information

Keywords
Arctic charr, sex reversal, endocrine disrupting chemicals, xenobiotic induced sex reversal, environmentally induced sex reversal, temperature induced sex reversal.

Project title
Endocrine diSruptor SEx ChangE (ESSEnCE)

Year
2017

Project leader
Marc Anglès d'Auriac, NIVA

Geographical localization of the research project in decimal degrees (max 5 per project, ex. 70,662°N and 23,707°E)
Bear Island (Bjørnøya) Lakes Laksvatn 74.48957º N, 19.06897º E, Ellasjøen 74.38438º N, 19.01335º E, Stevatnet 74.46913 º N, 19.0369 º E and Spælvatnet 74.46945 º N, 19.06007 º E; Continental Northern Norway lake Skogsfjordvatn 69.94641 º N, 19.15949 º E.

Participants

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Flagship
Hazardous Substances

Funding Source

The project was funded with 300 KNOK from FRAM.

Summary of Results

The qPCR method previously developed for genetic sex determination of *Salmo salar* and *Salmo trutta* [1] was successfully demonstrated to be valid without modifications for *Salvelinus alpinus* (the Arctic charr).

A total of 538 Arctic charr individuals from Bear Island (Bjørnøya) and Continental Northern Norway were analyzed by qPCR for genetic sex determination. Of these, 496 gave unambiguous results and had phenotypic sex results available for interpretation. Of the remaining 42 individuals not included in the results, 30 were under 90mm length and prone to phenotype miscall, 6 did not amplify, 1 was empty, 2 did not have phenotypic data available and 3 produced ambiguous qPCR results. Ambiguous results may typically be observed with sample cross contamination. The results are presented in tables 1 to 4 for Bear Island and Table 5 for Continental Northern Norway.

The project aimed at investigating the possible correlation between pollution by endocrine-disrupting chemicals (EDC) and sex reversal for Arctic charr (*Salvelinus alpinus*) by comparing genotypic sex with phenotypic sex from two populations associated either to "pristine" or "polluted" lakes. Feminization of males in which case genetic males present a female phenotype is expected to be associated with EDC pollution. The results we have obtained show sex-reversal although with different patterns than anticipated.

On Bear Island Lake Ellasjøen was selected for being associated to EDC contamination, whereas Lake Laksvatn, Stevatnet and Spelvatnet were expected to be pristine. Feminization of males was indeed observed at relatively high levels (9,7%) for Lake Ellasjøen whereas Lakes Stevatnet and Spelvatnet showed no feminization of males (See table 1 and 3). However, lake Laksvatn which is geographically downstream from lakes Stevatnet and Spelvatnet and also theoretically non polluted compared to lake Ellasjøen shows the highest level of feminization of males at 16% (see table 2). In Continental Northern Norway lake Skogsfjordvatn shows a relatively low level of feminization of males at 5,2%

(see table 5).

The other unanticipated result is the presence of masculinisation of females, both in Lakes from Bear Island and Continental Northern Norway. In the literature this type of sex reversal, which we had not initially anticipated, has been associated to harsh conditions such as increased temperatures, hypoxia or pH changes [2].

Table 1. Bear Island Lake Ellasjøen results, percentage discrepant calculated against relevant gender genotypic number

Salvelinus alpinus		**Genotype**		**Total**
		Male	**Female**	
Phenotype	**Male**	56	**4 (10,5%)**	61
	Female	**6 (9,7%)**	34	39
Total		62	38	**100**

Table 2. Bear Island Lake Laksvatn results, percentage discrepant calculated against relevant gender genotypic number

Salvelinus alpinus		**Genotype**		**Total**
		Male	**Female**	
Phenotype	**Male**	20	**0 (0%)**	20
	Female	**4 (16,0%)**	20	24
Total		24	20	**44**

Table 3. Bear Island Stevatnet and Spelvatnet

Salvelinus alpinus		**Genotype**		**Total**
		Male	**Female**	
Phenotype	**Male**	17	**1 (2,1%)**	18
	Female	**0 (0%)**	46	46
Total		17	47	**64**

Table 4. Bjørnøya overall results with Stevatnet and Spelvatnet, percentage discrepant calculated against relevant gender genotypic number

Salvelinus alpinus		**Genotype**		**Total**
		Male	**Female**	
Phenotype	**Male**	93	**5 (4,8%)**	98

	Female	**10 (9,7%)**	100	110
Total		103	105	**208**

Table 5. Continental Northern Norway Skogsfjordvatn

S. alpinus		**Genotype**		**Total**
		Male	Female	
Phenotype	Male	148	**3 (2,3%)**	151
	Female	**8 (5,2%)**	135	**144**
Total		155	138	**288**

References

1. Anglès d'Auriac, M.B., H.A. Urke, and T. Kristensen, *A rapid qPCR method for genetic sex identification of Salmo salar and Salmo trutta including simultaneous elucidation of interspecies hybrid paternity by high-resolution melt analysis.* Journal of Fish Biology, 2014. **84**(6): p. 1971–1977.

2. Baroiller, J.F. and H. D'Cotta, *The Reversible Sex of Gonochoristic Fish: Insights and Consequences.* Sexual Development, 2016. **10**(5-6): p. 242-266.

Master and PhD-students involved in the project

None

For the Management

The laboratory analysis for the project was completed in November 2017 and we therefore unfortunately were not able to apply for a FRAM follow up study in 2018 (deadline 01.11.17). We believe that the surprising results we have obtained would warrant new studies implicating the same species as well as others to further explore the concept of using sex reversal detection for environmental disturbances understanding and monitoring.

Published Results/Planned Publications

We aim at publishing the results in a peer reviewed journal (i.e. Journal of Biology of Reproduction), and an abstract was submitted to SETAC 2018 in Rome, Italy.

Budget in accordance to results

Yes, more individuals than targeted were analyzed.

Could results from the project be subject for any commercial utilization

No

If Yes

Conclusions

Sex reversal has been observed both in the direction of feminization of males, associated to EDC pollution, and masculinization of females which has been associated with temperature increases as well as hypoxia and pH changes and possibly other types of stresses. With the notable exception of lake Laksvatn, Lake Ellasjøen did show high levels of feminization of males compared to Lake Stevatnet, Spelvatnet and also lake Skogsfjordvatn in Continental Northern Norway. Phenotype miscall may be suspected to bias the results although reported sex reversed individuals were mainly large individuals (100 to 523mm). Interestingly the other type of sex reversal, masculinization of females, was also found in lakes from both Bear Island and Continental Northern Norway, possibly indicating the presence of other types of stresses for these fish populations.

The patterns we have found show the presence of sex reversal in a wider proportion of the population than anticipated. Although these patterns are not yet explained, we believe this study shows the potential of this type of analysis for unveiling and studying the impact of environmental changes to fish populations.