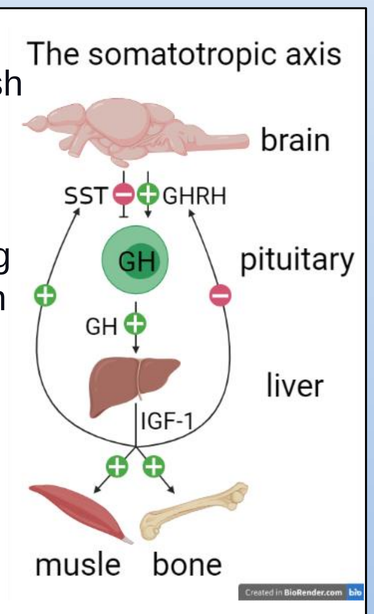
Enhanced growth in transgenic zebrafish line

Noam Cohen, Naama Mizrahi and Berta Levavi-Sivan

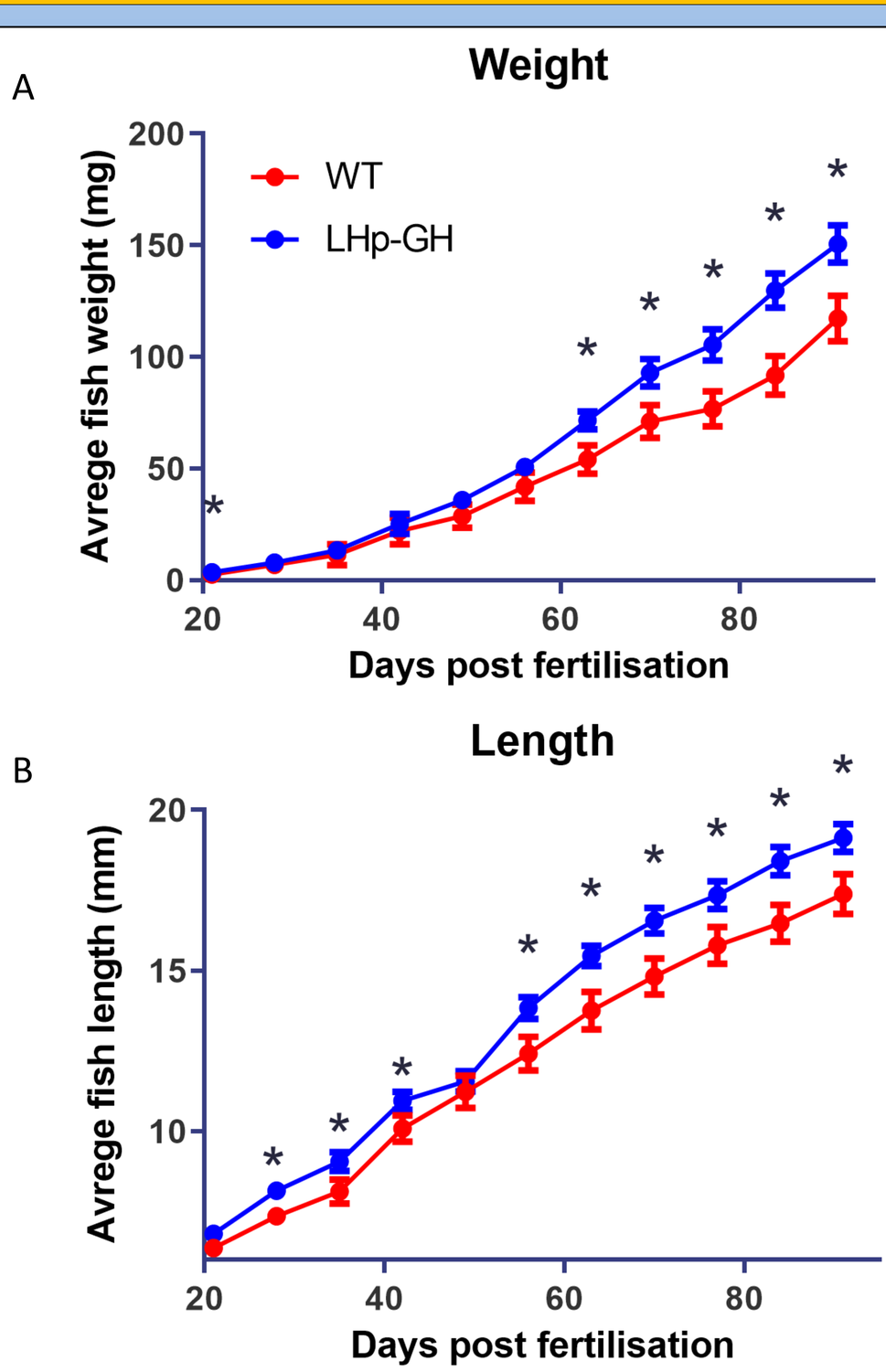
The Robert H. Smith Faculty of Agriculture, Food and Environment, Department of Animal Sciences, The Hebrew University of Jerusalem, Rehovot, Israel.

Background

In vertebrates, growth is mainly regulated by hormones along the somatotropic axis. Studies in fish have shown that overexpression of growth hormone (GH(increases body weight and size. We have previously created a transgenic zebrafish line that expresses GH under the regulation of the Luteinizing hormone(LH) promoter (LHp-GH), both expressed in the pituitary. The objective of this study was to characterize the somatic growth and reproductive status of the transgenic fish line using physiological parameters, and gene expression in the brain and pituitary. We hypothesize that the coupling of GH expression to LH will cause an accelerated growth and a decline in reproductive processes.

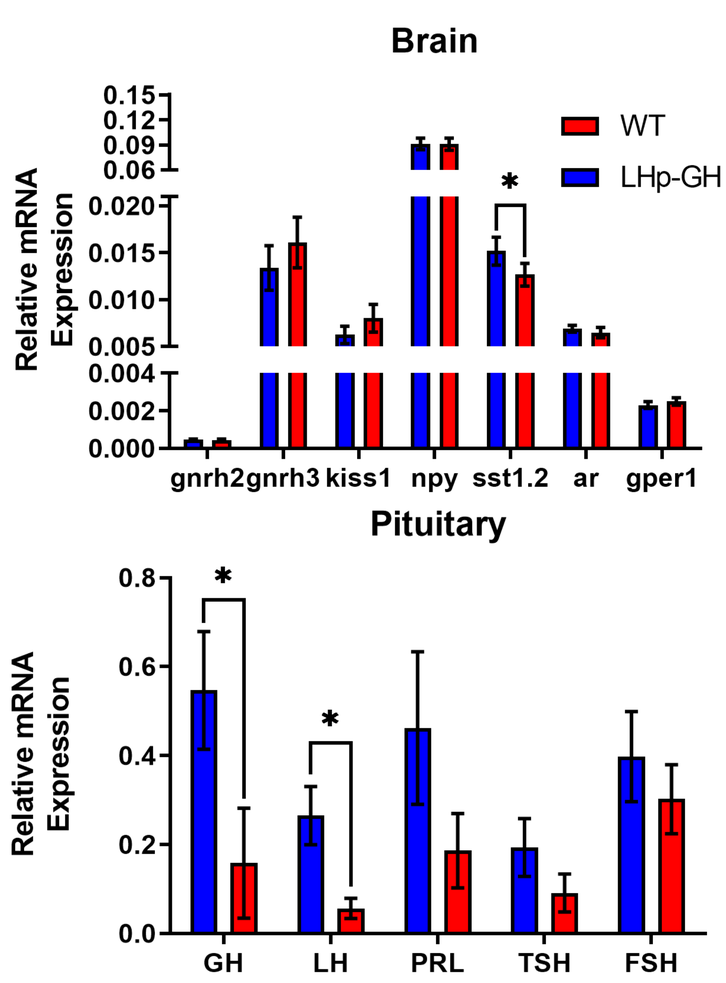


Results



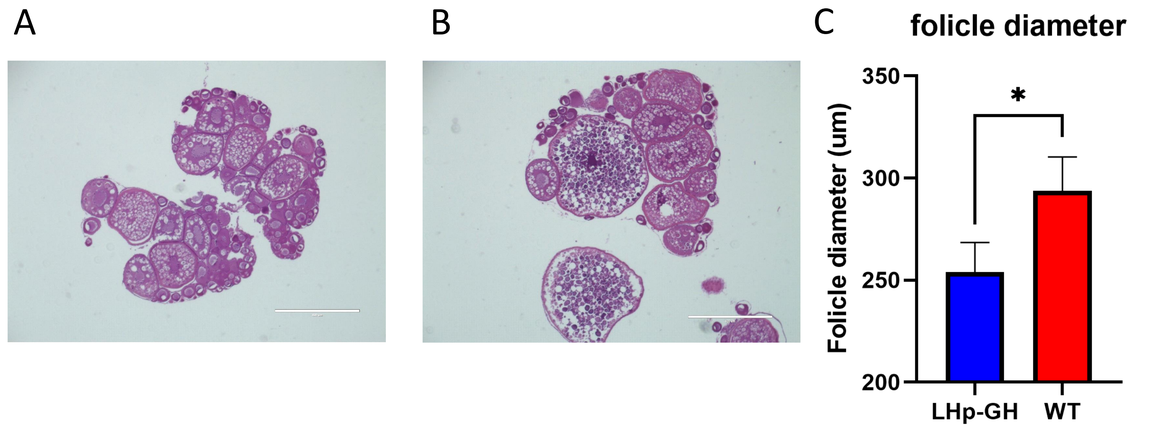
Accelerated weight and length gain in LHp-GH Fish in comparison to wild type (WT).
Both groups were reared for 3 months in the same conditions and fed in excess (>5% bw). **A**) Weight was measured once a week using analytical scales (±0.1mg). **B**) length was measured using image analysis in ImageJ Fiji. Statistical analyses for both measurements were conducted using JMP pro-15, *t-test* (n is different for each week due to mortality) with a significance level of 5%. Significant differences are marked with an asterisk.

Results



mRNA expression of different neuropeptides in LHp-GH and WT fish brain.
Expression levels of the various genes were measured using real-time RT-PCR analysis. Expression levels were normalized to the housekeeping gene ef1a. The differences between the expression levels of the various genes in LHp-GH and WT were analyzed using *a t-test* (LHp-GH n=15, WT n=9) with significance level of 5%. Significant differences are marked with an asterisk.

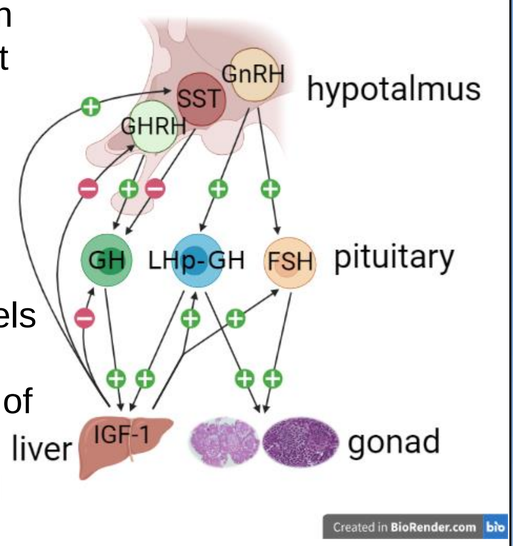
mRNA expression of different hypophyseal hormones in LHp-GH and WT fish pituitary.
Expression levels of the various hormones were measured using real-time RT-PCR analysis. The level of the expression was normalized to the housekeeping gene ef1a. The differences between the expression levels of the various genes in LHp-GH and WT were analyzed using *a t-test* (LHp-GH n=7, WT n=4) with significance level of 5%. Significant differences are marked with an asterisk.



Significant Decline of follicles size in female LHp-GH fish.
Follicles diameter was measured using image analysis of H&E stained histology slides. **A**) A picture of a female LHp-GH gonad slide for visualization of follicle size (scale bar length 400 µm). **B**) A picture of a female WT gonad slide for visualization of follicle size. **C**) The 10 largest follicles in each slide were measured and then analyzed using *a t-test* (LHp-GH n=60, WT n=40) with significance level of 5%. Significant differences are marked with an asterisk.

Conclusions

- Accelerated growth alongside a decline in follicle development in LHp-GH fish might suggest a diversion of resources from reproductive processes to somatic processes.
- We suggest a potential model which can integrate the physiological and hormonal changes we saw in LHp-GH fish. GH levels are elevated due to additional secretion from LH cells which causes upregulation of IGF-1 which in return downregulates GH expression in GH cells but upregulate LH and LHp-GH expression in LH cell.



Future research

- Determining the GH, LH and FSH pituitary content using specific ELISA.
- Determining IGF-1 expression in the LHp-GH fish liver.
- In vivo experiment using CCK and sGnRH analog injection in order to evaluate how manipulation on the Hypothalamus-pituitary-gonad (HPG) axis will interact with the LHp-GH construct In the transgenic fish.
- Create LHp-GH transgenic Nile tilapia and characterize its growth and reproductive to produce an improved fish for aquaculture.