

## Introduction

The inorganic phase of bone is composed of phosphorus and calcium (Bonucci 2012). In post-metamorphic zebrafish, bone mineralization, crucial for skeletal development, relies on dietary intake of phosphorus (Cotti et al., 2020). However, the impact of dietary phosphorus during the critical larval period of rapid skeletal development remains unexplored. Early nutritional deficiencies can lead to skeletal deformities (Koumoundouros 2010) in both farmed and lab-raised fish, which are a major welfare and economic concern in aquaculture.

**Aim: Study the effects of dietary phosphorus under different calcium levels on:**

1. Skeletal development (mineralization rate, abnormalities) [larvae]
2. Gene expression of osteoblastic markers [larvae]
3. Response of the vertebral column against swimming-induced lordosis [juveniles]

## Materials and methods

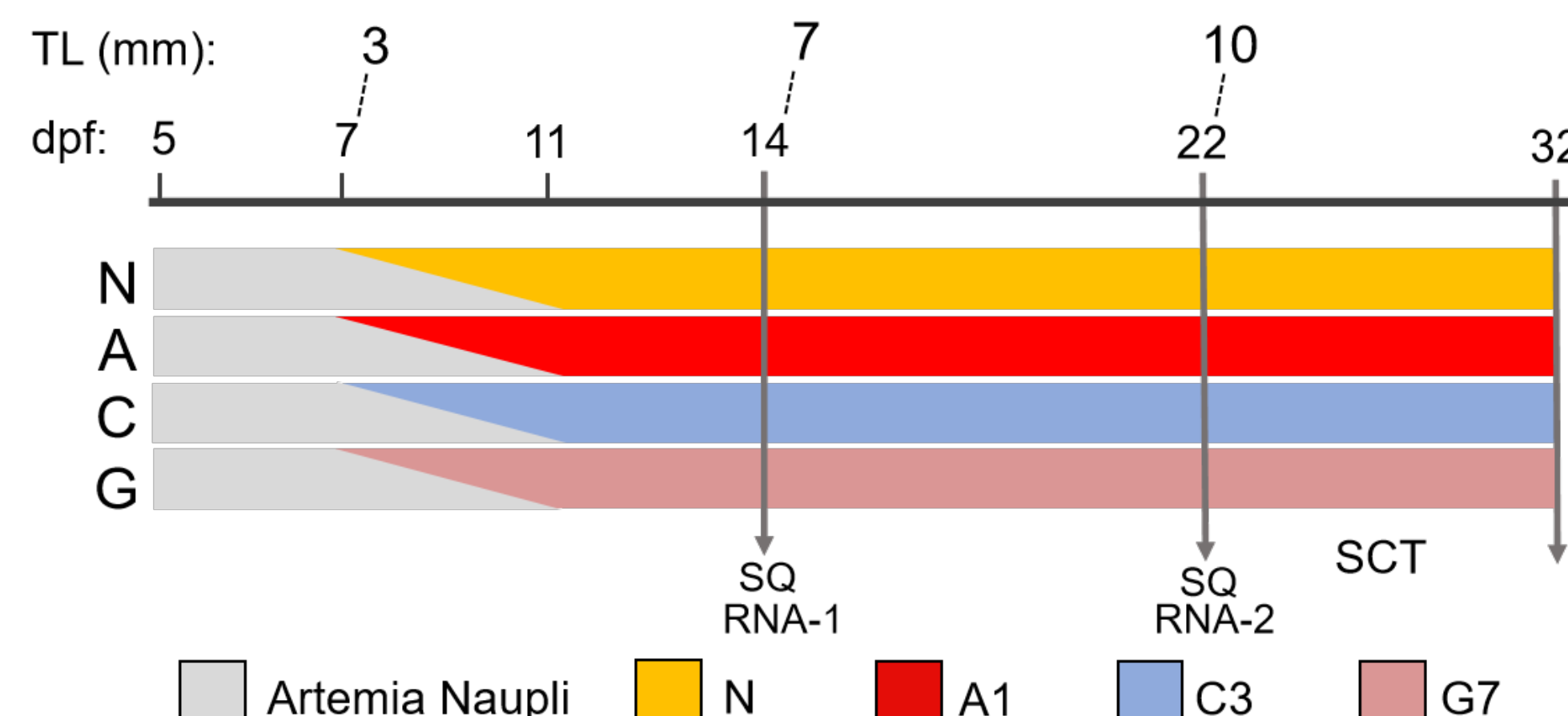


Figure 1. Experimental design. Trials were performed in triplicate.

## Samplings - Analyses

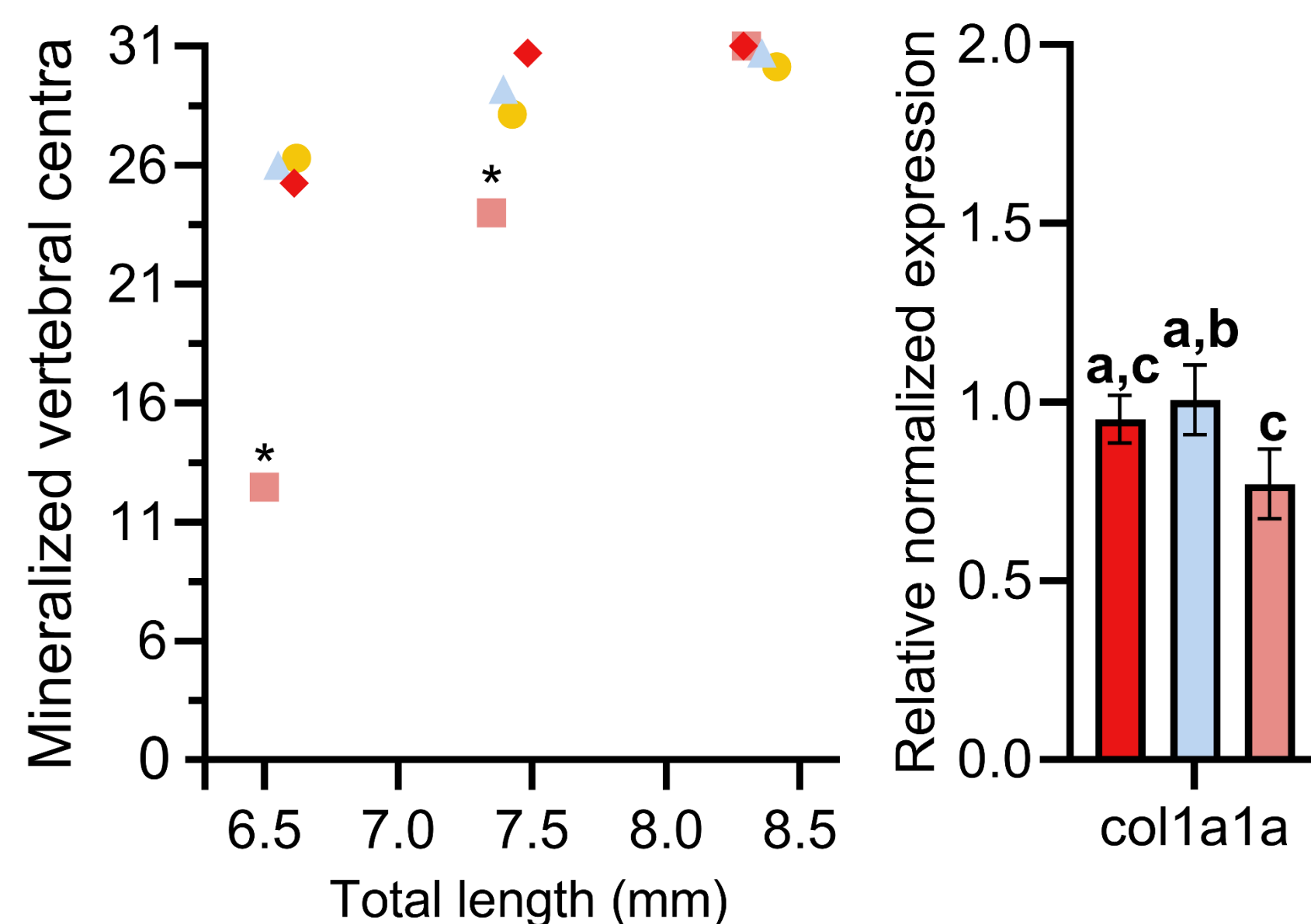
- Vertebral column mineralization rate (at ~7 mm TL).
- Gene expression analysis (at 7.0-7.5 mm TL).
- Skeletal abnormality scoring (at ~10 mm TL).
- Swimming challenge test (SCT) for haemal lordosis (at 10-11 mm TL).

Diet	P%	Ca%	Ca/P
A	3.20	1.67	0.52
C	2.20	1.90	0.86
G	2.22	1.62	0.73

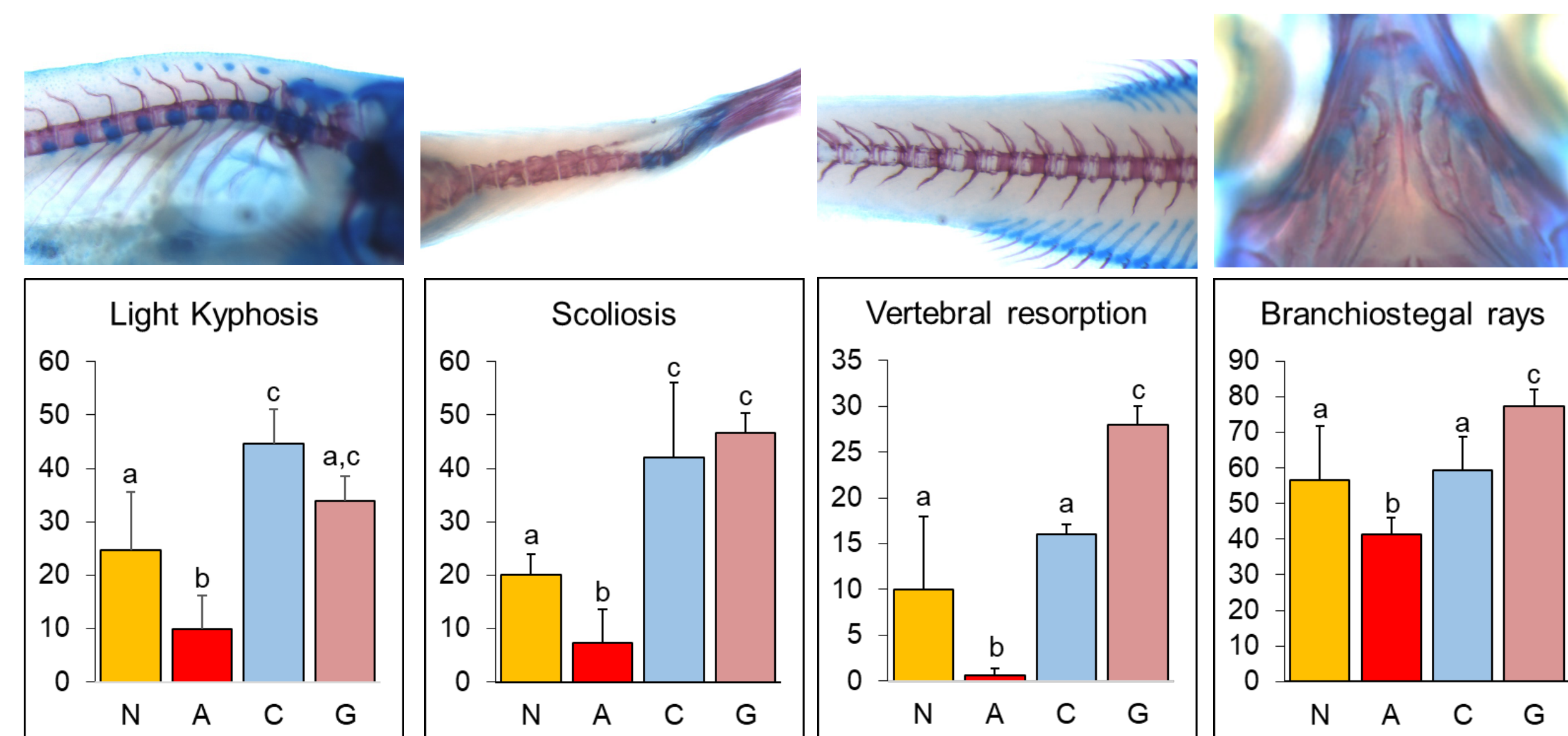
Figure 2. Phosphorus and calcium levels of the experimental diets formulated, modifying commercial fish meal and adding monosodium phosphate.

## Results

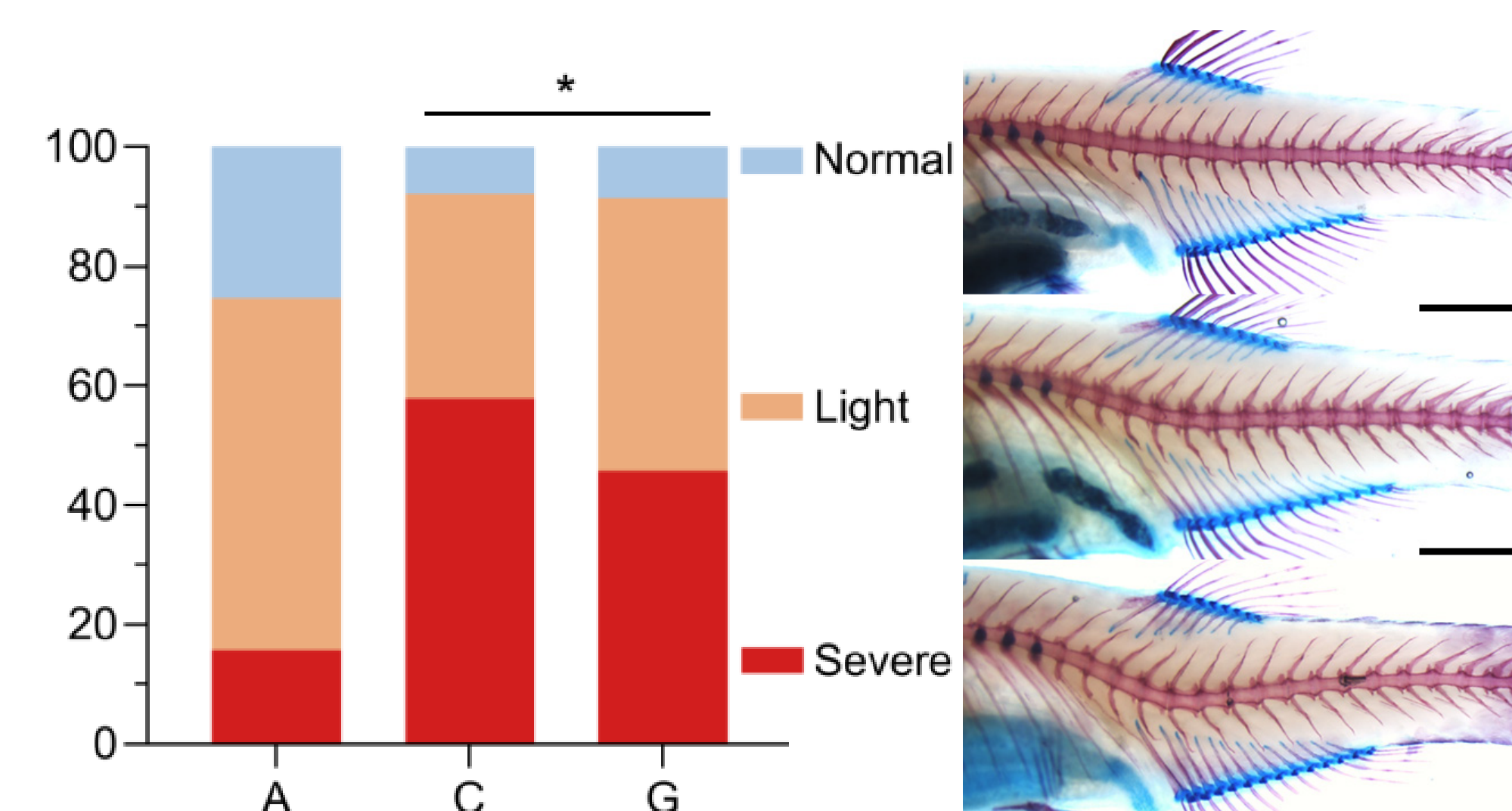
Low P and Ca delayed vertebral bone mineralization and matrix formation



High P reduced abnormality rates; High Ca/P mitigated some low-P effects



High P reduced swimming-induced lordosis rates



## Discussion

- Consistent with existing literature, a low-P diet leads to poorly mineralized or non-mineralized osteoid in endoskeletal elements (Witten et al., 2019; Cotti et al., 2024). However, our novel finding is that low-P also delays bone mineralization and matrix formation during early larval development.
- The initially reduced mineralization rate was associated with an elevated frequency of skeletal abnormalities in the following developmental period (present study).
- Another novel finding is that, the high-P diet was associated with a stronger vertebral column and a reduced incidence of swimming-induced lordosis post-metamorphosis.

## References

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3. Cotti, S., Di Biagio, C., Huyseune, A., Koppe, W., Forlino, A. and Witten, P.E., 2024. Matrix first, minerals later: fine-tuned dietary phosphate increases bone formation in zebrafish. *JBMR plus*, 8(8), p.ziae081.
4. Koumoundouros, G., 2010. Morpho-anatomical abnormalities in Mediterranean marine aquaculture. *Recent advances in aquaculture research*, 661(2), pp.125-148.
5. Witten, P. E., Fjellidal, P. G., Huyseune, A., McGurk, C., Obach, A., and Owen, M. A. G. (2019). Bone without minerals and its secondary mineralization in Atlantic salmon. *J.*