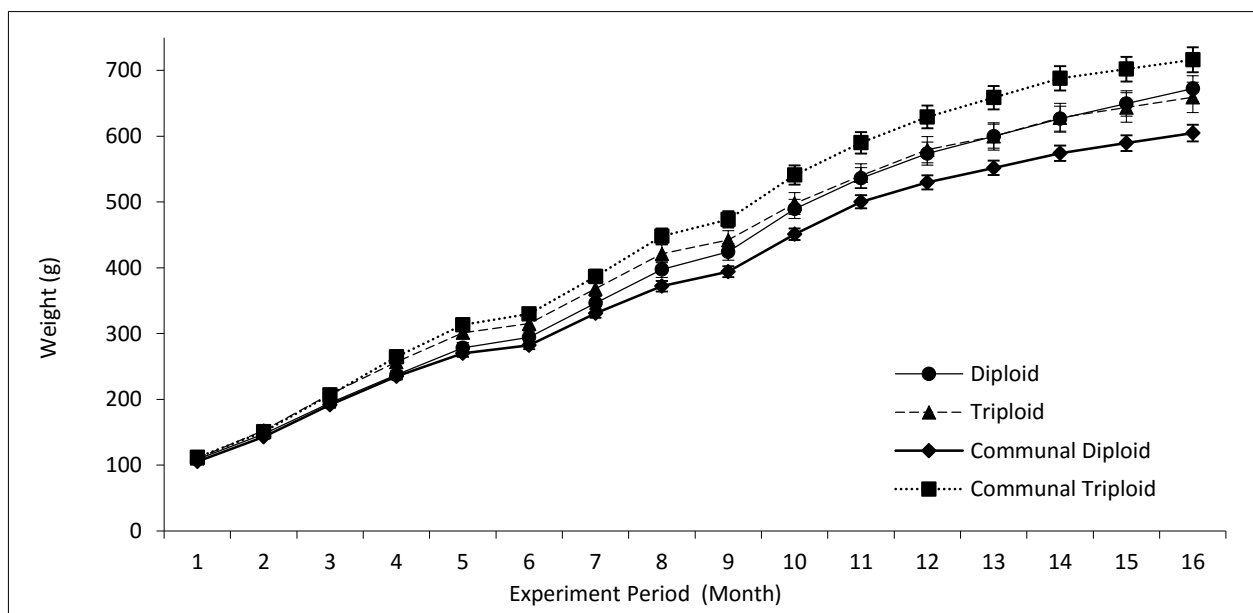


Figure 1. The weight increase of turbot *S. maximus* during 15 months rearing period at 18°C. Diploid (●), triploid (▲), communal diploid (◆) and communal triploid (■).

diploid communal groups (0.48 ± 0.01). However, similar SGR values were found in diploid, triploid and, communal groups (0.50 ± 0.01) ($P < 0.05$). Similar to SGR values, diploid (533.58 ± 12.85), triploid (513.88 ± 12.15), and communal groups (522.53 ± 12.83) had statistical similar WGR values. In the meantime, WGR was the highest (558.07 ± 19.02) in triploid communal groups while diploid communal groups had the lowest WGR (486.97 ± 16.30). Similarly, communal diploid fish displayed relatively the lowest LGR (79.91) while communal triploid fish had the highest LGR (86.44). LGR of the other groups were found to be similar ($P < 0.05$) (Table 1.).

Discussion

Triploid induction has a different effect on fish growth and feeding behaviours. It depends on various factors such as fish size, fish species, and rearing conditions (either separately or communal). Superior effect of triploid fish was observed during sexual maturity due to sterility, along with the period of puberty, triploidy did not lead to any weight increase. Studies indicated that triploids grew similarly to diploids in many teleost species (Fernandez-Pato et al. 1996; Cal et al. 2006, Maxime 2008; Piferrer et al., 2009). Arai (2001) reported that under independent rearing conditions, the growth of triploid rainbow trout, *Oncorhynchus mykiss* and ayu, *Plecoglossus altivelis* are nearly the same as that of diploids. Turbot also exhibits similar growth for both ploidy during juvenile and growing phase (Piferrer et al., 2003; Cal et al. 2006; Domingues et al., 2019). In the present study, at the end of 15 of months rearing the mean weight of turbot, *S. maximus* reached 600-700 g from 100 g. Based on the growth and feeding patterns, it has pointed out that, performance of separately reared diploid and triploid turbot are similar. Like previous studies, this study demonstrated that before the onset of sexual maturation, ploidy did not significantly affect the growth of diploid and triploid turbot, which were reared at separate tanks but in similar environment.

The growth and feeding behaviour of triploid fish reared under the communal condition with their diploid is uncertain, and in literature, there is no information on the triploid turbot. Only several published studies have shown that the ploidy did not affect the growth of barfin

flounder, *Verasper moseri* (Mori et al., 2006) and loaches, *Misgurnus anguillicaudatus* (Horei et al., 2004) in the communal rearing environment. On the other hand, in another study, it has been shown that triploid Atlantic salmon, *Salmo salar* grew better than their diploid partners when they were reared together (Galbreath et al., 1994). The main objective of this study was to determine cold-shock induced triploid turbot performances under communal rearing condition with their diploid counterparts. Therefore, in this study, the performance of triploid turbot in communal rearing with diploids was determined.

The effects of ploidy on fish physiology have not been fully elucidated (Cotter et al., 2000) and triploid fish exhibits different behavioural and physiological responses when compared with their diploid counterparts (Piferrer et al. 2009; Hernández-Urcera et al. 2012; Hernández-Urcera et al. 2016). Many studies indicate that the performance of ploidy is species-specific, and there are many different results obtained from various fish species (Arai, 2001; Felip 2001; Maxime 2008; Piferrer et al., 2009; Fraser et al., 2012). Moreover, the effect of ploidy on fish varies between individuals of the same species, even though they are reared under similar conditions (Galbreath et al., 1994; McGeachy et al., 1995). Therefore, in contrast with a previous study carried out with other species, the present study demonstrates that the growth and feed utilization of triploid turbot were higher than the diploid counterparts in communal rearing condition. The reason for triploid turbot growth to be superior to their diploid sibling under communal rearing is probably due to getting more feed or being more competitive during feeding.

The benefits of using triploid fish in fisheries management are; to avoid the problems associated with undesirable reproduction and ecological and genetic impacts of cultured fish on the wild stock (Piferrer et al., 2009; Fraser et al., 2012). The use of triploid fish also ensures that any escapees from aquaculture facilities are not able to reproduce in the wild. As an exotic species, sterile triploid fish may be cultured in the targeted region where fish do not exist naturally in that habitat. Thus, triploid fishes such as salmonids, ayu (*Plecoglossus altivelis*), loach (*Misgurnus anguillicaudatus*), and grass carp (*Ctenopharyngodon idella*) are used in releasing (Powell et al., 2009;

Table 1. Growth parameter indices of each group. Superscripts (a, b and c) indicate significant differences ($P < 0.05$).

Group	FCR	SGR	WGR	LGR
Diploid	1.22 ± 0.03^a	0.50 ± 0.01^b	533.58 ± 12.85^b	83.27 ± 1.12^b
Triploid	1.21 ± 0.01^a	0.50 ± 0.01^b	513.88 ± 12.15^b	82.97 ± 1.16^b
Communal	1.21 ± 0.02^a	0.50 ± 0.01^b	522.53 ± 12.83^b	83.18 ± 1.20^b
Diploid Communal	-	0.48 ± 0.01^a	486.97 ± 16.30^a	79.91 ± 1.58^a
Triploid Communal	-	0.51 ± 0.01^c	558.07 ± 19.02^c	86.44 ± 1.77^c

FCR-Feed conversion rate, SGR-Weight-Specific growth rate, WGR-Weight gain ratio, LGR-Length gain ratio.

Beaumont et al., 2010). Also, one more common use of triploidy is sport fishery. Similarly, the release of triploid turbot may be useful because of a low level of genetic diversity of the turbot populations (Han, Lee, Jung, Aydin, & Lee, 2018; Prado et al., 2018; Firdin et al., 2020). However, there are still tremendous information gaps in the performance of triploid fish, particularly when they have been released or escaped into wild environments (Fraser et al., 2012). More research is still needed on this topic to understand triploid fish performance either in communal or wild environments.

Conclusion

The impact of escapees from aquaculture is of general concern for the sustainability of the natural resource. Triploidy is under consideration to avoid this concern because of its sterility. Despite this advantage, there is a debate on the effect of release or escapee triploid fish in a wild environment. In this study, the performance of triploid turbot in communal rearing with diploids was determined. Before the onset of sexual maturation, Ploidy did not have a major effect on diploid and triploid turbot growth, which were grown in different tanks but under similar conditions. On the other hand, under communal rearing conditions, the growth and feed utilization of triploid turbot were found to be higher than the diploid counterparts. The reason for triploid turbot growth to be superior to their diploid sibling under communal rearing is probably due to getting more feed or being more competitive during feeding. Nevertheless, further research on this subject is required to understand the performance of triploid fish under communal condition and in a wild environment. Finally, it should be highlighted that triploid turbot may impact the wild population when they release to the same environment in terms of feeding and growth. Thus, to use of triploid fish in releasing programme should be under consideration.

Ethical Statements

All triploidy process and experimental protocols have been approved by Veterinary Control and Research Institute, Animal Experiments Local Ethics Committee (No;22-003/2) under the General Directorate of Agricultural Research and Policy surveillance.

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Author Contributions

IA: Conceptualization, funding acquisition, project administration, methodology, formal analysis, investigation, writing-review & editing.

Conflict of interest

The author declares that there are no conflicts of interest regarding the publication of this article

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