SKELETOGENESIS OF TENCH (*TINCA TINCA*) REARED IN EXTENSIVE AQUACULTURE: TOWARDS A HIGH QUALITY PRODUCTION STANDARDS

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Introduction

Tench is a freshwater fish cultured for centuries in Central and Southern Europe. Its **production is limited** in quantity (around 1400 t) and value (4.5 M USD). In Spain and Italy tench (80-120 g) had a high value (16-18 € kg^{-1}; Parisi et al., 2014), and is considered a **promising new species** for aquaculture diversification since it can **tolerate low levels of oxygen**, a **wide range of temperatures** (10-34 ºC), **vegetable dietary sources** and **manipulation**. Although no specific studies have been done, the high rate of **skeletal deformities** stands as one of the main **bottlenecks** (Parisi et al., 2014).

Since skeletal deformities **hinders production efficiency, decrease animal welfare and product value** (Boglione et al., 2013), **determining** the incidence of deformities at early juvenile stage when reared in ponds (the most common rearing system) will reveal the real **impact of deformities** on its production. **Describing the sequence of skeletogenetic events** (when and how skeletal structures are formed) will allow to identify proper rearing conditions and feeding protocols to be applied.

The aim of the present study was to **implement an acid-free double staining protocol** for an accurate and detailed description of main skeletal deformities and the skeletogenetic process during tench larval development in traditional extensive systems.

Deformity results

The **54.14 %** of sampled fish had at least one deformity, being **4 the mean deformity load** per specimen.

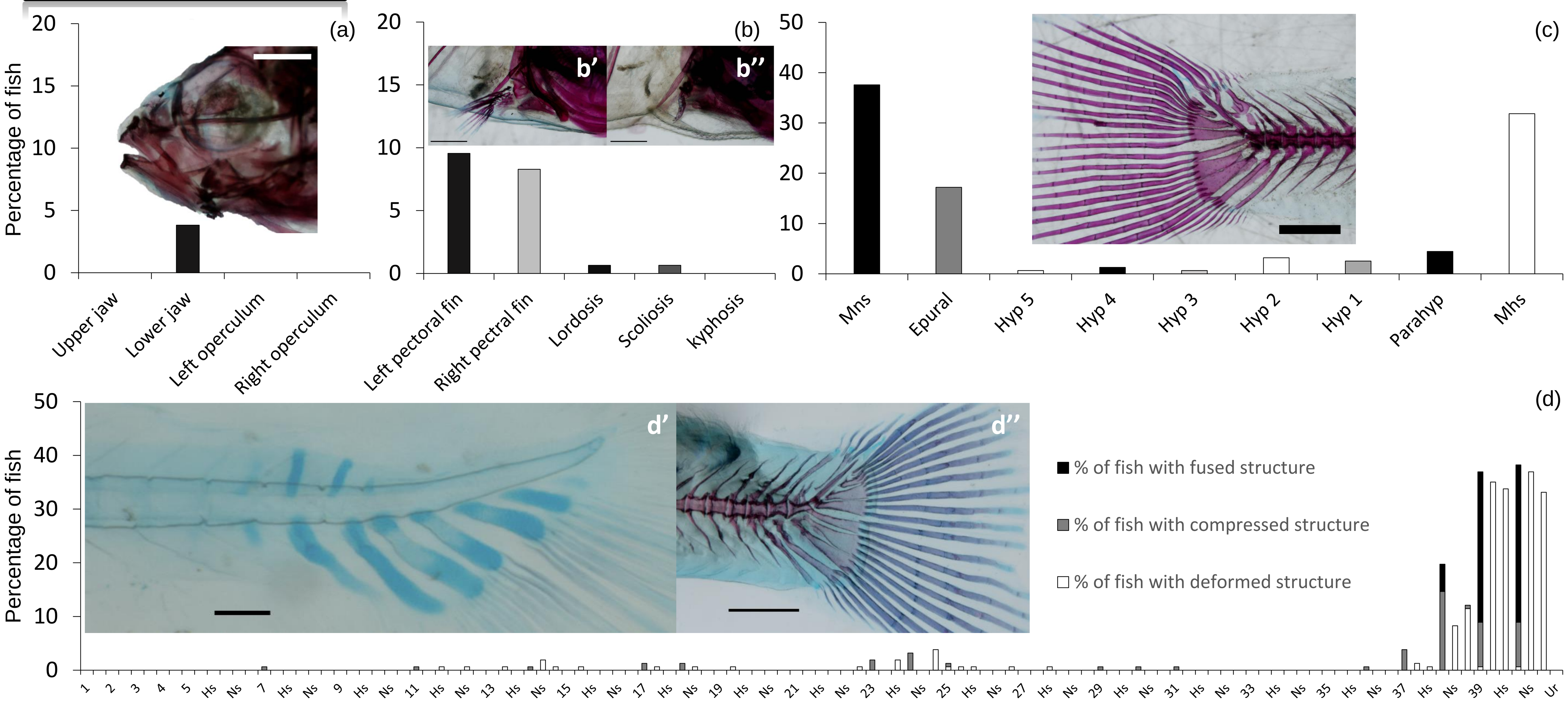


Figure 2. Incidence of deformities at the head (a), the trunk (b), and the caudal fin (c) regions, and the vertebral axis (d). Photos showing examples of short lower jaw (a), presence (b') and absence (b'') of pectoral fin (b), deformed epural and modified neural spine (c), and formation of caudal fin complex (d') and fused pre-ural vertebrae (d''). Scale bar = 1mm.

Sampling and staining protocol

Larvae/juveniles (>15 per sampling time) were sampled at 2, 5, 7, 30, 42, 65 and 85 days post-fertilization (dpf). Description of skeletogenesis and incidence of deformities were done with an adapted protocol from Fernández et al. (2018).

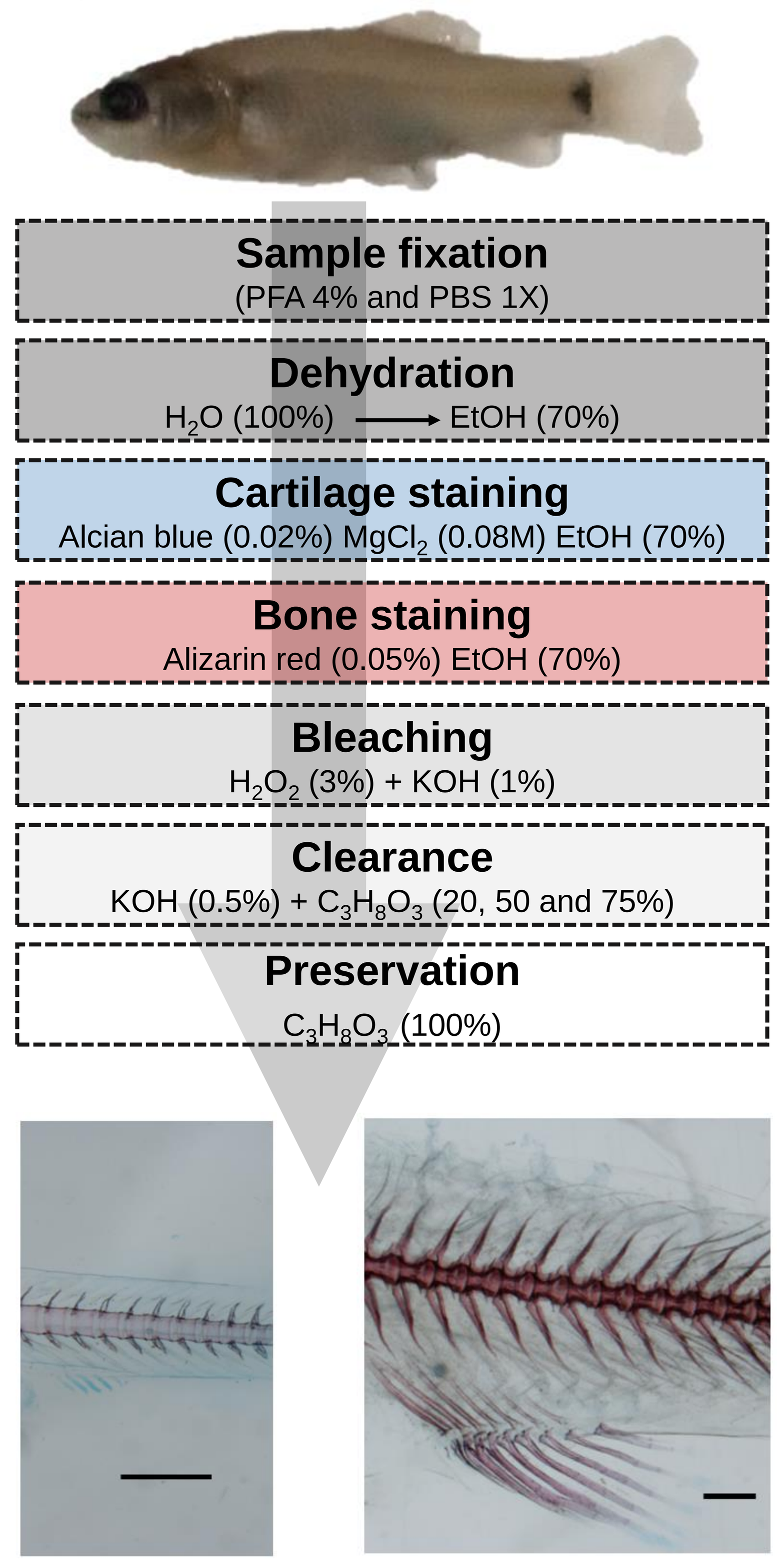
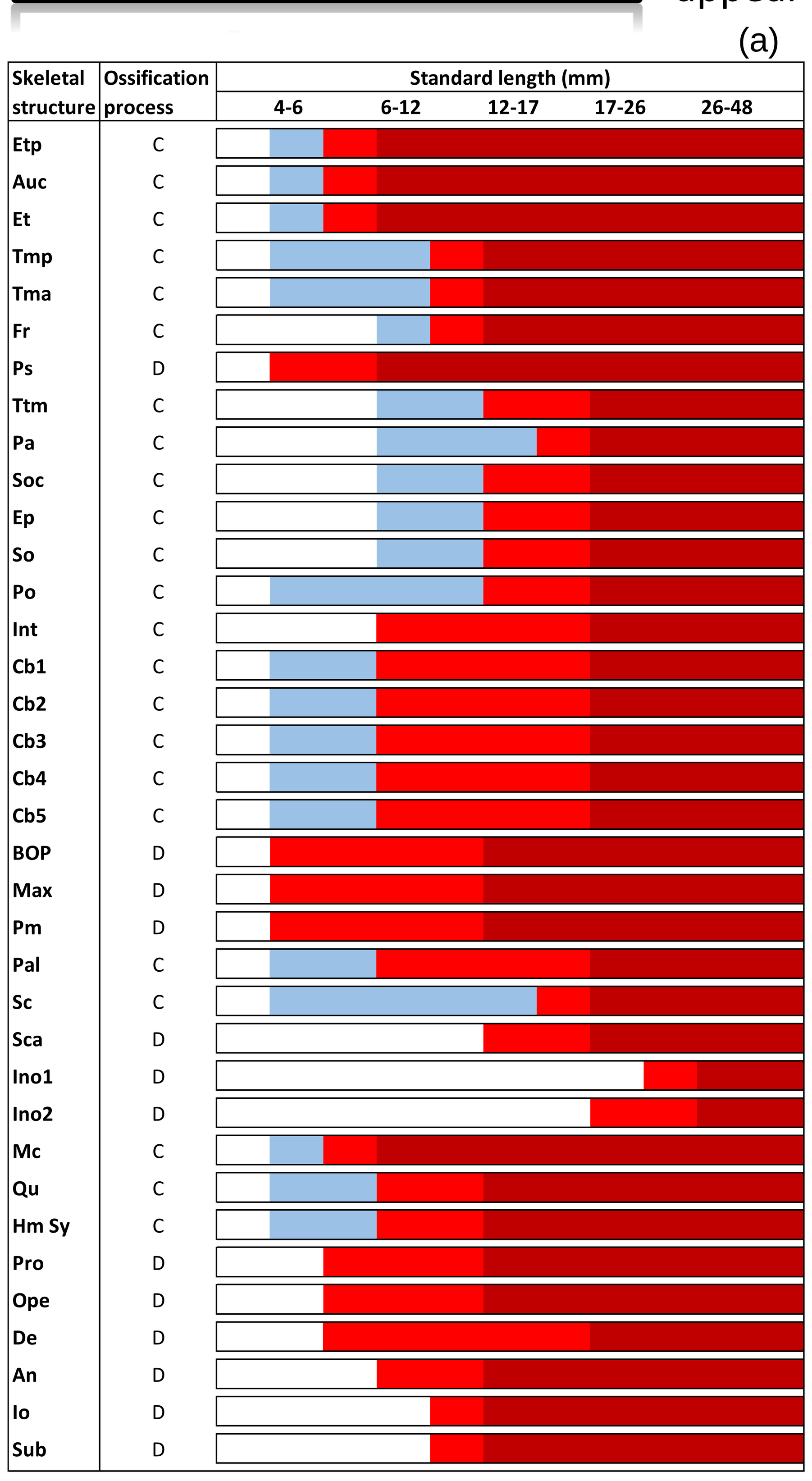
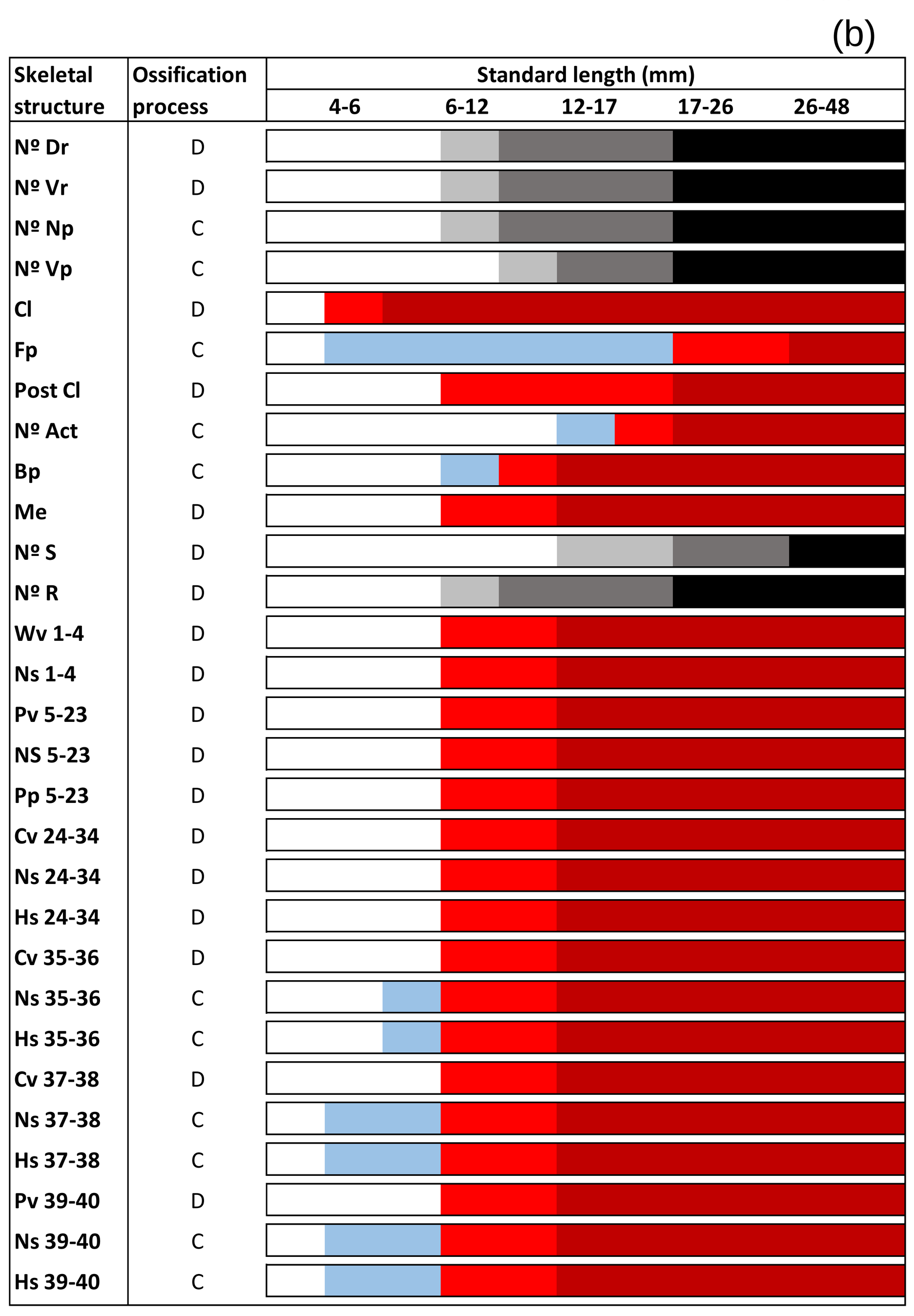


Figure 1. List of the specific steps and solutions for acid-free double staining protocol. Note the images of tench larvae before the protocol was applied (top) and the particular result for vertebral bodies along development (bottom). Scale bar = 0.5 mm.

Skeletogenesis results

The **skeletal formation** of tench **progressed very rapidly**, most skeletal structures being **almost complete at 26 mm** (85 dpf). **First structures** to appear were those related with **cranial structures** (upper and lower jaws, operculum and cleithrum) being followed by **caudal fin structures**.





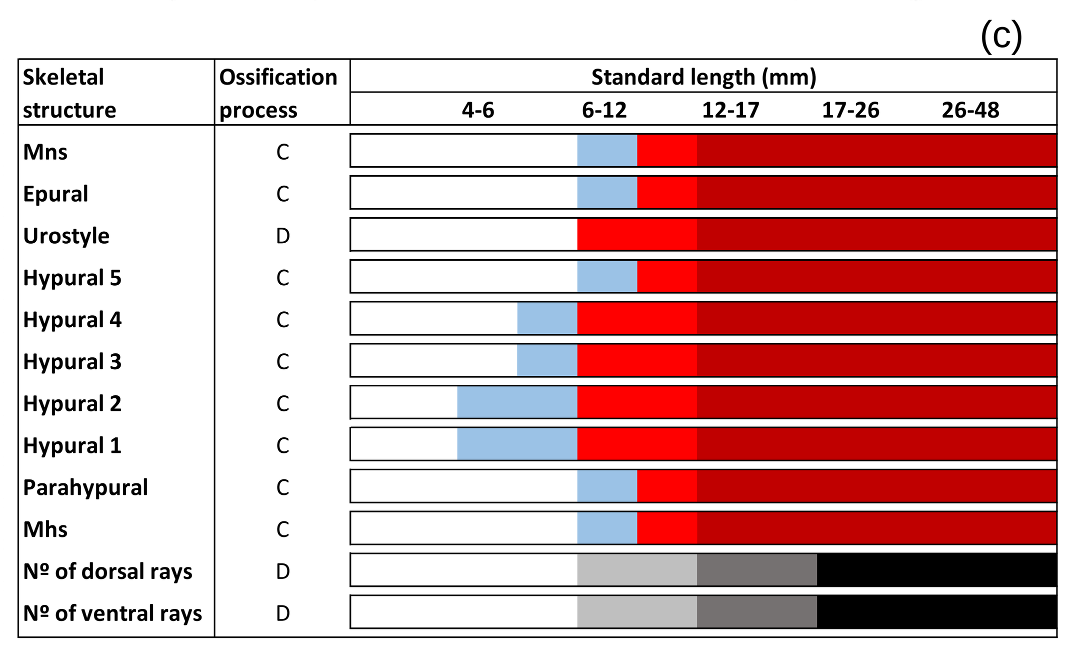


Figure 3. Skeletal formation of main structures of the cranium (a), trunk (b) and caudal fin complex (c). Color lines: *white*, structure was still not formed; *blue*, structure was in cartilage stage; *light red*, structure was slightly mineralized; *dark red*, structure was fully mineralized; *light grey*, first elements were appearing; *dark grey*, half of the elements were formed; *black*, all the elements composing this structure were formed and mineralized; *C*, chondral ossification; and *D*, dermal ossification. Nomenclature from Bird and Mabee (2003) and Cubbage and Mabee (1996).

References

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Conclusions

- A **high % of deformed fish** was found (54.14 %).
- A **wide type of deformities** (jaw, vertebral and caudal fin deformities) were found.
- Some fish had no pectoral fins (8.3-9.5 %).
- A **high incidence of vertebral fusion** in the pre-ural vertebrae (28-29 %) was observed.
- **Skeletal formation progressed very rapidly**, with first elements mineralizing at 4 mm (2 dpf) and skeleton being almost completed at 26 mm (85 dpf).

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