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Selection experiments to alter the sex ratio in rainbow trout (*Oncorhynchus mykiss*) by means of temperature treatment

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ABSTRACT

A selection experiment in rainbow trout (*Oncorhynchus mykiss*) was initiated to verify if the proportion of females after temperature treatment could be significantly altered by selection. Rainbow trout spawners out of six populations were used to produce a diallel cross in which all populations were used as male as well as female parents. The 95 families of this breeding base were tested for their temperature sensitivity as follows. After an incubation period of 42 days, alevins of each family were subdivided into a treatment group and a full sib control group (12 °C), each consisting of 300 fish. Temperature treatments were carried out with 18 °C for 30 days. Each treatment and corresponding control group was raised separately until an age of 9 months post fertilization. Then a random sample of 10 fingerlings out of each control group was kept for later selection decisions. All other fish from the treatment and control groups were sexed by microscopic inspection of gonad squashes. The families were ranked according to the percentage of females, and families with the highest ($n=6$) and lowest percentage of females ($n=6$) in their sex ratios after temperature treatment were selected to produce two divergent lines (high and low line). After one generation of selection the temperature-treated groups in the high line showed a female percentage of 58%, whereas the low line had an average female proportion of 44%. The realized heritability was 0.63 in the high and 0.71 in the low line. This study provides the first evidence that the sex ratios in temperature-treated groups of rainbow trout can be selected as a quantitative trait. Additionally, in the meiotic gynogenetic offspring derived from a female spawner, which showed low percentages of females after temperature treatment in previous matings, 16% males were observed in the temperature-treated group. Thus, there seem to be two possible consumer- and environment-friendly ways to increase significantly the proportion of females in rainbow trout: directly via selection of families which show a high percentage of females in their sex ratios after temperature treatment to build up a corresponding temperature sensitive line or indirectly via neo-males derived from temperature treatments of gynogenetic offspring.

1. Introduction

The production of large rainbow trout (*Oncorhynchus mykiss*) over 1.2 kg body weight has become a growing commercial interest in the world. The major bottleneck in this field is the early maturity of the male rainbow trout relative to female. Thus, all female rainbow trout stocks are preferred, which are currently produced by the use of neo-males in matings with normal females. The growing concerns for food security make the prevalent approach of producing neo-males via the oral application of testosterone to sexual indifferent fry less desirable. The finding of a sex control alternative based on a safe, consumer and environmentally friendly method is a major challenge for the production of all female populations (Azuma et al., 2004). Recent studies in Nile tilapia (*Oreochromis niloticus*) have shown that rearing temperature may modify the phenotypic sex of an individual and consequently alter the sex ratios of the offspring. This temperature sensitivity during the sexual undifferentiated phase has a genetic background (Baroiller et al., 2009).

In *Oncorhynchus* species, previous studies have also shown that under certain rearing conditions, environmental factors (such as temperature) may modify the phenotypic sex of an individual and consequently alter the sex ratios of the offspring. Azuma et al. (2004) found males in genetically all female sockeye salmon (*Oncorhynchus nerka*), when the authors raised the temperature from 9 °C to 18 °C for longer periods during embryonic and alevin stages. Craig et al. (1996) observed an increase of females in temperature-treated offspring also in sockeye salmon, when a temperature shift of 8.3 °C–9.7 °C to 10.4 °C–12.0 °C was applied during incubation and alevin stage (40–86 dpf). Nagler et al. (2001) also reported that sex reversal in chinook salmon (*Oncorhynchus tshawytcha*) from the Columbia River could occur from an exposure to fluctuating temperatures. In rainbow trout, Magerhans et al. (2009) observed differences between and within

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populations with regard to thermal responsiveness of sex ratios, maternal and paternal effects on temperature dependent sex ratios, and nearly identical results of repeated matings, which are similar to the observations in Nile tilapia (Baroiller et al., 1995; Tessema et al., 2006). In Nile tilapia, Wessels and Hörstgen-Schwark (2007) could show in a two generation selection experiment that thermosensitivity to sex differentiation is a heritable trait. Hence, in the present investigations with rainbow trout, the anticipated response to selection regarding sex ratios in temperature-treated groups was tested in an up and down selection experiment.

2. Materials and methods

Rainbow trout spawners from six populations were used to produce a diallel cross, in which all populations were used as male as well as female parents. All spawners were kept in ponds at the Experimental Trout Farm Relliehausen (University of Göttingen, Germany) with a final stocking density of 10 kg/m^3 (water temperature min 2 °C in winter and max 16 °C in summer, O_2>8 mg/l, pH 6.5–8.0, NH_3<0.01 mg/l, NO_2<0.01 mg/l and NO_3<10.0 mg/l). The broodstock was given standard trout pellets twice per day (Trouvit F-8 Pro Aqua Repro, crude protein 46%, crude fat 16%). Single pair matings of individually tagged spawners were carried out to produce full sib families for temperature experiments. In contrast to the spawners used, produced progenies were hatched and reared indoors by the use of well water of constant quality and temperature of 12 °C ± 0.3 °C.

The 95 families which were used as a breeding base were tested for their temperature sensitivity in the following way. Fertilized eggs and hatched alevins were kept in Zuger glasses at a water temperature of 12 °C ± 0.3 °C till day 42 post fertilization (dpf). At that time, alevins were counted and each progeny batch was separated into a treatment and a full sib control group, each consisting of 300 fish (n_1). The temperature treatment was carried out according to the recommendations of Magerhans et al. (2009). The full sib control groups were kept at 12 °C ± 0.3 °C water temperature whereas the treatment groups were subjected to a thermal treatment at 18 °C water temperature for 30 days. Temperature treatment was carried out in two semi-closed recirculation systems, each of 1000 l volume with a separate temperature regulation (thermo-regulator 3000 W), bio filter and three compartments (each 300 l volume with air blower 44 l/min). In each compartment four net cages (25 l) were installed, and in each net cage one full sib progeny was kept. The thermoregulators did not exceed a maximum deviation of 0.2 °C to guarantee the specific temperature of 18 °C required for the experimental batch. Water temperature was checked three times daily and all other water parameters were checked twice a week. Alevins were gradually acclimatized to 18 °C temperature at the onset of the treatment and to 12 °C at the end of the treatment period by a slow up and down temperature regulation over a period of 48 h in order to reduce fish losses. According to the procedure developed for family testing at the experimental trout farm (Morkramer et al., 1985; Hörsten-Schwark et al., 1986; Hörstgen-Schwark, 1993) the corresponding full sib control groups were kept continuously at 12 °C in Zuger glasses.

First feeding fry were given Trouvit Pro Aqua Brut diet (crude protein 57%, crude fat 15%) to apparent satiety. After temperature treatment, fry in the treatment and in the corresponding full sib control groups were counted again (n_2) to determine the survival rates as (n_2:n_1) × 100. Both groups were then standardized to 150 fish each and placed in cylindrical tanks (volume 80 l each), which were incorporated in semi-closed recirculation systems of 35 tanks each. These systems were used and described in detail by Morkramer et al. (1985) and Hörstgen-Schwark (1993). Each semi-closed recirculation system had a volume of 10 m^3 (rearing and water regeneration unit) and was supplied with about 30 m^3 of fresh well water per day. The water temperature was constant at 12 °C ± 0.2 °C. Five months post fertilization, treatment and control groups were fed three times daily (Trouvit Select 1P, crude protein 46%, and crude fat 15%). Nine months post fertilization, when the fish reached a weight of approximately 30 g, they were subjected to sexing. The well water parameters in the trout breeding facilities, measured during the whole experimental period, were within the following range: O_2>8 mg/l; pH 6.5–7.5, NH_3<0.001 mg/l, NO_2<0.005 mg/l and NO_3<5.0 mg/l. Before sexing started, a random sample of 10 fish from each full sib control group was kept as putative breeders for the next generation. All other fish were killed by an overdose of anaesthetic. Sexing was accomplished by microscopic observation of gonad squashes (Guerrero and Shelton, 1974) of each fish in the treatment and in the corresponding full sib control groups.

The chi-square, goodness-of-fit test was applied for analysis of the sex ratios of the full sib control groups. For the verification of a normal distribution of sex ratios within each line and generation the Kolmogorov–Smirnov test was applied. For the analysis of differences in sex ratios and survival rates between treatment and control groups for significance the χ^2, goodness-of-fit test (P<0.05, 0.01, 0.001) of the statistical program SPSS Version 14 was used. Then families were ranked accordingly to the percentage of females and those with the highest (n = 6) and lowest (n = 6) percentage of females in their sex ratios after temperature treatment were selected to produce two divergent lines (high and low line). Similar to selection experiments in Nile tilapia to increase the proportion of males by temperature treatments (Wessels and Hörstgen-Schwark, 2007) means were weighted according to the number of offspring in each group before the selection differential and response to selection for the sex ratios in temperature-treated groups were estimated.

The realized selection differentials (S_R) showing the mean superiority of selected families (in % females) were obtained for each line by the subtraction of the weighted average sex ratio of the base population (G0) from the weighted selected families' mean (data not shown). Standardized realized selection differentials (S_R in SD) were obtained by dividing the realized selection differential (S_R) by the corresponding line's (population) standard deviation (SD) of the sex ratio in temperature-treated groups. The realized response to selection (ΔG_R) was calculated for both lines separately by the subtraction of the weighted mean sex ratio of the first generation of selection (G1) from the breeding base (G0). Realized selection responses in the first generation (ΔG_R) in relation to the applied selection (S_R) were given by realized heritabilities (h^2_R) for the high and the low line according to Falconer and Mackay (1996).

Furthermore 10 fish were taken randomly out of a random sample of 12 different treatments and corresponding full sib control groups in G0 and raised group-wise in tanks under standardized conditions until 18 months post fertilization. The fish were then sexed and their body weights recorded.

Additionally, a meiotic gynogenesis was conducted with a female spawner showing low percentages of females in offspring after temperature treatment in previous matings. Meiotic gynogenesis was induced according to the method of Chourrout and Quillet (1982) and a haploid control was kept in order to verify the success of the induced gynogenesis. Gynogenetic offspring were divided into a treatment and a full sib control group and handled in the same way as the full sib progeny from single pair matings.

3. Results

Table 1 gives an overview on mean survival rates and sex ratios in the treatment and full sib control groups of the breeding base (G0) and in the high and the low line of the first generation of selection (G1). The number of families within the high (n = 18) and the low line (n = 14) in G1 differed according to the availability of ripe female spawners in the selection lines in the course of this experiment. In general, survival rates of treatment groups were significantly higher