Study of Arctic charr brain gene expression linked with behaviour

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Introduction

The Arctic charr occupies different habitats and several morphs can be found in sympatry. Its cognitive abilities and behaviour may be shaped according to evolutionary history and current ecological factors (Sørensen *et al.*, 2013) implying differential expression patterns of genes linked with spatial cognition variation between populations. **The overall goal of this project is to study expression differences of cognition-linked genes** (Tab. 1) **in several brain structures of Arctic charr morphs.** For this purpose, we have designed and tested primer pairs for these genes.

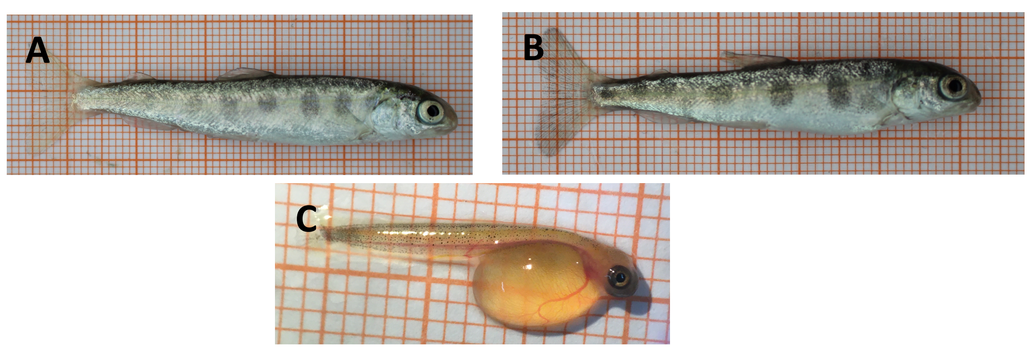

Figure 1 – Three Arctic charr morphs. **A**: Anadromous morph from Fljótaá river, **B**: Large benthic morph from Þingvallavatn lake and **C**: Brown morph newborn (before first feeding) from Vatnshlíðarvatn lake.

Methods

- Identification of Arctic charr orthologs by BLAST search using 15 salmonids genes as probes.
- Search for primers using Primer-BLAST NCBI:
  - ✓ One primer spanning an exon-exon junction or the pair on two different exons separated by an intron of at least 1 kb.
  - ✓ Tm = 60°C, amplicon size in the 100-200 bp range.
  - ✓ Absence of amplification of unexpected products.
- Primer tests by qPCR using hatched VB larvae dilutions.

When primers for all genes are validated, the gene expression can be monitored (Fig. 2).

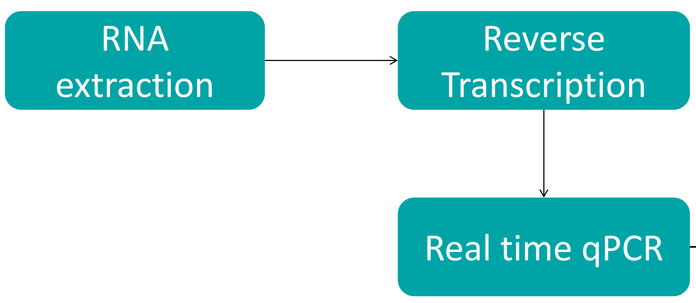

Figure 2 – Experimental workflow of the study of the gene expression.

Table 1 – List of targeted genes grouped by function.

Neurogenesis	Synaptic plasticity	Epigenetic	Stress regulation
pcna	*reelin*	*pp1*	*gr*
bdnf	*arc1*	*calcineurin*	*mr*
neurod1		*crebbp*	*mao*
erg1		*mecp2*	*dbh*
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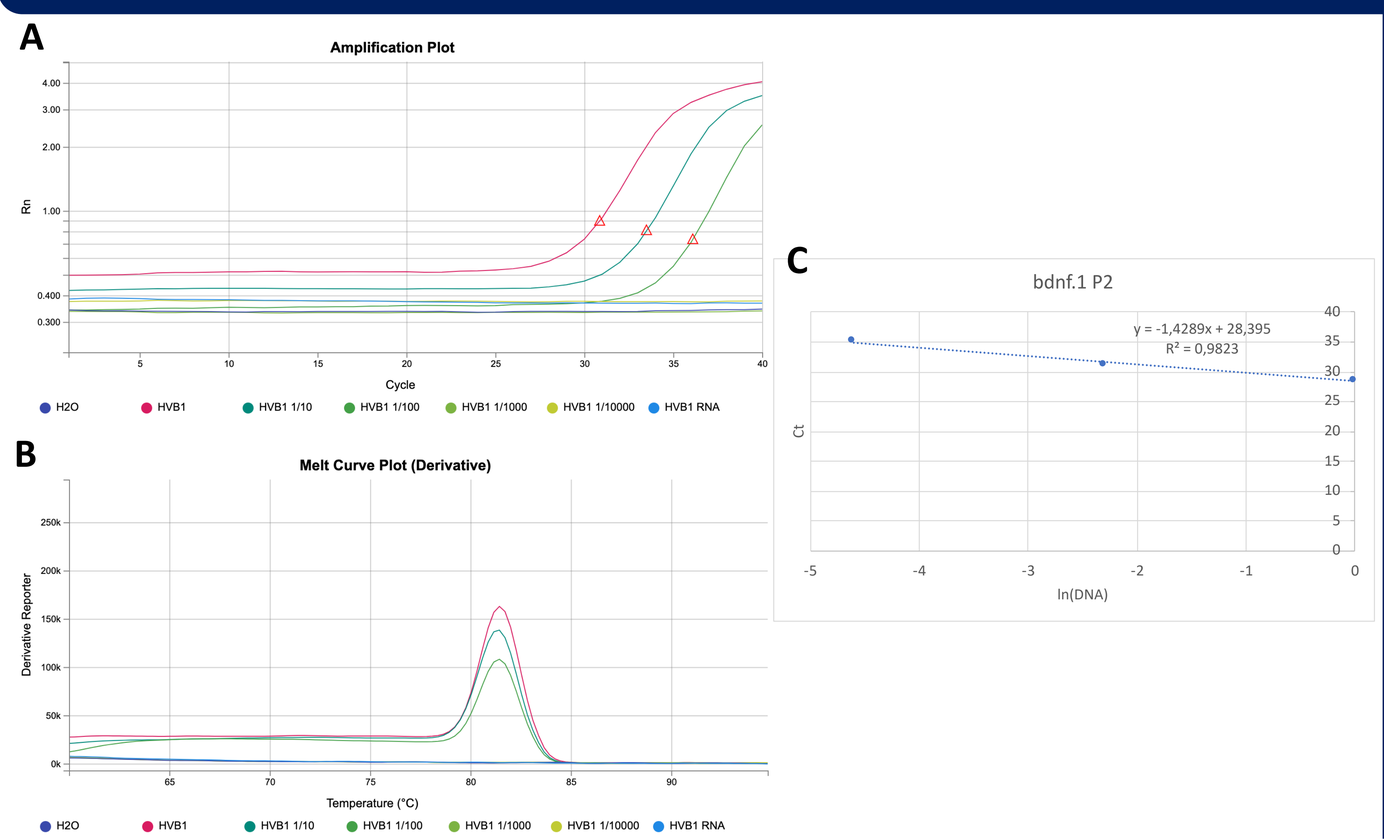
Preliminary Results and Perspectives



34 designed pairs were designed and tested (Fig. 3) and the PCR efficiency was calculated.

Preliminary results show different issues in the experiment:

- weak expression of some genes in tested tissues
- an efficiency of primers higher than 2
- genomic DNA amplification.

For now, at least 3 pairs were validated, 6 were considered as bad and the others will be tested in new conditions (with other tissues, with an other primer concentration (500 nM to 200 nM)). Primers for 3 genes need to be designed again.

Figure 3 – Example of graphs obtained for one pair validated for *bdnf* gene. **A:** Amplification plot. Red triangles indicate the Ct. **B:** Melt curve. **C:** Linear curve of the threshold cycle number (Ct) according to the logarithm of the dilution of DNA. The efficiency was equal to 2.01 ($E = 10^{-1/slope}$).

References:

Sørensen, Christina, Ida B. Johansen, et Øyvind Øverli. « Neural Plasticity and Stress Coping in Teleost Fishes ». *General and Comparative Endocrinology* 181 (janvier 2013): 25-34. https://doi.org/10.1016/j.ygcen.2012.12.003.

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