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Persistent unequal sex ratio in a population of grayling (Salmonidae) and possible role of temperature increase

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Abstract

Environmental changes can influence major demographic parameters by affecting individual behavior, growth, or survival. In some fish, water chemistry or temperature can influence sex determination or create sex-specific selection pressures. The resulting population sex ratios are hard to predict from laboratory studies if the environmental triggers interact with other factors, while in field studies, sex ratios are often determined as part of another study and singular extra-ordinary observations may hence be particularly prone to selective reporting. By conducting long-term monitoring, these problems can largely be avoided. We studied a population of grayling (*Thymallus thymallus*) that has been monitored since 1948. We found a shift in the operational sex ratio from around 65% males before 1993 to around 85% from 1993 to 2011. The observed sex ratios correlated with the water temperatures that the fish experienced in their first year of life. A life stage-specific analysis revealed that the sex ratios were best explained by the average temperature that juvenile fish experienced during their first summer. The abundance of grayling is declining, but we found no evidence for a strong genetic bottleneck that could explain the apparent lack of an evolutionary response to the unequal sex ratio. Other studies found no evidence of endocrine disruptors in the study area. Our findings therefore suggest that temperature-sensitive development affects population sex ratio and thereby contributes to population decline.

Introduction

River-dwelling fish often have little possibility of moving to avoid non-optimal environmental conditions and can hence be sensitive to changes in water temperature or chemistry. If tolerance to stressors is sex-specific (e.g. Perry et al. 2005), or if genetic sex determination is reversed by environmental conditions (Baroiller et al. 2009), environmental changes can have demographic and evolutionary effects that may sometimes be counterintuitive (Cotton & Wedekind 2009). Such effects may not be detected easily in natural fish populations if, for example, non-random exploitation obscures demographic effects of environmental changes. However, laboratory-based studies on the possible demographic effects of, for example, temperature can be misleading (Ospina-Alvarez & Piferrer 2008).

We analyzed 63 years of monitoring data (1948-2011) on sex ratio in a population of grayling (*Thymallus thymallus*; Salmonidae) whose spawning area has been protected since 1952. The temperature in the region encompassing the spawning area increased abruptly in 1987/1988 (Hari et al. 2006). We observed a shift in the operational sex ratios (OSR = the proportion of males among all breeders during the time when egg-bearing females with mature eggs could be caught at the spawning site) that may have contributed to an otherwise unexplained decrease in abundance (Wedekind & Küng 2010), and we tested for possible links between the OSR and the water temperature.

If a population cannot change its geographic distribution, phenotypic plasticity or evolutionary adaptation might be the only way a threatened population can persist (Hoffmann & Sgrò 2011). Magerhans et al. (2009) found significant paternal and maternal effects on temperature-dependent sex determination in aquaculture stocks of another salmonid species, and analogous genetic effects on sex-specific tolerance to environmental stressors seem possible (Perry et al. 2005; Hutchings 2006). A genetic bottleneck could therefore reduce the adaptive capacity of the population and contribute to changes in demography, for instance in OSR. We therefore also tested whether genetic bottlenecks could have occurred in recent years.

Methods

The study population has its main feeding territory in Lake Thun in Switzerland and spawns in its outlet, the river Aare (between the lake and a weir 1.3 km downstream). Since 1948, samples of spawning fish have been caught with nets about three times per week around the spawning season in March and April. Yearly catches during spawning season range from 51 to 2085 grayling (median = 630), were low before fishing efforts were standardized in 1962, and have been declining from the 1970s to 2009 (Wedekind & Küng 2010). The large majority of these fish were released back to the spawning site after their gametes were collected (by stripping), but in some years, especially in the first years of the program, some males (range 0 – 95%) and some females (range 0 – 85%) were lost due to handling or released downstream of the weir and hence separated from their original spawning population (Fig. 1a). In 1992, 2006, and 2008, fish were marked by clipping the adipose fin or removing scales in order to determine whether the same individuals were captured multiple times. On average, 15.0% of the males (range 3.8%-22.3%) and 0.4% of the females (range 0%-1.9%) were caught twice during the same spawning period. We used these averages to correct the OSR estimates for all years. Using instead the lowest or the highest observed number of multiple captures would not change our conclusions and would only slightly shift the OSR estimates (e.g. in 2011 from 0.861 to 0.877 or 0.851, respectively). We used Spearman rank order correlation coefficients (r_s) to test for changes over time, and to compare yearly OSR with the number of sampled females from 1948 to 2009 (as reported in Wedekind & Küng 2010), also including 2010 and 2011.

The water temperature at the spawning site of our study population has been continuously recorded at 1-min intervals since April 1970, and the timing of spawning is known for each year. We could therefore determine the average temperature that embryos and juveniles were exposed to at different developmental stages, here defined in intervals of 500 ATU (accumulated temperature units = degree days). Wedekind and Küng (2010) found two major changes in the temperature environment of embryos and juveniles during the last decades. First, because of a temperature-linked shift in the timing of spawning and the fact that water temperatures rose more slowly in early spring than in late spring, embryos and hatchlings have been exposed to increasingly colder temperatures during their first 500 ATU. Second, summer temperatures, i.e. the temperatures that juveniles are exposed to during 1500-2000 ATU, have been increasing. Most grayling in our samples are four, five, or six years old (mean = 5.0, SD = 1.2; Wedekind & Küng 2010). We therefore conducted a multiple regression analysis (in JMP 8.0.1) relating the OSR of each year to the average temperature that embryos and juveniles had experienced five years earlier during each of the four intervals of their first 2000 ATU of development (starting every year with the day when half the spawners of that year had been sampled). We also performed a forward step-wise regression predicting OSR from the average water temperatures of the current or the six previous years to evaluate the significance of the temperature during the first year of life as compared to later years. Because extraordinarily high temperatures were recorded in 2003 (Fig. 1b), we estimated the OSR of the 2003 cohort on 712 spawners that were sampled in 2008 and 2009 and aged on the basis of growth rings on scales. Age was estimated independently by two observers and analyzed separately per observer.

We determined the genotypes of 169 spawners collected in 2007 (Table 1) by polymerase chain reactions (PCR) to test whether genetic bottlenecks occurred in the past and to estimate the effective population size (N_e) of the population in 2007. Each PCR reaction (10 µl) was composed of 2.5 µl of DNA template (extracted from fin samples with DNeasy Tissue Kit, Qiagen), 1 × PCR buffer, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.5 µM of each primer and 0.25 units of Taq DNA polymerase (Qiagen). We used the following PCR profile: initial denaturation at 94°C for 3 min, 30 cycles of 30 s at 94°C, 30 s at the annealing temperature described in Gum et al. (2003), 30 s at 72°C, and a final extension at 72°C for 5 min. We ran PCR templates on an ABI 3100 automated DNA sequencer, used GENEMAPPER (Applied Biosystems) to score alleles, analyzed number of alleles and gene diversity (H_S) in FSTAT 2.9.3 (Goudet 1995), and used BOTTLENECK (Piry et al. 1999) to test whether recent bottlenecks had occurred. We assumed a two-phase mutation model with the settings recommended by Piry et al. (1999) for microsatellite data (95% single-step mutations and 5% multiple-step mutations with variance among multiple steps = 12).

To estimate N_e we used a method based on linkage disequilibrium implemented in the software LDNE (Waples & Do 2008). Because we did not sample all the cohorts from the population, we only used the individuals from the cohort with the greatest number of individuals (the 5 years old, n = 60). This analysis provided an estimate of the effective number of male and female breeders (N_b) that we multiplied by the generation time (estimated as the average age of sampled fish, i.e. 4.97) to calculate N_e (Fraser et al. 2007). We applied the rule proposed by Waples and Do (2010) when sample size is > 25 by not considering alleles at frequency < 0.02. We did not include the locus Str73 in these analyses because it significantly deviated from Hardy-Weinberg equilibrium (Table 1).

Results

Both, the OSR and the average spring and summer temperature increased over the observational period (Fig. 1a, b). The increase in OSR was correlated to the decline in the number of females ($r_s = -0.63$, $n = 58$, $p < 0.0001$). The OSR was best explained by the average temperatures during the early developmental stages of these spawning fish, i.e. around 5 years ago (Fig. 1c). Our OSR estimates of the 2003 cohort were 84.9% and 88.5% and corresponded to the OSR observed after 1992 (Fig. 1a). The average temperature that juveniles experienced during 1500 and 2000 ATU, i.e. during summer, was the covariate most strongly associated with the OSR five years later (Table 2).

Genotypes of 168 individuals were successfully identified. A significant deviation from Hardy-Weinberg equilibrium was detected for only one locus (Str73, Table 1), probably due to the occurrence of null alleles. Average allele number was 4.78 and average gene diversity was 0.42 (Table 1). We found no evidence for a significant recent bottleneck (using the criteria of Piry et al. (1999); both including the locus Str73 in analysis or not). N_b for the year 2007 was estimated to be 158.6, and $N_e = 788.2$.

Discussion

Male grayling stay longer at the spawning place than females and are hence more likely to be caught by our sampling methods. Therefore, OSR estimates of > 0.5 can be expected even when sex ratios are equal. However, we found a distinct shift in water temperatures followed by high OSRs that persisted over 19 years (1993 to 2011). The ability of populations to genetically adapt to such unequal OSR depends on the selection coefficient, the population size and its genetic variability, and the reproductive excess (e.g. the number of eggs per female; Hoffmann & Sgrò 2011). Unequal operational sex ratios are likely to cause strong selection, and the reproductive excess is large (thousands of eggs per female and year). The current genetic diversity of the study population is moderate but within the range of European grayling populations (Gum et al. 2003; 2009). The apparent lack of an evolutionary response to unequal OSR supports the view that many salmonid populations may not be able to adapt to changing temperature (Fraser et al. 2007).

Consuegra and de Leaniz (2007) studied another salmonid and found significant variation in the sex ratios of populations that were in close geographic proximity. They suggested that spatial and temporal variation in OSR within populations would lead to sex-specific dispersal. However, connectivity between populations of many river-dwelling fish has been reduced. This is also true for our study population that is largely separated from a neighboring one by a weir. Both the observed low N_e and low genetic exchange between populations may contribute to the observed lack of an evolutionary response to unequal OSR.

In fish, OSR can be changed by temperature or toxicants that either affect sex determination or induce sex-biased mortality. Our study population shares its main feeding area, Lake Thun, with several whitefish (*Coregonus* sp.) populations that displayed an extraordinarily high prevalence of gonadal deformations in the early 2000s (Bernet et al. 2009) (so far, graylings of our study population have not been inspected for gonadal deformations). Because the lake serves as drinking water reservoir for the region and cities downstream, several studies then focused on the occurrence and trends of potential endocrine disruptors in the water, the sediments, and in the tissue of affected and non-affected whitefish (e.g. Liedtke et al. 2009; Bogdal et al. 2010). So far, no study has found contamination levels that could explain the malformations. Moreover, Bernet et al. (2009) raised whitefish from a population with gonadal deformation in spring water and on artificial food and found similar gonadal abnormalities as observed in the wild. These findings suggest that toxicants are not responsible for the unequal OSR we observed.

The average water temperature at the outlet of Lake Thun, i.e. the temperature that embryos and juveniles are exposed to, has increased by about 1.5°C from 1970 to 2011. Wedekind and Küng (2010) observed a shift to earlier spawning in the study population from 1948 to 2009. This shift has several effects: first, it leads to a decelerated temperature increase during the first 500 ATUs. It is therefore increasingly colder for embryos and hatchlings. At these developmental stages, salmonids are typically exposed to microbial pathogens and show behavioral and immunological reactions (Jacob et al. 2010) that are often temperature-dependent (CW, unpublished results) and could be sex-specific (Perry et al. 2005). This critical period also seems to be the time at which gonads go through the labile period of sex determination that can, in some salmonids, be affected by temperature (Baroiller et al. 2009; Magerhans et al. 2009). However, our analyses suggest that OSR is not significantly affected by environmental changes during these early developmental stages, and recent experiments suggest that temperature does not affect sex determination in grayling (MP and CW, unpublished results). Instead, the increasing summer temperatures seem to affect OSR, probably by sex-specific survival or sex-specific growth that influences later survival (Hutchings 2006). Not much is known about sex-specific behavior or growth and their potential temperature-dependence during that time, and it is unclear what kind of evolutionary response could be expected in response to the shifted OSR. At least, the temperature effect does not seem to be linear, as the extraordinarily warm summer in 2003 did not further skew the sex ratio.

Increased temperatures may contribute to the observed decline of many salmonid populations (Clews et al. 2010). Our findings suggest that sex-specific tolerance to high summer temperatures directly or indirectly accelerate extinction rates. The persistence of an unequal OSR suggests that probability of extirpation can be reduced by focusing on evolutionary processes in population management, e.g. by improving the connectivity between populations.

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Table 1. Properties[a] of nine microsatellite loci[b] used to estimate the genetic diversity in the study population of grayling in Lake Thun, Switzerland.

	A	H_S	F_{IS}
SSOSL311	2	0.09	-0.05
BFRO005	2	0.31	-0.002
BFRO006	5	0.47	-0.07
BFRO0011	7	0.73	-0.02
Ogo2	8	0.43	-0.01
BFRO004	2	0.18	-0.05
BFRO0010	6	0.42	0.11
One9	4	0.37	0.07
Str73	7	0.79	0.26[c]

[a] A = number of alleles per locus; H_S = gene diversity; F_{IS} = inbreeding coefficient

[b] See Gum et al. (2003) and references therein for details on the primers.

[c] $p < 0.05$ as tested in FSTAT 2.9.3 (Goudet 1995) with 1000 permutations.

Table 2. Multiple regression analysis of the average temperatures experienced by naturally spawned embryo and fry on the operational sex ratio that was recorded 5 years later (full model: $F_{4,31} = 5.6$, $p = 0.002$).

	Parameter estimate (SE)	t	p
Intercept	-0.07 (0.32)	-0.21	0.84
0-500 ATU*	0.001 (0.03)	0.05	0.96
500-1000 ATU	-0.01 (0.02)	-0.66	0.52
1000-1500 ATU	-0.002 (0.01)	-0.13	0.90
1500-2000 ATU	0.06 (0.01)	4.26	<0.001

* Developmental stages are separated into consecutive periods of 500 accumulated thermal units (ATU).

Figure 1. Operational sex ratio (OSR) and water temperature at the spawning place of the study population. (a) The OSR since 1948 (increasing over time: $r_s = 0.62$, $p < 0.001$). Grey and black points indicate years in which at least 30% and 50%, respectively, more males than females were lost or released downstream of a weir after stripping. (b) The average water temperature at the spawning ground between March 1st and August 31st (increase over time: $r_s = 0.65$, $p < 0.0001$). The shaded area marks the first five years after an abrupt change to higher temperatures in 1987/88 (as observed all over Switzerland, see Hari et al. 2006). During the marked 5 years, temperatures stayed high but OSR were relatively low. (c) The amount of variation in the observed OSR explained by the average temperatures of the current and previous years, as determined in a forward stepwise regression analysis. All models explain significant variance in the OSR (model including only the mean temperature five years ago: $F_{1,34} = 32.7$, $p < 0.0001$; model including all factors: $F_{7,27} = 8.6$, $p < 0.0001$). Filled symbols indicate models that are significantly improved (F always > 4.8, p always < 0.05) by adding the latest covariate, open points and dashed lines indicate no statistically significant model improvement.

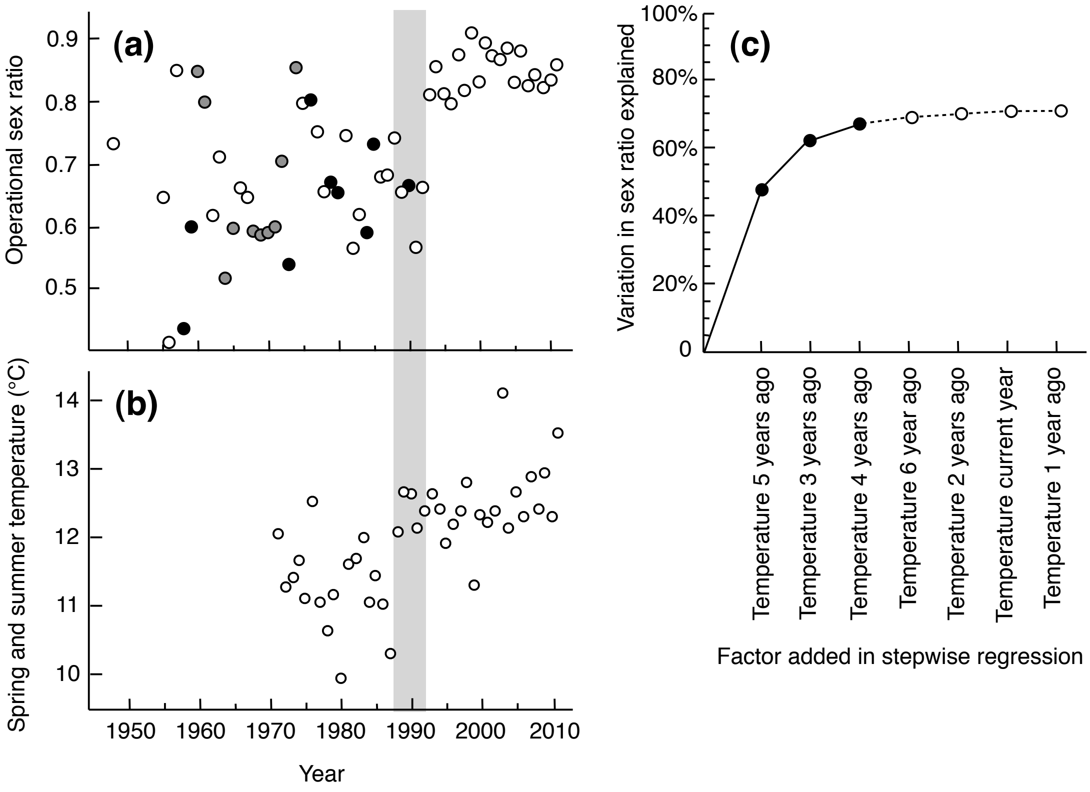

Figure 1