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Teleost Skeletal Plasticity: Modulation, Adaptation, and Remodelling

P. Eckhard Witten¹ and Brian K. Hall²

The vertebrate skeleton is a plastic organ system and the skeleton of teleosts is no exception. Epigenetic factors during development influence skeletal anatomy, mechanical properties, and meristic characters. The adult teleost skeleton undergoes changes connected to mechanical adaptation, repair, mineral homeostasis, sexual maturation, and aging. Vestiges, rudiments, atavisms, hyperostotic bones, additional tooth rows, and variable numbers of vertebral bodies are prominent examples of variable characters. Morphological changes require changes of skeletal structures on the cellular level, including modulation, transdifferentiation, and remodelling. Alterations at the cellular level are best understood by acknowledging those characters that distinguish teleost skeletons from mammalian skeletons: (a) the absence of osteocytes in most species of teleosts; (b) abundant mononucleated osteoclasts that perform non-lacunar bone resorption; (c) a phosphorous- rather than a calcium-driven mineral homeostasis; and (d) a variety of tissue types intermediate between bone and cartilage. This brief account of teleost skeletal plasticity shows that the teleost skeleton is a lifelong plastic organ system. Using examples, also from our own studies, we provide examples of skeletal plasticity at various hierarchical levels.

THE skeleton (Greek, the dry matter) as opposed to soft tissues is often viewed as the invariant component of the vertebrate body. In this review we provide examples that the dry matter in teleost fishes is variable, as it is the case for other vertebrates. Taxonomists and developmental biologists concerned with skeletal development of model organisms often view the skeleton as invariant over the time frame of their analyses (Bolker, 2014). In contrast, disciplines such as evolutionary developmental biology, biomechanics, physiology, or immunology have a more dynamic view on the skeleton.

As a consequence of its many functions there is no doubt that the vertebrate skeleton is a dynamic organ that is subject to lifelong changes (Hall, 1990; Witmer, 1995). In addition to its mechanical functions (protection, locomotion, feeding), the skeleton fulfills many other roles (Ruff et al., 2006). For example, early in development the teleost axial skeleton represented by the notochord is an important signalling center. Later, the mineralized skeleton is a regulator of the animals' mineral metabolism, and an organ that actively produces several hormones. The skeleton is a place for lipid storage (Stemple, 2005; Witten and Huysseune, 2009; Hall, 2015), takes part in acoustic reception and sound production (Parmentier et al., 2011), and is an organ system that generates both transitory and permanent secondary sexual characters (Chardon and Vandewalle, 1997; Witten and Hall, 2003) to give only a few examples. In this short review we mainly focus on alterations of the skeleton that are morphologically evident. We provide examples about different types and levels of teleost skeletal variability and plasticity. Our account is far from being complete regarding teleost species that show known types of skeletal alteration. Many more examples from more species can be found in the literature. Examples for the different types of teleost skeletal plasticity in the literature are, however, often scattered or consist of a footnote about 'developmental noise.' Our aim is to emphasize that the teleost skeleton is, in all life stages, a variable and plastic organ. Any type of skeletal based analysis should take this into account. Certainly much more can be said about mechanisms and consequences of skeletal plasticity. General information about the plasticity of the

vertebrate skeleton can be found in Johnston and Wilson (2002), Ruff et al. (2006), Currey (2010), and Hall (2015). While several of the physiological and molecular mechanisms behind skeletal plasticity are known, many are still being discussed for teleosts and for all other vertebrates (Hall, 1983, 1989; Witten and Huysseune, 2009; Gunter et al., 2013; Harris et al., 2014). Modularity and heterochrony are mechanisms that facilitate the plasticity of skeletal structures (Kimmel, 2014). A matter of ongoing discussion is the consequence of the different types of skeletal plasticity for macroevolution and speciation (Greenwood, 1965; Meyer, 1987a; Pigliucci et al., 2006; Hall, 2011). The importance of developmental plasticity for evolution and speciation has been reviewed by Mary Jane West-Eberhard (West-Eberhard, 2003, 2005a, 2005b) and by Danos and Staab (2010).

Functional adaptation of teleost bones.—Parts of our knowledge that the skeleton is not the invariant dry matter goes back to the studies of Roux (1881) and Wolff (1892) who observed that internal bone spongiosa in mammalian long bones grows to adapt to the requirements of mechanical load. The principle that epigenetic factors elicit phenotypic plasticity in skeletal structures is now widely accepted (Hall, 2005). It became known as 'Wolff's law of bone transformation' but as explained by Ruff et al. (2006) 'bone functional adaptation' appears to be a more appropriate term.

'Bone functional adaptation' is commonly observed in teleosts and well documented (Greenwood, 1965; Fiaz et al., 2010; Grünbaum et al., 2012; Currey and Shahar, 2013; Fig. 1). Various experiments present evidence that morphology, structure, and patterns of gene expression in teleost jaw bones change in response to alternate feeding conditions (Meyer, 1987a, 1987b; Huysseune et al., 1994; Hegrenes, 2010; Albertson et al., 2003; Gunter et al., 2013; Gunter and Meyer, 2014). In response to hard food items, Huysseune et al. (1994) demonstrated that increased mechanical load causes adaptive remodelling of pharyngeal jawbones in the cichlid *Astatoreochromis alluaudi*. Hard food items elicit the development of more and stronger internal bone trabeculae.

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In addition, continuous tooth replacement generates broader, molariform teeth. Meyer (1987a, 1987b) showed that the type of food changes the shape of the entire head in juveniles of the cichlid *Cichlasoma managuense*. As shown by Parsons et al. (2012, 2014), in cichlids the processing of hard food items induces the local production of increased levels of Bmp4, a potent determinant of jaw shape differences not only in teleosts. Likewise, manipulation and local down regulation of the Wnt signalling pathway has significant consequences for jaw shape of cichlids. Mechanical loading affects not only bone growth and shape in adult and juveniles, it also affects early teleost bone development (Fiaz et al., 2012). Endurance swim training experiments with the cyprinid *Danio rerio* show the acceleration of ossification in the endoskeletal elements of tail skeleton. In trained fish, hypurals ossified at 20 days post fertilization, whereas in non trained fish ossification occurred only 35 days post fertilization (van der Meulen, 2005). Fiaz et al. (2012) could show that even earlier in development zebrafish skeletal tissues respond to swim training. In animals trained from 5 to 14 days post fertilization, the formation of cartilage and bone structures in the head and tail region as well as the formation of anal and dorsal fin elements was accelerated by up to three days. In a study with the salmonid *Salvelinus alpinus*, Grünbaum et al. (2012) showed that swim training has a larger effect on the acceleration of bone development than on cartilage development. In another salmonid species, Deshamps et al. (2009) raised the bone mineral content of vertebral bodies in rainbow trout (*Oncorhynchus mykiss*) by increasing the animals' swimming activity. Exercise influences many organ systems and metabolic functions in teleosts, as reviewed by Palstra and Planas (2011). Thus, in addition to mechanical load other epigenetic factors influence the rate of skeletal development and mineralization. One example are nutritional factors other than minerals, such as vitamins and fatty acids. Even the composition of the gut microflora can accelerate or delay skeletal development (Izquierdo et al., 2013; Maradonna et al., 2013; Boglione et al., 2013).

Apart from acceleration or delay, epigenetic factors can initiate bone development. One example for the latter is the ossification of tendons as reaction to tensile load. Tendons in mammals ossify at insertion points into cartilage or bone (Benjamin et al., 2000). An example discussed by Danos and Staab (2010) for a bone that mineralizes in reaction to mechanical forces is the kinethmoid. The kinethmoid connects the premaxilla with the neurocranium. This bone provides extended mouth protrusion and is a synapomorphy that unites cypriniforms (Staab et al., 2012). The kinethmoid develops as a cartilage within a ligament, a type of skeletal element that could be considered as a sesamoid (Hall and Witten, 2007). In zebrafish the kinethmoid starts to ossify at its ligamentous insertion points, at the time when the animals perform increased premaxillary protrusion (Staab and Hernandez, 2010). Danos and Staab (2010) hypothesize that a jaw muscle (adductor mandibulae) modification resulted in changes in the force regime within the heads of a cypriniform ancestor. This change could have ultimately led to the epigenetic origin of the kinethmoid bone from a tendon. Danos and Staab (2010) support their hypothesis, about a load-related epigenetic origin of bones from tendons, by pointing out that intermuscular bones (intramembranous bones) found in the myosepta of teleosts are completely embedded in the myosepta and do not connect

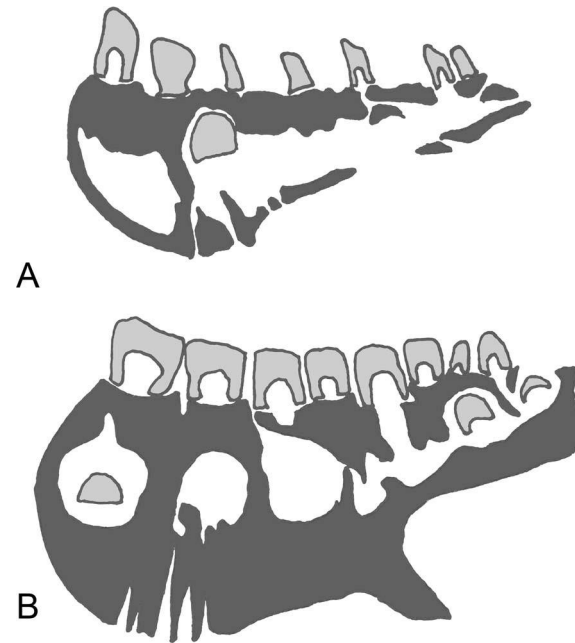


Fig. 1. Adaptation of internal bone structures and tooth morphology in the lower pharyngeal jaw in response to mechanical load in the cichlid *Astatoreochromis alluaudi*. (A) Animal raised on soft food. (B) Animal raised on hard food. In this animal the amount of bone (dark gray) in the lower pharyngeal jaw increases and the teeth (light gray) acquire a molariform shape. Modified after Witten and Huysseune (2009), see also Huysseune et al. (1994) for further details.

to other bones. These intermuscular bones are homologous to lateral and epineural myoseptal tendons (Gemballa and Britz, 1998). Transformation of the periosteum into cartilage was proposed by Liem (1973) as pivotal to the evolution of cichlid pharyngeal jaws. The basipharyngeal joint of these jaws is proposed to have evolved as a reaction to the sliding of the ventral surface of the cranial base against the upper surface of the pharyngeal jaw. Liem (1973) points out that the development of a cartilaginous diarthrotic joint between bone elements that slide against each other is a relatively simple and rapid ontogenetic, and therefore evolutionary, process.

Roux (1881) and Wolff (1892) observed functional adaptation of bones on malformed skeletal elements that rearrange internal structures. Fiaz et al. (2010) emphasize that similar observations can be made on deformed teleost skeletal elements. In the moronid *Dicentrarchus labrax* that suffered from lordosis, Kranenbarg et al. (2005) showed that an increase in strain in deformed vertebral bodies was compensated for by a change in bone architecture and by increased bone formation. In adult salmon, after the pathological fusion of two vertebral bodies, remodelling and rearrangement of bone structures can generate one normal shaped and normal structured vertebral body (Witten et al., 2006; Fig. 2). Sambraus et al. (2014) show that the same process of vertebral fusion occurs in wild salmon.

The plastic response of teleost skeletal structures to changing load regimes accords with observations made on tetrapods and most basal extant actinopterygians (Standen et al., 2014). Remarkably, this response is not only observed in more basal teleosts with cellular bone (osteocyte containing bone) but also in more advanced teleosts with acellular (anosteocytic) bone (Meyer, 1987a, 1987b; Huysseune et al., 1994; Kranenbarg et al., 2005; Hegrenes, 2010). From what

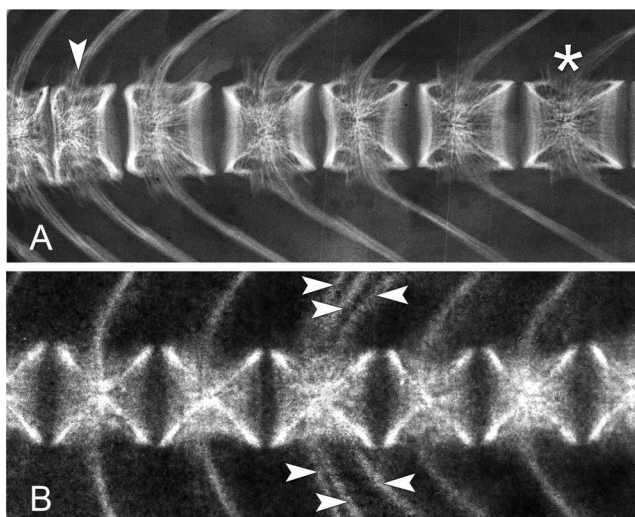


Fig. 2. Remodelling and plasticity of vertebral bodies in Atlantic salmon (*Salmo salar*). (A) The elongation of adjacent vertebral bodies (white asterisk) compensates for the compression of vertebral bodies (white arrowhead). (B) Compression as shown in (A) can lead to the complete fusion of vertebral bodies. The figure shows the result of a complete fusion, a process in which three fused vertebral bodies are remodeled into one normal shaped vertebral body, indicated by supernumerary haemal and neural arches (white arrowheads). Modified after Witten et al. (2006), see also this reference for developmental stages of vertebral fusion; see Sambraus et al. (2014) for the occurrence of vertebral body compression and fusion in wild Atlantic salmon.

we know about osteocyte function in mammals, osteocytes and their elaborate meshwork of interconnected cell processes are responsible for the detection of strain and mechanical load. Consequently, osteocytes are believed to guide the process of bone remodelling in response to external forces (Burger et al., 1995, 2003; Bonewald, 2004; Franz-Odenaal et al., 2006; Bonucci, 2009). We currently do not know how load reception functions in advanced teleosts with acellular bone. The same question must be asked for early developmental stages of more basal teleosts since their skeleton is initially also free of osteocytes (Sire et al., 1990; Huysseune, 2000). These observations have been made on early stages of bone formation in *Danio rerio*, *Oncorhynchus mykiss* and in the callichthyid *Hoplosternum littorale* (Ann Huysseune, pers. comm.). Fiaz et al. (2010) considered the role of the notochord as a mechanosensor. Vatsa et al. (2006) showed that mammalian osteoblastic and osteocytic cells *in vitro* are sensitive to mechanical load. Likewise, mammalian odontoblasts are considered to have sensory capacity (Magloire et al., 2004; Allard et al., 2006). Thus, in teleosts with acellular bone, osteoblasts and bone lining cells that reside on the bone surface are obvious candidate cells for sensing mechanical load. This idea is supported by the finding that in some teleost species with acellular bone, the cells on the bone surface extend cytoplasmic cell process into the matrix, similar to mechano-sensing osteocytes. Meunier (2011) describes this skeletal tissue as primary canaliculated bone and provides the example of *Lethrinus nebulosus* (Lethrinidae, Perciformes) as a representative of this type of acellular bone.

Vertebral body numbers.—The number of somites in vertebrates is often considered to be species-specific and thus constant (Gomez et al., 2008; Schröter and Oates, 2010; Pourquié, 2011). Accordingly, the number of somite-derived

vertebrae is a meristic feature that is considered to be a valuable character in taxonomic studies of teleost fishes (Ferreri et al., 2000). Yet, Darwin commented using vertebral bodies as example, “it seems to be a rule that when a part or organ is repeated many times in the same individual the number is variable” (Darwin, 1859:160); see also Hall (2010) for an in-depth review of Darwin’s view about skeletal plasticity.

It is indeed known that variations in meristic counts occur within a population of teleosts as a result of different rates of development (Smirnov et al., 2006), often related to variations of the environmental temperature (Fowler, 1970; Jordan, 1982). For chum salmon (*Oncorhynchus keta*), Murray and Beacham (1989) identified a time window at which temperature changes appears to be critical for the number of vertebrae that develop. Moreover, there can be considerable variation concerning the number and shape of axial skeletal elements that support the caudal fin (Bensimon-Brito et al., 2012a). Several studies report a temperature-dependent induction of variation in teleosts on the somite level or on the level of vertebral bodies (Mitton and Koehn, 1976; Swain, 1992; Brooks and Johnston, 1994; Johnston and Wilson, 2002; Favaloro and Mazzola, 2003; McDowall, 2003; Ando et al., 2008; Sfakianakis et al., 2011; Yurtseva et al., 2014). Brooks and Johnston (1994) show that the pleuronectid *Pleuronectes platessa* reared at low temperature have more somites in comparison to embryos raised at higher temperatures. In a heat shock experiments at 37°C, targeting embryos of *Danio rerio* with 2–14 somites, Connolly and Hall (2008) induced somite malformations and a cessation of somite development. Mitton and Koehn (1976) and Johnston and Wilson (2002) reported a positive correlation between temperature and number of vertebrae over a large temperature range. In his comprehensive treatise, Fowler (1970) shows that in many teleost species higher vertebra numbers are obtained on either side of the optimum temperature. In confirmation of Fowler (1970) several studies record an increase in vertebral body numbers in teleosts at lower temperatures (Brooks and Johnston, 1994; Ando et al., 2008; Sfakianakis et al., 2011; Fig. 3). Since *Danio rerio* became a model organism for genetic and developmental biology, information about variable vertebral numbers in this species is available from different sources. Excluding the compound centra of the urostyle, the taxonomic work of Sanger and McCune (2002) indicates that the genus *Danio* has 30 to 32 vertebrae; according to Meyer et al. (1995) the vertebral number in *Danio rerio* ranges 29 to 32. Ferreri et al. (2000) count between 30 and 34 vertebral bodies in wild type zebrafish and 30 to 32 vertebral bodies in a laboratory strain of *Danio rerio*. Bird and Mabee (2003) count 30, Bensimon-Brito et al. (2012b) 31, and Morin-Kensicki et al. (2002) 28 vertebrae. A surprisingly low and essentially invariant number of only 26 vertebrae were reported by Schröter and Oates (2010). Sfakianakis et al. (2011) raised zebrafish at four different temperatures (22, 25, 28, and 31°C). In line with the findings of Brooks and Johnston (1994) on embryos of *Pleuronectes platessa*, Sfakianakis et al. (2011) found that the number of vertebrae was significantly higher in the lower temperature group (22°C). In accordance with Fowler’s model (Fowler, 1970), the authors also observed a slight increase of vertebral body numbers in the high temperature group (31°C).

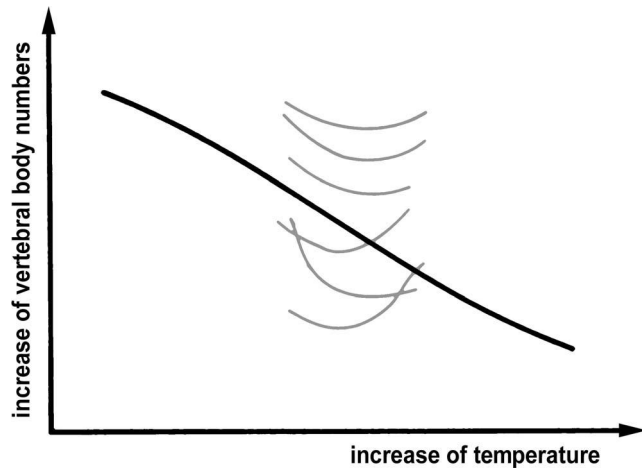


Fig. 3. The relationship between temperature and the number of vertebral bodies according to Fowler (1970). The bold black line represents the general relationship between vertebral numbers in teleost fishes and temperature. At lower rearing temperatures more vertebral bodies develop. Less vertebral bodies develop at higher temperatures. For particular species, a U-shaped relationship between temperature and vertebral body number has been observed (light gray lines). The minimum number of vertebral bodies develops at the species-specific temperature optimum with a tendency to increase vertebral body numbers at lower and at higher temperatures. Modified after Fowler (1970).

An interesting observation, albeit not temperature related, was made by Gorodilov (2004). Development of the caudal-most somites can explain some of the observed variations of vertebral numbers. In *Oncorhynchus kisutch* several caudal-most pairs of somites degenerate during development. Gorodilov (2004) designates these as pseudosomites. Unlike true somites, pseudosomites apparently do not give rise to subsequent metameric structures, such as myotomes and vertebrae. Moriyama et al. (2012) describe a similar process in the adrianchthyid *Oryzias latipes* that they designate as fusion of the caudal-most somites. If pseudosomites (somite fusion) occur in members of phylogenetically distant teleost genera such as *Oncorhynchus* and *Oryzias*, it could well be a general process in teleost skeletal development.

Vestiges and rudiments.—When Darwin commented on the variability of vertebral numbers, he also remarked that “rudimentary parts are apt to be highly variable” (Darwin, 1859:160). The presence, absence, and variation of rudimentary skeletal elements can be readily observed in the caudal fin skeleton of teleosts (De Schepper et al., 2004; Harris et al., 2014). We use the term vestiges and rudiments following the definition from Hall (2003): Rudiments are incompletely formed features found only in embryos; vestiges are remnants of ancestral features that also persist in adults.

The teleost caudal fin endoskeleton is the result of a progressive reduction of a number of skeletal elements compared with the ancestral situation (Gosline, 1961; Eastman, 1979; Schultze and Arratia, 2013; Wiley et al., 2015, in this volume). The heterocercal tail found in more basal representatives of actinopterygian fishes such as *Amia* (Arratia and Schultze, 1992) eventually transformed into the homocercal tail during the early evolution of teleosts (Schultze and Arratia, 2013). Skeletal development of the internally asymmetric teleost caudal fin is a complicated

process to which most of the animal’s skeletal tissues contribute: notochord, notochord sheath, cell rich cartilage and hyaline cartilage, perichondral, endochondral, and intramembranous bone as well as dermal bone (Witten and Huysseune, 2007). Caudal vertebral bodies fuse into the urostyle. Neural and haemal arches (respectively spines) of these vertebral bodies regress and/or transform into epurals and hypurals. Epurals and hypurals support the external fin rays (lepidotrichia) that can also be supported by haemal and neural spines of preural vertebral bodies. Preural vertebral bodies also tend to fuse, an observation made in *Danio rerio* (Bensimon-Brito et al., 2012a). Some of the observed variation in vertebral body numbers certainly relate to the fusion (or multiple fusion) or non-fusion of preural vertebral bodies. In *Salmo salar* such fusions are not malformations (Witten et al., 2009). For zebrafish (*Danio rerio*) we have a detailed account about fusion events, vestiges, and rudiments that occur during normal development. According to Bensimon-Brito et al. (2012a), ural vertebral bodies 1 and 2 usually develop as one structure from early on, but in 4% of the animals the former intervertebral space is present as a vestige before it disappears through fusion later in development. Unrelated to this fusion process, a vestige of hypural 3 occurs in the position of the diastema, a gap in the series of hypurals that corresponds to the lack of hypural 3 (see also Wiley et al., 2015, in this volume). 15% percent of all zebrafish studied by Bensimon-Brito et al. (2012a) display a vestige of hypural 3. The vestige may be a separate cartilage or a cartilaginous extension of the following hypural (Fig. 4). 23% of the preural vertebral bodies have extra neural spine rudiments. A detailed study by Grünbaum and Cloutier (2010) shows similar variations during the development of the caudal skeleton in *Salvelinus alpinus*. De Schepper et al. (2004) document the considerable amount of intraspecific variation in the caudal and pre-caudal skeleton of African clariids.

More than rudiments: atavisms and taxic atavisms.—The occurrence of atavisms in salmonids was already described by Day (1887). The term ‘atavism’ refers to the reappearance of a lost character that is not present in the parents or in the recent ancestors of the organisms (Hall, 1984). Rare atavistic anomalies in individual specimens can be considered as variations. In some instances, atavistic features become permanent characters in what is known as taxic atavism (Stiassny, 2003). A permanent skeletal character established through taxic atavism *sensu stricto*, is not a subject for a discussion about skeletal plasticity, although such a repairing skeletal structure can be subject to its own variation. As pointed out by Stiassny (1992), taxic atavism is a mechanism of considerable importance in generating morphological variation within clades. Moreover, rudiments can be atavistic characters. Below we present some examples that demonstrate that there can be blurred borderlines between the variable appearance of rudiments and the permanent reappearance of an ancestral character.

In addition to the variations in the caudal skeleton of *Salvelinus alpinus*, Grünbaum and Cloutier (2010) also show that hypural 7 and the fourth uroneural are taxic atavism in salmonids. Additional examples of taxic atavisms from other vertebrate taxa (including advanced teleosts) and other organ systems (fins, muscles, skull bones) are discussed by Raikow et al. (1979), Stiassny (1992), Meyer (1999), Gatesy et al. (2003), and Hall (2007). An interesting case is the

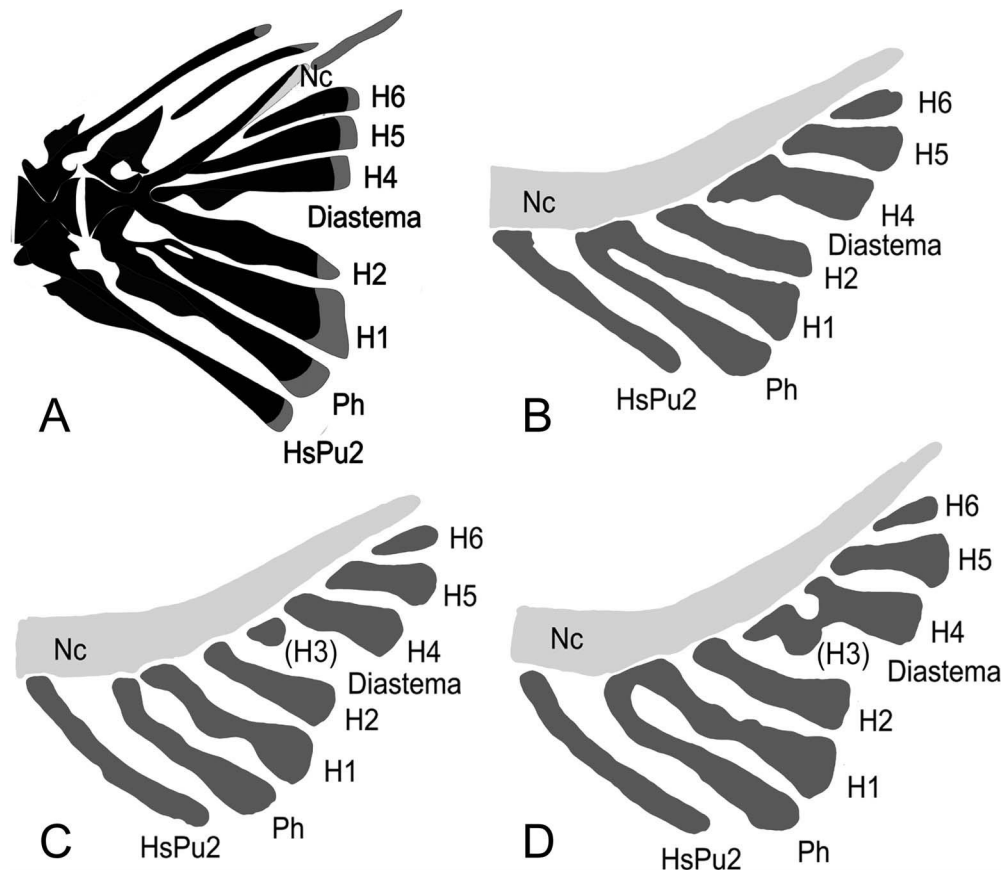


Fig. 4. Variation in caudal fin endoskeleton development in *Danio rerio*, modified after Bensimon-Brito et al. (2012a). (A) The fully developed caudal fin endoskeleton contains modified haemal arches, respectively haemal spines, named hypurals. A gap in the row of hypurals is represented by the diastema. (B) Characteristic development of cartilaginous hypural anlagen in an animal of 6 mm standard length. The basis of H4 is typically broad (anteriorly extended). (C) A variation of hypural development shows the rudiment of H3 instead of a broadened basis of H4. (D) Similar developmental variation as in (C), but the cartilaginous rudiment of H3 is connected to H4. See Bensimon-Brito et al. (2012a) for a detailed description and for other variations (rudiments and vestiges) during caudal fin development of *D. rerio*. Bone, black; cartilage, dark gray; notochord light gray; H, hypurals; Ph, parhypural; HsPu2, haemal spine of preural vertebral body 2; Nc, notochord. The nomenclature of elements follows the new polyural interpretation according to Schultze and Arratia (2013) and Wiley et al., 2015, in this volume.

reappearance of a fourth row of pharyngeal teeth in the cyprinid *Barbus paludinosus* that has been described as a possible case of taxic atavism by Golubtsov et al. (2005). In a natural population of *Barbus intermedius*, about 10% of the animals have a four-rowed dentition instead of a three-rowed dentition (Shkil et al., 2010). The authors show that the occurrence of the fourth row can be heritable since laboratory-reared offspring from parents with a four-rowed dentition had the same dentition, and if one of the parents had a four-rowed dentition the percentage of four-rowed offspring was significantly higher than 10%. In further laboratory experiments, Shkil et al. (2010) also showed that high levels of thyroid hormone cause a decrease in the number of tooth rows from three to two. In contrast, thyroid hormone inhibition increased the number of tooth rows from three to four (Fig. 5).

Tooth replacement patterning and variation.—Above we have briefly discussed diet-related changes in tooth shape in teleosts with a heterodont dentition as well as the appearance and disappearance of complete tooth rows. Below these levels there can be also variation in the patterning of tooth replacement. In mammals, teeth are initiated at well-defined locations corresponding to incisor, canine, premolar, and molar regions, respectively. The

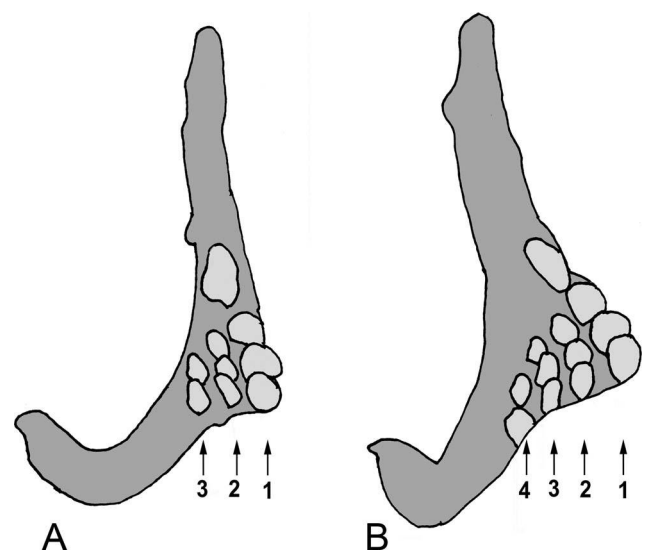


Fig. 5. Atavistic reappearance of a 4th tooth row, modified after Shkil et al. (2010). (A) The pharyngeal jaws of *Barbus intermedius* are characterized by the presence of three rows of teeth. (B) 10% of the animals in a population have a 4th tooth row. Shkil et al. (2010) show that this natural phenomenon can also be obtained in experiments by the inhibition of thyroxin.

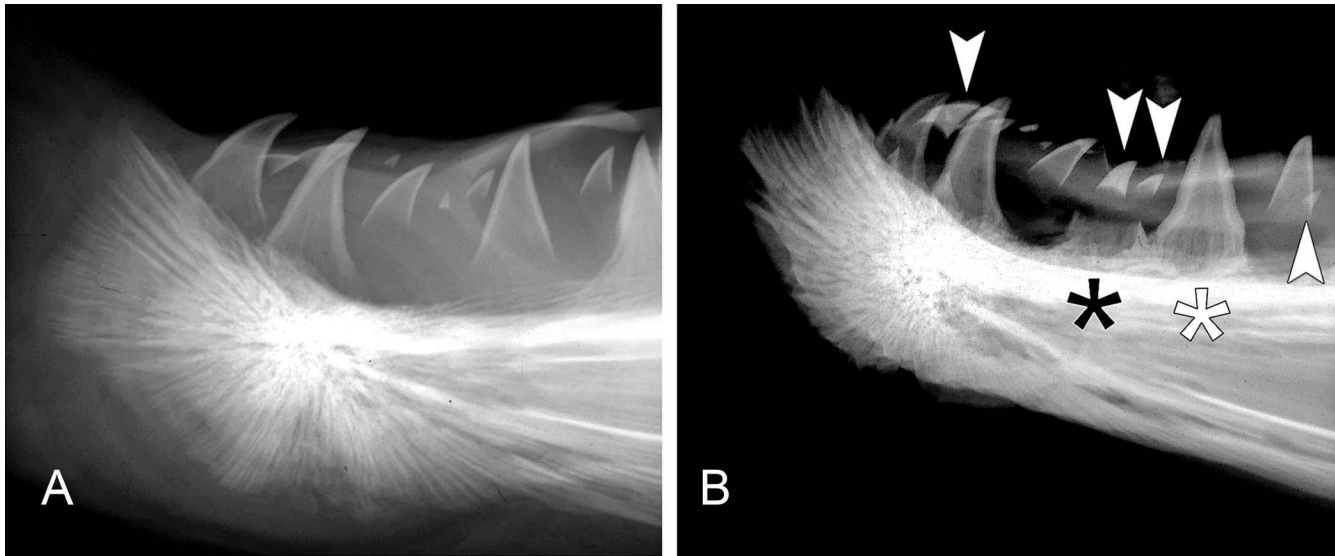


Fig. 6. (A) X-ray, showing the regular patterning of the dentition in wild Atlantic salmon, modified after Huysseune et al. (2007). The position of a functional tooth is followed by a developing replacement tooth in the next tooth position, followed by a tooth anlage and an old tooth in resorption in the subsequent position. (B) X-ray showing that the highly regular patterning of tooth replacement (no edentulous stage prior to spawning) is lost in salmon that stay in the river over the winter after spawning. Several functional teeth are completely resorbed (white arrowheads). Other functional teeth are neither resorbed nor shed, indicated by the complete mineralization of the tooth basis (white asterisk). The black asterisk points to the basis of a resorbed tooth. Regular tooth patterning is restored in animals that return to the sea after the winter. See also Witten et al. (2005).

pattern of the dentition is species specific (Van der Heyden et al., 2000). Because of considerable interspecific variation and assumed strong linkage with diet (and thus small intraspecific variation) these variations in tooth replacement patterns in teleosts have been extensively used in systematics (Huysseune and Sire, 1998). Smith-Vaniz and Carpenter (2007) caution, however, that the comparison of the dentition of a large number of members of the Carangidae group ('crevalle jacks') reveals an almost complete continuum of dentition types, making it questionable if the dentition can be used as a taxonomic character.

In most teleost species, once established, the replacement pattern appears to be maintained as the 'default' state. Still, variations and modifications in tooth replacement pattern are commonly observed, suggesting that tooth replacement is under local control at the level of the initiation of replacement teeth (Huysseune and Witten, 2006). The observations on juvenile and adult *Danio rerio* published by Van der Heyden et al. (2001) indicate that there are quite some variations in the order (patterning) of tooth replacement. Van der Heyden et al. (2001) propose an uneven change in length of the replacement cycle among the different loci to account for the shift in the tooth replacement cycle during development. The ventral pharyngeal tooth row in zebrafish has five tooth positions, designated as 1V to 5V. Early in development the pattern of tooth replacement is (2V–4V)–(1V–3V–5V); teeth together in a pair of brackets are typically replaced synchronously. In juvenile and adult zebrafish, the pattern of tooth replacement shifts to the formula (5V–2V)–(3V)–(1V–4V). Likewise, Huysseune and Witten (2006) found frequent examples in salmon where teeth on the dentaries were in a state of development different from what could be expected on the basis of a theoretical replacement tooth pattern with similar stages of development every third position. The frequency of tooth replacement, and thus the patterning, might decrease with increasing age, when teeth in individual loci have gone through an increasing number of replacement

cycles. This is indeed the case in *Amia* (Miller and Radnor, 1973) and in crocodylians (Edmund, 1962) and thus possible also in teleosts.

Salmo salar shows that the typical pattern of tooth replacement can be lost temporarily. *Salmo salar* has a regular tooth replacement pattern and minor variations are observed, such as the lack of a functional tooth, or a more advanced state of development of two adjacent positions in a tooth row (Huysseune et al., 2007; Huysseune and Witten, 2008). Moreover, and contrary to previous reports, tooth replacement in *Salmo salar* continues regularly also during the reproduction period. There are no indications for tooth loss prior to spawning and no special breeding teeth have been identified (Witten et al., 2005). However, in Atlantic salmon that stay during the entire winter in the river after spawning (Miramichi River, New Brunswick, Canada), tooth replacement comes to a halt and the regular pattern of tooth replacement is lost. Some teeth are resorbed while the bases of other teeth continue to mineralize. In the spring when the animals move back to the sea, the dentition has lost a recognizable patterning (Witten et al., 2005; Fig. 6). Since repetitive spawning animals arrive at the next spawning event with a well-patterned dentition, the cessation of tooth replacement and loss of the regular tooth replacement patterning can only be temporary events (Huysseune et al., 2007).

Hyperostosis and secondary sexual characters.—Another variable teleost skeletal character is hyperostosis. Hyperostosis is localized bone expansion through extensive periosteal membrane bone formation (Witten and Huysseune, 2009). Hyperostosis occurs late in life (Fig. 7). There is a great amount of variability concerning shape, degree, number, and time of occurrence of hyperostotic bone extensions, well documented by Smith-Vaniz et al. (1995). At the same time, hyperostosis is a regular occurrence. When and where it will develop can be predicted and is species specific. Predictability and site-specificity of hyperostosis in a number

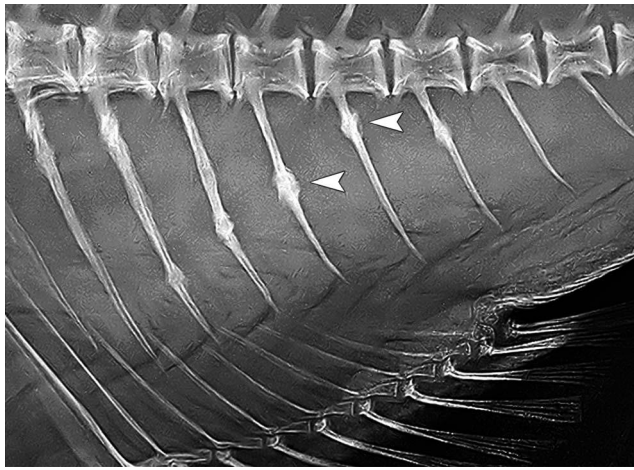


Fig. 7. X-ray, showing the start of hyperostotic bone formation at haemal arches and haemal spines in *Pagrus pagrus*, 41.7 cm SL. The X-ray shows vertebral bodies 13–20. See Smith-Vaniz et al. (1995) and Smith-Vaniz and Carpenter (2007) for a comprehensive treatment of the regular occurrence of hyperostotic bone outgrowth.

of marine teleosts led Smith-Vaniz et al. (1995) to propose that hyperostosis is a regular late event in the animal's life and not a pathological condition (osteoma), as suggested by Schlumberger and Lucke (1948) and by Giarratana et al. (2012). In agreement with Smith-Vaniz et al. (1995) and Smith-Vaniz and Carpenter (2007), Jawad (2013) also argues that one should not consider hyperostosis as a pathological condition because its occurrence can be species-specific and that the development of hyperostotic bone during ontogeny is a regular event. Hyperostosis is certainly both, a species specific and a variable character. Smith-Vaniz and Carpenter (2007) describe the strikingly inconsistent occurrence of hyperostosis of the third rib in the carangid *Caranx caninus*. Likewise, a high degree of variability concerning the number and shape of hyperostotic bone expansions is found in the thrichiurid *Lepidopus caudatus* (Giarratana et al., 2012). The functional significance of hyperostosis is not known. Hyperostotic bone outgrowths represent foci of extensive bone remodelling, even in marine teleosts with an otherwise quiescent acellular skeleton (Smith-Vaniz et al., 1995; Meunier, 2002). This raises the question of an involvement of hyperostotic bones in the animals' mineral metabolism (Witten and Huysseune, 2009). Smith-Vaniz and Carpenter (2007) discuss whether the presence or absence of hyperostotic rib extensions in adult *Caranx caninus* could be sex-linked.

The formation of the kype in male *Salmo salar* is not hyperostosis, but is an example for sex-linked periosteal bone outgrowth that also occurs late in life (Witten and Hall, 2002, 2003). Sexually premature juvenile males do not develop the structures (Gillis et al., 2006). Different from hyperostotic bone outgrowth, the kype is only permanent in parts. Distal parts of the kype are resorbed after spawning; proximal parts are preserved and constitute the bases for the development of a new and larger kype at the next spawning event. The size of the kype is variable, depending on the animal's age, on how long the animal stays out in the sea prior to spawning, and on how many times the animal has spawned (Witten and Hall, 2003). Another example for a secondary sexual skeletal character that can be of variable size are the extended ventral lepidotrichia at the caudal fin of male poeciliid *Xiphophorus helleri* (swordtail). There is

a hypothesized trade-off between female preference for long tail extensions (swords) and predation that increases survival of males with shorter tail extensions (Basolo and Wagner, 2004). Removing the sword from male *Xiphophorus helleri* of an inbred aquarium strain did not accelerate burst swimming. This led Baumgartner et al. (2011) to conclude that the costs associated with bearing a sword are complex and not simply related to predator escape. In nature, however, this secondary sexual skeletal character can vary in different populations of the species (Basolo and Wagner, 2004).

Modulation, transdifferentiation, and remodelling.—Above we have listed examples for variations of the skeleton that affect the individual fish at some stage in life. Changes can be observed on the anatomical and micro-anatomical level. Not different from other vertebrates, the teleost skeleton also is subject to lifelong changes at the microscopical level: There are changes in the cellular composition of skeletal elements and changes in skeletal matrix composition (Witten and Huysseune, 2009). At the cellular level, three mechanisms have been defined that change the skeleton. According to Hall (2005) these are: (1) Modulation, a temporary change in cell behavior, structure, and/or the type of matrix that is produced by the cell; (2) Metaplasia, a lasting transformation. The transformation of a cell into another cell type without intermediate dedifferentiation; (3) Remodelling, the resorption of a skeletal tissue and its replacement by the same tissue or by another tissue type. Remodelling turns bone from an inert mineralized supporting tissue to a vital and metabolically active organ. The knowledge that teleost skeletal tissues undergo lifelong changes in response to external and internal cues is old; it is the basis for age determination of teleosts by skeletochronology. According to Meunier and Panfili (2002), the Swedish monk Hederström (Hederström, 1759) was the first to propose in an 18th Century treatise that vertebral rings in fish can be counted to estimate their age.

Compared to tetrapods, the teleost skeleton has a number of distinctive features. In short, in addition to the endoskeleton, there is a well-developed dermal skeleton. Two skeletal systems (endo- and dermal skeleton) exist with a large degree of independence concerning function and evolution (Hall, 2014). In the endoskeleton, vertebral body anlagen are not cartilaginous. Vertebral bodies arise by the segmented mineralization of the notochord sheath (Huxley, 1859; Arratia et al., 2001; Bensimon-Brito et al., 2012b). Compared to mammals, additional skeletal tissue subtypes are recognized in teleosts as part of the regular skeleton (Hall and Witten, 2007; Witten et al., 2010). Benjamin (1990) describes seven categories of cartilage: (1) hyaline cell cartilage, (2) Zellknorpel, (3) fibro/cell-rich cartilage, (4) elastic/cell-rich cartilage, (5) cell-rich hyaline cartilage, (6) matrix-rich hyaline cartilage, and (7) scleral cartilage. Cartilaginous tissues are not restricted to the endoskeleton. Secondary cartilage and chondroid cartilaginous tissues develop on cranial dermal bones (Benjamin, 1989; Beresford, 1993; Huysseune, 2000; Witten and Hall, 2002). Chondroid bone—cartilage cells incised in bone matrix—occurs in basal teleosts with osteocyte-containing bone and in advanced teleosts with acellular bone, and should not be confused with cellular bone (Beresford, 1981, 1993; Huysseune and Sire, 1990; Huysseune and Verraes, 1990; Meunier and Huysseune, 1992; Witten and Hall, 2002; Gillis et al.,

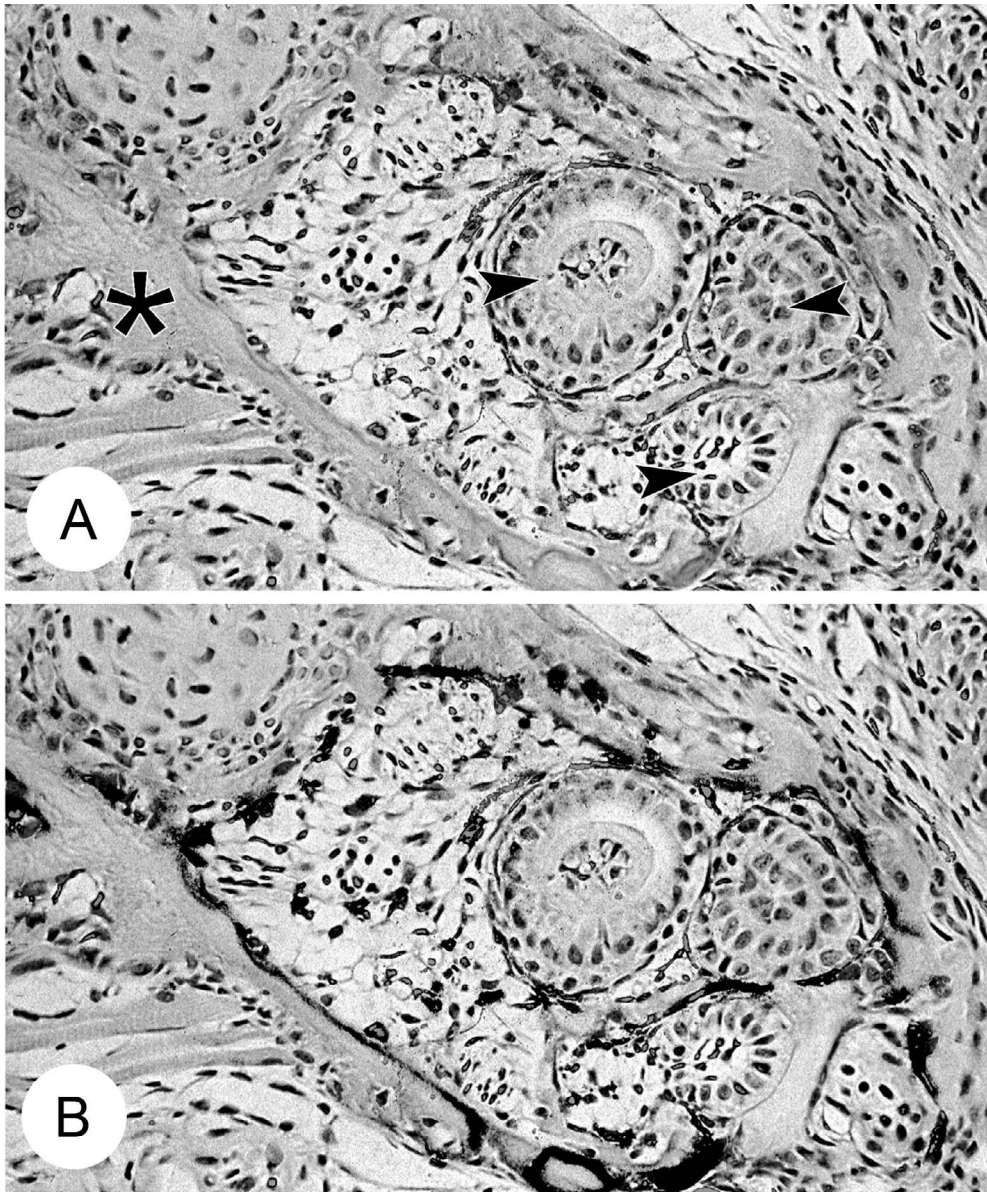


Fig. 8. (A) Intramedullary tooth development at the dentary of the cichlid *Oreochromis niloticus*. Histological section stained with haematoxylin. Alveolar bone, black asterisks; developing teeth, black arrowheads. (B) Development and tooth eruption require bone resorption and bone remodelling. The plain histological staining in (A) does not reveal signs of resorption: multinucleated osteoclasts and resorption lacunae are not visible. The identification of osteoclasts by demonstrating the osteoclast specific enzyme tartrate resistant acids phosphatase (TRAP) reveals the presence of bone resorbing cells and the location of bone resorption (black staining, red in original preparation). See Witten and Huysseune (2009) for more details and further references.

2006). The majority of teleost species that belong to derived lineages lack osteocytes inside the bone matrix. Their bone tissue is designated as acellular bone (Kölliker, 1859; Moss, 1961a, 1961b, 1962). Acellular bone was long considered as metabolically inactive for two main reasons (reviewed by Witten and Huysseune, 2009): a) the frequently observed absence of multinucleated bone resorbing cells (osteoclasts), and b) several experiments revealed that teleost acellular bone is not involved in the animals' calcium metabolism. We now know that acellular bone is being remodeled like cellular bone. If multinucleated osteoclasts are absent, remodelling is achieved by small mononucleated cells (Witten, 1997; Fig. 8). Recent studies show that main molecular regulators of mammalian osteoclast function, such as RANK and RANK-ligand, are active in *Oryzias latipes* (acellular bone) and apparently highly conserved among

vertebrates (To et al., 2012). Skeletal development and allometric growth, mechanical adaptation, a phosphorous-driven skeletal mineral metabolism, repair of fractures, and mechanical adaptations are some of the cues that trigger the change the internal skeletal structures in teleosts. Many, if not all, observed morphological skeletal alterations also change to the internal skeletal composition. A few examples from aforementioned morphological alterations:

Mechanical load not only triggers the development of more bone and broader teeth in cichlid jaws, it also triggers the development of chondroid bone. This chondroid bone is located at the upper pharyngeal jaw where the jaw articulates with the base of the neurocranium (Huysseune, 1986; Huysseune and Verraes, 1986). Osteogenic cells can start to produce cartilage instead of bone, thus generating another type of chondroid bone, as for instance the kype in male

Atlantic salmon (Witten and Hall, 2003). Secondary cartilaginous tissues can develop on cranial dermal bones late in life on skeletal elements that do not have a cartilaginous precursor (Benjamin, 1989; Beresford, 1993; Huysseune, 2000). Hyperostotic bones in advanced teleosts with acellular bone are again cellular and osteocyte containing. Hyperostotic bone is remodeled by multinucleated osteoclasts, an osteoclast type otherwise not common in advanced teleosts with acellular bone (Smith-Vaniz et al., 1995). Early in ontogeny, bones of all teleosts are acellular, also the bones of basal teleosts that later develop cellular bone (Sire et al., 1990; Huysseune, 2000), possibly a case of extreme heterochrony (Witten et al., 2004). First generation teeth are without dentine tubules, atubular dentine. Subsequent replacement teeth are then composed from regular orthodentine (Sire et al., 2002). Still some teleosts, such as *Salmo salar*, completely lack dentine tubules as adults also (Huysseune and Witten, 2008).

Concluding remarks.—The teleost skeleton is a plastic organ system. We have provided examples from different hierarchical levels that show that the structure, shape, number, presence, and degree of mineralization of skeletal elements in teleost fishes can vary in response to epigenetic factors. Adult teleost skeletons undergo changes related to mechanical adaptation, repair, mineral homeostasis, sexual maturation, and aging. Morphological changes require a change of skeletal structures on the cellular level, changes that involve transdifferentiation and remodelling. Such changes are best understood in view of the major characteristics that distinguish teleost skeletons from mammalian skeletons, which are the absence of osteocytes in most species, abundant mononucleated osteoclasts that perform non-lacunar bone resorption, a phosphorous-driven mineral homeostasis, and the presence of tissues intermediate between bone and cartilage. In nature, several changes may simultaneously affect the skeleton of one individual. An animal that displays an atavistic skeletal character may have a vertebrate count that deviates from other individuals in the group. Adaptation of bone to mechanical load, alterations in dentition and internal remodelling of the skeleton may take place. Late in life the same individual may develop hyperostotic bones or skeletal-based secondary sexual characters such as a kype. Clearly the teleost skeleton is a dynamic and life-long changing tissue and should be understood as such.

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