Physiology and morphology of clonal Atlantic salmon
– Influence of Incubation temperature, ploidy, and zygosity

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1. Background:

High individual variation often obscure data in biological experiments. Clonal fish are genetically identical and can be used to reduce variability. Differences will then solely be caused by environmental factors.

Atlantic salmon is important in aquaculture. Clonal lines were recently established at the Institute of Marine Research, Norway. These could become important tools for numerous fundamental and applied research purposes.

2. Purpose:

We aimed to measure metabolic rate traits and morphology of hearts and otoliths in clonal Atlantic salmon using diploid, triploid, and homozygous groups incubated at 4 or 8 °C.

Our hypotheses included:
- Incubation at 4 °C would provide wild-like phenotypes with healthier otoliths and hearts that correlated with improved metabolic performances.
- Homozygous fish would be more deformed and perform worse physiologically owing to being functionally inbred.
-Less individual variation would be observed in clonal groups when compared to outbred Atlantic salmon.

3. Methods:

Clonal Atlantic salmon groups were made using established lines (Hansen et al., 2020) (**Table 1**). Metabolic rates and hypoxia tolerance were measured with respirometry (**Photo**). Heart morphology traits were quantified as described by Engdal et al. (2024). The proportion of the two calcium carbonate polymorphs, vaterite and aragonite, were quantified in otoliths. Adipose fins were clipped for DNA microsatellite analyses using 19 different markers.

Clonal groups	Explanation	Weight (g)	Length (cm)	Condition
Diploid	Two pairs of chromosomes from different parents (heterozygous).	171 ± 5	24 ± 0.2^{ab}	1.20 ± 0.01^{a}
Triploid	Three pairs of chromosomes from two parents.	169 ± 7	23.9 ± 0.4^{a}	1.23 ± 0.37^{a}
Homozygous	Two pairs of chromosomes from one parent (functionally inbred).	170 ± 4	24.1 ± 0.2^{a}	1.22 ± 0.01^{a}
Slow diploid (4 °C)	Same as the other diploid group except for being incubated at 4 °C.	172 ± 6	25.2 ± 0.3^{b}	1.07 ± 0.01^{b}

Table 1. *Conal fish groups and their size parameters at the time of experimental trials. N = 16.*

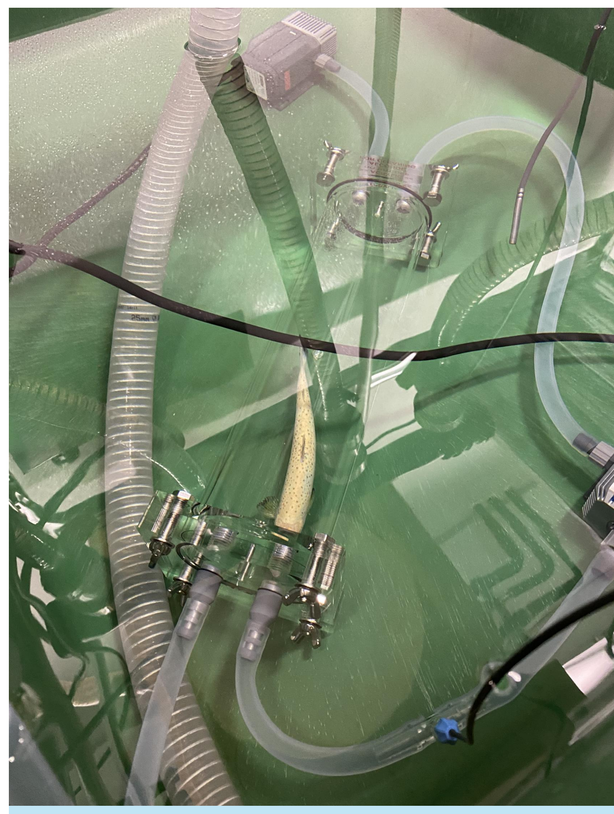
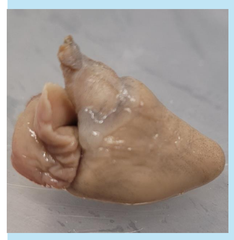


Photo: *Respirometry setup to measure oxygen uptake as proxy for aerobic metabolic rates in clonal Atlantic salmon.*

Fig. 2. *Heart morphology parameters in clonal Atlantic salmon groups.*



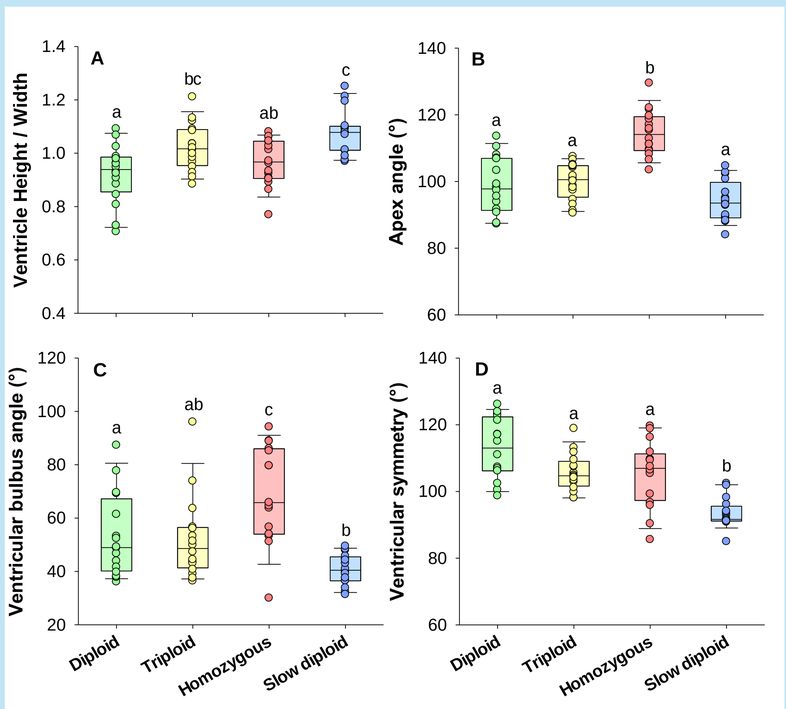
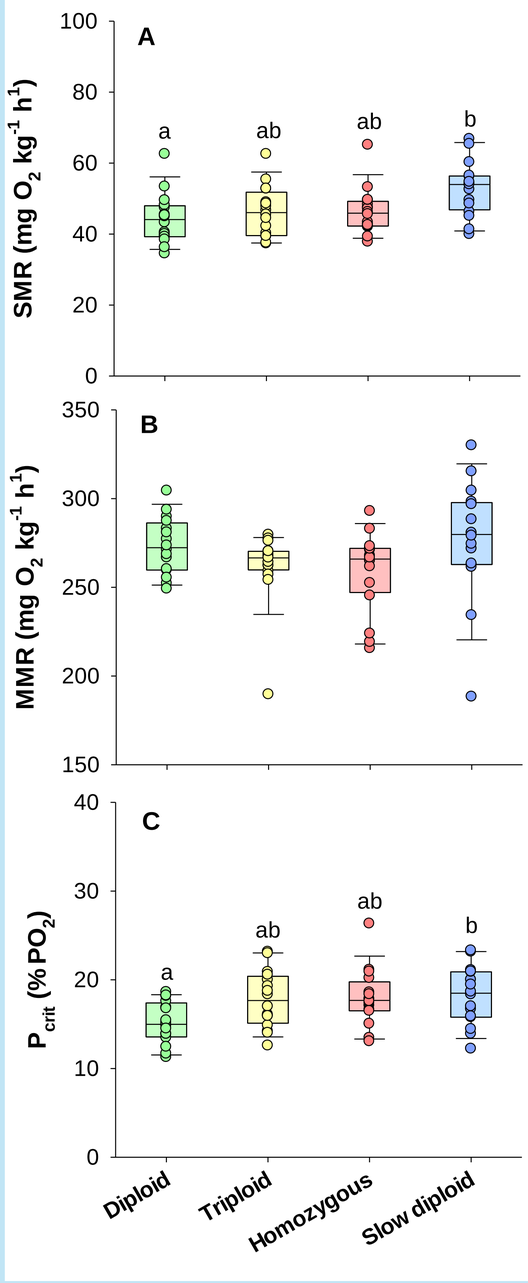


Fig. 1. *Standard metabolic rate (SMR) (**A**), maximum metabolic rate (MMR) (**B**), and the critical oxygen tension (P_{crit}) (**C**) in clonal Atlantic salmon groups.*



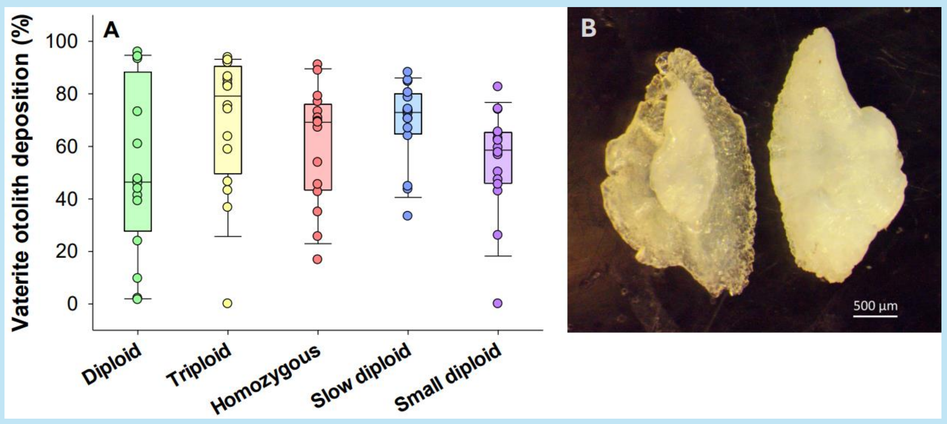


Fig. 3. ***A**: Vaterite otolith deposition. "Small diploid" refers to the smaller part of the bimodal size distribution in the Slow diploid group. **B**: Deformed otolith with vaterite (outer) and aragonite (central) (left), and a healthy otolith only with aragonite (right).*

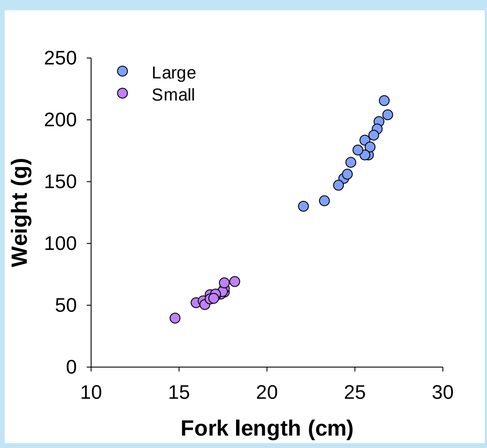


Fig. 4. *Bimodal size distribution in the slow growing diploid group incubated at 4 °C. Approximately one-third of the group were smaller sized.*

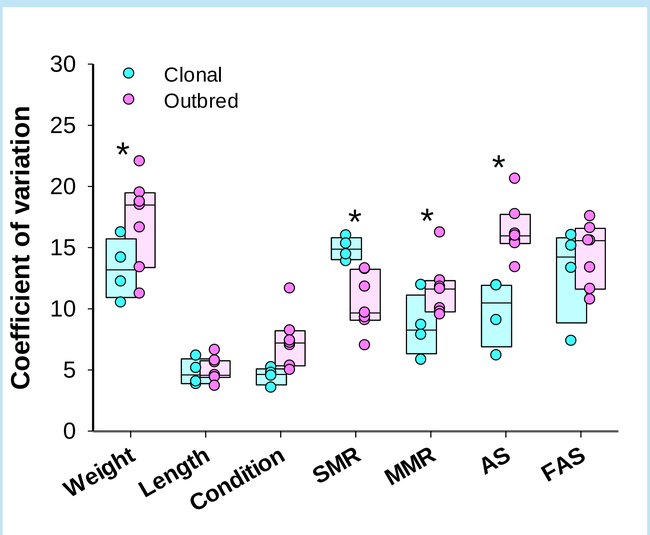


Fig. 5. *Coefficient of variation for size and metabolic rate parameters in clonal groups compared to outbred groups. SMR and MMR, Standard and maximum metabolic rate; AS, Aerobic scope; FAS, Factorial aerobic scope.*

4. Results and discussion:

Microsatellite markers confirmed clonal status, correct ploidy, and zygosity. Fish incubated at 4 °C were growing much slower and experiments were performed 9 months later than in the other groups.

Metabolic rates were mostly similar between groups (**Fig. 1**). Only notable differences were higher standard metabolic rates and P_{crits} in diploids incubated at 4 °C compared to at 8 °C. This likely reflected recent growth history over the summer as opposed to the other groups tested in late autumn.

Heart morphology varied distinctly between groups (**Fig. 2**). As hypothesized, slower development at 4 °C resulted in morphology traits resembling wild phenotypes, exemplified by more triangular shaped ventricles (higher ventricle height to width ratios), lower ventricular symmetry, and a lower ventricular bulbus angle. This suggests a strong environmental influence for these traits. Homozygous fish also had distinct heart phenotypes such as higher apex and ventricular bulbus angles. However, heart morphology traits were not correlated with metabolic rate traits (not shown).

All groups had high vaterite proportions, implying deformed otoliths (**Fig. 3**). This may be a fish welfare issue, as abnormal otoliths cause hearing loss and impaired swimming balance. Slower growth trajectories were previously found to reduce vaterite proportions (Reimer et al., 2017), but this was not the case here.

A bimodal size distribution emerged in the group incubated at 4 °C (**Fig. 4**). Bimodal size distributions are well-known to occur in the wild. Depending on size in autumn, larger fish migrate to sea and smaller fish overwinter in the river. Thus, minor size variation at a critical time can have major impact on life-histories.

Coefficients of variation were overall lower in clonal groups compared to outbred groups, as hypothesized (**Fig. 5**). However, this was not consistent for all traits. Substantial data variation was also still observed in clonal Atlantic salmon which cannot be ascribed to genetics but instead originate from random factors.

5. Conclusion:

We measured physiological and morphological traits of interest in clonal Animal salmon groups. Using clonal fish did not completely solve prevailing challenges of high individual variation in experiments. We still conclude that clonal fish models can be useful, not least as any variation unrelated to genetics can provide novel insights of environmental effects on important phenotypic outcomes.

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Key references:

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- Hansen et al. (2020). Production and verification of the first Atlantic salmon (*Salmo salar* L.) clonal lines. *BMC Genetics* **21**, 71.

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