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RESEARCH PAPER

Sex Ratio Response to High Temperature During Early Development of Rainbow Trout (*Oncorhynchus mykiss*) in Farmed Stocks

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Abstract

The objective of the study was to investigate the effect of high temperature on sex ratios of early stage rainbow trout (*Onchorhynchus mykiss*) in commercially farmed populations from distinct hatcheries. Almost 32500 larvae from five different farms were used in experiments as thermal groups and their corresponding controls. Temperature treatments were conducted at 17.5°C for 40 days starting at day 10 after hatching. In only one population, the sex ratio was skewed significantly in favor of females in thermally treated group (67.5%), when compared to control group (52.1%) and there was deviation from the expected 1:1 ratio. In the treatment group the females grew 8.3% more than males. However there was no repeatiblity and any skewness in sex ratio of progenies from photoperiod induced spawning. Survival rates were not affected by the thermal treatments. The results showed that the increasing of femaleness of farmed stocks is possible by thermal treatment in rainbow trout and which depends on percentage of thermosensitive individuals rather than genetic background of population.

Keywords: *Onchorynchus mykiss*, temperature, sex ratio, farmed stocks.

Erken Gelişim Döneminde Uygulanan Yüksek Sıcaklığın Gökkuşağı Alabalığının (*Oncorhynchus mykiss*) Çiftlik Stoklarındaki Cinsiyet Oranına Etkisi

Özet

Çalışmanın amacı birbirinden uzak kuluçkahanelerde ticari olarak yetiştirilen alabalık (*Onchorhynchus mykiss*) popülasyonlarında erken dönemde uygulanan yüksek sıcaklığın cinsiyet oranları üzerine etkisini araştırmaktır. Denemelerde sıcaklık ve kontrol grupları olmak üzere beş farklı çiftlikten yaklaşık 32500 larva kullanılmıştır. Sıcaklık denemeleri 17.5°C' de 10 günlük larvalara 40 gün olarak uygulanmıştır. Kontrol grubu (%52.1) ve beklenen teorik cinsiyet oranı (1:1) dikkate alındığında, çalışılan popülasyonların sadece birinde sıcaklık cinsiyet oranı üzerinde dişilik yönünde (%67.5) önemli bir etki oluşturmuştur. Aynı popülasyonda dişiler erkeklere göre %8.3 daha fazla gelişmiştir. Buna rağmen bu sonucun popülasyon genelinde tekrarlanabilirliğinin ve fotoperiyot uygulanan stoklarda ise benzeri cinsiyet eğiliminin olmadığı anlaşılmıştır. Sıcaklık uygulamaları yaşama oranlarını olumsuz etkilememiştir. Bu sonuçlar kültürü yapılan stoklarda popülasyonun genetik geçmişinden çok termal duyarlılığa sahip bireylerin sayısına bağlı olarak mümkün olabileceğini göstermiştir.

Anahtar kelimeler**:** *Onchorynchus mykiss*, sıcaklık, cinsiyet oranı, çiftlik stokları.

Introduction

Manipulation of sex and rearing of monosex female salmonids are most advantageous for aquaculturists. Genetic manipulation or hormonal treatments are the major methods of sex control in fish but it is not practical to apply during every spawning season. Further, the hormones can be harmful to both human health and aquatic environment.

Many studies have indicated the effect of temperature on the phenotypic sex in fish but this phenomenon is not very widespread (Ospina-Alvarez and Piferrer 2008). Although, several investigations demonstrated the temperature effect on egg size, embryonic development and performance in salmonids (Beachem and Murray 1990; Ojanguren *et al*., 1999). Specific exact knowledge on the relationship between temperature and sex differentiation is limited and variable. Baroiller *et al*., (1999) assumed that salmonids would be non-thermolabile in gonadal sex determination, whereas

Craig *et al*., (1996) and Azuma *et al*., (2004) suggested that temperature affects the sex ratio during the early life stage in sockeye salmon (*O. nerka*) and in rainbow trout (Magerhans *et al*., 2009). However, temperature based genetic variation is seen between strains, populations but also between families within the same population (Baroiller *et al*., 2009; Magerhans *et al*., 2009). The huge variations in sex ratios of progenies from the same breeders have been related to the varying temperature (Baras *et al*., 2001). Variable sex ratio among families (or among individuals) has suggested that poly-factorial influences on sex determination may cancel the sex-determining activity of the sex chromosomes (Devlin and Nagafama, 2002). Moreover, existences of different Y chromosomes in different salmonid species (Volff *et al*., 2007) show evidence that they have not conserved sex determination mechanism. The effect of temperature on sex-ratio seems to exist in rainbow trout depending on exposed temperature and proportion of females can be increased via selection (Magerhans *et al*., 2009). The cultured stocks are genetically affected by various historical events, as the hatchery practises can dramatically affect the genetic composition through founder effects, inbreeding and selection and these changes can sometimes be seen already after one generation (Bezault *et al*., 2007; Tiira *et al*., 2005). In this regard, repeatability of sex response to temperature is very important and the role of thermosensitivity has not been rigorously investigated in farmed stocks. Therefore, the present study was designed to test thermosensitivity in sex-differentiation among farmed stocks.

Materials and Methods

The samples of fish (*Oncorhynchus mykiss*) were procured from six distant hatcheries to realize varied populations according to the genetic background and rearing environment. Each broodstock containing 500–1500 breeders, which has a specific genetic background and environmental conditions, have been kept under farming conditions more than 20 years. In three of the hatcheries photoperiodic control have been used in manipulation of spawning for out-of-season production once a year. Akhan and Canyurt (2005) reported very high genetic variability (ranged between 0.659 and 0.752) among broodstocks used in the hatcheries located in the regions. The characteristics of the broodstocks and their environmental characteristics are presented in Table 1.

"Experiment 1" was carried in natural spawning season to determine the differences among the populations (P1, P2, P3, P4 and P5). Eyed eggs were transferred to a hatchery where the thermal treatments were carried out. Hatched larvae (9–10 days post-hatch, dph) originating from each hatchery (20 mating per batch per hatchery) were randomly separated into two groups: heated group exposed to 17.5±0.5°C of water temperature, which resulted in better survival rates than other tested water temperatures (19-21 ºC) in preliminary studies, control siblings were raised at ambient water temperature in the hatchery. The fish selected for thermal treatments were transferred in plastic fish bags containing the hatchery water (8-11 ºC, 100 fish per bag) into 60L buckets. Due to disease risks of hatcheries that might cause the loss of all experimental fish, each group of the three buckets was used for one population and replicates and was connected to a semi-recirculated water system separately, which includes filter pump, an aerator to maintain the oxygen concentration over 5 ppm and a heating system (3000 W) with thermostat. The temperature was gradually increased in 2 hours for progressive thermal acclimation. The water flow in the buckets was set at 2 L min^{-1}, and the reserved water was changed 3 times per day by allowing constant flow of fresh water into system for keeping the water quality. An identical design was used for the control groups (3 buckets per population). Water temperatures were checked three times a day and dead fish were removed daily. Commercial rainbow trout feed (47% crude protein) were distributed ad libitum by hand four times a day. The experiment lasted 40 days at age of 50 dph, after Krisfalusi and Nagler (2000) demonstrated the labile period in sex determination of rainbow trout ranged from hatching to 24 dph. In the second experiment (experiment 2), three populations originating from broodstocks under photoperiod (P1, P3, P5; to check the effect of photoperiod induced spawning on sex ratio of the population) and ten family mating from the population (P3) that showed femaleness in "experiment 1" (to determine the repeatability) were randomly selected and examined in next out-of-season and natural spawning periods.

During the first experiment period one replicate of P5 population was almost dead, caused by interruption of water supply and therefore the replication was taken out. Following the thermal treatment period, the fish were stocked into 125-L cylindrical tanks (10°C, 146 fish) and reared under ambient thermal regime (10-15°C) for visiual sexing of gonads at 150 dph by microscope observation. According to the sex proportion in "experiment 1" at the time of sexing, P2 and P3 (as nonbiased and biased population) were selected and further reared until 210 dph to compare the growth rates between the sexes at marketing size. For this purpose, randomly selected 80 fish from each replication of these populations were stocked into twelve subdivided units (1x1x0.5m) in four concrete ponds (3x1x0.5m) supplied with 5 L min^{-1} water.

Sexing was carried out by microscopic observation of the gonads (Guerrero and Shelton 1974), after administration of a lethal dosage of anaesthetic (2-Phenoxyethanol). When sexes could not be identified visually, gonads were histologically

Table 1. Studied populations from hatcheries with geographic locations and environmental conditions

Population	Hatchery Location	Lat. /Long.	Water temp. in farm (°C)	History of hatchery and broodstock
P1	Manisa	38°36′ N 27°26′ E	11 – 20	Water temperature (11-13 °C) in hatchery is relatively higher than the other hatcheries. No photoperiod manipulation
P2	Fethiye	36°79′ N 29°31′ E	8 – 9	Photoperiod manipulation has been used for production of out-of-season eggs.
P3	Fethiye	36°76′ N 29°40′ E	8 – 17	A photoperiod induced spawning in a year-round and water temperature increases 17°C in summer season.
P4	Kütahya	39°25′ N 29°59′ E	7.5 – 9	There is no photoperiod manipulation.
P5	Mugla	36°91′ N 28°79′ E	8.5 – 9.5	Mixed strains (originating from USA). A photoperiod manipulation for spawning control.

examined (Takashima *et al*., 1980). Differences in sex-ratios among populations were analyzed by using a chi-square test for k independent samples, followed by a chi-square goodness-of-fit test of each population against the 1:1 sex-ratio. The effect of the thermal treatment on sex ratio for significance was analysed by comparison of the treatment groups and corresponding controls using a 2×2 contingency Chi-square (χ^2) test. It was also used to compare survival rates. Weight comparisons were assessed by Student's t-test.

Results

There were no significant differences among replicates within populations as well as between treatment and control groups when comparing survival rates (Table 2) ($P>0.05$). Bacterial and parasitic diseases detected in both treated and sibling controls have caused significantly higher mortalities in some of the populations viz: P1, P3 and P5. In general, there were high survival rates (>71%) in groups without disease problems during all experimental phases.

Sex ratios were similar among replicates within each population and non-significant χ^2 values were obtained in all of the treatment and control groups ($P = 0.05$). Replicates were, therefore pooled to obtain an overall sex ratio-value for each population. The observed sex ratios in thermal treatment groups and sibling controls are presented in Table 2. About thirteen thousand fish were sexed in total and the proportion of females in thermal treatment groups varied from 46.6 to 67.5% at 17.5°C while the femaleness of 57.7% was the highest rate in the sibling control groups. A relationship was obtained between the population variable and temperature towards sex ratio (χ^2 = 39.459, df = 4, alpha=0.001), whereas the differences among the control groups were not significant ($P>0.05$, χ^2=4.46 df=4). There was no significant deviation from a balanced (1:1) sex ratio in most of the groups ($P>0.05$), except the fish from hatchery P3, where the proportion of females was significantly higher both in the thermal and control groups in experiment 1 and thermal groups in "experiment 2". In the same population, when comparisons were made among groups raised under different water temperature regimes, skewness toward female was significantly higher in thermal groups ($P<0.01$; χ^2 value of 6.89) of the "experiment 1". However, the difference was not observed in the "experiment 2".

To determine the effect of phenotypic sex on growth, all fish in the control and treated groups from hatcheries P2 and P3 were weighed at 210 dph (Table 3). There was no growth dimorphism between the sexes, neither in the control nor in the treated groups in P2 ($P>0.05$), whereas in P3, the dimorphism is significant in both cases ($P<0.05$). There was also significantly higher growth gain for fish in thermal treated groups than in control groups for both populations ($P<0.001$).

Discussion

The study investigated the influence of water temperature (17.5°C) which did not cause a high mortality in heated groups, on rainbow trout considering the varied farmed stocks anticipated to have selective genetic backgrounds and may effects on sex differentiation. Only limited investigation on small size groups of broodstock was carried out that showed temperature did not affect sex ratio in rainbow trout (Baroiller *et al*., 1999). However, recently, Magerhans *et al*., (2009) obtained strongly female biased sex ratio (57.5-60.6%; control: 48.8-51.2) in progenies from four different rainbow trout populations which were exposed to 18 °C during 30 days post hatching. This is similar to the present results which showed markedly female biases (67.5%) in progeny sex ratio in a farmed population. Similar femaleness effect (60–80%) was exhibited by Craig *et al*., (1996) in different populations of sockeye salmon (*O. nerka*) when eggs were exposed to high temperature (12 °C against control of 9 °C). However, Magerhans *et al*., (2009) have found a strongly male biased population (64%). Although, the temperatures and treatment time used in the studies were almost

Table 2. Female ratios and survival rates (SR) in function of population and temperature treatment, chi-squared (χ^2) analysis comparing sex ratios of experimental groups for each population (pooled replicates) with a theoretical 1:1 sex ratio and their controls

Population		Temperature (°C)	SR, % (at 40 dph)	Sexed (*n*)	Female (%)	χ^2 (control)[a]	*P*	χ^2 (1:1)[b]
P1	T1	17.3 ± 0.2	54,4	234	46.6	0.187	0.665	1.094
	C1	8.0 ± 0.5	51,9	164	48.8			0.098
P2	T2	17.5 ± 0.2	96,4	855	53.1	0.544	0.461	3.285
	C2	8.0 ± 0.5	98,0	668	51.2			0.383
P3	T3	17.4 ± 0.2	53,7	397	67.5	6.89**	0.008**	48.668***
	C3	8.0 ± 0.5	57,0	281	57.7			6.580*
P4	T4	17.3 ± 0.2	92,1	435	49.7	0.472	0.492	0.021
	C4	8.0 ± 0.5	94,6	355	52.1			0.634
P5	T5	17.5 ± 0.2	48,8	184	49.5	0.140	0.708	0.022
	C5	8.0 ± 0.5	53,3	230	51.3			0.157
P2-PIS	T2-PIS	17.7 ± 0.2	76,7	675	48.6	0.330	0.566	0.535
	C2-PIS	11.3 ± 1.0	80,2	696	50.1			0.006
P3-PIS	T3-PIS	17.5 ± 0.2	70.9	623	56.5	2.268	0.132	10.530**
	C3-PIS	11.3 ± 1.0	68.7	592	52.2			1.142
P5-PIS	T5-PIS	17.5 ± 0.2	96.1	850	51.6	0.132	0.716	0.922
	C5-PIS	11.3 ± 1.0	97.4	851	52.5			2.173
P3-REP	T3-REP	17.4 ± 0.2	93.2	2724	52.4	0.939	0.333	6.396*
	C3-REP	8.0 ± 0.5	94.8	2741	51.1			1.357

T=Treatment, C=Control
Significance: *P<0.05; **P<0.01; ***P<0.001
a=χ^2 test between treated groups and their corresponding controls, *P*= p values for χ^2 controls,
b= χ^2 test between groups and theoretical 1:1 sex ratio

Table 3. Fish body weights (the means and standard deviations (±SD) from replicates)

	P2			P3		
Groups	females	males	mixed	females	males	mixed
Thermal	206.1±2.3[a]	203.6±2.8[a]	204.9±2,5[a]	235.7±1.8[c]	218.2±2.4[d]	227.0±10.3[cd]
Control	170.3±2.4[b]	169.9±2.3[b]	170.1±1,9[b]	180.6±1.5[e]	171.8±1.3[b]	176.2±4.9[eb]

Within a row, means with a letter in common are not significantly different (α=0.05)

similar, high temperature (17.5 °C; controls: 8-11 °C) did not cause a biased sex ratio toward male in the studied farmed stocks. This is similar to the results of Craig, Foote and Wood (1996) who have not found male biased sex ratio in different populations though sex reversal from genetic females to functional males was found in sockeye salmon by Azuma *et al*., (2004) when fish (40-150 dpf) were exposed to high temperature (18 °C) in relation to controlled temperature at 9 °C.

Differences in direction of sex and in deviations among the studies and populations may be linked to genetic variations or differences in exposed temperatures. The direction of sex was mostly to femaleness in the studies that may have been an evidence of femaleness is favourable sex in salmonids under treatment of high temperature. However, the highest deviations vis-a-vis control groups was varied among the studies from 15% in the present study to 20% in Magerhans *et al*., (2009) and 28% in Craig *et al*., (1996) respectively. These results showed that temperature could alter the sex ratio in different salmonid broodstocks and the thermolability was formed during those in these previous studies without any attribution to higher mortality of one sex. In the present study, the disease, which was observed in both groups of thermal treatment and corresponding controls (8 °C) in several populations, caused low survival rates and there was no skewness in sex ratio such as the populations of P1 and P5 showed 1:1 sex ratio in both cases. The experiments were conducted with the same water supply, the same condition of high temperature exposure was applied, and therefore the skewed sex ratio in the groups and populations cannot be attributed to high mortality percentage or water supply.

It is believed that sex determination in salmonids is strictly under genetic control but sometimes can be shifted towards predominance of one sex caused by some other factors (Brykov *et al*., 2008) and there are significant differences in fish the ratios of one river to another have been observed among the populations (Gilk *et al*., 2005). Thus, the control group of P3 hatchery might have showed a tendency towards femaleness. However, in practice, this might have caused partly by spontaneous selection during the summer season (photoperiod) when juveniles have been exposed the high water temperatures (13-17°C) due to seasonal fluctuations. Likelihood factor was exhibited by selection of thermo-sensitive families altering the sex ratio in progenies of rainbow trout in Magerhans and Hörstgen-Schwark (2010). The authors reported that by temperature treatment proportion of females can be increased or decreased in selection lines. In this regard, water supply temperature, breeding systems and selection of thermos-sensitive individuals are prominent factors in deciding the sex proportions in the farmed stocks. Similarly, comparative studies were conducted on different domestic stocks and on wild breeders originating from well differentiated thermal habitats in order to investigate both genetic and environmental components of sex determination in Tilapia (Abucay *et al*., 1999; Bezault *et al*., 2007). The authors reported that natural thermal environments may differentially affect the sex determination system in fish.

Salmonids have distinct sex chromosomes and therefore interaction between temperature and genotype related to the sex chromosomes or to the sex differentiation are still unclear. Quillet *et al*., (2002) reported that the environmental impacts are likely to occur when the primary sex-determining factors are bypassed in rainbow trout. Therefore, complexity of genetic sex determination was expressed as karyotyp mosaic of the Salmonidae (Penman and Piferrer, 2008), and extensive chromosome polymorphism in rainbow trout (Hartley and Horne 1984). Lastly, this was concluded by Ospina-Alvarez and Piferrer (2008) as the genotypic sex determination plus temperature effects in salmonids. Some differences in variability of thermosensitivity have been observed between populations as wells as inter-familial variation, which points to important parental effects and genotype-environment interactions (Bezault *et al*., 2009). Such parental effect has also been found in rainbow trout by Magerhans *et al*., (2009).

Four of the farmed stocks did not show any distortion in the study similar to the report of Baroiller *et al*., (1999), while one population showed no temperature effect in Magerhans *et al*., (2009). In this case, frequency of occurrence and repeatability of the response of sex ratios to temperature are very important. Magerhans *et al*., (2009) observed high repeatability with femaleness (max 67.2%; control: 52.3) in full sib progenies produced from repeated single pair mates in rainbow trout. In the present study, population P3 was tested for repeatability of female-biased sex ratio but only one of ten familes produced higher proportion of female progenies, which did not increased the femaleness of the population significantly. The results demonstrated that the percentage of thermo-sensitive breeders and progenies is more important factor to select or increase the femaleness in the farmed stocks as photoperiod induced spawning and different environmental conditions of hatcheries showed similar and balanced sex ratios among the populations. When the repeatability is achieved and performed practically in selective breeding, juvenile rearing can be planed directly in farms or seasons with naturally available water temperature (17-18 ^{o}C) to increase the femaleness without energy cost.

Moreover, at the end of the experiment growth performance of females was 7.5% better than males in heated group. Even more remarkable is that the observed growth of heated group was 22.5% more than full sib control. In conclusion, the results suggested that exposure to high temperature during early development is able to induce gonadal sex differentiation depends on individual variation based on genetic background and thermosensitivity in rainbow trout. Further investigations of the relations found between femaleness and individual response to high temperature, selective breeding are warranted to optimize temperature dependent sex control in farmed stocks.

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