

The Kype: An Assessment of Presence and Absence, Composition, Structure, and  
Evolution of a Spawning-related Lower Jaw Modification in Living and Fossil Salmonids

by

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## **Dedication**

Pour ma famille.

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## Abstract

When in their spawn-run, several salmonids (salmon, graylings, etc.) develop a kype. The kype is a spawning-related lower jaw modification composed of a connective tissue mass supported by a framework of chondroid and Sharpey-fibre bone skeletal needles. However, while previous research considered the structure and composition of the kype in Atlantic salmon, little is known of the kype in other salmonids, or indeed which other salmonid species are kype-bearing. Additionally, the evolutionary origins of the kype and of spawning-related morphological changes in its associated structures and tissues are also unknown. Physical examinations, Micro-CT assessments, literature surveys, and histological analyses of living and fossil material reveal that a kype is found only in eusalmonines (trout, salmon, and chars), with the exception of *Hucho*. The lack of kype in *Hucho* supports reassessments of the phylogenetic location and monophyly of this genus. The relocation of *Hucho* is also supported by genus-specific, life-history stage, and life history mode variation in the trends in lower jaw fat, epithelium, and blood vessel morphology evident in all salmonids. The combination of Sharpey-fibre and chondroid bone, tissues previously thought to be only associated with the kype skeleton, is present across Salmonidae to some extent. Establishment of the origins of these tissues requires further research as to the conditions of ancestral groups. This project represents the first time that a family-wide histological assessment of the salmonids has been done and uses both living and fossil material to do so.

## **List of abbreviations used**

### ***Collections facilities***

<b>CMN</b>	Canadian Museum of Nature (Ottawa, Ontario, Canada)
<b>CMNFI</b>	Canadian Museum of Nature Fish collection (Ottawa, Ontario, Canada)
<b>CMNFV</b>	Canadian Museum of Nature Fossil Vertebrate collection (Ottawa, Ontario, Canada)
<b>CMNZ</b>	Canadian Museum of Nature Zoological collection (Ottawa, Ontario, Canada)
<b>RBCM</b>	Royal British Columbia Museum (Victoria, British Columbia, Canada)
<b>UOF</b>	University of Oregon Museum of Natural and Cultural History
<b>UW</b>	University of Washington Fisheries collections (Seattle, Washington, USA)
<b>UWBM</b>	Burke Museum of Natural History and Culture (Seattle, Washington, USA)
<b>ZIN</b>	Zoological Institute of the Russian Academy of Sciences (Moscow, Russia)
<b>ZP</b>	Zabrina Prescott personal collection (Dalhousie University, Halifax Nova Scotia, Canada)

### ***Procedural abbreviations***

<b>HBQ</b>	Hall-Brunt Quadruple histology stain
<b>MAS</b>	Masson's Trichrome histology stain
<b>Micro-CT</b>	Micro-computer tomography
<b>NBFS</b>	10% Neutral buffered formal solution, fixative
<b>SL</b>	Standard (snout to vent) length

## Glossary

<b>Anadromous</b>	Hatching and living, as a juvenile, in freshwater conditions, migrating to, and maturing in, the ocean, and returning to (typically the same) freshwater system to spawn.
<b>Defleshed specimen</b>	Specimen preserved as a dried skeleton, with soft tissue having been removed; also “skeletonized specimen.”
<b>Dentary</b>	Tooth-bearing bone on the lower jaw. When fused, two dentaries are fused at a symphysis. In salmonids, the dentary tends to be relatively triangular in shape.
<b>Fleshed specimen</b>	Specimen preserved with soft tissue intact, fixed with 10% neutral buffered formal solution or equivalent, kept in long term 70% ethanol storage; also “Fresh specimen.”
<b>Fresh specimen</b>	See “Fleshed specimen.”
<b>Freshwater living</b>	Living wholly in a freshwater system, no oceanic, marine, estuarine, or coastal migration. May, however, migrate within freshwater systems, particular to spawn.
<b>Greyscale</b>	The value of each pixel within an image carries an intensity that is expressed along a threshold of minimum (black) to maximum (white).
<b>Iteroparous</b>	An animal capable of repeat spawning or breeding events, able to reproduce multiple times during its lifetime. Usually due to environmental conditions, illness, overuse of resources, injury, and other factors, not all members of an iteroparous population or species will survive to have multiple breeding events.
<b>Landlocked</b>	See “freshwater living”; typically refers to a population that is physically cut off from salt water.
<b>Lateral Line</b>	Also “lateral line canal system.” The lateral line is a mechanosensory system that allows a fish to perceive changes in the direction of water flow.
<b>Maxillae</b>	See “maxillary.”
<b>Maxillary</b>	Long, tooth-bearing bone of the upper jaw; forms the sides of the upper jaw. Plural: maxillae.



<b>Non-anadromous</b>	See “freshwater living.”
<b>Post-spawning</b>	Period following spawning period. Semelparous species are in senescence while, in iteroparous species, animals are in a ‘recovery state’ of increased resource need in consequence of pre-spawning starvation and high demand resource allotment. In animals that develop kype, this is a period of kype demineralization and resorption, with a decrease in the structure and integrity of the kype skeleton.
<b>Premaxillae</b>	See “premaxillary.”
<b>Premaxillary</b>	Short, tooth-bearing bone of the upper jaw; forms the anteriormost part of the sides of the mouth, part of the face, and the lower boundary of the nares (nostrils). Plural: premaxillae.
<b>Pre-spawning</b>	Period of starvation and migration. In later stages of pre-spawning, this is also a time of increased aggression and hierarchy establishment in both males and females, nest building, and completion of sexual maturation. Animals experience high resource demand and low resource intake, resulting in reallocation of existing resources such as fat stored in the dorsal ‘hump’ of many species and demineralization of many aspects of the skeleton (including vertebrae and certain skull bones). In animals that develop kype, this is a period of kype development, with an increase in the structure and integrity of the kype skeleton.
<b>Resident population</b>	See “freshwater living”; typically indicates a non-migratory population.
<b>Semelparous</b>	Animals that breed or spawn a single time during the course of their lives.
<b>Skeletonized specimen</b>	See “Defleshed specimen.”
<b>Spawning</b>	Period of egg fertilization, with previously established hierarchies dictating where eggs are placed and which dominant males fertilize the eggs of which females. This is also when “sneaker” males or “jacks,” unbeknownst to dominant males, sneak near nests and fertilize eggs. In animals that develop kype, this is the period where the kype is most extensive and well-developed.

**Symphysis**

A fibrocartilaginous fusion between two bones. It is a type of cartilaginous joint, specifically a secondary cartilaginous joint. For example, in many teleosts including the salmonids, the dentaries are fused by a symphysis at their anterior tip to form the lower jaw.

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## CHAPTER 1 – Introduction

The family Salmonidae (Fig. 1.1) includes a variety of taxa that are largely restricted to the northern hemisphere (Behnke, 2002; Nelson, 2006; Scott and Crossman, 1973). There are three subfamilies within the Salmonidae: Coregoninae (whitefishes), Thymallinae (graylings), and Salmoninae (Nelson, 1994, 2000; Stearley and Smith, 1993). Salmonines include *Eosalmo driftwoodensis*, the extinct “dawn salmon;” *Brachymystax*; and the eusalmonines. The Eusalmoninae includes a variety of economically, socially, and culturally important fishes such as trout and salmon (Ames and Maschner, 1999; Barker, 1651; Baum, 1997; Campbell and Butler, 2010; Carlson, 1998; Nettle, 1857; Nelson, 1994, 2006). Previous research including work by Tchernavin (1938) and by Witten and Hall (2002, 2003) identified some species of eusalmonines as being of interest due to their formation of a spawning-related lower jaw modification composed of secondarily developing tissues: the kype.

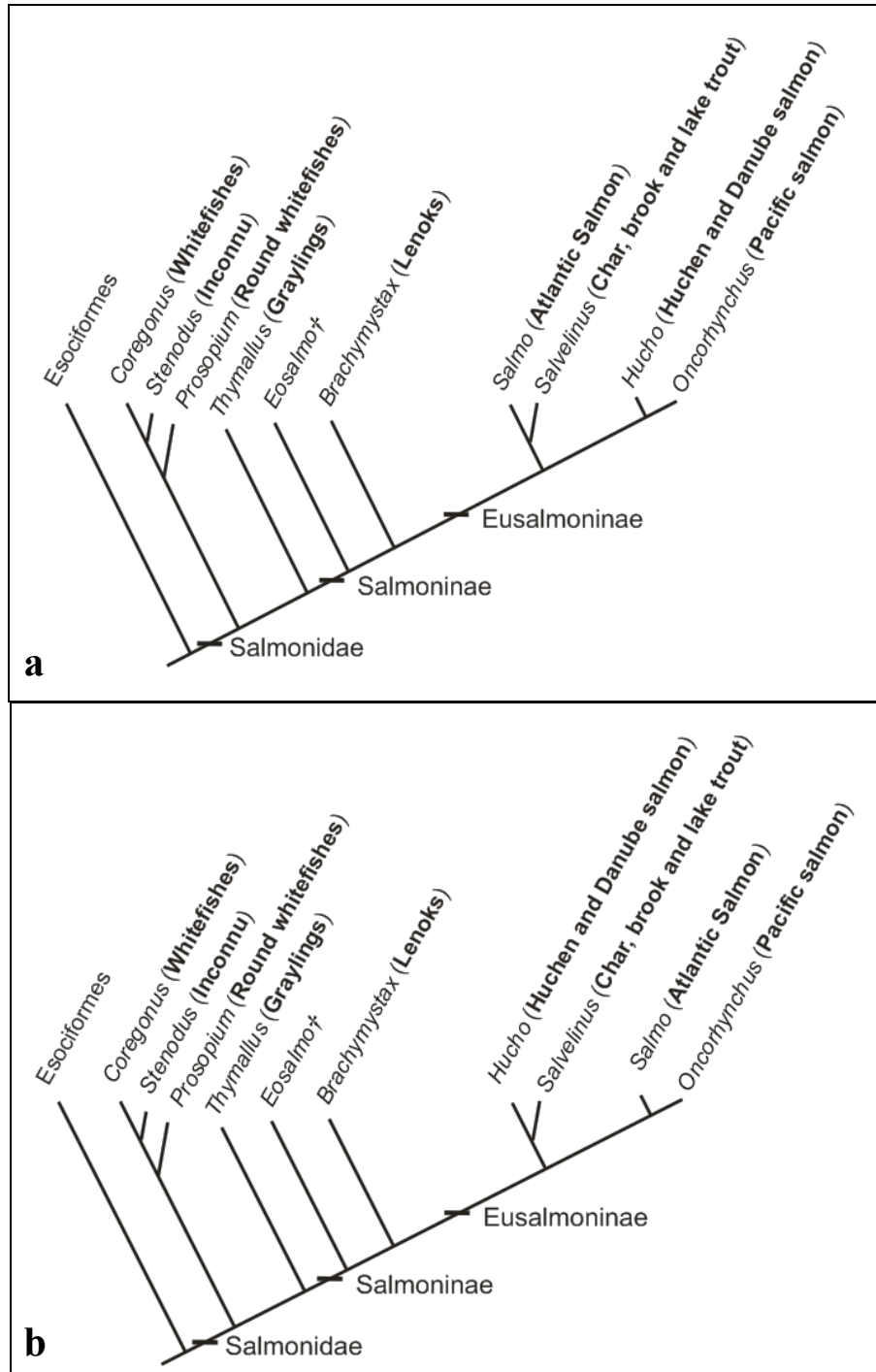


Figure 1.1 – Two previously published salmonid phylogenetic trees. a) Based predominantly on molecular analysis, this tree displays *Hucho-Oncorhynchus* and *Salmo-Salvelinus* as sister groups. Modified from Crespi and Fulton (2004). b) Morphologic analysis frequently places *Hucho-Salvelinus* and *Salmo-Oncorhynchus* as sister groups. Modified from Stearly and Smith (1993).

This project represents the first family-wide assessment of the structure, organization, and composition of the tissues of the salmonid lower jaw. In this thesis, I

will establish the trends evident in structure, organization, and composition of the tissues of the lower jaw across the salmonids. In particular, patterns in the presence and absence of the secondarily developing kype are identified. Using evidence from both living and fossil specimens, the origins of the kype and of the secondarily developing tissues associated with it will be established. Furthermore, I will evaluate patterns in the evolution of these tissues and structures that have implications for salmonid phylogenetic relationships.

### ***Salmonid natural history***

The family Salmonidae belongs to the Salmoniformes, fossil evidence of which, and of some related groups, has been identified from Cretaceous period (Stearly and Smith, 1993; Taverne, 1981). However, the earliest salmonid fossil, *Eosalmo driftwoodensis*, is known from the Eocene of western North America, approximately 50 million years ago (Behnke, 2002; Wilson and Li, 1999). This earliest salmonid is marked by the narrow, elongated body form, the presence of an adipose fin, and the variety of other characteristics which define the Salmonidae (for a comprehensive morphological description of this clade, refer to Gill, 1894; Holčík et al., 1988; Stearley and Smith, 1993; or Nelson, 1994, 2006. For a description with greater focus on molecular data, see Crespi and Fulton, 2004 or Crête-Lafrenière et al., 2012).

The phylogenetic tree of the salmonids as a whole is generally well understood, with consensus between many molecular and morphological studies placing *Hucho* (Danube trout and salmon), *Salmo* (Atlantic salmon), *Salvelinus* (trout and char), and *Oncorhynchus* (Pacific salmon) within the Eusalmoninae (Fig. 1.1). However, when Figure 1.1a, constructed using molecular data, and Figure 1.1b, constructed from morphological data, are compared, it is apparent that, while morphological data favours *Hucho-Salvelinus* and *Salmo-Oncorhynchus* sister groups, many molecular studies suggest alternate phylogenies, including one that favours eusalmonine sister groups of *Hucho-Salmo* and *Salvelinus-Oncorhynchus*. These alternative phylogenetic trees are, in part, the result of a different pool of information to draw from. Morphological analysis can incorporate evidence from fossil material, while molecular analysis relies solely on living material.



Another source of contention in the salmonid phylogeny lies in the appropriate location of *Hucho*. While the many morphological and molecular phylogenetic trees place *Hucho* within the Eusalmoninae, whether as the sister group to *Salvelinus* or not, others argue that *Hucho* belongs with more basal salmonines, outside of the eusalmonines (Crespi and Fulton, 2004; Crête-Lafrenière et al., 2012; Jonsson and Jonsson, 2011; Nordern, 1961; Oleinik and Skurikhina, 2008; Ramsden et al., 2003). While several data support the relocation of *Hucho*, skull morphology (Nordern, 1961) and genetic similarity to *Brachymystax* (Crête-Lafrenière et al., 2012; Oleinik and Skurikhina, 2008), as well as geographic distribution and behaviour (Holçik et al., 1988) are of particular interest to relocation arguments. Further studies of morphological features, including the present study, may resolve some of the discrepancies between these trees. Alternative phylogenies built using morphological, molecular, and behavioural data are discussed in greater detail in Chapter 2, with each of the chapters in this thesis considering the implications of these other possible trees.

### **Eusalmonine life-cycle**

Of note when considering the salmonid phylogenetic tree is the divergence between freshwater and anadromous lineages (McDowall, 1997). With the exception of some populations of brown trout (*Salmo trutta*) and certain Pacific salmon (*Oncorhynchus*) known to spawn in brackish water (Behnke, 2002; Jonsson and Jonsson, 2011; Willson, 1997), all salmonids spawn in freshwater. Despite spawning in freshwater, many Eusalmoninae species are obligate or optional anadromous organisms (Behnke, 2002; McDowall, 1997; Scott and Crossman, 1973; Willson, 1997). As a result, many eusalmonine adults spend the majority of their lives in the ocean (McDowall, 1997; Nelson, 1994, 2006). The majority of other salmonids are solely freshwater organisms with no ocean-going stage in their life cycle (Nelson, 2006), although there are some anadromous populations of *Coregonus* (whitefishes), *Stenodus leucichthys* (inconnu), one species of *Prosopium* (round whitefishes), and several populations of *Thymallus arcticus* (Arctic grayling) (Behnke, 2002; McPhail and Lindsey, 1970; Nelson, 2006; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993; Willson, 1997).

Perhaps one of the best understood and most representative of the anadromous salmonid life cycles is that of the Atlantic salmon (*Salmo salar*; Fig. 1.2). The life history of Atlantic salmon includes alevin, fry, parr, smolt, and mature adult life history stages (Aas et al., 2011; Jonsson and Jonsson, 2011). Hatching from their eggs as “alevin” or “sac fry” hatch individuals remain in their nursery redds for 3-6 weeks before becoming fry and leaving the gravel nests. Fry develop into well-camouflaged “parr.” These parr will either stay in their nest stream for 1-3 years, becoming silvery-coloured, ocean-going “smolts,” or become “precocious parr” (Thorstad et al., 2011).

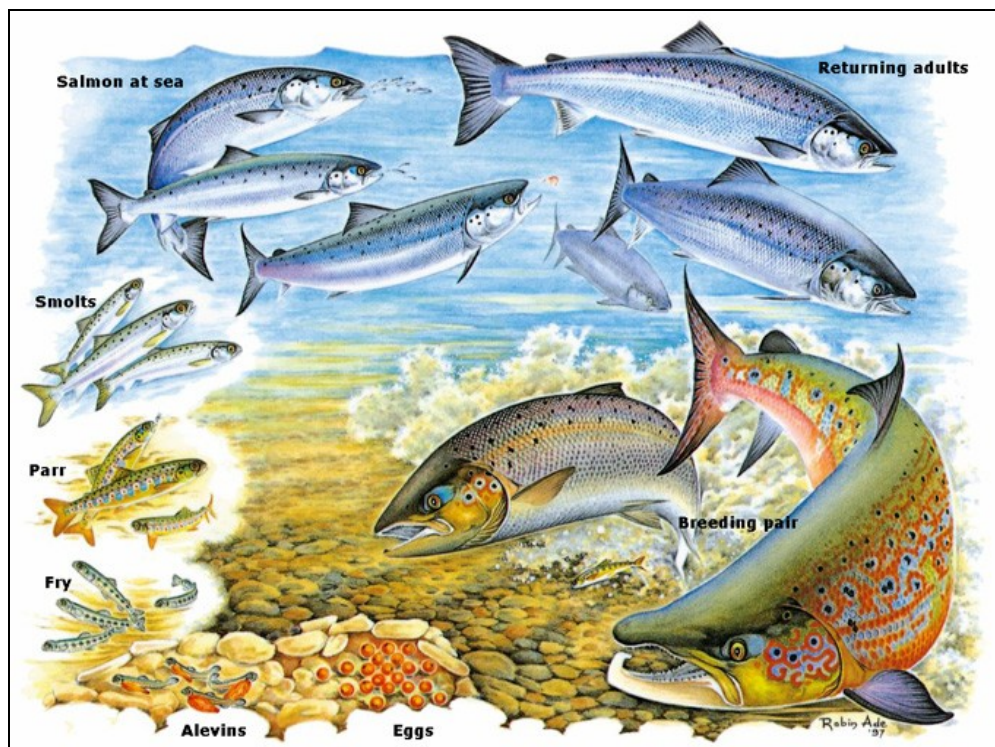


Figure 1.2 - Life-cycle of the anadromous eusalmonine *Salmo salar* (Atlantic salmon). Image by Robin Ade for the Atlantic Salmon Trust.

Precocious parr devote their resources to reaching sexual maturity as swiftly as possible, sometimes never leaving their freshwater stream. Following a breeding season, these precocious parr may either remain as sexually mature parr or undergo smoltification (Thorstad et al., 2011). Unlike the solely freshwater parr, smolts migrate to the ocean, developing into mature salmon within two to three years.

Upon reaching sexual maturity in the ocean, adults return to their native streams to spawn (Thorstad et al., 2011). Migration begins in late spring and early summer with spawning individuals reaching their respective streams by September and, depending on genetic and environmental conditions (including latitude), spawning from September to February. Between the arrival of adults to their nursery streams and spawning, competition between individuals leads to the establishment of a breeding hierarchy (Fleming, 1998; Haugland et al., 2011).

Individuals that survive the breeding season are called “kelts” or “black salmon” (Briggs, 1953; Thorstad et al., 2011). Kelts either over-winter in the nursery stream, conserving their energy before returning to the ocean, or return to the ocean directly. Survivorship in post-spawning individuals is characteristic of *Salmo* and *Salvelinus* (Behnke, 2002; Bley and Moring, 1988) and of *Hucho* (Esteve et al., 2009a, 2009b; Fukushima, 1994). These genera are iteroparous, meaning that they will breed multiple times in their lifetimes. The trout of *Oncorhynchus* (*O. mykiss* and *O. clarki*) are also iteroparous and have survivorship ratios similar to, and sometimes greater than, those of the other eusalmonines (Behnke, 1992, 2002; Fleming, 1998; Scott and Crossman, 1973; Willson, 1997). However, the salmon of *Oncorhynchus* (for example *O. nerka*, *O. gorbuscha*, and *O. kisutch*) are semelparous and, as such, are genetically programmed to die following spawning (Thorstad et al., 2011).

When mature eusalmonines travel to their breeding streams during the migratory period, adults experience a variety of changes to their physiology, body chemistry, and morphology (Behnke, 2002; Thorstad et al., 2011). These changes are most pronounced in males of *Oncorhynchus* and *Salmo*, but occur in both sexes and in virtually all species of Eusalmoninae to some degree (Moyle and Cech, 1996). Physiological changes include modifications in blood chemistry (Robertson et al., 1961), decreases in fat and protein content of tissues (Aksnes et al., 1986), an increase in plasma cortisol and thyroxine production (Pickering and Christie, 1981), and seasonal variation in gonadotrophin (Crim et al., 1975). Changes to morphology include gonad maturation (Aksnes et al., 1986), brighter and more vibrant colouration, an increase in body depth, particularly in Pacific salmon as a result of increased hump height, and an increase in adipose fin length (Hendry and Berg, 1999).

Of particular interest to this study are the spawn-run related modifications to the craniofacial skeleton. These craniofacial modifications are quite pronounced, leading to resorption of some bones and tissues such as the gill cover and postorbital, as well as growth of others such as the premaxillary and dentary (Fig. 1.3; Hendry and Berg, 1999; Witten and Hall, 2003). The combination of resorption and growth of bony tissues leads to an increased snout length in both males and females (Hendry and Berg, 1999). These modifications to the craniofacial skeleton culminate in the growth of an unusual combination of tissues and in the formation of a kype (Witten and Hall, 2003).

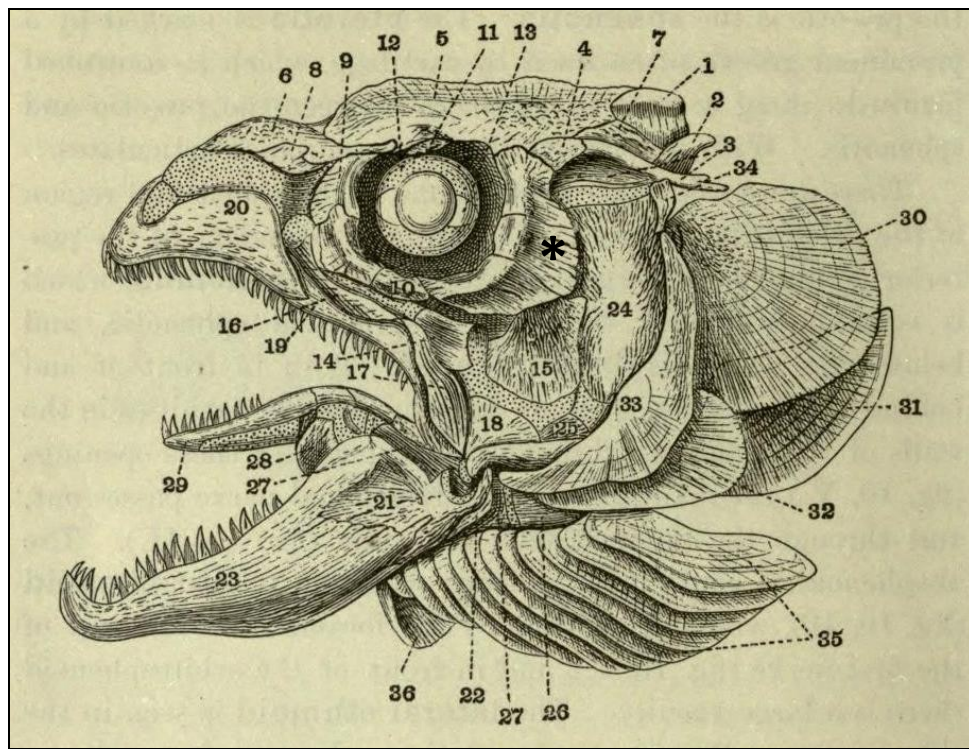


Figure 1.3 - Lateral view of Atlantic salmon (*Salmo salar*) skull. Structures of note: (asterisk) Post-orbital, (20) Premaxilla, (23) Dentary (with kype), (30) Operculum or “gill cover.” Image from Reynolds (1897).

### *The kype*

The kype is a spawning-related elongation and modification of the lower jaw (Witten and Hall, 2002, 2003). This lower jaw modification is a secondary sexual characteristic, a hook-like structure that develops as an extension of the most anterior portion of the dentary just prior to and during the initial stages of the spawn-run (Fig. 1.4; Mottley, 1936; Morton, 1965; Fukushima, 1994; Esteve et al., 2009a, 2009b).

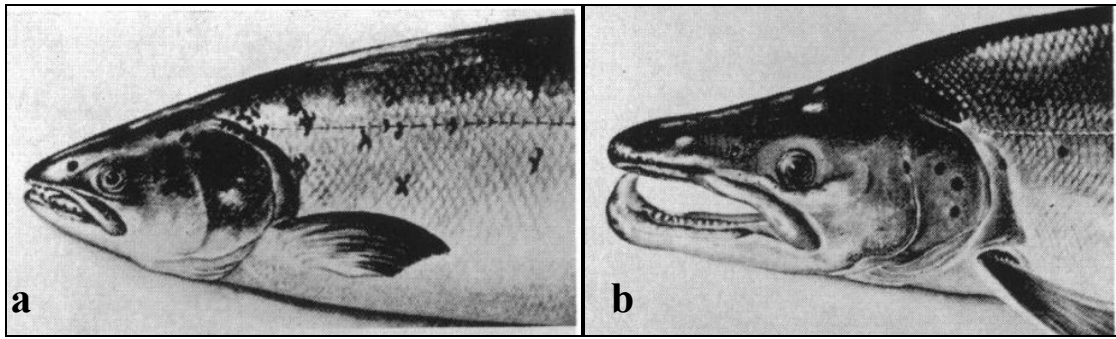


Figure 1.4 - Atlantic salmon head morphology. a) Prespawning and b) spawning males. Image modified from Vladykov (1953).

During formation of the kype, a variety of adjacent tissues and structures in the craniofacial skeleton undergo modification, in many ways compensating for the growth of the hooked lower jaw. These modifications may represent integrated changes in the upper and lower jaws and include the resorption of the post-orbital and the operculum noted above, as well as, depending on genus, compensatory modification in the upper jaw to accommodate for the hooked form of the kype (Lewis and Van Vliet, 2008; Witten and Hall, 2002). In many species of *Oncorhynchus*, altered curvature and elongation of the upper jaw compensate for kype growth and lead to the formation of a snout-like structure (Fig. 1.5 b; Morton, 1965). Furthermore, alteration to tooth structure and a reduction of soft lower jaw tissue reveals ‘breeding teeth’ in many salmonines (Witten et al., 2005). In Atlantic salmon, these teeth are feeding teeth already present in the jaw and are merely revealed to a greater extent as soft gum tissue regresses during kype formation (Witten et al., 2005). While Atlantic salmon do not develop true breeding teeth, the large anterior teeth prevalent in Pacific salmon may or may not be homologous to those in *Salar*, meaning that *Oncorhynchus* may indeed develop breeding teeth as part of their secondary sexual development.

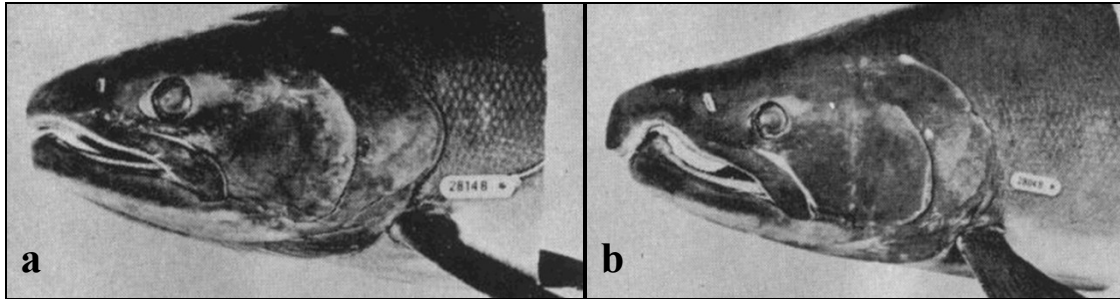


Figure 1.5 - *Oncorhynchus kisutch* head morphology. a) Pre-spawning and b) spawning males. Note the elongation of the upper jaw in addition to that of the lower jaw. Image modified from Vladykov (1962).

Histological analysis of the kype of Atlantic salmon conducted by Witten and Hall (2002, 2003) reveal that the kype is composed of a combination of two bony tissues: type I chondroid bone (hereafter “chondroid bone”) and Sharpey-fibre bone (Fig. 1.6). Kype-related modifications to the lower jaw and to its associated tissues are the result of the growth of, and modification to, this unusual combination of tissues (Witten and Hall, 2002, 2003).

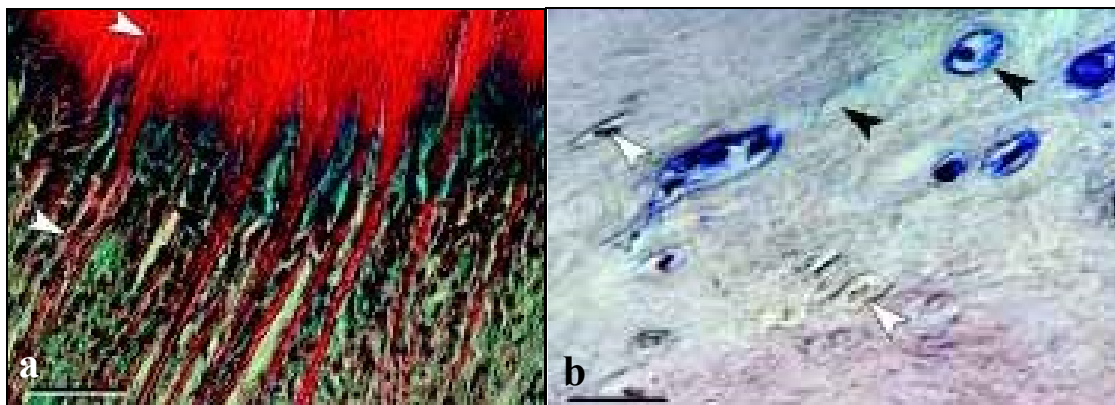


Figure 1.6 – Kype associated tissues. a) Sharpey-fibre bone. Needle-like spicules (white arrows) that extend from the periosteum of the dentary into the cartilage-rich tissues of the kype. b) Chondroid bone. A cartilage and bone intermediate tissue. Note the blue stain highlighting the collagen-II being secreted by chondrocytes embedded within surrounding matrix (black arrows). Also present are osteocyte-like cells (white arrows). Images from Witten and Hall (2002).

Chondroid bone is a cartilage/bone intermediate tissue that shares an initial developmental pathway with secondary cartilage (Beresford, 1981). Secondary cartilage is cartilage that develops in an embryo after the primordial cartilaginous skeleton has formed and persists post-natally or after hatching in birds (Bailleul et al., 2012; Beresford, 1981). This secondarily developing tissue can be differentiated from primary cartilage

using histological techniques because primary cartilage secretes only type II collagen while secondary cartilage presents with both collagen type I and type II (Bailleul et al., 2012; Witten and Hall, 2002, 2003). Furthermore, secondary cartilage lacks the linear organisation of primary cartilage and is found on a pre-existing bone's articulating surface.

Developing on pre-existing membrane bones, secondary cartilage frequently forms at sites under high mechanical influence such as suturing, articulation, and connection sites of ligaments and muscles (Bailleul et al., 2012). Secondary cartilage is induced as a means of buffering the local system when, during osteogenesis, periosteal cells respond to mechanical stress by switching their differentiation mode to chondrogenesis (Hall, 2005). As such, secondary cartilage forms from a periosteal precursor that can also differentiate into bone cells (Beresford, 1981; Hall, 2005).

For many years, chondroid bone was discussed synonymously with secondary cartilage (Beresford, 1981) and was argued to be an ossified form of secondary cartilage (Gussen, 1968; Moss, 1958). Sharing a similar developmental pathway as secondary cartilage, chondroid bone is a cartilage/bone intermediate tissue that develops from osteogenic precursors (Beresford, 1981; Hall, 2005; Huysseune, 1986; Huysseune and Verraes, 1986; Meunier and Huysseune, 1992). This intermediate tissue is formed when cartilage-secreting chondrocytes become embedded in an osteoid matrix secreted by local periosteum following secondary chondrogenesis (Gillis et al., 2006; Hall, 2005; Witten and Hall, 2003).

The formation of chondroid bone may occur from incomplete ossification of the primordial cartilaginous precursor (i.e.: incomplete endochondral ossification), yielding type II chondroid bone. Like secondary cartilage, chondroid bone may also occur from secondary chondrogenesis within the periosteum, a transdifferentiation of cells from osteoblast to chondroblast, yielding chondroid bone type I (Beresford, 1981; Gillis et al., 2006). Both ontogenetic pathways lead to a mineralized bony matrix that contains chondrocytes and osteocytes in addition to collagen types I and II (Gillis et al., 2006; Goret-Niçaise and Dhem, 1987). In fact, Beresford (1981) identified chondroid bone as being a cartilage/bone intermediate tissue that frequently contains chondrocyte-like cells within a bony matrix, associated with the new cartilage and bone with which it develops.

As a result, chondroid bone can be identified in histology when a chondrocyte-like cell, actively secreting collagen types I or II, is located within an osteoid matrix (Witten and Hall, 2002, 2003; Hall, 2005).

Contrary to the cartilage/bone intermediate identities of both secondary cartilage and chondroid bone, Sharpey-fibre bone is a bony tissue comprised of bundles of uncalcified and calcified collagenous fibres (“Sharpey’s fibres”) that extend from the periosteum (Hall, 2005; Witten and Hall, 2003). Found in several classes of vertebrates and in a variety of skeletal elements, Sharpey-fibres serve as connective tissues, attaching muscles to insertion sites, binding teeth to periosteum, or suturing cranial bones (Hall, 2005; Johnson, 2005; Kuroiwa et al., 1994; Retzlaff et al., 1982). These fibres permit some movement of bound structures, thereby accommodating for mechanical stress (Retzlaff et al., 1982). In histology, Sharpey-fibre bone is predominantly identified simply by its relatively unusual fibrous structure and its continuation from the periosteum of a bone matrix into surrounding connective tissue (Hall, 2005; Witten and Hall, 2002).

The combination of chondroid bone and Sharpey-fibre bone in the kype yields a basal architecture of fast-growing skeletal needles encased within a connective, chondroid tissue mass. Though Sharpey-fibres are frequently present in zones of attachment on the dentary, such as where teeth are bound to the jaw (Kuroiwa et al., 1994; Johnson, 2005), chondroid bone presence in the dentary region is noteworthy. However, chondroid bone originates from secondary chondrogenesis, thereby following a similar initial developmental pathway as secondary cartilage, a tissue that has been identified in the dentary of a eusalmonine (Atlantic salmon) in the past (Gillis et al., 2006). Gillis et al. (2006) determined that secondary cartilage is present in the tissues of parr, sexually immature Atlantic salmon. The presence of secondary cartilage in immature individuals, and the developmental similarity that this tissue share with chondroid bone, suggest that kype-related tissues emerge in individuals long before their kype begins to develop, and that during its evolution, the structure of the kype co-opted tissues already present in the dentary.

In addition to the rapid growth of the kype, further remodelling and reworking of the lower jaw occurs following spawning. While the majority of *Oncorhynchus* species die following spawning, many iteroparous species of *Salmo* and *Salvelinus* undergo a



remodification to their lower jaw (Behnke, 2002). During this remodification, many of the secondary sexual characters that formed in advance of spawning are resorbed and reduced back to their ‘normal’ (pre-spawning) conditions. Among the structures that are modified are the craniofacial elements, including the kype. For example, in Atlantic salmon many portions of the kype are demineralised and resorbed into the dentary post-spawn (Fig. 1.7; Witten and Hall, 2003). As tissue is resorbed, the numbers of needle-like Sharpey-fibres extending from the periosteum of the dentary are reduced even as bone density in associated structures such as the dentary itself declines.

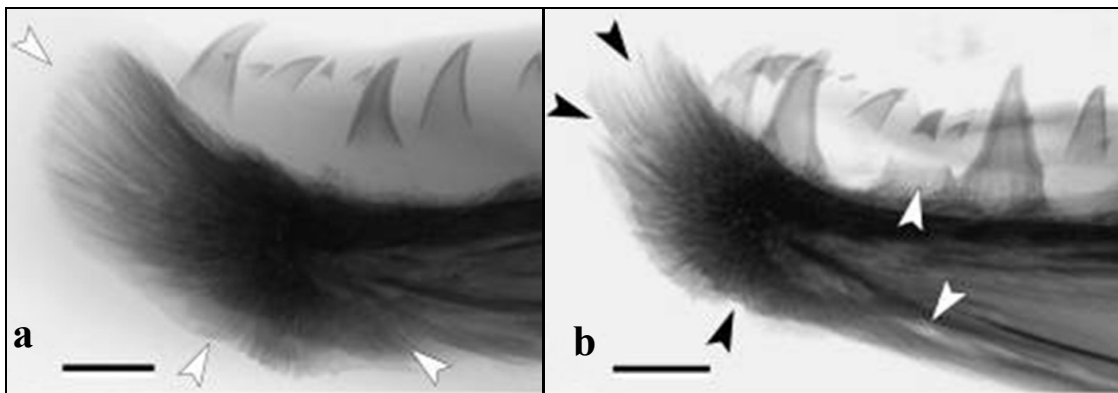


Figure 1.7 - Atlantic salmon kype before and after spawning. a) Pre-spawn, robust Sharpey-fibres (white arrows) jut from the apical portion of the dentary into the surrounding connective tissue. b) Post-spawn, Sharpey-fibres (black arrows) and bone density (white arrows) are reduced as a result of demineralisation and resorption of tissues. Images from Witten and Hall (2003).

Although resorption, demineralisation, and other alterations lead to a substantial decrease in the Atlantic salmon kype, not all portions of the kype are lost following spawning (Morton, 1965; Vladykov, 1953). This maintenance of some aspects of the kype structure means that following each subsequent breeding season an individual’s kype may become increasingly exaggerated (Morton, 1965). As a result, the oldest and largest males often have the largest and most elaborate kype.

### Research objectives

This thesis is divided into four data chapters. In Chapter 2, I discuss which genera within the Salmonidae display a feature resembling the kype of the Atlantic salmon. This will be done using gross anatomical inspections of fresh, or “fleshed” and skeletonized, or “defleshed” specimens as well as Micro-computer tomography (Micro-CT) assessments

of representatives of several genera. These assessments will attempt to establish evidence of the presence and absence of kype-like structures and tissues across the salmonids and begin to establish the structure and organization of tissues in the lower and, to a lesser extent, upper jaw. A literature survey incorporating both scientific and non-scientific resources will be used to supplement and confirm trends identified from specimens examined in this study.

Having established conditions and trends within the Salmonidae at the structural level, within Chapter 3 I will assess the features of the lower jaw of these fishes using histological assessments of tissues. I will consider variation within genera, incorporating comparisons of several species within the same genera at the same life history stage as well as making comparisons between anadromous and landlocked taxa. Finally, I will examine and compare any patterns in tissue morphology across all salmonid genera.

In Chapter 4, physical examinations, Micro-CT assessments, and a survey of the literature will be used to determine if a record of kype growth is preserved in the fossil record as well as if transitional states are represented in fossil material. In Chapter 5 paleohistological analysis will be used to establish evidence of kype growth and of the composition and organization of the tissues of the dentary. In both Chapter 4 and 5, I will use assessments of fossils and comparisons between fossil material and the living specimens of Chapters 2 and 3 to answer to consider evolutionary trends in salmonids relevant to the kype and attempt to determine when and how the kype evolved.

Chapter 6 summarizes the results discussed in the data chapters and considers possible areas of future research that may supplement this study.

### **Research Significance**

In addition to being the first family-wide assessment of the tissues of the salmonid lower jaw, both fossil and living specimens are examined and compared to one another in an effort to examine evolutionary patterns. This study is the first time kype presence and absence is directly examined in representatives of each salmonid lineage and also represents the first histological, paleohistological, and Micro-CT assessments of many of those lineages. As a result, this project provides the unique opportunity of establishing

many of the conditions and trends present in the lower jaw across this family of fish, revealing information on spawning related modifications across the salmonids.

## Chapter 2 – Presence and absence of the kype in living salmonids

### Introduction

As early as 1915, Dr. V. Tchernavin, at the time a naturalist of the Far-East Expedition of the Russian Department of Fisheries, began to notice that many spawning *Salmo* (Atlantic salmon) and *Oncorhynchus* (Pacific salmon) individuals experienced dramatic morphologic changes during their spawn-runs. Dr. Tchernavin would go on to become an expert on these morphologic changes, among them modifications in the craniofacial skeleton that resulted in prominent “rostra” on the upper jaws and “hooking” of the lower jaw (Tchernavin, 1938). In his research, Tchernavin described the lower jaw elongation and build-up of connective tissue at the anterior tip of the dentary that is known as the “kype”.

The kype, a secondarily developing characteristic found in several spawning salmonids (whitefish, grayling, salmon, trout, and chars), is a spawning-related elongation and modification of the anterior portion of the lower jaw resulting in a more-or-less prominent hook (Fig. 2.1; Morton, 1965; Stearley and Smith, 1993). Although the development of a kype has been used as a taxonomic feature since at least the early 1950s (Vladykov, 1953, 1954) and arguably much earlier (Day, 1887), the function, development, and many other aspects of the kype are poorly understood.

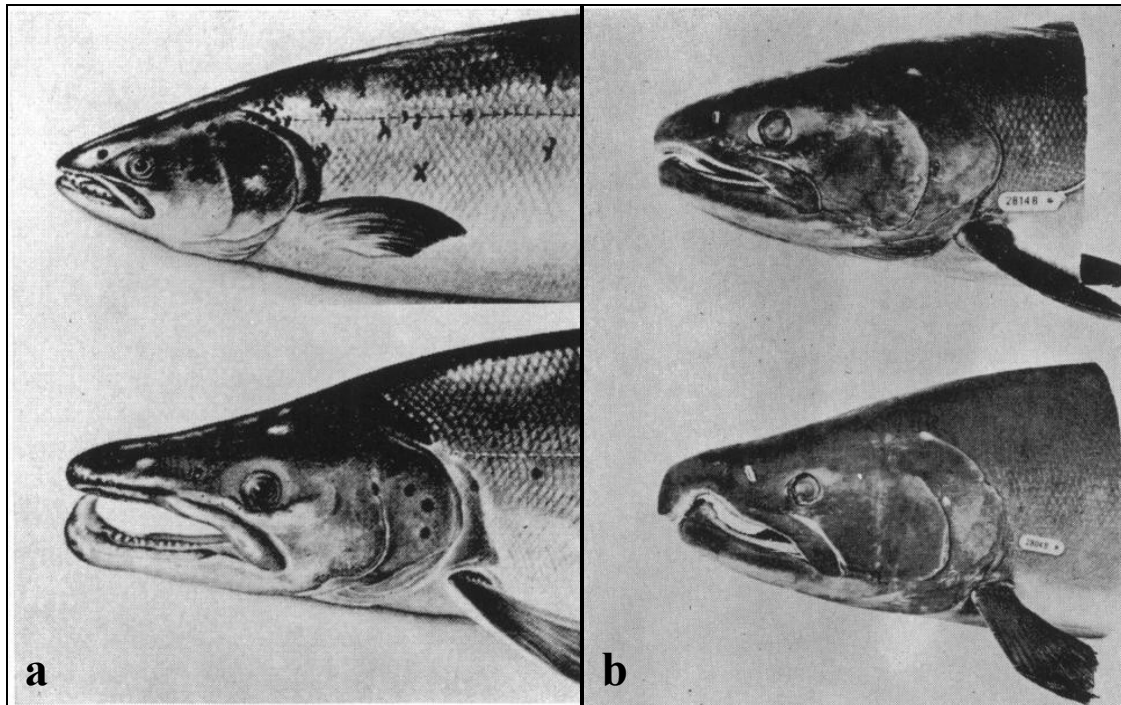


Figure 2.1 – A comparison of kypes in *Salmo* and *Oncorhynchus*. The kype is a secondarily-developing and spawning-related lower jaw modification found in many species of salmonids including (a) Atlantic salmon (*Salmo salar*) (Vladykov, 1953), and (b) coho salmon (*Oncorhynchus kisutch*), a Pacific salmon (Vladykov, 1962). Images depict pre-spawning (above) and spawning (below) males.

The kype is thought to play a number of roles in sexual selection (Jonsson and Jonsson, 2011; Morton, 1965). The kype may aid in the physical competition between spawning males over females and between females over nest sites, potentially serving as a weapon (Behnke, 2002; Jonsson and Jonsson, 2011). However, although contusions and wounds may be found on both living and dead animals, suggesting that combat has indeed taken place, actual sightings of individuals engaged in physical combat are relatively rare (Behnke, 2002; Morton, 1965). Instead, salmonids are typically able to establish hierarchies of dominance using non-violent displays and behavioural cues (Fleming, 1998; Fleming and Gross, 1989, 1994). Rather than serve as a weapon, the kype may be used as a means of maintaining hierarchy between males and between females rather than be a method of establishing those hierarchies. The kype also seems to play a role in mate selection, with females opting to spawn with males displaying the most dramatic secondary sexual characteristics, including the most prominent kype (Briggs, 1953; Morton, 1965). The growth of a prominent kype appears to be correlated with older, larger, and more dominant males. The kype may even play a role in female

reproductive behaviour, with some researchers suggesting that the kype is used by females to modify and to manipulate gravel and bottom sediment during nest-building (Thorstad et al., 2011). However, the true function of the kype remains unknown.

The precise trigger for kype development is also poorly understood. Other animals that have diadromous lifecycles, spending different portions of their lives in salt- and freshwater systems, such as sturgeon and lamprey, do not experience secondary sexual modifications in colour, behaviour, and morphology experienced by many salmonids (Gross et al., 1988; Harris et al., 2005; McDowall, 1997; Sulak and Randall, 2002; Vecsei, 2000). Some research has linked kype development in salmonids to hormonal changes in the body resulting from a switch from salt- to freshwater (Butts et al., 2012; Willson, 1997). However, this link has not yet been well substantiated by other studies, nor does it account for the differences in the kype development of non-anadromous species and populations.

While some earlier studies would indicate that the kype is a cartilagenous expansion (Stearley and Smith, 1993), recent work on Atlantic salmon, *Salmo salar*, by Witten and Hall (2002, 2003) has revealed much about the structure of the kype in this species. The skeleton of the kype incorporates chondroid bone and Sharpey fibres arising from the periosteum of the dentary bone, creating a spur-like network that supports an anterior connective tissue mass (Witten and Hall, 2002, 2003). However, kype structure in many other salmonid species, kype presence or absence, and extent of kype development remain unclear. The current study expands on the work of Stearley and Smith (1993), who used two kype characters (presence or absence and extent) when building their phylogenetic tree.

In other studies that have used the presence or growth of kype as a taxonomic feature, co-occurring modifications in the upper and lower jaws have also been noted (Morton, 1965; Scott and Crossman, 1973; Tchernavin, 1938; Valdykov, 1953, 1954, 1962). For example, modifications to the upper jaw that may compensate for changes in the lower jaw have been found (Behnke, 2002; Morton, 1965). Patterns in the development of these upper jaw features within each kype-developing taxa, their presence or absence across the salmonids, and their function remain to be determined.

This chapter will outline the presence or absence of the kype across the salmonids and will associate patterns in life history mode, behaviour, and morphology with presence or absence and extent of development of the kype. Such patterns may help explain the evolution and distribution of this character within a complex, and often controversial, phylogenetic tree (Stearley and Smith, 1993). Additionally, I will try to answer several questions about the presence or absence of the kype within the salmonid clade, the extent to which the kype develops, and the co-occurrence of features that are associated with the kype. I will also explain how patterns of kype growth may be used to validate and test current, proposed salmonid phylogenetic trees.

### **Methods and Materials**

A variety of techniques were used in an effort to garner as much information as possible in regards to the presence or absence of a structure resembling a kype, as well as other conditions in the upper and lower jaws of salmonids. These techniques include: physical examination of specimens, Micro-computer tomography scan analysis, and literature review.

#### ***Physical examination of specimens***

Representative specimens of twenty-three salmonid species (Table 2.1) were gathered from the field and from the collections and archives of several museums. The specimens collected in the field, namely six mature lake trout (*Salvelinus namaycush*) individuals, animals from a landlocked population in the Kogaluk River drainage system of Labrador (see McKraken et al. 2013), were collected and frozen by D. Ruzzante (Dalhousie University). These four specimens were not fixed prior to examination, though tissues of interest were fixed after examination. Following fixation, tissues were dehydrated in progressively higher ethanol solutions before being placed in 70% ethanol storage.

**Table 2.1 – Taxonomic grouping of relevant salmonid species including scientific and common names. Common names listed here are the most commonly used name for the species in general. Where possible, common names that differ with life history mode are listed. Compiled from Behnke (2002), Holčík et al. (1988), Roberge et al. (2002), Scott and Crossman (1973), and Stearley and Smith (1993).**

Sub-family	Genus	Species	Common name	
Coregoninae	<i>Coregonus</i>	<i>clupeaformis</i>	Lake whitefish	
	<i>Stenodus</i>	<i>Leucichthys</i>	Inconnu	
	<i>Prosopium</i>	<i>Williamsoni</i>	Mountain whitefish	
Thymallinae	<i>Thymallus</i>	<i>Arcticus</i>	Arctic grayling	
Salmoninae	<i>Brachymystax</i>	<i>Lenok</i>	Lenok; Asiatic or Manchurian trout	
(Eusalmoninae)	<i>Hucho</i>	<i>Hucho</i>	Huchen; Huchen trout; Danube salmon	
		<i>Perryi</i>	Sakhalin or sea-run taimen; Japanese huchen; Ito	
	<i>Salvelinus</i>	<i>Fontinalis</i>	Brook trout	
		<i>Namaycush</i>	Lake trout	
		<i>namaycush x fontinalis</i>	Splake	
		<i>Leucomaenis</i>	Iwana; white-spotted char	
		<i>Confluentus</i>	Bull trout	
		<i>Alpinus</i>	Arctic char	
		<i>Salmo</i> (Atlantic salmon and trout)	<i>Trutta</i>	Brown or sea-run (anadromous) trout
			<i>Salar</i>	Atlantic salmon (non-anadromous individuals may be called landlocked, lake, Ouananiche, or Sebago salmon, depending on population)
	<i>Oncorhynchus</i> (Pacific salmon and trout)	<i>Clarki</i>	Highly variable. Most commonly cutthroat trout	
		<i>mykiss</i>	Highly variable, but most commonly Landlocked: rainbow trout Anadromous: steelhead	
		<i>Kisutch</i>	Coho salmon	
		<i>Tshawytscha</i>	Chinook salmon; also king salmon	
		<i>keta</i>	Chum salmon; also dog and calico salmon	
		<i>gorbuscha</i>	Pink or humpback salmon	
		<i>nerka</i>	Anadromous: Sockeye salmon Non-anadromous: Kokanee	

Museum collection specimens were received preserved in one of two ways, being either dried skeletons (“defleshed” specimens) or fixed whole specimens in 70% ethanol (“fleshed” specimens). Due to the constraints of transportation and shipping, only the heads of the specimens anterior to the operculum were available for examination. Information as to specimen loaning institutes, collection date, collection site, and other relevant details about specimens can be found in Appendix A, while a list of the number and the species of specimens examined is recorded in Appendix B. Where not given, relative life history stage was estimated based on the snout to vent length (SL) and the



time of year when animals were collected. Where it was not possible to determine life history stage due to absence of collection date or SL information, animals were denoted as being “mature” or “immature” based on relative size.

Relative life-history stages are denoted as “pre-spawning,” “spawning,” and “post-spawning.” Pre-spawning animals are developing toward sexual maturity and have entered a period of starvation and, in non-resident animals, migration. This is also the life history stage when male-male and female-female hierarchies are established and secondary sexual characteristics develop. Spawning animals have entered into the reproductive stage and have achieved sexual maturity and are experiencing the greatest amount of sexual dimorphism, including that of the kype size and complexity. The period following reproduction, post-spawning is the period of high mortality when animals have increased resource requirements to compensate for pre-spawn starvation. This is also the period when secondary sexual characteristics, including kype, are diminishing in size and extent.

Using the conditions found in *Salmo salar* as a guide (Witten and Hall 2002, 2003; Tchernavin, 1938), specimens were examined for evidence of kype, in so far as a modification in the bony tissue (typically yielding a honeycomb-like meshwork) or an accumulation of soft tissue at the anterior-most lower jaw resulting in a hook-like structure. Other craniofacial structural changes, particularly those in the upper jaw compensating for a growth in the kype, were also considered.

#### ***Micro-computer tomography (Micro-CT) Scanning***

Several ‘fresh’ specimens were also Micro-CT scanned as a means of obtaining information on internal structures and patterns of tissue density, in addition to the external structures assessed by gross anatomical review.

Specimens were scanned at the National Research Council Canada Biomedical MRI Research Lab (Halifax, NS) using the Triumph X-O CT system of a Lab PET 4 machine. Specimens were placed on the scanning bed, following which the bed was mechanically shifted into the main housing of the scanning system. With the specimen in place, the position of the rotating X-ray gantry was adjusted so that the specimen and bed would both be cleared during the scan and scanned at the highest resolution and

magnification possible. Typically, the position of the gantry in relation to the specimen resulted in 1.3 to 3.5 times magnification, or 178 to 66 micron ( $\mu\text{m}$ ) resolution. Scans were made using radiation of 80 kilovoltage (kVp) with 512 projections over a 360 degrees rotation. Scanning information for specific specimens (including species, specimen ID code, and magnification and resolution of scans) is shown in Table 2.2.

**Table 2.2 – Micro-CT scanning information for modern specimens. Voltage is in units of peak kilovoltage (kVp), resolution is in micrometres ( $\mu\text{m}$ ).**

	Specimen ID	Condition	Voltage (kVp)	Magnification	Resolution ( $\mu\text{m}$ )	
<i>Brachymystax</i>						
-	<i>B. lenok</i>	UW 022131	Fleshed	70	3.5	66
<i>Hucho</i>						
-	<i>H. hucho</i>	UW 022130	Fleshed	80	2.0	115
<i>Salmo</i>						
-	<i>S. salar</i>	UW 019807	Fleshed	80	2.0	115
-	<i>S. salar</i>	CMNFI-1980-0181.1	Defleshed	80	3.0	77
<i>Oncorhynchus</i>						
-	<i>O. gorbuscha</i>	UW 16027	Fleshed	70	1.3	178
-	<i>O. nerka</i>	UW 005624	Fleshed	80	2.0	115
-	<i>O. nerka</i>	CMNFI-1977-0277.1	Defleshed	80	3.0	77

After specimens were scanned, projections from individual scans were digitally stacked using the Lab PET Triumph operating system, resulting in files that could be opened and manipulated with standard imaging software; the public domain software Image J was used for this project. Following adjustments for contrast and yield, scans could be used to reconstruct three-dimensional renderings of the specimens and portions of scans could be analyzed as two-dimensional slices.

### ***Literature review***

A literature survey of academic and more “popular” literature was used to provide an authoritative review of the conditions of kype within populations, species, and genera. In assessments of kype presence, only material that directly noted the kype or that noted alterations in the anterior craniofacial skeleton, specifically in the lower jaw but, to a

lesser extent, the upper jaw as well, was considered. Literature was also used to gather information as to life history, habitat, and spawning conditions across the Salmonidae.

## **Results**

### ***Physical examinations of specimens***

The heads of representative specimens for species in each group within the Salmonidae were examined. The following section details the craniofacial morphology of specimens, with particular focus on the aspects of the anterior lower jaw. Description of colouration was not considered due to the loss of true pigments during the fixation process. Specimens are described in the order of their phylogenetic position (Fig. 2.2). During each examination, particular focus was paid to the jaw bones of each specimen, namely the dentaries, maxillae, and premaxillae (Fig. 2.3), though other characteristics such as the size of the eye, head, and mouth were also considered so as to standardize relative size of structures of interest. As a means of giving context, included below is a brief description of the life history characteristics of each subfamily and, where appropriate, genus. The results of these physical examinations are summarized in Table 2.3.

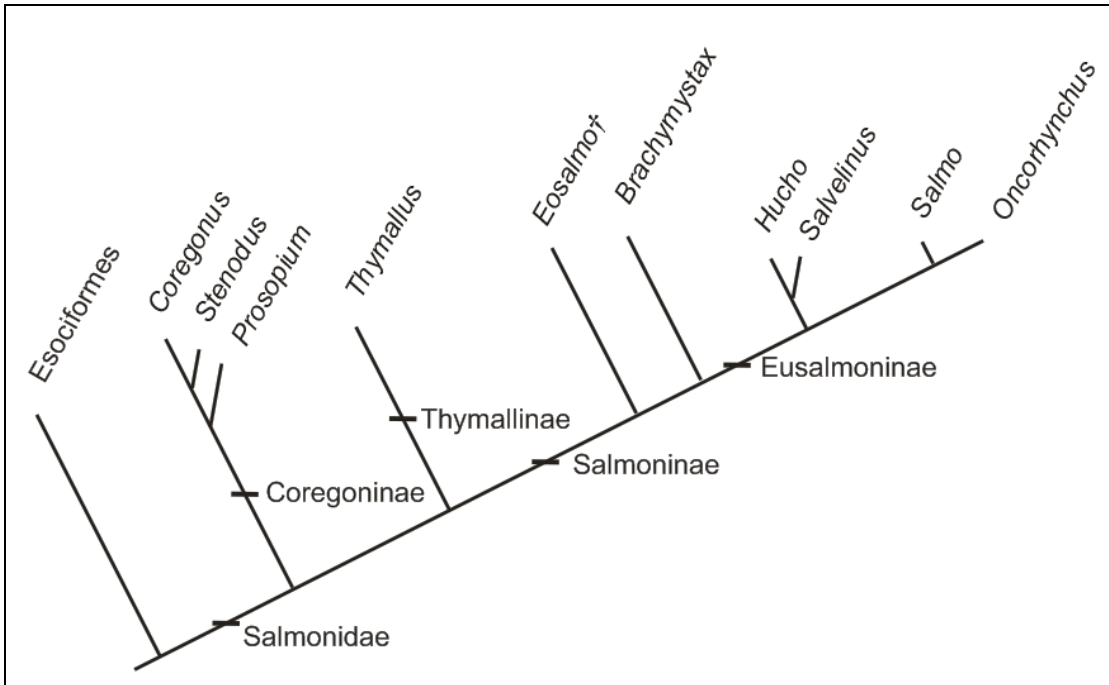


Figure 2.2 – Salmonid phylogenetic tree indicating the three salmonid subfamilies Coregoninae (whitefishes), Thymallinae (grayling), and Salmoninae [false and true (Eusalmoninae) salmon and trout]. Modified from Stearley and Smith (1993) and based on morphological evidence.

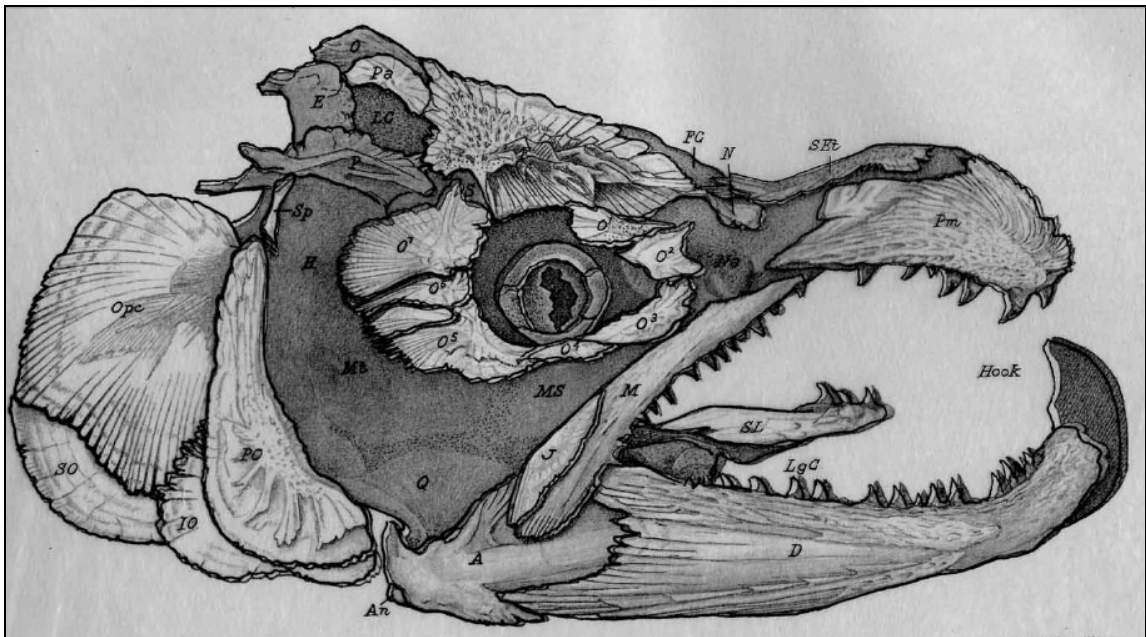


Figure 2.3 – Skull bones of *Salmo salar* in late stages of kype development. Premaxillary (Pm), Maxillary (M), and dentary (D) are particularly important to the present study. From Tchernavin (1938).

**Table 2.3 – Summary of kype presence and absence in living specimens.**

Genus and species	Specimen ID	Condition	Life history stage	SL (mm)	Sex	Kype present (P) or absent (A)
<i>Coregonus</i>						A
- <i>C. clupeaformis</i>	UW 05587	Fleshed	Spawning	440	Male?	A
<i>Stenodus</i>						A
- <i>S. leucichthys</i>	CMNFI-Z004302	Defleshed	Pre-spawning	660	Male	A
<i>Prosopium</i>						A
- <i>P. williamsoni</i>	UW 005260	Fleshed	Spawning	370	Male	A
- <i>P. williamsoni</i>	CMFI-1969-0203.1	Defleshed	Post-spawning	254	Unsexed	A
<i>Thymallus</i>						A
- <i>T. arcticus</i>	CMNFI 1977-ID undesignated	Defleshed	Pre-spawning	390	Unsexed	A
<i>Brachymystax</i>						A
- <i>B. lenok</i>	UW 022131	Fleshed	Immature	126	Unsexed	A
<i>Hucho</i>						A
- <i>H. hucho</i>	UW 022130	Fleshed	Post-spawning	473	Male	A
- <i>H. hucho*</i>	ZIN 7872	Fleshed	Mature	358	Unsexed	A
- <i>H. perryi*</i>	ZIN 13160	Fleshed	Spawning	395	Unsexed	A
- <i>H. perryi*</i>	ZIN (ID unknown)	Defleshed	Mature	598	Unsexed	A
<i>Salvelinus</i>						P
- <i>S. fontinalis</i>	UW 28888	Fleshed	Mature	285	Unsexed	A
- <i>S. fontinalis</i>	CMFI 1973-0255.1	Defleshed	Pre-spawning	215	Unsexed	A
- <i>S. namaycush</i>	ZP 2013-07-01(A)	Fleshed	Mature, pre-spawning?	880	Unsexed	A
- <i>S. namaycush</i>	ZP 2013-07-02(B)	Fleshed	Mature, pre-spawning?	Unrecorded (670-880)	Unsexed	A

Genus and species	Specimen ID	Condition	Life history stage	SL (mm)	Sex	Kype present (P) or absent (A)
<i>Salvelinus</i>						P
- <i>S. namaycush</i>	ZP 2013-07-03(C)	Fleshed	Mature, pre-spawning?	Unrecorded (670-880)	Unsexed	A
- <i>S. namaycush</i>	ZP 2013-07-04(D)	Fleshed	Mature, pre-spawning?	670	Unsexed	A
- <i>S. namaycush</i>	ZP 2013-07-05(E)	Fleshed	Mature, pre-spawning?	Unrecorded (670-880)	Unsexed	A
- <i>S. namaycush</i>	ZP 2013-0706(F)	Fleshed	Mature, pre-spawning?	Unrecorded (670-880)	Unsexed	A
- <i>S. namaycush</i>	UW 020761	Fleshed	Pre-spawning	Unrecorded	Unsexed	A
- <i>S. namaycush</i>	CMNFI 1982-0385	Fleshed	Mature	400	Unsexed	A
- <i>S. namaycush</i>	CMNFI 1958-0100A.1	Defleshed	Post spawning	Unrecorded, large	Male	A
- <i>S. namaycush</i> / <i>S. fontinalis</i> hybrid ("splake")	CMNFI 1976-0187.1	Defleshed	Immature	342	Unsexed	A
- <i>S. leucomaenis</i>	UW 042437	Fleshed	Pre-spawning	216	Unsexed	A
- <i>S. confluentus</i>	UW 20760	Fleshed	Pre-spawning	500	Male	P
- <i>S. alpinus</i>	CMNFI 1968-1262.1	Fleshed	Spawning	410	Unsexed	P
- <i>S. alpinus</i>	CMNFI 1961-0229	Fleshed	Pre-spawning, spawning	470	Male	P
- <i>S. alpinus</i>	CMNFI 1977-0348.1	Defleshed	Mature	380	Male	P?
- <i>S. alpinus</i>	CMNFI 1979-1001.1	Defleshed	Immature	Unrecorded, large	Unsexed	A
- <i>S. alpinus</i>	UW 041192	Fleshed	Immature (nearly mature?)	410	Immature (nearly mature?)	A
- <i>S. alpinus aureolus</i>	UW 020740	Fleshed	Immature (nearly mature?)	~300	Unsexed	A
<i>Salmo</i>						P
- <i>S. trutta</i>	UW 005350	Fleshed	Pre-spawning	290	Male	P
- <i>S. salar</i>	UW 019807	Fleshed	Mature	250	Unsexed	A
- <i>S. salar</i>	CMFI 1980-0181.1	Defleshed	Spawning	610	Male	P

Genus and species	Specimen ID	Condition	Life history stage	SL (mm)	Sex	Kype present (P) or absent (A)
<i>Oncorhynchus</i>						P
- <i>O. clarki</i>	UW 028776	Fleshed	Pre- spawning	242	Unsexed	P?
- <i>O. clarki</i>	CMNFI 1973-0393.1	Fleshed	Pre-spawning	221	Unsexed	A
- <i>O. mykiss</i>	UW 016073	Fleshed	Immature	~200	Unsexed	A
- <i>O. mykiss aguabonita</i>	UW 015967	Fleshed	Spawning	310	Female	P?
- <i>O. kisutch</i>	UW 015091	Fleshed	Pre-spawning	Unrecorded, large	Female?	A
- <i>O. kisutch</i>	CMFI Z000804	Defleshed	Spawning	Unrecorded, large	Male	P
- <i>O. tshawytscha</i>	UW 015893	Fleshed	Pre-spawning	330	Unsexed	A
- <i>O. keta</i>	UW 13664	Fleshed	Pre-spawning	Unrecorded, large	Male	A
- <i>O. gorbuscha</i>	UW 16027	Fleshed	Spawning	430	Male	P
- <i>O. gorbuscha</i>	CMNFI 1984-0169.1	Fleshed	Mature, pre-spawning?	235	Unsexed	A
- <i>O. nerka</i>	UW 005624	Fleshed	Mature	367	Unsexed	A
- <i>O. nerka</i>	CMNFI 1977-0277.1	Defleshed	Spawning	Unrecorded, large	Male	P

\*Received photographs and/or X-Ray images of specimens, but did not have physical access to specimens

## **Coregoninae**

Coregonids are typically freshwater living, though some populations of certain species are at least partly anadromous, making moderate to extensive migrations to brackish conditions (Behnke, 1972; Scott and Crossman, 1973). The coregonids are almost exclusively iteroparous, spawning multiple times over the course of their lives (Scott and Crossman, 1973). Survivorship and repeat spawning frequency varies across genera (Lindsey and Woods, 1970). Members of this subfamily tend to migrate in the summer, almost always broadcast spawning in lakes and sometimes in rivers during the fall (Lindsey and Woods, 1970; Scott and Crossman, 1973); they do not build or recycle nests (Willson, 1997). There tends to be little sexual dimorphism among the coregonids, though some genera may have minimal dimorphism evident just prior to and during spawning, with some males growing breeding or nuptial tubercles (“pearl organs”) on their flanks (Behnke, 2002; Lindsey and Woods, 1970; Scott and Crossman, 1973).

### ***Coregonus clupeaformis* (lake whitefish)**

UW 05587 (fleshed); Figure 2.4; spawning male

Specimen has a small, sub-terminal mouth with maxillae extending to just anterior of anterior margin of the large eye. Upper and lower jaws terminate at an equal distance from the eye, though the maxillae are somewhat curved and is slightly longer. There is no evidence of teeth or any suggestion of a kype.



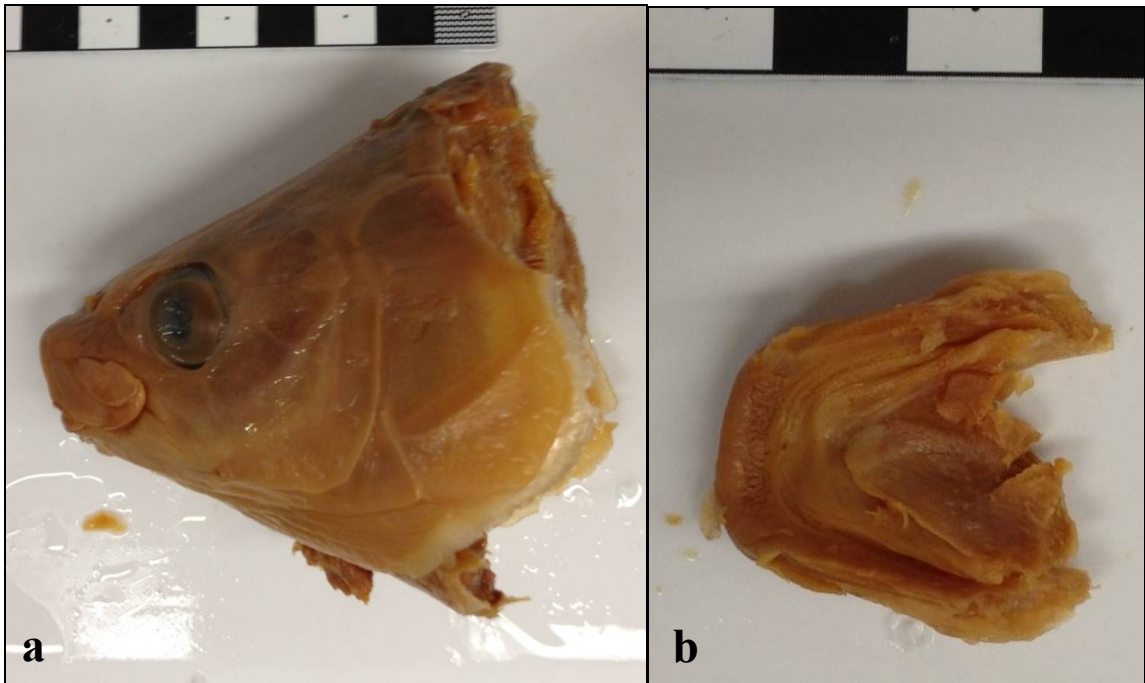


Figure 2.4 – Fleshed *Coregonus clupeaformis* UW 05587, (a) head and (b) lower jaw.

***Stenodus leucichthys (inconnu)***

CMNFI-Z004302 (defleshed); Figure 2.5; pre-spawning male

Many, very small teeth are present on the premaxillae, maxillae, dentaries, and vomer. Teeth are so fine and delicate that they are difficult to distinguish, particularly on the dentaries where they are localized to the anterior-most aspect. Maxillae and dentaries are relatively long and robust, particularly the dentaries. The dentary symphysis, the fibrocartilaginous joint between the two lower jaw bones (Hall, 2005), remains prominent, and the two dentaries are well-fused. There is no visible evidence of a kype.



Figure 2.5 – Defleshed *Stenodus leucichthys* CMNFI-Z004302, lower jaw in (a) dorsal and (b) medial view.

***Prosopium williamsoni* (mountain whitefish)**

UW 005260 (fleshed); pre-spawning male

This specimen has a small head and a small, subterminal mouth. The maxillae extend to just posterior of the anterior margin of the large eye. There is no visible dentition, nor is there any soft tissue build up at the anterior aspect of the lower jaw that would suggest a kype.

CMNFI-1969-0203.1 (defleshed); Figure 2.6; pre-spawning, unsexed

Skull elements of this specimen are rather small and the majority of the skull remains articulated, making examination difficult. However, the mouth is small and subterminal, with maxillae extending to just posterior of the anterior orbit margin. The dentaries are relatively robust, and there is little evidence of teeth on any of the visible jaw bones. The symphysis of the lower jaw remains fused, with no evidence of kype-related reworking or honeycombing adjacent to it.

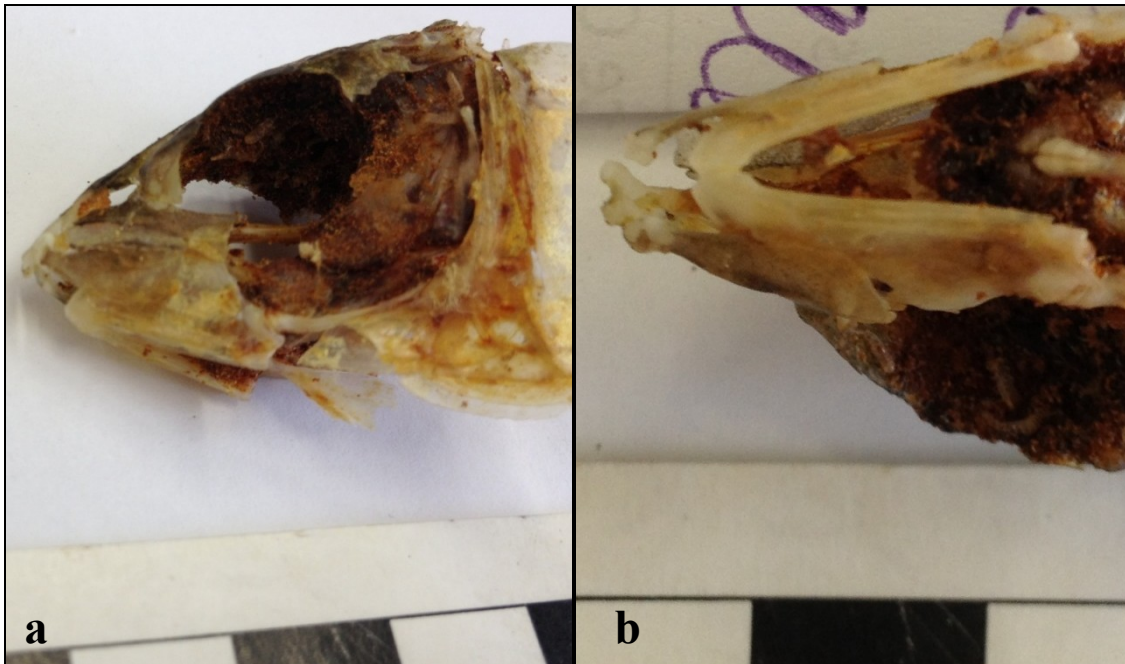


Figure 2.6 – Defleshed *Prosopium williamsoni* CMNFI-1969-0203.1, skull in (a) lateral and (b) ventral view.

These specimens do not display evidence of kype development in Coregoninae.

### **Thymallinae**

Thymallinae contains the single genus *Thymallus*, which in turn contains approximately four different species and over twenty sub-species. Relatively little variation in spawning behaviour amongst the different groups exists, with the majority of species being defined by geographic distribution rather than major differences in behaviour or morphology (Armstrong, 1986; Scott and Crossman, 1973). Given this relative lack of variation, the

following description of *Thymallus arcticus* (Arctic grayling) is typical of the genus *Thymallus* and of the subfamily Thymallinae

*Thymallus arcticus* is primarily freshwater living, though some Asian populations may be at least partly anadromous (Armstrong, 1986; Scott and Crossman, 1973). Migration and spawning occur in spring and summer (March to July) (Roberge et al., 2002; Scott and Crossman, 1973), but timing is very dependent both on ice break-up (Beauchamp, 1990; Craig and Poulin, 1975; Roberge et al., 2002; Scott and Crossman, 1973) and on water temperature (Behnke, 2002). *T. arcticus*, and other Thymallinae, are iteroparous and relatively long lived, living up to 22 years (Beauchamp, 1982, Scott and Crossman, 1973; Willson, 1997), though such longevity is rare (Behnke, 2002). Spawning does not occur each year for all populations (Alt, 1969; Willson, 1997). When spawning does occur, nests are not used (Roberge et al., 2002). However, there is some parental care in that eggs are deposited in the interstitial space of gravel and rock (Behnke, 2002) or are buried in the silt at the bottoms of lakes and, more particularly, streams (Roberge et al., 2002; Scott and Crossman, 1973; Willson, 1997). Males are territorial, and females spawn in the territory of their chosen male (Morrow, 1980; Willson, 1997). Contrary to many other basal salmonids, *Thymallus* does display dramatic sexual dimorphism at the time of spawning (Scott and Crossman, 1973). While this is seen even more dramatically in the Mongolian grayling (*T. brevirostris*; Berg, 1962), *T. arcticus* males have more vibrant colouration than females and may have larger and longer pelvic and dorsal fins, particularly at the time of spawning (Carlander, 1969; McPhail and Lindsey, 1970).

***Thymallus arcticus* (Arctic grayling)**

CMNFI-1977-ID undesignated (defleshed); Figure 2.7; pre-spawning, unsexed  
Jaw bones are very small and thin. Small, needle-like teeth are present on all jawbones and are absent from palatine and vomer. Dentaries are rather short and squat, while premaxillae are relatively wide. There is no evidence of honeycombing or reworking at the anterior of the dentaries and, therefore, no evidence of a kype.

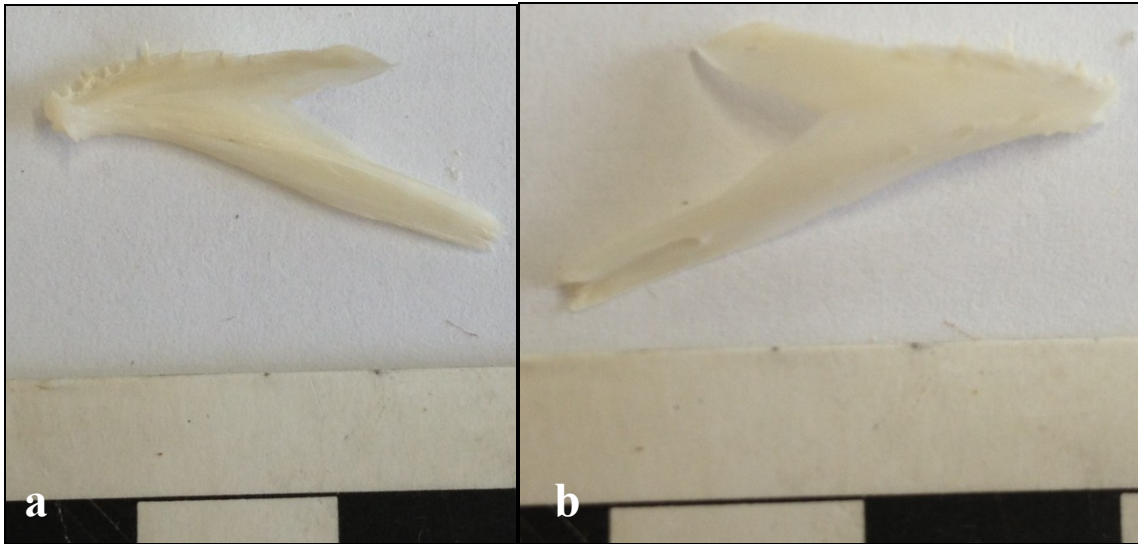


Figure 2.7 – Defleshed *Thymallus arcticus* CMNFI-1977-ID undesignated, right dentary in (a) medial and (b) lateral view.

Absence of a kype in this specimen would suggest that thymallines do not develop a kype.

### **Salmoninae**

Conditions in this subfamily are highly variable and are outlined by genus, below.

#### ***Brachymystax***

As noted by Esteve and McLellan (2008), English-language publications that discuss the behaviour of *Brachymystax* are relatively rare. Those that are available tend to focus on the “typical” *Brachymystax* species, *B. lenok*.

*Brachymystax lenok* is solely freshwater living (Hendry and Stearns, 2004; Nelson, 1994, 2006; Willson, 1997). This species experiences very minimal migrations within its freshwater systems during the spring, spawning soon after arrival at spawning site (Mitrofanov and Petr, 1999). Many populations may engage solely in vertical migrations, particularly those populations residing in lakes, migrating from moderately deep water at lake centres to shallower waters near the shore (Kifa, 1974; Mitrofanov and Petr, 1999). Spawning time is highly depending on ice-melt, and some populations and sub-species may spawn in February or March (Mitrofanov and Petr, 1999; Zhao and

Zhang, 2009). *B. lenok* is iteroparous (Mitrofanov and Petr, 1999). Maximum lifespans of at least 9 years have been recorded, though this species may live considerably longer (Mitrofanov and Petr, 1999). Survivorship and repeat spawning frequencies are unknown. When spawning, this species makes use of nests and prefers cold, fast moving, shallow rivers with gravel substrates (Baimukanov, 1996; Esteve and McLellan, 2008; Kifa, 1974; Mitrofanov and Petr, 1999; Xu et al., 2009). Females select their nest site within the territories defended by their males of choice (Esteve and McLellan, 2008).

*Brachymystax* displays minimal sexual dimorphism, even during the spawn-run (Esteve and McLellan, 2008; Mitrofanov and Petr, 1999). Spawning males may experience moderately more vibrant colour changes than do females, but behaviour is the only reliable way to distinguish the sexes (Esteve and McLellan, 2007, 2008). This behaviour includes females digging nests and testing nests by probing with their anal fin, while males quiver next to females to stimulate egg-laying.

***Brachymystax lenok* (lenok)**

UW 022131 (fleshed); Figure 2.8; immature, unsexed

The head is small, with a relatively rounded snout. A terminal mouth is also small and the maxillae extend no further than the midpoint of the very large eye. No overt evidence of teeth is apparent, which is expected from a juvenile. Dentaries are very delicate, with a rounded tip that shows no evidence of connective tissue build-up or other features suggestive of a kype.

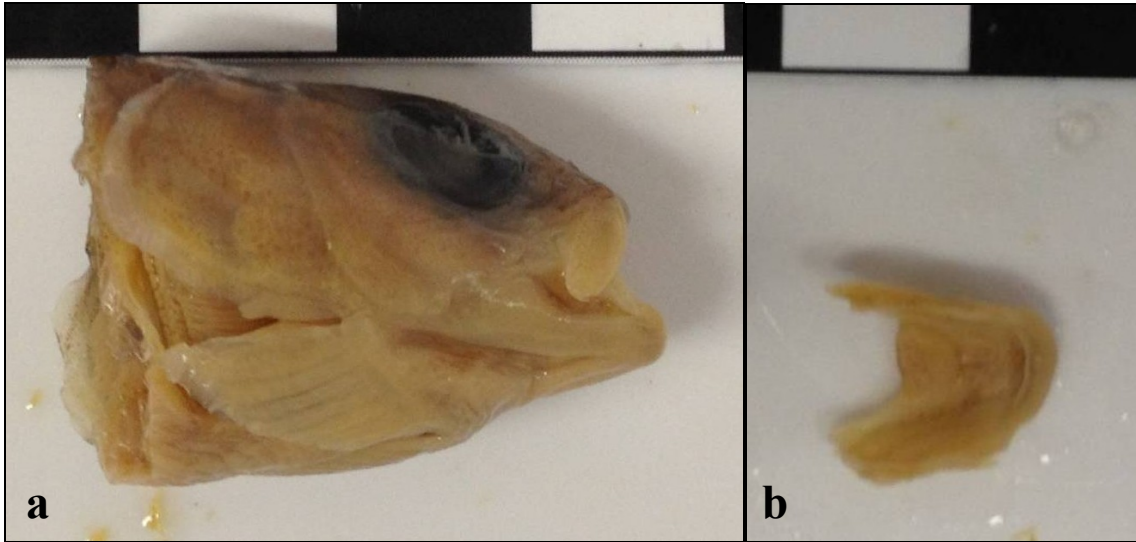


Figure 2.8 – Fleshed *Brachymystax lenok* UW 022131 (a) head and (b) lower jaw.

## Eusalmoninae

### *Hucho*

To give context, the phylogenetic relationships of the species of the *Hucho* genus are shown in Figure 2.9.

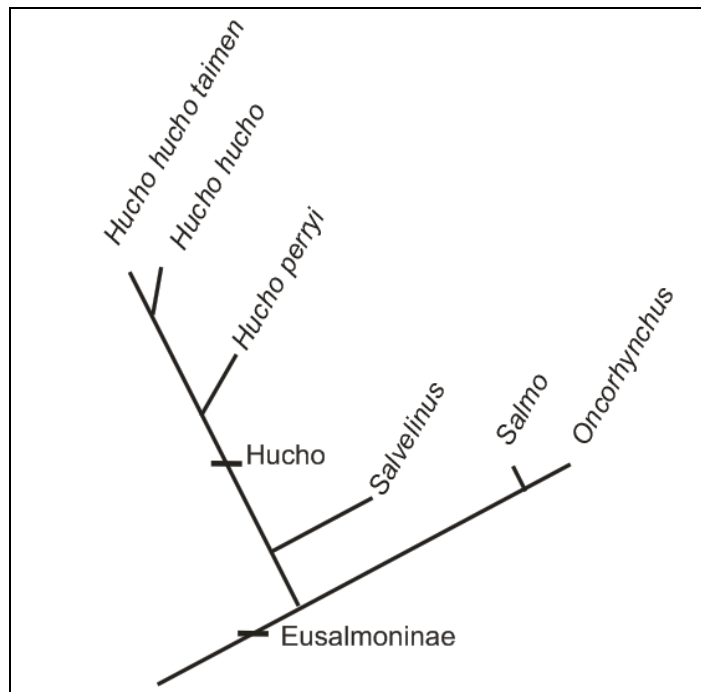


Figure 2.9 – Eusalmonine phylogenetic tree with particular focus on the genus *Hucho*. Modified from Holčík et al. (1988); Stearley and Smith (1993) and based on morphological evidence.

As with *Brachymystax*, primary literature for *Hucho* is rarely written in English (Esteve et al., 2009a). Nonetheless, information as to the various characters and behaviours of this genus is available. Unfortunately, this information is often generalized. The species of this genus are highly variable in behaviour, habitat, life history mode, and morphology; the following is a simplified overview of conditions within this genus.

While *Hucho hucho* (Huchen) is exclusively freshwater living (Hendry and Stearns, 2004; Willson, 1997), *Hucho perryi* (Sakhalin taimen) has relatively few non-anadromous populations and tends towards anadromy spending much of its life in coastal or brackish waters (Fukushima, 1994; Gilroy et al., 2010; Hendry and Stearns, 2004; Kawamura et al., 1983; Holčík, 1982; Holčík et al., 1988). Migrations may be extensive or moderate, depending on the population and on the geography of a given region (Fukushima, 1994; Kimura, 1966). In some populations of *H. hucho*, migrations may be as short as from one riverbank to another or from a river into an adjacent tributary (Holčík et al., 1988; Witkowski, 1988). Furthermore, not all populations migrate, and some may stay in their home streams and rivers for their entire lives (Dulmaa, 1999; Holčík et al., 1988; Mitrofanov and Petr, 1999). Migration occurs in spring and summer, usually from mid-March to July, and is very dependent on latitude and water temperature (Berg, 1962; Fukushima, 1994; Holčík et al., 1988; Kimura, 1966). Once animals have reached the spawning ground, they will begin almost immediately to engage in nest building and subsequent spawning (Esteve et al., 2009a; Holčík et al., 1988; Witkowski, 1988).

*Hucho* species are iteroparous and relatively long-lived, with a maximum lifespan of 10 years for *H. hucho* and 16 years for *H. perryi*, though even longer lifespans are likely (Esteve et al., 2009; Fukushima, 1994; Holčík et al., 1988). Animals are capable of spawning once per year, but many populations spawn only once every three years. Males do not engage in precocious spawning behavior (Esteve et al., 2009b). Thus, rather than having alternative life histories where some males devote their resources to reaching sexual maturity as quickly as possible, all males mature “normally” and achieve sexual maturity following typical development.

*Hucho* species are typically nest builders and prefer to spawn in shallow rivers and streams with sand to rock substrates (Esteve et al., 2009a; Fleming, 1998; Fukushima,



1994; Holčík et al., 1988), though lake-living populations spawn on rocky and sandy substrates near-shore (Fleming, 1998; Holčík et al., 1988). While no dimorphism outside of spawn-run is exhibited by this species, some colour changes during spawning do occur (Holčík et al., 1988). These colour changes result in males with copper-red flanks and bluish-green heads, while females become uniformly olive-green to greyish (Esteve et al., 2009a, 2013; Holčík et al., 1988). There is some variation in about 10% of spawning individuals, however, with females becoming more brightly coloured than their male counterparts (Esteve et al., 2009a, 2013). There is also some evidence that male and female *H. hucho* individuals may develop breeding tubercles near the anal fin, but this evidence is not well substantiated (Holčík et al., 1988). However, the most reliable way to distinguish males from females is by using behaviour: males quiver against females to stimulate egg-laying, while females build and probe nests, similar to what was seen in *Brachymystax* (Esteve et al., 2009b; Esteve et al., 2013).

### ***Hucho hucho* (Huchen)**

UW 022130 (fleshed); Figure 2.10; post-spawning male

The head is large, long, and compressed dorsally. The terminal mouth is large, approximately half the length of the head. Maxillae extend well posterior of posterior margin of a relatively small eye. Maxillae and dentaries are robust. Well-developed, sharp teeth are present on, vomer, premaxillae, maxillae and dentaries. The anterior aspect of the lower jaw is rounded and shows no external features suggestive of a kype.

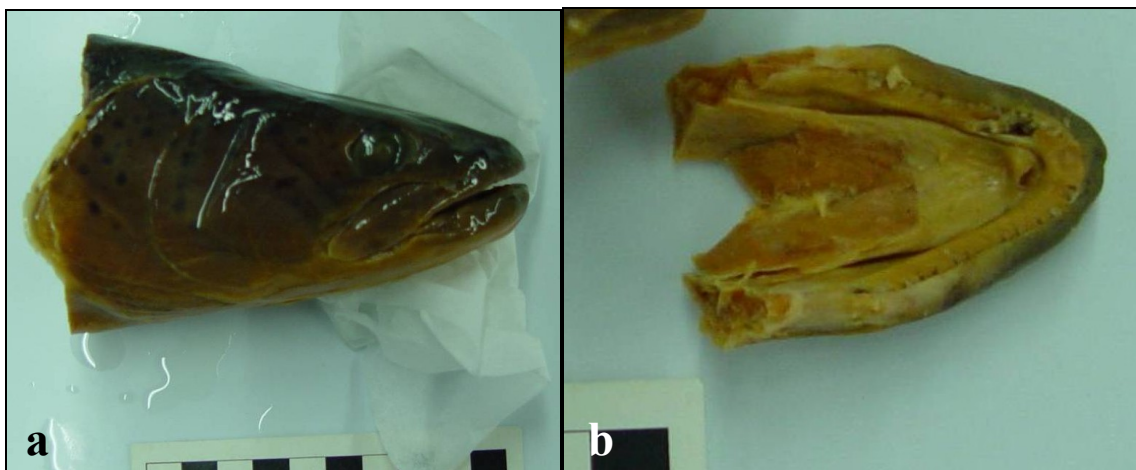


Figure 2.10 – Fleshed *Hucho hucho* UW 022130 (a) head and (b) lower jaw.

ZIN 7872 (fleshed); Figure 2.11; mature, unsexed

Again, the head is large and dorsally compressed. The terminal mouth is large and maxillae extend well posterior of eye, which is of a moderate size. Robust maxillae and dentaries are tooth bearing, and the X-ray image reveals that these teeth are large, particularly on the maxillae. The symphysis of the lower jaw is rounded, and neither the image of the head nor the X-ray reveal evidence of a kype.

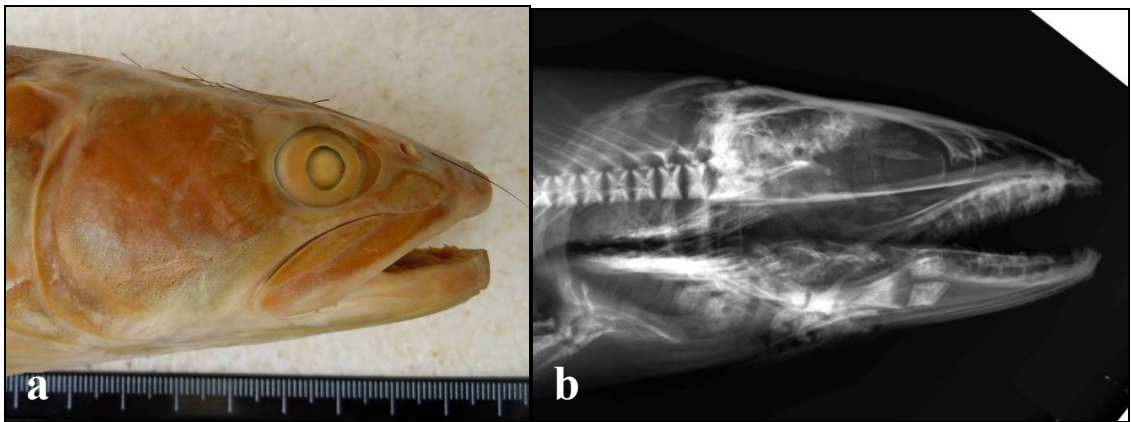


Figure 2.11 – Fleshed *Hucho hucho* ZIN 7872 (a) head and (b) skull (X-ray).

### ***Hucho perryi* (Sakhalin taimen)**

ZIN 13160 (fleshed); Figure 2.12; spawning, unsexed

The head is large and dorsally compressed, though less dramatically so than in *Hucho hucho*. The terminal mouth is large, with maxillae extending posterior of posterior eye margin. Small but robust teeth are present on maxillae and, albeit less so, dentaries. There is no evidence of connective tissue build-up or, in X-ray, skeletal features that would suggest the presence of a kype.

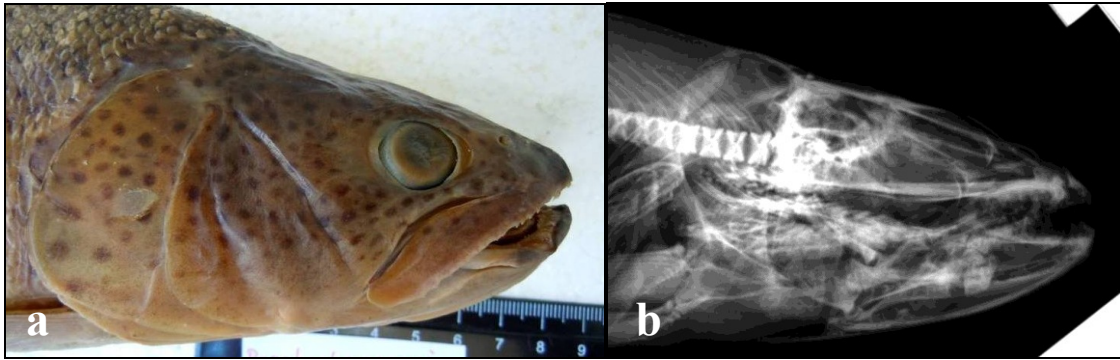


Figure 2.12 – Fleshed *Hucho perryi* ZIN 13160 (a) head and (b) skull (X-ray).

ZIN ID unknown (defleshed), Figure 2.13; mature, unsexed

Dentaries are large and robust, with well-developed, sharp teeth. The bony tissue of the dentaries appears, without exception, smooth and compact. There is no honeycombing or spongy texture that would be indicative of a kype.



Figure 2.13 - Defleshed *Hucho perryi* ZIN ID unknown, right (above) and left (below) dentaries.

### ***Salvelinus* (trout and char)**

Please refer to Figure 2.14 for the phylogenetic relationships of the species of *Salvelinus*.

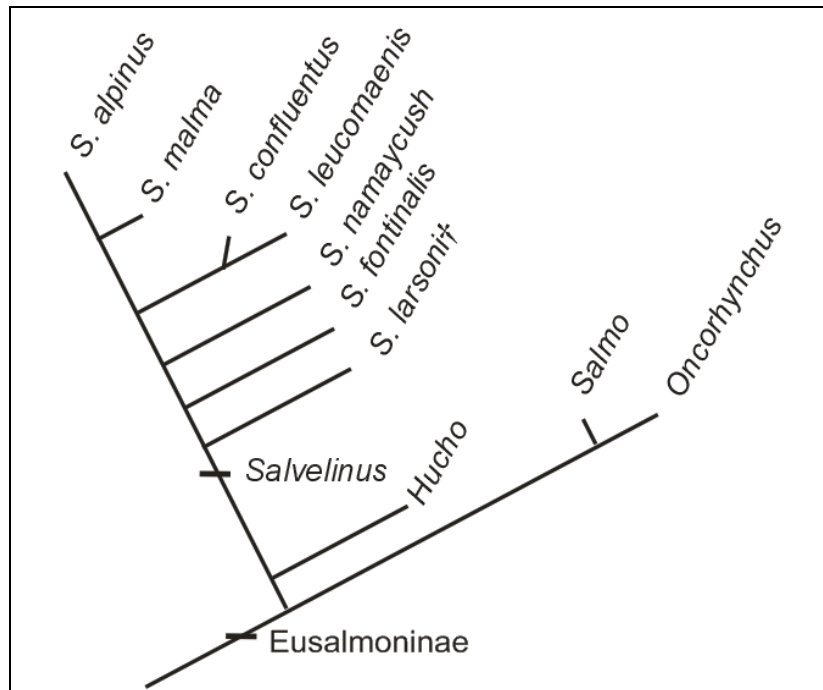


Figure 2.14 - Eusalmonine phylogenetic tree with particular focus on the genus *Salvelinus*. Modified from Stearley and Smith (1993) and Crane et al. (1994), and based on morphological evidence.

*Salvelinus* species vary widely in habitat and migration. However, anadromy is a dominant characteristic within the genus, but rather substantial populations of resident and freshwater living individuals are also evident (Balon 1980; Behnke, 2002; Willson, 1997). With the majority of species migrating in the late summer and early fall, members of *Salvelinus* tend to spawn in mid to late fall, though there is a great deal of variation (Balon, 1980; Scott and Crossman, 1973; Willson, 1997). The genus *Salvelinus* is entirely iteroparous, with a high prevalence of repeat spawning, though percentages of survivorship and multiple reproductive events vary across different species and populations (Armstrong, 1965; Balon, 1980; Fleming, 1998; Willson, 1997). Fish of this genus tend to spawn in gravel-bottomed rivers and streams or on rocky lake and estuary coastlines and reefs, though nest or redd use is highly variable across species (Armstrong, 1965; Balon, 1980; Fleming, 1998). Following spawning, females undulate over the nest, presumably to aerate the eggs, before covering the nest (Esteve et al., 2011; Wilson and

Williams, 2010). Prior to and following spawning, these animals display little in the way of sexual dimorphism (Behnke, 2002; Stearley and Smith, 1993). However, immediately prior to and during spawning, colouration, differences in kype growth, and other, less obvious changes are such that differentiating males from females may be done easily (Behnke, 2002).

***Salvelinus fontinalis* (brook trout)**

UW 28888 (fleshed); Figure 2.15; mature, unsexed

The head is very large, relative to the animal's SL, and has a blocky, square appearance. The mouth is terminal and also large, with long and prominent jaws and a maxillae extending posteriorly beyond the eye. Small teeth are present on premaxillae, maxillae, and the anterior dentary, but no evidence of dentition is apparent rearwards in the mouth. There is no evidence of a kype.

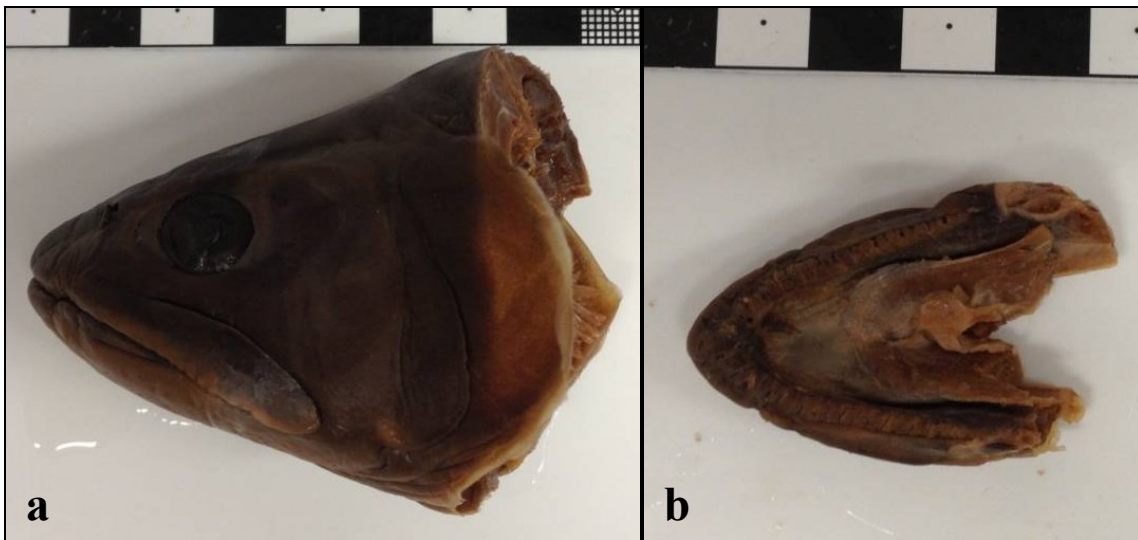


Figure 2.15 – Fleshed *Salvelinus fontinalis* UW 28888 (a) head and (b) lower jaw.

CMNFI-1973-0255.1 (defleshed); Figure 2.16; pre-spawning, unsexed

The bones of the mouth and jaws, in particular the maxillae and even more so the dentary, are relatively large, robust, and long. Teeth are present on the premaxillae, maxillae, and dentaries, but are needle-like and very fine. There is no evidence of a kype.

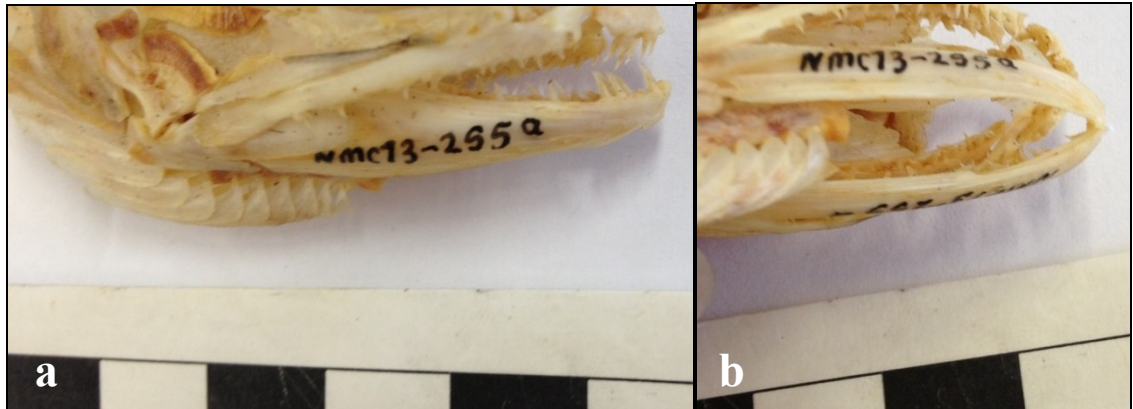


Figure 2.16 – Defleshed *Salvelinus fontinalis* CMNFI-1973-0255.1, lower jaw in (a) lateral and (b) semi-ventral view.

***Salvelinus namaycush* (lake trout)**

ZP 2013-07-01(A) (as representative of the full ZP collection; fleshed); Figure 2.17; pre-spawning, unsexed

The head is large, long, and pointed. The eyes are neither large nor small and are located near the top of the head, which is rather flat. The terminal mouth is large, with the maxillae extending posterior to the posterior margin of the eye. Both upper and lower jaws are robust, with teeth covering the premaxillae, maxillae, dentaries, and head of the vomer (or roof of the mouth). There is no evidence of a kype.

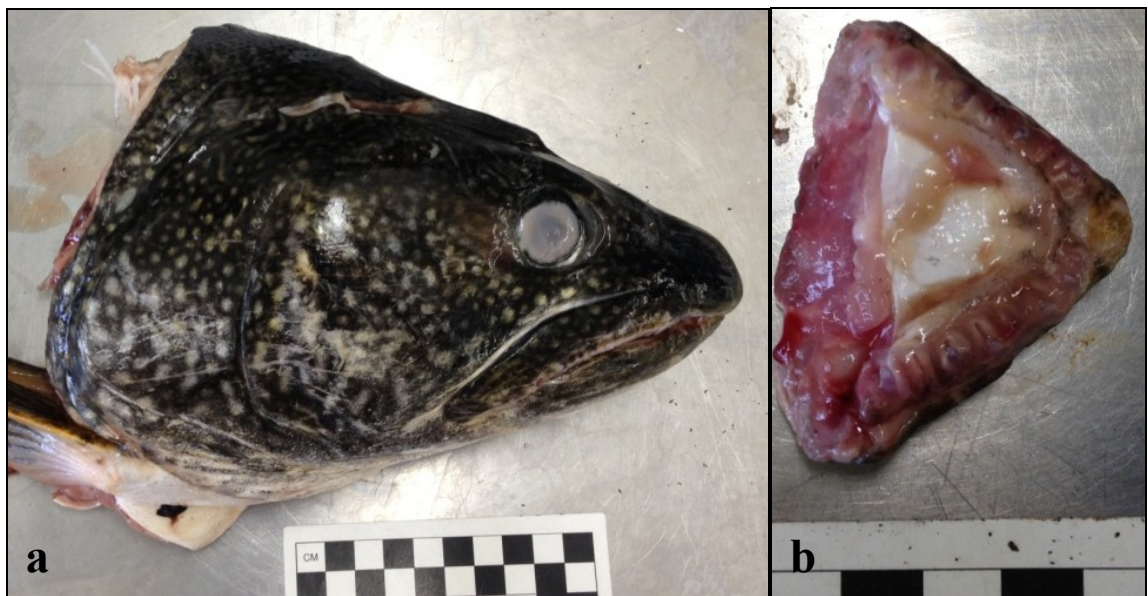


Figure 2.17 – Fleshed *Salvelinus namaycush* ZP 2013-07-01(A) (a) head and (b) lower jaw.

UW 020761 (fleshed); Figure 2.18; pre-spawning, unsexed

This specimen is similar to the conditions of ZP 2013-07-01(A), though the head seems smaller and, perhaps, less robust. Nonetheless, the head is quite large, with prominent, tooth-bearing jaws in a large terminal mouth and with no evidence of a kype.



Figure 2.18 – Fleshed *Salvelinus namaycush* UW 020761 (a) head and (b) lower jaw.

CMNFI 1982-0385 (fleshed); Figure 2.19; mature, unsexed

Again, this specimen is similar to ZP 2013-07-01(A), but is perhaps more similar in stature and robustness to UW 020761. Once again, there is no evidence of a kype.

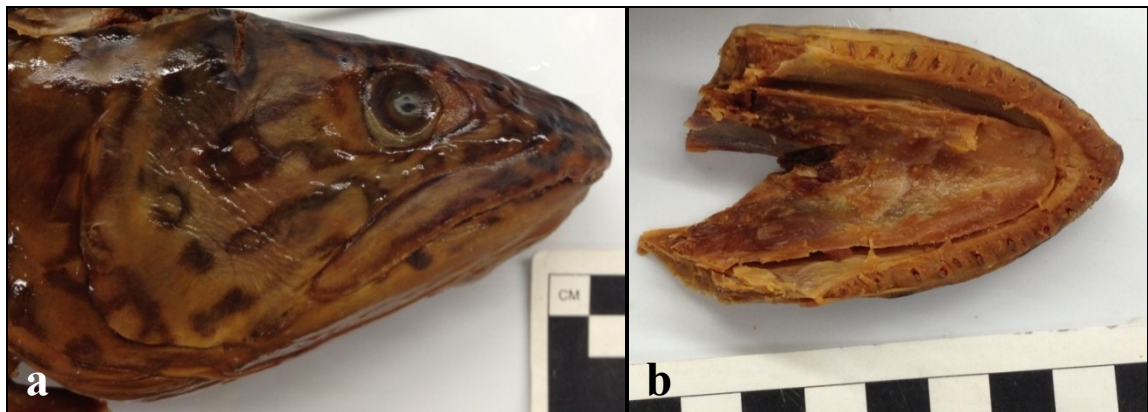


Figure 2.19 - Fleshed *Salvelinus namaycush* CMNFI-1982-0385 (a) head and (b) lower jaw.

CMNFI-1958-0100A.1 (defleshed); Figure 2.20; post-spawning male

Jaw bones are robust and large, particularly so in the dentary, where the triangular shape of the salmonid dentary is less apparent than in other *Salvelinus* species and is instead massive and blocky. Dentition is extensive, with robust teeth appearing on the dentaries, maxillae, premaxillae, and the head of the vomer. There is no evidence of the honeycombing or spongy meshwork of the kype skeleton, though there is evidence of the cartilage mass connecting the right and left dentaries at the symphysis.



Figure 2.20 – Defleshed *Salvelinus namaycush* CMNFI-1958-0100A.1, right dentary in (a) medial and (b) lateral view.

### ***Salvelinus namaycush x fontinalis* (“splake”)**

CMNFI-1976-0187.1 (defleshed); Figure 2.21; immature, unsexed

The dorsal anterior features, particularly those of the upper jaw, the operculum, and in terms of the moderate size of the eye are similar to what is found in *Salvelinus namaycush*. However, the prominence and extension of the lower jaw is much more similar to what is seen in *Salvelinus fontinalis*. Mouth is large, with minimal dentition,



albeit this is more extensive than was the case in the fresh *S. fontinalis* specimens. There is no evidence of any elements of a kype skeleton.

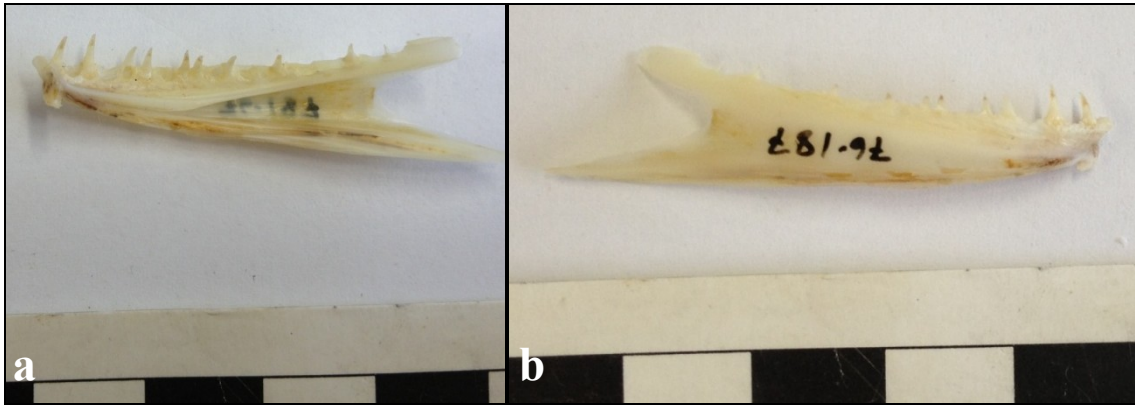


Figure 2.21 – Defleshed *Salvelinus namaycush x fontinalis* CMNFI-1976-0187.1, right dentary in (a) medial and (b) lateral view.

### ***Salvelinus leucomaenis* (Iwana)**

UW 042437 (fleshed); Figure 2.22; pre-spawning, unsexed

This specimen has a large, broad, and sturdy head with a large terminal mouth. The upper jaw is robust and curved, with maxillae extending posterior to the posterior margin of the eye. There is a very small “notch” or separation in the premaxillae that may compensate for the small nodule present at the symphysis of the lower jaw. This nodule is continuous with the dentaries and does not appear to resemble the hook of a kype, or even the soft tissue aggregation of a developing kype.

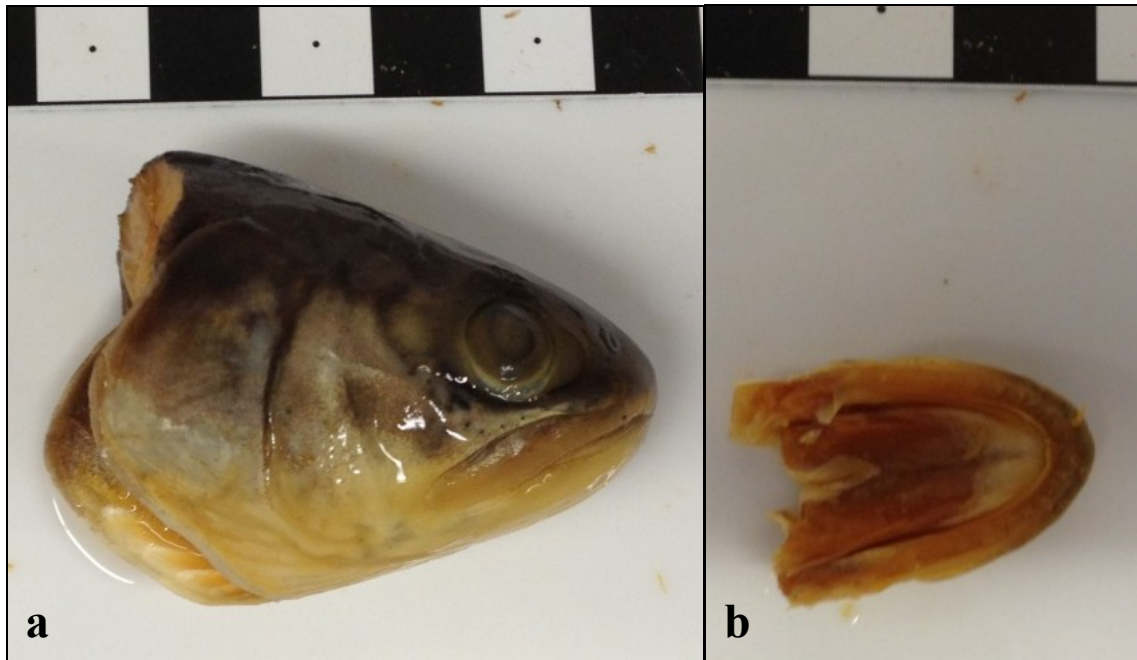


Figure 2.22 – Fleshed *Salvelinus leucomaenis* UW 042437 (a) head and (b) lower jaw.

***Salvelinus confluentus* (bull trout)**

UW 20760 (fleshed); Figure 2.23; pre-spawning male

The head is rather small and flattened dorsally, with large eyes. The mouth is large, with maxillae extending posterior to the posterior margin of the eye. Jaws are large and robust, with teeth present on the premaxillae, maxillae, and dentaries. A nodule at the symphysis of the dentaries composed of a build up of soft tissue is present, indicative of kype. However, this nodule extends anterior and does not hook upwards. There is also little evidence of alterations to the anterior ventral aspect of the dentaries. These factors may indicate that the kype, if present, was still developing at the time of capture.

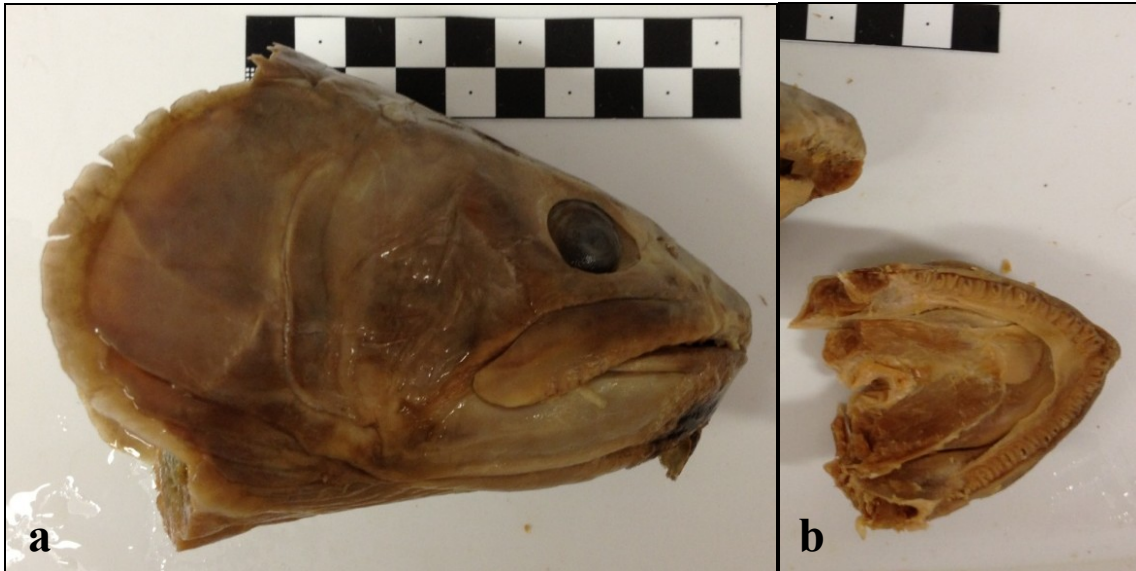


Figure 2.23 - Fleshed *Salvelinus confluentus* UW 20760 (a) head and (b) lower jaw.

***Salvelinus alpinus* (Arctic char)**

UW 041192 (fleshed); Figure 2.24; immature, unsexed

The head is small, rather delicate, with a “pointed” shape, large eyes, and a relatively large terminal mouth. Maxillary extension posteriorly reaches past the anterior edge of the eye. Teeth are present on upper and lower jaws, on the vomer, and on the tongue. A kype is absent in this individual.



Figure 2.24 – Fleshed *Salvelinus alpinus* UW 041192 (a) head and (b) lower jaw.

CMNFI-1968-1262.1 (also representative of CMNFI-1961-0229; fleshed); Figure 2.25; spawning male

Conditions are similar to those of UW 041192, with the addition of a prominent, fleshy kype on the anterior lower jaw, as well as a compensatory separation in the premaxillae.



Figure 2.25 - Fleshed *Salvelinus alpinus* CMNFI-1968-1262.1 (a) head, (b) lower jaw, (c) right jaw in sagittal view, and (d) frontal view of the head showing notch in upper jaw compensatory for presence of the kype.

CMNFI-1977-0348.1 (defleshed); Figure 2.26; mature, unsexed

The presence of evidence for a kype is unclear. There are some fine projections out from the dentary that might be a rudimentary or developing kype, but these features are not substantial.

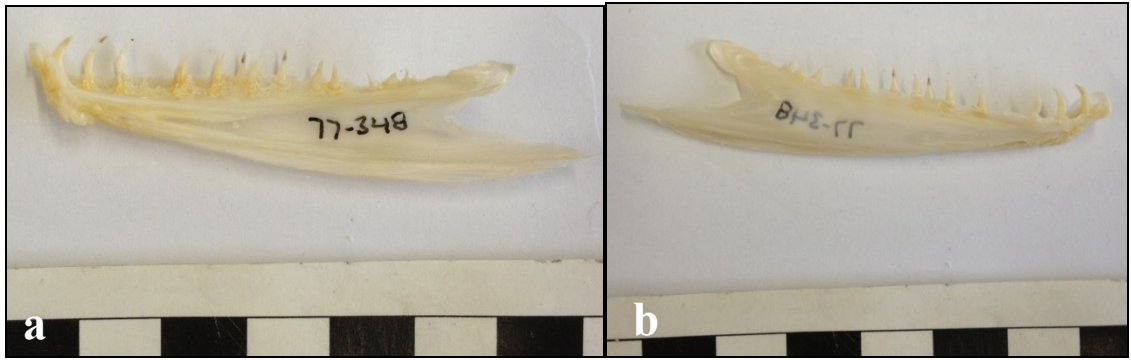


Figure 2.26 – Defleshed *Salvelinus alpinus* CMNFI-1977-0348.1, right dentary in (a) medial and (b) lateral view.

CMNFI-1979-1001.1 (defleshed); Figure 2.27; immature male

Bones are fine and delicate. Dentaries and premaxillae are fairly long, and the anterior dentary shows no convincing evidence of reworking or honeycombing resulting from a kype.

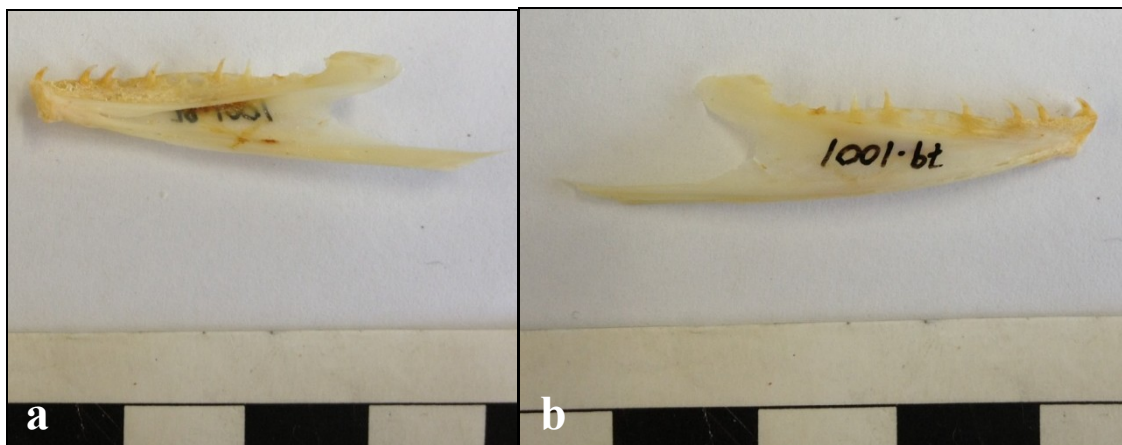


Figure 2.27 – Defleshed *Salvelinus alpinus* CMNFI-1979-1001.1, right dentary in (a) medial and (b) lateral view.

### *Salvelinus alpinus aureolus*

UW 020740 (fleshed); Figure 2.28; immature, unsexed

The head of this specimen is fairly large, considering its SL. The terminal mouth is large, and maxillae extend posterior to the posterior margin of the eye. The lower jaw is prominent, with a slightly pointed or triangular shape. There is no convincing evidence of kype elements.

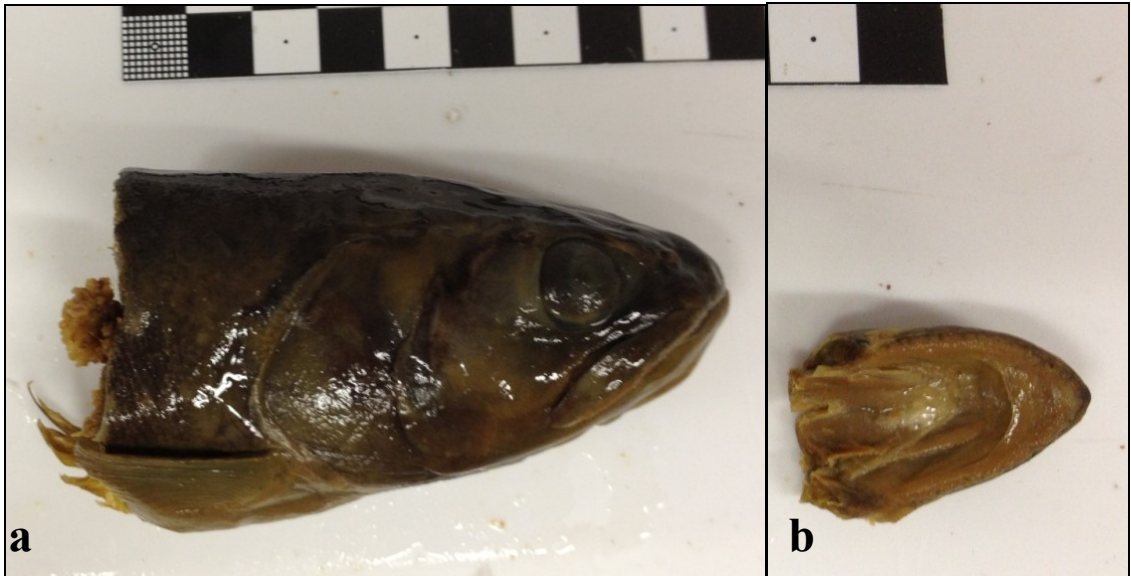


Figure 2.28 – Fleshed *Salvelinus alpinus aureolus* UW 020740 (a) head and (b) lower jaw

### *Salmo* (Atlantic salmon and trout)

Please refer to Figure 2.29 for the phylogenetic relationships of the species of *Salmo*.

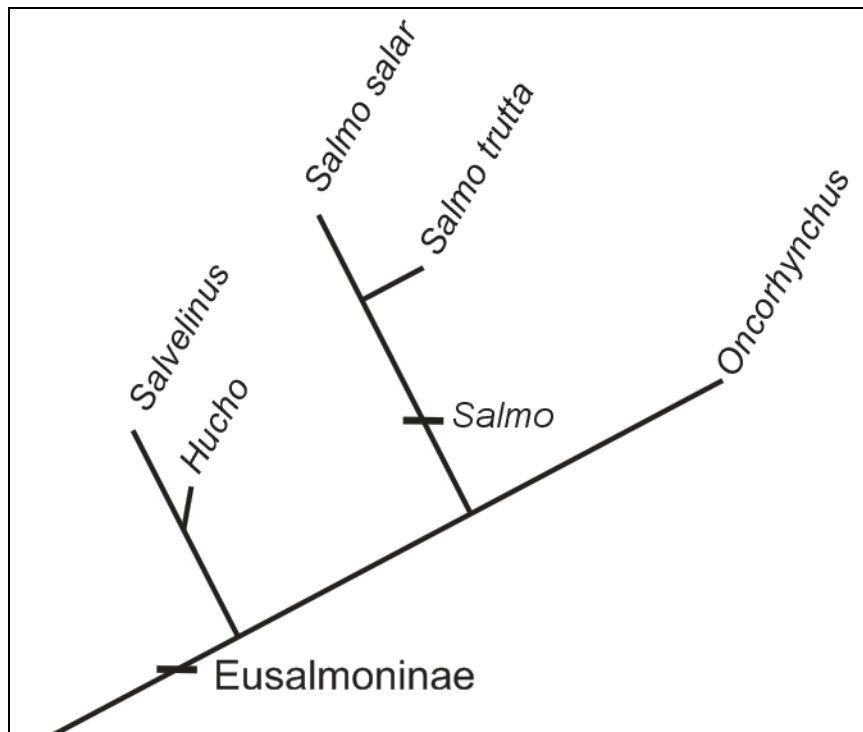


Figure 2.29 - Eusalmonine phylogenetic tree with particular focus on the genus *Salmo*. Modified from Stearley and Smith (1993) and based on morphological evidence.

Though this genus contains a number of isolated sub-genera and -species, only two widely distributed and recognized species, *S. salar* and *S. trutta* exist (Behnke, 2002; Jonsson and Jonsson, 2011; Nelson, 2006). The *Salmo* genus shows relatively little variation in terms of morphological and behavioural characteristics. Both *S. salar* and *S. trutta* are anadromous and iteroparous. However, *S. salar* has very low post-spawn survivorship, with only 0.7-42.5% of animals in an anadromous population experiencing repeat spawning and 30% in non-anadromous populations (Piggins, 1968; Baglinière, 1985, Bley and Moring, 1988; Ducharme, 1969).

Both *S. salar* and *S. trutta* migrate in the later part of the summer and spawn within nests typically constructed in gravel-bottomed rivers and streams. Females perform nest-covering behaviour immediately after spawning (Esteve et al., 2011; Summers, 1996; Wilson and Williams, 2010). Neither *S. salar* nor *S. trutta* display much in the way of sexual dimorphism except in the period directly before and at the time of spawning, when males experience more vibrant changes in body colour, kype development, and increased aggression (Behnke, 2002; Jonsson and Jonsson, 2011; Willson, 1997).

### ***Salmo trutta***

UW 005350 (fleshed); Figure 2.30; pre-spawning male

The head is small and pointed. This specimen has a large mouth with maxillae that extend well posterior to the posterior margin of the eye. The jaws have well-developed teeth. There is evidence of a kype, with a bulbous, anterior soft tissue mass on the relatively slender dentary. Furthermore, this specimen exhibits other spawning-related craniofacial modification, with the head displaying the typical triangular shape of a spawning brown trout.

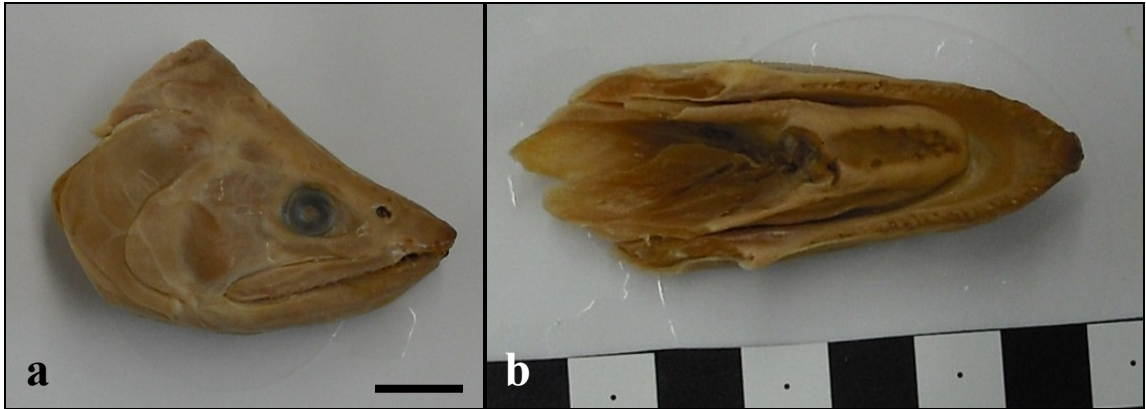


Figure 2.30 - Fleshed *Salmo trutta* UW 005350 (a) head and (b) lower jaw. Scale bar in (a) is 2 cm in length.

***Salmo salar***

UW 019807 (fleshed); Figure 2.31; mature, unsexed

This specimen has a relatively small head with a large terminal mouth. Maxillae extend posteriorly to rear margin of eye. Upper and lower jaws (premaxillae, maxillae, and dentaries) have needle-like teeth. The dentary is fairly small and slender, with a rounded anterior margin and no evidence of a kype.

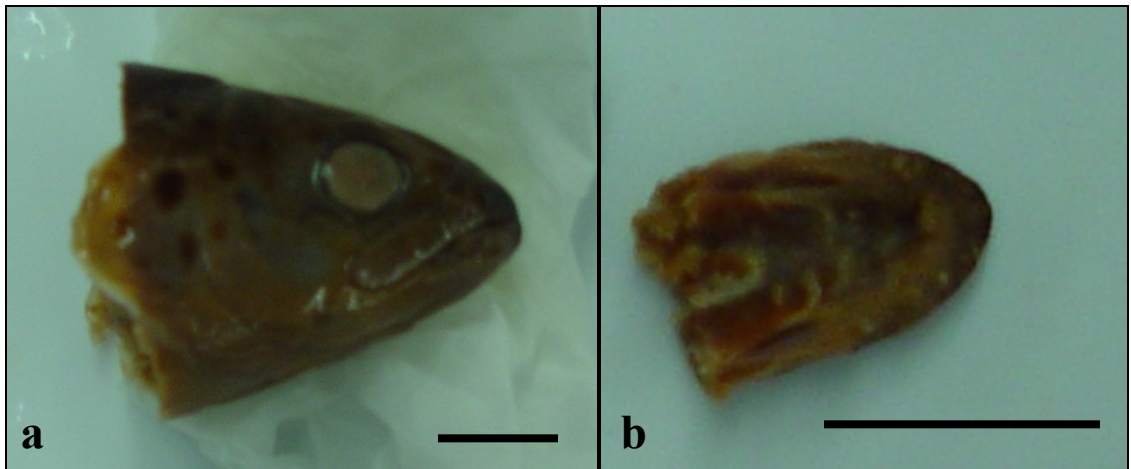


Figure 2.31 - Fleshed *Salmo salar* UW 019807 (a) head and (b) lower jaw. Scale bars are 2 cm in length.

CMNFI-1980-0181.1 (defleshed); Figure 2.32; spawning male

As one could predict from UW 019807, the craniofacial skeleton of this specimen is fairly delicate, with slender jaw bones and teeth. However, a thickening of the anterior dentary and a complicated anterior bony meshwork reveals evidence of a kype.





Figure 2.32 - Defleshed *Salmo salar* CMNFI-1980-0181.1, right dentary in (a) medial and (b) lateral view.

***Oncorhynchus* (Pacific salmon and trout)**

The phylogenetic relationships of the species of *Oncorhynchus* are shown in Figure 2.33.

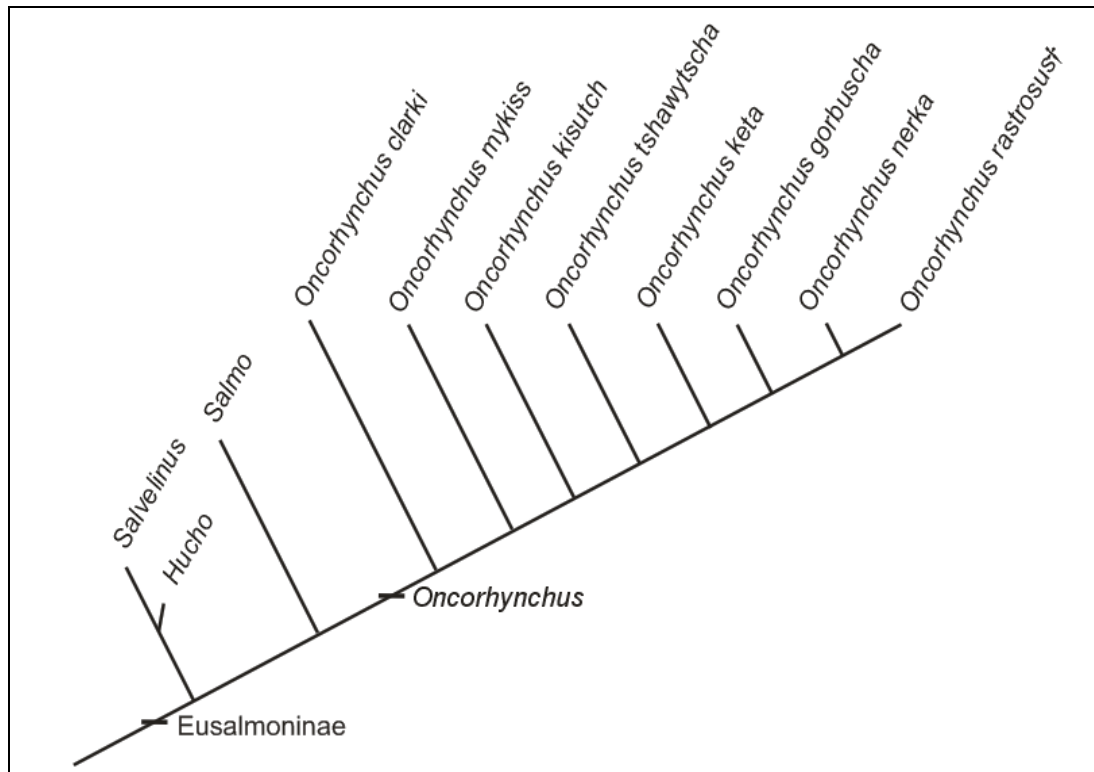


Figure 2.33 - Eusalmonine phylogenetic tree with particular focus on the genus *Oncorhynchus*. Modified from Stearley and Smith (1993) and based on morphological evidence.

*Oncorhynchus* species are predominantly anadromous and are rarely landlocked or non-anadromous (Behnke, 1992, 2002; Scott and Crossman, 1973; Willson, 1997). The majority of species have long-distance migrations and long-term sea runs (Groot and Margolis, 1991, Healey, 1986). In Pacific salmon, such as *O. gorbuscha*, *O. nerka*, or *O. kisutch*, these migrations occur from spring to summer, with animals spawning in late summer and early fall (Willson, 1997). Trouts such as *O. mykiss* and *O. clarki*, on the other hand, migrate in the late summer and fall, spawning in the winter. Some populations inhabiting southern rivers may spawn in the fall (Behnke, 1992). As a rule, Pacific salmon die after spawning, with very few exceptions (Behnke, 2002; Healey, 1991; Ricker, 1972; Willson, 1997). However, many Pacific trout populations tend to have at least some iteroparity (Behnke, 2002; Scott and Crossman, 1973).

With the exception of some populations of *O. nerka*, all *Oncorhynchus* species build nests prior to spawning (Scott and Crossman, 1973; Willson, 1997). In some cases, males even help in the digging process, though this behaviour may be due to

displacement reactions (Esteve, 2007). Gravel bottomed streams and lakes are the preferred sites for nest building, but some populations prefer boulder or silt substrates (Quinn and Foot, 1994; Scott and Crossman, 1973; Willson, 1997). Semelparous females cover their nests immediately following spawning (Esteve et al., 2011; Wilson and Williams, 2010) and guard their nest for days to weeks until just prior to or at the moment of their deaths (Scott and Crossman, 1973). Males in some populations of some species may select the spawning site and defend the nest.

Though males may be slightly larger than females of the same age, there is little sexual dimorphism prior to spawning (Behnke, 2002). At the time of spawning, the upper and lower jaw modification (“snout” and kype) are most dramatic in males, as is colouration, dorsal hump height, and adipose fin length (Morton, 1965; Keenleyside and Dupuis, 1988; Willson, 1997). Other changes, such as a behavioural increase in aggression, may also be evident, particularly in males (Groot and Margolis, 1991).

***Oncorhynchus clarki***

UW 028776 (fleshed); Figure 2.34; pre-spawning, unsexed

The head is relatively small, with large eyes and a large, terminal mouth. Maxillae extend past posterior margin of the eye. Dentaries are long. Large, robust teeth are present on the dentaries, premaxillae, and maxillae as well as on other mouth elements such as the tongue. There does not appear to be any extension or other modification in the tissues of the upper jaw, but the presence of a firm node of connective tissue at the symphysis of the lower jaw may be suggestive of a developing or of a poorly developed kype.

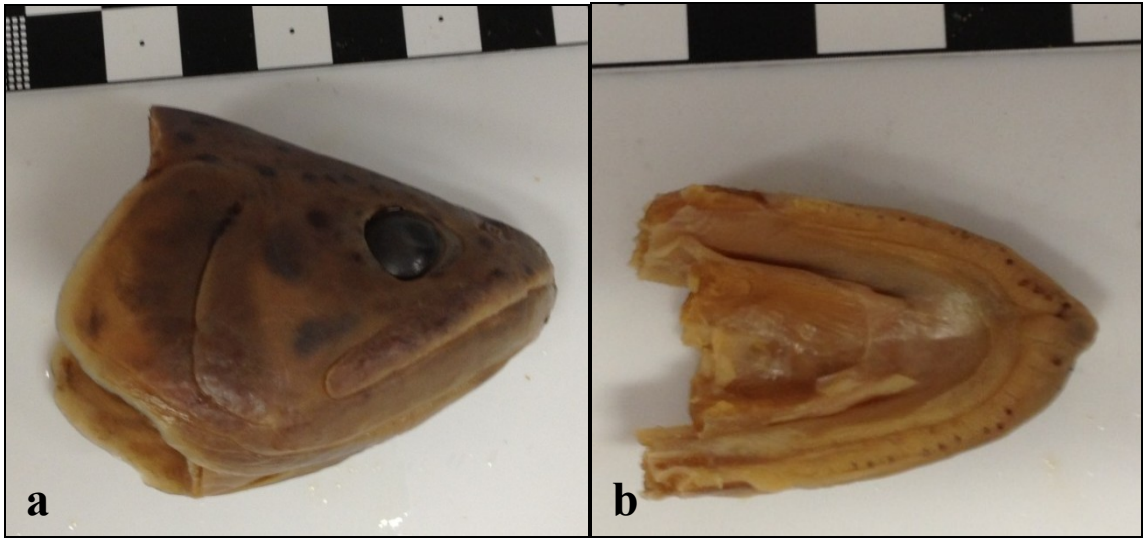


Figure 2.34- Fleshed *Oncorhynchus clarki* UW 028776 (a) head and (b) lower jaw.

CMNFI-1973-0393.1 (fleshed); Figure 2.35; pre-spawning, unsexed

Once again, the head is relative small, with eyes even larger than those seen in UW 028776. Maxillae extend to posterior of the posterior margin of the eye. Dentaries are shorter than was seen in UW 028776 and do not extend as anteriorly as the maxillae. The lower jaw lacks the node of soft tissue seen in the other fleshed *O. clarki* specimen. There is also a lack of any evidence of modification in the tissues of the upper jaw. Thus, it would appear that this specimen does not have a kype.

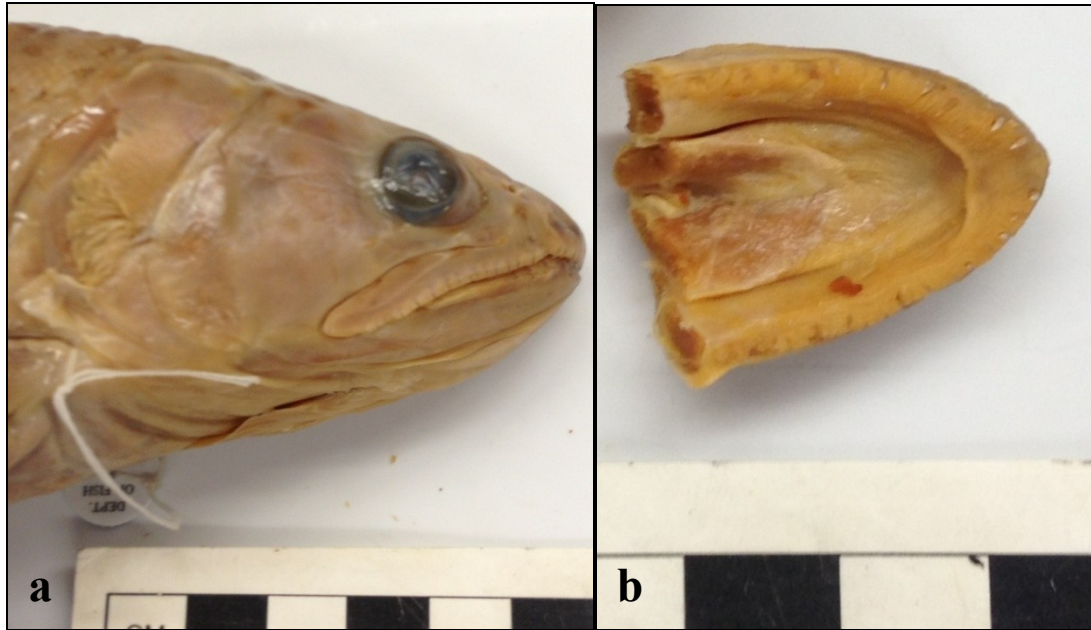


Figure 2.35 - Fleshed *Oncorhynchus clarki* CMFI-1973-0393.1 (a) head and (b) lower jaw.

***Oncorhynchus mykiss***

UW 016073 (fleshed); Figure 2.36; immature, unsexed

The head is small, squat, and rounded anteriorly. A terminal mouth is relatively large, though smaller than seen in other *Oncorhynchus* species. The maxillae extend to the posterior margin of the rather large eye. Teeth are present on upper and lower jaws, tongue, and vomer, but are small. The jaws have a blunted appearance and are relatively short. There does not appear to be any modification in the upper or lower jaws that would suggest the presence of a developing kype.

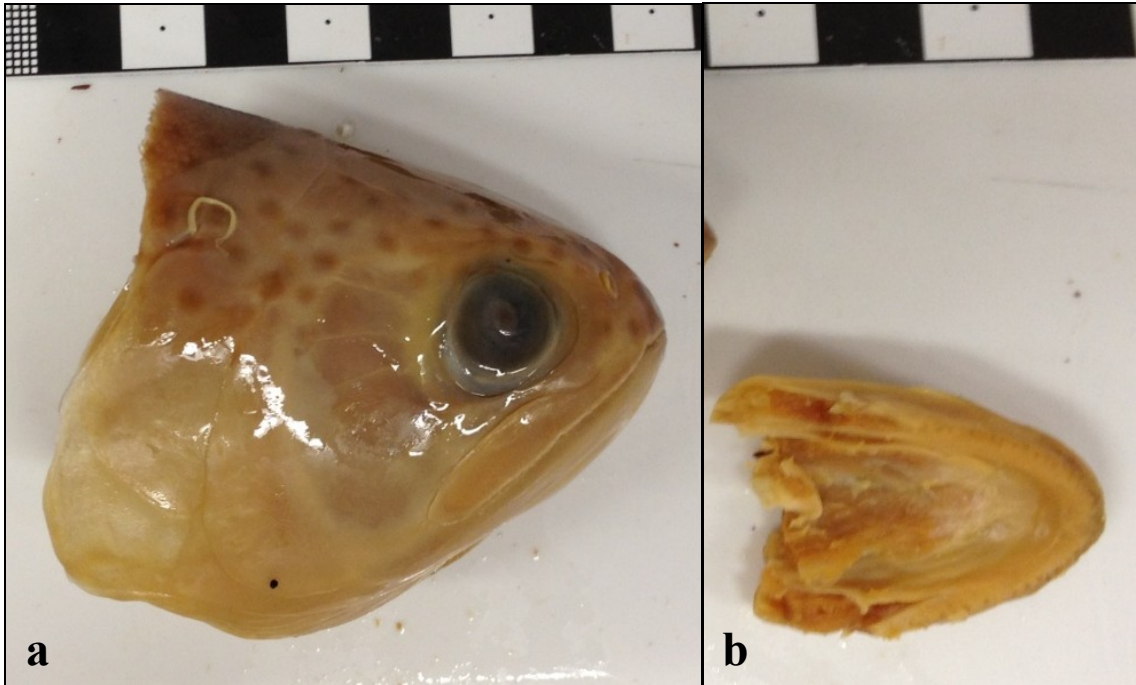


Figure 2.36 - Fleshed *Oncorhynchus mykiss* UW 016073 (a) head and (b) lower jaw.

***Oncorhynchus mykiss aguabonita***

UW 015967 (fleshed); Figure 2.37; spawning female

Once again, the head is small and has a squat, rounded appearance. The mouth is somewhat larger than in UW 016073, with maxillae extending posterior to the posterior margin of the relatively large eye. However, the jaws are relatively short. The lower jaw is somewhat longer than the upper jaw, resulting in the appearance of an “underbite.” This longer dentary and a minor build-up of soft tissue at the symphysis of the lower jaw may suggest the presence of a kype, but if a kype is present, it is not very pronounced. Relatively small teeth are present on upper and lower jaws, tongue, and vomer.

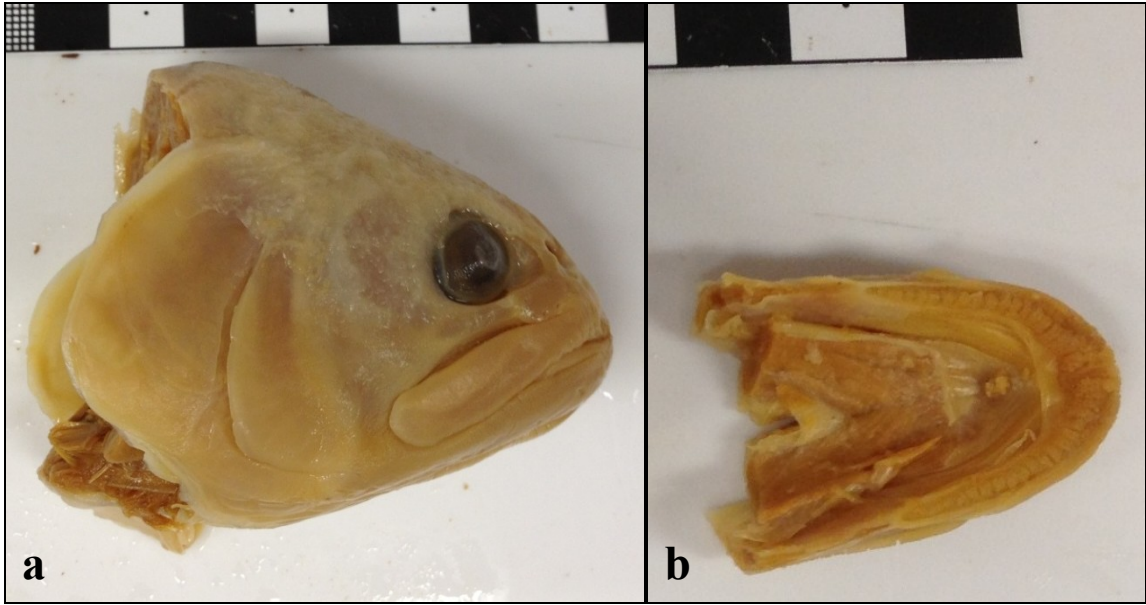


Figure 2.37 – Fleshed *Oncorhynchus mykiss aguabonita* UW 015967 (a) head and (b) lower jaw.

***Oncorhynchus kisutch***

UW 015091 (fleshed); Figure 2.38; pre-spawning female(?)

The head is relatively large and conical. Though the upper jaw is slightly blunted, it is without the fleshy, blunted snout seen in even non-spawning males of this species, suggesting that this animal is a female. The terminal mouth is large, with maxillae extending past the posterior margin of the moderately large eye. Well-developed, sharp teeth are present on the dentaries, premaxillae, maxillae, tongue, and vomer. Connective tissue masses are absent from the upper and lower jaws, and there does not appear to be any modifications to the anterior jaw tissues suggestive of a snout or of a kype.

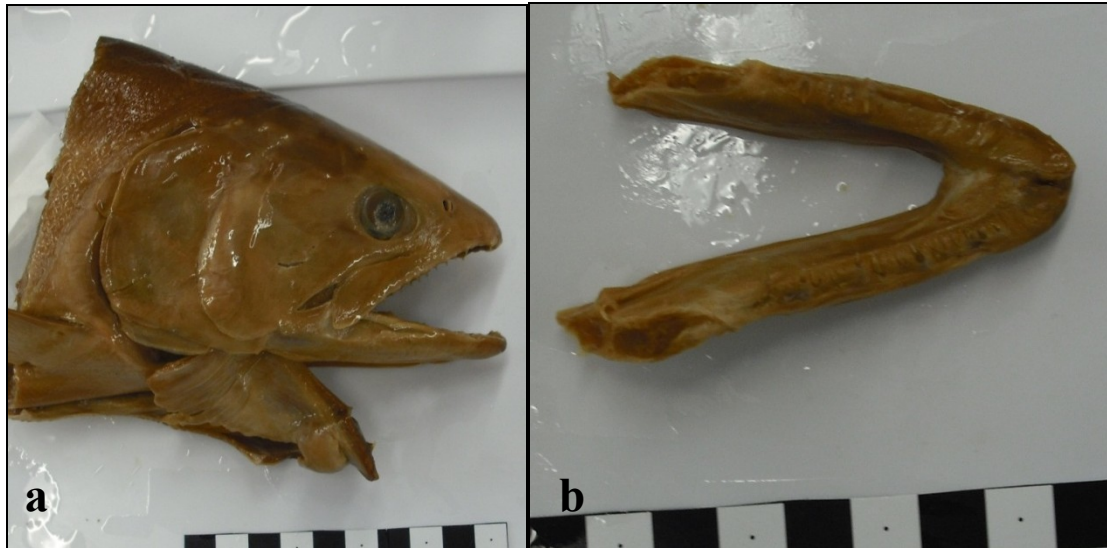


Figure 2.38 - Fleshed *Oncorhynchus kisutch* UW 15091 (a) head and (b) lower jaw.

CMNFI-Z000804 (defleshed); Figure 2.39; spawning male

The dentary and maxillae are large and long, with well-developed, sharp, posterior oriented teeth. The thin maxillae exhibit a slight curvature. The dentary is also relatively thin and delicate looking posteriorly, but thickens anteriorly, where the smooth bone of the compact dentary give way to the honey-combing features of a well-developed kype. The kype is associated with large, sharp, robust teeth as well as rough, spongy textures on the bone itself.





Figure 2.39 - Defleshed *Oncorhynchus kisutch* CMNFI-Z000804, right dentary in (a) medial and (b) lateral view.

***Oncorhynchus tshawytscha***

UW 015893 (fleshed); Figure 2.40; pre-spawning, unsexed

The head is small and somewhat conical. This individual has a large, terminal mouth, with maxillae extending posterior to the posterior margin of the eye. This animal has long jaws, with dentaries extending further forward than maxillae. Teeth are not very large, but are robust and sharp and are present on the dentaries, maxillae, premaxillae, and tongue. There is no connective tissue build-up or other morphological features suggestive of a kype.

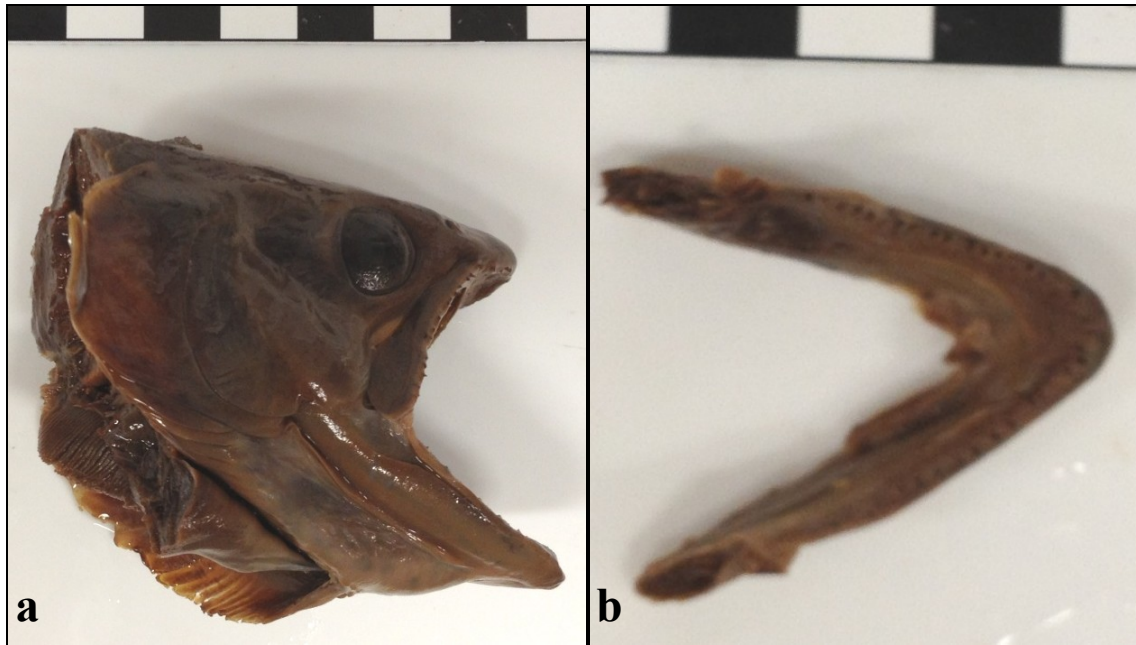


Figure 2.40 - Fleshed *Oncorhynchus tshawytscha* UW 15893 (a) head and (b) lower jaw.

***Oncorhynchus keta***

UW 13664 (fleshed); Figure 2.41; pre-spawning male

The head is small and narrow, with an overall conical shape. The mouth is terminal and large, with maxillae extending posterior to the posterior margin of the eye. The dentaries may be longer than the maxillae, but it is a subtle difference. Sharp, relatively small teeth are present on the jaws, tongue, and vomer, though these teeth are larger towards the symphysis of the lower jaw. There is not, however, any evidence of tissues or modifications suggestive of a kype on either the upper or lower jaws.

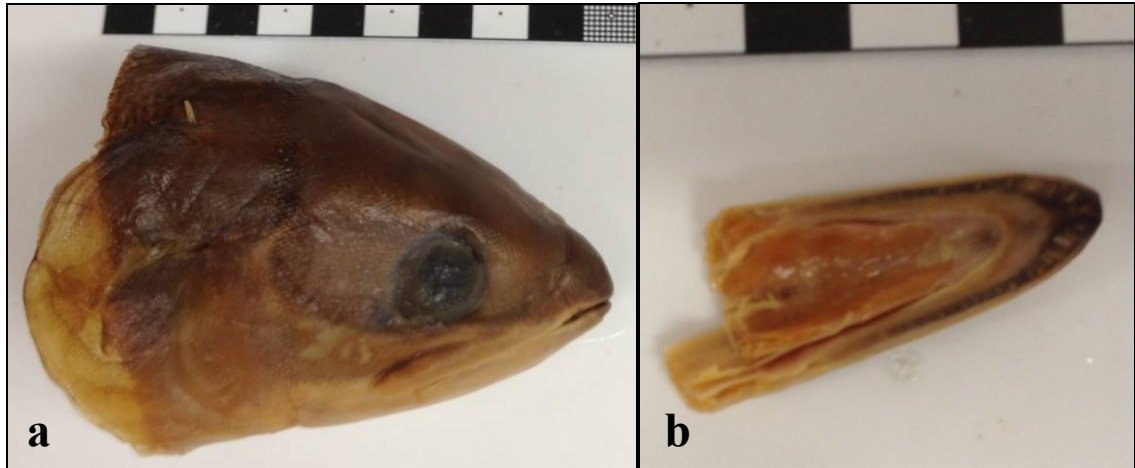


Figure 2.41 – Fleshed *Oncorhynchus keta* UW 13664 (a) head and (b) lower jaw.

### *Oncorhynchus gorbuscha*

UW 16027 (fleshed); Figure 2.42; spawning male

The head is large, conical, and nearly triangular. The terminal mouth is large, with maxillae extending well posterior of posterior margin of relatively small eye. Jaws are of equal length and each displays relatively large, sharp, well-developed teeth. Teeth are also found on the tongue and vomer. Teeth on the dentaries increase in size anteriorly, where an anterior extension of the jaw displays a well-developed kype. This kype is paired with a large, curved snout on the upper jaw, which also bears large teeth.

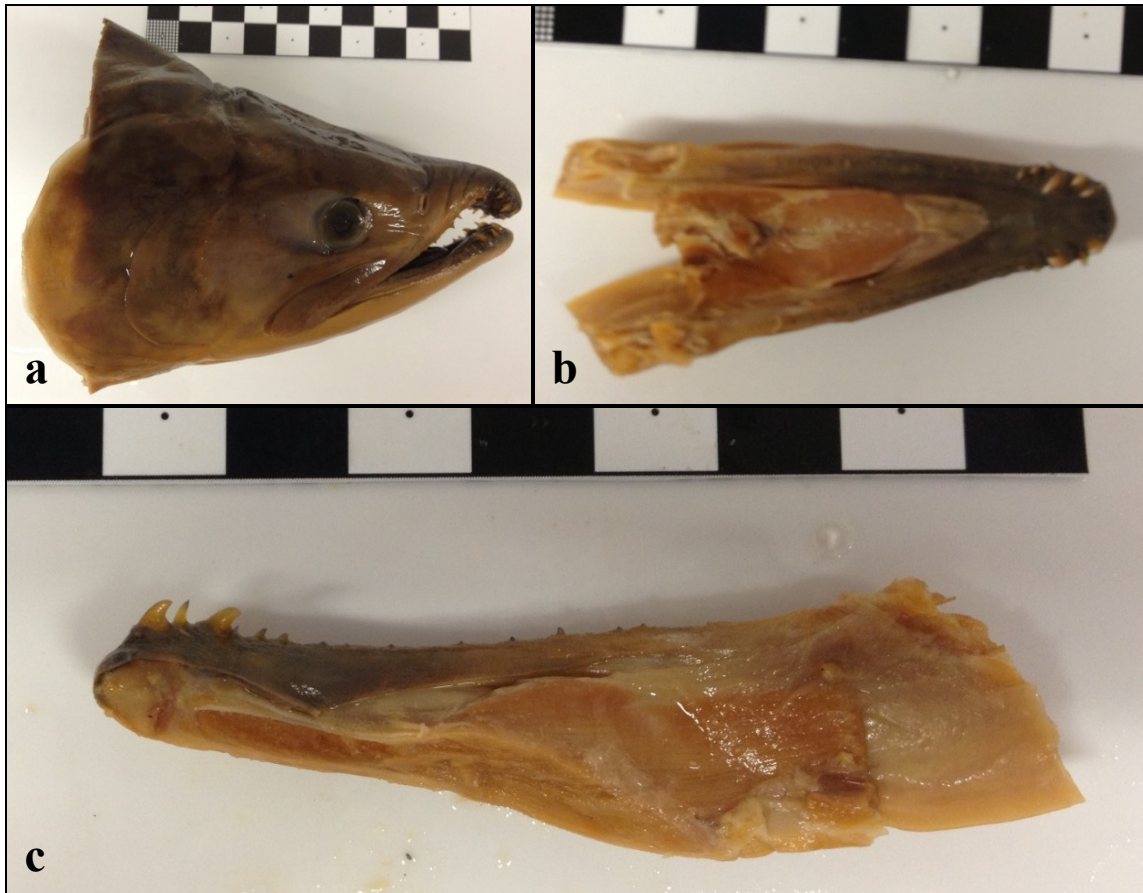


Figure 2.42 - Fleshed *Oncorhynchus gorbuscha* UW 16027 (a) head, (b) lower jaw, and (c) right jaw in sagittal view.

CMNFI-1984-0169.1 (fleshed); Figure 2.43; mature, unsexed

This specimen is partially skeletonized, though many features are still apparent. The head is relatively small and pointed. The mouth is large and terminal, with maxillae extending posterior to the posterior margin of a relatively large eye socket. The dentaries are shorter than the maxillae. Well-developed teeth are present on the upper and lower jaws, but they are relatively small in comparison to the anterior teeth of UW 16027. There is little connective tissue remaining at the anterior tip of the lower jaw, but what soft tissue is present does not display evidence of a kype-related build up, nor does the absence of honey combing in what bone is visible.

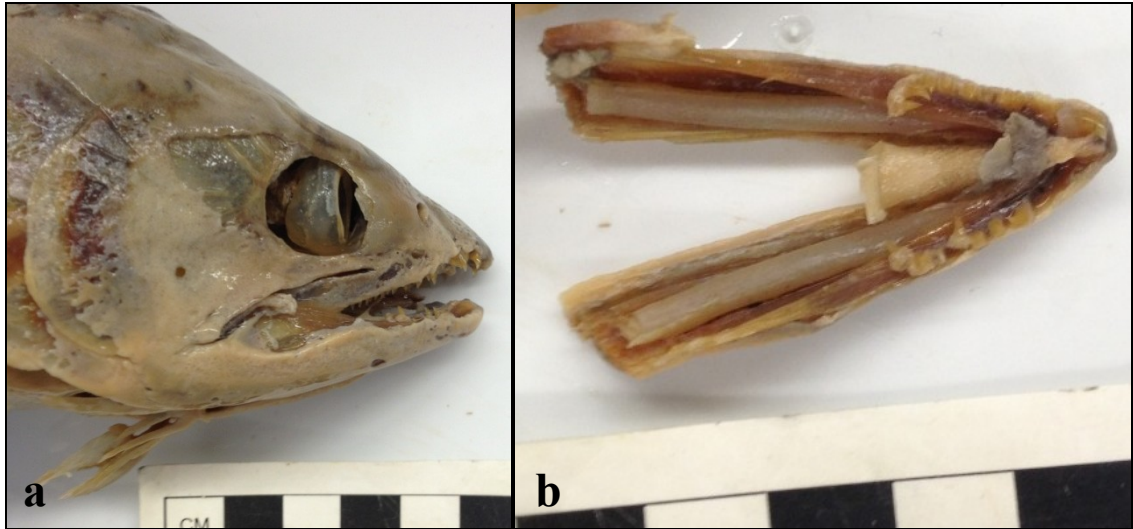


Figure 2.43 - Fleshed (partially skeletonized) *Oncorhynchus gorbuscha* CMNFI-1984-0169.1 (a) head and (b) lower jaw.

### *Oncorhynchus nerka*

UW 005624 (fleshed); Figure 2.44; mature, unsexed

The head is relatively small, with a conical shape. Mouth is terminal and rather small compared to other *Oncorhynchus* specimens, with a maxillae that extends to just the posterior margin of a small eye. Upper and lower jaws are the same length, with small but robust teeth. Teeth are also found on the tongue and vomer. The anterior aspects of the upper and lower jaws are blunted and without any of the connective tissue or boney features that would suggest a kype.

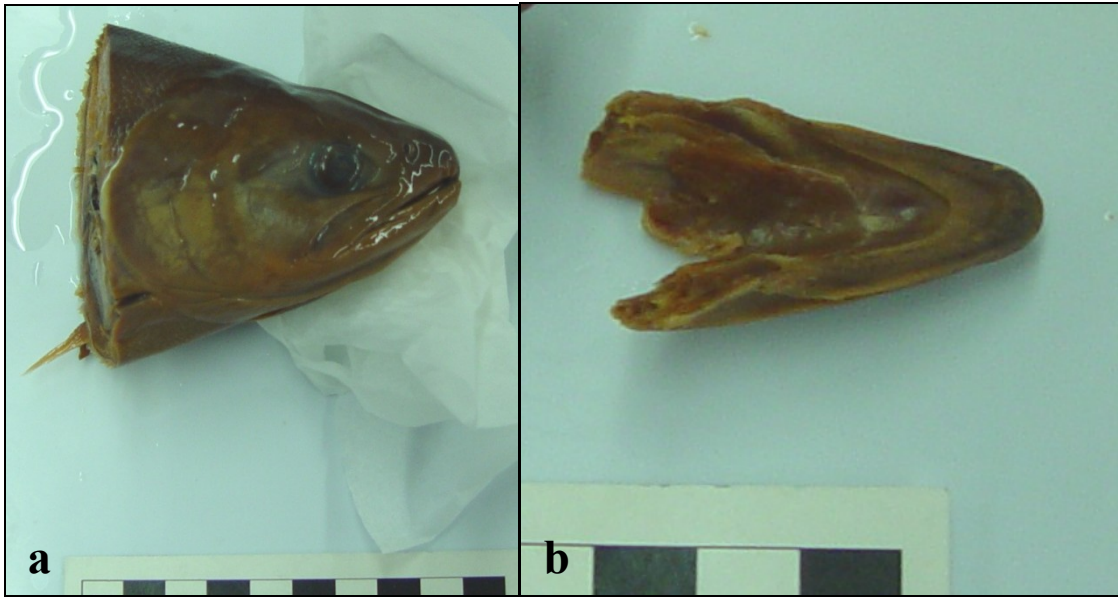


Figure 2.44 – Fleshed *Oncorhynchus nerka* UW 005624 (a) head and (b) lower jaw.

CMNFI-1977-0277.1 (defleshed); Figure 2.45; spawning male

The maxillae and dentary are large, slender, and delicate seeming, with relatively small but sharp teeth. The delicate maxillae are somewhat curved. Toward the anterior of the dentary, much in the same way as with the *O. kisutch* specimen, the smooth texture of the compact dentary gives way to a rougher texture with a spongy appearance. This texture is associated with a grouping of very large teeth clustered at the very tip of the dentary, which give the kype of this specimen a hooked appearance.



Figure 2.45 - Defleshed *Oncorhynchus nerka* CMNFI-1977-0277.1, right dentary in (a) medial and (b) lateral view.

Evidence of a kype is found in many salmonines. More specifically, with the absence of a kype in *Brachymystax lenok*, it appears that kype-development is a trait specific to Eusalmoninae, although it is lacking in *Hucho*.

#### ***Physical description summary***

Several patterns in terms of kype presence and absence emerged from physical examination of a wide range of specimens. These patterns include a phylogenetic relationship with kype development and differences in the compensatory features of the upper jaw in each genus.

Phylogenetic position reflects kype presence. Structures and tissues suggestive of a kype were not found in any of the more basal salmonids, including members of the

Coregoninae, Thymallinae, and *Brachymystax*. While absent in many pre-spawning and immature individuals, a kype is found in all genera included in the Eusalmoninae, with the exception of *Hucho*. Namely, *Salvelinus*, *Salmo*, and *Oncorhynchus* were kype-bearing (Fig. 2.46). The lack of a kype in *Hucho* may suggest a loss (or reversal) of the kype bearing character or, as noted in Chapter 1, may indicate that the phylogenetic position of *Hucho* should be reconsidered. This concept of reassessing *Hucho*'s taxonomic position will be discussed further in the "Literature survey" and "Final discussion and conclusions" sections, below.

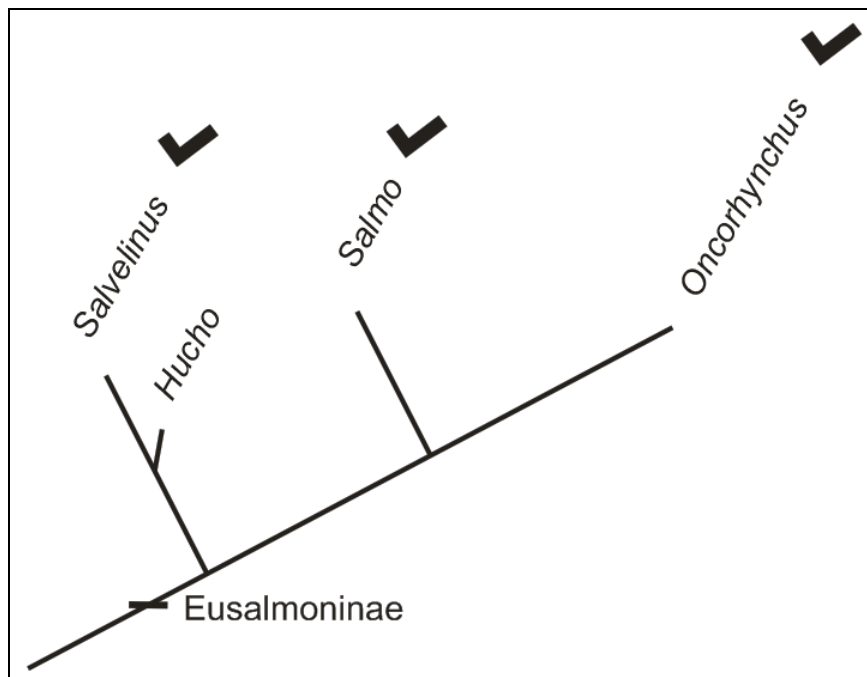


Figure 2.46 - Eusalmonine phylogenetic tree with kype developing genera indicated by the check mark. *Salvelinus*, *Salmo*, and *Oncorhynchus* appear to be kype developing, while *Hucho* is not. Modified from Stearley and Smith (1993).

In addition to a phylogenetic pattern of kype presence, physical examinations also reveal a phylogenetic pattern in terms of compensation for kype growth. As is briefly noted in the literature (Behnke, 2002; Scott and Crossman, 1973), trends in kype development on the lower jaw are balanced by patterns in the development of compensatory features on the upper jaw. While *S. salar* develops a hole in the soft tissue of the upper jaw that the hook-like structure of the kype fits into (Tchernavin, 1938; Witten and Hall, 2002), *Oncorhynchus* and *Salvelinus* use different methods to



compensate for the presence of a kype. As seen in *O. gorbuscha* (UW 16027), *Oncorhynchus* develops an accumulation of soft tissue at the anterior aspect of the upper jaw. Furthermore, as was seen in the defleshed *O. kisutch* (CMNFI-Z000804) and *O. nerka* (CMNFI-1977-0277.1), the maxillae of some kype-bearing *Oncorhynchus* individuals are also slightly curved. This accumulation of soft tissues and curvature of the upper jaw bones yields a curved snout that the kype fits within. On the other hand, as is demonstrated in two of the *S. alpinus* specimens (CMNFI-1968-1262.1 and CMNFI-1961-0229), *Salvelinus* individuals instead develop a modification in the spacing of their premaxillae (and to a much lesser degree, their maxillae) creating a “notch” that the kype slips into. Regardless of their method, each of the three genera here discussed develops compensatory features that often permit the mouths of animals with a dramatic kype to close, yielding a seal that the animal uses to push water over the gills. These compensatory features will be discussed in the “Micro-CT scanning summary,” “Literature survey,” and “Final discussion and conclusions” sections below.

### ***Micro-CT scanning***

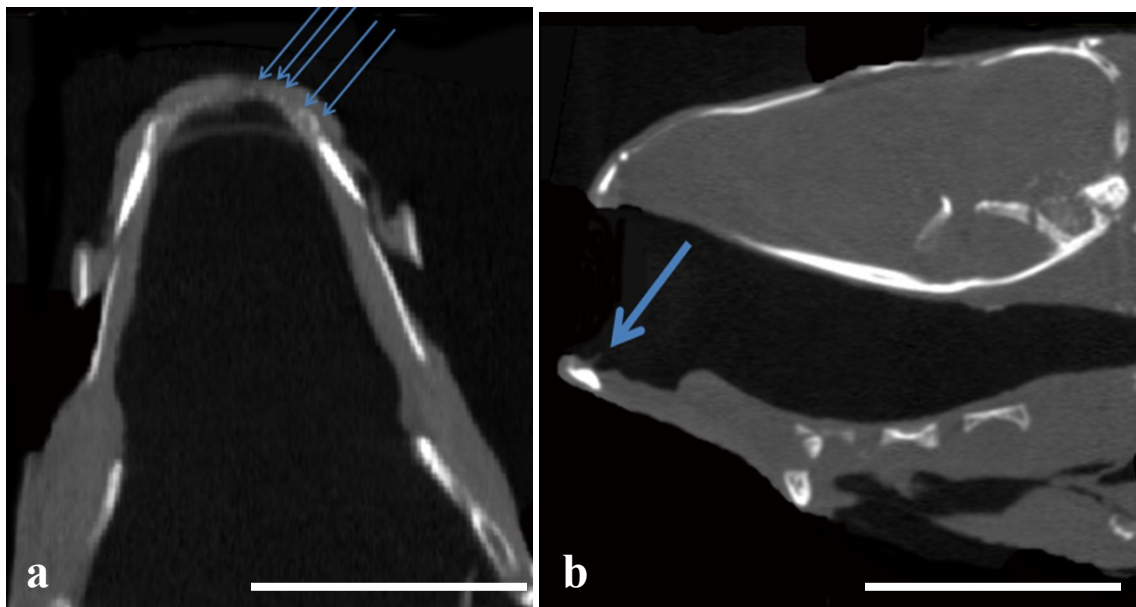
Due to constraints in accessing the Lab PET 4 machine, I scanned only representative specimens of my genera of interest. However, scanned at similar voltages and at magnifications appropriate to their dimensions as outlined in Table 2.2, the following specimens reveal a great deal about the conditions within the craniofacial skeleton of salmonids. The differences in intensity within the scans (or “grayscale”) represent the differences in the densities of the various elements of the scanned objects. As radiation passes from the radiating bed of the CT machine through the object being scanned, the different elements of that object will block X-ray waves below certain amplitudes. Essentially, this means that a dense feature will block more radiation than an object of lower density (i.e. the object of greater density will have higher attenuation), because only very high amplitude radiation will be able to pass through it. The less radiation that passes through an object, the less the reception bed of the CT machine will be stimulated. As a result, a very dense object will display as very pale to white in CT imaging, particularly if the parameters of the image is set such that the grayscale gradient of the entire image falls between the densest (white) and least dense (black) object within the

frame of interest. Assuming that grayscale patterns in the image are representative of tissue density in the elements of the skull, then density can be used as a qualitative proxy for patterns of mineralization.

***Brachymystax lenok***

UW 022131 (fleshed); Figure 2.47

As can be seen in the transverse section through the skull of this specimen (Fig. 2.47 a), contrary to initial morphological assessment, there is evidence of teeth on the lower jaw, some of which are indicated by blue arrows. That these teeth are present but have not yet erupted from the epithelium of the jaw would suggest that this individual was nearing maturity when it was caught, given that only adults of this species have teeth (Alekseyev et al., 2003; Holčík, 1982).



**Figure 2.47 – Micro-CT scan of *B. lenok* UW 022131. (a) Transverse section through lower jaw. Blue arrows indicate the presence of some teeth. Margin of dentary bone is distinct, with little gradation into the surrounding grey of the soft tissue. (b) Sagittal section through right side of head, just lateral to the symphysis, showing the lack of gradation from the dentary into surrounding tissues. The margin of the dentary is distinct with the exception of one area that is being influenced by the presence of the tooth (the tooth is indicated by the blue arrow). Scale bars are 1 cm.**

It is also evident from this image that there is little variation in the tissues of and around the anterior dentary. A uniform grey, soft tissue surrounds the nearly uniform white of the symphysis and dentaries. There is no transition from harder to softer tissues, nor is there any evidence of projections out from the dentary. This lack of gradation into

surrounding tissues is perhaps easier seen in sagittal section (Fig. 2.47 b), where we also see a portion of one of the dentary teeth, indicated by the blue arrow. In this sagittal view, we see that the margins of the dentary are clear, with the only disruption to the smooth dentary boundary being the result of the tooth root. This distinct dentary margin is apparent through each layer of the scan.

### ***Hucho hucho***

UW 022130 (fleshed); Figure 2.48

The robustness of the bones of the jaw inferred from the physical examinations is evident. The dentary and maxillae are dense, nearly uniformly so. Large sharp teeth are present on both the upper (Fig. 2.48 a, green arrows) and lower (Fig. 2.48 b, blue arrows) jaws, as well as on the premaxillae and the tongue (red arrows).

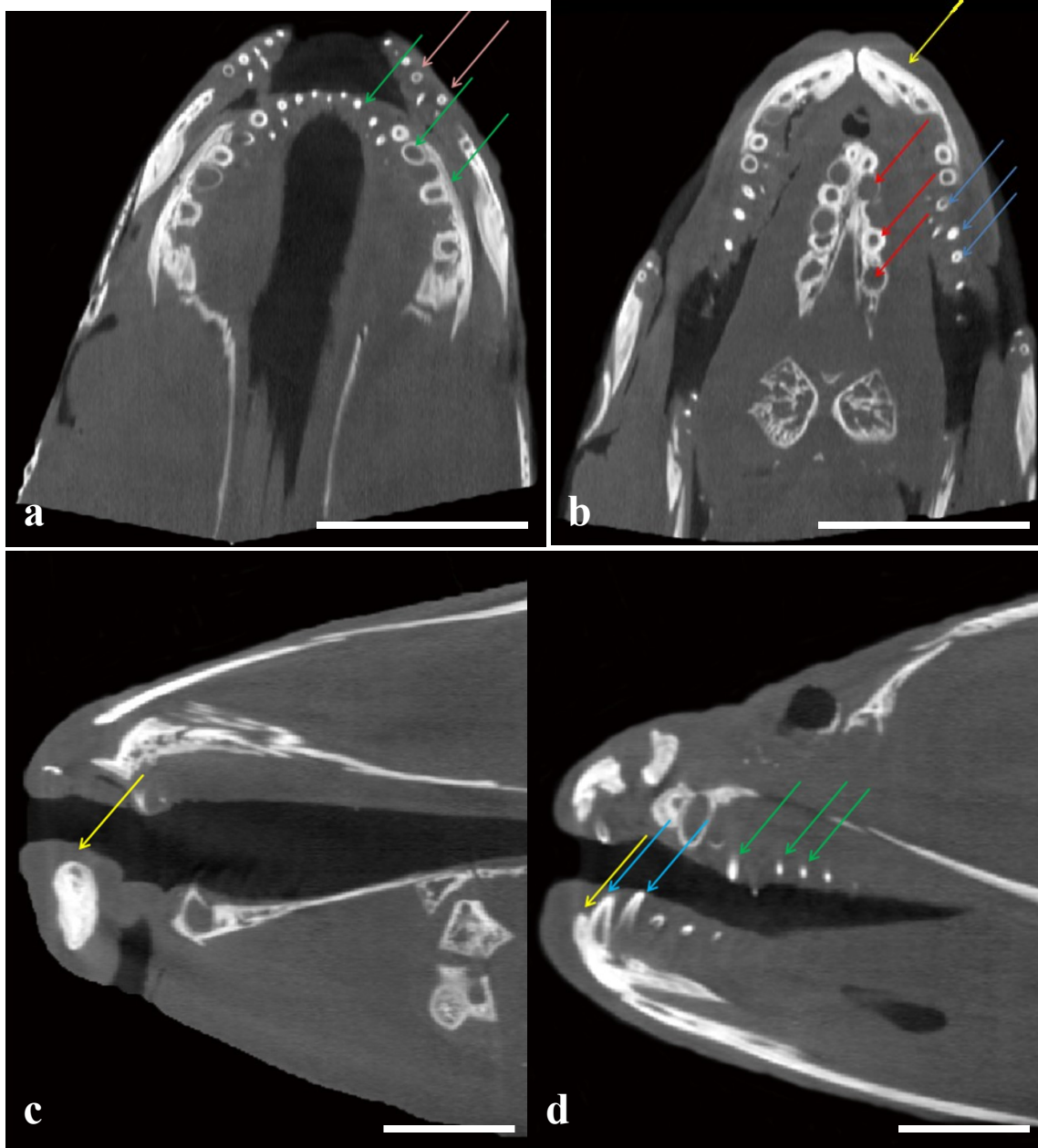


Figure 2.48 – Micro-CT scan of *Hucho hucho* (UW 022130). (a) Transverse section through upper jaw. Prominent teeth are apparent on maxillae (green arrows) and premaxillae (pink arrows). Jaw bones are robust. (b) Transverse section through lower jaw. Large, robust teeth on tongue (red arrows) and dentary (blue arrows) are obvious, as is the distinct margin of the dentary (yellow arrow). The transition between the dentary and lower jaw symphysis and the soft tissue of the rest of the jaw is abrupt. (c) Sagittal section through the anterior part of the head. Sharp contact between lower jaw symphysis and remainder of tissues (yellow arrow) is even more distinct from this perspective. (d) Sagittal section through the head midway between the symphysis and the lateral margin of the jaw. The contrast between the bone of the dentary and the soft tissue surrounding it remains distinct throughout the lower jaw (yellow arrow). Note the large, sharp teeth on upper (green arrows) and lower (blue arrows) jaws. Scale bars are 2 cm.

As was seen in *Brachymystax*, there is a distinct boundary between the dense, compact bone of the dentaries and the surrounding soft tissue of the lower jaw (Fig. 2.48

b-d, yellow arrows). Though there is evidence of tissues of different densities within the cartilage mass at the symphysis (Fig. 2.48 c), there is no evidence of the gradation from dense bone, through less mineralized tissues, to soft tissue, nor is there evidence of spurs radiating from the dentary that would suggest the presence of kype. This lack of evidence for kype is also true for the remainder of the jaw, such as at the midpoint between symphysis and the lateral margin of the jaw (Fig. 2.48 d).

***Salmo salar***

UW 019807 (fleshed); Figure 2.49

There is no evidence of a hole in the soft tissue of the upper jaw noted by Tchernavin (1938) and Witten and Hall (2002) that would indicate a developing kype (Fig. 2.49). Jaw bones appear relatively slender and delicate (Fig. 2.49 a-c), particularly when compared to the jaw bones of *H. hucho*. The margins of the dentary are somewhat blurred, but are distinct from the surrounding connective tissue of the jaw. Epithelium and connective tissue surrounding the jaw bones is thin when compared to *Hucho* and *Brachymystax*. The trends of thin epithelium and connective tissue and somewhat blurred margins continue throughout the jaw (Fig. 2.49 c).

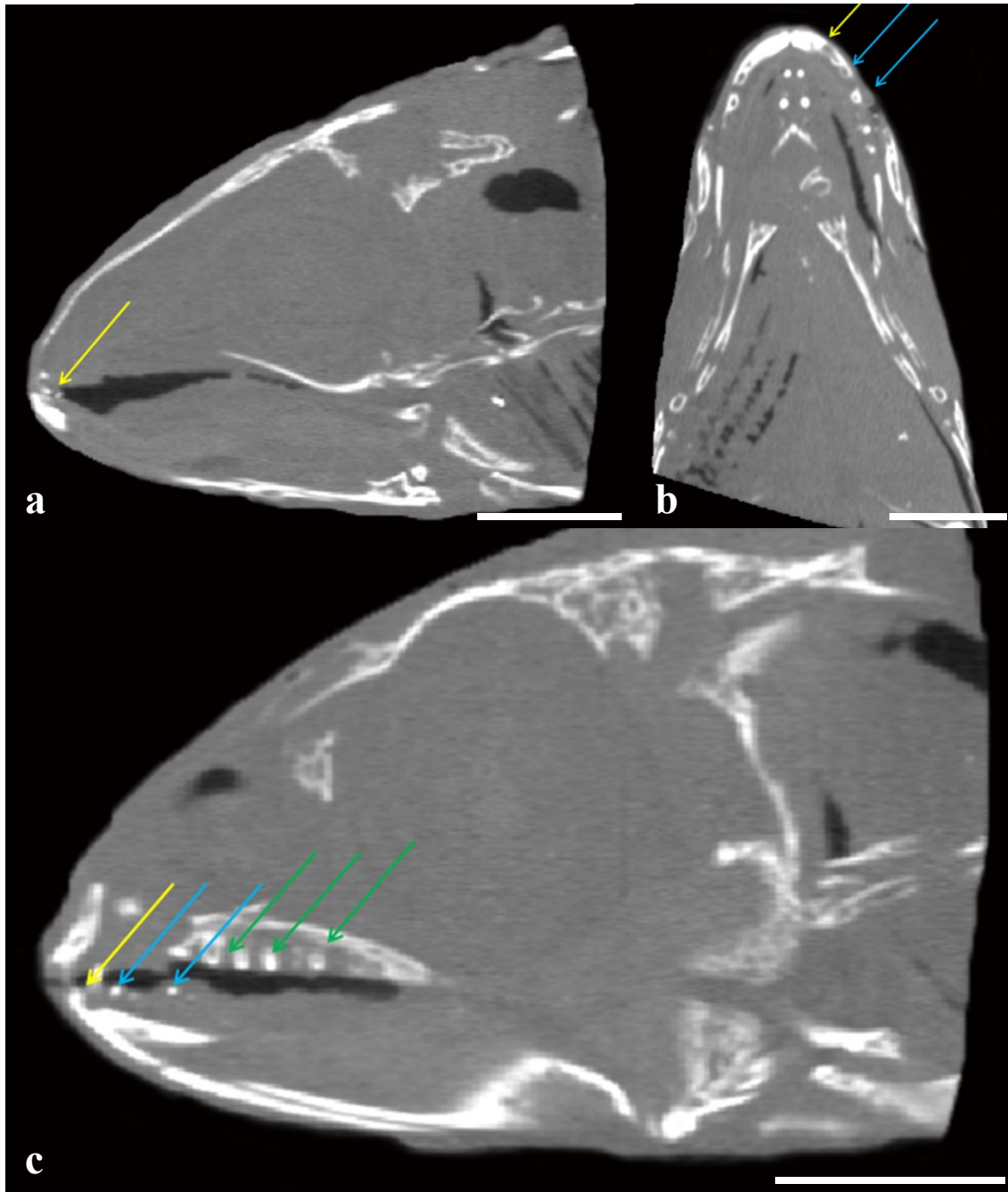
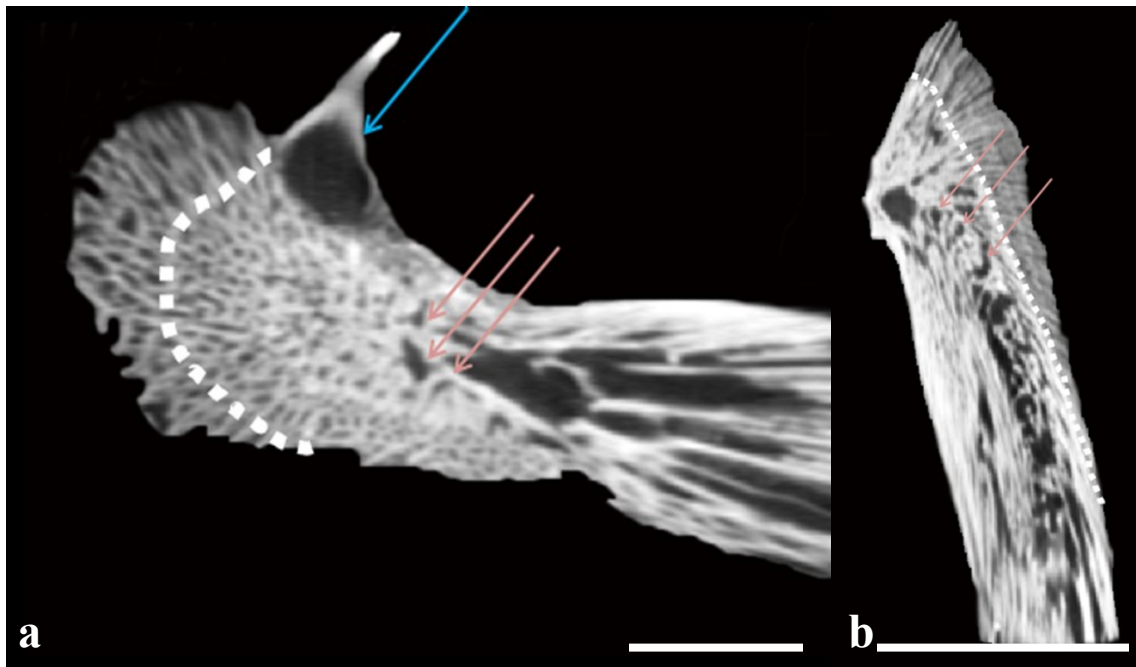


Figure 2.49 – Micro-CT scan of *Salmo salar* (UW 019807). (a) Sagittal section through head at the symphysis (yellow arrow). Epithelium and connective tissue surrounding the jaw bones are not as thick and margins of the dentaries are not as distinct as in *Hucho* and *Brachymystax*, but transition is still abrupt. There is no evidence of a “hole” in the soft tissue of the upper jaw noted by Tchernavin (1938) and Witten and Hall (2002). (b) Transverse section through lower jaw. Dentary seems relatively delicate and thin. Many teeth (blue arrows) are present. Margins of the dentaries are somewhat blurred, but nonetheless distinct from surrounding tissue. Epithelium and adjacent connective tissue is very thin when compared to *Hucho* and *Brachymystax*. (c) Sagittal section through head to the right of the symphysis, approximately halfway between symphysis and lateral margin of the head. Many teeth are present on dentary (blue arrows) and maxillae (green arrows). Margins of the dentaries are slightly fuzzy, but are still distinct. Epithelium and connective tissue surrounding jaw bones are very thin. Scale bars are 1 cm

CMNFI-1980-0181.1 (defleshed); Figure 2.50

Prominent needle-like features extending from the margins of the dentary bone are immediately apparent in both Figures 2.50 a and b. These extensions are present along a significant portion of the dentary margin (Fig. 2.50 b), but are thickest and most extensive anteriorly (Fig. 2.50 a). While it is possible to distinguish the margin of the compact dentary from these needles (Fig. 2.50, white dashed lines), it is relatively difficult to do so and the transition between the needles and the bone of the dentary is gradual.



**Figure 2.50 – Micro-CT scan of *S. salar* (CMNFI-1980-0181.1). (a) Sagittal section through dentary. The most obvious feature is the array of needle-like skeletal features extending from the dentary. The dentary margin is very obscured by this array and is indicated by the white dotted line. Other prominent features of this specimen include the amount of empty space within the bone, including several features that were the sites of large blood vessels (pink arrows) that can be tracked through the different slices of the scan. Also present is a large, sharp tooth with an extensive pulp cavity (blue arrow). (b) Transverse section through dentary. The margin of dentary is outlined with white dashed line. Skeletal needles are prominent and cover majority of area visible on anterior lateral margin of dentary. Scale bars are 0.5 cm.**

Also apparent in these images are large, sharp tooth (Fig. 2.50 a, blue arrow) and a variety of blood vessels (Fig. 2.50 a and b, pink arrows). These blood vessels can be tracked through several slices of the Micro-CT scan and form an intricate, almost dendritic meshwork of tunnels through the specimen. While animals without kype would

of course have blood vessels, the presence of so numerous a collection of large blood vessels is indicative of the large blood supply required to grow and maintain a kype.

***Oncorhynchus gorbuscha***

UW 16027 (fleshed); Figure 2.51

The margin of the dentary of this specimen is blurred and has a great deal of gradation from the dense, compact bone of the dentary out into the soft tissue of the surrounding jaw (Fig. 2.51 a-c). Furthermore, projections from the dentary into surrounding tissues are obvious on Figure 2.51 a, as is the thicker connective tissue region buffering the anterior tip of the lower jaw. These projections out from the dentaries are even more apparent in sagittal section (Fig. 2.51 b).



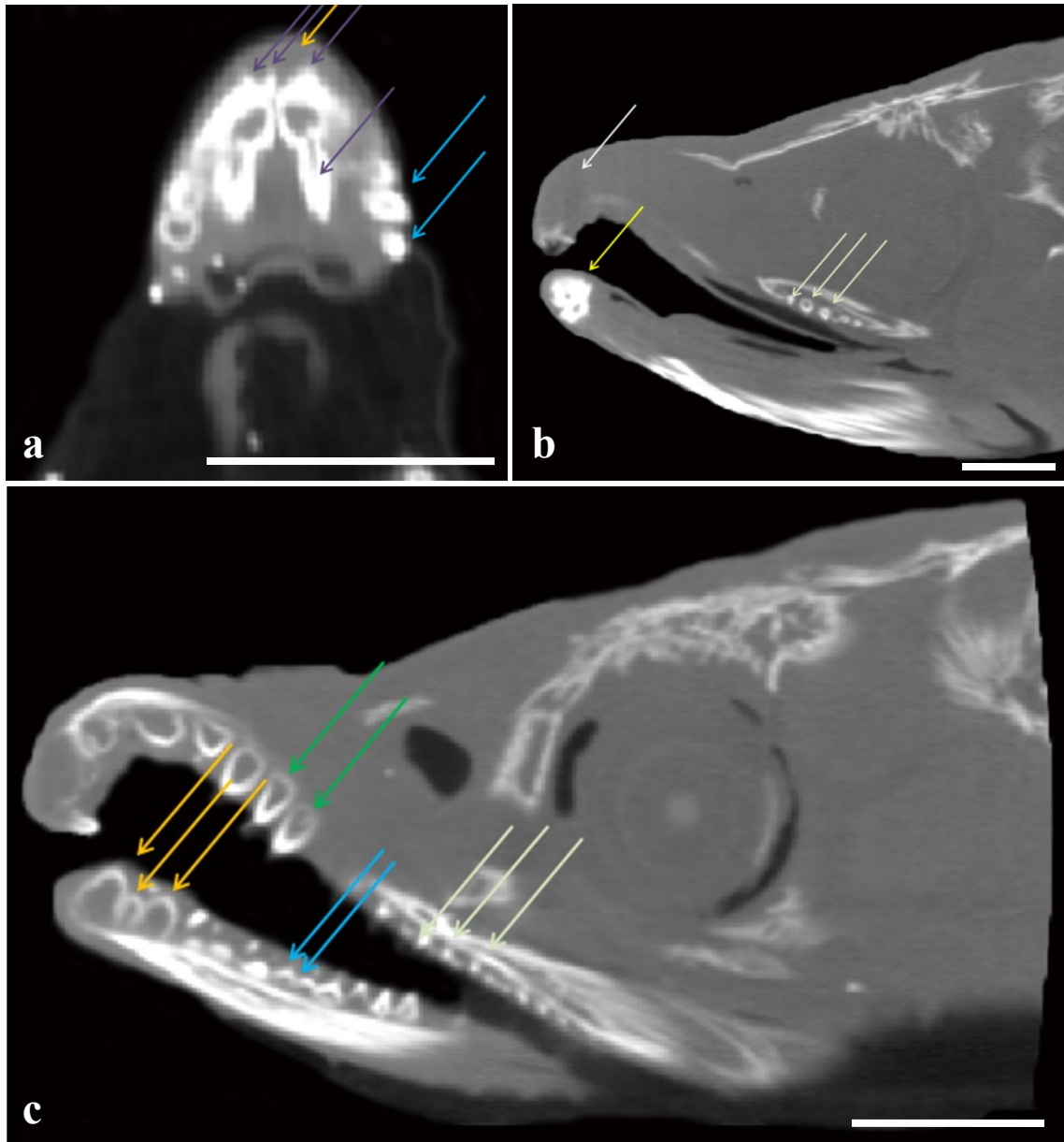


Figure 2.51 – Micro-CT scan of *Oncorhynchus gorbuscha* (UW 16027). (a) Transverse section through lower jaw just above dorsal surfaces of dentaries. Several teeth are visible at anterior (blue arrows), but the most obvious feature of this image is the lack of contrast at the margin of the dentary. Margins are very fuzzy and several projections jutting out from dentary anteriorly and posteriorly (purple arrows). Epithelium and connective tissue overall are very thin, but there is a thicker connective tissue region directly anterior to the symphysis (orange arrow). (b) Sagittal section through the right side of the head, midway between symphysis and anterior dentition. Contours of dentary are blurred (yellow arrow), with many complex mineralization and textural patterns within bone and cartilage mass. There are also many skeletal processes extending from the dentary. The soft tissue of upper jaw has formed a pronounced snout that overhangs the kype of the lower jaw (white arrow). Some teeth are also visible on the vomer (light green arrows). (c) Sagittal section through the right side of the head near the lateral margin. A large number of sharp and robust teeth are visible on both the upper (green) and lower (blue) jaws as well as on the vomer. These “normal” teeth are associated with putative “breeding” teeth at the anterior aspect of the dentary (orange arrows) that are larger and seem to be less dense than the smaller, more posterior teeth. The substantial number of large teeth on both the upper and lower jaws belies the delicate appearance of jaw bones, which appear to be slender, thin, and fragile. Scale bars are 2 cm.

In sagittal section, it is also possible to see the modifications to the upper jaw that compensate for the changes in the lower jaw that result in kype, forming a snout. In Figure 2.51 b, the curve of soft tissue that forms the snout is readily apparent, as are some teeth on the vomer (light green arrows). Further through the scan, towards the lateral margin of the head, both the upper (Fig. 2.51 c, green arrows) and the lower jaws (blue arrows) display a number of well-developed teeth (Fig. 2.51 c). Amongst these teeth are several on the anterior tip of the dentary that are much larger than those teeth more posterior of them (orange arrows). These enormous teeth appear to have a spongier interior and lower density than other teeth present in this specimen.

### ***Oncorhynchus nerka***

UW 005624 (fleshed); Figure 2.52

In transverse view, the abrupt shift from mineralized bone to soft tissue is apparent (Fig 2.52 a). In addition to a lack of gradual transition and intermediate regions, there are also no needle-like processes extending from the bone out into surrounding tissues, and thus, no kype, even a poorly developing one. Furthermore, the connective tissue and epithelium of the lower jaw is thin, as was seen in the fleshed *S. salar* (UW 019807; yellow arrow).

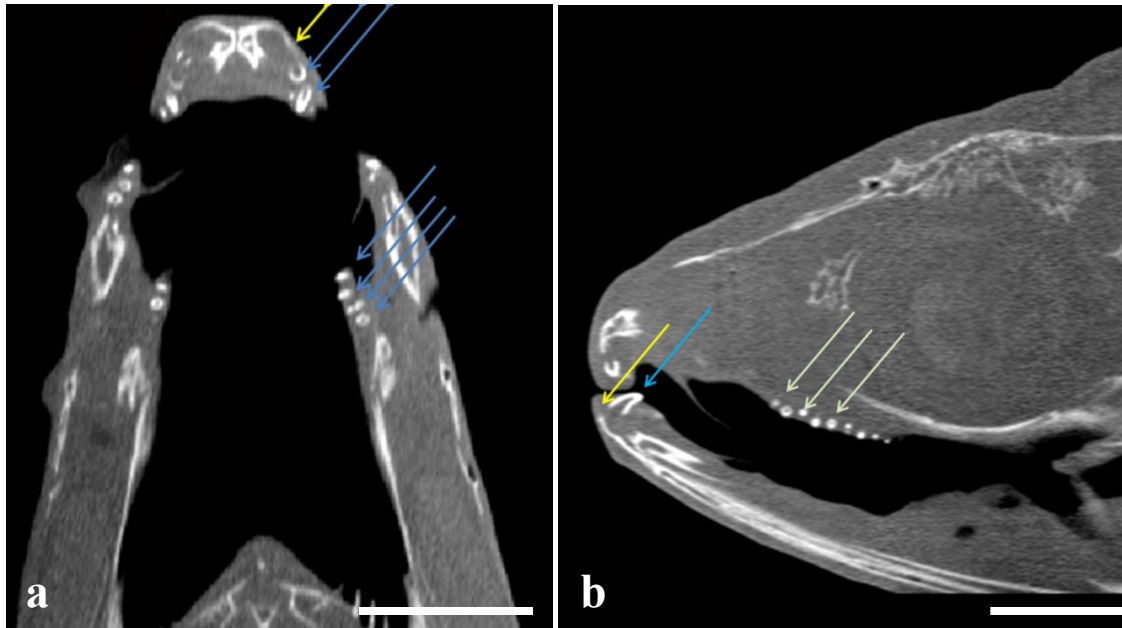


Figure 2.52 – Micro-CT scan of *Oncorhynchus nerka* (UW 005624). (a) Transverse section through lower jaw. Many teeth are present on dentary (blue arrows). The margins between the surrounding connective tissue and the dentary and the symphysis (thin yellow arrow) are distinct. The connective tissue and epithelium of the lower jaw are very thin. (b) Sagittal section through head to the right of the symphysis. Many teeth are present on dentary (blue arrows) and the vomer (light green arrows). Margins of the dentaries are distinct. Anterior epithelium and associated connective tissues are very thin (yellow arrow). Scale bars are 1 cm.

These trends are also apparent in sagittal section (Fig. 2.52 b). Unlike *O. gorbuscha*, (UW 16027), which displayed a curve in the bone and soft tissue of the upper jaw forming a snout, this specimen has upper and lower jaws of equal length. These jaws are also straight and do not appear to have a pronounced curve, contrary to what was seen in *O. gorbuscha*. Furthermore, a number of teeth are present on the dentaries (blue arrows), vomer (light green arrows), and maxillae.

CMNFI-1977-0277 (defleshed); Figure 2.53

Transverse sections (Fig. 2.53 a and b) through the dentary reveal several interesting features. While the most prominent feature on the kype of *S. salar* (CMNFI-1980-0181.1; Fig. 2.50) was extensive skeletal needles protruding from the compact dentary bone, the kype of *O. nerka* instead displays many teeth, including several very large, anterior “breeding teeth” (orange arrows) of lower density and with a deeper root than the remainder of the dentition (blue arrows). However, some skeletal needles are present, though they are localized to the anteriormost portion of the bone (white arrows).

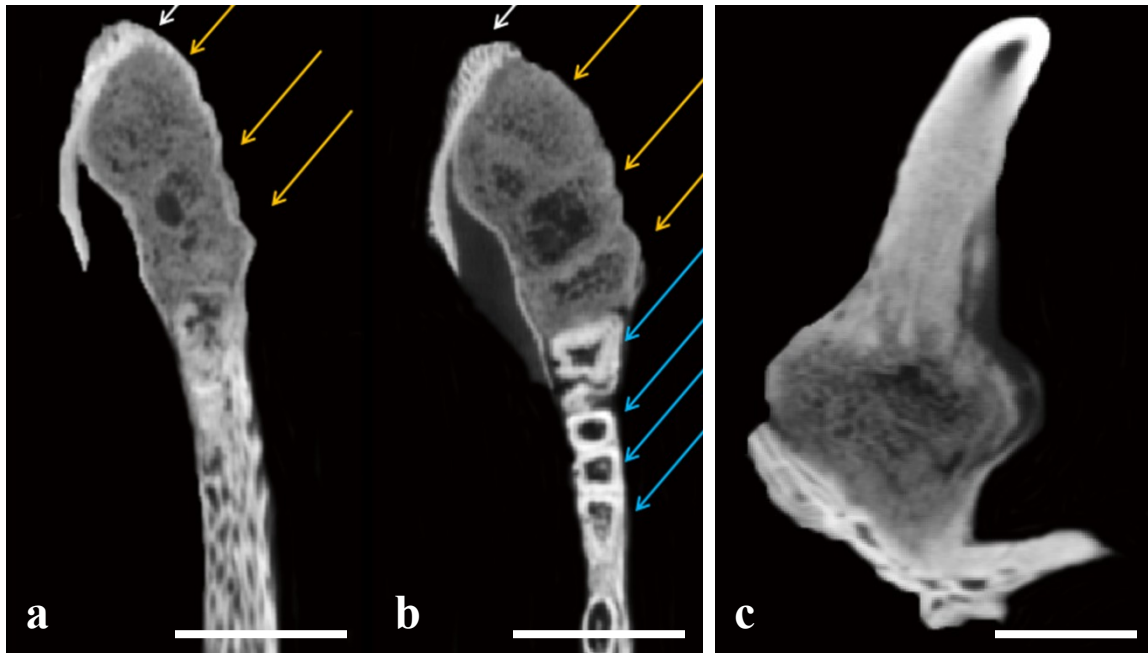


Figure 2.53 – Micro-CT scan of *O. nerka* (CMNFI-1977-0277 ). Transverse sections through dentary (a) below and (b) above bedding axis of non-breeding teeth. Several exceptionally large teeth (orange arrows) are visible anterior of “normal” teeth (blue arrows). These very large, anterior teeth are braced on the anterior side by a small region of skeletal needles similar to those seen in the defleshed *Salmo* specimen (CMNFI-1980-0181.1) (Fig. 2.4a and b) (white arrows). These “breeding” teeth have a deeper root than do the other, “normal” dentition and also appear to be spongier in texture and less dense than the other teeth. (c) Sagittal section through the anteriormost aspect of the dentary. The 1<sup>st</sup>, large breeding tooth rests on a thin and delicate band of compact dentary and is braced anteriorly by the sparse, needle-like extensions of the kype. The base of the tooth is very spongy in texture, and the shaft of the tooth seems compact and dense. Scale bars are 0.5 cm.

In sagittal section (Fig. 2.53 c), the very deep-rooted breeding teeth rest on a thin, delicate band of dentary bone. Anteriorly, the small region of needle like features extends from the dentary a short distance away, barely clearing the extension of the breeding tooth. The tooth has a very spongy base and a more compact, denser shaft.

#### ***Micro-CT summary***

The features discussed above were determined using grayscale patterns as a proxy for tissue density and, in turn, mineralization. As determined by Enax et al. (2013) in their assessment of crocodile teeth, this use of grayscale makes it possible to develop qualitative assessments of patterns in the skeletal morphology of the heads of these specimens. However, a quantitative assessment may also be possible if we attempt to match grayscale values to the different tissues of interest. This grayscale matching method is used as a diagnostic tool in medicine, for instance in diagnosing osteblastoma

and other tumours (Feldman et al., 2003; Fleischer et al., 1978; Walsh et al., 1979), coronary artery stenosis (Grant et al., 2003), and a variety of other conditions including jaundice (Taylor et al., 1979) and pyelonephritis (Lee et al., 2012). It may be feasible to use density patterns, and thereby mineralization patterns, to determine if the tissues previously found to be associated with kype development, namely chondroid bone and Sharpey-fibre bone, are present in various specimens.

Upon establishing a grayscale gradient in *O. gorbuscha* (Fig. 2.54) and comparing it to one in *H. hucho* (Fig. 2.55), it is evident that the patterns in grayscale values are different between the two animals. The grayscale plot of a sagittal section through the right side of the head of *O. gorbuscha* reveals a gradual increase in density from the margin of the jaw through the soft tissue surrounding the dentary and kype, through the tissues of the kype, and into the compact and dense dentary bone. This gradual increase in density suggests that transitional and intermediate tissues lie between the low density soft tissue and the high density compact bone. A grayscale plot through a similar section in *H. hucho* reveals instead an abrupt transition from the low-density soft tissue and the high density dentary lying within it. Without a gradual slope in the grayscale plot, there is no evidence of transition and intermediate tissues between the two regions and tissue types along the plotline.

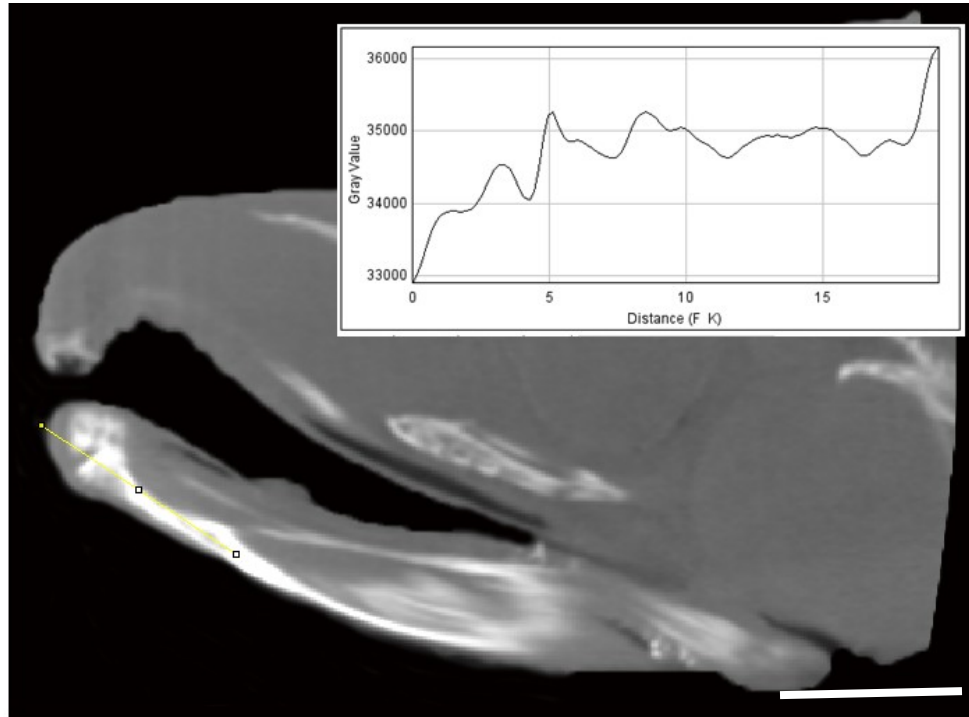


Figure 2.54 - Grayscale plot using a sagittal section through the right side of the head of *O. gorbuscha* (UW 16027) one third of the distance from the symphysis to the lateral margin of the head. The contours of the plot follows the density (and thereby mineralization) of the tissues crossed by the yellow line. The plot is zeroed at the margin on the lower jaw. The plot displays a gradual increase in the density of the tissues the selection line is passing through, suggesting that transitional and intermediate tissues lie between the soft tissue of the jaw and the mineralized, compact bone of the dentary. Scale bar is 2 cm.

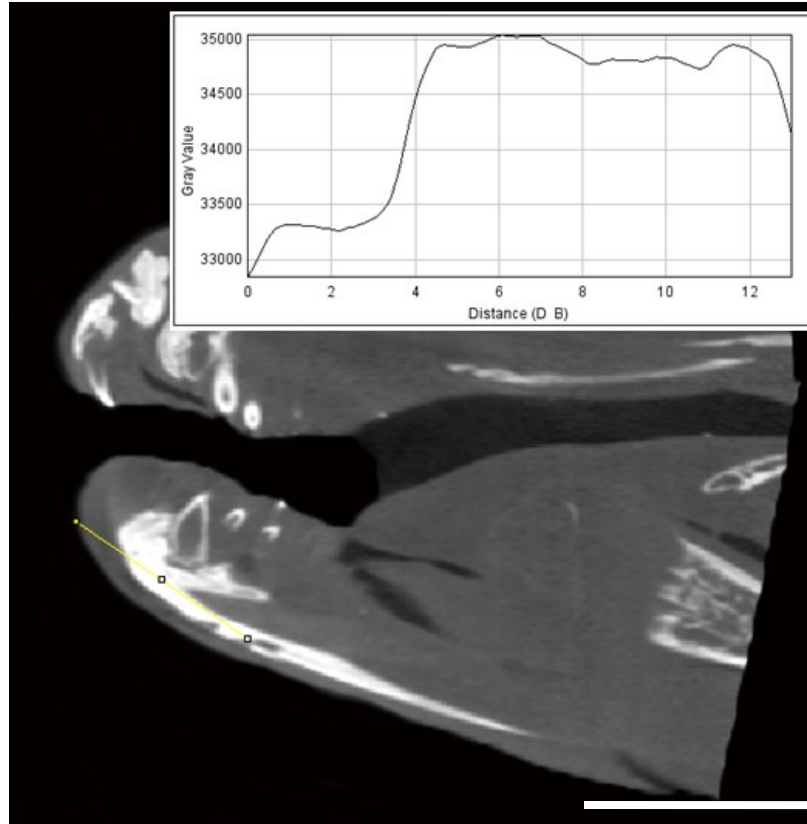


Figure 2.55 - Grayscale plot using a sagittal section through head of *H. hucho* (UW 022130) at approximately one third of the distance from the symphysis to the lateral margin of the head. The contours of the plot follows the density (and thereby mineralization) of the tissues crossed by the yellow line. The plot is zeroed at the margin on the lower jaw. The plot displays an abrupt increase in the density of the tissues the selection line is passing through. There is a distinct shift from low to high density tissues, suggesting a lack of transitional and intermediate tissues between the soft tissue of the jaw and the mineralized, compact bone of the dentary. Scale bar is 2 cm.

While the slopes of the grayscale plots provide convincing evidence for and against the presence of intermediate tissues, or at the very least tissues of intermediate densities between the soft tissues and the compact dentary bone within it, there are also differences between the grayscale values within the tissues of two different sections of the same specimen. As we can see when we contrast the first image of *O. gorbuscha* in Figure 2.54 with one from a slightly more medial slice of the same specimen (Fig. 2.56), there is a less gradual transition from surrounding soft tissue to compact dentary. The transition from soft tissue to dense bone is much more step-wise in the medial slice than in the lateral slice.

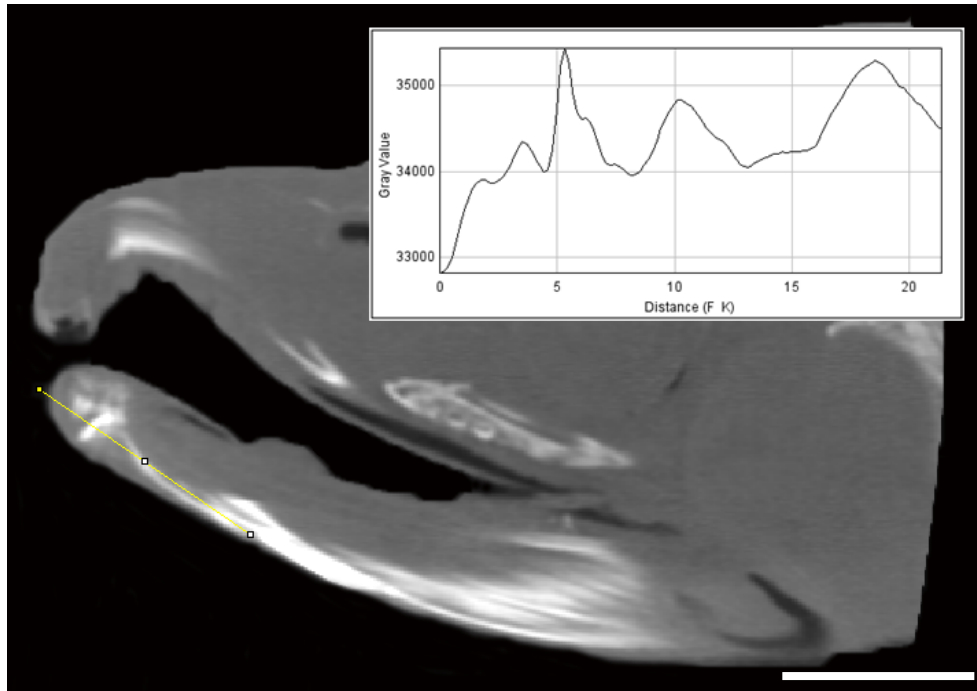


Figure 2.56 - Grayscale plot using a sagittal section through the right side of the head of *O. gorbuscha* (UW 16027) at a position slightly medial to one third of the distance from the symphysis to the lateral margin of the head. The contours of the plot follows the density (and thereby mineralization) of the tissues crossed by the yellow line. The plot is zeroed at the margin of the lower jaw. In this portion of the head, there is a less subtle shift from the less dense soft tissue of the jaw and the mature, mineralized, compact dentary bone. However, there remains a section between the two tissue types where there is shift from areas of less to areas of more mineralization, a shift that is much more gradual than is seen in the *Hucho* grayscale plot.

These differences in grayscale plot within the same animal may be explained by slight variations in tissue organization between the tissues of different portions of the jaw, but, nonetheless, they complicate the issue of attempting to use grayscale to diagnose kype-related tissues within a scan. This grayscale diagnosis of kype tissues is a process that would need extensive validation using studies involving a large number of known kype-bearing and non-kype bearing animals of similar size and preservation, pin-pointing, if possible, the specific attenuation of individual tissues in the fish jaw. Tissues of interest in this validation process include compact bone, alveolar bone (tooth attachment bone), Sharpey fibres, Sharpey-fibre bone, chondroid bone, and soft tissues.

While grey-scale plots may not yet be a viable means of quantitatively assessing the kype-related morphology of these specimens, relative and qualitative assessments of grey-scale values reveal a great deal as to conditions within kype- and non-kype bearing animals. These conditions include gradients in anterior tissue density and anterior



structural evidence of a kype as well as differences in the structure of the kype and of elements related to the kype in different genera.

At the anterior tip of the lower jaw of several specimens, a gradient was present and ranged from dense, compact dentary bone into less dense material, out into the grey soft tissue and epithelium of the tissues surrounding the dentary. Furthermore, some of these animals also display evidence of an irregular anterior dentary surface, with projections and extensions of material that is denser than surrounding connective tissue. These specimens reflect those animals found to have structural evidence for kype upon physical examination. In animals without these gradients and projections, no evidence of kype had been found.

In addition to anterior tissue gradients and skeletal projections, extensive evidence of differences in the development and structure of kype in *Salmo* and *Oncorhynchus* was obvious. These differences include different compensatory modifications in the upper jaw, different types of teeth at the anteriormost dentary, and differences in the extent of skeletal needles extending from the dentary of a kype-bearing animal.

In looking at the different compensatory upper jaw modifications, as noted previously in the “Physical examination” section above, Witten and Hall (2002) found that *Salmo* individuals develop a hole in the soft tissue of the upper jaw that the kype slips into. Contrarily, *Oncorhynchus* uses a rather different compensatory mechanism: a “snout”. The structure of this snout is revealed in sagittal sections of *O. gorbuscha* (UW 16027; Fig. 2.51, 2.54, 2.56), where an accumulation of soft tissue at the anterior tip of the upper jaw balances the kype-related elements of the lower jaw. This accumulation of soft tissue forming the curve of the snout is supported by a curve in the maxillae, which is decorated with an array of large, sharp teeth.

Another interesting feature in *Oncorhynchus* specimens are the large, spongy breeding teeth located at the anterior tip of the dentary. These teeth, seemingly of lower density than the other teeth posterior of them, are well exhibited by the *O. gorbuscha* (UW 16027) and *O. nerka* (CMNFI-1977-0277) specimens examined here and are absent in the *Salmo salar* specimens. As with UW 16027 and CMNFI-1977-0277, the largest teeth found in the *S. salar* specimen (CMNFI-1980-0181.1) were located on the anterior edge of the dentary. However, these large teeth in CMNFI-1980-0181.1 did not have a

different texture or density when compared with other teeth on the jaws. These differences between the anteriormost teeth found in *Oncorhynchus* and *Salmo* are of particular interest when one considers previous work that revealed that, contrary to what had previously been thought (Aas et al., 2011; Scott and Crossman, 1973; Tchernavin, 1938), “breeding teeth” in *S. salar* are actually present throughout an animal’s life and are only more exposed by spawning-related changes in the soft tissue of the jaw (Witten and Hall, 2005). No such research on the genus *Oncorhynchus* has been performed. However, when comparing the non-kype-bearing *O. nerka* (UW 005624) to the kype-bearing specimens, these putative spongy, breeding teeth are not present in the absence of kype, perhaps suggesting that, unlike to *S. salar*, breeding teeth do develop in spawning *Oncorhynchus* individuals. More research, particularly using an extensive *Oncorhynchus* growth series study that complements the previous work done by Witten and Hall on *S. salar*, is needed to truly establish if breeding teeth develop in spawning *Oncorhynchus* individuals.

Finally, while there appears to be a dramatic change in the dentition of spawning *Oncorhynchus* individuals, there is a relatively minimal change in terms of the extent of skeletal needles protruding from the dentary. When comparing the defleshed *S. salar* and *O. nerka* specimens, the *S. salar* specimen has a profusion of skeletal needles that extends from the anterior aspect of the dentary to several centimetres down the dentary shaft. These needles are long and densely packed, making the margin of the compact dentary at times difficult to distinguish. In contrast, the extent of the skeletal needles in *O. nerka* is marginalized and the region of the dentary covered in needles is localized to the very anteriormost aspect, anterior of the putative breeding teeth. Furthermore, these needles are relatively short, barely clearing the margins of the tooth root.

### ***Literature survey***

Having gathered information as to kype presence and absence in salmonid fishes using physical examinations and Micro-CT assessment, I then used an extensive literature review to supplement this information. In this review, I sought direct reference to kype presence or absence, as well as photographs or sketches of animals with a kype. However, because not all authors included images of spawning animals, are necessarily familiar

with the kype, or would make note of it even if a kype was present on a given animal, I also tentatively considered several terms to be positive indicators of kype growth. These terms included references to a secondary elongation of the lower jaw, craniofacial sexual dimorphism, changes in the profile of the head during the spawn-run, and presence of compensatory features in the upper jaw.

The results of the literature review are summarized in Table 2.4. Please note that the literature listed in Table 2.4 includes references that did not specifically mention the term “kype” or the positive indicator terms noted in the previous paragraph. These references are included because they aid in the understanding of known conditions in each group. Also outlined below are clarifications on and more detailed descriptions of kype presence in several species and genera of interest.

**Table 2.4 – Summary of literature survey of kype presence or absence across the Salmonidae.**

<i>Genus species</i>	Kype present?	Literature
Coregoninae	No	Behnke, 2002; Nelson, 2006; Scott and Crossman, 1973; Stearley and Smith, 1993; Willson, 1997
<i>Coregonus</i>	No	Alt, 1969; Behnke, 1972; Cavender, 1970; Eckmann, 1991; Ihssen, et al., 1981; Jonsson and Jonsson, 2011; Nelson, 2006; Scott and Crossman, 1973; Stearley and Smith 1993; Willson, 1997;
- <i>C. clupeaformis</i>	No	Airaksinen, 1968; Alt, 1969; Carlander, 1969; Cavender, 1970; Eckmann, 1991; Hendry and Stearns, 2004; Ihssen et al., 1981; Nelson, 2006; Schulz and Freyhof, 2003; Scott and Crossman, 1973; Stearley and Smith, 1993; Svårdson, 1979; Willson, 1997
- <i>C. laurettae</i>	No	Cavender, 1970; Hendry and Stearns, 2004; Lee et al., 1980; McPhail and Lindsey, 1970; Morrow, 1980; Nelson, 2006; Riede, 2004; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993
- <i>C. lavaretus</i>	No	Cavender, 1970; Eckmann, 1991; Geller and Guede, 1989; Hartmann, 1984; Hendry and Sterns, 2004; Krause and Palm, 2000; Nelson, 2006; Ruhlé, 1986; Stearley and Smith, 1993
<i>Stenodus</i>		
- <i>S. leucichthys</i>	No	Alt, 1969; Alt, 1973; Alt, 1977; Brown et al., 2007; Dymond, 1943; Eckmann, 1991; Howland et al., 2000; Nelson, 2006; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993; Walters, 1955; Willson, 1997

<b>Genus species</b>	<b>Kype present?</b>	<b>Literature</b>
<i>Prosopium</i>	No	Norden, 1970; Scott and Crossman, 1973; Stearley and Smith, 1993; Willson, 1997
- <i>P. williamsoni</i>	No	Behnke, 2002; Brown, 1952; Hagen, 1970; McPhail and Lindsey, 1970; Nelson, 1994, 2006; Nener et al., 1995; Norden, 1970; R.L. & L. Environmental Services Ltd., 1995, 1997; Roberge et al., 2002; Scott and Crossman, 1973; Willson, 1997; Vladykov, 1970
- <i>P. cylindraceum</i>	No	Brown et al., 2007; Dryer, 1966; Mraz, 1964; Nelson, 1994, 2006; Norden, 1970; Normandeau, 1969; Rawson, 1951; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993
<i>Thymallus</i>		
- <i>T. arcticus</i>	No	Alt, 1969; Armstrong, 1986; Beauchamp, 1990; Behnke, 2002; Berg, 1962; Carlander, 1969; Craig and Poulin, 1975; Harper, 1948; MacPhail and Lindsey, 1970; Morrow, 1980; Nelson, 1994, 2006; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993; Willson, 1997
<i>Brachymystax</i>		
- <i>B. lenok</i>	No	Alekseyev et al., 2003; Baimukanov, 1996; Edo et al., 2000; Esteve and McLellan, 2008; Hendry and Stearns, 2004; Holčík, 1982; Kifa, 1974; Mitrofanov and Petr, 1999; Nelson, 1994, 2006; Stearley and Smith, 1993; Willson, 1997; Xu et al., 2009; Zhao and Zhang, 2009
<i>Hucho</i>		
- <i>H. hucho</i>	No	Blanc et al., 1971; Drenski, 1948; Dulmaa, 1999; Esteve et al., 2009a; Esteve et al., 2013; Fleming, 1998; Hendry and Stearns, 2004; Holčík et al., 1988; Ladiges and Voigt, 1965; Mitrofanov and Petr, 1999; Schindler, 1935; Stearley and Smith, 1993; Willson, 1997; Witwowski, 1988
- <i>H. perryi</i>	Indeterminate	Berg, 1962; Cavender, 1980; Edo et al., 2000; Esteve et al., 2009b; Fukushima, 1994; Hendry and Stearns, 2004; Gilroy et al., 2010; Holčík, 1982; Holčík et al., 1988; Kawamura et al., 1983; Kimura, 1966; Stearley and Smith, 1993

<b>Genus species</b>	<b>Kype present?</b>	<b>Literature</b>
<i>Salvelinus</i>	Yes; “notch” compensatory feature in upper jaw	Armstrong, 1965; Balon, 1980; Fleming, 1998; Haugland et al., 2011; Johnson, 1980; Stearley and Smith, 1993; Willson, 1997
- <i>S. fontinalis</i>	Yes	Balon, 1980; Behnke, 2002; Blanchfield and Ridgway, 1997; Dutil and Power, 1980; Fleming, 1998; Gaboury, 1980; Hazzard, 1932; Hutchings, 1993, 1994; Jonsson and Jonsson, 2011; Lewis and Van Vliet, 2008; McAfee, 1966; Morton, 1965; Power, 1980; Saunders and Power, 1970; Vladykov, 1956; Willson, 1997
- <i>S. namaycush</i>	No (though is capable) <sup>1</sup>	Arai et al., 2005; Balon, 1980; Cavender, 1980; Chereshnev et al., 2002; Esin and Sorokin, 2012; Esteve et al., 2011; Gritsenko, 2002; Gritsenko and Churikov, 1976; McKraken et al., 2013; Patterson, 1968; Sato, 2007; Sato and Watanabe, 2004; Savvaitova et al., 2007; Volubuev, 1987; Yamamoto et al., 1999
- <i>S. namaycush</i> X <i>fontinalis</i> hybrids (“splake”)	Yes (usually only in large anadromous males)	Behnke, 2002; Bjornn, 1991; Block, 1955; Brenkman and Corbett, 2005; Brenkman et al., 2001; Cavender, 1978; Dunham et al., 2011; Fraley and Shepard, 1989; Monnot et al., 2008; Moyle, 2002; Muhlfield and Marotz, 2005; Rieman and McIntyre, 1996; Willson, 1997
- <i>S. leucomaenis</i>	Yes (with some controversy)	Armstrong, 1965; Balon, 1980; Behnke, 2002; Cuerrier and Schultz, 1957; DeRoche and Bond, 1957; Eschmeyer, 1955; Fleming, 1998; Johnson, 1972; Jonsson and Jonsson, 2011; Loftus, 1958; Martin and Olver, 1980; Miller and Kennedy, 1948; Morton, 1965; Paterson, 1968; Pichugin et al., 2006; Rawson, 1947; Royce, 1951; Séguin and Roussel, 1968; Tomkins, 1951; Willson, 1997
- <i>S. confluentus</i>	Yes	Balon, 1980; Behnke, 2002; Berst et al., 1980; Berst and Spangler 1970; Buss and Wright, 1958, Graham, 1996; Pratt, 1992; Shepard et al., 1984
- <i>S. malma</i>	Yes	Armstrong, 1974; Armstrong and Morrow, 1980; Balon, 1980; Behnke, 2002; Maekawa and Hino, 1986; Maekawa et al., 1993; Morton, 1965; Scott and Crossman, 1973; Willson, 1997
- <i>S. alpinus</i>	Yes, rarely in non-anadromous forms	Balon, 1980; Behnke, 2002; Dempson and Green, 1985; Dutil, 1984; Fleming, 1998; Haugland et al., 2011; Johnson, 1980; Johnston, 2002; Jonsson and Jonsson, 2011; Jonsson et al., 1988; McCart, 1980; Morton, 1965; Nordeng, 1961; Willson, 1997

<sup>1</sup> *Salvelinus namaycush* does not typically develop a kype. However, extremely rare specimens from some populations displaying kype-like structures have been identified, indicating that this species is capable of kype growth (Martin and Olver, 1980; Willson, 1997).

<i>Genus species</i>	<b>Kype developing?</b>	<b>Literature</b>
<i>Salmo</i>	Yes, “hole” compensatory feature in the upper jaw	Behnke, 2002; Fleming, 1998; Jonsson and Jonsson, 2011; Stearley and Smith, 1993; Willson, 1997
- <i>S. trutta</i>	Yes	Barlaup et al., 1994; Behnke, 2002; Campbell, 1977; Elliot, 1984; Evans, 1994; Fleming, 1998; Jones and Ball, 1954; Jonsson and Jonsson, 2011; Jonsson and L’Abée-Lund, 1993; L’Abée-Lund and Hindar, 1990; L’Abée-Lund et al., 1989; L’Abée-Lund et al., 1990; Willson, 1997
- <i>S. salar</i>	Yes	Aas et al., 2011; Allen et al., 1972; Baglinière, 1985; Baglinière, et al., 1990; Baglinière, et al., 1991; Barlaup et al., 1994; Baum, 1997; Beall and Marty, 1983; Behnke, 2002; Belding, 1934; Bley and Moring, 1988; Carlander, 1969; Crisp and Carling, 1989; Dempson et al., 1986; Ducharme, 1969; Fleming, 1996, 1998; Fleming et al., 1997; Gibson, 1993; Glebe et al., 1979; Gustafson-Greenwood and Moring, 1991; Heggberget, 1988; Heggberget et al., 1988; Jonsson and Jonsson, 2011; MacDonald, 1970; Metcalfe, 1998; Metcalfe and Thorpe, 1992; Mills, 1989; Peterson, 1978; Piggins, 1968; Saunders, 1967; Thorstad et al., 2011; Webb and McLay, 1996; Willson, 1997
<i>Oncorhynchus</i>	Yes, “snout” compensatory feature on upper jaw	Behnke, 2002; Cavender, 1970; Groot and Margolis, 1991; Healey, 1991; Keenleyside and Dupuis, 1988; Morton, 1965; Quinn and Foote, 1994; Ricker, 1972; Scott and Crossman, 1973; Stearley and Smith, 1993; Vladykov, 1962; Willson, 1997
- <i>O. clarki</i>	Yes	Behnke, 1992, 2002; Brown and Mackay, 1995; Burgner et al., 1992; Fleming, 1998; Irving, 1955; Johnston, 1982; Jones, 1972, 1973, 1974, 1975, 1976; Morton, 1965; Ricker, 1972; Roberge et al., 2002; Scott and Crossman, 1973; Shepard et al., 1984; Stearley and Smith, 1993; Sumner, 1953; Tipping, 1981; Trotter, 1989; Willson, 1997
- <i>O. mykiss</i>	Yes	Bangham and Adams, 1954; Barlaup et al., 1994; Behnke, 1992, 2002; Bley and Moring, 1988; Briggs, 1953; Burgner et al., 1992; Busby et al., 1996; Cavender, 1970; Clemens et al., 1939; Crisp and Carling, 1989; Fleming, 1998; Hartman et al., 1962; Horncastle, 1981; Hunter, 1973; Knapp and Vredenburg, 1996; Leider et al., 1986; Lindsey et al., 1959; Lohr, 1996; Mackey et al., 2001; Morton, 1965; Orcutt et al., 1968; Ricker, 1972; Roberge et al., 2002; Shapolov and Taft, 1954; Smith, 1955, 1960; Stearley and Smith, 1993; Stenton, 1963; Willson, 1997

<i>Genus species</i>	Kype developing?	Literature
<i>Oncorhynchus</i>		
- <i>O. kisutch</i>	Yes	Behnke, 2002; Briggs, 1953; Fleming, 1998, Fleming and Gross, 1994, Halupka et al., 1996; Healey and Prince, 1995; Holtby and Healey, 1990; Morton, 1965; Ricker, 1972; Roberge et al., 2002; Sandercock, 1991; Scott and Crossman, 1973; Shapovalov and Taft, 1954; Stearley and Smith, 1993; Swain and Holtby, 1989; van den Berghe and Gross, 1989; Willson, 1997
- <i>O. tsawytscha</i>	Yes, typically moderate	Barlaup et al., 1994; Behnke, 2002; Bernier et al., 1993; Brett, 1995; Briggs, 1953; Butts et al., 2012; Chapman, 1943; Dauble et al., 1999; Fleming, 1998; Fraser River Action Plan, 1995; Healey, 1991; Roberge et al., 2002; Roni and Quinn, 1995; Scott and Crossman, 1973; Unwin et al., 1999; Willson, 1997
- <i>O. keta</i>	Yes	Beauchamp, 1984; Behnke, 2002; Brett, 1995; Fleming, 1998; Halupka et al., 1995a; Johnson et al., 1971; Keenleyside and Dupuis, 1988; Morton, 1965, 1980; Roberge et al., 2002; Salo, 1991; Sano, 1959; Scott and Crossman, 1973; Schroder, 1981; Stearley and Smith, 1993; Willson, 1997
- <i>O. gorbuscha</i>	Yes	Behnke, 2002; Brett, 1995; Dye et al., 1986; Fleming, 1998; Halupka et al., 1995b; Heard, 1991; Roberge et al., 2002; Scott and Crossman, 1973; Smirnov, 1975; Willson, 1997
- <i>O. nerka</i>	Yes	Behnke, 2002; Brett, 1995; Burgner, 1991; Chebanov et al., 1984; Fleming, 1998; Foerster, 1968; Foote and Larkin, 1988; Ford et al., 1995; Fukushima and Smoker, 1998; Gilhousen, 1960; Hendry and Berg, 1999; Hendry et al., 1998; Hendry and Quinn, 1997; McPhee and Quinn, 1998; Morton, 1965; Murray et al., 1989; Quinn and Foote, 1994; Ricker, 1938, 1972; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993; Willson, 1997

Species and genera of interest include:

### ***Hucho***

#### ***Hucho hucho* (Huchen)**

Although some researchers have mentioned finding kype in *H. hucho* (Ladiges and Vogt, 1965; Schindler, 1935), this assessment is not well accepted and is generally believed to

be in error (Holčík et al., 1988). As such, *H. hucho* is not generally believed to be kype-bearing (Holčík et al., 1988; Stearley and Smith, 1993).

***Hucho perryi* (Sakhalin taimen).**

While some popular sources, in particular the blog of one Japanese fisherman, would indicate that *H. perryi* does develop at least a rudimentary kype (Fig. 2.57; Anonymous, 2007), formal morphological and taxonomic work suggests instead that kype development is absent in this species (Holčík et al., 1988; Stearley and Smith, 1993). However, because of the dearth of English-language publications on this species, it is very difficult to confirm or deny the presence of kype in *H. perryi*.

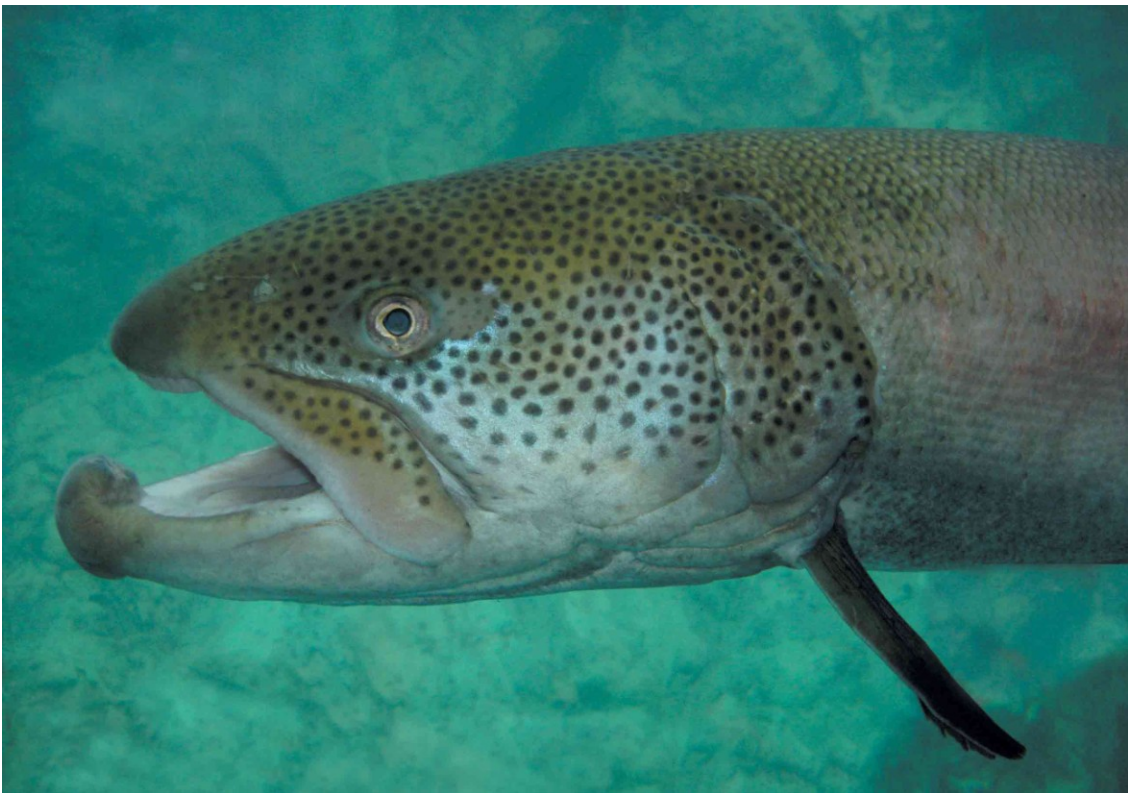


Figure 2.57 - *Hucho perryi* with an anterior connective tissue mass and gaping mouth posture suggestive of the presence of a kype. (From <[http://19809452.at.webry.info/200705/article\\_2.html](http://19809452.at.webry.info/200705/article_2.html)>, accessed December 23, 2013).



***Salvelinus* (trouts and chars)**

Largely kype bearing, this genus compensates for the hooking upward of the lower jaw with a separation in the premaxillae, creating a “notch” that the kyped lower jaw can slip into (Balon, 1980; Behnke, 2002; Haugland et al., 2011; Stearley and Smith, 1993).

***Salvelinus fontinalis* (brook trout)**

*Salvelinus fontinalis*, except in small, freshwater living animals, almost always develop a prominent kype prior to spawning (Jonsson and Jonsson, 2011; Morton, 1965; Stearley and Smith, 1993).

***Salvelinus namaycush* (lake trout)**

Extremely rarely, individuals may develop a kype; however, this species does not develop a kype though it is capable of doing so (Behnke, 2002; Martin and Olver, 1980; Morton, 1965; Stearley and Smith, 1993; Willson, 1997).

***Salvelinus namaycush x fontinalis* (“splake”)**

Fertile hybrids of *Salvelinus namaycush* and *S. fontinalis*, many of the traits and characters of these animals are variable, though typically intermediate between the conditions of their parents (Behnke, 2002; Berst et al., 1980; Buss and Wright, 1958). As such, the kype is not always found in these animals, though it is usually present in large, anadromous males (Berst et al., 1980; Berst and Spangler, 1970).

***Salvelinus leucomaenis* (lake trout)**

As with the majority of *Salvelinus* species, *S. leucomaenis* appears to develop a kype, with both the kype and compensatory notch in the upper jaw being prominent in both sexes (Savvaitova et al., 2007). However, some researchers propose that kype development may be absent in this species (Stearley and Smith, 1993) or may depend on resource availability and life history selection (Esin and Sorokin, 2012).

### ***Salvelinus confluentus* (bull trout)**

In *Salvelinus confluentus*, the kype tends to be pronounced in spawning animals and, in males, may begin to develop well before the initiation of the spawning migration (Behnke, 2002; Balon, 1980). The kype is typically subtle in females.

### ***Salvelinus malma* (dolly varden)**

Both males and females develop kype, though it is much more prominent and “dramatic” in males (Armstrong and Morrow, 1980; Behnke, 2002; Morton, 1965).

### ***Salvelinus alpinus***

*Salvelinus alpinus* is variable in terms of life history mode, physical characteristics, spawning behaviour, morphology, and, to an extent physiology (Behnke, 2002; Johnson, 1980; Jonsson and Jonsson, 2011; McCart, 1980; Willson, 1997). While *S. alpinus* individuals may develop a prominent kype, it is rarely seen in non-anadromous animals (Johnson, 1980; Morton, 1965).

### ***Salmo* (Atlantic salmon and trout)**

The *Salmo* genus shows relatively little variation in terms of morphological and behavioural characteristics, with both *S. trutta* and *S. salar* developing prominent kype (Behnke, 2002; Eliot, 1984; Jonsson and Jonsson, 2011). To compensate for the growth of the kype, a “hole” or “hollow” in the soft tissue of the upper jaw develops concurrently (Behnke, 2002; Eliot, 1984; Jonsson and Jonsson, 2011; Tchernavin, 1938; Witten and Hall, 2002).

### ***Salmo trutta***

Both males and females of *S. trutta* tend to develop a kype, albeit a relatively non-dramatic one in females (Barlaup, et al., 1994; Behnke, 2002; Campbell, 1977).

### ***Salmo salar***

As with *S. trutta*, both female and, more particularly, male *S. salar* individuals develop a prominent kype (Baum, 1997; Behnke, 2002; Jonsson and Jonsson, 2011; Thorstad et al., 2011).

### ***Oncorhynchus* (Pacific salmon and trout)**

Almost all species and sub-species of *Oncorhynchus* develop kype (Behnke, 2002; Groot and Margolis, 1991; Scott and Crossman, 1973; Stearley and Smith, 1993). However, kype development is rare in non-anadromous and non-migratory populations. The modifications to the lower jaw that result in a kype are paired with an elongation of the upper jaw to form a “snout” (Scott and Crossman, 1973). Prominent kype in this genus co-occur with large, often dog-like teeth at the tip of the lower jaw, particularly in *O. keta* and *O. nerka* (Behnke, 2002; Scott and Crossman, 1972).

### ***Oncorhynchus clarki* (cutthroat trout)**

*O. clarki* is highly variable, with many subspecies that, depending on a number of factors including latitude, life history mode, and preferential habitat, display a variety of different morphologies and behaviours (Roberge et al., 2002). Although *O. clarki* does exhibit kype development (Behnke, 1992; Morton, 1965; Stearley and Smith, 1993), the kype may be minimal or absent in small (Willson, 1997) or non-anadromous individuals (Scott and Crossman, 1973).

### ***Oncorhynchus mykiss* (rainbow trout)**

*O. mykiss* is also highly variable, with many sub-species and a wide geographical distribution (Behnke, 1992, 2002). As with *O. clarki*, individuals of *O. mykiss* typically develop kype (Behnke, 1992; Morton, 1965; Stearley and Smith, 1993), but the structure may be minimal to absent in smaller (Willson, 1997) or non-anadromous individuals (Behnke, 2002).

***Oncorhynchus kisutch* (coho salmon)**

The kype in *O. kisutch* is prominent, particularly in males (Behnke, 2002; Morton, 1965; Scott and Crossman, 1973; Stearley and Smith, 1993; Willson, 1997).

***Oncorhynchus tshawytscha* (Chinook salmon)**

While *O. tshawytscha* does develop a kype, it may be rather moderate in size and scope (Behnke, 2002; Butts et al., 2012; Stearley and Smith, 1993).

***Oncorhynchus keta* (chum or dog salmon)**

*O. keta* develops a prominent kype and large snout during the spawn-run (Behnke, 2002; Morton, 1965; Stearley and Smith, 1993). Concurrently, large, dog-like teeth develop on the lower jaw, which give this species its common name (“dog salmon;” Behnke, 2002; Salo, 1991; Scott and Crossman, 1973).

***Oncorhynchus gorbuscha* (pink salmon)**

The kype of *O. gorbuscha* develops as a prominent elongation of the lower jaw without much of the hooking upward seen in other eusalmonines (Behnke, 2002; Heard, 1991; Scott and Crossman, 1973).

***Oncorhynchus nerka* (sockeye salmon)**

Spawning *O. nerka* often display well-developed kype and snout (Behnke, 2002; Morton, 1965; Scott and Crossman, 1973; Stearley and Smith, 1993). However, the kype may be minimal or absent in small or landlocked (“kokanee”) individuals (Willson, 1997).

***Literature survey summary***

Assessments taken from the literature validate the findings made during physical examination of specimens and Micro-CT assessments. No evidence that basal salmonids (Coregoninae and Thymallinae) or *Brachymystax* develop a kype was found in the literature. Furthermore, although two researchers noted the presence of kype in *Hucho hucho* (Ladiges and Voigt, 1965; Schindler, 1935), this is not generally accepted by other

researchers (Holčík et al., 1988). With the exception of *Hucho*, all eusalmonine genera develop kype as a secondarily developing, spawning-related character. Even those eusalmonines that do not typically develop kype such as *S. namaycush* are capable of doing so (Behnke, 2002; Martin and Olver, 1980). In animals that do not always develop kype, such as *S. alpinus*, *O. clarki*, and *O. mykiss*, kype is often found in large, anadromous individuals even if it is absent in smaller or freshwater living ones.

This link between anadromy and kype development translates not only across a given genus or species but also across Eusalmoninae as a whole. The largest and most best developed kype is found in animals that are anadromous, particularly those with a semelparous life history, as can be seen in the physical examinations and Micro-CT assessments noted above. *O. kisutch*, *O. gorbuscha*, *O. keta*, and *O. nerka* arguably develop the most prominent kype amongst all eusalmonine species. Even in iteroparous kype-bearing genera, kype is rarely found in non-anadromous spawners, and species that display lower post-spawn survivorship (*S. salar*, for example) develop much more prominent kype than species within the same genera with higher post-spawn survivorship (for instance, *S. trutta*). That being said, repeat spawners often have larger and more complex kype than do first time spawners (Behnke, 2002; Hendry and Stearns, 2004; Jonsson and Jonsson, 2011).

It is worth noting that, while reports of kype in *Hucho hucho* are not well-accepted, there is very little evidence for or against kype development in *Hucho perryi*. While there has been some suggestion from popular sources that kype is found in *H. perryi*, the work of Holčík et al. (1988) and Stearley and Smith (1993) would seem to contradict the idea of kype in *Hucho perryi*. However, these authors address *Hucho* as a whole or focus on *Hucho hucho* and do not consider *H. perryi* individually. If kype is found in *H. perryi*, that character would support an alternative Eusalmonine phylogeny, one that divides the current *Hucho* into *Hucho* (*Hucho hucho* and *Hucho taimen*) and the *Parahucho* (*Parahucho perryi*). This potential alternative phylogeny is discussed in more detail in the conclusions.

## **Discussion and conclusions**

Spawning salmonids experience many physiological changes when making the switch from salt- to freshwater habitats (Butts et al., 2012; Willson, 1997). That these spawning salmonids also experience a variety of behavioural and morphological changes makes their spawn-run nothing short of incredible. These morphological changes include altered colouration, changes in fin length, increased body depth, and many other changes that may be particular to species or, may be encompassed by whole genera (Behnke, 2002; Berg, 1962; Carlander, 1969; Esteve et al., 2009a, 2013; Holčík et al., 1988; McPhail and Lindsey, 1970; Scott and Crossman, 1973).

Among these morphological changes are those modifications in the craniofacial skeleton that result in both the kype and in the compensatory changes in the upper jaw that accommodate the kype (Behnke, 2002; Scott and Crossman, 1973). In this study, I sought to answer several questions about the distribution of kype presence or absence within the salmonid clade, the extent and variation in kype structure within and across genera, and the co-development of features that are associated with the kype. The following provides some discussion and a summary of my results.

### ***Kype distribution and the salmonid phylogenetic tree***

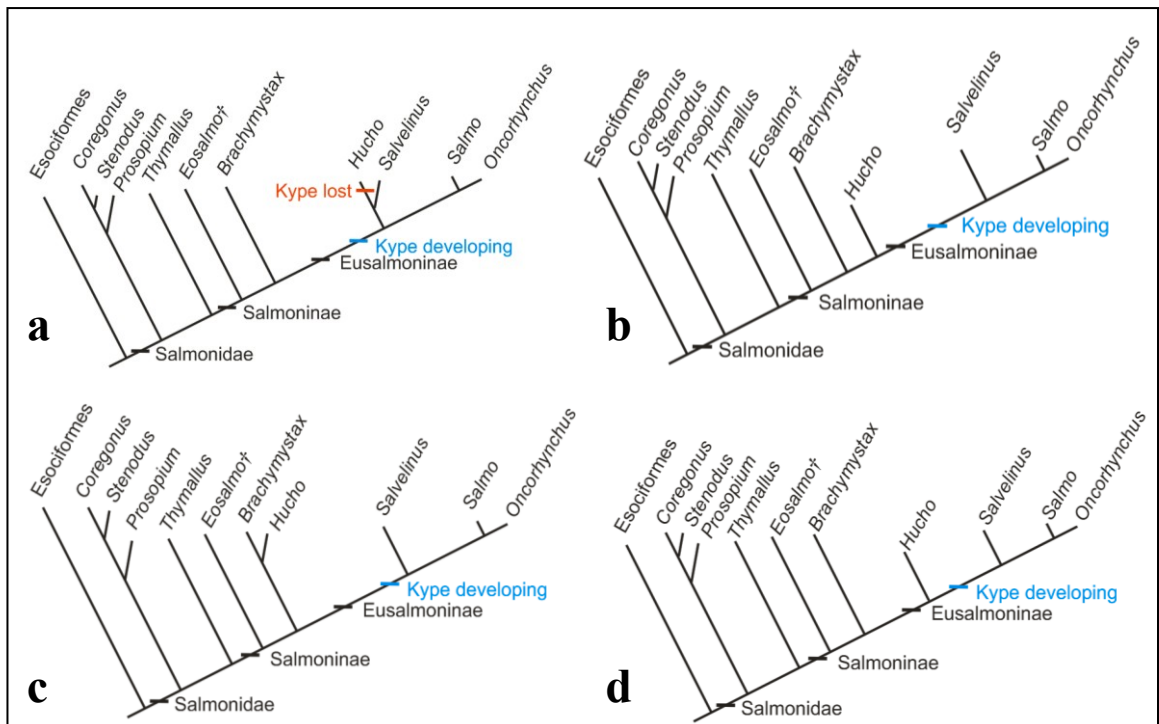
Using data taken from a combination of physical examinations, Micro-CT assessments, and extensive literature survey, kype is only found within the Eusalmoninae, namely in *Oncorhynchus*, *Salmo*, and *Salvelinus*, and is absent in all other, more basal salmonids including *Hucho*.

This distribution of kype brings up several questions regarding the evolution of this unusual feature and of the salmonid clade. While the evolutionary history of kype will be discussed in greater detail in Chapters 4 and 5 when the fossil record will be taken into account, it is possible to discuss some of the issues surrounding the salmonid phylogenetic tree using information summarized in this chapter.

As was briefly discussed in Chapter 1, there remains controversy surrounds the phylogenetic position, and monophyly, of the genus *Hucho* (Crespi and Fulton, 2003; Jonsson and Jonsson, 2011; Oleinik and Skurikhina, 2008). *Hucho* is often grouped within the Eusalmoninae (Stearley and Smith, 1993) and was early in its classification

even placed within *Salmo* (Esteve et al., 2009a; Esteve et al., 2013; Holčík et al., 1988). However, recent molecular work indicates that *Hucho* is in fact more closely related to *Brachymystax* and does not belong in the Eusalmoninae (Crespi and Fulton, 2004; Jonsson and Jonsson, 2011; Oleinik and Skurikhina, 2008; Ramsden et al., 2003). This molecular work supports morphologic work that considers *Hucho* to be an intermediate between *Brachymystax* and the eusalmonines (Holčík et al., 1988; Nordern, 1961).

If we add the likely position of “kype development” as a phylogenetic characteristic to morphologic-, molecular-, and behavioural- based phylogenetic trees, first discussed in Chapter 1, it may be possible to test the validity of the different trees (Fig. 2.58a-c). Based on the apparent absence of kype in *Hucho*, a tree where *Hucho* lies outside of the eusalmonines is likely the most parsimonious. However, a reanalysis of the morphologic characters used to assess the phylogenetic relationships of the salmonids, one that included kype characters such as presence and extent, would be required to confirm parsimony.



**Figure 2.58** – Examples of different phylogenetic trees with “kype-bearing” character added. Morphologic-based trees (a) modified from Stearley and Smith (1993) and (b) modified from Nordern (1961). Molecular-based trees, (c) modified from Crespi and Fulton (2003) and (d) from Oleinik and Skurikhina (2008). As predicted, the most parsimonious trees are those where *Hucho* lies outside of the eusalmonines.

While the absence of evidence for kype development in *Hucho hucho* and *Hucho taimen* supports the relocation of the genus *Hucho* to a position outside of the eusalmonines, the possibility that *H. perryi* develops kype, albeit a possibility that is relatively unsubstantiated, confuses the issue. How can a tree that would break up a monophyletic group be parsimonious? One explanation for the possible presence of kype in *Hucho perryi* is that the genus *Hucho* is not monophyletic at all.

For decades, the controversy over the phylogenetic position of *Hucho* has been paired with a controversy regarding *Hucho*'s monophyly. Many researchers have placed *H. perryi* within another genus entirely, *Parahucho* (Hendry and Stearns, 2004; Holčík et al., 1988; Nelson, 2006). Using several techniques including morphologic (Holčík et al., 1988), molecular (Phillips et al., 1995; Shed'ko et al., 2005), and behavioural (Esteve et al., 2009b, 2011; Wilson and Williams, 2010) means, an increasing pool of evidence supports clustering *Parahucho perryi* with *Salmo* and *Oncorhynchus* (Fig. 2.59). If *H. perryi* does develop kype, this may be further support for the repositioning of *H. perryi* within its own genus and relocating it to cluster within the crown eusalmonines. Indeed, depending on where *Hucho*'s location on the phylogenetic tree is determined to be, *Parahucho perryi* may even represent an intermediary species, either between kype-developing and non-kype-developing eusalmonines or between salmonines (no kype) and eusalmonines (kype). Further work that assesses the characteristics of the possible *Parahucho* genus is required.



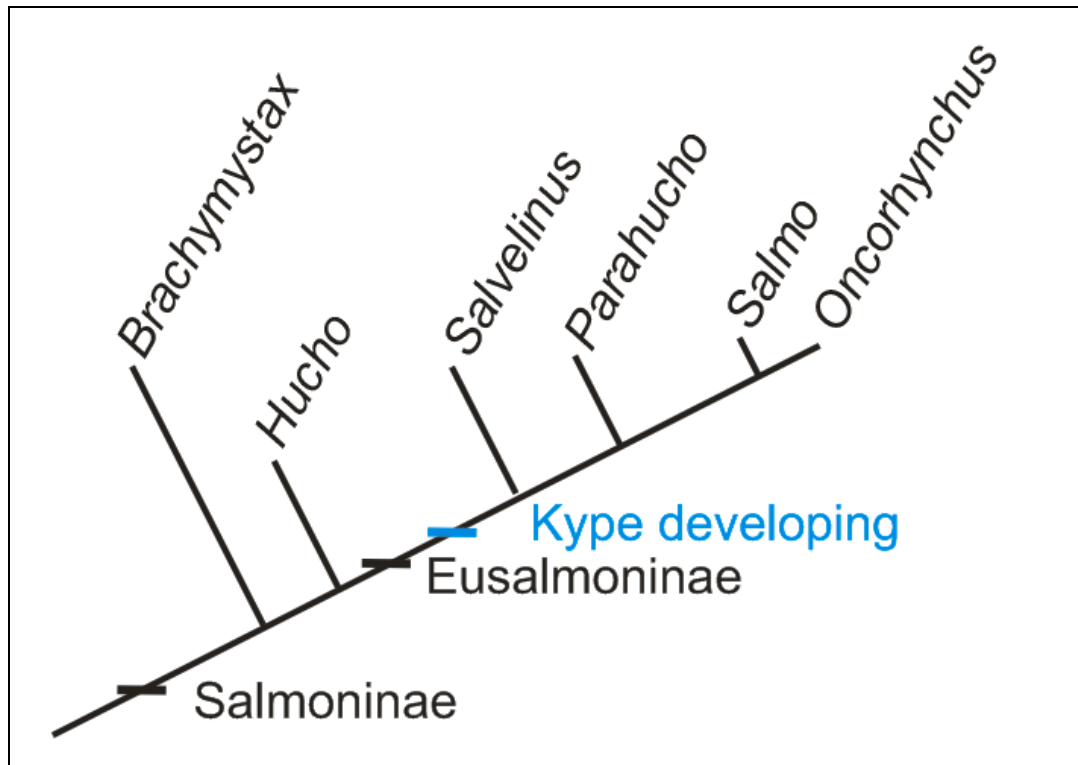


Figure 2.59 – “Kype developing” character mapped on a salmonine supertree built using a compilation of morphological, molecular, and behavioural data showing *Parahucho perryi* clustered with *Salmo* and *Oncorhynchus*. Modified from Wilson and Williams (2010).

### ***Patterns in the size and extent of kype structure***

Upon performing physical examination and Micro-CT assessments of available specimens, as well as taking into consideration previous research, the largest and best developed kype were found in *O. kisutch*, *O. nerka*, *S. salar*, and *O. gorbuscha*. This conclusion matches what is seen in much of the literature (Behnke, 2002; Fleming and Gross, 1994; Scott and Crossman, 1973), though the development of kype in *O. keta*, as described in other research, warrants its addition to this list (Johnson et al., 1971). However, there is some conflict between my list of extreme kype developers and that of Stearley and Smith (1993). While many of the same species were analyzed in both studies, Stearley and Smith (1993), found that only *Salmo salar* and *S. trutta* displayed extreme kype development. The contrast between the findings of Stearley and Smith (1993) and my own is partially the result of slightly different definitions for the term “kype.” While Stearley and Smith (1993) defined the kype as being a cartilaginous extension of the lower jaw, I considered extent of kype development to include

compensatory changes in the upper jaw, as well. Furthermore, while the analysis of Stearley and Smith (1993) relied only on the hooking upward of the kype, a trait that is prominent in the genus *Salar*, I also assessed breeding teeth and, using Micro-CT analysis, extent of skeletal needle growth. My assessment, as a result, reveals that the “type 1” *Oncorhynchus* species, as defined by Stearley and Smith (1993) and *Salmo salar* develop the most prominent kype and other spawning-related changes to the upper jaw.

Each of my extreme kype developers are predominantly anadromous and are either semelparous (*O. kisutch*, *O. nerka*, *O. keta*, and *O. gorbuscha*) or have very low survivorship (*S. salar*, with a repeat spawning frequency of 0.7-42.5%, but most frequently approximately 1-10%; Willson, 1997). As such, the largest and best developed kype is found in animals with a semelparous (or near-semelparous), anadromous life history mode. Why exactly kype development is maximized in anadromous, semelparous species is not clear. Resident, landlocked, and non-migratory animals, especially in stream-spawning populations and species, develop minimal or no kype (Behnke, 2002; Scott and Crossman, 1973; Willson, 1997), as was seen in *O. mykiss aguabonita* (personal observations; Willson, 1997). In fact, secondary sexual characteristics in general are more developed in larger, anadromous animals as opposed to smaller individuals, dwarf morphs, freshwater residents, and precocious individuals, in each species considered in this and other studies (Fleming and Gross, 1989; Scott and Crossman, 1973; Stolz and Schnell, 1991; Willson, 1997). Perhaps the explanation for this trend lies in the availability of resources in different habitats.

The benefits conferred by an ocean life history phase have been proposed as one reason for the evolution of a diadromous life history in sturgeons (Harris et al., 2005; Sulak and Randall, 2002), lampreys (Gross et al., 1988; McDowall, 1997), and salmonids (Hutchings and Morris, 1985; Miller and Brannon, 1982; Stearley, 1992; Willson, 1997). This is in part due to the increased availability of resources in the temperate ocean setting, an availability which allows animals to grow more quickly and achieve greater sizes than their freshwater living relatives (Gross et al., 1988; McDowall, 1997; McDowall, 2001; Willson, 1997). However, the dangers and energy demand of an ocean run life history phase with extensive migration increases the rate of adult mortality. This high adult mortality favours high reproductive effort meted into a single life history event (Fleming,

1998). This selection for semelparity results in increased sexual competition and, in turn, increased sexual dimorphism (Willson, 1997).

All eusalmonine populations that do not develop a kype or develops a minimal kype resides permanently in freshwater (Behnke, 2002; Fleming and Gross, 1989; Johnson, 1980; Martin and Olver, 1980; Morton, 1965; Scott and Crossman, 1973; Stolz and Schnell, 1991; Willson, 1997), meaning that they do not have the access to the large amount of resources available in the ocean and do not experience selection for a high degree of sexual dimorphism. Thus, on the one hand, anadromous animals have access to a greater pool of resources that they can use toward primary and secondary sexual development, but, because of the high demands of their selected life history mode and the high sexual demand associated with it, use up all of these resources in a single or very few reproductive events. However, while kype development may be related to physiological or hormonal changes in the body due to the switch from salt- to freshwater during the spawn-run (Butts et al., 2012; Willson, 1997), as of now, the exact stimuli for kype development is unclear. Until the trigger for kype development is better understood, pinpointing the cause for the extent of kype growth is likely impossible.

### ***Differences in kype morphology and kype-related features***

The morphology of the kype and the features associated with it was different in each of the three kype-bearing genera. In *Salmo*, the kype was composed of a prominent array of long, fibril- and needle-like extensions that covered a large region of the anterior dentary. This large expanse of needle-like elements supported a large soft-tissue build-up on the lower jaw. Thus, the combination of these two elements results in a curved hook at the anterior aspect of the lower jaw. This hooked kype is, in turn compensated for by the development of a hole in the soft tissue of the upper jaw. The hooked kype fits into this hole, permitting the mouth to close and allowing *Salmo* to maintain the seal that allows a fish to push water over its gills.

In *Salvelinus*, as with *Salmo*, spawning animals develop a large, curved hook on the lower jaw, albeit of a gentler curve than in *Salmo*. The effects of the development of this large kype are mitigated by a modification in the spacing of the premaxillae and, to a

lesser degree, the maxillae. These modifications result in a notch that the kype can slip into, once again allowing the animal to maintain a respiratory seal.

In *Oncorhynchus*, the extent of needle-like elements protruding from the dentary is rather small. These elements are also rather short and barely surpass the margins of the well-developed anterior teeth. It is in fact these enormous teeth that are the dominant elements of the *Oncorhynchus* kype. In comparing the overall appearance of the *Oncorhynchus* (Fig. 2.60 a and b) versus the *Salmo* (Fig. 2.60 c and d) kype, the major differences between the two lie in the extent of skeletal needles and in the size of these anterior teeth. Where the profusion of needle-like elements from the *S. salar* dentary results in an abrupt hook, the rather minimal needle-like extensions and large teeth of the *O. nerka* dentary result in kype with a gentler slope. These teeth appear to have a deeper root and lower density than surrounding teeth, and do not appear to be present in non-spawning specimens (Fig. 2.52).

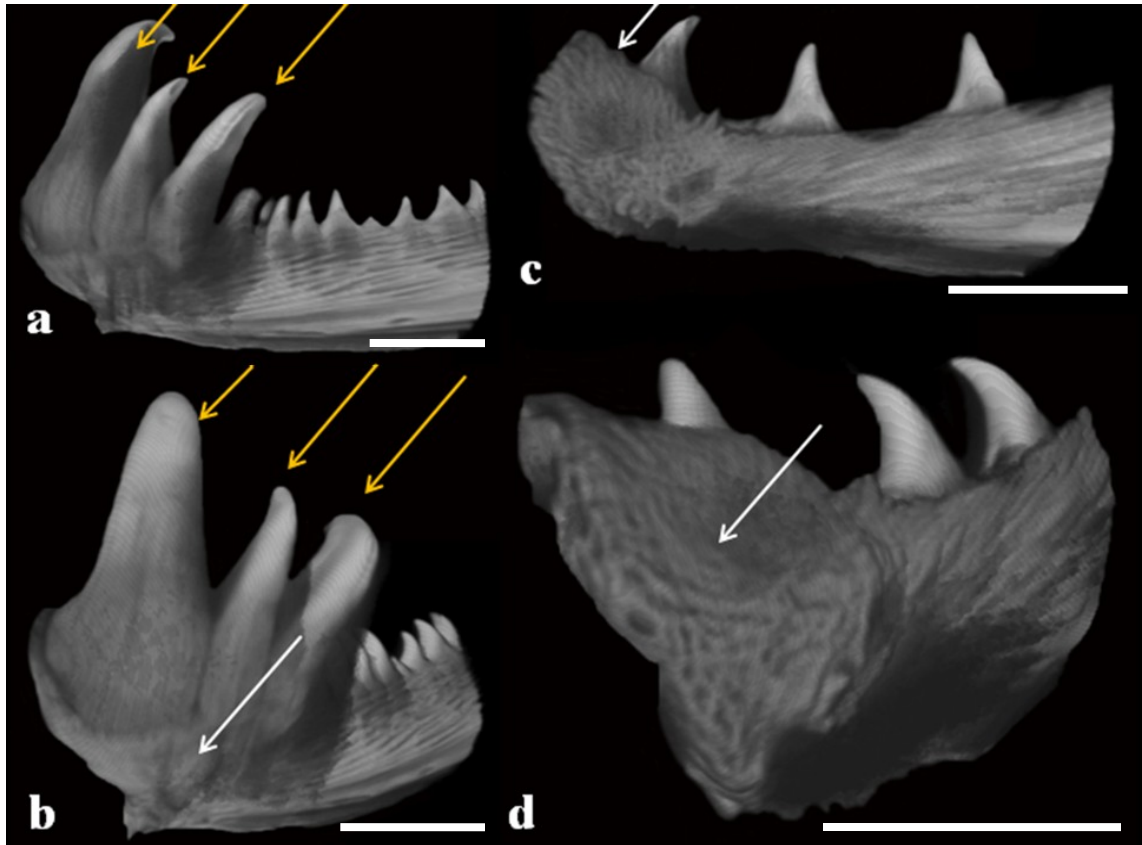


Figure 2.60 – Three dimensional reconstructions of defleshed specimens. (a) Lateral and (b) anterior views of *O. nerka* (CMNFI-1977-0277). (c) Lateral and (d) anterior views of *S. salar* (CMNFI-1980-0181.1). The anterior teeth of *O. nerka* (orange arrows) are enormous compared to those of *S. salar*, though *O. nerka* has very little in the way of skeletal needle extensions in comparison to those of *S. salar* (white arrows), having but a thin margin at the anterior edge of the kype. Scale bars are 1 cm.

Also found in *Oncorhynchus* is a buildup in the soft tissue at the anterior of the upper jaw. Additionally, the maxillae become slight curved. Together, the combination of maxillae curvature and soft tissue accumulation results in a prominent snout. The bend in the snout compliments the slight curve of the relatively straight dentary, and, except in extreme cases, permits the mouth to close and the respiration seal to be maintained.

### *Sources of error*

Wherever possible, I mitigated sources of error in this study. However, as in any study, possible sources of error exist. Although I used an extensive literature survey to mitigate the effects of small sample size and of any sampling bias that might have occurred, due to specimen availability these issues nonetheless remain. I did have access to a wide range of species representing many of the salmonid genera, but I had relatively few individuals,

many of which were at a variety of life history stages. I tried to account for this small sample size by incorporating material in both fleshed and defleshed state of the most mature specimens that I could, but collection, preservation, and sampling bias remain, each of which are magnified by the small sample size. Small sample size is a particular issue in my assessments of basal salmonids, where I gained access to a very small number of specimens.

I also wasn't able to control at what life history stage animals were caught. Relative life history stages were established for physical specimens using accepted SL estimates for maturity. However, individuals may be smaller than the norm and still attain sexual maturity or may be very large and not yet be mature. This is particularly the case in animals such as *Salmo salar* that employ alternate life histories, including precocious parr, where seeming juvenile fish as small as 8-10 cm in length may achieve maturity (Jonsson and Jonsson, 2011; see also *Salvelinus alpinus*, whose non-anadromous forms can achieve maturity at 10 cm, Johnson, 1980). However, most non-anadromous salmonids will achieve sexual maturity at a younger age and at a smaller size than anadromous populations of the same species (Johnson, 1980, for example). Furthermore, complete information as to collection date, SL, and sex was not always available for all physical specimens, increasing the potential for inaccurate estimates. That being said, even if specimens diagnosed as "immature" were sexually mature, these precocious parr would not be expected to developed kype, so concerns about labelling a specimen as "immature" when it was in fact a precocious parr may be irrelevant. Nevertheless, in order to truly pinpoint which animals have a kype, one would need to sample a large number of specimens from each species and from different populations at the same life history stage, something outside of the scope of this project.

Finally, I attempted to mitigate the effects of my small sample size and inability to get access to all of the specimens in their spawning life history stage that I would have wanted to have by turning to the literature. However, I cannot be sure that my assessment of the literature, or indeed the literature itself, was not incomplete. The term "kype" or one of the positive indicator terms describing changes in the anterior craniofacial skeleton of a spawning individual needed to be used by the authors in order for me to consider any one study a positive response for kype presence. However, authors may not have referred

to such features because these elements were irrelevant to the study or because the authors did not recognize the craniofacial modifications that were in fact present. Furthermore, while one or even several specimens examined by the authors did not display kype, other specimens of the species or population might. However, because I was able to pool multiple resources, including this wide literature survey, I believe I have mitigated error and bias as much as possible.

### **Summary**

- The kype is found exclusively within the eusalmonines. The lack of kype in *Hucho* supports research suggesting that *Hucho* does not belong within Eusalmoninae. Presence of a kype in *Hucho perryi* would help confirm that *Hucho* is not monophyletic and should instead be divided into *Hucho* (*H. hucho* and *H. taimen*) and *Parahucho* (*Parahucho perryi*). If this is the case, *Parahucho perryi* may need to be repositioned within the salmonid phylogenetic tree such that it is an outgroup of the *Salmo-Oncorhynchus* sister group. However, parsimony analysis is required to confirm this hypothesis.
- The largest and most dramatic kype is found within species and populations that are anadromous, in particular those with low, minimal, and no post-spawning survivorship (i.e. are anadromous and semelparous). The growth of a more substantial kype in these animals may be due to a combination of factors. First, there is higher resource availability in oceans, meaning that anadromous animals have more resources at their disposal. Secondly, semelparous individuals have skewed resource allotment, spending nearly all of their available resources on the development of primary and secondary sexual characteristics and keeping little or no resources in reserve for post-spawn survival.
- Kype structure is somewhat different in *Salmo*, *Salvelinus*, and *Oncorhynchus*. While in each case the dentary changes morphology, this change is much better developed in *Salmo* and *Salvelinus*, where an extensive needle-like array encompasses a large portion of the dentary. In *Oncorhynchus*, these needle-like extensions are relatively minimal and appear to be localized to anterior to several large breeding teeth. However, while the skeletal changes in the kype are

somewhat more minimal in *Oncorhynchus* than in either *Salmo* or *Salvelinus*, the combination of connective tissue build-up, breeding teeth development, and skeletal modifications result in a kype that is, overall much more dramatic.

- Another major difference in kype development lies in the other craniofacial modifications during the spawn-run, one of which compensates for the development of kype, ostensibly to allow kype-bearing animals to maintain the seal that allows them to push water over the gills. This compensatory feature, found on the upper jaw, is different in each genus. While *Salmo* develops a hole that the kype slips into, *Salvelinus* develops a notch formed when the premaxillae and maxillae separate slightly. *Oncorhynchus*, on the other hand, builds-up soft tissue at the tip of upper jaw and a slight curve in the maxillae. The combination of soft tissue and curved maxillae results in a snout with which the kype dovetails.

In this chapter, trends in the presence and absence of kype across the salmonids, the extent and variation in kype development within each of the kype-bearing genera, and the co-development of features that are associated with the kype were considered. Having established behavioural and life history mode characteristics, as well as gross anatomical, morphological, and structural conditions across the Salmonidae, Chapter 3 presents the conditions within the kype at the tissue and cellular level. In Chapter 3, I will also attempt to answer the question of whether the structure of kype or the tissues of which it is composed evolved first.



### **Chapter 3 – Histological assessment of tissues of the lower jaw and of the kype in living salmonids**

#### **Introduction**

As shown in Chapter 2, many of the eusalmonines with an anadromous life history, particularly those that are semelparous, develop kypes that are better developed and substantial than those of their non-anadromous counterparts. The prominent kype of anadromous *Salmo*, *Salvelinus*, and *Oncorhynchus* are likely possible because of the many resources gleaned from a marine ecosystem that would provide them with more nutrients (Gross et al., 1988; McDowall, 1997; McDowall, 2001; Willson, 1997). The size and complexity of the kype in semelparous species is a result of prioritization of those resources: in animals that will die following their first spawn, the investiture of resources into optimizing the secondary sexual characteristics that make them attractive to possible mates will increase their fecundity. That these semelparous fish will die following spawning negates their need to keep resources in reserve for post-spawn survival. As shown in Chapter 2, several trends in the macroscopic structure and morphology of the kype can be determined from physical examination and Micro-CT assessment. However, in order to determine how the cellular composition and organization of these kype reflect their distinctive morphology and the differences in that morphology across kype-bearing genera, histological analysis is required. In order to undergo that analysis, a better understanding of the tissues found in salmonids, and teleosts in general is also needed.

As outlined in Witten et al. (2010), the variety of intermediate tissues in teleost fish results in a level of tissue complexity that makes categorization and identification of these tissues difficult. In comparison to the small number of tissue types present in mammals, fish display a large number and range of skeletal tissue types ranging from cartilage to bone (Witten and Hall, 2002; Hall and Witten, 2007). While the majority of intermediate tissues in mammals are transient or are symptomatic of pathology, fish display non-aberrant, permanent intermediate tissues that have characteristics indicative of some combination of two or more of cartilage, bone, dentine, and enamel tissues (Beresford, 1981; Witten and Hall, 2010). In fact, there are several types of each of these

four categories; fifteen different types and sub-types of teleost cartilage alone have been proposed (Benjamin, 1990; Benjamin et al., 1992).

Given the complexity of the tissues of teleost fish in general, it is no surprise that the tissues specific to the kype of Atlantic salmon are comprised of a combination of tissues and structures. As was briefly mentioned in both Chapter 1 and Chapter 2, the kype of Atlantic salmon is composed of a massive buildup of anterior connective tissue supported by a spongiose and spur-like network of interwoven skeletal elements (Witten and Hall, 2002, 2003). These skeletal elements are, in turn, composed of a combination of Sharpey-fibre bone and chondroid bone, described in detail in the “Kype” section of Chapter 1.

While the cellular and tissue composition of the kype of Atlantic salmon is fairly well understood, virtually nothing is known as to the organization and composition of the tissues of other kypes, or indeed of the lower jaw of salmonids, in general. In this chapter, I will use histological analysis of representative specimens to assess what tissues are present in the lower jaw in general and, where present, in the kype specifically across the salmonid phylogenetic tree. I will compare these tissues phylogenetically and in relation to patterns found in physical examinations and in Micro-CT assessments of specimens (Chapter 2). I will focus my assessments on the composition of the kype, where present, and on other tissues and structures within the jaw that are of interest in the study of kype presence and absence and kype growth. These other tissues include teeth, epithelium, fat accumulations, and blood vessel networks. To fully understand the patterns and trends in the composition and structure of the kype and of the other tissues of the lower jaw, I will compare the tissues of the lower jaw of members of the same species in pre-spawning and spawning life history stages. I will also contrast the tissues of individuals of the same or different species of different life history stages and modes.

I hypothesize that the tissues of the kype and the other tissues of the lower jaws of other salmonids will be similar in composition to those of Atlantic salmon, as described by Witten and Hall (2002, 2003). In particular, I suggest that the Sharpey-fibre bone and chondroid bone of which the Atlantic salmon kype is composed will also be found to compose the kype in other species. To my knowledge, this study represents the first family-wide histological assessment of the salmonids. Furthermore, the following

includes some of the first skeletal histological data of many salmonid species with the exception of *Salmo salar* (Atlantic salmon) including, but not limited to *Hucho hucho*, *Brachymystax lenok*, *Coregonus clupeaformis*, and many species of *Salvelinus*.

### **Methods and materials**

Using the fleshed representative specimens of many salmonid species as were described in Chapter 2 and as are outlined in Appendix A, a histological phylogenetic survey was conducted. This survey used a wide assortment of salmonid species to identify the different tissues of the lower jaw. In particular, this survey was used to establish if it is possible to discern patterns in the structure and composition of tissues depending on environment (freshwater living versus anadromous), life history stage (juvenile versus adult, spawner versus non-spawner), and phylogenetic position (basal versus derived).

While museum specimens had been fixed previously using formaldehyde, formalin, or an equivalent fixative, specimens collected in the field (ZP 2013-07-01(A) to (F)) were fixed in 10% neutral buffered formal solution (NBFS) for 24-36 hours. Fixed specimens were then treated to the same procedure outlined by Witten and Hall (2002, 2003) for their Atlantic salmon specimens.

Specimens were dissected such that the lower jaw was removed from the remainder of the head. Lower jaws were then bisected at the midline, and the right half of each jaw was decalcified in a neutral (pH 7.0), 10% EDTA, 0.1M TRIS base buffered solution for 72 hours. Due to their large size, the ZP 2013-07 specimens required decalcification for 96 hours. Following decalcification, tissues of interest were dehydrated in a graded ethanol series and were embedded in Paraplast.

Once embedded, all right jaws were serially sectioned from the midline in 10  $\mu$ m sagittal sections. Specimens were sectioned in their entirety, resulting in 62 to 432 slides, depending on the specimen. These sections were then stained with either Masson's Trichrome (MAS) (Flint and Lyons, 1975) to distinguish collagen, bone cells, and mineralized bone or with Hall-Brunt Quadruple Stain (HBQ) (Hall, 1986) for bone, cartilage, chondroid bone, and to demonstrate collagen fibres. Interpretations of sections in this study were guided by the assessments of the kype and kype-related tissues in Atlantic salmon by Witten and Hall (2002, 2003). Images taken from the studies of

Witten and Hall (2002, 2003), such as that shown in Figure 3.1 showing the tissues and structures of interest in kype-bearing Atlantic salmon, were used as guidelines when assessing conditions and characteristics of other salmonids. All slides of each specimen were examined, with the photos included in this chapter showing the regions of most interest in each specimen.

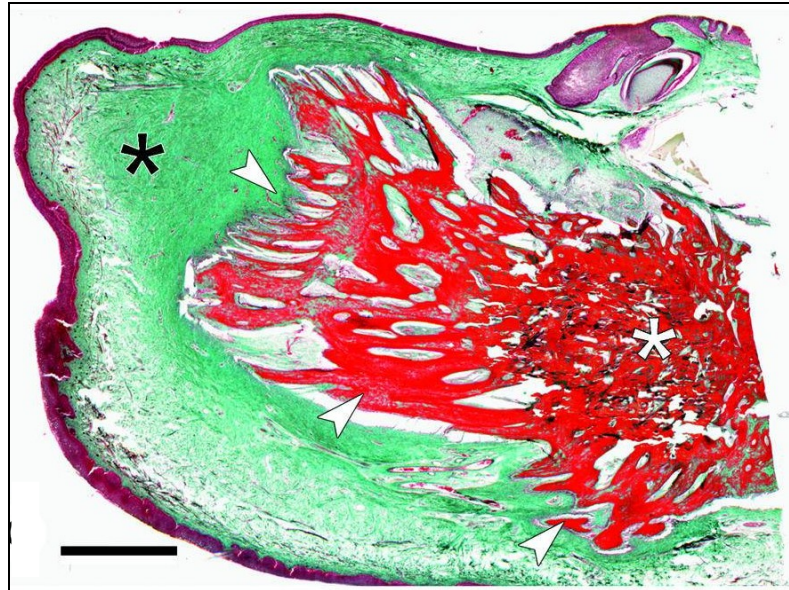


Figure 3.1 – Tissues of interest in kype-bearing *Salmo salar*. Black asterisk indicates anterior connective tissue mass, white arrows delineate contours of spongiöse kype skeleton, and white asterisk indicates compact dentary bone. Image taken from Witten and Hall (2003). Scale bar is 2 mm.

As I did not have control over the fixation and preservation of the many museum specimens used in this study, in some cases, inadequate fixation, dehydration to the point of brittleness, and other negative effects of long-term ethanol storage may have damaged the integrity of the tissues of these specimens. These negative effects appear to have had the greatest effect on some of the more basal specimens examined, including *Prosopium williamsoni* and *Coregonus clupeaformis*, as well as *Hucho hucho*, resulting in slides with lifted and torn tissues. However, as will be discussed, these sections nonetheless display a great deal of information as to the composition and organization of the tissues in these specimens.

## **Results**

### ***Phylogenetic survey of skeletal tissues in the salmonid lower jaw***

The following outlines the conditions of the lower jaw and, where present, of the kype in several salmonid genera. One or two specimens are described for each genus. Tissues and structures of interest are discussed in the context of their species or genera, as appropriate.

### **Coregoninae**

Similarly to what is seen in kype-bearing fish, coregonids have very thick and dense anterior connective tissue regions (Fig. 3.2 a and b, asterix). These connective tissue regions contain many blood vessels, making them very well vascularised. However, while the massive nature of the anterior connective region is similar in structure to that found in Atlantic salmon, the morphology of the dentary is not. The dentary of kype-bearing animals displays a number of spur-like processes that extend out into their respective anterior connective tissue masses. The dentaries of these coregonids, on the other hand, lack those spur-like extensions of bone and intermediate tissues (Fig. 3.2 a and b). As a result, these fish do not display evidence of the growth of a kype skeleton.

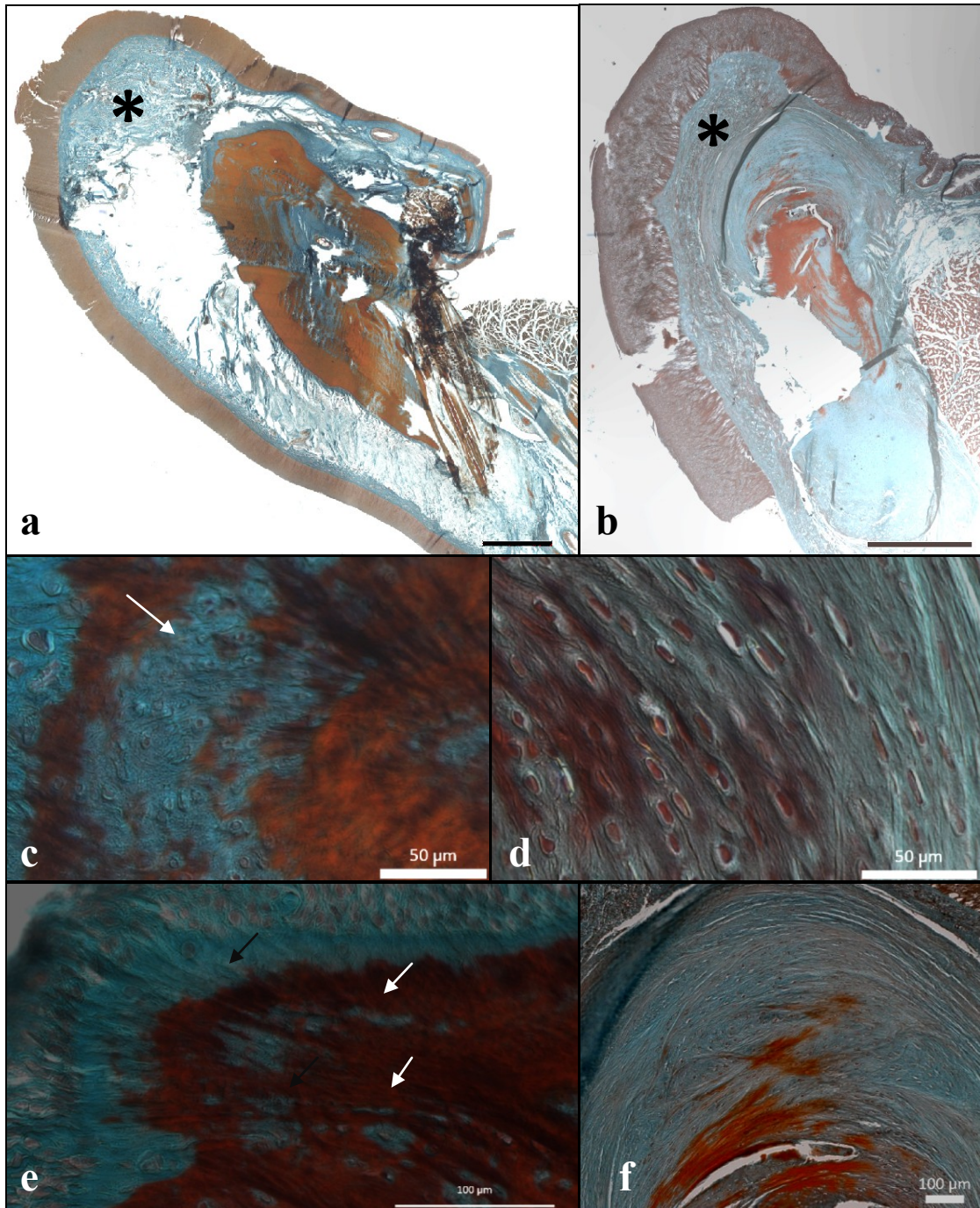


Figure 3.2 – Lower jaw skeletal tissues of the Coregoninae (MAS). (a) *Prosopium williamsoni* and (b) *Coregonus clupeaformis* in sagittal section. The thick connective tissue mass at the anterior tip of the jaw (asterixes) is not supported by spurs of bone or by intermediate tissue from the dentary. (c) *P. williamsoni* anterior dorsal margin of the dentary. Actively secreting chondrocytes aggregate in amorphous groups (white arrow). (d) Chondrocytes aggregate in strings on the *C. clupeaformis* anterior dorsal dentary margin. Chondrocytes groups may be very dense and very actively secreting, to the point that they disguise the contours of the bone. (e) *P. williamsoni* anterior dorsal dentary. Many small fibres (black arrows), several of which have strings of chondrocytes associated with them (white arrows) blur the margin of the mineralized bone of the dentary. (f) The dorsal dentary margin of *C. clupeaformis* displays many fibrous elements. These fibres may be associated with extensive and complex patterns of mineralization in part resulting from the many chondrocytes within this region. Unless otherwise indicated, scale bars are 1000μm.

While the structure of the kype appears to be absent in the coregonids, analysis of tissues at the margins of the dentary reveal several interesting features. Pairs and groups of actively secreting and dividing chondrocytes are located within the interior and at the margin of the dentary (Fig. 3.2 c and d, white arrows). These cartilage-secreting chondrocytes lack cellular processes and aggregate in amorphous groups and in strings aligned with collagen fibres that penetrate deep into the dentary (Fig. 3.2 c-e). In some cases, the dentary is so densely packed with chondrocytes that the secretions of the chondrocytes disguise the margins of the bone (Fig. 3.2 d and f). The presence of these actively secreting and dividing chondrocytes within the bone of the dentary is suggestive of the chondroid bone found in the kype of Atlantic salmon.

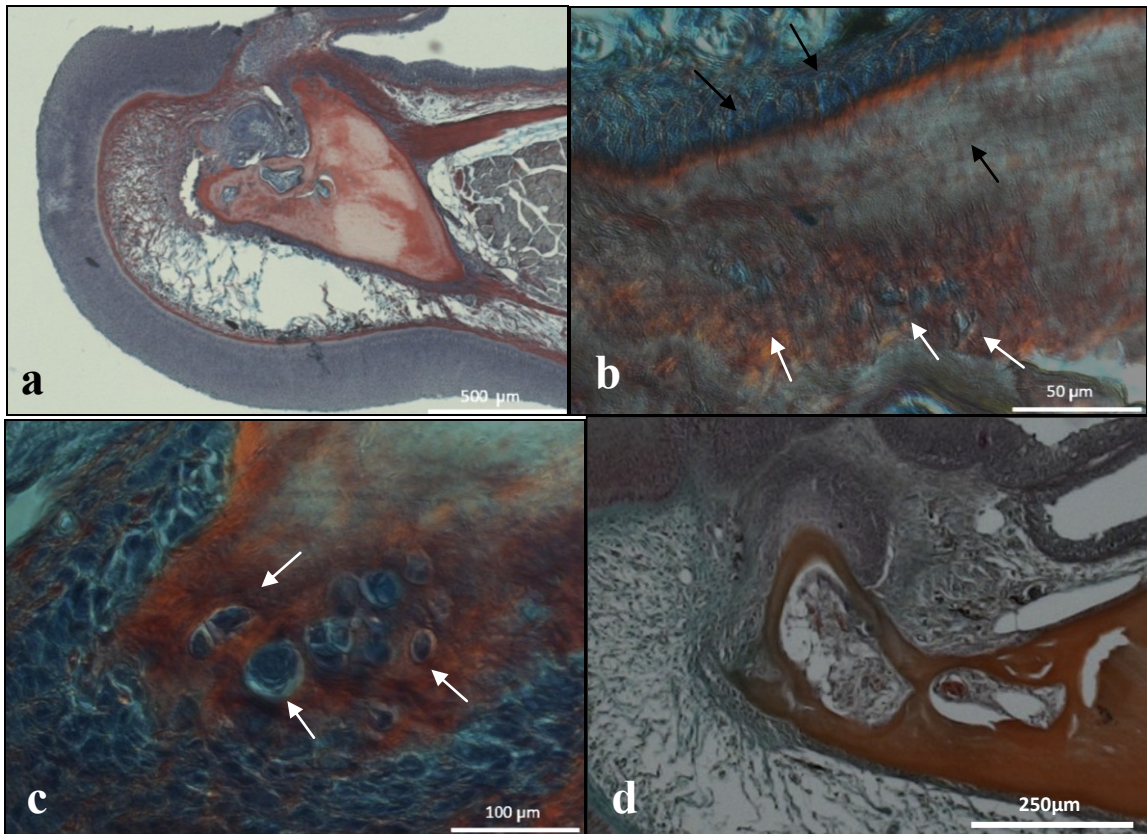
Also present in the examined coregonids are small, mineralized fibres that extend from the interior of the dentary into surrounding connective tissue (Fig. 3.2 e and f, black arrows). These fibres are often associated with strings of chondrocytes (Fig. 3.2 e and f) and may also be associated with complex patterns of mineralization (Fig. 3.2 f).

## **Salmoninae**

### ***Brachymystax***

A single, immature *Brachymystax lenok* specimen was examined in this study. This specimen was rather small and displayed none of the structures associated with kype growth. The anterior connective tissue is relatively thin throughout the specimen and there was no evidence of spurs extending from the dentary (Fig. 3.3 a). Examination of the margins of the dentary revealed a periosteum that, although typically very distinct from connective tissue, at times displayed fibrous extensions that led from the dentary into the surrounding connective tissue, particularly in anterior regions (Fig. 3.3 b, black arrows). Several nests of chondrocytes are found in regions adjacent to these fibrous elements. These chondrocyte cells are round, surrounded by halos of newly secreted material, lack cell processes, and are actively dividing (Fig. 3.3 c, white arrows). In some sections, particularly in those where Meckel's cartilage is beginning to come into plane, these chondrocytes may aggregate in actively secreting and dividing groups at the interior

of the dentary. The presence of unerupted teeth in histological section supports Micro-CT evidence that this specimen was nearing maturity at the time of its capture (Fig. 3.3 d).



**Figure 3.3** – Lower jaw skeletal tissues of *Brachymystax lenok* (HQB and MAS). (a) In low magnification sagittal section, neither an anterior connective tissue mass nor the spur- and needle-like skeletal extensions from the dentary that are suggestive of a kype are present (HBQ). (b) Periosteum is very distinct throughout specimens, though fibrous extensions from the dentary into the surrounding connective tissue are evident in some regions, particularly anteriorly (some examples are indicated with black arrows) (HBQ). Chondrocytes are sometimes found in association with these fibres (white arrows) (c) and may also form groups at the interior of the dentary (white arrows) (HBQ). These chondrocytes are well-rounded, surrounded by halos of newly secreted material, lack cell processes, and are actively dividing. (d) Several unerupted teeth are also present in this specimen (MAS).

## Eusalmoninae

### *Hucho*

As with *Brachymystax*, a single *Hucho hucho* specimen was examined for this study. The structure and organization of the tissues of this mature, post-spawning male are in many ways very similar to those of more basal salmonids. As with *Brachymystax* and the coregonids, there is very little evidence of an anterior connective tissue mass in *Hucho*



(Fig. 3.4 a). However, unlike more basal salmonids, the epithelium of *Hucho* is relatively thin. While there are some extending elements on the anterior ventral margin of the dentary (Fig. 3.4 b), these are neither needle- or spur-like nor do they display much in the way of a band of non-mineralized bone matrix at their tips, as described by Witten and Hall (2002) in Atlantic salmon.

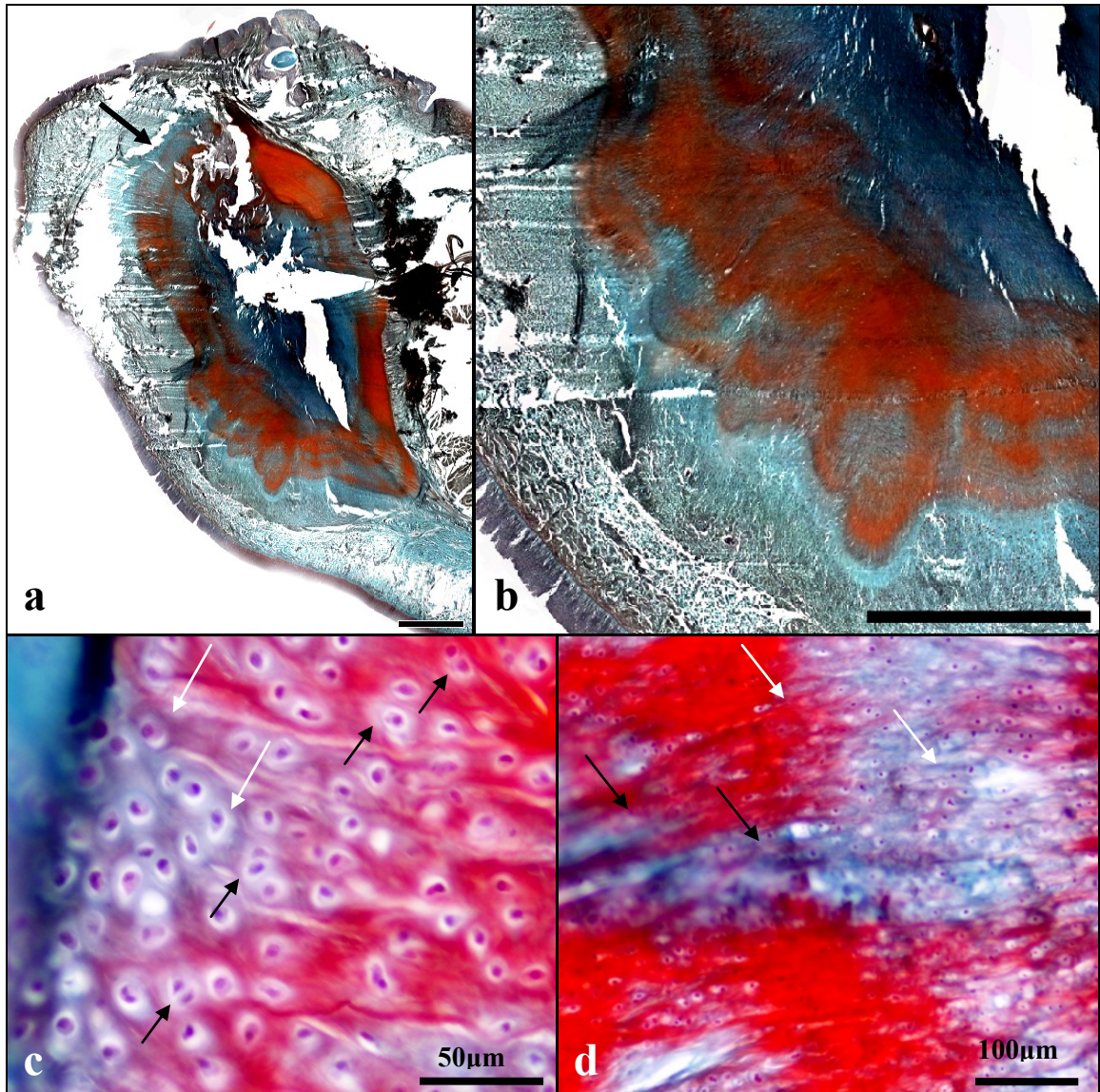


Figure 3.4 – Lower jaw skeletal tissues of post-spawning *Hucho hucho* (MAS). In (a) sagittal section, black arrow indicates anterior dorsal region of alternating mineralized and demineralized bands. (b) Anterior ventral region of extensions from the dentary. These features do not display notable kype-like features such as thick bands of non-mineralized bone matrix at their tips. (c) Chondrocytes embedded in bone matrix. Several of these are newly dividing (black arrows) or are surrounded by halos of newly secreted matrix (two examples are indicated by white arrows). (d) Anteriorly, chondrocytes aggregate in strings (two examples indicated by white arrows) around mature Sharpey fibres (black arrows). Unless otherwise indicated, scale bars are 1000µm.

Within the anterior dorsal portion of the specimen, complex mineralization and demineralization patterns result in an almost bull’s eye-like appearance (Fig.3.4 a, black arrow). Within this bull’s eye region, a very large number of chondrocytes are present (Fig.3.4 c). These chondrocytes display typical chondrocyte morphology, lacking cell processes, and are densely packed within the mineralized bone in aggregations of two or

more. Several cells have been preserved in the act of dividing (Fig. 3.4 c, black arrows) and are surrounded by more mature, actively secreting chondrocytes that are ringed in halos of new matrix (white arrows). Anteriorly, these chondrocytes aggregate in strings that extend through the alternating mineralized and non-mineralised regions of the dentary bone (Fig. 3.4 d, white arrows), at times congregating around thicker, more mature Sharpey fibres (Fig. 3.4 d, black arrows).

### ***Salvelinus***

The species within *Salvelinus* can be divided into either kype-bearing or non-kype-bearing individuals. In kype bearing specimens such as anadromous *S. alpinus* (CMNFI 1968-1262.1), an enormous anterior connective tissue mass dominates the lower jaw (Fig. 3.5 a, asterix). This connective tissue mass, absent in basal specimens such as *Brachymystax lenok* (Fig. 3.3 a) is well supported by several spurs that extend from the dentary in an array from the anterior dorsal tip to the posterior ventral margin (Fig. 3.5 a, black arrows). The structure of this kype skeleton displays several elements of interest. At the tips of dentary spurs are thick bands of non-mineralized material (Fig. 3.5 b, white arrow). Also found at the tips of these spurs are chondrocytes aggregating in strings (black arrows). Associated with these chondrocyte strings are fibres that extend from the mature, mineralized bone, through the non-mineralized bands, and into surrounding connective tissue (white arrowheads).

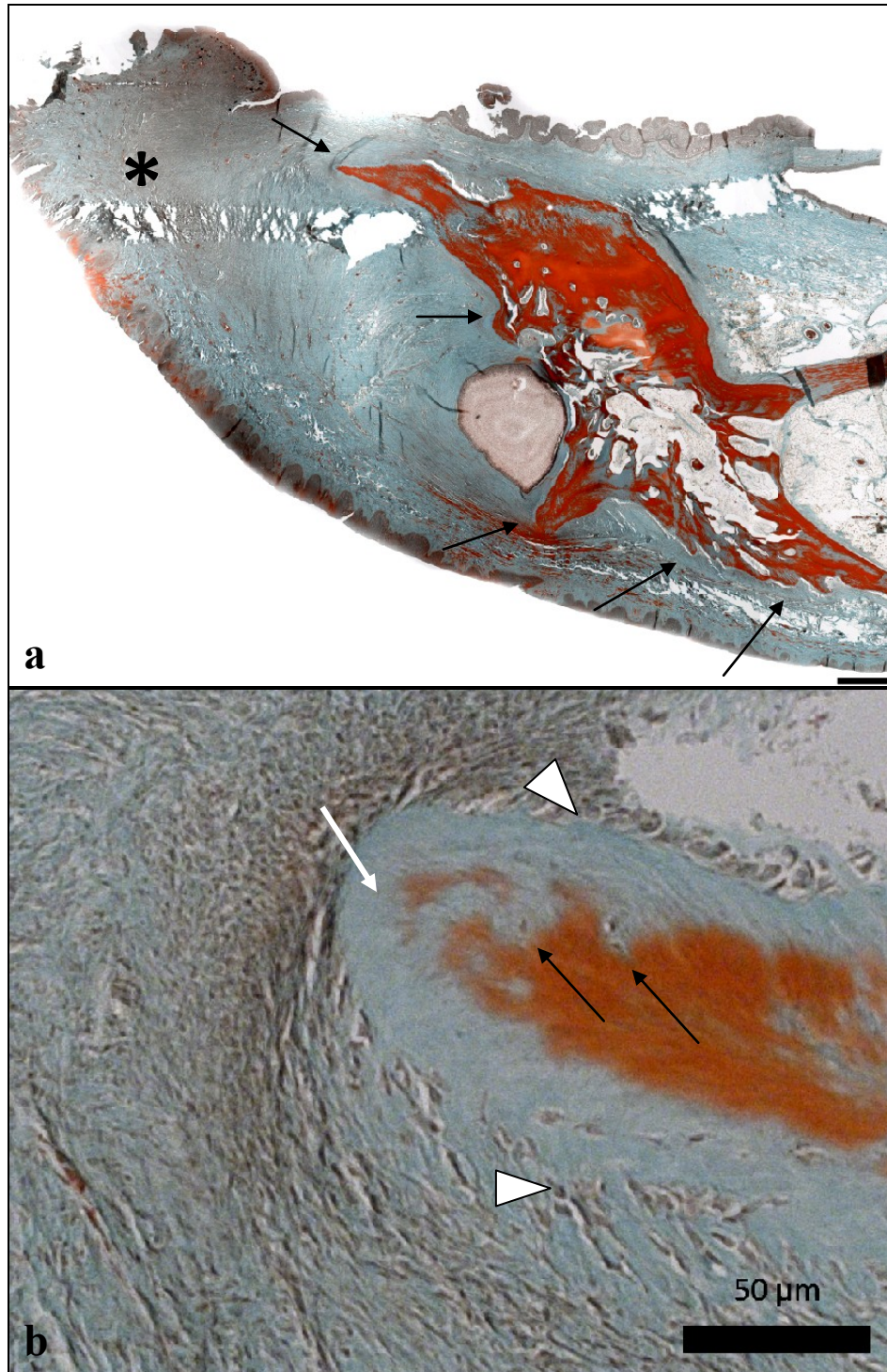


Figure 3.5 – Lower jaw skeletal tissues of kype-bearing *Salvelinus alpinus* (MAS). In (a) sagittal view, many features of the kype structure seen in Atlantic salmon are visible. A massive anterior connective tissue (asterisk) is supported by several spurs of bone extending from the dentary (several of the more prominent skeletal spurs are indicated by black arrows). (b) At the tips of the dentary spurs, a broad band of non-mineralized tissue (white arrow), strings of chondrocytes (black arrows), and extensive fibrous elements (white arrow heads indicate two of these regions) are apparent. Unless otherwise indicated, scale bars are 1000µm.

Non-kype-bearing *Salvelinus* individuals include those animals that are immature or non-spawning members of species that do grow a kype when spawning, such as *S. fontinalis* (UW 28888) (Fig. 3.6 a), as well as individuals that do not appear to develop a kype at any stage in their life history, such as *S. namaycush* (ZP 2013-07-01(A)) (Fig. 3.6 b) or non-anadromous *S. alpinus*. In these individuals, an anterior connective tissue mass may be very minimal, as in immature *S. fontinalis* (Fig. 3.6 a), or may be more extensive, as in *S. namaycush* (Fig. 3.6 b, asterisk). While the more extensive anterior connective tissues of species like *S. namaycush* may be supported by structural elements extending from the bone of the dentary (Fig. 3.6 b, black arrows), these elements do not resemble the spurs and needles of the kype skeleton. Rather, these extensions from the dentary tend to be very thick and rounded or curved and are similar to the elements extending from the dentary of *Hucho*. Structural evidence of a kype skeleton is not found in immature individuals such as *S. fontinalis* (UW 28888).

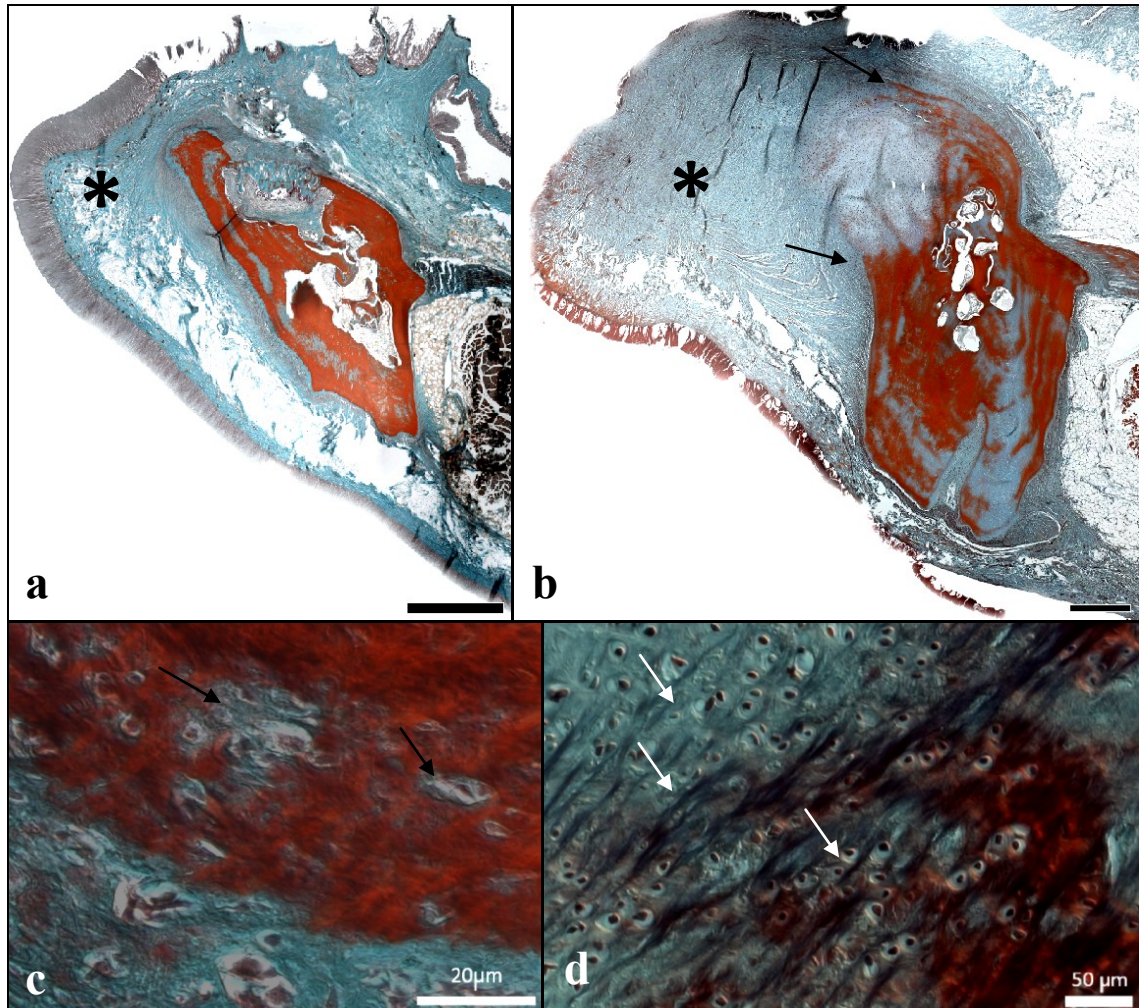
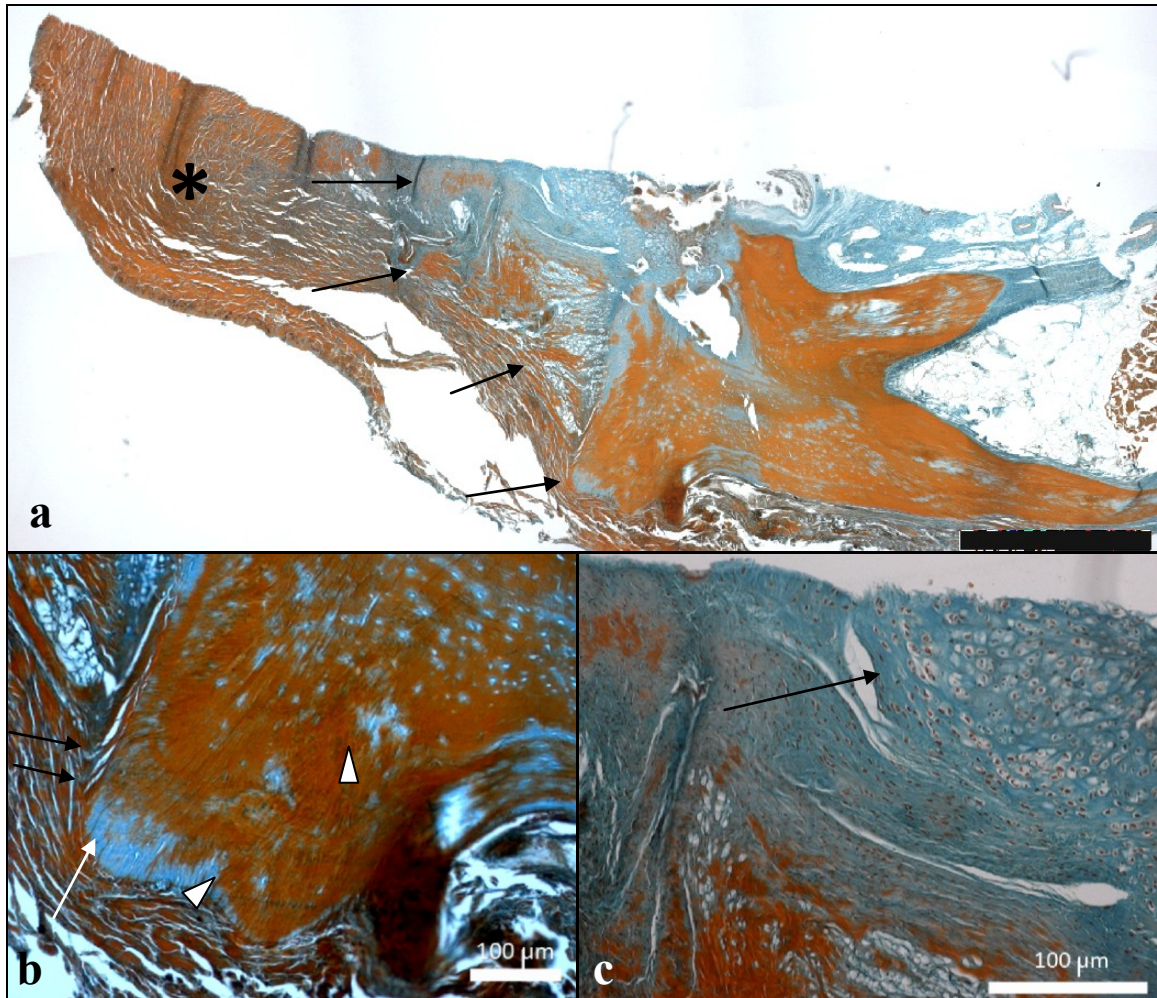


Figure 3.6 - Lower jaw skeletal tissues of non-kype-bearing *Salvelinus* specimens. (a) Non-spawning *S. fontinalis* (UW 28888) and (b) *S. namaycush* (CMNFI 1982-0385) in sagittal view. Anterior connective tissue is minimal in *S. fontinalis*, but may be more extensive in *S. namaycush* (asterixes). This more extensive connective tissue in *S. namaycush* may be supported by elements extending from the dentary (black arrows), but these elements do not resemble the kype skeleton found in kype-bearing individuals. (c) Extensive aggregations of chondrocytes within the mature dentary bone are frequently evident (*S. fontinalis*, black arrows), as are (d) strings of chondrocytes aggregating around fibres extending from the interior of the dentary into the surrounding connective tissue (*S. namaycush*, some examples indicated by white arrows). Unless otherwise indicated, scale bars are 1000µm.

While evidence of a kype is absent in non-kype bearing *Salvelinus* individuals, as with the basal salmonid and *Hucho* specimens described above, both chondroid bone and Sharpey-fibre bone may be present. Particularly in anterior ventral regions of the dentary, extensive aggregations of newly divided and more mature, actively secreting chondrocytes are apparent (Fig. 3.6 c, black arrows). In some cases, these chondrocytes aggregate around fibres extending from the dentary bone into surrounding connective tissue (Fig. 3.6 d).

### ***Salmo***

Spawning *Salmo* specimens show many of the traits found in spawning *Salvelinus* specimens. In brown trout (*Salmo trutta*), an anterior connective tissue mass (Fig. 3.7 a, asterix) is well supported by spur-like extensions of bone and intermediate tissue from the dentary (black arrows). These extensions are often tipped with bands of non-mineralized material (Fig. 3.7 b, white arrow), much as is found in *S. salar*, and are the sites of complex interactions between fibres extending from the periosteum and associated connective tissue (black arrows). These spurs also contain a range of small regions of actively secreting and dividing chondrocytes (Fig. 3.7 b, white arrow heads) as well as regions very densely packed with chondrocytes (Fig. 3.7 c). In some cases, particularly dorsally, these chondrocytes are so actively secreting that the contours of the spurs are disguised by a surplus of newly secreted, non-mineralized material that surrounds the actively dividing chondrocytes (Fig. 3.7 c).



**Figure 3.7 – Lower jaw skeletal tissues of spawning *Salmo trutta* (MAS).** In (a) sagittal view, a massive anterior connective tissue dominated the lower jaw (asterisk) and is support by numerous spur-like extensions of bone and intermediate tissue, reminiscent of a kype skeleton (black arrows). (b) Spur-like extensions are tipped with a layer of non-mineralized material (white arrow). At the margins of these extensions, fibrous elements extend from the interior of the spur into surrounding connective tissue (black arrows). Some of these spurs may contain small numbers of actively secreting chondrocytes (b, white arrowheads), but others (c) appear to be almost entirely composed of actively secreting and dividing chondrocytes. Unless otherwise indicated, scale bars are 1000 $\mu$ m.

Contrarily, but as is the case with immature *Salvelinus* specimens, non-spawning *Salmo* individuals do not display any structural evidence of the kype skeleton (Fig. 3.8 a). The anterior connective tissue is very thin and dense, at times appearing to be composed of closely packed, cord- and fibre-like elements. In some areas of the jaw, these fibres extend from or penetrate into the compact bone of the dentary (Fig. 3.8 b, white arrows) and are associated with strings of chondrocytes (black arrow). This penetration or extension of the dentary is particularly prevalent in those sections adjacent to the margin



of the hyaline cartilage-displaying region of the symphysis, approximately one quarter of the distance between the midline and the first tooth. Chondrocytes may also occur as aggregations of actively dividing and secreting cells at the interior of compact, mature bone (Fig. 3.8 c).

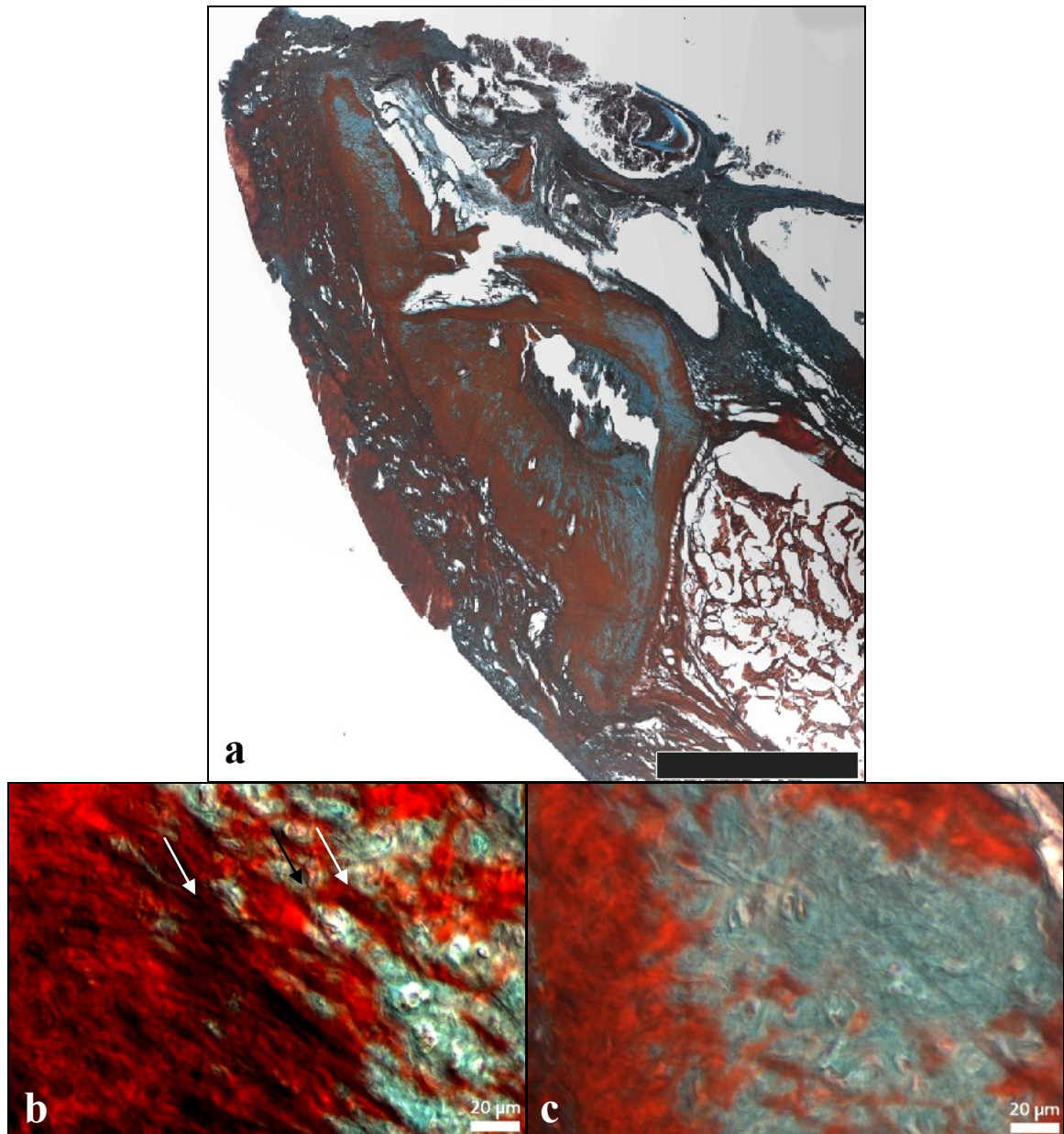


Figure 3.8 – Lower jaw skeletal tissues of non-spawning *Salmo salar* (MAS). In (a) sagittal view. (b) Fibrous elements extend from or penetrate into the dentary (some examples indicated by white arrows) and may be associated with strings of chondrocytes (one example indicated by a black arrow). (c) Aggregations of chondrocytes also occur at the interior of the bone of the dentary. Unless otherwise indicated, scale bars are 1000µm.

### ***Oncorhynchus***

Once again in *Oncorhynchus*, the contrast in structure and composition of the lower jaw between individuals that do and do not have a kype is very apparent. In kype-bearing, spawning *O. gorbuscha*, a massive anterior connective tissue region (Fig. 3.9 a, asterix) is supported by a near-spongiose network of spurs extending from the dentary. The margin of this network is indicated by black arrows in Figure 3.9 a. These spurs are very extensive and radiate from the dorsal surface of the dentary to the posterior ventral margin. At the tips of individual spurs are bands of non-mineralized material containing many very small fibres and strings of chondrocytes extending from the periosteum into the surrounding connective tissue (Fig. 3.9 b). Chondroid bone is also in evidence, with many aggregations of chondrocytes actively secreting and dividing at the interior of the dentary bone (Fig. 3.9 c).

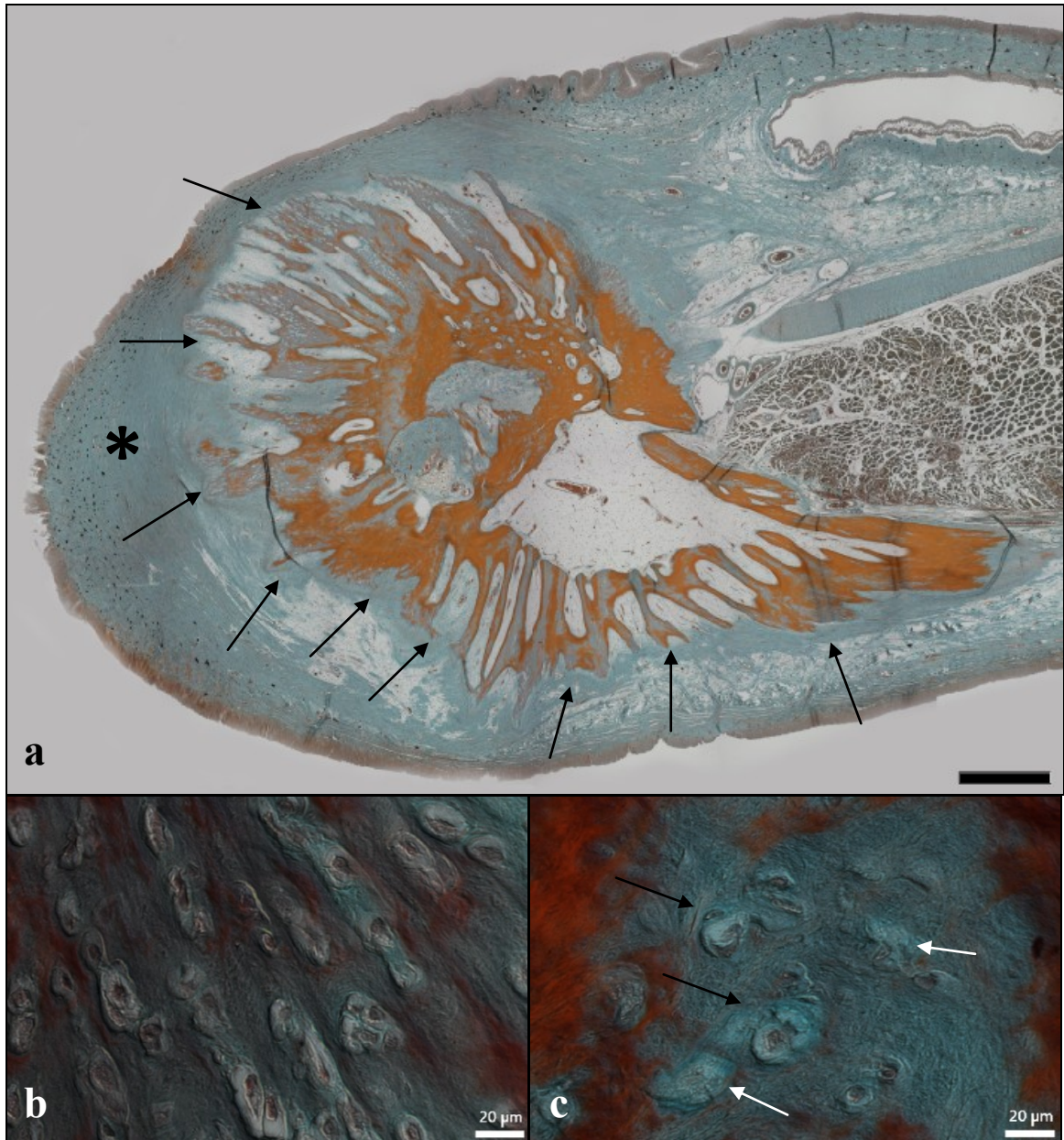


Figure 3.9 – Lower jaw skeletal tissues of kype-bearing, spawning *Oncorhynchus gorbuscha* (MAS). In (a) sagittal view, a large anterior connective tissue mass (asterix) is supported by an extensive array of skeletal needles and spurs (the margin of this array is indicated by black arrows). (b) The tips of the dentary spurs are marked by a thick band of non-mineralized material containing many small fibres and strings of chondrocytes extending from the periosteum into surrounding connective tissue. (c) Chondroid bone is present at the interior of the dentary where actively dividing (some examples indicated by white arrows) and secreting (some examples indicated by black arrows) chondrocytes aggregate together. Unless otherwise indicated, scale bars are 1000µm.

Non-kype-bearing individuals like non-spawning *Oncorhynchus nerka* do not have the large, anterior connective tissue mass seen in kype-bearing individuals (Fig. 3.10 a). Also absent are the spurs and needles extending from the dentary that would serve to

support such a tissue mass. However, the periosteum of the dentary is blurred by innumerable fibrous elements extending from the interior of the dentary into surrounding connective tissue (Fig. 3.10 b, white arrow). Several of these fibres are associated with strings of chondrocytes, forming immature Sharpey fibres that penetrate deep into the dentary (Fig. 3.10 c, some examples are indicated with white arrows). Also present within the bone at the interior of the dentary are aggregations of paired, newly divided chondrocytes (Fig. 3.10 d, some examples are indicated with black arrows) surrounded by other, mature chondrocytes (some examples are indicated with white arrows).

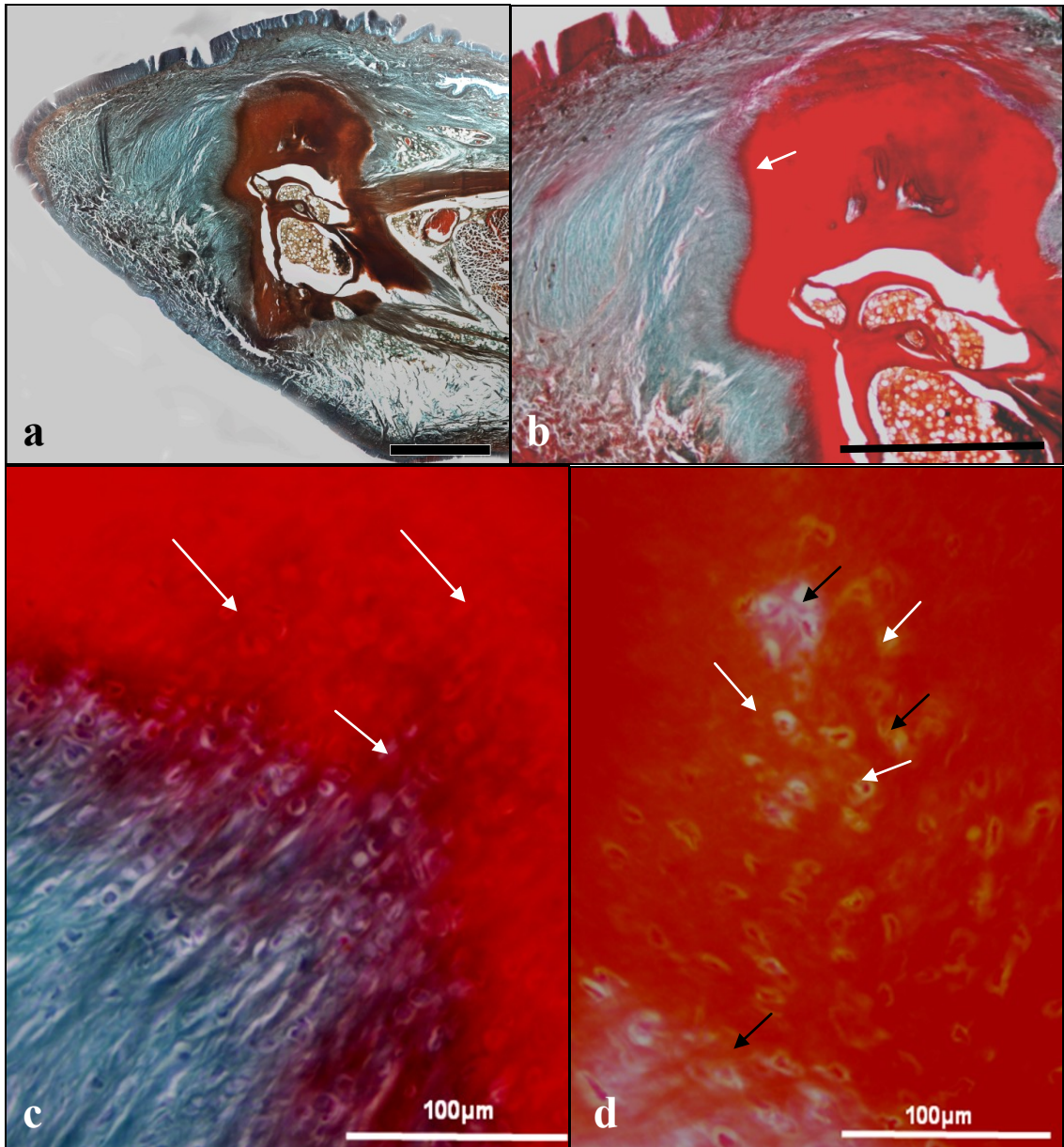


Figure 3.10 – Tissues of non-spawning, non-kype-bearing *O. nerka* (MAS). In (a) sagittal view, the trademark anterior connective tissue mass supported by spurs of bone and intermediate material extending from the dentary found in kype-bearing individuals are absent in this specimen. (b) The periosteum surrounding the dentary (white arrow) is blurred by many fibres extending from the interior of the bone into adjacent connective tissue. Some of these fibres (c) are associated with strings of chondrocytes (some examples are indicated by white arrows). (d) Chondrocytes are also present as aggregations of paired, newly divided cells (some examples indicated by black arrows) surrounding more mature, more actively secreting cells (some examples indicated by white arrows) within the bony material of the dentary at its interior. Unless otherwise indicated, scale bars are 1000µm.

Due to the distinct differences in the tissues of kype-bearing and non-kype-bearing individuals, it is relatively easy to determine when a kype is present in an organism using histological sections even when physical examinations are inconclusive.

For example, while physical examinations of *O. mykiss aguabonita* and *O. clarki* (UW 028776) in Chapter 2 were both inconclusive for kype presence, histological analysis of *O. m. aguabonita* reveals evidence of several kype-related structures and tissues. An anterior connective tissue mass (Fig. 3.11 a, asterix) is supported by several extensions from the dentary (black arrows). That this tissue mass is small and its associated supportive spurs are relatively few in number is likely due to the fact that this specimen was still developing its kype at the time of its capture.

Although kype-related structures are evident in *O. m. aguabonita*, *O. clarki* (UW 028776) displays very little evidence of a kype (Fig. 3.11 c). While the anterior connective tissue mass of this specimen may be somewhat larger than in other pre-spawning *O. clarki* specimens such as (CMNFI 1973-0393.1) (Fig. 3.11 b), there is very little or no evidence of supportive spurs extending from the dentary. However, both the kype-bearing *O. m. aguabonita* and the non-kype-bearing *O. clarki* (UW 028776 and CMNFI 1973-0393.1) display evidence of strings of chondrocytes at the margins of the dentary (Fig. 3.11 d, black arrows), often associated with fibrous elements extending into surrounding connective tissue (white arrows). Chondrocytes are also present at the interior of the dentary bone, aggregating in groups and pairs of actively dividing and secreting cells (Fig. 3.11 e).

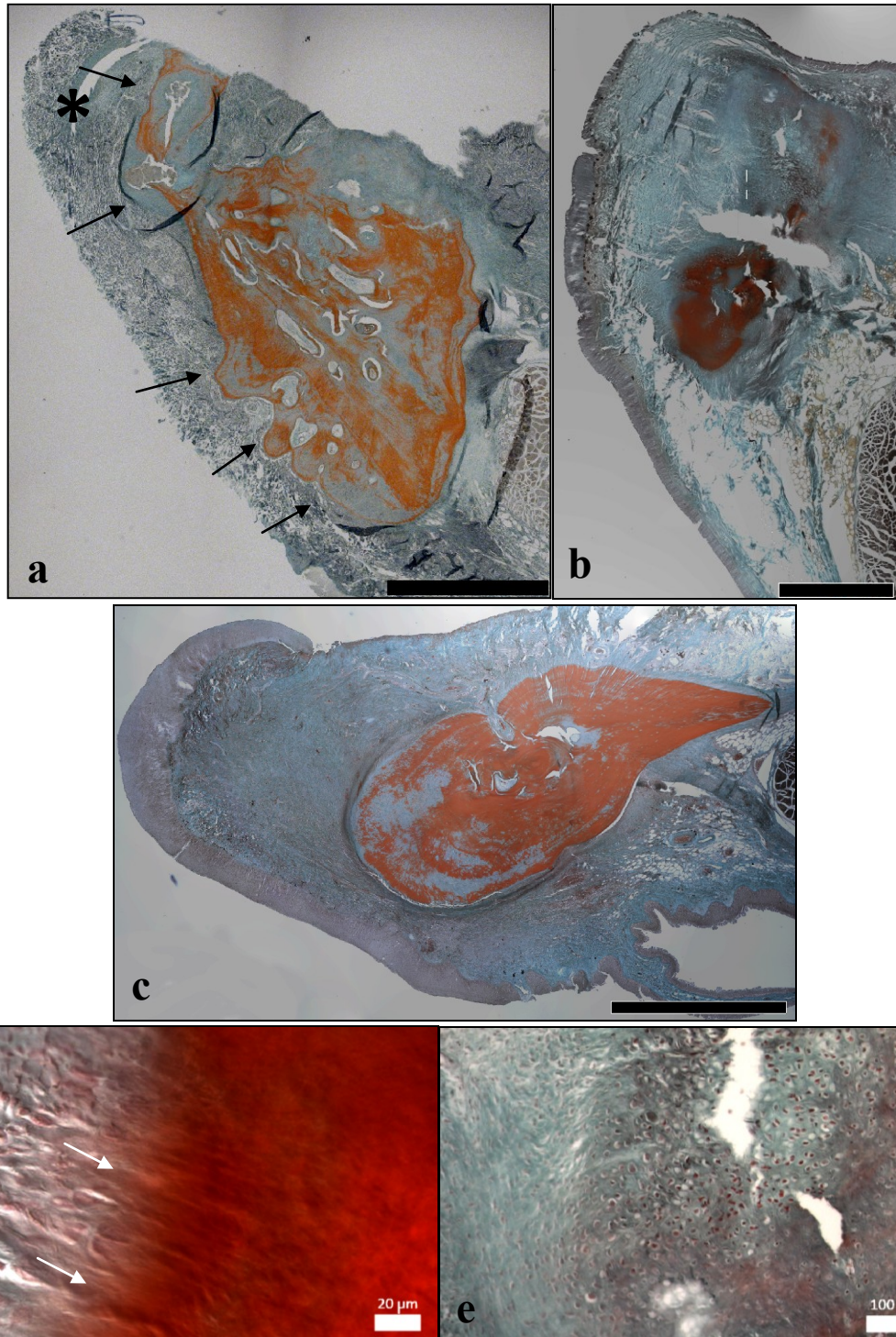


Figure 3.11 – Distinguishing an enigmatic kype or subtle (MAS). Sagittal views of (a) spawning *Oncorhynchus mykiss aguabonita*, and pre-spawning *Oncorhynchus clarki* (b) CNFI 1973-0393.1 and (c) UW 028776. While *O. m. aguabonita* displays an anterior connective tissue mass (asterisk) supported by several extensions from the dentary (black arrows), these structures are absent in the *O. clarki* specimens. However, (d) strings of chondrocytes (some examples indicated by black arrows) aggregating with fibrous extensions at the margins of the dentary (white arrows) and (e) aggregations of actively secreting and dividing chondrocytes within the bone of the dentary are evident in all *Oncorhynchus* specimens examined. Unless otherwise indicated, scale bars are 1000 $\mu$ m.

*Phylogenetic survey summary*

While rudimentary structures extending from the jaw are found in animals that do not appear to grow a kype (*Hucho hucho*), the structure and organization of the kype skeleton is found only in mature, spawning eusalmonines (Fig. 3.12). However, Sharpey-fibre and chondroid bone are present in all salmonids, regardless of their phylogenetic position or life history stage. These tissues are found in both kype-bearing and non-kype-bearing individuals including those that have not yet grown a kype, as well as those that are not believed to be capable of growing a kype (i.e. basal salmonids).



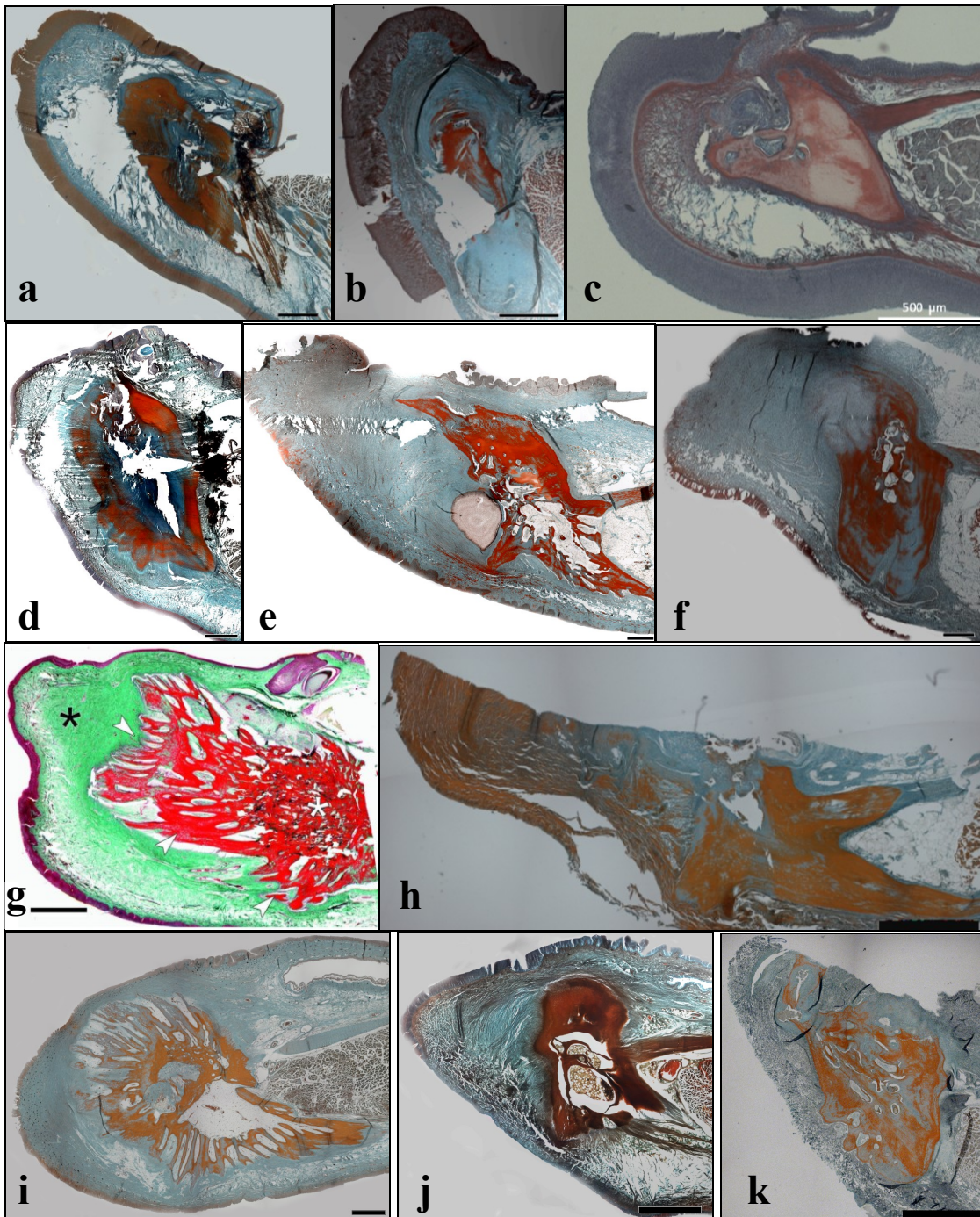


Figure 3.12 – Comparison of salmonid lower jaw skeletal structure. (a) *P. williamsoni*, (b) *Coregonus clupeaformis*, (c) *Brachymystax lenok*, (d) *Hucho hucho*, (e) *Salvelinus alpinus*, (f) *S. namaycush*, (g) *Salmo salar* (from Witten and Hall, 2003), (h) *S. trutta*, (i) *O. gorbuscha*, (j) *O. nerka*, and (k) *O. mykiss aguabonita*. Spur-like extensions from the dentary composed of a combination of chondroid bone and Sharpey-fibre bone are present in spawning Eusalmonines (e, g-i, and k), absent in non-spawning eusalmonines and basal salmonids (a-c, f, and j), and may be present, if enigmatic in *Hucho hucho* (d). Scale bars are 1000 $\mu$ m.

Tissues and structures associated with the presence of kype are easily distinguished in histological analysis. Thus, while an enigmatic or developing kype may not be apparent during physical examination of specimens, histology is a reliable means of confidently determining if a kype is or is not present, as shown in assessments of *Oncorhynchus mykiss aguabonita* and *O. clarki*. The structure of a kype was even found in animals that displayed no structural evidence of a kype in physical examination, as in *O. kisutch* (Fig. 3.13). Kype presence and absence, as well as the presence and absence of the kype-associated tissues Sharpey-fibre and chondroid bone, in specimens examined in this study as confirmed by histological analysis is shown in Table 3.1.

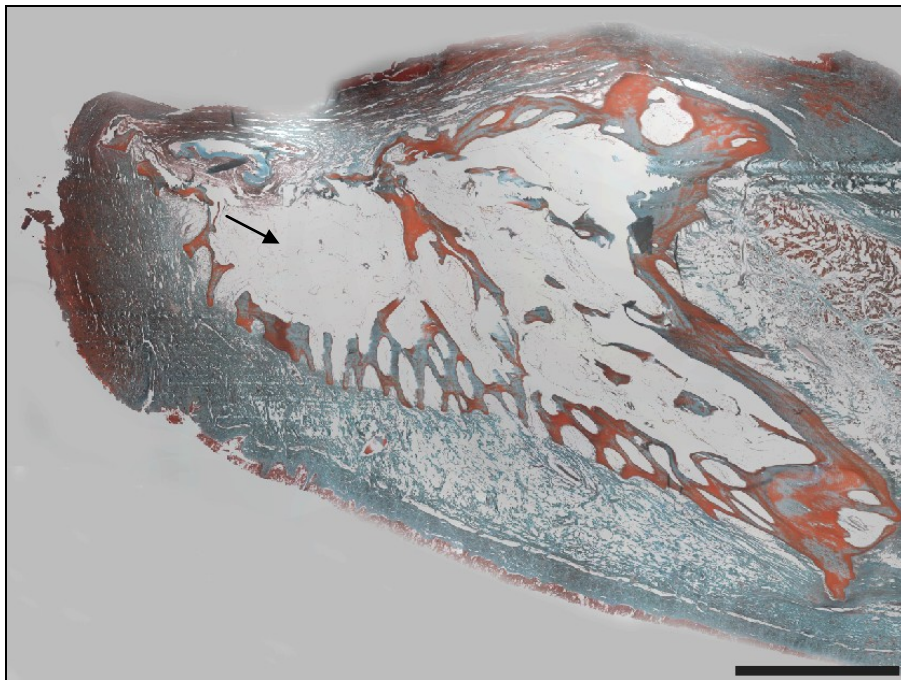


Figure 3.13 – Kype skeleton of *Oncorhynchus kisutch* (MAS). Evidence of a kype skeleton at the tissue level may be present in individuals even when macroscopic evidence of a kype is absent, as in *O. kisutch*. This specimen displays the short, squat skeletal needles of the typical *Oncorhynchus* kype skeleton. Also evident in this section is the large fat pocket at the interior of the dentary (black arrow). Scale bar is 1000µm.

Table 3.1 – Presence and absence of a kype and of “kype-associated” tissues (tissues previously found in combination only in association with a kype; chondroid and Sharpey-fibre bone) in specimens examined using histology.

<i>Genus species</i>	Specimen ID	Kype present (P) or absent (A)	Sharpey-fibre and chondroid bone present (P) or absent (A)
<i>Coregonus</i>		A	P
- <i>C. clupeaformis</i>	UW 05587	A	P

<b>Genus species</b>	<b>Specimen ID</b>	<b>Kype present (P) or absent (A)</b>	<b>Sharpey-fibre and chondroid bone present (P) or absent (A)</b>
<i>Prosopium</i>		A	P
- <i>P. williamsoni</i>	UW 005260	A	P
<i>Brachymystax</i>		A	P
- <i>B. lenok</i>	UW 022131	A	P
<i>Hucho</i>		A	P
- <i>H. hucho</i>	UW 022130	A	P
<i>Salvelinus</i>		P	P
- <i>S. fontinalis</i>	UW 28888	A	P
- <i>S. namaycush</i>	ZP 2013-07-01(A)	A	P
- <i>S. namaycush</i>	ZP 2013-07-02(B)	A	P
- <i>S. namaycush</i>	ZP 2013-07-03(C)	A	P
- <i>S. namaycush</i>	ZP 2013-07-04(D)	A	P
- <i>S. namaycush</i>	ZP 2013-07-05(E)	A	P
- <i>S. namaycush</i>	ZP 2013-0706(F)	A	P
- <i>S. namaycush</i>	UW 020761	A	P
- <i>S. namaycush</i>	CMNFI 1982-0385	A	P
- <i>S. leucomaenis</i>	UW 042437	A	P
- <i>S. confluentis</i>	UW 20760	P	P
- <i>S. alpinus</i>	CMNFI 1968-1262.1	P	P
- <i>S. alpinus</i>	CMNFI 1961-0229	P	P
- <i>S. alpinus</i>	UW 041192	A	P
- <i>S. alpinus aureolus</i>	UW 020740	A	P
<i>Salmo</i>		P	P
- <i>S. trutta</i>	UW 005350	P	P
- <i>S. salar</i>	UW 019807	A	P
<i>Oncorhynchus</i>		P	P
- <i>O. clarki</i>	UW 028776	A	P
- <i>O. clarki</i>	CMNFI 1973-0393.1	A	P
- <i>O. mykiss</i>	UW 016073	A	P
- <i>O. mykiss aguabonita</i>	UW 015967	P	P
- <i>O. kisutch</i>	UW 015091	P	P
- <i>O. tshawytscha</i>	UW 015893	A	P

<i>Genus species</i>	Specimen ID	Kype present (P) or absent (A)	Sharpey-fibre and chondroid bone present (P) or absent (A)
<i>Oncorhynchus</i>		P	P
- <i>O. keta</i>	UW 13664	A	P
- <i>O. gorbuscha</i>	UW 16027	P	P
- <i>O. gorbuscha</i>	CMNFI 1984-0169.1	A	P
- <i>O. nerka</i>	UW 005624	A	P

### ***Other tissues of interest***

A consideration of the structure of the kype is necessary when establishing presence or absence of kype in a given individual, species, or genus. However, as was found in Chapter 2, an understanding of the other tissues and structures found in the lower jaw is also important when distinguishing phylogenetic trends in kype growth. The following will consider several tissues of interest in the lower jaw of salmonids. These tissues include teeth, epithelium, fat, and blood vessels.

### ***Teeth and supporting tissues***

With the exception of the *Coregonus clupeaformis* specimen, teeth were found in each specimen examined. As was previously indicated by Micro-CT scan (Fig. 2.47), even the immature *Brachymystax lenok* specimen bore teeth, which suggests that this specimen was nearing maturity at the time of its capture, despite its small size (Holčík et al., 1988). That these teeth in *Brachymystax* had not yet erupted from the epithelium does, however, confirm that it was not yet mature.

While teeth were present in nearly all specimens, there was some variation in dentition. In the majority of specimens only “feeding teeth” were present (Fig. 3.14 a and b). These teeth are relatively slender, typically triangular in shape, and are typically connected to the dentary via alveolar bone (as in *Hucho hucho*, Fig. 3.14 a and *Brachymystax*, Fig. 3.14 b), a dense extension of the dentary that is honeycombed with nerves and blood vessels, or simply rest on the compact bone of the dentary (as in *Brachymystax lenok*, Fig. 3.14 b). In *Oncorhynchus*, however, these feeding teeth (Fig. 3.14 c, black arrows) are joined by much larger, more fang-like “breeding teeth” (white arrows).

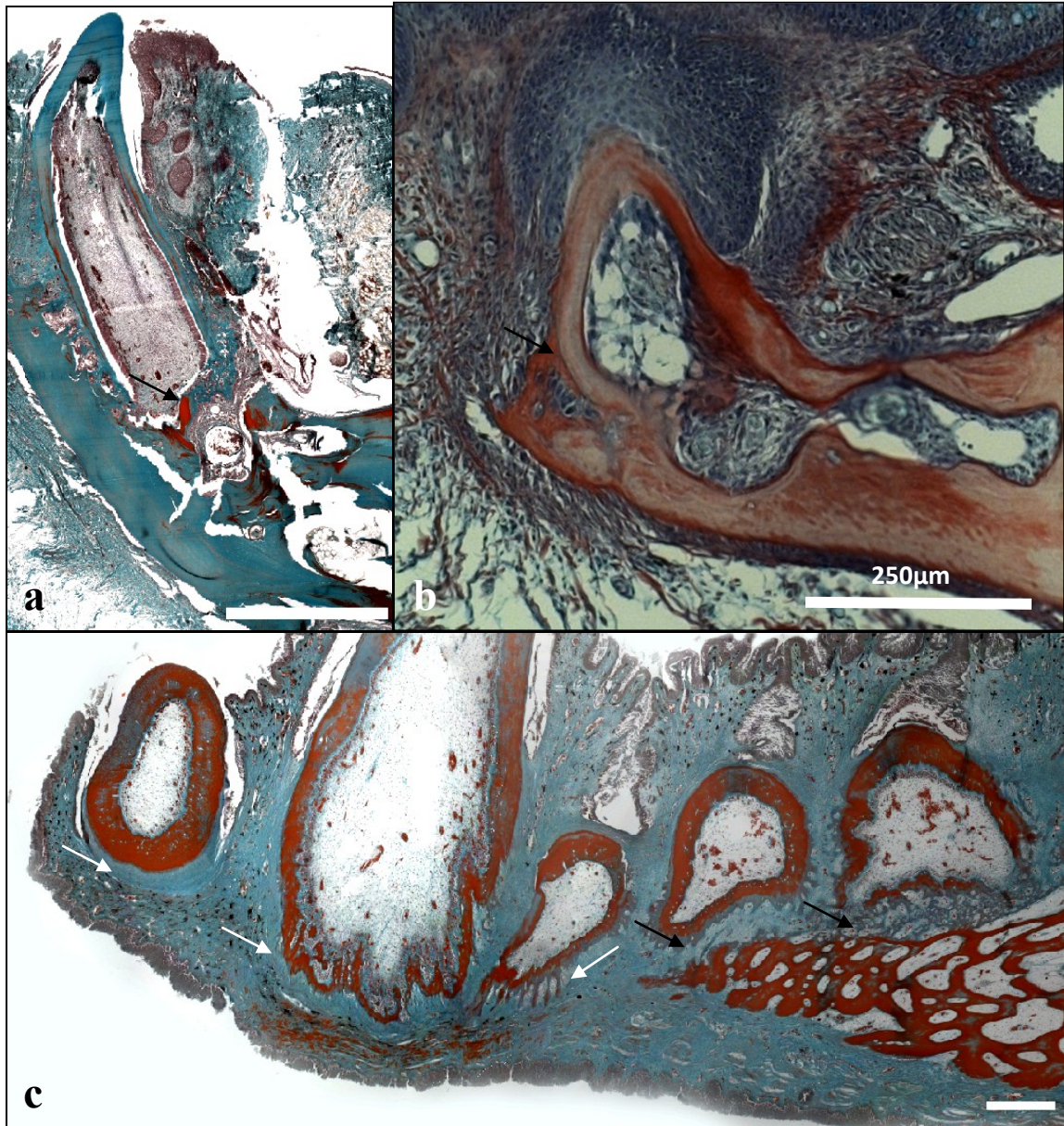


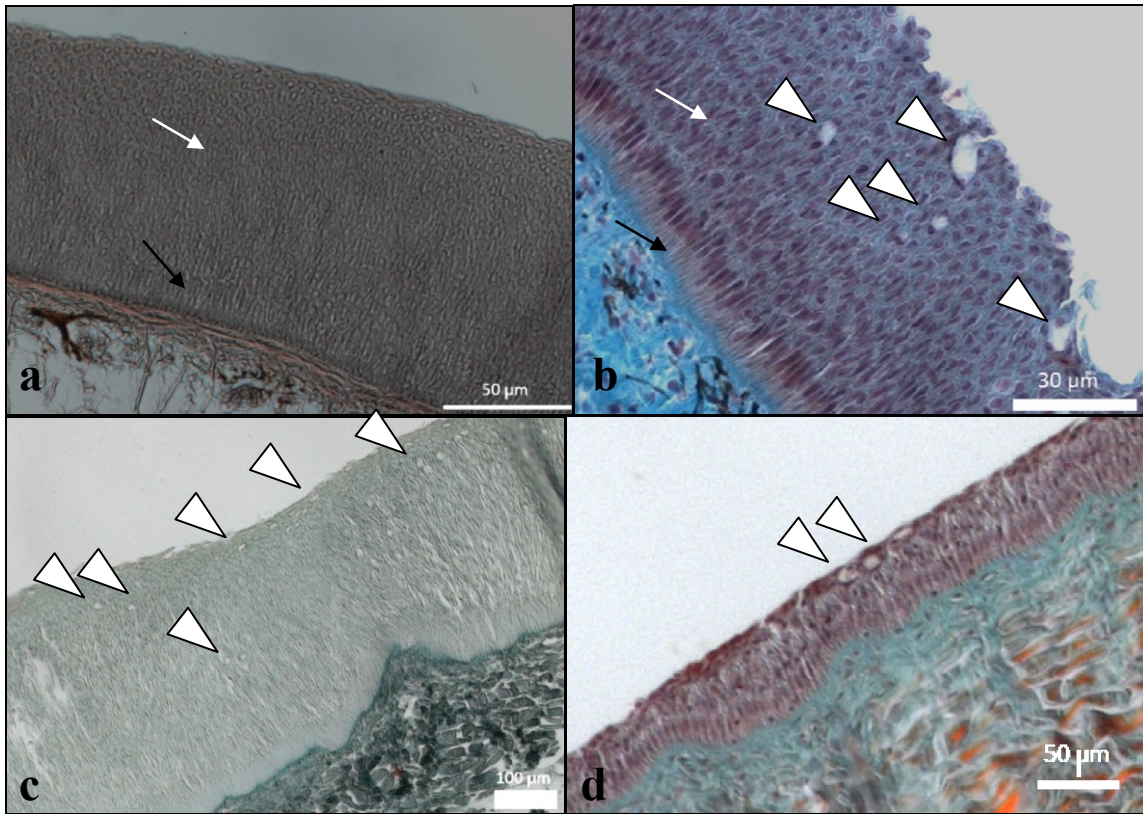
Figure 3.14 –Salmonid teeth (MAS and HBQ). Feeding teeth in (a) *Hucho hucho* (MAS), (b) *Brachymystax lenok* (HBQ), and (c) *Oncorhynchus gorbuscha* (black arrows) are attached to teeth via alveolar bone (MAS). Breeding teeth in *Oncorhynchus gorbuscha* (white arrows), resting on a spongy base. Breeding teeth are larger than feeding teeth, with thicker, less mineralized walls, and rest on large, spongy bases that are supported by thin strips of compact dentary bone. Unless otherwise indicated, scale bars are 1000µm.

These breeding teeth, first discussed in this thesis in the physical examinations and Micro-CT assessments of spawning *Oncorhynchus* specimens (Chapter 2), differ a great deal from feeding teeth, even within the same individual. Breeding teeth are much larger than the other teeth present on the dentary and have spongier, less dense roots. While the feeding teeth rest directly on the dentary, either on compact bone or alveolar

bone, breeding teeth rest on a very large, spongy base that, in turn, is only secondarily connected to the dentary. These enormous bases replace the majority of the anterior dentary bone, particularly near the lateral margin of the jaw.

### *Epithelium*

In many of the specimens examined, particularly in the coregonids, *Brachymystax*, and *Hucho*, the epithelium tends to be very thick (Fig. 3.15 a and b). In *Brachymystax*, in fact, the epithelium is arguably the major component of the lower jaw (Fig. 3.3 a). Furthermore, this epithelium is very well organized. A region of slender cells organized in parallel striations near the basement membrane transitions into one of fatter, rounded cells toward the exterior of the tissue (Fig. 3.15 a-d, white arrows).



**Figure 3.15 – Salmonid epithelium (MAS and HQB).** (a) *Brachymystax lenok* (HBQ) and (b) *Hucho hucho* (MAS) epithelium are thick and very well-organized. Tissues are divided into a polarized region composed of slender cells stacked in parallel adjacent to the basement membrane and the adjacent connective tissue (white arrows) and a region with fatter, rounded cells (black arrows). (c) While not as thick or as well-organized as in more basal salmonids, the epithelium in spawning Eusalmonines, such as *Oncorhynchus mykiss aguabonita* (MAS), is thicker than in non-spawning individuals of the same species, such as (d) immature *O. mykiss* (MAS). Spawning salmonids, regardless of phylogenetic position, tend to have more glands and mucous cells (some example are indicated by white arrowheads) in their epithelium and adjacent connective tissue than do non-spawners.

The epithelium of *Salvelinus*, *Salmo*, and *Oncorhynchus* are not as thick or as well organized as in more basal salmonids. However, the epithelium of spawning individuals, particularly when near the symphysis, is thicker and than in non-spawning ones (Fig. 3.15 c and d). Additionally, regardless of genus or phylogenetic position, the epithelium of spawners frequently contains a greater number of globular mucous cells and glands (Fig. 3.15 a-d, white arrowheads). Spawners also display more pigment cells in both the epithelium and in the connective tissue adjacent to the basement tissue of the epithelium than is the case in non-spawners (please refer to spawning *O. gorbuscha* Fig. 3.10). However, because of the secondary alteration of the epithelium resulting in this increase in pigment cells and mucous cells, the epithelium of non-spawning individuals tends to be better organized than that of spawning individuals.

### *Fat*

Many salmonid specimens displayed a number of fat accumulations in their bone and muscle (Fig. 3.16, examples are indicated by black arrows). These fat accumulations ranged in size from virtually non-existent in, for instance, immature *Brachymystax lenok* (Fig. 3.16 a) to being one quarter of the size of the visible dentary, as in spawning *S. alpinus* (Fig. 3.16 b) and *O. gorbuscha* (Fig. 3.16 c). In pre-spawning, anadromous *Oncorhynchus* such as *O. kisutch* (UW 015091), this fat pocket is, arguably, the major component of the dentary (Fig. 3.13, black arrow). The number and size of these fat accumulations were larger in *Coregonus clupeaformis*, *Salvelinus alpinus*, *S. fontinalis*, *Salmo trutta*, *Oncorhynchus kisutch*, *O. tshawytscha*, *O. keta*, and *O. gorbuscha* specimens than in the other fish examined. Furthermore, when both a mature and an immature specimen were assessed, fat accumulations were larger and more numerous in individuals that were more mature, as shown in *S. alpinus* in Figure 3.16 c and d. These smaller fat accumulations in juveniles are usually associated with scalloped dentary margins resulting from rapid growth of skeletal elements.



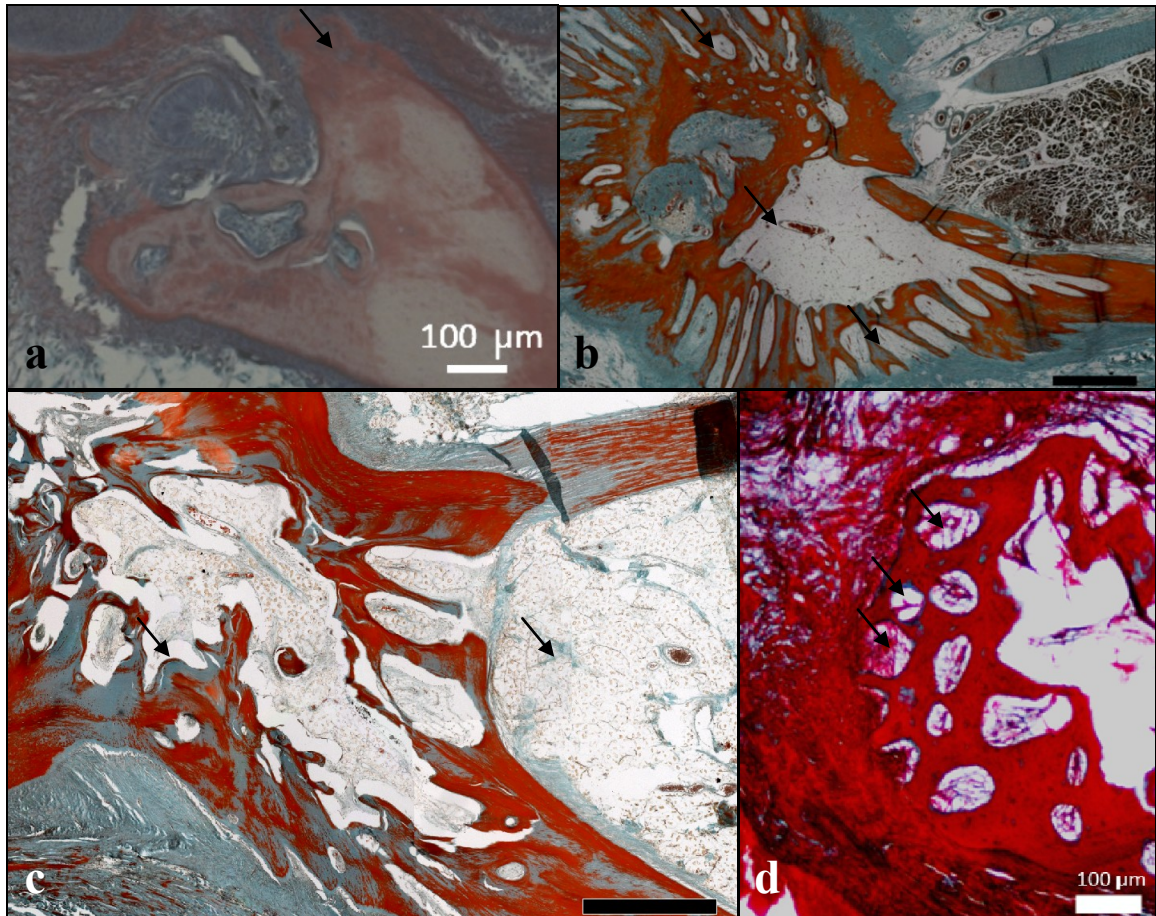


Figure 3.16 – Salmonid fat pockets (HBQ and MAS). (a) Fat pockets in *Brachymystax lenok* (HQB) are virtually non-existent, but may comprise a region greater than one quarter of the size of the visible dentary, as in (b) spawning *Oncorhynchus gorbuscha* (MAS) and (c) spawning *Salvelinus alpinus* (MAS) (black arrows). (d) As seen in immature *S. alpinus* (MAS), immature individuals of the same species have much smaller fat pockets than those found in more mature and spawning individuals. Fat pockets in juveniles are also usually associated with scalloped dentary margins resulting from rapid growth. Unless otherwise indicated, scale bars are 1000µm.

#### *Blood vessels and sinuses*

Blood vessels are widespread in each specimen, as is shown in *O. gorbuscha* in Figure 3.17 a, black arrows. Occurring both within the dentary itself and within the muscle and connective tissue that surrounds it, blood vessels form an intricate and extensive network throughout the lower jaw. The extensive nature of these networks is particularly apparent in spawning individuals such as *Salvelinus alpinus* (CMNFI-1968-1262.1) and *Oncorhynchus mykiss aguabonita*, where the many blood vessels seen in other specimens are joined by vascular sinuses (Fig. 3.17 b and c, white arrows).

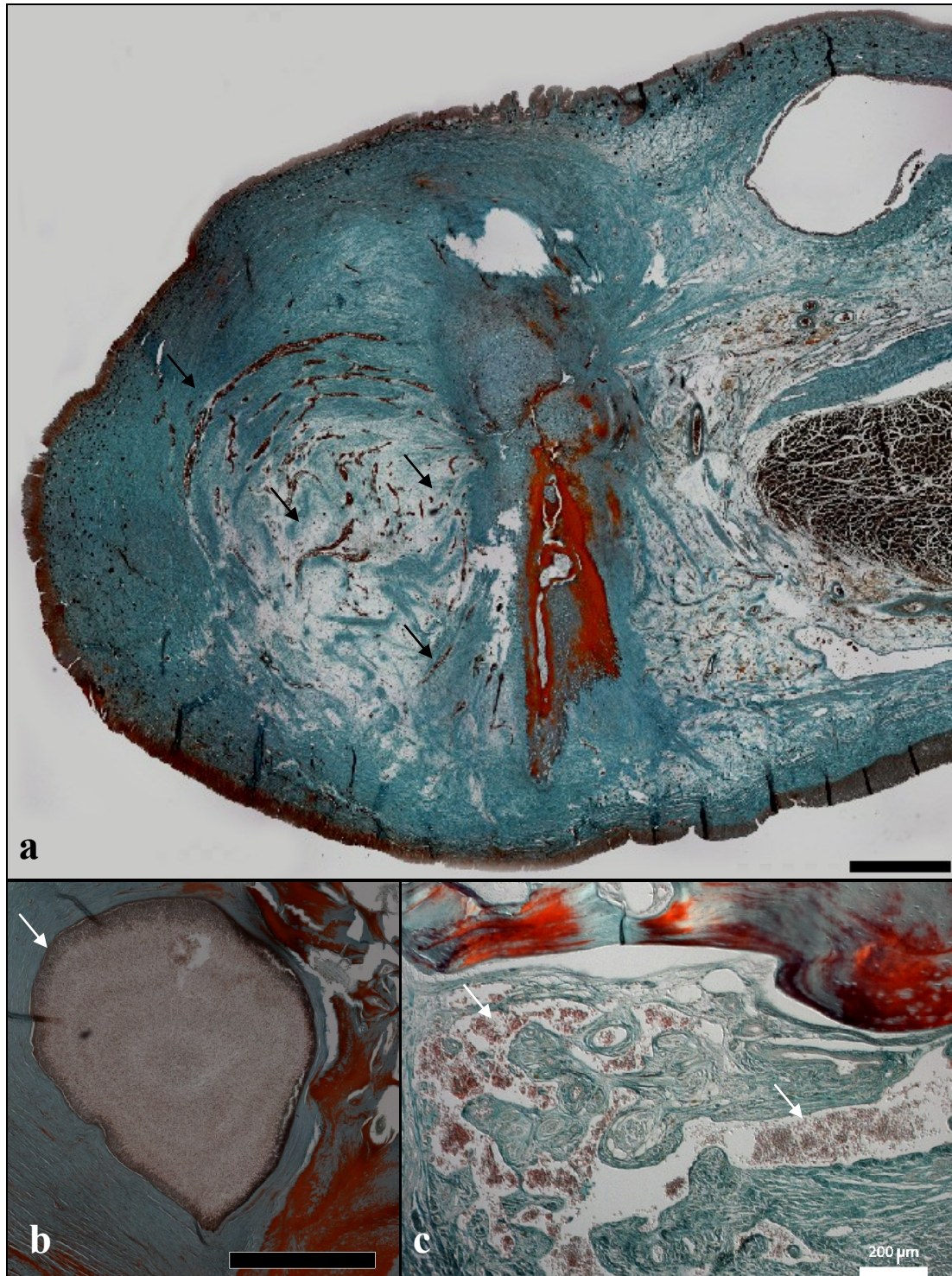


Figure 3.17 – Salmonid blood vessels (MAS). (a) Blood vessel networks are extensive in the salmonids, including in the tissues of *Oncorhynchus gorbuscha* (several examples of blood vessels are indicated with black arrows). In several specimens, vascular sinuses may also be present as in (b) *S. alpinus* and (c) *O. mykiss aguabonita* (white arrows). Unless otherwise indicated, scale bars are 1000 $\mu$ m.

## **Discussion and conclusions**

Sexual maturation results in a number of changes in physiology and morphology. Most relevant to this study, some of these changes may result in the establishment of secondarily developing skeletal and soft tissue elements (Hall, 2005). In many mammals, transformational growth within the genitals results in the development of an *os penis* or penile bone (for a description of this feature in rats, please refer to: Murakami and Mizuno, 1984, 1986; Yamamoto, 1989). In deer and their relatives, sexual maturity of males (and of female reindeer) is marked by the growth of antlers (Hall, 2005; Witten and Hall, 2002). While penile bones have a complex histogenesis that is in many ways similar to of limb development (Hall, 2005), antler development is similar to that of the kype (Witten and Hall, 2002).

Antlers and kype skeletons develop periosteally at a specific site on the dermal skeleton: the apical dentary in the kype and the pedicle of the frontal bone in antlers (Hall, 2005; Witten and Hall, 2002). Additionally, metaplastic conversion from an initial cartilaginous phase results in the development of bone in both of these secondarily developing features. The conversion of rapidly growing cartilaginous tissue into bone results in the rapid growth, but minimal material cost, of both kypes and antlers.

In addition to displaying metaplastic conversion and secondary development, the kype is often associated with the emergence of other tissues and structures of interest in the lower jaw and appears to have a phylogenetic component (personal observations; Witten and Hall, 2002, 2003). As shown in Chapter 2 and in the results section of this chapter, a kype is found only in certain genera of eusalmonines. However, tissues of interest, including those previously associated with kype growth, are present even when a kype is not. Furthermore, the structure and organization of the kype and of the other tissues in the lower jaw vary depending on a number of factors, some of which relate to phylogenetic position or genus, but others that appear to be due to life history and mode.

To conclude this chapter, I will discuss the concepts of the phylogenetic relationships in kype growth and in growth and structure of the tissues of the lower jaw, attempting to place them in an evolutionary context. I will begin by considering histological evidence for the presence or absence of a kype, before assessing the presence of Sharpey-fibre and chondroid bone across the salmonids. I will then assess the

implications of the “other tissues of interest” before comparing the tissues and structure of the kype in the different kype-bearing genera. I will close this chapter with an assessment of sources of error and a brief summary.

### ***Lower jaw and kype structure, composition, and evolution***

A dramatic and dynamic structure, the kype has appeared in native art and legend for centuries and is one of the most recognizable features of spawning salmon, trout, and char (Ames and Maschner, 1999; Campbell and Butler, 2010). However, prominent and recognizable as this structure is, as shown in the study of *Oncorhynchus mykiss aguabonita* in this chapter, confirmation of presence of a developing or enigmatic kype may require histological analysis. As was found in assessments of *Oncorhynchus clarki* specimens, kype-like features visible during physical examination may not, in fact, represent kype growth at all (Fig. 3.11). Furthermore, microscopic, histological evidence of the presence of a kype may occur when no macroscopic, structural evidence of a kype is apparent, as in *O. kisutch* (UW 015091, Fig. 3.13).

Even as evidence of a kype is not always apparent upon physical examination of a given specimen, Sharpey-fibre and chondroid bone, tissues found in previous work to be associated with the presence of a kype (Witten and Hall, 2002, 2003) may be present even in animals that do not have a kype. Although they were most prominent and best developed in kype-bearing *Salvelinus*, *Salmo*, and *Oncorhynchus* individuals, these tissues were present in each salmonid specimen examined in this study, regardless of phylogenetic position or life history stage.

As noted in Chapter 1, Sharpey-fibre bone and chondroid bone are present in the skeleton of many animals, including teleost fish. Sharpey-fibres are, in fact, frequently found in the dentary of many animals such as, as discussed by Johnson (2005) and Kuroiwa et al. (1994), the rat. In the dentary, Sharpey fibres provide additional support and strength in zones of attachment such as those connecting teeth to the dentary bone. However, the presence of chondroid bone in the dentary, particularly when in combination with Sharpey-fibre bone, is unusual. Evidence of these tissues, previously only found in combination where they were associated with the presence of a kype, in species that are non-kype-bearing suggests that the kype evolved via co-option of pre-

existing tissues. Examination of even more basal salmoniformes is required to determine when Sharpey-fibre and chondroid bone evolved. Furthermore, inclusion of fossil material, as will be shown in Chapters 4 and 5, may also reveal more information as to basal conditions not seen in living species and genera.

#### *Tissues of the kype*

As with other the tissues of interest, the tissues and structure of the kype are also illustrative of conditions within eusalmonines, varying depending on genus and on life history stage and life history mode. When contrasting tissues and structures in the different kype-bearing genera, a genus-specific trend emerges. *Salvelinus* (Fig. 3.5 a) and *Salmo* (Fig. 3.8a) both favour a kype with many slender skeletal needles associated with large and small nests of chondrocytes. These needles support a large anterior connective tissue mass with thick epithelium.

In *Oncorhynchus* individuals (Fig. 3.10), an extensive array of short, robust skeletal needles support a mass of anterior connective tissue, much like the more slender needles of *Salmo* and *Salvelinus*. However, these needles tend to be much more squat and minimal in area than in other kype-bearing genera (Fig. 3.10 and Fig. 3.13). Instead, the most prominent feature of the *Oncorhynchus* kype is the very large anterior breeding teeth (Fig. 3.14d). These teeth were first seen in the physical examinations and Micro-CT assessments of Chapter 2, but are also very much in evidence in histological section, as is discussed in greater detail in above.

While the same tissues and structures are present in each kype-bearing genus, with the exception of the enormous breeding teeth in *Oncorhynchus*, the size and number of the elements vary. Thus, the dominant character of the kype of *Oncorhynchus* is breeding teeth while in *Salmo* and *Salvelinus*, the kype is dominated by skeletal needles. However, because only one spawning *Salvelinus* specimen was examined in this study and because I am not aware of previous histological work that considered the kype in *Salvelinus*, further study of spawning *Salvelinus* individuals may reveal that, in fact, the kype of *Salvelinus* and *Salmo* differ greatly.

In addition to the kype skeleton, resulting from Sharpey-fibre bone and chondroid bone growth, other tissues and structures of interest are found in many of the salmonids

examined in this study. Described in the “Other tissues of interest” section, above, the presence and parameters of breeding teeth, adipose tissue, epithelium, and blood vessels and sinuses imply a great deal as to the life history and growth of salmonids.

### *Teeth*

Although “normal,” feeding teeth were found in the majority of specimens examined, breeding teeth were found only in pre-spawning and spawning *Oncorhynchus*. These fang-like breeding teeth are substantially larger than feeding teeth even within the same specimen and do not resemble the anterior teeth of *Salmo*.

These anterior teeth in *Salmo* were once believed to be homologous to the breeding teeth of *Oncorhynchus* (Tchernavin, 1938). However, the anterior teeth of *Salmo* have since been shown to be feeding teeth present throughout an individual’s life that become more exposed during the spawn-run due to changes in the soft tissue of the lower jaw (Witten and Hall, 2005). *Oncorhynchus* breeding teeth, on the other hand, are found only in spawning individuals and are absent in juveniles and non-spawners (personal observations ZP; Tchernavin, 1938).

Rather than being directly connected to the jaw as in feeding teeth, the shaft of an *Oncorhynchus* breeding tooth rests on a spongiose base. One breeding tooth base is fused to the tooth base of an adjacent breeding tooth until, eventually, these teeth bases are collectively supported by a slender extension from the dentary. Tooth bases are part of the tooth and one cannot be separated from the other without the risk of damaging both (Tchernavin, 1938). The strip of bone upon which the tooth base lies extends from the dentary as part of the spawning-related lower jaw extension (Morton, 1965; Tchernavin, 1938).

The minimal support conferred by this thin strip of bone raises the question of whether these teeth are actually functional in a physical capacity. In addition to the weak connection of these teeth to the dentary, Tchernavin (1938) argued that the kype was too much in the way for breeding teeth to be able to serve much of a purpose. Furthermore, Briggs (1953), when discussing the reproductive behaviour of *Oncorhynchus kisutch*, noted that the physical conflict rarely occurred between males, with threat displays and aggressive behaviour determining the victor of two rivals. This description of hierarchy

determination is in agreement with a number of other researchers whose work was discussed in the introduction of Chapter 2, each of whom proposes that, while the kype may be used to maintain hierarchy and may play a role in mate selection, it is not used as a weapon (Behnke, 2002; Fleming, 1998; Fleming and Gross, 1989, 1994). As such, the use of the kype, and the breeding teeth, of *Oncorhynchus* in sexual selection would appear to be limited to reproductive displays and not to physical altercations.

To truly assess if individuals would be physically able to make use of their breeding teeth, force-tension, hydrodynamic, and other functional analyses would be required. However, the issue of the functionality of breeding teeth may be irrelevant. This irrelevance is partly due to the lack of evidence supporting accounts of physical altercations where individuals used their kype and breeding teeth as a weapon, but is also due to the demands of the spawn-run. By the time that *Oncorhynchus* individuals have begun to develop breeding teeth or a kype, they have already entered a period of starvation and senescence. Breeding teeth, therefore, appear to function as a visual cue in establishment and maintenance of hierarchies and in mate selection, but not as weapons in physical competition or as tools in prey capture. However, this hypothesis of breeding teeth being used in hierarchy establishment requires further testing to confirm.

### *Fat*

While only individuals within *Oncorhynchus* displayed evidence of large, spongy based breeding teeth, virtually all specimens had small and large accumulations of adipose tissue in their bone and muscle. As noted in the “Other tissues of interest” section, these fat accumulations were virtually non-existent in immature fish such as *Brachymystax lenok* (Fig. 3.16a). In pre-spawning individuals on the other hand, particularly in species that make long spawning migrations such as *O. kisutch* (Fig. 3.13), these fat accumulations can be of significant size. The lack of fat accumulations in immature individuals is likely because juveniles preferentially allocate their available resources to growth and to achieving maturity. These still-maturing fish simply do not have extra resources that can be stored as fat.

Contrary to the dearth of large areas of adipose tissue in juvenile and immature fish, the large fat accumulations found in prespawning fish is partly due to the allocation

and storage of energy reserves. Several researchers have found that pre-spawning salmonids, particularly eusalmonines, may keep large amounts of lipids and fats in their muscles and bone (Jobling et al., 1998; Jørgensen et al., 1997; Phleger et al., 1989; Phleger et al., 1995). In pre-spawning Pacific salmon nearing entry of freshwater systems, the neurocranium is composed of 0.5-6.3% lipid, while the vertebral centra may be composed of as much as 2.9-13% (Phleger et al., 1989). In contrast, post-spawning, river-caught salmon contain only 0-1.4% lipid in their neurocrania and 0-1.6% in their centra.

As spawning salmonids, once in their migration phase, enter a period of starvation and senescence (Groot and Margolis, 1991; Jørgensen et al., 1997; Vladykov, 1953), the rapid use of these skeletal lipids during the spawn-run is hardly surprising. Particularly in anadromous fish, migration may occur over a span of several weeks. Over that time, the only source of energy that these fish have lies in the resources that they were able to accumulate, and store, during their feeding phase. In *Oncorhynchus kisutch*, one specimen of which displayed a dentary that was, in some areas, almost completely in-filled by adipose (Fig. 3.13), migrating populations have been known to travel distances of 240km (Godfrey, 1965) to 2,200km (Bryan, 1973) upstream to spawn in higher altitude tributaries (Sandercock, 1991). Such an extensive journey would require large energy stores.

The distances travelled by *O. kisutch* are in contrast to those travelled by more basal salmonids such as *Brachymystax lenok* as well as basal eusalmonines like *Hucho hucho* during their spawning migrations. Many *Brachymystax* populations have minimal migrations (Kifa, 1974; Mitrofanov and Petr, 1999). In particular, those populations residing in lakes will migrate from moderately deep water at lake centres to shallower waters near the shore. The migrations of *H. hucho* are also very short, in many populations being only a few tens of metres or kilometers from one river bank to the other or into an adjacent tributary (Holčík et al., 1988; Witkowski, 1988). While pre-spawning specimens of *Hucho* and *Brachymystax* were not available for assessment in this study, it would be very interesting to see how the fat content in these minimal migrators differed from species with extensive migrations, such as the species of *Oncorhynchus*. Presumably, the longer the migration and the more strenuous the migratory route, the



more resources an individual needs to store in order to survive and the larger the fat accumulations found in their muscles and bones will be.

A further explanation for the large volume of adipose in the tissues of derived eusalmonines, as will be discussed below in contrasting anadromous and non-anadromous specimens and as was introduced in Chapter 2, lies in resource availability. Anadromous species have access to the more productive, resource rich temperate ocean habitat (Gross et al., 1988; McDowall, 1997; McDowall, 2001; Willson, 1997). This availability of resources ensures that these anadromous individuals are able to accumulate more energy stores than is the case in the often more basal, non-anadromous salmonids. These fat accumulations will be discussed further in Chapter 4.

### *Epithelium*

Another contrast between basal and more derived salmonids lies in the thickness of the epithelium in these specimens (Fig. 3.15). As was first seen in the Micro-CT scans of Chapter 2, basal salmonids often have thick, well-organized epithelium. Contrarily, the epithelium of the derived eusalmonines (*Oncorhynchus*, *Salmo*, and *Salvelinus*) is much thinner. This contrast in epithelium thickness reflects a change from the ancestral form of epithelium and may be the result of different reproductive behaviour. As noted above, kype-developing individuals are able to establish hierarchy and dominance using non-violent displays (Behnke, 2002; Briggs, 1953; Fleming, 1998; Fleming and Gross, 1989, 1994). The use of secondarily modified craniofacial skeleton features and fin length in those displays ensures that violence and physical altercations are kept to a minimum. In many basal salmonids, however, spawning-related secondary modification of the body, including those to colouration or fin size or length, is very minimal (Willson, 1997). Indeed, basal salmonid sexual dimorphism may be virtually nonexistent.

In *Coregonus* and *Proposopium*, moderate differences in male and female body size (Carlander, 1969; McPhail and Lindsey, 1970) and the growth of breeding tubercles (Ihssen et al., 1981; Norden, 1970; Scott and Crossman, 1973) are the only distinguishing features between spawning males and females. On the other hand, in *Thymallus arcticus*, males have more vibrant colouration than females and may have larger and longer pelvic and dorsal fins, particularly at the time of spawning (Carlander, 1969; McPhail and

Lindsey, 1970). However, in *Brachymystax*, changes in colouration are unreliable in distinguishing males from females and reproductive behaviour (i.e. nest-digging) is the only sure method of properly identifying sex (Esteve and McLellan, 2007, 2008). In fish where reproductive displays are minimal, physical altercations may be the only means of establishing dominance within a spawning cohort. A thick epithelium in these basal salmonids may ensure that damage to tissues resulting from injury is kept to a minimum.

This hypothesis of thicker epithelium in basal salmonids is supported by evidence in *Hucho* that would suggest that the epithelium of spawning males becomes thicker and rougher than in non-spawning individuals (Holčík et al., 1988). The epithelium of females, who are less prone to violent altercations than males but who may experience more abrasive damage to epithelium during nest digging, remains relatively thin but is thicker than in non-spawning individuals. While the thicker epithelium of males minimizes the seriousness of combat injury, the thinner epithelium of females is protected by an increase in the number of superficial goblet cells on its surface. These goblet cells are filled with a mucous that defends the thinner skin of females from abrasive injury during nest-digging as well as from subsequent infection. The roughness of the skin in males ensures better contact with the slippery skin of females during spawning.

Similarly to *Hucho*, thickening epithelium and an increase in epithelial goblet cells are traits found in many brood-hiding and nest-digging salmonids (Pickering, 1977). These traits have been previously identified in *Salmo trutta* (Stoklosowa, 1966, 1970) and in many *Salvelinus* species (Pickering, 1977). While the thickness of the epithelium in these more derived salmonids does not approach that of more basal genera, it, and a concurrent increase in mucous-filled cells, may serve the same protective function as in basal salmonids. These modifications to the epithelium would protect the tissues of a spawning eusalmonine during rare bouts of physical conflict, from injury during nest building, and from crowding or abrasion against rocks during migration. However, because the epithelium of basal salmonids is very thick even when not in spawn, factors in addition to that of spawning-related altercations are at work. Further study is needed to assess the conditions of basal and more derived salmonid epithelium and to establish patterns in the changes in organization and thickness of that epithelium over the course of an individual's life.

### *Blood vessels and vascular sinuses*

The final tissue of interest examined in this study is that of blood vessels and sinuses (Fig. 3.17). While many blood vessels were present in the tissues of the lower jaw of both basal and more derived salmonids, they were particularly prevalent in the tissues of spawning and pre-spawning individuals. As such, rather than exhibit a phylogenetic trend, as is the case with epithelium, these blood vessel networks appear to be related to life history stage. In near spawning and spawning individuals, extensive blood vessel networks were joined by several small and large vascular sinuses (Fig. 3.17 b-d), further increasing the blood supply of the bone, connective tissue, and epithelium of the lower jaw. The extent of this blood supply network ensures that the many changes in the skeleton, epithelium, fat content, dentition, and other tissues relating to spawning in these animals do not fail for lack of resources. Additionally, any injuries sustained during this physically demanding and dangerous period in a fish's life history, many of which were discussed above, would require many resources in order to heal. The many blood vessels and sinuses of this network allow salmonids, regardless of their phylogenetic position, to alter and maintain their spawning-related modifications, including that of the kype and to heal spawning- and migration-related injuries. Blood vessels will be discussed further in Chapter 4.

### ***Comparison of tissues and structures of interest: Life history stage and mode***

As is the case with the kype of different genera, there is variation in the tissues of the lower jaw and of the kype in animals at different stages in their life history or in animals that employ different life history modes. Thus, tissues vary depending on the age and size of individuals considered, as well as between anadromous and non-anadromous or iteroparous and semelparous individuals, even within the same species. For instance, in comparing larger, more mature individuals such as *Salvelinus alpinus* (CMNFI-1968-1262.1) to smaller, assumedly less mature individuals such as *S. alpinus* (UW 41192), CMNFI-1968-1262 has a higher number of large and small blood vessels as well as a vascular sinus (Fig. 3.17b). Additionally, more mature individuals have a higher fat content in bone and muscle (Fig. 3.16 c, d), as well as, as is shown in *Oncorhynchus mykiss aguabonita* versus *O. mykiss*, a thicker epithelium (Fig. 3.15 c and d). Particularly

when comparing spawning and non-spawning individuals, spawners also tend to have a higher number of pigment cells in their thickened epithelium and in adjacent connective tissue (Fig. 3.15 e and f).

The contrast in blood vessel networks, fat content, epithelium thickness, and pigment cell presence between larger, more mature and smaller, less mature fish is likely due to priorities in resource allotment and physical requirements of an individual's life history stage. Immature, smaller fish allocate their resources to growth and to achieving sexual maturity (Aksnes et al., 1986; Armstrong, 1965; Behnke, 1992; Hagen, 1970; Jørgensen et al., 1997). Contrarily, more mature individuals, preparing for migration and spawning, allocate their resources to favour their forthcoming reproductive behaviour and exertions. The large volume of adipose tissue in the fat accumulations in and around the dentary in spawning individuals offers a ready source of resources (Phleger et al., 1989; Phleger et al., 1995).

While a thicker epithelium in spawning individuals as opposed to non-spawning ones is in contrast with some previous work in brown trout (*Salmo trutta*) (Stoklosowa, 1966, 1970), this earlier work did not consider the epithelium of the jaw but instead of the trunk of the body. A thicker epithelium will help cushion the lower jaw against injury resulting from physical competition with other individuals and from overcrowding and abrasion against rocks and substrates during migration. The extensive blood vessel network in spawners will ensure that resources will quickly reach target tissues, providing a steady supply of energy and ensuring that any injuries that are sustained are able to heal quickly.

Many of the trends found between animals of different life history stages are also evident when comparing resident or non-anadromous individuals of the same species to anadromous ones. Anadromous individuals often have more blood vessels, higher fat content (particularly when comparing pre-spawning specimens), and thicker epithelium than non-anadromous individuals. These trends are most noticeable when contrasting animals with long migrations such as anadromous *S. alpinus* (Fig. 3.5a) or *O. gorbuscha* (Fig. 3.10a) to those of the same genus with little or no migration within their freshwater system, such as *S. namaycush* (Fig. 3.6) and hatchery raised *O. m. aguabonita* (Fig. 3.11a). Furthermore, the kype is much larger and better developed in anadromous

animals. The skeleton of the kype in *O. gorbuscha* as compared to *O. m. aguabonita*, for instance, displays a greater array of longer, more robust skeletal needles as well as being marked by higher number of actively secreting and dividing chondrocytes.

While several explanations for the contrast between the tissues of interest in anadromous and non-anadromous individuals exist, it is probably the result of a combination of factors. First, fish experiencing long migration also experience higher energy demand and greater chance of injury. The increased energy demand and longer period of starvation that are coupled with a more extensive, lengthier migration would require a greater store of energy in the form of fat deposits. A more extensive network of blood vessels would ensure that tissues receive a steady supply of these resources. Furthermore, this more extensive blood vessel network would also provide an individual with the necessary resources to heal from an injury quickly. The thicker epithelium found in anadromous fish may help prevent against injury resulting from crowding or attacks from other fish during migration or from grazing or collision with rocks or the substrate of the stream within which fish are swimming. However, in the event of injury, a ready blood supply would ensure that an injury would be quickly healed.

Another explanation for the contrast between anadromous and non-anadromous fish is the higher availability of resources in animals with an ocean phase. As discussed in Chapter 2 and briefly above, resources in the temperate ocean setting are much more abundant than in freshwater systems (Gross et al., 1988; McDowall, 1997, 2001; Willson, 1997). In addition to allowing animals to grow more quickly and achieve greater sizes than their freshwater living relatives, this resource availability also provides anadromous animals with resources to build, and sustain thicker epithelium, greater fat storage, and more extensive kype skeletons.

As with comparison of animals of different life history stages and migration modes, a contrast between semelparous and iteroparous individuals also reveals a number of differences in the tissues of the lower jaw, specifically of the kype. In particular, semelparous individuals display much more extensive skeletal modification than their iteroparous counterparts. Semelparous *O. gorbuscha* (Fig. 3.10a) and *O. kisutch* (Fig. 3.13 and 3.17) as well as iteroparous *S. salar* (from the work of Witten and Hall, 2002, 2003), which has very low post-spawn survivorship, display a wider array of skeletal

needles and modification of the compact dentary than do iteroparous *O. m. aguabonita* (Fig. 3.11a), *Salmo trutta* (Fig. 3.8a), and *S. alpinus* (Fig. 3.5a).

While some of these differences are tempered by the non-anadromous habit of *O. m. aguabonita* or genus-specific traits that limit comparison between genera, the tissues of semelparous individuals nonetheless reinforces the trend noted upon physical examination. The kype of semelparous and near-semelparous individuals tend to be much more extensive and dramatic than in iteroparous specimens. As discussed in more detail in Chapter 2, because semelparous species do not have to keep resources in reserve for survival following spawning, they are able to allocate more resources to growing a more extensive and dramatic kype. In *S. salar*, the cost of so large and complex a kype may be one of a number of confounding issues that limits post-spawn survivorship.

The large and extensive kype in *S. salar* may also be due to repeat spawning. While this was a concept that I was not able to explore in the current study, the maintenance of some aspects of the kype following the spawn-run has long been identified in iteroparous species (Morton, 1965; Tchernavin, 1938; Vladykov, 1953; Witten and Hall, 2003). This maintenance of some portion of a previous kype allows repeat spawners to grow subsequently larger and more dramatic kype. In Atlantic salmon, the skeletal alteration to the lower jaw in repeat spawning males is so extensive that, even with the development of a compensatory hollow in the tissue of the upper jaw, animals are not able to fully close their mouths (Witten and Hall, 2002). A study that compared the structure and composition of the kype in known repeat spawners versus those of semelparous, one-time spawners may be able further distinguish trends in kype growth and complexity.

There is variation in the tissues and structures of the lower jaw and of the kype depending on life history stage and mode. Generally speaking, more mature, migratory, individuals tend to have thicker epithelium, more blood vessels and sinuses, and higher fat content than less mature and resident animals. Furthermore, semelparous eusalmonines displayed more extensive remodelling of the lower jaw than iteroparous ones, yielding a higher number of breeding teeth and a greater extent of skeletal needles (Fig. 3.9 a versus Fig 3.11 a).

These trends reinforce those found in Chapter 2 regarding the overall structure and extent of growth of the kype. In both the current chapter and in Chapter 2, migratory (anadromous), semelparous individuals tend to grow larger and more extensive kype than their non-migratory or iteroparous kin. However, as I was not able to access known repeat spawners for this study, I was not able to consider how maintenance of elements of the kype between spawning periods in iteroparous individuals affects the size of the kype and the complexity of its tissues. As such, these patterns only consider first time spawners. Further study of the conditions of the kype in repeat spawners is needed in order to assess how the maintenance of portions of the kype between spawning events affects its structure and composition.

### ***Testing patterns in the modifications of tissues of the salmonid lower jaw***

There are several trends prevalent when comparing the composition and organization of the lower jaw across the salmonid tree and between individuals of different life history modes and of different ages. While a kype is only found in spawning members *Salvelinus*, *Salmo*, and *Oncorhynchus*, the “kype-related” tissues (Sharpey-fibre bone and chondroid bone) are found in all eusalmonines. Furthermore, the kype is largest and most complex in anadromous, semelparous or near-semelparous species and individuals. There is some variation within the kype-bearing eusalmonines, with the kype skeletons of *Salmo* and *Salvelinus* being dominated by skeletal needles and that of *Oncorhynchus* being dominated by enormous breeding teeth and their associated bases. Breeding teeth are not found in any other salmonid lineage.

Patterns in phylogenetic position extend to other tissues of interest in the lower jaw. While fat accumulations are larger in eusalmonines, epithelium is much thicker in basal salmonids and in *Hucho*. However, large fat accumulations in eusalmonine specimens may be the result of the more resource rich anadromous lifestyle rather than being a phylogenetic attribute. There does not appear to be a phylogenetic trend in blood vessel networks, and vascular sinuses are found in both eusalmonines and basal salmonids. To test for phylogenetic trends in these salmonid tissues, comparisons with other bony fish such as members of the Esociformes is required.

Figure 3.18 displays two possible salmonid phylogenetic trees that have had the characteristics of the salmonid tissues of interest in the lower jaw placed in the appropriate locations. By positioning the origins of these tissues of interest on these trees, it is possible to assess patterns in tissues of interest and to test possible salmonid phylogenetic trees. As shown in Figure 3.18, patterns in the tissues of interest, including the kype, fit well to an established salmonid phylogenetic tree. However, it is also obvious that the more parsimonious tree, at least in regards to the characters assessed in this study, is one that separates *Hucho* from the other eusalmonines. This conclusion further supports the hypothesis expressed in Chapter 2 that a relocation of *Hucho* may be required. However, a parsimony analysis that combines assessment of kype- and non-kype-related parameters is required to confirm this.



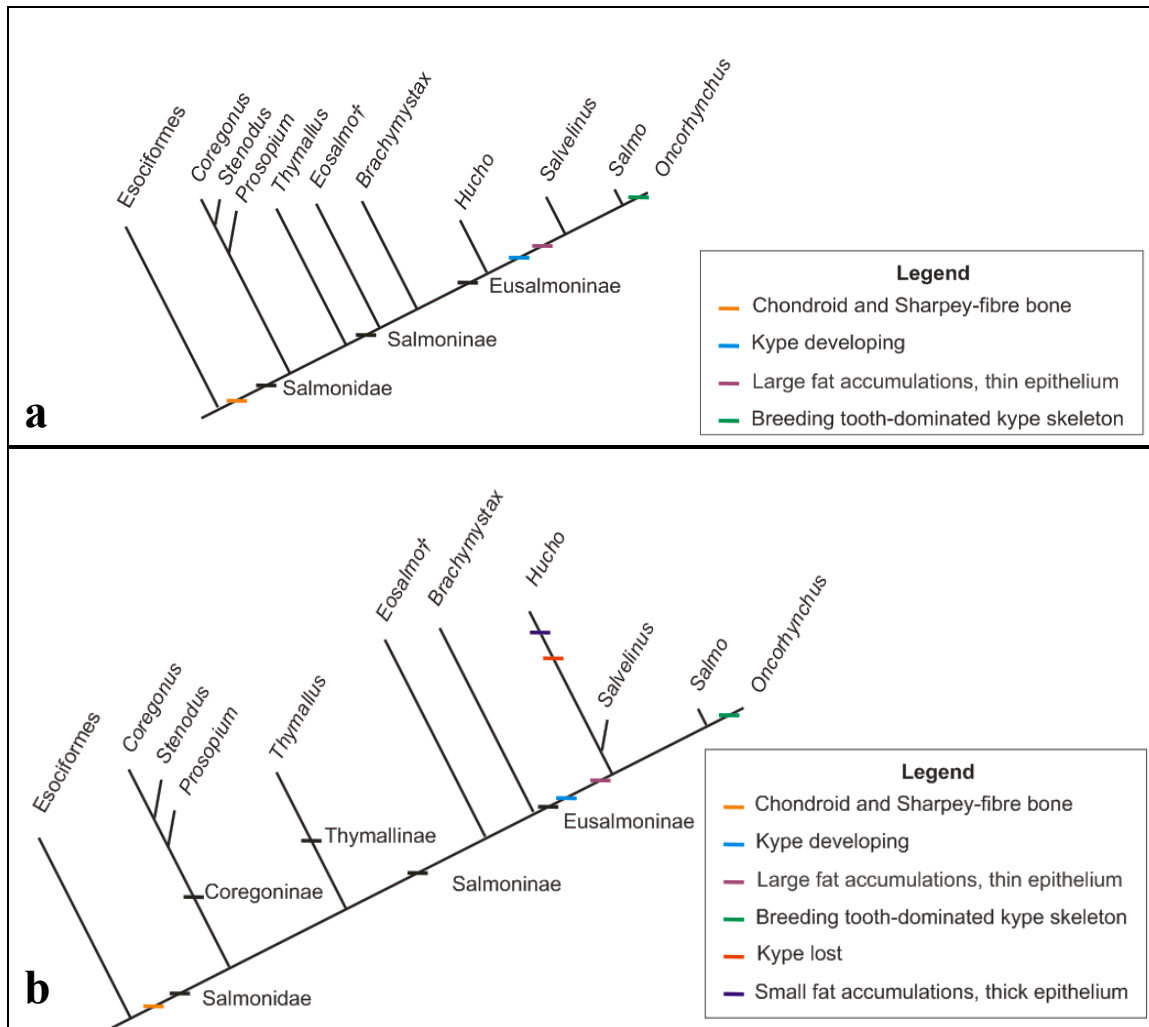


Figure 3.18 – Summary phylogenetic tree showing trends in tissues of the lower jaw. These possible trees place *Hucho* (a) with (modified from Stearley and Smith, 1993) and (b) outside of (modified from Oleinik and Skurikhina, 2008) the other eusalmonines. On each tree, chondroid bone and Sharpey-fibre bone is found in combination in all salmonids to some extent. Kype is only found in Eusalmonines, but is absent in *Hucho*. While *Salvelinus* and *Salmo* kype skeletons are dominated by skeletal needles, that of *Oncorhynchus* is dominated by large breeding teeth and their associated bases. Breeding teeth are not found in any other salmonid lineage. Large fat accumulations are found in eusalmonines in particular, though this may be result of a more resource rich life history mode than of a phylogenetic cue. Thick epithelium is found in basal salmonids and in *Hucho*. The addition of other tissues of interest to these trees support the hypothesis expressed in Chapter 2 (See Fig. 2.58) that a tree that separates *Hucho* from *Salvelinus*, *Salmo*, and *Oncorhynchus* is more parsimonious.

It is also important to note however, that life history stage and life history mode or environment are also important factors when considering patterns in the growth and modification of the kype and of other tissues of interest in the lower jaw. Epithelium was thicker, fat accumulations were larger, and vascular networks were more extensive in spawning and anadromous individuals, even within the same genus or species. As a result, the trends in tissue growth and modification based on phylogenetic position noted

above may in fact be complicated by other cues. In order to fully understand the trends and patterns that can be identified in the growth and modifications of the tissues and structures of interest in the salmonid lower jaw, including the kype, one must take into account phylogenetic position, life history stage, and life history mode.

### ***Sources of error***

Several of the sources of error listed in Chapter 2 hold true in this chapter as well. Small sample size and lack of control over life history stages, in particular, negatively impact this study. To truly assess many of the concepts and trends addressed in this chapter, a larger sample size of known life history stage, including that of repeat spawning, would be required. For instance, the combination of small sample size and approximations of life history stage in this study result in comparisons between different groups of kype-bearers that may be skewed. I may be comparing the kype of an individual that had few resources that they were able to allocate for kype growth, resulting in a small kype with few modifications to the lower jaw, to an animal with many available resources allocated to growing a much more substantial kype. Additionally, due to incomplete specimen data, it was not possible to determine the sex of some specimens. As females tend to develop much smaller, less complex kypes than males of the same size (Behnke, 2002; Scott and Crossman, 1973; Tchernavin, 1938; Vladykov, 1953), some of the differences in kype structure and extent between two specimens may be due to sex.

Another possible source of error lies in my lack of control over the fixation and preservation of museum specimens. The use museum specimens permits a phylogenetic analysis of a wide range of specimens and species, including several from locations around the world that I would not otherwise have access to. However, improper fixation and preservation of specimens may lead to loss or degradation of tissues resulting from incomplete or inadequate fixation and preservation thereby resulting in loss of important data.

While these sources of error are not insubstantial, throughout this study I attempted to draw comparisons within specific, appropriate groups. For instance, I assessed conditions in pre-spawning and spawning, anadromous *Oncorhynchus* individuals as separate from non-spawning, anadromous *Oncorhynchus* individuals. By

focussing my attentions as specifically as possible, I believe I have mitigated the negative effects of my small sample size, the wide-ranging life history stages of my specimens, and my lack of some specimen information as much as possible.

While not a source of error in this study, I only investigated the tissues of the lower jaw and did not examine the tissues of the upper jaw. Not having considered the histology of the tissues of the upper jaw, as well, I am not able to assess some of the trends in genus-specific kype morphology noted in Chapter 2. Additional work that took into these tissues into account would result in a better understanding of how the kype of *Salmo*, *Salvelinus*, and *Oncorhynchus* differ from one another. Furthermore, unknown spawning-related modification in the upper jaws of other salmonids would be revealed.

### ***Summary***

- The kype is a secondarily developing structure that is found only in members of Eusalmoninae. However, tissues previously believed to be associated with the kype are found even when the kype is not. The presence of kype-associated tissues in non-kype-bearing salmonids suggests that the kype evolved when pre-existing structures and tissues already present in the lower jaw were co-opted to form the kype.
- The kype may be very subtle, and evidence of a kype may not be apparent except in histological examination
- Many other tissues relating to the growth of a kype, as well as tissues that are important to the survival and behaviour of a spawning salmonid, are apparent in histological section. These tissues included breeding teeth, fat in muscle and bone, epithelium thickness, and the presence of blood vessels and sinuses. Further work is required to assess the implications of these tissues and to assess how these tissues change prior to and during the spawning season.
- While the same tissues and structures are present in the kype of each kype-bearing genus, dimensions and number of these elements vary. The dominant character of the kype of *Oncorhynchus* is breeding teeth. The kype in *Salmo* and *Salvelinus* is dominated by a large number of slender skeletal needles.

- The tissues of the lower jaw and of the kype vary in animals of different life history mode and stage. More mature, migratory, individuals have thicker epithelium, more blood vessels and sinuses, and higher fat content than less mature and resident animals. Semelparous animals experience more extensive remodelling of the lower jaw than iteroparous animals. Migratory (anadromous), semelparous individuals tend to grow larger and more extensive kype than non-migratory or first-time spawning, iteroparous individuals.
- Trends in the growth and modification of tissues of interest in the lower jaw, including those associated with the kype, fit easily to a salmonid phylogenetic tree. However, a tree that separates *Hucho* from the other eusalmonine genera requires steps and is a better fit to the new data than one that keeps *Hucho* within the eusalmonines. This conclusion supports the hypothesis expressed in Chapter 2 that a relocation and possible division of *Hucho* is required.

The overall structure of the lower jaw and the kype of modern salmonids were considered in Chapter 2. In the present chapter, the composition of those structures was determined using histological analysis. In these first two chapters, many trends and patterns relating to phylogenetic position and lifestyle were established using assessment of these tissues and structures. However, more information is needed before drawing conclusions as to the evolution of these elements. Chapter 4 will determine if evidence of kype presence and growth survives in the fossil record and if it is possible to use this fossil evidence to assess transitions in the evolution of the structure and composition of the kype and of the other tissues of interest in the salmonid lower jaw.

## Chapter 4 – Presence and absence of the kype in fossil salmonids

### Introduction

As shown in Chapters 2 and 3, studying the structure and composition of the kype and of the dentary in living specimens reveals a great deal as to patterns and conditions of these elements in living salmonids. It is possible to infer certain aspects of kype evolution by tracking these conditions in modern tissues across the salmonid genera, provided a robust phylogeny is available. However, additional evidence is needed to support these inferences and to establish trends in the evolution of the kype in the salmonid phylogenetic tree. This evidence can be found in the fossil record.

Several researchers have noted the ways by which research using fossil data can supplement studies of Recent organisms (Albert et al., 2009; Cloutier, 2010; Cobbett et al., 2007; Grande, 1985; Hilton and Grande, 2008). Among these are the landmark works by Wilson (1992) and Wilson and Williams (1992), which considered freshwater fossils, including *Eosalmo*, the first known true salmonine, in particular. Both Wilson (1992) and Wilson and Williams (1992) also note several ways in which the use of fossil evidence to supplement Recent data can contribute positively to phylogenetic study. For instance, fossils usually represent new taxa and may be helpful in testing the assumptions of phylogenetic trees built using Recent specimens. Furthermore, fossils often demonstrate transitional states that cannot be determined from study of Recent organisms. While recent organisms represent the “after” snapshots following a sequence of the evolution of divergent traits, fossils may represent steps along that sequence. To take this concept of fossils as representatives of transitional states one step further, fossils can be a critical aid in determining the order in which derived traits evolved. Thus fossils greatly improve our understanding of characters and traits found in living organisms.

Making use of fossil evidence, the data in this chapter serve two purposes. First, I want to demonstrate how the physical examination and Micro-CT assessment methods used to assess living specimens in Chapter 2 might be modified and used in paleontological analysis, and secondly, I will use these methods to answer questions as to the evolutionary history of the kype.

While the overall structural- and tissue-level trends from Chapters 2 and 3 have

already elucidated a great deal as to conditions in living salmonids, many questions remain as to the arguably less derived conditions of ancient salmonids. Does a record of kype growth and of other tissues in the lower jaw survive in fossil representatives and relatives of living species? Is it possible to use this record to discern a transition from the basal conditions of the Coregoninae and Thymallinae to the more derived traits in Salmoninae? From basal to more derived conditions within the same genus or species?

This chapter will consider the conditions found in fossilized salmonids using physical examinations, Micro-CT assessments, and literature survey. In this chapter, I compare the structural conditions in fossil specimens with those found in the living salmonids described in Chapters 2 and 3.

### **Methods and Materials**

As with Chapter 2, several methods were used to gather information from the available specimens and material. These methods include physical examination, Micro-CT assessment, and literature survey.

#### ***Physical examination of specimens***

Similarly to Chapter 2, several specimens representing different salmonid genera were gathered from the collections and archives of museums in Canada and the United States. However, while the specimens of Chapter 2 represented living material, the specimens described in this chapter are all paleontological. Information as to specimen loaning institutes, relative age of specimens, collection site, and other relevant information can be found in Appendix D. A list of the number of specimens examined for each genus and species is recorded in Appendix E. Wherever possible, maturity and life history stage were estimated based on snout to vent length (SL) and, in some cases, depositional environment.

Physical examinations of dry fossils were conducted in much the same way as was done for the defleshed living specimens of Chapter 2. Dentaries were examined for modifications in bony tissue that resulted in a honeycomb meshwork or profusion of needle-like elements from compact bone. Evidence of spawning-related changes in terms of the large, fang-like teeth seen in the modern spawning *Oncorhynchus* from Chapter 2

were also taken into consideration.

### ***Micro-computer tomography (Micro-CT) Scanning***

As with the modern specimens of Chapter 2, fossils were scanned with a Lab PET 4 machine and a Triumph X-O CT system using the methodology outlined previously. The only alteration to the method outlined in Chapter 2 is the addition of a wrapping of protective gauze padding around the fossils before they were placed on the specimen bed. Scanning information of specific specimens (including species, specimen ID code, and magnification and resolution of scans) are presented in Table 4.1. For large specimens, such as the *Oncorhynchus rastrosus* material, only the anteriormost portion of the specimen was scanned.

**Table 4.1 - Micro-CT scanning information for fossil specimens. Voltage is in units of peak kilovoltage (kVp), resolution is in micrometres (µm).**

<b><i>Genus species</i></b>	<b>Specimen ID</b>	<b>Voltage (kVp)</b>	<b>Magnification</b>	<b>Resolution (µm)</b>
<i>Stenodus</i>				
- <i>S. leucichthys</i>	CMNFV-48886	80	3.5	66
<i>Salvelinus</i>				
- sp.	UOF-26785	80	3.25	71
- sp.	UOF-26786	80	2.75	84
- sp.	UOF-26787	80	2.75	84
<i>Oncorhynchus</i>				
- sp.	UOF-26773	80	3.0	77
- sp.	UOF-26782	80	2.26	102
- sp.	UOF-31389	80	4.0	58
- <i>O. nerka</i>	UWBM-87121	80	3.0	77
- <i>O. (Smilodonichthyes) rastrosus</i>	UWBM-50816	80	1.7	136
- <i>O. (Smilodonichthyes) rastrosus</i>	UWBM-71908a	80	2.3	100
- <i>O. (Smilodonichthyes) rastrosus</i>	UWBM-71908b	80	2.3	100

Following scanning, two-dimensional slices and three-dimensional renderings were assessed for patterns of density and mineralization. Fossil material was compared with defleshed modern material in an effort to assess the validity of the scanning process. Comparison between fossil and defleshed living specimens also permits better identification of features and elements found in the fossil material that are the result of taphonomic and diagenetic alterations following deposition as opposed to features present

in the animal during its life. Such diagenetic alterations include squashing, stretching, and torsion of fossils.

### ***Literature review***

Following physical examination of specimens and Micro-CT assessment of material, the literature survey conducted in Chapter 2 was considered and was supplemented. These supplements yield a survey of literature relevant to the species assessed in this chapter that are known only from the fossil record.

## **Results and Discussion**

### ***Physical examinations and Micro-CT assessments***

In an effort to efficiently assess the craniofacial morphology of each specimen examined in this study, physical examinations and, where available for a given specimen, Micro-CT assessments are described together. In regards to physical examinations, in specimens that preserve portions of the anterior skeleton in addition to the dentary, particular attention was paid to the jaw bones, namely the dentaries, maxillae, and premaxillae, though other characteristics are also considered, much as in the physical examinations discussed in Chapter 2. Also similarly to Chapter 2, specimens are described in order of their phylogenetic position (Fig. 4.1). Wherever possible, known life history traits, physical characteristics, and other aspects of fossil species are provided; please refer to Chapter 2 for more information as to extant species. Where interpretations of fossils are shown, the colour code indicating the different skeletal elements remains constant in each interpretation. Furthermore, jaw bones are labelled as follows: dentary (De), maxillary (Mx) and premaxillary (Px).



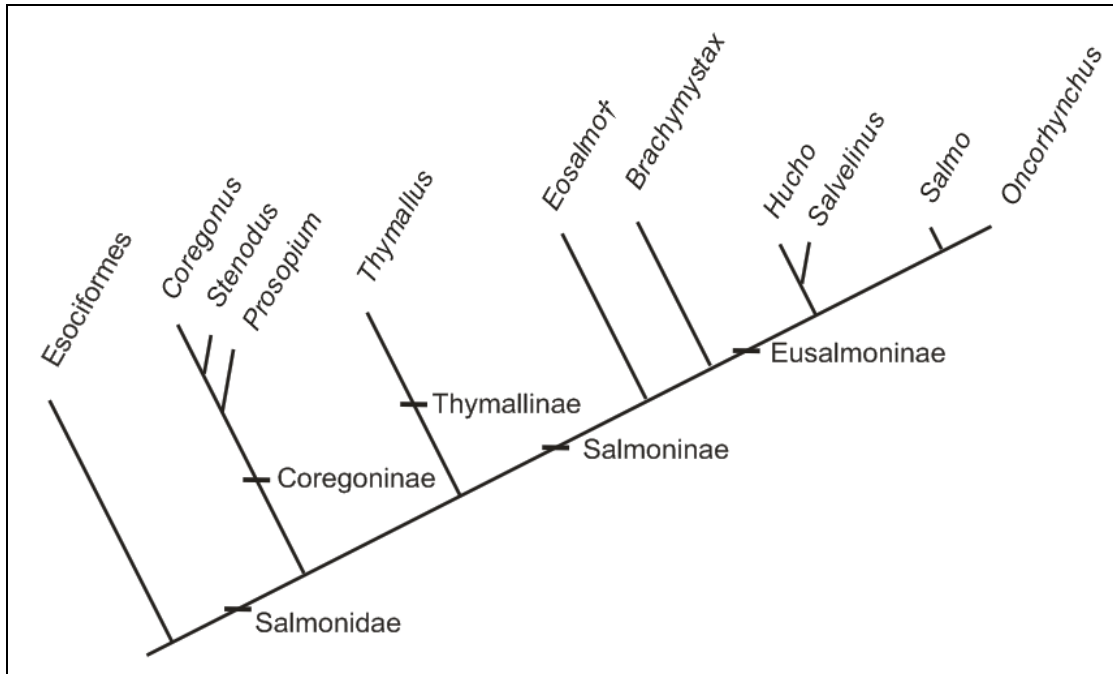


Figure 4.1 – Salmonid phylogenetic tree indicating the three salmonid subfamilies Coregoninae (whitefishes), Thymallinae (grayling), and Salmoninae [false and true (Eusalmoninae) salmon and trout]. Modified from Stearley and Smith (1993).

In terms of Micro-CT assessments, given the small size of the resting bed around which the X-ray gantry of the Lab PET 4 machine used in the current study rotates, only those specimens that had been prepared out of the surrounding sediment were scanned. Using voltages and magnifications outlined in Table 4.1, the same qualification of grayscale values as was used to assess patterns of mineralization in Chapter 2 was used to determine trends and patterns in morphology of the following specimens.

## Coregoninae

### *Stenodus leucichthys* (Quaternary, Old Crow River, Yukon)

CMNFV-48886; physical examination: Figure 4.2, Micro-CT assessment: Figure 4.3

Specimen is fairly robust, though somewhat slender and thin, and is long. Although the posterior portion of the dentary has broken off, the anterior portion is intact and in good condition and the posterior portion displays the sweeping wing first seen in the modern *Stenodus leucichthys* (CMNFI-Z004302) specimen from Chapter 2.



Figure 4.2 - Quaternary *Stenodus leucichthys* CMNFV-48886 (Old Crow River, Yukon), left dentary in (a) medial and (b) lateral view. The black arrows indicate the anterior orientation.

Three-dimension reconstruction of CMNFV-48886 reveals the presence of several openings on the lateral and medial surfaces of the bone. While some of these openings on the lateral surface of this specimen occur in a line and represent the pores of the mandibular lateral line canal (Fig. 4.3 a-c, light blue arrows), others, including those on the marginal surface (Fig. 4.3 a-c, dark blue arrows), lead into channels that form networks within the bone (Fig. 4.3 e-g, green arrows). These channels were likely the locations of blood vessels. In posterior view, the interior structure of the bone is evident, revealing many hollows and open spaces (Fig. 4.3 d-f, orange arrows). This hollow- and channel-rich internal structure is even more apparent when looking at two-dimensional slices of the scan (Fig. 4.3 e-g). These slices reveal that while the posterior portion of the bone is interspersed with large hollows, the anterior portion is very dense and compact. Furthermore, the channels in the bone first seen in three-dimensional view are also apparent in these two-dimensional slices. It is in fact possible to track the channels in the bone through several sagittal sections. These channels have a very complex network within the bone, supporting the supposition that they are the remnants of the foramen and canals that accommodated blood vessels. There are no fibrous or needle-like extensions on the exterior of the bone, nor is there any anterior honeycombing within the bone, that would suggest evidence of a kype in this specimen.

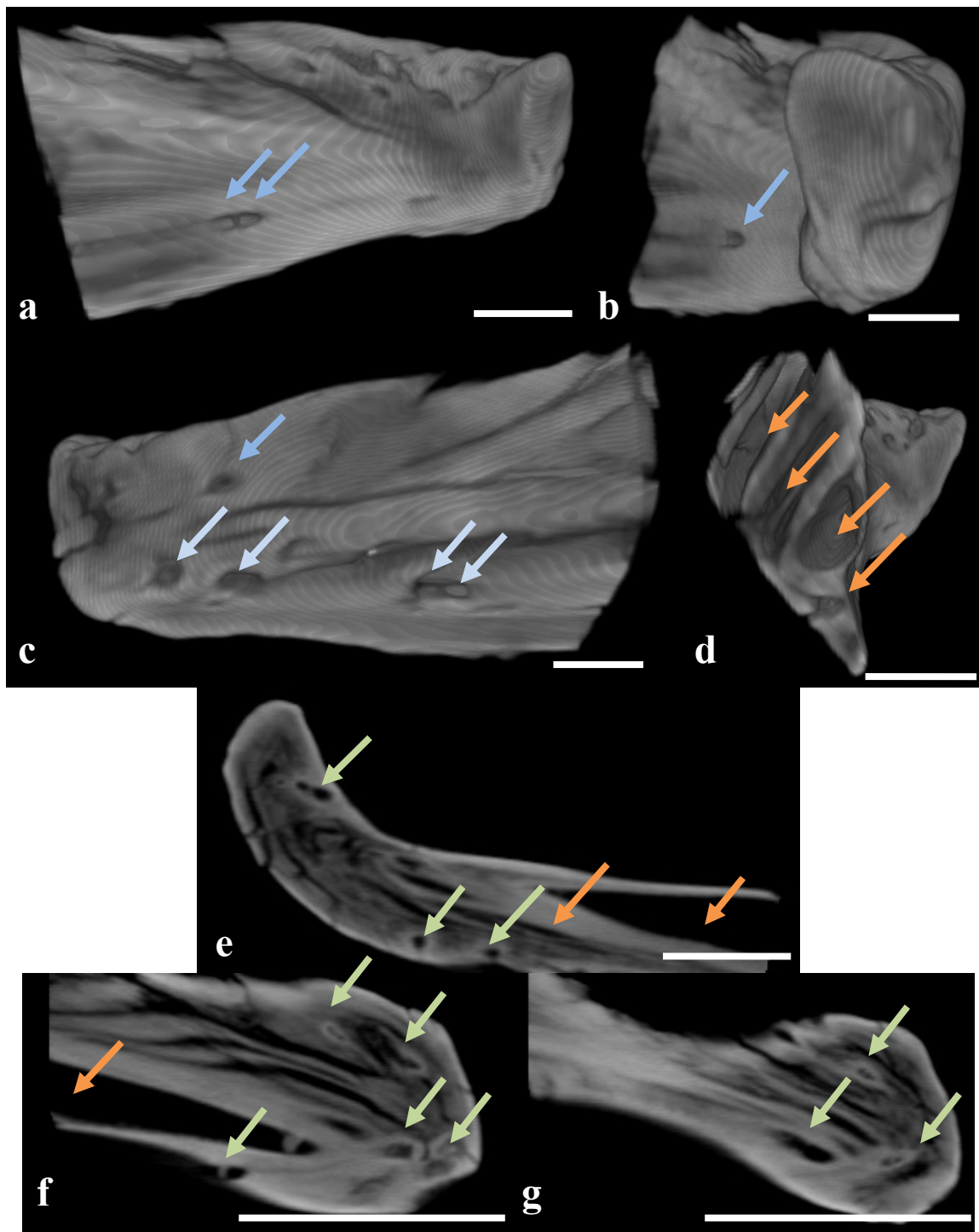


Figure 4.3 –Micro-CT scan of Quaternary *Stenodus leucichthys* CMNFV-48886 (Old Crow River, Yukon), left dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slices with (e) transverse section midway through the dentary and with sagittal section (f) midway and (g) near lateral edge. The openings of several channels into the bone are present on the lateral and medial surface (blue arrows) and can be tracked through the interior of the bone (green arrows). Some of these lateral openings occur in a line and represent the pores of the lateral line canal (light blue). Also, the many hollows found in the posterior of the bone (orange arrows) disappear anteriorly, where the bone become very dense and compact. Scale bars are 0.5 cm.

## **Salmoninae**

### ***Eosalmo***

The Eocene *Eosalmo* genus contains a single recognized species: *E. driftwoodensis*, the “dawn salmon” (Behnke, 2002; Stearley and Smith, 1993). This species is the oldest known and most basal salmonine fish (Behnke, 2002; Nelson, 2006; Wilson, 1977).

Being basal, this species has many of the traits typically found in less derived salmonids, including, based on depositional environment, a freshwater habit (Wilson and Williams, 1992; Stearley, 1989). With a wide range of different-sized organisms from juvenile to mature being found in the same lake, it is believed that this species remained solely in freshwater systems and did not employ an anadromous life history mode (Wilson, 1977; Wilson and Williams, 1992; Stearley, 1989). In light of how frequently specimens are found in fluvial and lacustrine deposits in British Columbia and northern Washington, these animals were likely abundant within their freshwater systems (Behnke, 2002; Wilson, 1992; Wilson and Williams, 1992).

### ***Eosalmo driftwoodensis* (“dawn salmon”)**

#### **Eocene, Driftwood Canyon, British Columbia**

RBCM.EH 2011.006.0001; Figure 4.4

The specimen was taphonomically altered and elements of the skull are difficult to distinguish. Interpretation of discernible elements (Fig. 4.4 b) would suggest that the head is relatively small in comparison to the rest of the body. The dentary (rose) and maxillary (orange) are short and robust, and the mouth is small. There may be the imprint of large teeth at the dorsal dentary surface (white outline), but evidence for this dentition is minimal. Although it is not clear if the anterior aspect of the dentary is still present on this specimen, preserved parts of the dentary do not display any evidence of a kype.



Figure 4.4 – Eocene *Eosalmo driftwoodensis* RBCM.EH 2011.006.0001 (Driftwood Canyon, British Columbia) (a) without and (b) with interpretation of relevant discernible anterior skeletal elements. Colour key is provided. Relatively long, robust dentary (rose) and maxillary (orange) would have resulted in a moderately large mouth, with maxillaries likely extending midway through the orbit of the eye. The imprint of large dentary teeth may be visible (white outline). Jaw bones are labelled dentary (De), maxillary (Mx), and premaxillary (Px).

### **Eocene, Klondike Formation**

UWBM-82489; Figure 4.5

As with RBCM.EH 2011.006.0001, this specimen experienced a great deal of post-depositional disturbance, resulting in post-mortem rearrangement of the skeletal elements. That being said, many of the bones are distinguishable, as can be seen in Figure 4.5 b. Perhaps the most easily diagnosed elements are the tooth-bearing bones: the dentary (Fig. 4.5 b, rose), maxilla (orange), and premaxilla (peach). Each of these bones bears many small, sharp teeth and is also robust. The mouth was likely of a moderate size, with maxillary extending to at least midway through the orbit. The dentary is large and has a smooth surface, without any of features associated with the presence of a kype.

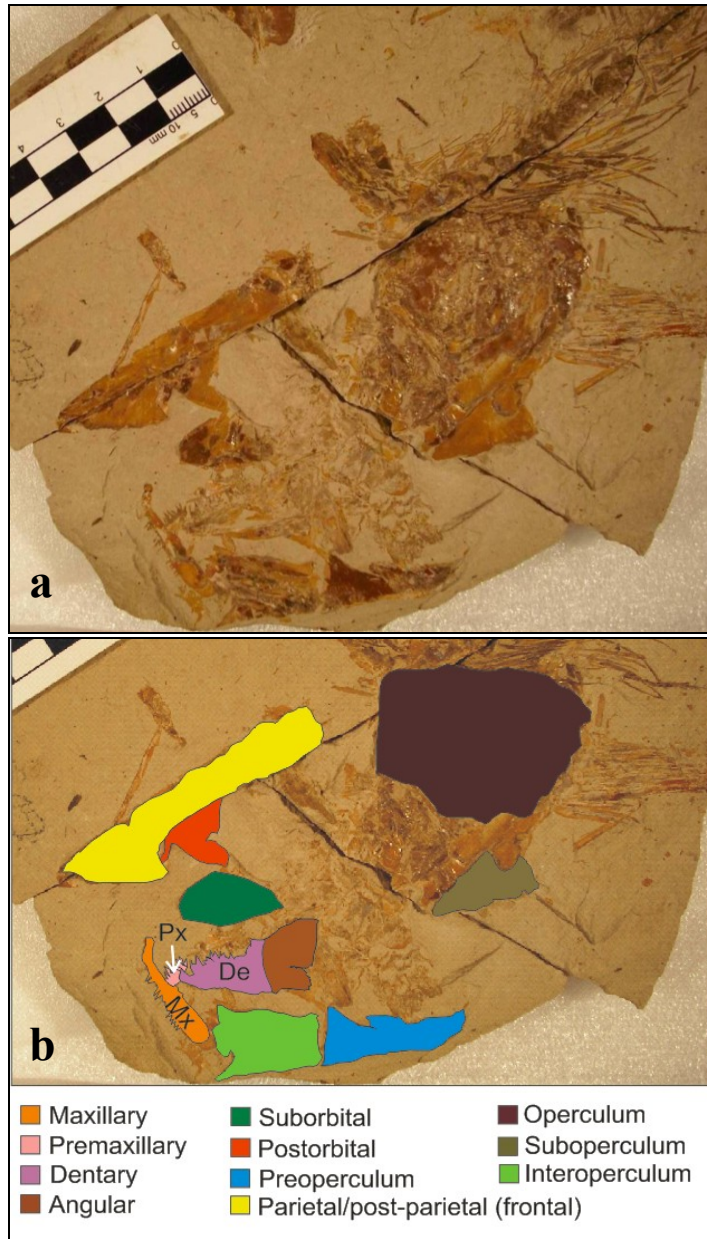


Figure 4.5 – Eocene *Eosalmo driftwoodensis* UWBm-82489 (Klondike Formation) (a) without and (b) with interpretation of relevant discernible skeletal elements. Colour key is provided. Jaw bones are labelled dentary (De), maxillary (Mx) and premaxillary (Px).

### *Salvelinus*

#### *Salvelinus* sp.

All 3 of the following specimens were taken from the same site (UO2227 Worden, Oregon, USA).

## Miocene, Worden, Oregon

UOF-26785; physical examination: Figure 4.6, Micro-CT assessment: Figure 4.7

This specimen consists of the anterior of the right dentary. This bone is very robust, with the same sturdy, squat appearance seen in the defleshed *Salvelinus namaycush* specimen from Chapter 2 (CMNFI-1958-0100A.1). Several teeth are evident and while approximately half of these are indicated only by the remnants of their roots (Fig. 4.6 a, red arrows), at least three teeth display portions of the tooth shaft, as well (pink arrows). Ventral to these teeth, there is a robust, shelf-like “ledge” that runs the length of the specimen (yellow arrow).

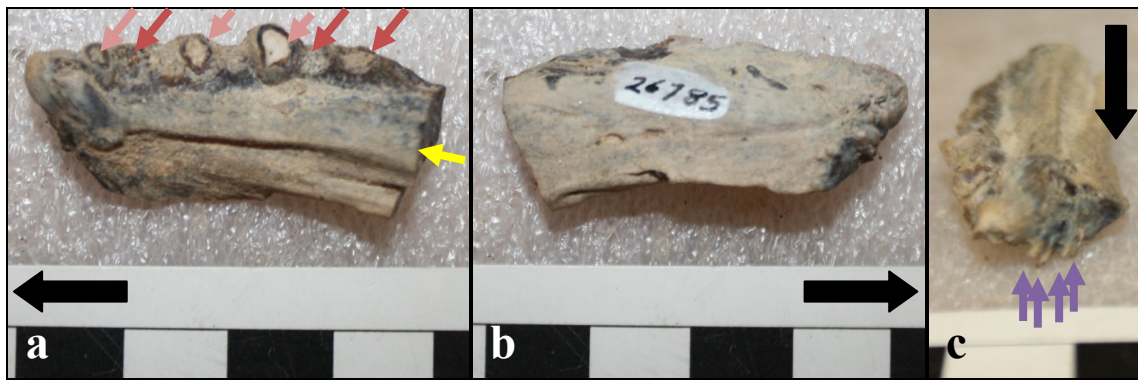


Figure 4.6 – Miocene *Salvelinus* sp. UOF-26785 (Worden, Oregon), right dentary in (a) medial, (b) lateral view, and (c) anterior view showing minute skeletal extensions protruding from anterior. The black arrows indicate the anterior orientation. The remnants of several teeth are evident (red arrows), including at least three that display a small portion of their shaft (pink arrows). A shelf-like ledge ventral to this dentition region runs the length of the specimen (yellow arrow), and several delicate extensions protrude from the anterior lateral edge (purple arrows).

On the lateral and anterior edges of the specimen, delicate extensions of bone protrude from the dentary (purple arrows). These extensions form a starburst of small, slender nodes. These nodes appear to represent an alteration to the surface of the dentary that may be evidence of a kype.

These nodular and fibrous textures identified in physical examination of this specimen are very much in evidence in Micro-CT scan (Fig. 4.7 b, c, e-g, purple arrows). These features, located on the anterior lateral edge, are very numerous and prominent. The outer margins of the nodular like elements appear to be of higher density than their interior, with a relatively gradual transition between the two densities. The patterns in density and near-uniform continuity of these structures with the remainder of the dentary is very similar to the kype skeletal needles seen in defleshed, living specimens.



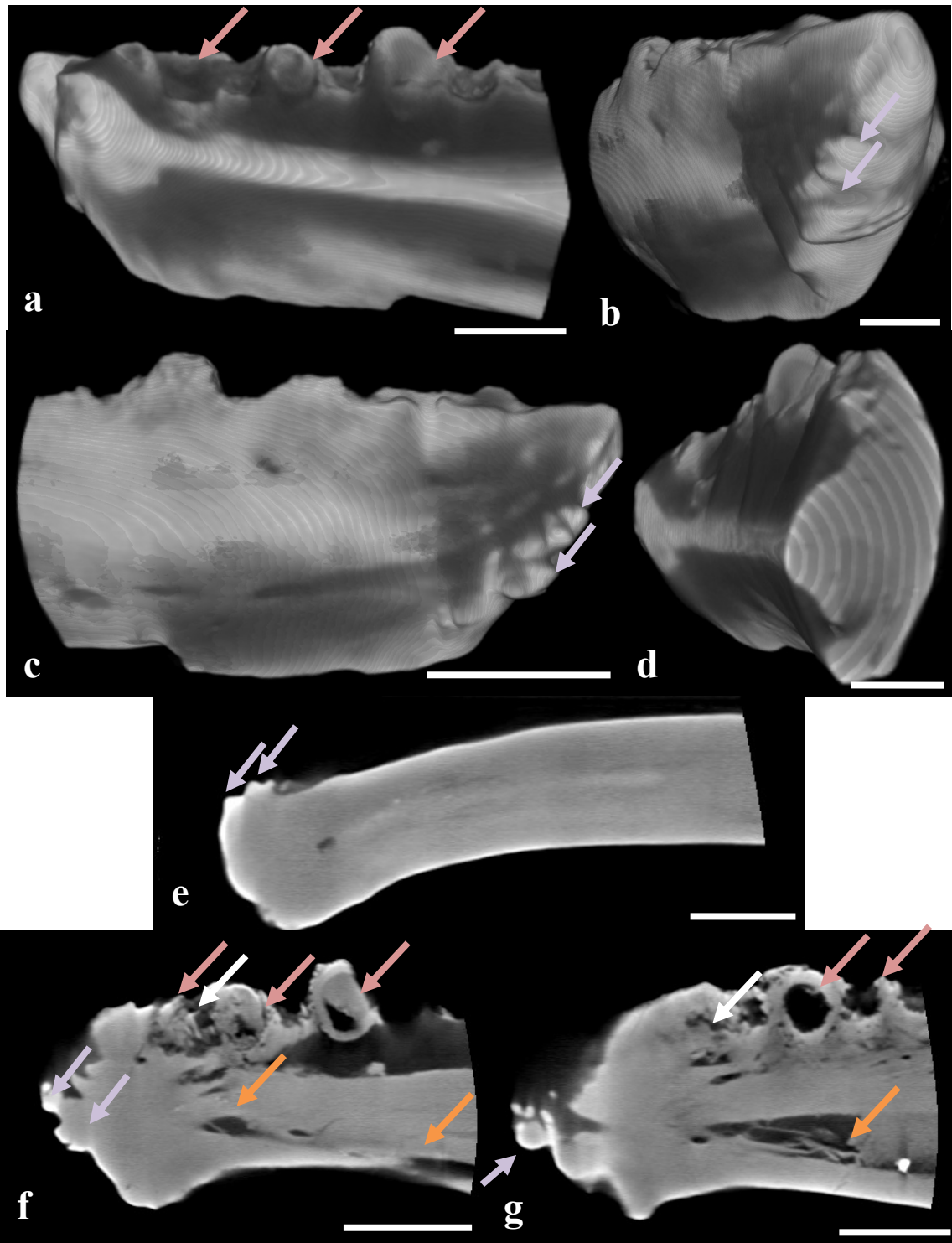


Figure 4.7 – Micro-CT scan of Miocene *Salvelinus* sp. UOF-26785 (Worden, Oregon), right dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slices with (e) transverse section midway through the dentary and with sagittal section (f) midway and (g) near lateral edge of dentary. On the anterior lateral margin of the dentary, many prominent, nodular features are evidence (purple arrows). Also present are several, hollow teeth (red arrows). This specimen is almost universally dense, with some channeling near the lateral margin (orange arrows) and some near-spongy matrix surrounding the teeth (white arrow). Scale bars are 0.5 cm.

Also in evidence are several teeth, each of which appears to be absent of any infilling (Fig. 4.7 a, f-g, red arrows). This specimen shows no evidence of the many channels and hollows within the *Stenodus* fossil. Instead, UOF-26785 is almost uniformly dense and compact, though a number of large hollows are present near the lateral edge in sagittal section (Fig. 4.7 f-g, orange arrows).

### Miocene, Worden, Oregon

UOF-26786; physical examination: Figure 4.8, Micro-CT assessment: Figure 4.9

This specimen is very similar in size and shape to UOF-26785. The robust, anterior aspect of a right dentary, this fossil displays a ledge of bone that runs the length of the specimen similar to that of UOF-26785 (Fig. 4.9 a, yellow arrow) as well as the roots of several teeth and a portion of the shaft of at least one. Although this specimen is almost entirely composed of compact, smooth bone, towards its anterior, a rough texture and several nodular processes extend from the bone (Fig. 4.8 c, purple arrows). These processes are similar to the elements seen in UOF-26785 and may represent preserved evidence of a kype.

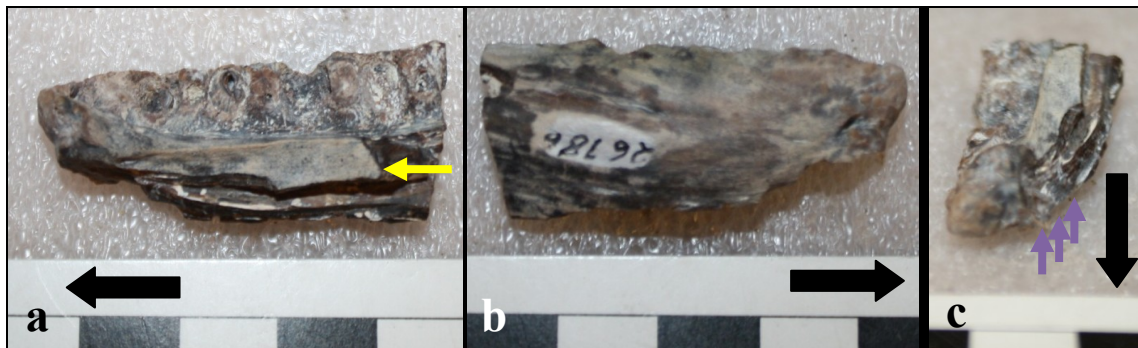


Figure 4.8 – Miocene *Salvelinus* sp. UOF-26786 (Worden, Oregon), right dentary in (a) medial, (b) lateral view, and (c) anterior view showing minute skeletal extensions protruding from anterior. The black arrows indicate the anterior orientation. Nodular textures near the anterior lateral edge of the bone are indicated by purple arrows. The shelf-like feature seen in UOF-26785 is also evident in this specimen (yellow arrow).

These anterior lateral nodular features are also visible in Micro-CT scan (Fig. 4.9 b, c, e, and f, purple arrows). These features are not as numerous as in UOF-26785 and are also of a more uniform density, although the very dense margin and gradation into less dense material seen in UOF-26785 is present. These nodular, fibrous elements are oriented similarly to and have a structure that greatly resembles the kype skeletal needles of defleshed living specimens.

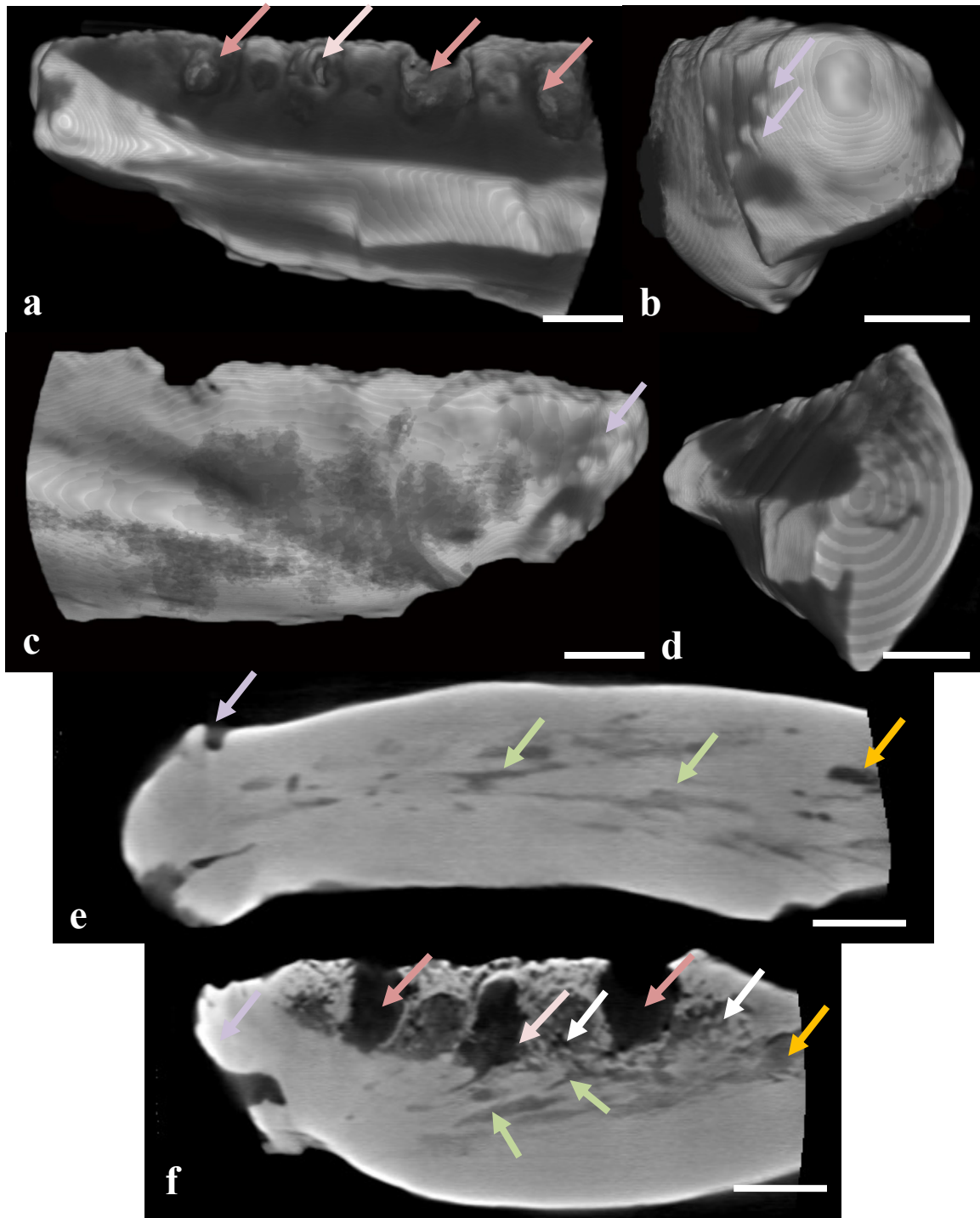


Figure 4.9 – Micro-CT scan of Miocene *Salvelinus* sp. UOF-26786 (Worden, Oregon), right dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slices with (e) transverse section midway through the dentary and (f) sagittal section midway through dentary. Nodular textures are present on the anterior lateral margin (purple arrows). The specimen is relatively uniform in density, but several channels exist at the interior that travel the long axis of the bone (green arrows) and the bone around the toothed region is almost granular in appearance (white arrows). The remnants of several teeth are also evident (red arrows). The second tooth (pink arrow) has a small portion of its shaft remaining. At least one hollow is present posteriorly (orange arrow). Scale bars are 0.5 cm.

As with UOF-26785, the remainder of the dentary is also relatively uniform in density, but with far more in the way of channels along the length of the bone (Fig. 4.9 e and f, green arrows). At least one large hollow (Fig. 4.9, e and f, orange arrows) is also visible. Also, near the tooth region, there is a portion of the dentary that has a spongy, almost granular appearance (Fig. 4.9 f, white arrows). While all of the teeth of this specimen have been lost, their roots remain (Fig. 4.9 a and f, red arrows) and, in the case of the second tooth, a small portion of the shaft may be present, as well (Fig. 4.9 a and f, pink arrow).

### Miocene, Worden, Oregon

UOF-26787; physical examination: Figure 4.10, Micro-CT assessment: Figure 4.11

The anterior portion of a large, robust left dentary, this specimen displays many of the features seen in UOF-26785 and UOF-26786. Three complete teeth (Fig. 4.10 a, red arrows), one partial tooth (pink arrow) and at least two tooth sites (white arrows) are visible on this specimen. These teeth rest above a shelf-like process similar to what was seen in UOF-26785 and UOF-26786 (yellow arrow).



Figure 4.10 – Fossil *Salvelinus* sp. UOF-26787, left dentary in (a) medial, (b) lateral view, and (c) anterior view showing minute skeletal extensions protruding from anterior. The black arrows indicate the anterior orientation. Several teeth are visible, including three complete (red arrows) and one partial tooth (pink arrow), as well as two tooth sites (white arrows). Also evident are the shelf-like feature extending the length of the bone (yellow arrow) and the nodular-like textures on the anterior surface (purple arrows). Specimen is of Miocene age and was taken from Worden, Oregon.

Also visible is a rough texture on the anterior aspect of this specimen (Fig. 4.10 c, purple arrows). This texture is the result of several nodular processes extending from the ventral and lateral margins of the anterior dentary. As with UOF-26785 and UOF-26786,

these nodular elements may be evidence of a kype.

Once again, these nodular features are visible in Micro-CT scan and extend from the surface of the dentary (Fig. 4.11 b, c, e, and f, purple arrows). These features are very dense at their exterior and grade into less dense material toward the interior of the dentary. As with the other *Salvelinus* specimens, these features greatly resemble the skeletal needles of living kype-bearers.

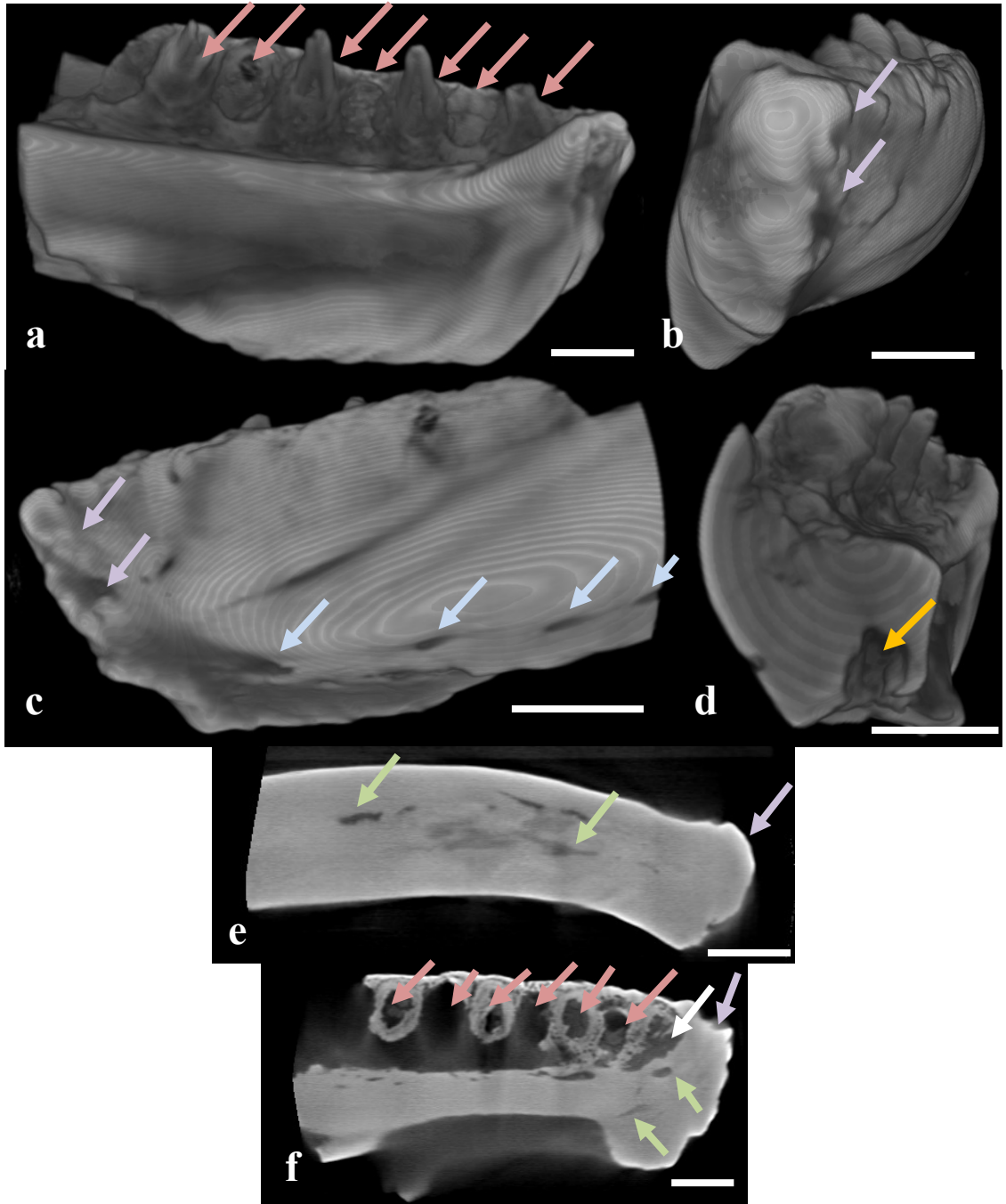


Figure 4.11 – Micro-CT scan of Miocene *Salvelinus* sp. UOF-26787 (Worden, Oregon), left dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slices with (e) transverse section midway through the dentary and (f) sagittal section midway through dentary. Nodular features are present on the anterior lateral margin of the bone (purple arrows). These features are of higher density at their margins and grade into lower density. The density of the specimen as a whole is fairly uniform though, as with the other *Salvelinus* specimens, several channels at the interior of the dentary run along the long axis of the bone (green arrows). Many nearly complete and damaged teeth are present in this specimen (red arrows) and are surrounded by the sponge-like density patterns seen in UOF-26785 and UOF-26786 (white arrow). Also present are several channels within the bone that indicate the remnants of the blood vessel network similar to those of the other specimens examined (green arrows). At least one hollow is present posteriorly (orange arrow). A row of pores on the lateral surface of the bone indicate the remnants of the mandibular lateral line canal (light blue arrows) that appear Scale bars are 0.5 cm.

Also evident in Micro-CT scan are the many complete and damaged teeth (Fig. 4.11 a and f, red arrows) that are surrounded by the spongy bone seen in the other *Salvelinus* specimens (Fig. 4.10 f, white arrows). As seen in CMNFV-48886, at least one hollow (Fig. 4.11 d, orange arrow), and channels (Fig. 4.11 e and f, green arrows) penetrate from the exterior margin of the bone into the dentary's interior, forming a meshwork of tunnels suggestive of a blood vessel network. These tunnels and channels also run along the long axis, as with UOF-26785, marring the near-uniform density at this specimen's interior. A row of pores on the lateral surface (4.11 c, light blue arrows) represent the remnants of the mandibular lateral line canal sensory system.

### ***Oncorhynchus***

#### ***Oncorhynchus* sp.**

#### **Miocene, Worden, Oregon**

UOF-26773; physical examination: Figure 4.12, Micro-CT assessment: Figure 4.13

This small, slender specimen represents a nearly complete left dentary. A small portion of the anterior tip is missing, as is the posterior articulating process. Two teeth (red arrows) are visible, as are several tooth sites (not visible in the figure). Due to a conglomeration of sediment adhering to the specimen, the surface of the fossil has an artificial rough texture. Close examination of the bone itself, however, does not reveal any evidence of a kype.



Figure 4.12 – Miocene *Oncorhynchus* sp. UWBM-26773 (Worden, Oregon), left dentary in (a) medial and (b) lateral view. The black arrows indicate the anterior orientation. Two teeth (red arrows) are visible on the dorsal surface, as are several tooth sites (not visible in this image). The surface of this specimen is covered by a conglomeration of sediment.

Due to the fact that this specimen had not been fully prepared at the time it was scanned and so was covered with a layer of medium-grained sediment, there are a large number of artifacts distorting its surface, resulting in Micro-CT reconstructions with a “fuzzy” appearance. One feature that can be discerned from this scan, however, is the appearance of a spongy region of tissue located near the anterior dorsal margin of the bone (Fig. 4.13 e, white arrows). This tissue is in a similar position to the tissue found in adjacent to the teeth in the *Salvelinus* specimens examined in this study, but this tissue is indistinct and may be an artifact resulting from scanning distortions. Two small teeth are present in this specimen (Fig. 4.13 a and b, red arrows). There is no evidence of kype in this scan.



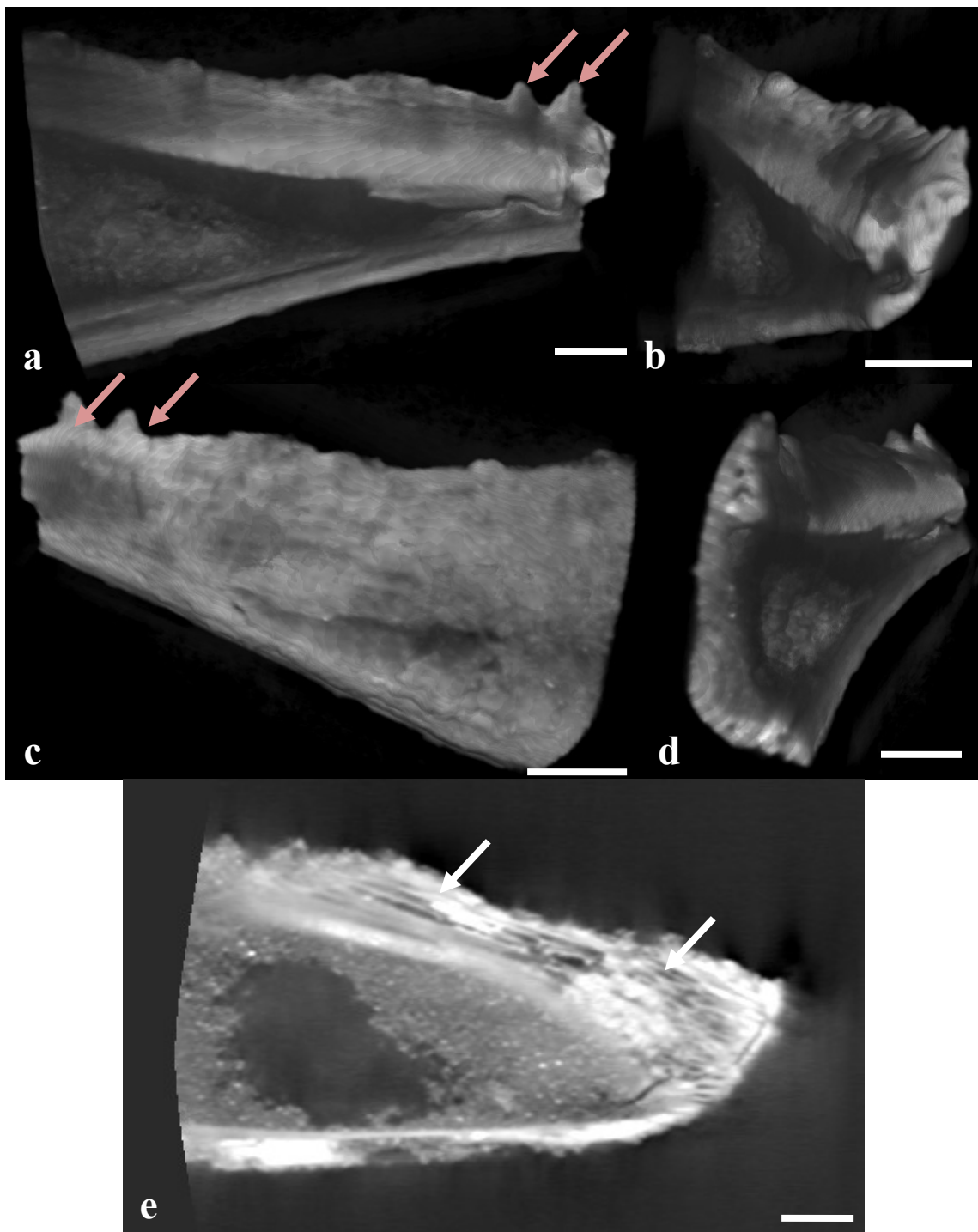


Figure 4.13 - Micro-CT scan of Miocene *Oncorhynchus* sp. UOF-26773, left dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slice with (e) sagittal section midway through dentary. This scan is very much distorted by the layer of medium-grained sediment covering the specimen at the time of scanning. However, two small teeth (red arrows) are visible, as is the appearance of spongy tissue toward the anterior dorsal margin of the bone (white arrows). This tissue may represent the same spongy tissue surrounding the tooth region seen previously in the *Salvelinus* specimens, but may also be the result of artifacts and distortions. Scale bars are 0.5 cm.

### Miocene, Jackass Butte, Oregon

UOF-26782; physical examination: Figure 4.14, Micro-CT assessment: Figure 4.15

This specimen is the anterior portion of a right dentary and is missing its anteriormost aspect. The contours of this specimen are smooth, with none of the rough textures that would suggest the presence of a kype. Slender and delicate, this specimen displays two complete teeth (Fig. 4.14, red arrows) and four partial teeth (pink arrows), including one, the most anterior, that became uprooted but remains associated with the specimen (white arrow).

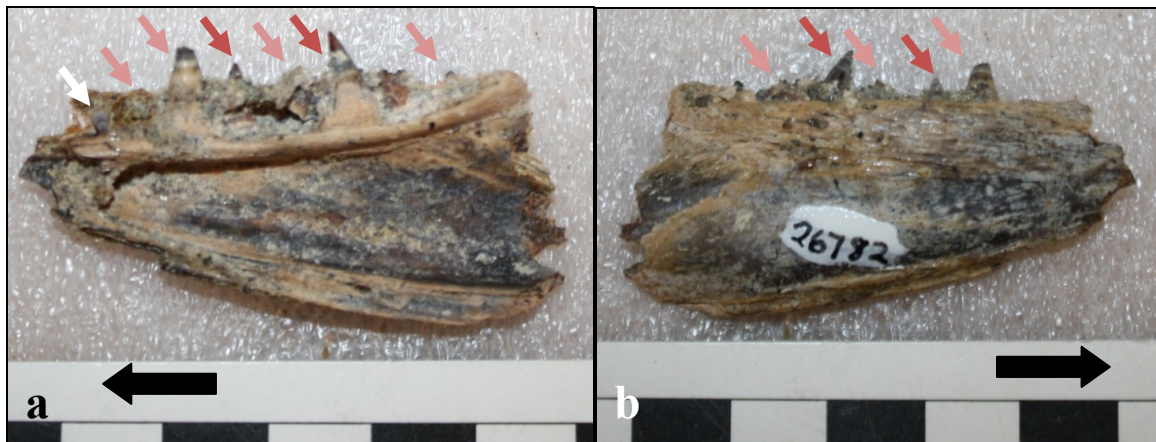


Figure 4.14 – Miocene *Oncorhynchus* sp. UOF-26782 (Jackass Butte, Oregon), right dentary in (a) medial and (b) lateral view. The black arrows indicate the anterior orientation. Several teeth are visible, including two complete teeth (red arrows), four partial teeth (pink arrows), and one tooth that was uprooted by remains associated with the specimen (white arrow).

These teeth include several that were only visible in two-dimensional sagittal view (Fig. 4.15 a-e, red arrows). Also evident is a rough texture on the anterior medial margin of the bone (Fig. 4.15 a, b, and d, yellow arrows). This texture appears to be the result of artifacts on the surface of the dentary and is not similar in form or location to the nodular kype-like textures seen in the *Salvelinus* specimens. A region of spongy tissue surrounding the teeth is also present in this specimen (Fig. 4.15 e, white arrows). This texture is very similar to that found in association with the teeth of the *Salvelinus* and other *Oncorhynchus* specimens, thus far. There is no evidence of a kype in this specimen.

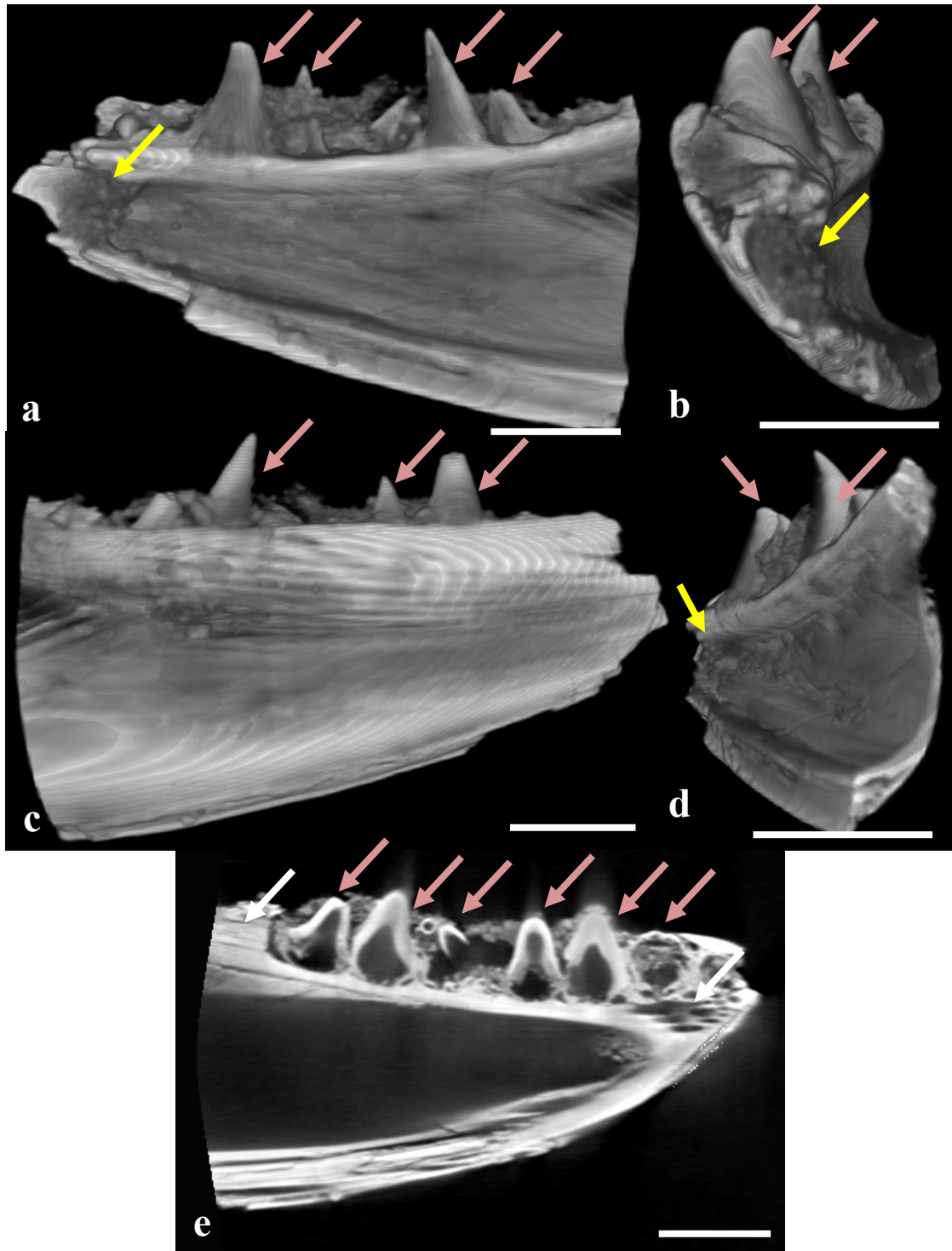


Figure 4.15 – Micro-CT scan of Miocene *Oncorhynchus* sp. UOF-26782 (Jackass Butte, Oregon), right dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slice with (e) sagittal section midway through dentary (and teeth). Several complete and damaged teeth are visible (red arrows). A rough texture on the anterior medial surface of the bone (yellow arrows) is also apparent, as is a spongy region of bone surrounding the teeth (white arrows). Scale bars are 1 cm.

### Miocene, Jackass Butte, Oregon

UOF-26789; physical examination: Figure 4.16, Micro-CT assessment: Figure 4.17

A small, delicate left dentary, this specimen is intact anteriorly but is missing a great deal of its posterior aspect (Fig. 4.16). There do not appear to be any teeth on this specimen. There also does not appear to be any textures or elements suggestive of a kype visible in physical examination. Rather, the anterior and lateral contours of the dentary are smooth.

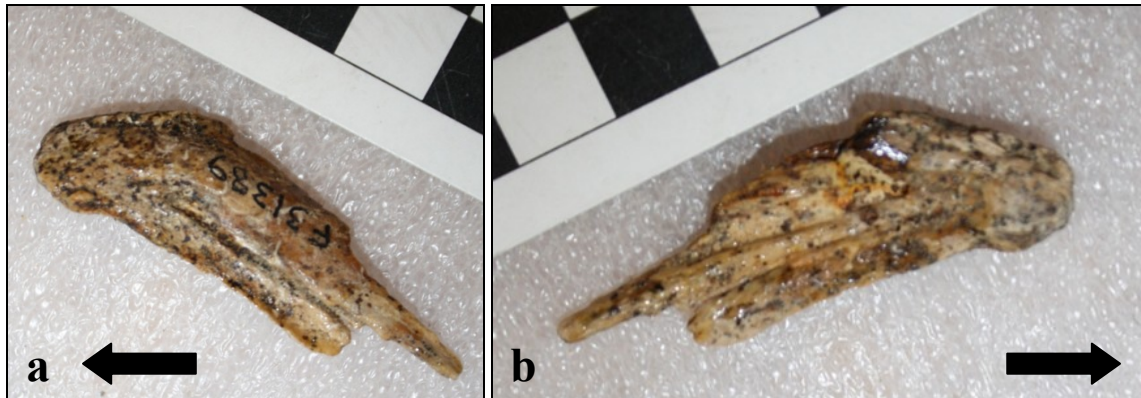


Figure 4.16 – Miocene *Oncorhynchus* sp. UOF-26789 (Jackass Butte, Oregon), left dentary in (a) medial and (b) lateral view. The black arrows indicate the anterior orientation.

A minimum of two channels from the exterior to the interior of the bone is visible on the Micro-CT scan, one entering the dentary on the medial margin and the other on the lateral (Fig. 4.17 a and c, blue arrows). While not as networked and extensive as was the case in CMNFV-48886, these channels may represent blood vessels. With the exception of the tunnels made by the presence of these channels (Fig. 4.17 e, green arrow), this specimen is of a near-uniform density, with few of the hollows (Fig. 4.17 d and e, orange arrows) and little evidence of the “spongy” material seen in other specimens (Fig. 4.17 e, white arrow). In fact, the dorsal region of non-uniform material found in this specimen (Fig. 4.17 e, white arrow) may be an artifact of a break within the bone rather than the tooth-associated spongy bone seen in other specimens. There is no evidence of a kype in this specimen.

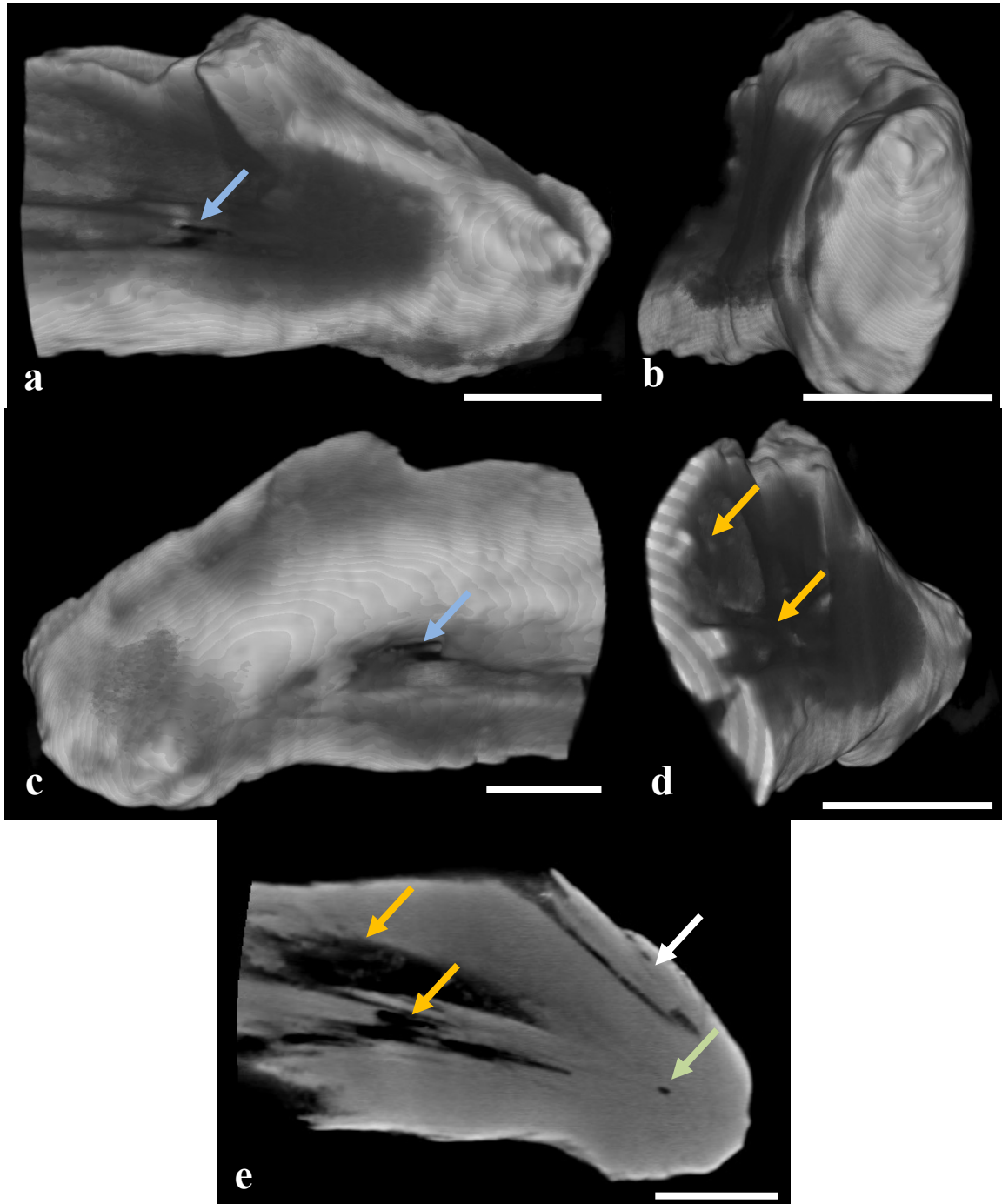


Figure 4.17 – Micro-CT scan of Miocene *Oncorhynchus* sp. UOF-31389 (Jackass Butte, Oregon), left dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slice with (e) sagittal section midway through dentary. Two channels enter the dentary, one on the lateral and the other on the medial margin (blue arrows). These channels are visible in sagittal section within the dentary (green arrow). The specimen is of near-uniform density, with very few hollows (orange arrows) and little evidence of the spongy material that has been found in association with the tooth region (white arrows). Scale bars are 0.5 cm.

## *Oncorhynchus nerka*

All four of the following specimens were taken from the Skokomish Valley, Washington.

### Pliocene, Skokomish Valley, Washington

UWBM-87112; Figure 4.18

This specimen represents the skull and anterior trunk of a mid-sized individual (Fig. 4.18). Although the specimen is missing the premaxilla and a portion of the anterior maxillary, the maxillary extends to posterior of the orbit, which would have resulted in a large mouth. Both the maxillary and the dentaries are long, slender, and seemingly delicate and have many small, sharp teeth. The dentaries, both of which are present on this specimen, each display two very large, rearward curving teeth at the anterior tip of the bones (red arrows indicate those of the right dentary). These large teeth are similar to those seen in both the fleshed and defleshed living specimens of *O. nerka* (and other *Oncorhynchus* specimens) from Chapter 2, though they are much smaller. Although minimal, there is also evidence of a rough texture on the anterior ventral surface of the left dentary. The combination of large “breeding” teeth and rough anterior textures in this specimen suggest the presence of a kype.

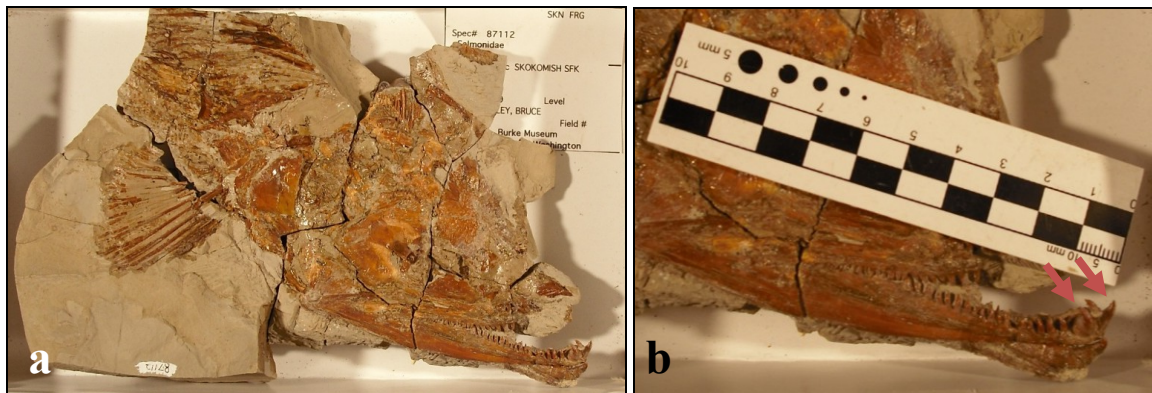


Figure 4.18 – Pliocene *Oncorhynchus nerka* UWBM-87112 (Skokomish Valley, Washington), anterior skeleton. (a) Entire specimen and (b) with focus on dentary. The dentaries both carry two large, rearward curving teeth (the red arrows indicate those of the right dentary).

### Early Pleistocene, Skokomish Valley, Washington

UWBM-87121; physical examination: Figure 4.19, Micro-CT assessment: Figure 4.20

This specimen is comprised of the nearly complete anterior portion of the left and right dentaries of a single individual. Both dentaries display many rather small, sharp teeth,

including a single, large tooth on the anterior tip of the right dentary (Fig. 4.19, red arrows). This tooth has presumably been lost on the left dentary. The texture on both dentaries becomes rougher towards the anterior, particularly in the case of the left dentary. This rougher texture is the site of several short, node-like fibrous elements that extend from the dentary surface. The presence of both a “breeding” tooth and rough, nodular anterior bone texture (purple arrows) suggests the presence of a kype in this specimen.

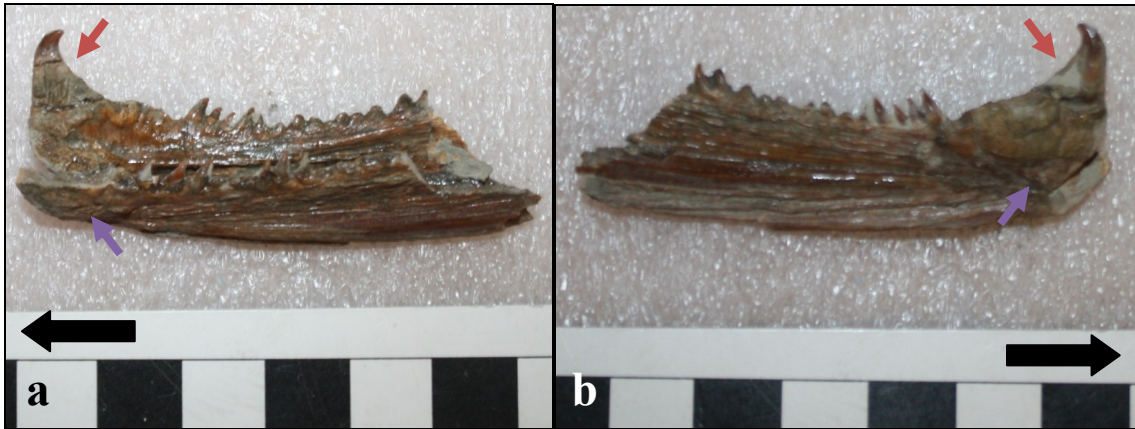


Figure 4.19 – Early Pleistocene *Oncorhynchus nerka* UWBM-87121 (Skokomish Valley, Washington), lower jaw. (a) left dentary in lateral view and right dentary in medial view and (b) right dentary in lateral view. The black arrows indicate the anterior orientation. A large breeding tooth is present on the right dentary (red arrows), though that of the left dentary has been lost. Very subtle, nodular textures are present on the anterior lateral dentary surface of both the left and right dentary (purple arrows).

As shown in Micro-CT scan, the large, anterior tooth on the right dentary (Fig. 4.20 a-d, pink arrows) rests on a bulky bed that bulges out from the rest of the smooth dentary (Fig. 4.20 a-d, yellow arrows). Within this tooth bed, a gradient at the anterior tip of the dentary ranges from very dense material on the exterior margin to less dense material posteriorly (Fig. 4.20 e, tooth base is outlined with white dash). This gradient is due to a shift from condensed material at the exterior margin of the bone, through a spongy region full of channels and small accumulations of empty space (Fig. 4.20 e, green arrows), to a compact, linearly arranged, posterior portion. While this linearly arranged, compact bone represents the mature, established dentary bone, the spongy anterior region appears to be the remnants of reworking of or addition to that mature bone, forming the tooth base. This spongy region contains many large and small channels and tunnels that form an intricate network that is possibly evidence of a complex blood vessel system that would have supplied the actively reworking anteriormost dentary. Even

in the more compact bone of the mature dentary, channels (Fig. 4.20 e, green arrows) and hollows (orange arrows) are numerous. The large breeding tooth with its associated base is very similar to the breeding teeth found in living, kype-bearing *Oncorhynchus* specimens.



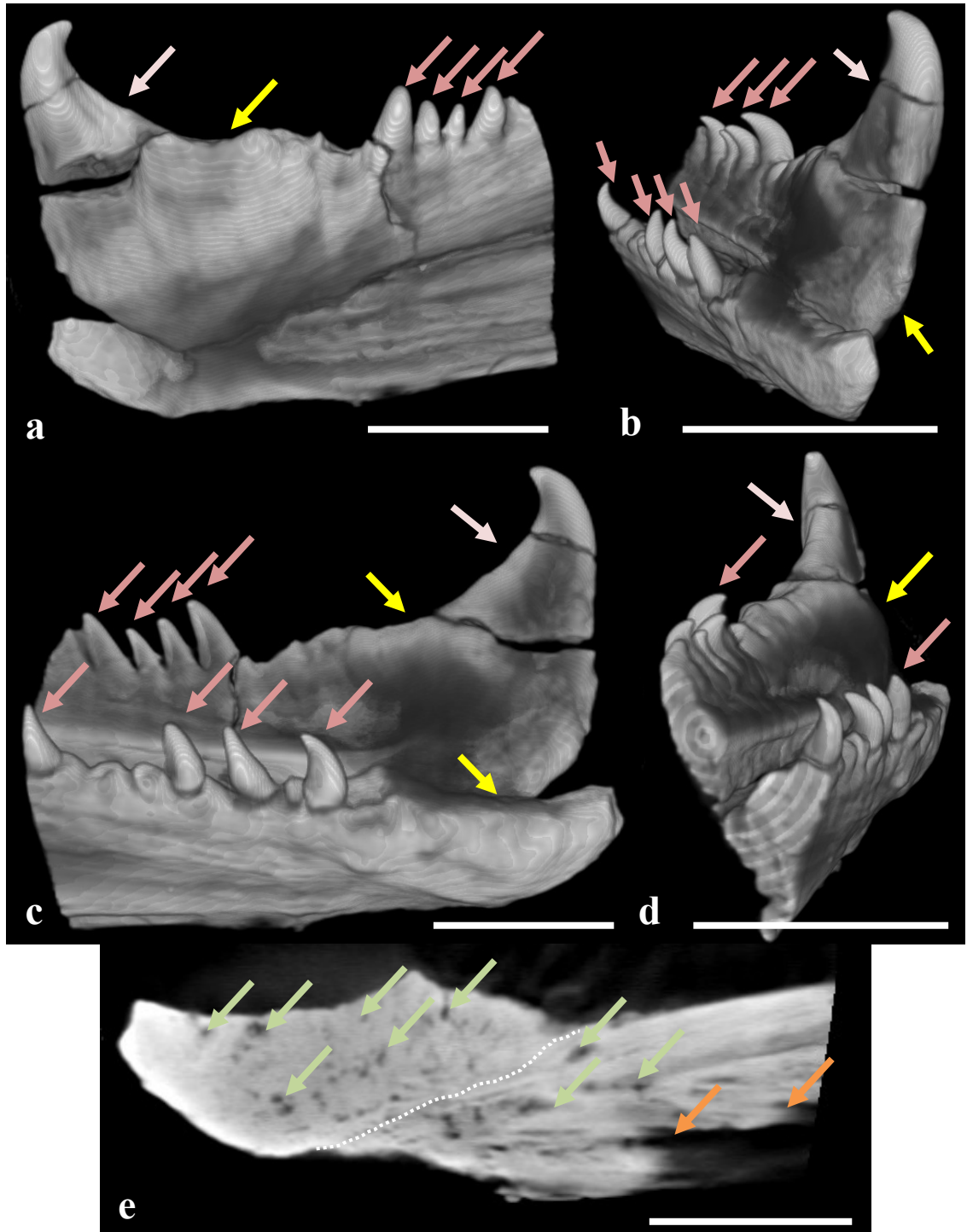


Figure 4.20 – Micro-CT scan of early Pleistocene *Onchorhynchus nerka* (Skokomish Valley, Washington), lower jaw. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slice with (e) sagittal section midway through left dentary. In addition to the many teeth apparent on both jaws, a very large anterior tooth (pink arrows), though lost from the left dentary, is connected to the right dentary via a bulky tooth bed (yellow arrows). In sagittal section, this tooth bed has a spongy structure that transitions into a more compact, linearly arranged bone posteriorly (transition is marked by the dashed line). Many channels (green arrows) and hollows (orange arrows) exist in both the spongy and in the more compact portions of the dentary. Scale bars are 1 cm.

### **Pliocene, Skokomish Valley, Washington**

UWBM-87122; Figure 4.21

Representing the anterior skeleton of a large individual, this specimen has experienced some post-depositional alteration in the craniofacial skeleton that makes some features difficult to distinguish. A relatively large skull terminates in a moderate-sized mouth, with maxillary (Fig. 4.21, orange) extending to at least the anterior margin of the orbit, following which the bone is covered by sediment. However, the dentary (rose) is at least as long as that of a modern specimen with similar proportions, suggesting that the maxillary would extend to at least the posterior margin of the orbit. The jaw bones were twisted in relation to one another such that the tooth-bearing edge of the dentary is covered by the maxillary and the tooth-bearing edge of the maxillary is pressed into the sediment, meaning that evidence of dentition is very sparse (Fig. 4.21b). There are, however some indistinct teeth near the dorsal surface and anterior tip of the dentary (blue arrows), though no evidence of very large “breeding” teeth exists. There is also no evidence of rough textures on the dentary. Lacking breeding teeth and extensions from the dentary, it would appear that there is no kype in this specimen.



Figure 4.21 – Pliocene *Oncorhynchus nerka* UWBM-87122 (Skokomish Valley, Washington) anterior skeleton (a) without and (b) with interpretation of relevant discernible skeletal elements. Posterior portion of the maxillary (orange) disappears into the sediment. Maxillary is oriented with dentition side pressed into the sediment and is positioned such that it covers the dentition side of the dentary (pink). Indistinct teeth are visible between the two bones (blue arrows), but no evidence of breeding teeth or rough textures are visible on the anterior dentary.

### **Pleistocene, Skokomish Valley, Washington**

UWBM-95842; Figure 4.22

This specimen is comprised of the nearly complete left dentary and portions of the maxillae and right premaxillary of a single specimen. The right premaxilla is represented by a single large tooth (Fig. 4.22, pink arrows), while the anterior portions of the maxillae are preserved. The maxillae are slender and covered on their ventral surfaces with many moderately sized, sharp teeth. Having been flipped during decay or scavenging, these ventral surfaces are oriented dorsally in respect to the other elements of this specimen. The dentary has been taphonomically altered, resulting in an unnatural bend in its length, yielding a sickle shape. However, the overall shape of this bone is unaltered and it is comprised of smooth, compact bone. The dentary displays many sharp teeth of a similar size to those of the maxillae, with the addition of three large, fang-like teeth at its anterior tip of almost the same size as the very large tooth on the premaxillary (red arrows). The bone around these teeth is of a rougher texture than the remainder of the dentary, but this may be an artifact of preservation or of preparation following this specimen's discovery. Nonetheless, the large "breeding" teeth on both the dentary and the premaxillary suggest that this individual was kype-bearing.



Figure 4.22 – Pleistocene *Oncorhynchus nerka* UWBM-95842 (Skokomish Valley, Washington), left dentary and portions of maxillae and right premaxillary. Many teeth are evident on both the dentaries and on the maxillae. Three large, fang-like teeth on the dentary (red arrows) and one on the premaxillary (pink arrow) are breeding teeth.

***Oncorhynchus (Smilodonichthys) rastrosus* (“sabre-toothed salmon”)**

Remains of the sabre-toothed salmon (*O. rastrosus*) have been found in western North America since at least 1917, but were only formally identified in 1972 by Cavender and Miller. In their descriptions of this species, Cavender and Miller (1972) discuss a very large late Miocene and early Pliocene fish that displays many primitive features that are similar to those found in more basal salmonids such as in Coregoninae. *O. rastrosus* had over 100 gill rakers and no teeth on the maxillae. A large number of long, closely spaced gill rakers and an absence of maxillary teeth have, in other animals including other salmonids like the Coregoninae, been linked to microphagy (Cavender and Miller, 1972; Eiting and Smith, 2007). As such, *O. rastrosus* was likely a planktivore and used its numerous, specialized gill rakers to strain plankton from the water column (Eiting and Smith, 2007).

Although at least one dwarf population that was likely landlocked has been identified from the Chalk Hills Formation of Idaho (Smith et al., 2000), *O. rastrosus* is believed to be predominantly anadromous. Found in marine and freshwater localities in

the northwest United States (Smith et al., 2000; Smith et al, 1982), and in California and Oregon in particular (Nelson, 2006), the majority of sabre-toothed salmon populations likely employed a life history mode similar to modern Pacific salmon, feeding in the ocean and migrating to freshwater systems during their spawn-run. Each of the specimens examined for the current study are from populations that are believed to be anadromous.

**Late Miocene, Gateway locality, Madras Formation**

UOF-3335; physical examination: Figure 4.23, Micro-CT assessment: Figure 4.24

A number of elements from this specimen were preserved, including vertebrae and many elements of the skull. Among these skull elements are the dentaries, premaxillae, and left maxillary. The vertebrae of this specimen display four annuli (blue arrows), suggesting that the animal was at least four years old at the time of its death (Fig. 4.23 a). This lifespan is what would be expected from spawning *O. nerka* and *O. gorbuscha* (Smith et al., 2007), which are believed to be this species' closest relatives (Cavender and Miller, 1972).

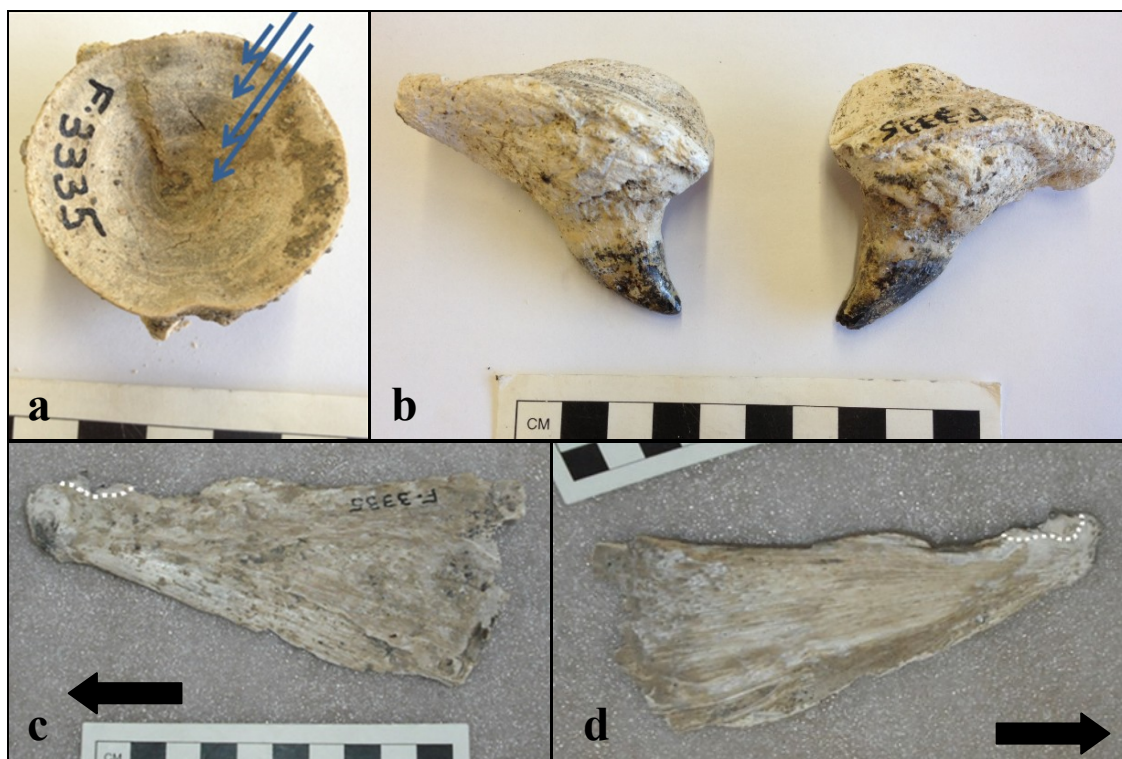


Figure 4.23 – Late Miocene *Oncorhynchus rastrosus* UOF-3335 (Gateway Locality, Madras Formation). (a) vertebra with four annuli, indicated by blue arrows. (b) left and right premaxillae with very large, pigmented putative breeding teeth. Right dentary in (c) medial and (d) lateral view with dashed line indicating contours of tooth bearing region. The black arrows indicate the anterior orientation.

Each of the jaw bones is large and robust, and although the maxillary (not shown) is relatively short, both the premaxillae (Fig. 4.23 b) and dentaries (Fig. 4.23 c and d) are large and long. The dentaries are nearly triangular and very thick, particularly in comparison to the more slender appearance of other *Oncorhynchus* species. Furthermore, unlike other *Oncorhynchus* species, which have dentaries with a smooth texture composed of compact bone, the dentaries of this specimen are striated with bony ridges. This surficial ridging echoes the internal composition of these bones. Looking into the bone along the axis of the bone where it has been broken, many chambers, tunnels, and runnels through the bone are apparent, giving it an almost corrugated, if not spongy, appearance.

Finally, while the maxillary of this specimen is toothless, the left dentary bears one and the right dentary bears two moderately sized teeth that are squat in appearance and have a large circumference at their base. These teeth rest on a large tooth –bearing region (Fig. 4.23 c and d, dashed white line) that is of a spongier appearance than the rest

of the dentary. However, these teeth are miniscule in comparison to the enormous teeth present on the premaxillae. Both premaxillae bear a single tooth that is so large that their root disrupts the shape of the bone they rest upon. Both of these premaxillary teeth curve rearward and are tipped with black pigment. While the large teeth on the premaxillae and dentaries may be breeding teeth, this specimen bears no other evidence of any of the skeletal elements associated with kype.

**Late Miocene, Gateway Locality, Madras Formation**

UOF-26799; Figure 4.24, please also refer to Cavender and Miller (1972)

The holotype of the species, this specimen includes the nearly complete and almost entirely articulated skull and anterior gill arches of one individual, with only the opercles and subopercles missing. The mouth of this animal is framed by large, robust jaw bones. As with UOF-3335, the maxillae are relatively short and somewhat slender in comparison to the massive dentaries. Each of these dentaries is approximately triangular and has a texture with many ridges running anterior to posterior. These dentaries also each display a small tooth-bearing site with two squat teeth with bases of large circumference. Although the shafts of the premaxillary teeth have been lost in this specimen, the premaxillae are large, sturdy elements that bear the evidence of large tooth base. With the exception of these breeding teeth, there is no evidence of compensatory elements in the upper jaw or of modification to the lower jaw that would suggest the presence of a kype.





Figure 4.24 – Late Miocene *O. rastrosus* UOF-26799 (holotype; Gateway Locality, Madras Formation) (a) several craniofacial elements, many of which are articulated. (b) Right dentary in medial view and (c) left dentary in lateral view.

#### Middle Pliocene, Arlington, Oregon

UWBM-50816; physical examination: Figure 4.25, Micro-CT assessment: Figure 4.26

This specimen, a nearly complete right dentary, shares many similarities with the dentaries of UOF-3335 and UOF-26799. It is triangular in shape, with many ridges on its surface and many tunnels and channels within its interior, though the dentary itself is of a relatively small size in comparison to the other specimens examined here. Unlike the dentaries of UOF-3335 and UOF-26799, the dentary of this specimen does not appear to bear any teeth. As with both UOF-3335 and UOF-26799, there is an absence of skeletal needles or fibrous textures anteriorly in this specimen as well as a lack of evidence of

kype.

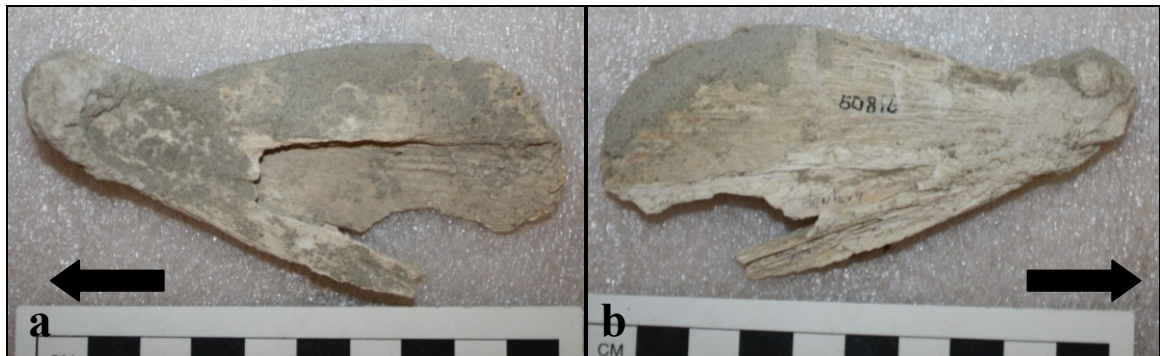


Figure 4.25 – Middle Pliocene *O. rastrosus* UWBM-50816 (Arlington, Oregon), right dentary in (a) medial and (b) lateral view. The black arrows indicate the anterior orientation.

In the Micro-CT scan, the surface of this specimen has many well-rounded contours (Fig. 4.26 b and c, yellow arrows). In sagittal section, this tooth bed is dense and has many complex internal patterns that are similar to those found in the tooth bed of UWBM-87121 and may indicate reworking of mature bone. Within this tooth bed are several channels that are indicative of an extensive blood vessel network (Fig. 4.26 e, green arrows) as well as evidence of two teeth that had not yet erupted from the bone (red arrows). Posteriorly, in what appears to be the more mature portion of the dentary, a large number of hollows create a near-honeycomb network within the bone (Fig. 4.26 d and e, orange arrows). There is no evidence of a kype in this specimen.

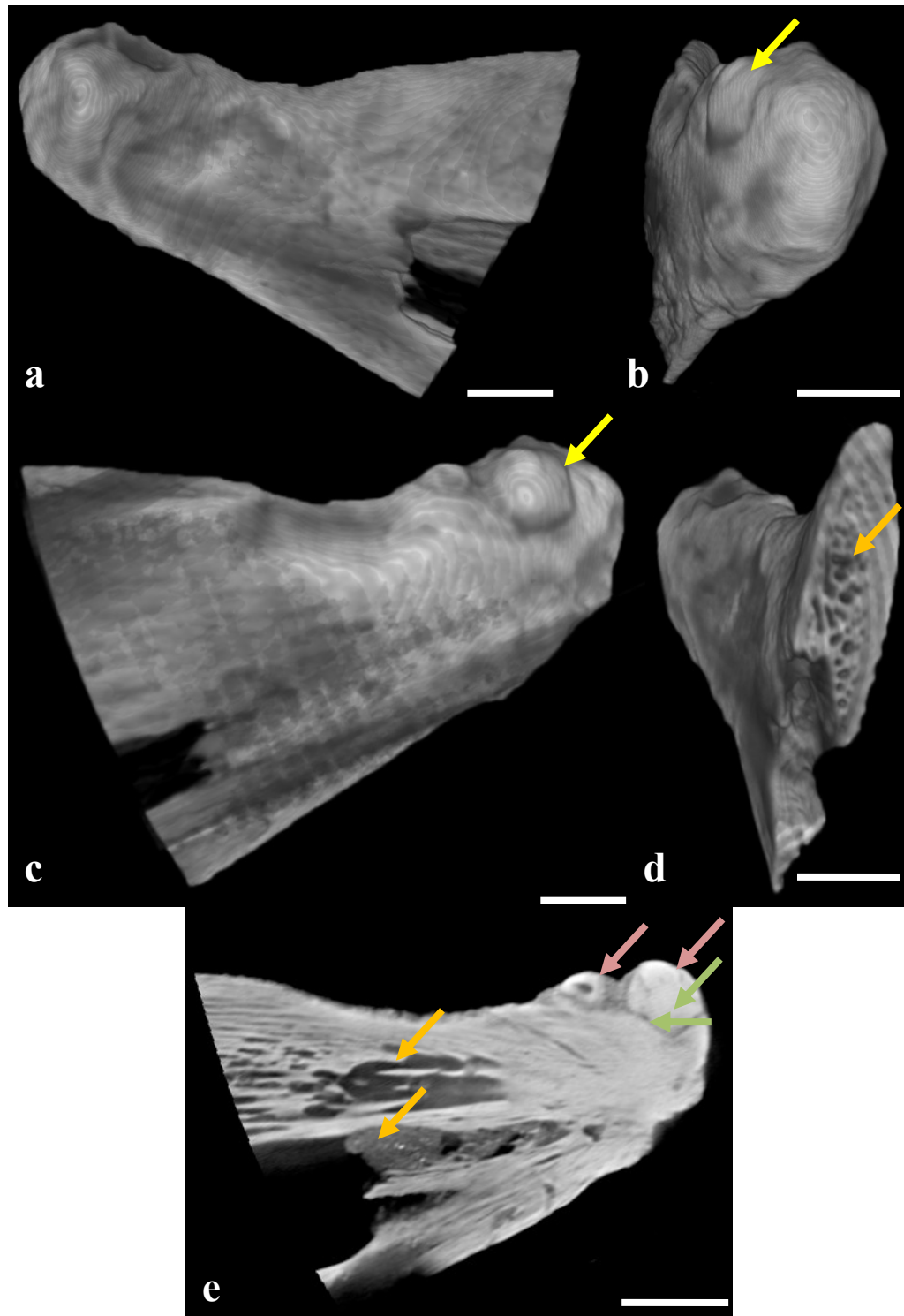


Figure 4.26 – Micro-CT scan of middle Pliocene *Oncorhynchus rastrosus* UWBM-50816 (Arlington, Oregon), right dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slice with (e) sagittal section midway through dentary. The surface of the bone has many well-rounded contours. The anteriorly, the tooth bed (yellow arrows) is very prominent. In sagittal section, this tooth bed contains many channels and complicated density patterns (green arrows). Posteriorly, many hollows (orange arrows) create a honeycomb-like network of chambers. Scale bars are 0.5 cm.

## **Pliocene Ringold Formation**

UWBM-71908

Specimen UWBM-71908 corresponds to two right dentaries of large size. To differentiate between the two, the identification numbers of these dentaries are amended to UWBM-71908a (the larger of the two specimens, being 136 mm in length) and UWBM-71908b (the smaller at 124 mm in length, including a portion of the posterior extension that has broken off).

UWBM-71908a; Physical examination: Figure 4.27, Micro-CT assessment: Figure 4.28

In addition to the ridged textures seen in other specimens of this species, the posterior lateral surface of this specimen shows a number of grooves. The walls of each of these grooves narrow anteriorly to a point, reaching an apex marked by small tunnels into the bone of the angular. It is likely that these features indicate channels for blood vessels and attachment sites for muscles and tendons, though without the complete articulating surface, these features are difficult to define.

Although overall similar in shape and structure to the other *O. rastrosus* specimens described in this chapter, this specimen bears a tooth site that is somewhat larger than those previously discussed (Fig. 4.27 b, yellow arrow). While it does not display a tooth, this region is a significant bulging out from the rest of the dentaries and would have served as a large surface from which a prominent tooth could extend. Anterior to this large tooth-bearing region of the dentary, the surface of the bone is marred by an interesting rough texture. This texture seems to be the result of several small, nodular features that extend no more than one or two millimeters from the surface of dentary. The Micro-CT and histological assessments below will help to determine whether these features are similar in structure to kype-related features seen in other specimens, including those in the *Salvelinus* material that represent potential kype remnants.



Figure 4.27 – Pliocene *O. rastrosus* UWBM-71908a (Ringold Formation), right dentary in (a) medial and (b) lateral view. The black arrows indicate the anterior orientation. The yellow arrow indicates the very large tooth base.

The many well-rounded contours seen in UWBM-50816 are also visible in Micro-CT scans of this specimen (Fig. 4.28 b and c, yellow arrows), as is the honeycomb-like network of hollows in the shaft of the dentary (Fig. 4.28 d and e, orange arrows). The tooth bed is large and, in sagittal view, full of many small, densely packed channels (Fig. 4.28 e, green arrows). Also present is a larger channel ventral to the tooth bed (Fig. 4.28 e, light green). There are, in fact, several channels that penetrate into the bone from the exterior, as seen previously in CMNFV-48886, UOF-26787, and UOF-31389 (Fig. 4.28 a and c, blue arrows). However, the most notable feature of this specimen is the rough, nodular texture present anterior to the tooth bed (Fig. 4.28 b and c, purple arrows). This texture is similar to what is seen in the *Salvelinus* specimens and, in sagittal section, would appear to have a similar density gradient (Fig. 4.28 e, purple arrow). As with the scans of the *Salvelinus* specimens, this region is dense at the exterior margin of the bone and becomes less dense moving interiorly. Furthermore, while this region appears to be continuous with the bone, there is some variation from the rest of the dentary in terms of its internal organization. It remains unclear if the nodular, fibrous elements of this portion of the bone are evidence of a kype.

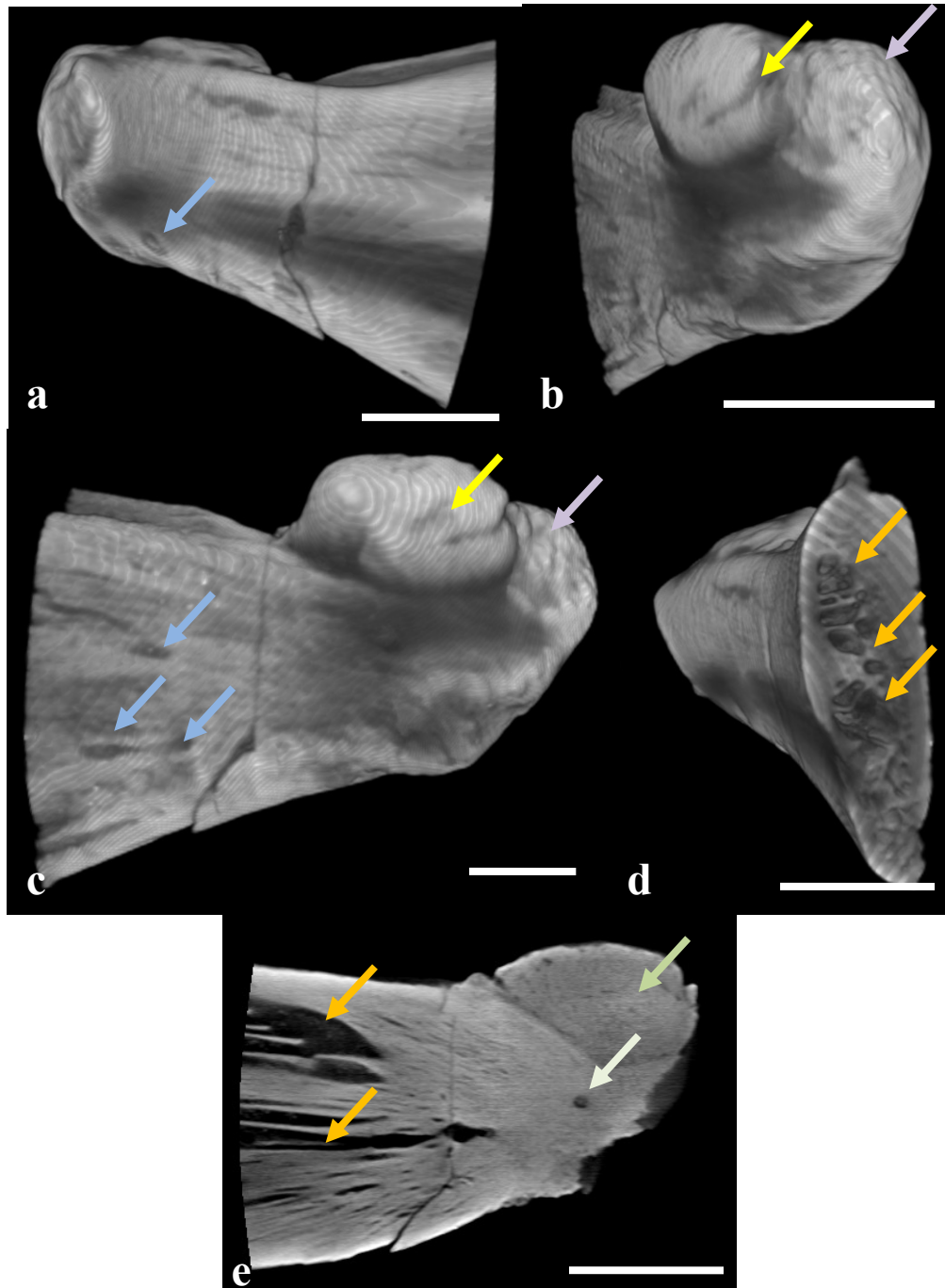


Figure 4.28 – Micro-CT scan of Pliocene *Onchorhynchus rastrosus* UWBM-71908a (Ringold Formation), right dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slice with (e) sagittal section midway through dentary. A large tooth bed (yellow arrows) is apparent and if filled with many small, densely packed channels. Ventral to this spongy region is a very large channel (light green). Several large channels penetrate the bone of the dentary from the exterior (blue arrows). Also present is a complex network of hollows within the shaft of the bone (orange arrows). On the anterior dorsal margin of the bone, a nodular surficial texture is very much in evidence (purple arrows). Scale bars are 0.5 cm.

UWBM-71908b; physical examination: Figure 4.29, Micro-Ct assessment: Figure 4.30

Although relatively small in size in comparison to the other *O. rastrosus* specimens, this specimen displays many similar features. The tooth-bearing site of this dentary, though small, is quite bulky and extends from the angular in much the same way as that of UWBM-71908a, yielding a large surface upon which a tooth would rest (Fig. 2.29 a, yellow arrow). This dentary appears to have been taphonomically altered and it is unclear if a tooth is present on its surface. If a tooth is present, the enamel on its surface has been lost and only its contours are preserved.



**Figure 4.29** – Pliocene *O. rastrosus* UWBM-71908b (Ringold Formation), right dentary in (a) medial and (b) lateral view. The black arrows indicate the anterior orientation. The yellow arrow indicates the site of a very large tooth base.

The surface of this fossil shows many of the ridges and channel-like textures seen in other specimens of this species. However, these surfaces are rather poorly preserved anteriorly. As with these anterior surficial textures, the textures of the symphysis and adjacent tissue are not well-preserved. That being said, as was the case in UWBM-71908a, there seems to be a rough texture anterior to the tooth-bearing region of the dentary present in this specimen as well. Though this texture does not display the distinct nodes seen in UWBM-71908a, this lack of distinction may be due to poor preservation of these features. As with UWBM-71908a, the Micro-CT and histological assessments to follow will help to better define this anterior surficial texture.

Micro-CT scans of this specimen are very similar to UWBM-71908a and to UWBM-50816. A large tooth bearing region (Fig. 4.30 b and c, yellow arrows) with many small, densely packed channels (Fig. 4.29 e, green arrows) dominates the anterior portion of this fossil, while a honeycomb network of hollows dominates posteriorly (Fig.

4.30 d and e, orange arrows). Several large channels penetrate from the external margin of the bone deep into the interior (Fig. 4.30 a and c, blue arrows), and a meshwork of channels within the bone (green arrows), including one very large channel ventral to the tooth bearing region (light green arrow), give the semblance of blood vessels. While the anterior dorsal nodular textures that stood out in UWBM-71908a are also present in this specimen (Fig. 4.30 b and c, purple arrows), they are much smaller, more localized, and less prominent. It remains unclear if these textures represent the remnants of a kype.



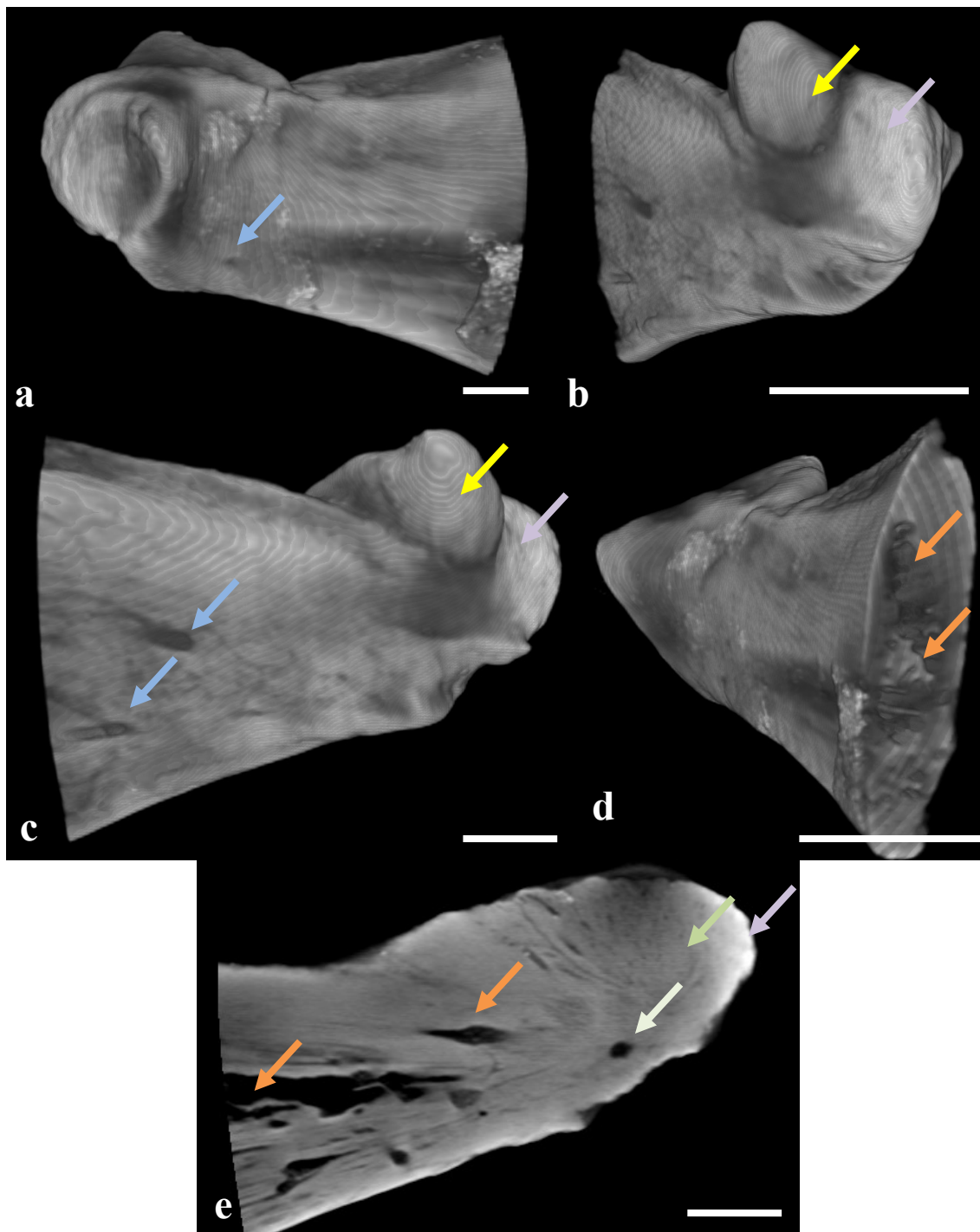


Figure 4.30 – Micro-CT scan of Pliocene *Onchorhynchus rastrosus* UWBM-71908b (Ringold Formation), right dentary. Three-dimensional reconstruction of fossil in (a) medial, (b) anterior, (c) lateral, and (d) posterior view. Two-dimensional slice with (e) sagittal section midway through dentary. The tooth-bearing region is very large (yellow arrows) and is composed of spongy bone filled with many small, densely packed channels (green arrows). Several hollows of varying sizes are present posteriorly (orange arrows), and several channels penetrate from the external margin of the bone into the interior (blue arrows), including one very large one ventral to the tooth bearing region (light green arrow). While the nodular textures seen in UWBM-71908b are present in this specimen, they are much subtler (purple arrows). Scale bars are 0.5 cm.

***Physical examination and Micro-CT summary***

The presence and absence of kype in each of these fossil specimens is summarized in Table 4.2.

**Table 4.2 – Assessment of kype presence or absence from physical examinations and Micro-CT analysis.**

	Specimen No.	Life history stage	Kype present (P) or absent (A)	
<i>Stenodus</i>				
-	<i>S. leucichthys</i>	CMNFV-48886	Unknown	A
<i>Eosalmo</i>				
-	<i>E. driftwoodensis</i>	RBCM.EH 2011.006.0001	Unknown	A
<i>Salvelinus</i>				
-	sp.	UOF-26785	Spawning?	P?
-	sp.	UOF-26786	Spawning?	P?
-	sp.	UOF-26787	Spawning?	P?
<i>Oncorhynchus</i>				
-	sp.	UOF-26773	Unknown	A
-	sp.	UOF-26782	Unknown	A
-	sp.	UOF-31389	Unknown	A
-	<i>O. nerka</i>	UWBM-87112	Spawning	P
-	<i>O. nerka</i>	UWBM-87121	Spawning	P
-	<i>O. nerka</i>	UWBM-87122	Spawning	A
-	<i>O. nerka</i>	UWBM-95842	Spawning	P
-	<i>O. (Smilodonichthyes) rastrosus</i>	UOF-3335	Unknown	A
-	<i>O. (Smilodonichthyes) rastrosus</i>	UOF-26799	Unknown	A
-	<i>O. (Smilodonichthyes) rastrosus</i>	UWBM-50816	Unknown	A
-	<i>O. (Smilodonichthyes) rastrosus</i>	UWBM-71908a	Unknown	A?
-	<i>O. (Smilodonichthyes) rastrosus</i>	UWBM-71908b	Unknown	A?

As in Chapter 2, the physical examination and Micro-CT assessment of specimens is illuminating, revealing a great deal of information in regards to kype growth. The examination of these fossils reinforces the phylogenetic trends in kype presence and absence across the salmonid clade first noted in Chapter 2.

### *Physical examination summary*

Following the physical examination of a range of fossil material, as was the case in Chapter 2, structural evidence of kype growth is found only in the Eusalmoninae. Although the defleshed *Stenodus leucichthys* (CMNFI-Z004302) was robust and had several prominent features including a well-fused symphysis, it showed no evidence of kype growth. As with its modern counterpart, the Quaternary *S. leucichthys* (CMNFV-48886) displayed no structural elements that would suggest the presence of kype. Similarly, neither of the *Eosalmo driftwoodensis* specimens examined in this study display features that are suggestive of kype.

Unlike these more basal salmonids, several of the eusalmonine specimens examined, namely the Miocene *Salvelinus* specimens and many of the Pliocene to Pleistocene *O. nerka* individuals, displayed kype-like structures. These structures included nodular, needle-like textures and, in the case of *O. nerka*, breeding teeth. Interestingly, unlike many of the other *Oncorhynchus* specimens examined in both this and the other chapters of this thesis that display prominent evidence of kype growth, presence of a kype in the late Miocene to middle Pliocene *O. rastrosus* specimens remains unclear. Two Pliocene *O. rastrosus* specimens (71908a and b) displayed textural features similar to those found in the Miocene *Salvelinus* specimens. Additionally, the late Miocene *O. rastrosus* bore large, robust teeth on their premaxillae and the anterior portions of their dentaries that are reminiscent of the breeding teeth seen in other *Oncorhynchus* species. A better understanding of the internal organization of these needle-like textures, breeding teeth, and other tissues of interest are possible by incorporating Micro-CT assessments.

### *Micro-CT assessment summary*

As was demonstrated in Chapter 2, Micro-CT technology is useful in collecting detailed information as to patterns within the tissues of fleshed and defleshed specimens without damaging them. In many cases, Micro-CT scanning provides information that is so detailed that it can be used to diagnose illnesses in living patients (Feldman et al., 2003; Fleischer et al., 1978; Grant et al., 2003; Lee et al., 2012; Taylor et al., 1979; Walsh et al., 1979). Micro-CT assessment is also of great use in fossil analysis and has been

used in the past to assess a variety of specimens such as Neanderthal dentaries (Smith et al., 2010), fossils within amber (Dierick et al., 2007; Penney et al., 2007), and Oligocene worm-bored whale bone (Kiel, 2010). There is also precedence for using Micro-CT in the study of fossil salmonid specimens. For instance, Eiting and Smith (2007) used Micro-CT scans of two Miocene *Oncorhynchus* species, *O. ketopsis* and *O. salax*, to determine the morphology of gill rakers in these fossil taxa.

Micro-CT assessments of fossils revealed several structures and textures of interest born by the ancient salmonid dentaries examined in this study. Among these structures and tissues are: breeding teeth in *Oncorhynchus nerka*, networks of channels and tunnels at the interior of bone (blood vessels), remnants of the lateral line canal system, spongy bone within tooth-bearing regions (alveolar bone), large hollows and honeycomb chambers at the interior of the dentary (fat pockets), and surficial, nodular textures at the anterior margin of several specimens that are suggestive of a kype.

#### *Breeding teeth*

The presence of a breeding tooth in the early Pleistocene *Oncorhynchus nerka* specimen (UWMB-87121, Skokomish Valley, Washington) is expected given those found in kype-bearing, spawning *Oncorhynchus* species alive today. This breeding tooth rests upon a large, spongy tooth base that transitions abruptly into the compact bone of the rest of the dentary (Fig. 4.20 e). In addition to resembling conditions in extant *Oncorhynchus* species, the breeding tooth and tooth base in UWBM 87121 resembles the structure of the kype skeleton in Atlantic salmon described in Witten and Hall (2002), who noted that the contact between compact bone of the dentary and the skeletal tissue of the kype is marked by an abrupt shift in bone type. At this contact, the lamellar bone of the compact dentary gives way almost immediately to the cancellous, spongy bone of the kype skeleton that is full of blood vessels.

#### *Blood vessels*

With the exception of Miocene *Salvelinus* specimen UOF-26785 and Miocene *Oncorhynchus* specimens UOF-26773, and UOF-26782, intricate networks of channels and tunnels are visible at the interior of the bone. While these blood vessel networks are

found throughout the dentaries of many of the specimens scanned, they are particularly prevalent in the breeding teeth bases of the *O. nerka* (UWBM-78121) and *O. rastrosus* specimens. These channels may be large in diameter (Fig. 4.27 e, light green arrow) or may be small and closely associated with other channels (Fig. 4.20 e, green arrows). Extensive networks of blood vessels would supply the tissues associated with them with a ready supply of resources, a supply that would be particularly important in individuals experiencing spawning-related secondary modifications to their skeleton and other tissues.

#### *Lateral line canal*

While some of the pores on the surfaces of the bone lead into the intricate blood vessel networks within the bone of the dentary (for example Fig. 4.3, blue arrows), other pores, occurring in a regularly spaced row on the lateral surface of the bone, represent the remnants of a lateral line canal system. This mechanosensory system is comprised of a canal that is open to the surrounding water via pores in the bone and epithelium (Webb, 2014). This canal contains a single row of neuromast receptor organs that are spaced such that one organ is located between two adjacent pores. Each neuromast receptor organ is composed of a ciliary bundle with a single long kinocilium in association with several short stereovilli on one side (Webb, 2014). Water flowing over these ciliary bundles will result in the movement of the kinocilium away from or toward the stereocilia, depending on the direction of water flow. As a result, the fish is sensitive to the subtle changes in water flow direction that may indicate an oncoming predator or evading prey.

#### *Alveolar bone and blood vessel channels*

While remnants of lateral line canals are discernible in only a few specimens, each specimen examined with the exception of the Quaternary *Stenodus leucichthys* specimen (CMNFV-48886) displays a region of tissue on the dorsal surface of the bone that was spongiose. This tissue region is similar to the tissues found in the same region in modern salmonids that contains numerous blood vessels and fat accumulations. In the fossil specimens, this tissue is full of channels that form an intricate, dendritic network within this tissue and in other areas of the dentary, meaning that this tissue was likely full of

many blood vessels. A rich blood supply would, as in the case in modern salmonids, have supported the growth and maintenance of teeth, repairing damaged dentition and, in *Oncorhynchus*, aiding in the growth of breeding teeth. The many hollows and complex density patterns within the dorsal, spongiöse tissues of these fossil specimens may be evidence of alveolar bone connecting teeth to the dorsal margin of the dentary. This hypothesis is supported by the absence (CMNFV-48886) and minimal evidence (UOF-26789) of these tissues in specimens that do not appear to have surviving teeth.

#### *Fat accumulation hollows*

Another interesting tissue found in this assessment is located within the interior of the shaft (ramus) of the dentary bone where regions full of hollows and empty spaces exist. These regions are found to some extent in the majority of specimens examined here and are typically found posteriorly in a given specimen. These tissues are more prevalent laterally than medially. Whereas the channels noted above are representative of blood vessels, these discrete, isolated encasements of empty space represent the sites of accumulations of fat. These fat accumulations are most prevalent in more mature and spawning specimens, but are particularly numerous in the *O. rastrosus* material (Fig. 4.28 e, Fig. 4.29 e, Fig. 4.30 e, orange arrows).

#### *Nodular textures*

The final tissue of interest found in this examination is the surficial textures evident in the *Salvelinus*, *O. nerka*, and *O. rastrosus* specimens. These textures were first noted in the physical examination of this chapter where they were found to be present on each *Salvelinus* specimen and on the *O. rastrosus* UWBM-71908a specimen, in particular, but also UWBM-71908b. These textures are nodular and range from needle-like, as in UOF-26785, to warty, as in UOF-26786. Composed of small elements, these textures tend to be localized to the anterior of the tooth bearing regions of the dentary.

In sagittal section, these textures are associated with interesting density gradients. The scans appear to be most dense where they are associated with these textures, following which the density gradient transitions into a lower density region of spongy bone, and finally into more dense and linearly organized bone at the interior of the

dentary. This transition is shown particularly well in UOF-86786 (Fig. 4.9f), UWBM-87121 (Fig. 4.20e), and UWBM-71908b (Fig. 4.30 e), but is present at some scale in each of the specimens bearing this rough, anterior dorsal texture. As was shown in Chapter 2, transitional gradients like this are often found in kype-bearing individuals, where the low density soft tissue transitions into the spongiöse intermediate tissue of the kype, and finally into the compact, very dense bone of the mature dentary. Thus, a gradual trend of low to high density material at the anterior portion of a salmonid dentary may be indicative of the presence of a kype, even a still developing one.

The transition between low to high density skeletal elements in these fossil specimens is marred by a dense exterior region adjacent to the nodular surficial textures. The highly mineralized nature of these exterior features may, however, be due to the fossilization process. During fossilization, spaces between skeletal elements are filled by minerals in the seeping ground water, even before partial or complete replacement of skeletal elements occurs (Prothero, 2004; Prothero and Schwab, 2013). Thus, rather than be evidence against the presence of a kype, the highly mineralized nature of these texture may be evidence of spongy or honeycombing kype-related skeletal elements present in the living individuals prior to their death and fossilization. Additionally, this dense exterior region may be a beam hardening artifact that is frequently observed in Micro-CT scans of the exposed surface of fossilized bone (Meganck et al., 2009). Paleohistological assessment in Chapter 5 may reveal further information as to the structure and composition of these features.

Micro-CT assessment provides detail as to the internal structure of these specimens that supplements that of the analysis of external and overall structure possible during physical examination. This technique is also useful as a means of preserving the original form of the fossils, down to exact measurements. This precision is something that is not permitted with casts, where slight fluctuations in moulding or casting material may result in minute changes in the parameters of a fossil. However, as useful and informative as Micro-CT scanning of fossil material is, it is limited by the material being scanned. A highly mineralized fossil or a fossil embedded in matrix may not permit scanning or may yield poor detail, as was the case with UOF-31389. Paleohistological analysis, as will be

discussed in Chapter 5, offers the opportunity of gathering even more detail as to conditions within the tissues of these specimens.

### *Literature review*

Following physical examinations of specimens and Micro-CT assessments, I used a literature review in order to supplement the information gathered from the examination of several fossil specimens collected for the purposes of this study. Please refer to Chapter 2, and Table 2.3, in particular, for greater detail as to extant organisms.

As with Chapter 2, I sought direct references to evidence for or against kype growth, as well as to positive indicator terms referring to a secondary elongation of the lower jaw, craniofacial sexual dimorphism, changes in the profile of the head during the spawn-run, and presence of compensatory features in the upper jaw. The results of this review are summarized in Table 4.3 and outlined in further detail below.



**Table 4.3 - Summary of literature survey of kype presence or absence in species known only from the fossil record.**

<i>Genus species</i>	Kype developing?	Literature
<i>Stenodus</i>		
- <i>S. leucichthys</i>	No	Alt, 1969; Alt, 1973; Alt, 1977; Brown et al., 2007; Cumbaa et al., 2010; Cumbaa et al., 1981; Dymond, 1943; Eckmann, 1991; Howland et al., 2000; Nelson, 2006; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993; Walters, 1955; Willson, 1997
<i>Eosalmo</i>		
- <i>E. driftwoodensis</i>	No	Ludvigsen, 1996; Stearley and Smith, 1993; Wilson, 1977, 1992; Wilson and Li, 1999; Wilson and Williams, 1992
<i>Salvelinus</i>	Yes; “notch” compensatory feature in upper jaw	Armstrong, 1965; Balon, 1980; Fleming, 1998; Haugland et al., 2011; Johnson, 1980; Stearley and Smith, 1993; Willson, 1997
- <i>S. larsoni</i>	No	Kimmel, 1975; Smith, 1975; Smith et al., 1982; Stearley 1992; Stearley and Smith, 1993
<i>Oncorhynchus</i>	Yes, “snout” compensatory feature on upper jaw	Behnke, 2002; Cavender, 1970; Groot and Margolis, 1991; Healey, 1991; Keenleyside and Dupuis, 1988; Morton, 1965; Quinn and Foote, 1994; Ricker, 1972; Scott and Crossman, 1973; Stearley and Smith, 1993; Vladykov, 1962; Willson, 1997
- <i>O. nerka</i>	Yes	Behnke, 2002; Brett, 1995; Burgner, 1991; Chebanov et al., 1984; Fleming, 1998; Foerster, 1968; Foote and Larkin, 1988; Ford et al., 1995; Fukushima and Smoker, 1998; Gilhousen, 1960; Hendry and Berg, 1999; Hendry et al., 1998; Hendry and Quinn, 1997; McPhee and Quinn, 1998; Morton, 1965; Murray et al., 1989; Quinn and Foote, 1994; Ricker, 1938, 1972; Roberge et al., 2002; Scott and Crossman, 1973; Stearley and Smith, 1993; Willson, 1997
- <i>O. rastronus</i>	No	Behnke, 2002; Cavender and Miller, 1972; Eiting and Smith, 2007; Nelson, 2006; Smith et al., 2000, 1982; Stearley and Smith, 1993

Species of interest include:

***Salvelinus* (trouts and chars)**

***Salvelinus larsoni***

This species was included as a representative of *Salvelinus* species known only from fossils. *Salvelinus larsoni* is a large, Mio-Pleistocene trout-like fish with characteristics similar to both *Hucho* and *Salvelinus* (Kimmel, 1975). There was no positive evidence of kype growth in *Salvelinus larsoni*.

## ***Oncorhynchus* (Pacific salmon and trout)**

### ***Oncorhynchus nerka* (sockeye salmon)**

Spawning *O. nerka* often display very well-developed kype and snout (Behnke, 2002; Morton, 1965; Scott and Crossman, 1973; Stearley and Smith, 1993). However, the kype may be minimal or absent in small or landlocked (“Kokanee”) individuals (Willson, 1997).

### *Literature review summary placed in context of examined specimens*

As was the case with the consideration of extant species in Chapter 2, the literature survey validates the findings of the physical survey and Micro-CT assessments of specimens included in this study. However, there are some interesting data that emerge as well that will be discussed below. There was no evidence of a kype in the more basal salmonids (*Stenodus* and *Eosalmo*), but the kype is present in the eusalmonines (*Salvelinus* and *Oncorhynchus*), with the exception of *Salvelinus larsoni* and *Oncorhynchus rastrosus*, neither of which have shown positive evidence of kype growth.

## **Final discussion and conclusions**

As has been determined by several researchers including Wilson (1992) and Wilson and Williams (1992), fossils can yield a wealth of information that can help support and reinforce data collected from extant specimens. Fossil evidence can be used to test phylogenetic trees constructed from modern evidence and can provide new information as to transitional states, including the order in which the sequence of transitions occurs (Wilson, 1992; Wilson and Williams, 1992). This chapter uses fossil specimens to expand on the information gathered as to presence and absence of the kype in salmonids. Using appropriately modified yet similar techniques as those used on the modern specimens of this study, this chapter demonstrates that physical examinations, Micro-CT assessments, and literature surveys can each be used to derive data from fossil material that is comparable to that found in modern material. This fossil data can be used to determine if a record of kype growth is present in fossil specimens and to establish if transitions from basal to more modern conditions at the family, genus, and species levels are evident in the

fossil record.

***A record of kype growth is preserved in fossilized relatives of modern, kype-bearing species***

As is described above in relation to the fossil specimens included in this study, a record of kype growth is preserved in the fossil record. This record may be subtle, as in the nodular textures of *Salvelinus* UOF-26785, UOF-26786, and UOF-26787, or prominent, as in the several kype- and breeding teeth-bearing *Oncorhynchus nerka* specimens (UWBM-87112, UWBM-87121, and UWBM-95842). Secondary reproductive modifications are also evident in several *O. rastrosus* specimens, where enormous premaxillary breeding teeth or minute nodular features on the dentary are present. The evidence of kype growth identified in the fossils included in the current study complements the findings of previous research such as those indicated in Table 4.3, while at the same time suggests that kype growth may have occurred in *O. rastrosus*, as will be discussed below.

***Using fossil material to discern transitions from basal to more derived states***

As noted in previous research such as that of Wilson (1992) and Wilson and Williams (1992), study of fossil specimens can both complement and supplement data collected from living organisms. In the case of the specimens described in this chapter, a number of trends and patterns emerge that make it possible to distinguish transitions from basal to more derived states. These transitions are evident in salmonids as a whole, as shown in describing the characteristics of *Eosalmo driftwoodensis*, within the same genus, as in *Salvelinus* and *O. rastrosus*, and within the same species, as in *O. nerka*.

***Transition from basal to more derived states in Salmonidae***

*Eosalmo driftwoodensis* is the oldest and most basal known salmonine (Behnke, 2002; Nelson, 2006; Wilson, 1977). Being so basal, this species displays a number of characteristics that are plesiomorphic and are shared by the basal salmonids of Coregoninae and Thymallinae (Wilson, 1977). As with most basal salmonids, *Eosalmo* was relatively small in size (Scott and Crossman, 1973; Behnke, 2002). Reaching approximately 30 cm at maturity, the youngest, smallest specimen known is only 15 cm

(Wilson, 1996). In addition to a small body size, these fish also had a small, squat head. Other plesiomorphic characteristics include short jaws and a small mouth, as well as an exclusive freshwater lifestyle. Furthermore, there is no record of this species growing a kype, as noted above and in previous research (Stearley and Smith, 1993; Wilson, 1977, 1992; Wilson and Li, 1999; Wilson and Williams, 1992).

While many of the characters found in *Eosalmo* are plesiomorphic, this species also displayed several synapomorphies of more derived salmonines. While the small size of the body is primitive, its elongate shape is not. Furthermore, the size and shape of the tail and of the scales are more similar to Salmoninae than to either Thymallinae or Coregoninae (Wilson, 1977, 1996). Thus, while *Eosalmo* displayed many primitive characteristics, it also bore many features that suggest a transition to the more derived conditions of Salmoninae.

#### *Transitions within genera: Salvelinus and Oncorhynchus*

As when considering a transition from basal salmonids to those that display more derived characters, analysis of basal and derived individuals of the same genus or species may also reveal trends in evolution, particularly of the evolution of the kype and of the other tissues of interest within the jaw. In particular, study of the specimens of *Salvelinus* and *Oncorhynchus* yields evidence of several interesting trends relating to kype evolution.

The Miocene *Salvelinus* specimens UOF-26785, UOF-26786, and UOF-26787 share many of the same characteristics as some of the more basal *Salvelinus* species such as the extinct, Miocene *S. larsoni* and the extant *S. namaycush*. For instance, the fossils are robust and have a similarly shaped shelf-like ledge on the inner surface of the dentary that is distinctive of less derived *Salvelinus* (Stearley and Smith, 1993). However, while *S. larsoni* and *S. namaycush* exhibit no evidence of kype growth, each of the fossil specimens examined here display evidence of features that are suggestive of a kype. While it is not possible to identify UOF-26785, UOF-26786, and UOF-26787 down to the species level using the anterior portion of the dentary alone, the presence of the aforementioned ledge on the inner dentary surface would suggest that these Miocene specimens represent basal *Salvelinus* individuals. If this is the case, the presence of kype-like elements on these basal *Salvelinus* fossils serves as evidence that kype is an ancestral

feature of this genus. Such evidence supports the phylogenetic positioning of *Salvelinus* within the Eusalmonine, and leads to doubts regarding the appropriateness of a kype-bearing *Salvelinus* and non-kype-bearing *Hucho* sister group.

Patterns of kype presence and absence in the fossil record would fit easily on a phylogenetic tree with a *Hucho-Salvelinus* sister group if the loss of kype growth in *Salvelinus larsoni* represented an ancestral loss at the base of the sister group that was maintained in basal *Salvelinus* species. Essentially, this would mean that the lack of kype growth in basal *Salvelinus* species such as *S. larsoni* may represent a transition from the plesiomorphic, non-kype-bearing condition shared by *Hucho*, *Salvelinus*'s potential sister group, to the more derived kype-bearing condition of other *Salvelinus* species, including the species of the *Salvelinus* fossil specimens examined in this study. However, this hypothesis of non-kype-bearing basal *Salvelinus* would either require a loss and reacquisition of the kype (Fig. 4.31) or the acquisition of kype in two clades: “advanced: *Salvelinus* and “*Salmo-Oncorhynchus*.”

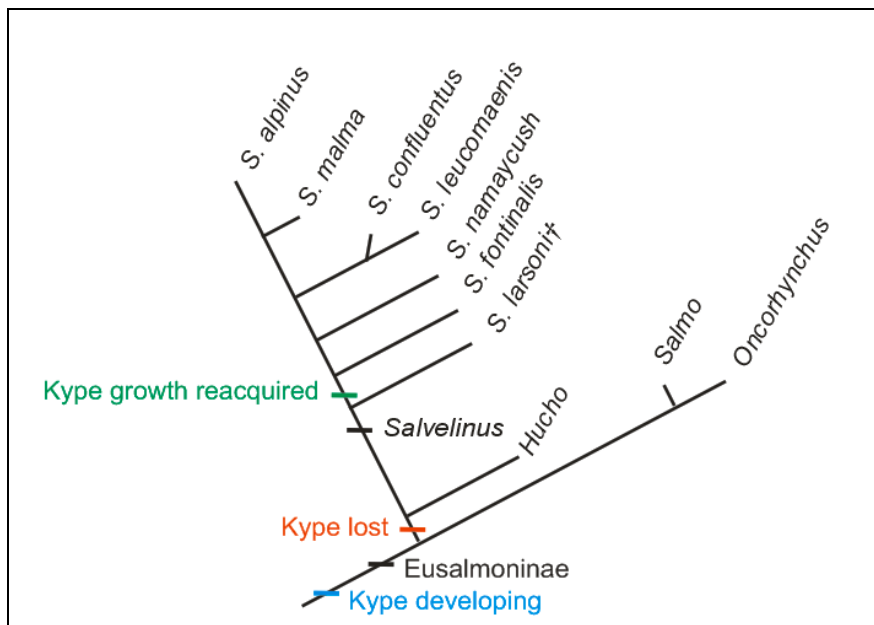


Figure 4.31 – Kype loss and reacquisition in the *Salvelinus-Hucho* sister group. Tree modified from Stearley (1990, 1992); Stearley and Smith (1993), and Crane et al. (1994).

While reacquisition of a kype in more derived *Salvelinus* or independent evolution of a kype in *Salvelinus* and *Salmo-Oncorhynchus* are possible, a simpler explanation for kype evolution is a relocation of *Hucho* and a loss of kype growth in *S. larsoni*. Indeed,

perhaps, as with *S. namaycush* (Martin and Olver, 1980), *S. larsoni* may simply not have grown a kype even though it had the capacity to do so. While *S. namaycush* does not grow a kype, previous research has found that these fish are able to grow a kype (Behnke, 2002; Martin and Olver, 1980). Although there is no evidence of kype growth in *S. larsoni*, it may, like *S. namaycush*, have been capable of kype growth. The evidence of kype-like features in the fossil *Salvelinus* specimens examined in this study, contemporaries of *S. larsoni*, support the hypothesis that kype growth is an ancestral character shared by *Salvelinus*, *Salmo*, and *Oncorhynchus*. If this is the case, it may serve as additional evidence that, as discussed in Chapter 2 and 3, a phylogenetic tree taking into account evidence for the repositioning of *Hucho* is required (Fig. 4.32).

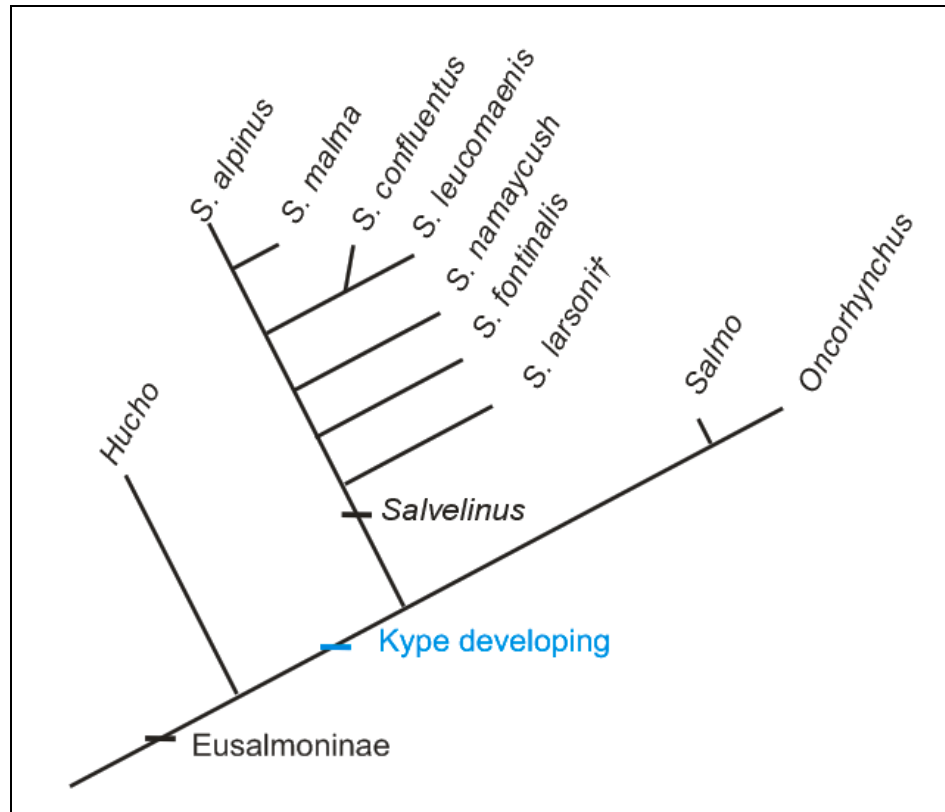


Figure 4.32 – Acquisition of kype growth character in Eusalmonine. Modified from Oleinik and Skurikhina (2008).

As shown here, the analysis of *Salvelinus* fossil material yields information as to the trends in kype evolution and phylogenetic relationships within the genus. Similarly, the study of fossil *Oncorhynchus* specimens reveals patterns and trend across the

*Oncorhynchus* genus and within species, as is shown in *O. rastrosus* and *O. nerka*.

As with the *Salvelinus* fossil material, *Oncorhynchus rastrosus* reveals evidence of transitions between basal and more derived conditions within the same genus. Characters exhibiting these transitions include dentition, kype growth, and fat accumulation. Unlike many of the modern *Oncorhynchus* species, *O. rastrosus* specimens display little dentition. In this species, a trend in tooth reduction that began subtly in more basal *Oncorhynchus* species is taken to extremes (Booke, 1975; Cavender and Mills, 1972); *O. rastrosus* is virtually toothless, with no maxillary teeth at all. However, what teeth this species has are very large. Several specimens display one or two large, robust dentary teeth and a single, extremely large tooth on each premaxillary. Often referred to as “breeding teeth” in the literature (Cavender and Miller, 1972; Eiting and Smith, 2007; Smith, 1981; Smith et al., 2000), these teeth are so large that they have resulted in the common name of this species: “Sabre-tooth salmon.” If, as was seen in other *Oncorhynchus* species, these teeth are breeding teeth and are associated with reproductive behaviour, they may raise some interesting questions as to the fate of the kype in the *Oncorhynchus* genus.

According to the literature, *Oncorhynchus rastrosus* did not grow kypes (Cavender and Miller, 1972). However, a lack of a kype in this species would be rather surprising. *Oncorhynchus* species, in particular anadromous species of this genus, display some of the best developed spawning-related craniofacial changes found in all salmonids. In several cases, these modifications result in extensive transformation of dentition, albeit with minimal associated needle-like extensions (Chapters 2 and 3, Behnke, 2002; Scott and Crossman, 1973). However, according to the literature and many of my assessments, kype growth is not found in *Oncorhynchus rastrosus*. Yet, curious features on the surface of the UWBM-71908 specimens echo the skeletal fibres and extensions found in other spawning salmonid species. Indistinct though these nodular features may be, they yield a texture and have a composition that is similar to those found in the fossil *Salvelinus* and *O. nerka* specimens examined in this study. Paleohistological assessment of these tissues is needed to determine if these nodular textures are evidence of kype presence.

Although the features found in UWBM-71908 a and b are restricted to a slightly different location than was the case in the *Salvelinus* specimens, being more dorsal than

ventral, they are found in a region where one would expect to find the skeletal tissues associated with an *Oncorhynchus* kype. While kype-bearing *Salmo* individuals display a starburst of skeletal needles extending from the anterior dentary to several centimetres on the lateral and ventral margins of the bone, spawning-related modification in the lower jaw of *Oncorhynchus* is dominated by the development of large fang-like teeth. These teeth are of lower density than the other teeth of the specimen and do not appear to be present in non-kype bearing individuals (see Chapter 2 “Micro-CT assessment discussion”). These breeding teeth are particularly large in *O. kisutch*, *O. keta*, *O. gorbuscha*, and *O. nerka*.

As is shown in Figure 2.33, reproduced below, *O. keta*, *O. gorbuscha*, and *O. nerka* are some of the closest relatives of *O. rastrosus*. While *O. rastrosus* may be a taxonomic outlier, possessing traits and characters that are unique to it alone, these characters may also present the culmination of evolutionary trajectory that began more basally. Perhaps the minimal skeletal fibres and large breeding teeth of *O. kisutch*, *O. keta*, *O. gorbuscha*, and *O. nerka* represent a transition from the plesiomorphic, skeletal needle-dominated kype of *Salvelinus* and *Salmo* to a condition where skeletal needles have been replaced in function by exceptionally large breeding teeth, as found in *O. rastrosus*. With the addition of these large breeding teeth, the rough, nodular textures found in UWBM-71908 a and b may in fact represent an extreme version of the *Oncorhynchus* kype.



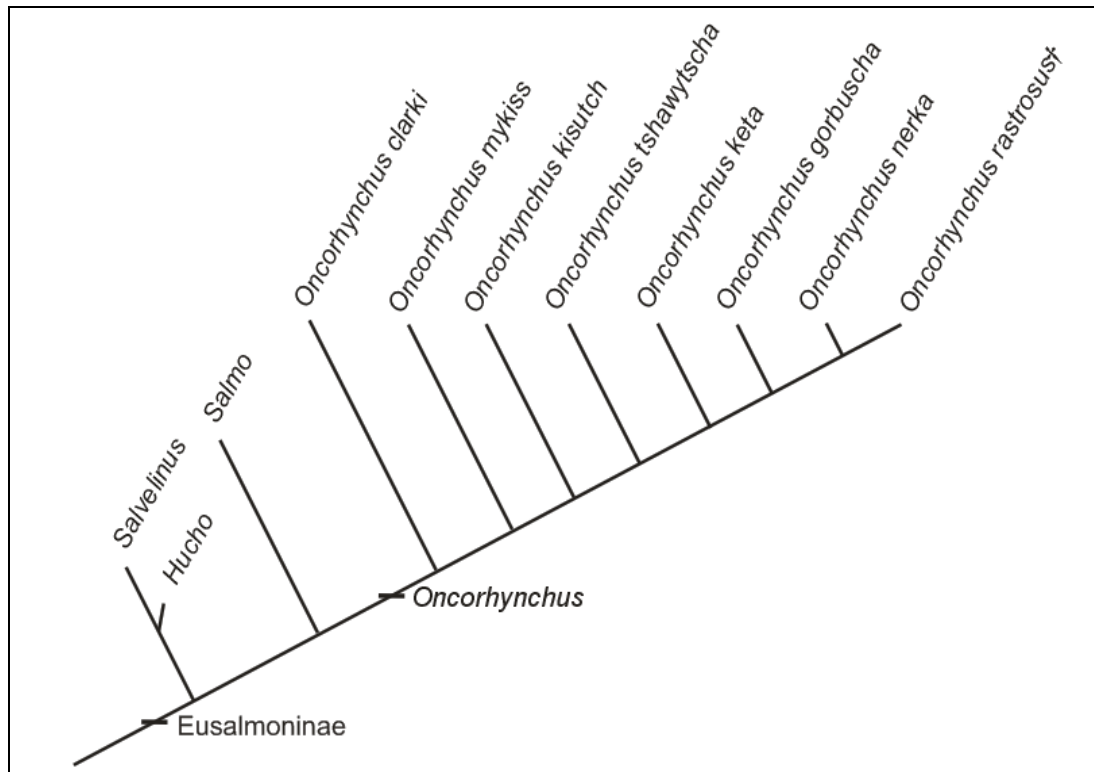


Figure 2.33 - Eusalmonine phylogenetic tree with particular focus on the genus *Oncorhynchus*. Modified from Stearley and Smith (1993).

As with breeding teeth and growth of a kype skeleton, *O. rastrosus* also shows a transition to arguably more derived conditions in the other tissues of interest within the lower jaw. Among these tissues are large numbers of enclosed fat accumulations. As shown in Chapters 2 and 3 in living salmonids and in the Micro-CT scans of the *Salvelinus* specimens and the other *Oncorhynchus* fossils, fat accumulations occur in virtually all salmonids to some degree. These accumulations tend to be particularly large and numerous in the most mature specimens, especially in spawning individuals. However, the structure of these tissues is taken to the extreme in *O. rastrosus* where they form a network of empty chambers that almost hollows out the whole of the posterior portion of the dentary. When establishing the new species of *O. rastrosus*, Cavender and Miller (1972) compared these hollow regions of the dentary bone in *O. rastrosus* to those found in *O. tshawytscha* and *S. salar*. In this comparison, the authors found that, while each species displayed evidence of a hollowing out of the shaft of the dentary, the dentary of *O. rastrosus* was much hollower than either of the other two specimens, though *O.*

*tshawytscha* was more honeycombed than *S. salar*. The honeycombed nature of the bones of *O. rastrosus* and, to a lesser degree, the other salmonines makes the bones of these fish lighter. Honeycombing bones with air pockets and fat accumulations lightens the skeleton of an individual significantly and may allow animals to grow large without detrimentally reducing the overall strength of their skeleton (Cavender and Miller, 1973). This maximizing of skeletal strength and minimizing of skeletal mass is also characteristic of the spongiosa in mammalian bone marrow (Parfitt, 1988).

In addition to the mass minimization and structural maximization of this honeycombing, as was discussed in Chapter 3, salmonids, particularly migrating and anadromous salmonids, store fat reserves in their muscles and bones as sources of energy (Cavender and Miller, 1972; Jobling et al., 1998; Jørgensen et al., 1997; Phleger et al., 1989; Phleger et al., 1995). That the networks of hollows and empty space in the specimens examined in this study are most prevalent in the largest animals and in those animals believed to be anadromous would suggest that these honeycombed dentary bones represent the remnants of fat pockets that served the dual purpose of lightening the skeletal load and of serving as resource reserves.

#### *Transitions within species: O. nerka*

While *O. rastrosus* serves to illustrate transitions in character states across the *Oncorhynchus* genus, *O. nerka* is a case study in transitions within a species. In *O. nerka*, differences in the structure and composition of the kype and its related structures are evident when comparing living and fossil specimens. The most pronounced differences between living and fossil specimens of *O. nerka*, and of other *Oncorhynchus* specimens lie in the comparatively minimal modification of the anterior jaw in kype-bearing individuals, particularly the relatively small number and size of the “breeding” teeth in fossil individuals.

The “breeding” teeth of living *O. nerka* (CMNFI-19 77-0277.1) when measured from the dentary to the tooth apex are more than three times the length of other teeth present on the dentary, while those of UWBM-87112 and UWBM-87112, for instance, are barely twice the length. Furthermore, while CMNFI-19 77-0277.1 displays three large breeding teeth, UWBM-87121 appears to have only one, though UWBM-87112 has at

least two. Although these differences in the complexity and size of the kype-related modifications in these living and ancient individuals may be the result of alteration of tissues during the fossilization process, they may also result from differing conditions between ancient and living individuals. These conditions include factors such as body size, sex, life history stage, and the potentially basal or more primitive condition of some of the fossil individuals examined in this study.

The comparatively minimal size of kype in these fossil specimens may be due to smaller body sizes in the fossil specimens. As has been suggested by several researchers (Behnke, 2002; Groot and Margolis, 1991; Scott and Crossman, 1973) and as was found in Chapter 2 of this thesis, the kype of smaller *Oncorhynchus* individuals is much smaller than those of larger ones. However, in comparing several of the skeletal elements of CMNFI-19 77-0277.1 to those of UWBM-87112, UWBM-87121, and UWBM-95842, any difference in body size between an animal with well-developed kype and these fossil specimens is minimal.

Another explanation for the smaller kype in UWBM-87112, UWBM-87121, and UWBM-95842 as compared to modern specimens of *O. nerka* may be that these animals represent females. While large, anadromous females in most of the eusalmonine species do develop many of the secondary sexual characteristics seen in males, these traits tend to be poorly developed when compared to their male counterparts (Behnke, 2002; Morton, 1965, 1980; Keenleyside and Dupuis, 1988; Willson, 1997). However, analysis of UWBM-87122, a large individual without any evidence of kype from the same deposit as UWBM-87112, UWBM-87121, and UWBM-95842 would suggest that, while this may be evidence of individual variation, females in this population did not develop kype at all. Although larger than UWBM-87112, UWBM-87121, and UWBM-95842, UWBM-87122 shows no evidence of spawning teeth or modification to the dentary suggestive of kype.

Rather than being an issue of body size or sex, perhaps the kype of the fossil specimens were still in development. As was found in Chapter 2, the presence of a developing kype is often difficult to impossible to discern. However, UWBM-87112, UWBM-87121, and UWBM-95842 were each from a post-spawn death assemblage in Skokomish Valley (Smith et al., 2007). Smith et al. (2007), in describing the specimens from this site, noted that several individuals displayed traits indicative of migration and

nest digging, going so far as to say that the positioning of individuals within the assemblage was similar to that of modern post-spawn die off.

If the smaller kype in the fossil specimens cannot be explained away by differences in body size, sex, or life history stage, it may be the result of the relative primitiveness of the specimens. Smith et al. (2007) noted that the fossils found at the Skokomish Valley assemblage site share many characteristics with *O. gorbuscha*. While Smith et al. (2007) identified these specimens as *O. nerka* on the basis of several traits such as the shape of the preoperculum and parietals, the length and shape of the fins, and the absence of a dorsal hump, many other characters such as the shapes of the dentary teeth and dermethmoid were variable to intermediate in the Skokomish fossils. Thus, while these specimens are believed to be *O. nerka*, that they display characteristics similar to those of *O. gorbuscha* suggests that they represent animals from a period shortly after the divergence of *O. nerka* and *O. gorbuscha*.

*O. gorbuscha* develops a kype that is straighter and less prominent than that of many other *Oncorhynchus* species (Behnke, 2002; Heard, 1991; Hendry and Stearns, 2004; Scott and Crossman, 1973). Additionally, the breeding teeth of *O. gorbuscha*, though large, are smaller than those of modern *O. nerka*. While *O. gorbuscha* has selected for colouration and hump height, the minimal size of kype in these early *O. nerka* individuals supports evidence that well-developed kypes and breeding teeth have been favoured over other secondary sexual characteristics in *O. nerka*. The fossil *O. nerka* specimens examined in this chapter may reflect the more plesiomorphic conditions of a smaller, less developed kype and smaller, fewer breeding teeth present in the species following the *O. gorbuscha*-*O. nerka* split.

As has been discussed previously, fossil evidence can be used to test phylogenetic trees constructed from modern evidence and can provide new information as to transitional states. Analysis of traits in *Eosalmo driftwoodensis* reveals transitions from the basal conditions of the Coregoninae and Thymallinae to the more derived traits of the Salmoninae. Furthermore, transitions from arguably more basal to more derived conditions in the same genus or species are evident when studying *Salvelinus* and *Oncorhynchus* material, particularly in relation to the evolution of kype growth. Not only

does comparison between fossil and living *Salvelinus* specimens reveal potential ancestral traits, but examination of these traits may reveal patterns that can be used to assess pre-existing phylogenetic trees. Analysis of *Oncorhynchus rastrosus* fossils may reveal trends in the evolution of spawning related tissues, including the kype and breeding teeth as well as fat accumulations. These trends may reveal an evolutionary trajectory that affected many of the more derived *Oncorhynchus* species or may reflect extreme modifications in one taxonomic outlier. Finally, in studying fossil *O. nerka*, transitions in character states within the same species from basal conditions following divergence from *O. gorbuscha* to more derived characters in living individuals are evident. These case studies, taken from the fossil record of the Salmonidae, reinforce the previous findings of researchers such as Wilson (1992) and Wilson and Williams (1992), who stated that analysis of fossil material can complement and reinforce data taken from the study of extant specimens.

### ***Sources of error***

As with the study of living specimens in Chapters 2 and 3, while sources of error were mitigated and minimized as much as possible, sources of error were still present. Some of these sources of error include small sample size; inability to accurately determine life history stage and, in some cases, species; and taphonomy.

As with living specimens examined previously in my thesis, the sample size of fossil specimens assessed was rather small. This small number of specimens is due, in large part, to preservation and collection bias. Salmonids do not tend to inhabit environments that favour preservation and fossilization (Jonsson and Jonsson, 2011; Scott and Crossman, 1973), thus salmonid fossils are relatively rare. When salmonid preservation does occur, individuals are usually residing in a relatively stable environment with low water energy. Such environments include the ocean, lagoons, lakes, and large, shallow rivers and streams with small currents. Those species that inhabit or migrate within environments with less stability and higher water energy, such as many of the more basal salmonids, tend to be preserved even less often. Furthermore, accessing specimens for collection from certain environments such as the ocean or lagoons is not always feasible, further reducing the number of specimens available for study. Thus, while further research that considered the structure and composition of the

lower jaws of a wider range and a larger number of specimens would be helpful in better understanding these fish, such a study may not be possible.

Again as with the living specimens I examined, while life history stage could be estimated for the majority of fossil specimens examined in this study, it was not always possible to distinguish these stages accurately. Loss of many important portions of individuals, including their soft tissues and parts of their skeleton, makes life history stage difficult to determine. As many of these missing elements are also taxonomically important, identification down to the species can also be very difficult, if not infeasible.

### ***Summary***

- The inclusion of physical examinations and Micro-CT analysis greatly improves the understanding of ancient salmonid lower jaw structure.
- Fossils can be incredibly useful in supplementing and complementing information gathered from living specimens, particularly by testing the assumptions of phylogenetic trees built using Recent specimens, demonstrating transitional states, and aiding in determining the order in which derived traits evolved.
- A record of kype growth is preserved in the fossil record and may be subtle or prominent. Physical examination and Micro-CT assessments reveal a great deal as to the structure of a remnant kype; however, paleohistological analysis is required in assessing composition of the kype and of other tissues of interest in the lower jaw.
- The structural elements and tissues in ancient kype bearers are similar to those of living ones. Large fat storages, many spaces for blood vessels, and enormous breeding teeth are much in evidence in fossil specimens.
- Transitions from basal salmonids to more derived salmonines, and, indeed, eusalmonines, can be identified in *Eosalmo*. Transitions from plesiomorphic to derived character states within the same genus or species can also be distinguished, as shown in *Salvelinus* and *Oncorhynchus*, particularly in *O. nerka* and *O. rastrosus*. Several of these trends are useful in understanding the evolution of the kype and of the tissues associated with it and may also be of use in validating alternative salmonid phylogenetic trees (Fig. 4.32).

Physical examination and Micro-CT assessment is useful in assessing kype presence and absence in the fossil record. Furthermore, these techniques provide an opportunity to assess transitional states from basal to more derived species in the salmonid family, as is the case with *Eosalmo*, and may also reveal transitions within genera or even within species. However, while physical examination and Micro-CT assessment result in evidence as to structural characteristics in these fossil specimens, paleohistology is required to assess composition of the kype and of tissues of interest in the jaw. Chapter 5 will use paleohistological analysis to do just that, as well as to attempt to establish when and how the kype evolved.

## Chapter 5 – Composition of the kype and other tissues of interest in fossil Eusalmoninae

### Introduction

In Chapter 4, physical examinations and Micro-CT assessments resulted in a better understanding of the structure of the lower jaw and of the kype in fossil salmonids. However, little is known about the tissue composition of these features. Histological analysis of living salmonids in Chapter 3 revealed evidence of a family-wide distribution of chondroid bone and Sharpey-fibre bone, tissues previously only found in combination in association with kype. Furthermore, several trends are seen in the extent and organization of epithelium, blood vessels, teeth, accumulations of fat, and the skeleton of the kype and its associated connective tissue mass that relate to life history stage and mode. More mature individuals, and spawners in particular, have larger fat accumulations and thicker epithelia than younger and non-spawning specimens. In *Oncorhynchus*, spawners also develop enormous breeding teeth that are absent in non-spawners. Furthermore, semelparous, anadromous eusalmonines tend to display a kype skeleton that is larger and more extensive than is the case in non-anadromous, iteroparous eusalmonines. Whether these trends hold true in ancient salmonids is unknown, as are any of the trends relating to phylogenetic position.

As discussed by Albert et al., (2007), Cobbett et al. (2007), Hilton and Grande (2008), Wilson (1992), and Wilson and Williams (1992), data collected from fossil specimens can expand on research using living and extant organisms. This expansion on research in living organisms using fossils is as true for paleohistology as it is for the methods used in Chapter 4. While soft tissues such as epithelium and connective tissue are long absent in fossil specimens, remnants of the skeletal tissues remain and preserve the composition of the original bone. Furthermore, as is evident in Micro-CT assessments, the remnants of fat accumulations, teeth, and blood vessel networks have also been preserved.

In this chapter, I will assess the composition of the tissues of the kype and of other tissues and structures of interest in the lower jaws of several fossil salmonids. I will use this assessment, in cooperation with data taken from Chapter 4 to reflect on trends in the



evolution of kype and lower jaw tissue structure and composition. I hypothesize that paleohistology will be of great use in revealing the characteristics of the tissues of the lower jaw in fossil salmonids. Specifically, I hypothesize that paleohistology can be used to determine if the same trends, and indeed the same tissues, found in the tissues in living salmonids are also present in ancient individuals. To this end, I will determine if chondroid bone and Sharpey-fibre bone are present in a given specimen. Furthermore, I will assess if patterns in the size, number, distribution, and organization of fat accumulations, blood vessels, and breeding teeth in fossil specimens resemble those of living individuals, particularly those of the same life history stage.

It is important to note that, to my knowledge, this study represents the first paleohistological assessment of fossil salmonids as a whole. Furthermore, several sections from each specimen were produced and examined, making it possible to assess the composition and structure throughout the lower jaw of these ancient fish.

### **Methods and materials**

In an effort to preserve the original structure and form of fossil material to be paleohistologically assessed, several casts were made of each fossil specimen prior to destructive sampling using standard moulding and casting procedures. Such casts are typically required by loaning institutions prior to destructive sampling, but are also useful in an ongoing study when one needs to confirm a particular element or feature in a specimen that has been destructively sampled.

Following the production of several replicas, I made paleohistological slides of specimens. This was done using a standard method as outlined in Appendix E, with sections being made along the long axis of the bone. Contrary to standard procedures, where one or two slides from a given specimen are made, I instead made multiple sections from each specimen. In so doing, I produced 5-18 slides per fossil, depending on the size of the specimen, which could be analyzed together to assess conditions in the tissues throughout the lower jaw.

When interpreting paleohistological slides, I relied heavily on comparisons to living specimens. For instance, in living specimens, I identified blood vessels as being relatively small channels through tissues that may sometimes lead to a vascular sinus. Fat

accumulations, on the other hand, are relatively large, discrete spaces that, while they may be connected to one another, are not connected through tunnels or channels to the exterior of the bone. Furthermore, while the edges of a blood vessel tend to be smooth and regular, the walls of a fat pocket or of a sinus are often rougher and more irregular. However, in a single section, a large blood vessel could be confused for a small fat pocket. Indeed, a fat pocket, regardless of its size, could be confused for a vascular sinus. By using the same identification methods as outlined in Chapter 3, bearing conditions in living specimens in mind, and comparing the paleohistological slides to slices from the Micro-CT scans, I was able to accurately differentiate between a variety of structures and better interpret the composition of tissues in fossil salmonid lower jaws.

The physical examinations and Micro-CT assessments in Chapter 4 permitted the evaluation of the lower jaw structures in the fossil specimens included in this study. Furthermore, the general composition of regions of bone could be considered using Micro-CT scanning. However, in order to appraise the composition of specific regions of tissues and of particular structures, paleohistological analysis is used. The following will consider the composition of tissues in the specimens of this study as specifically as possible, providing examples and context where appropriate. In particular, the current chapter will attempt to confirm structures and features seen in Micro-CT assessments of these fossils but will also establish the composition of those structures at the cellular level.

## **Results**

As was done in Chapter 3 for living specimens, a phylogenetic survey assessing the structure, composition, and organization of the tissues of the lower jaw was conducted for several eusalmonine fossils.

### ***Salvelinus* sp.**

The three *Salvelinus* specimens exhibit a number of characteristics in their structures and tissues that are similar both to one another and to their living counterparts. As with extant kype-bearing specimens, compact, laminar bone in the posterior dentary transitions into less uniformly organized and mineralized tissue anteriorly (Fig. 5.1 a, white arrow). In

contrast to the well-organized, osteocyte-rich tissues of the posterior compact bone (Fig. 5.1 b, inset shows osteocytes in detail), this anterior region displays much more complex patterns of mineralization and tissue organization (Fig. 5.1 c). These anterior tissues display concentric patterns that reflect the mineralized and non-mineralized patterns seen in living individuals (for example, near the anterior ventral margin of the bone of *S. namaycush* in Fig. 3.5 b) and may, particularly at the anteriormost margins, be nearly acellular (Fig. 5.1 d) or contain aggregations of large, well-rounded cell-like features lacking cell processes that often occur in closely-associated pairs or tightly knit groups (Fig. 5.1 e, black arrows). While it is not possible to discern the secretion halos typical of chondrocytes in living tissue, the structure and organization of these cells is similar to non-fossilized chondrocytes.

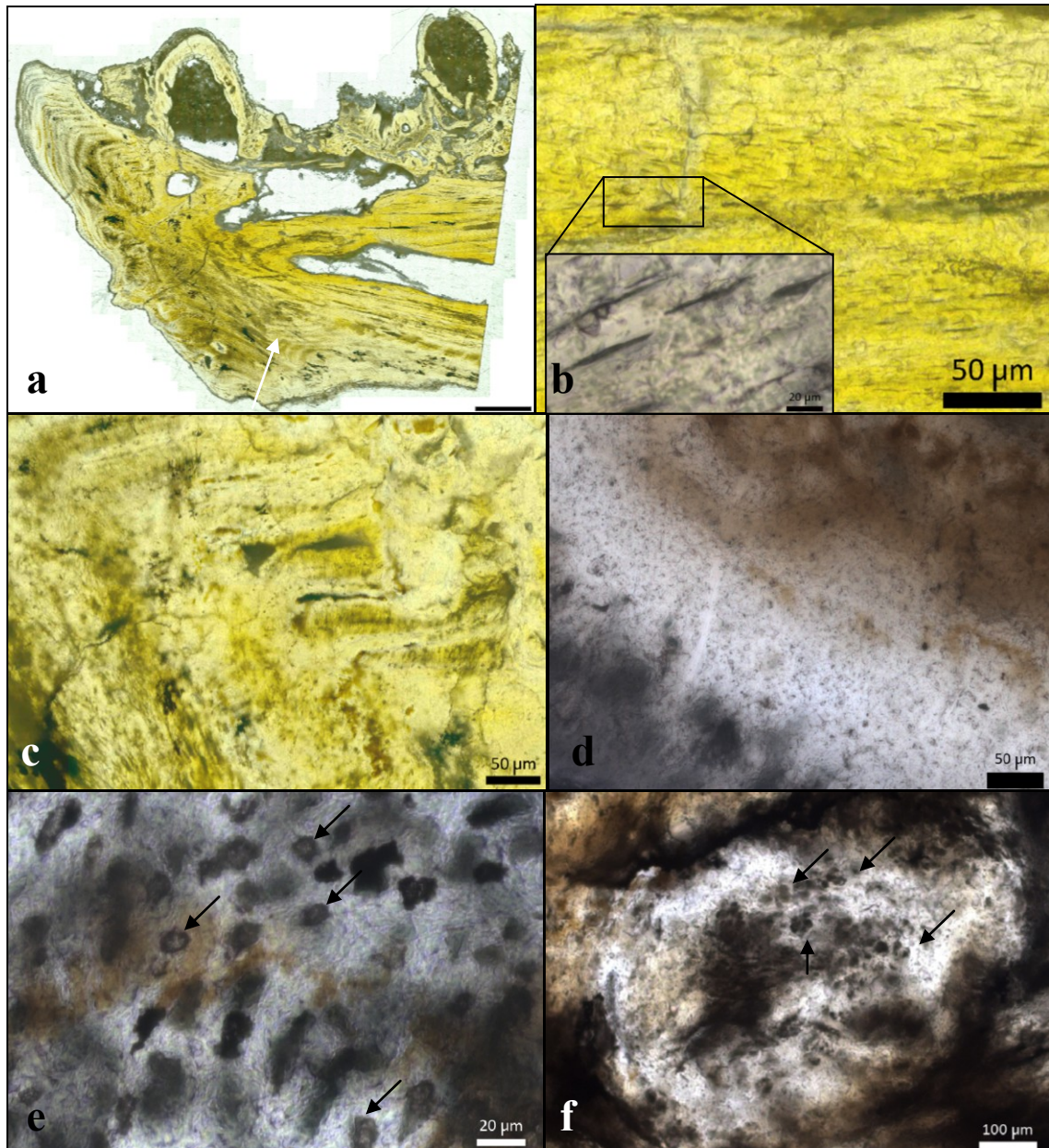


Figure 5.1 – Miocene *Salvelinus* (Worden, Oregon) tissues of interest. (a) A transition from compact laminar bone ventrally and posteriorly to a more complex, less organized anterior and dorsal tissue is evident (white arrow; UOF-26787). While (b) posterior regions are well-organized and osteocyte rich (inset), (c) anterior regions of tissue have much more complex patterns of mineralization and organization that often display concentric patterns similar to the mineralized/non-mineralized patterns in living individuals (UOF-26785). (d) These anterior tissue regions may be nearly acellular at the anterior margins of the bone (UOF-26785) or (e) may contain aggregations of well-rounded chondrocytes (black arrows; UOF-26785). (f) Chondrocytes (some examples indicated by black arrows) are also present at the interior of concentrically mineralized and organized structures (UOF-26785).

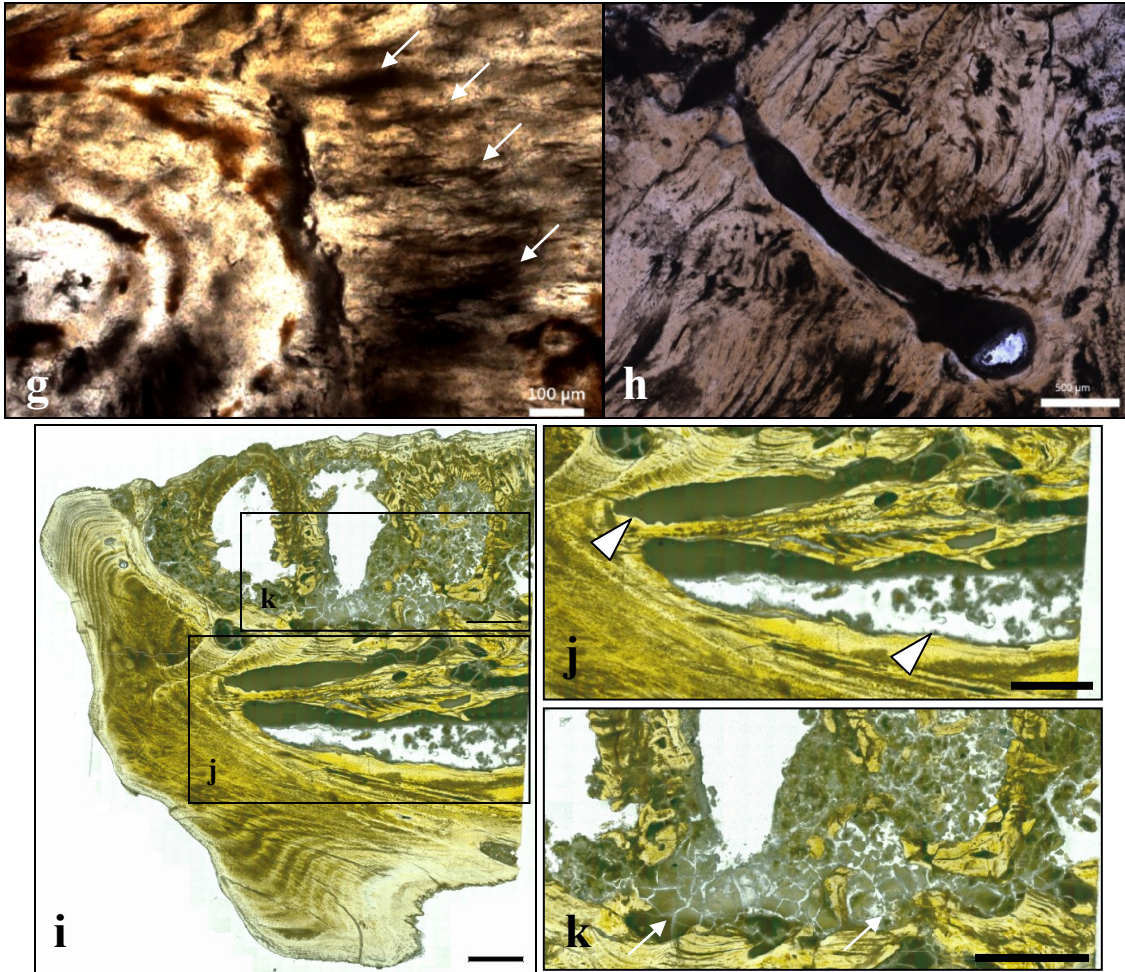


Figure 5.1 – *Continued.* (g) These concentrically mineralized and organized structures may display needle-like features that extend in an array into surrounding tissues (some examples indicated by white arrows; UOF-26785). (h) Channels representing blood vessels are evident at the interior of bone (UOF-26785). (i) Regions of secondary infilling where (j) large fat pockets penetrate the bone (white arrow heads) and (k) spongy alveolar bone connects teeth to the dentary (white arrows; UOF-26786) are evident. Unless otherwise indicated, scale bars are 2000 μm.

These well-rounded cells are also found at the centres of features exhibiting concentric patterns of mineralization (Fig. 5.1 f, black arrows). These features represent the internal, cross-sectioned structure of the nodular textures found in physical examinations and Micro-CT assessments. These concentrically organized features are often associated with fibrous, needle-like extensions that radiate into surrounding material (Fig. 5.1 g, white arrows), much like what is seen in the Sharpey fibres of the kype skeleton of a modern eusalmonine. Given the antero-ventral location of these chondrocyte-rich, concentrically organized structures, and their co-occurring array of needle-like extensions into surrounding tissues, I am interpreting these features as being

cross-sections through dentary spurs representing the remnants of a kype skeleton.

Other features of interest within the tissues of fossilized *Salvelinus* specimens include the many channels filled with a mineral that differs from that which composes the majority of the remainder of the specimen (Fig. 5.1 h). The presence of this second mineral within the bone of these specimens indicates that these channels represent a second taphonomic alteration, one that occurred prior to mineral replacement of the bone of the specimens during fossilization. This secondary infilling suggests that these channels were present in the living specimens and likely represent where blood vessels would have been.

Other regions of secondary infilling are also evident (Fig. 5.1 i). These regions are located at the interior of the bone and nearing the dorsal margin in association with the teeth. In both cases, many chambers, hollows, and empty spaces have been filled with what appears to be the same mineral as filled the blood vessel channels. The hollows at the interior of the bone (Fig. 5.1 j, white arrowheads) are oblong in shape and may be large. These interior chambers represent several fat accumulations, first identified in Chapter 3 in modern specimens (Fig. 3.14) and in the Micro-CT section of Chapter 4 (Fig. 4.20-22, orange arrows). The more dorsal hollows and chambers (Fig. 5.1 k, white arrows) is the spongiöse tissue first identified in the teeth-bearing regions of fish in the Micro-CT analysis of Chapter 4 (Fig. 4.20-22, white arrows). This tooth-associated material is similar in appearance and structure to the alveolar bone identified in Chapter 3 (Fig. 3.12 a-c). As in living salmonids, this spongy bone appears to connect teeth to the dentary.

### ***Oncorhynchus* sp.**

As with the *Salvelinus* specimens, the unknown *Oncorhynchus* specimens display a number of features typical of living individuals within *Oncorhynchus* specifically and in Eusalmoninae as a whole. First identified in Micro-CT analysis, these features include posterior laminar bone (Fig. 5.2 a) that is often well infiltrated with blood vessels (Fig. 5.2 b, black arrowheads). In each specimen, evidence of fat accumulations is virtually nonexistent.

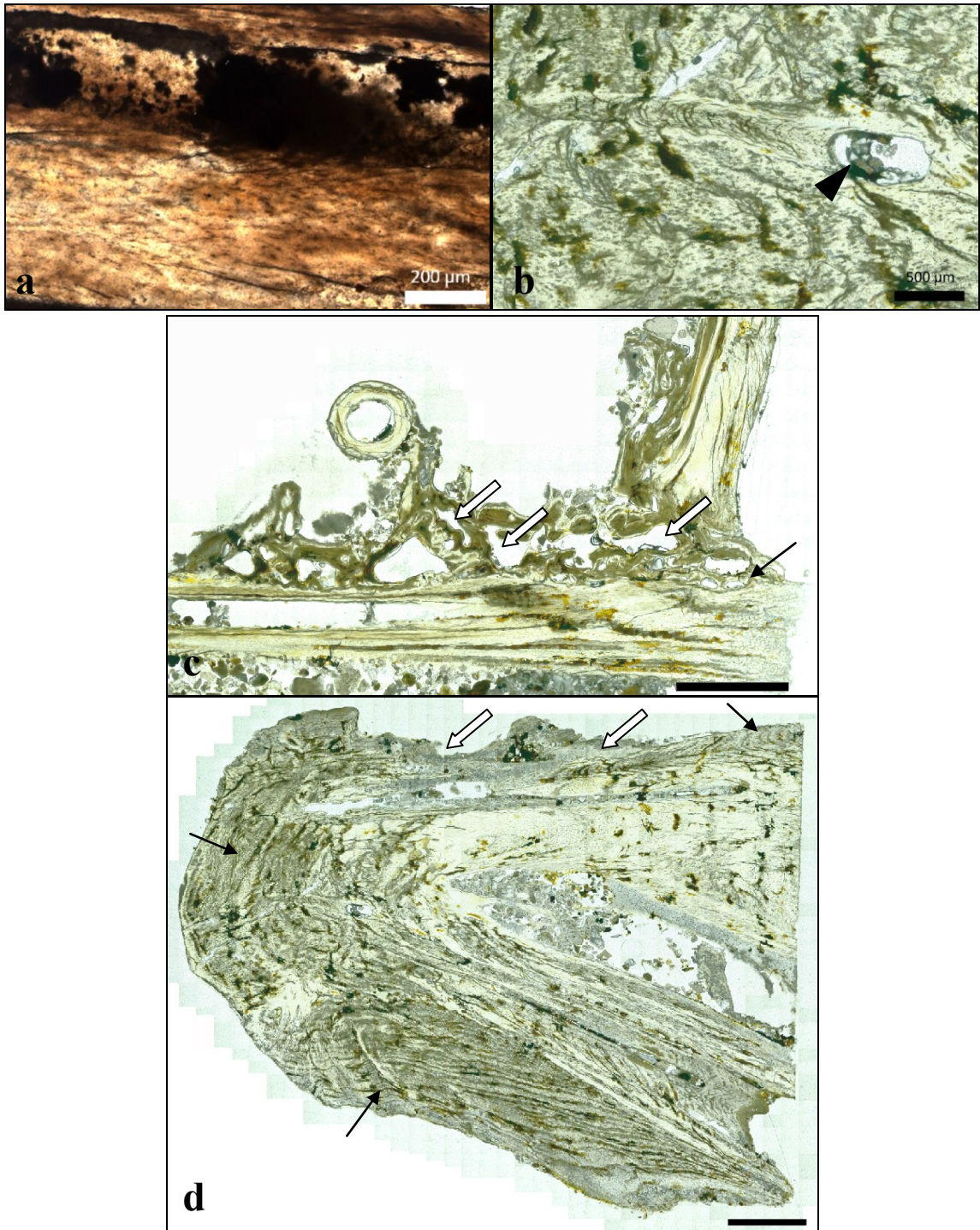


Figure 5.2 –Miocene *Onchorhynchus* tissues of interest. (a) Bone is well-organized linearly and is well-infiltrated by (b) channels for blood vessels (black arrow head; UOF-26782, Jackass Butte, Oregon). (c) A distinct transition between laminar bone and the spongier, alveolar bone associated with dentition is present in each specimen (black arrow; UOF-31389, Jackass Butte, Oregon). This is particularly apparent in UOF-26782, where alveolar bone was secondarily in-filled, making the complex loops and arches of this tissue obvious (white arrows). (d) Several juvenile characteristics are present, particularly in UOF-26782, where minimal dorsal alveolar bone (white arrows), a lack of teeth, and bands of newly secreted bone (black arrows) are evident. Unless otherwise indicated, scale bars are 2000 µm.

In each specimen, laminar bone and alveolar bone have a distinct transition zone (Fig. 5.2 c, black arrow). This alveolar bone was secondarily in-filled with a lighter, more transparent mineral, as with the *Salvelinus* specimens, above, resulting in a great deal of evidence as to the loops and arches of bone connecting teeth to the main dentary (white arrows). Unlike extant spawning *Oncorhynchus* individuals where the largest teeth are found anteriorly, these fossil individuals do not possess large, anterior breeding teeth.

The absence of breeding teeth, spurs extending from the dentary, and chondroid bone supports evidence from physical assessments and Micro-CT analysis that these *Oncorhynchus* specimens of unknown species were not spawning and were not kype-bearing. In fact, UOF-26789 displays many traits that suggest that it is a juvenile (Fig. 5.2 d). These traits include minimal dorsal alveolar bone (white arrows) and a lack of any preserved teeth, which may suggest that the alveolar bone connecting the teeth to the dentary was not well developed enough to withstand post-depositional disturbance and alteration. Furthermore, bands of newly laid down bone are evident in many portions of the dentary of UOF-26789 (black arrows). These distinct, continuous bands do not appear to have been reworked following formation, suggesting that this specimen was a rapidly growing juvenile.

### ***Oncorhynchus nerka***

The major component of this specimen is the enormous tooth base (Fig. 5.3 a, white arrow) that bears the extremely large, anterior breeding tooth (black arrow). Such a tooth was absent in the other, non-spawning *Oncorhynchus* specimens, above. The tooth base supporting this anterior breeding tooth is extremely well infiltrated with both blood vessels (Fig. 5.3 b, white arrow heads) and fat accumulations (Fig. 5.3 c, black arrows). Several channels for blood vessels (Fig. 5.3 d, black arrow heads) and large fat accumulations (Fig. 5.3 e, white arrow heads) are also present in the ventral and posterior laminar bone. The large number of channels for blood vessels and fat accumulations in this specimen was first described in its Micro-CT assessment, but is even more apparent in histological analysis.



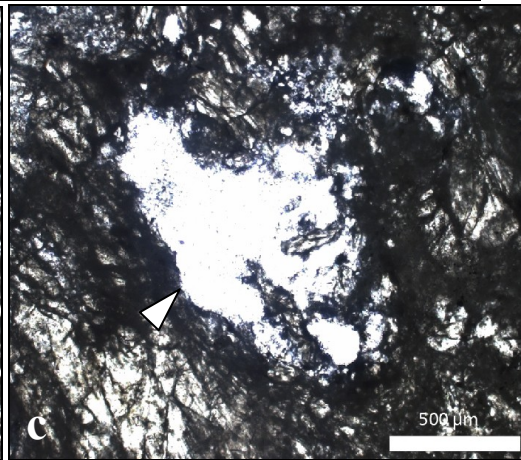
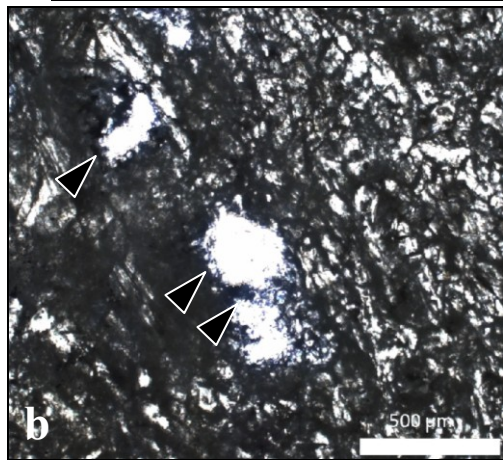
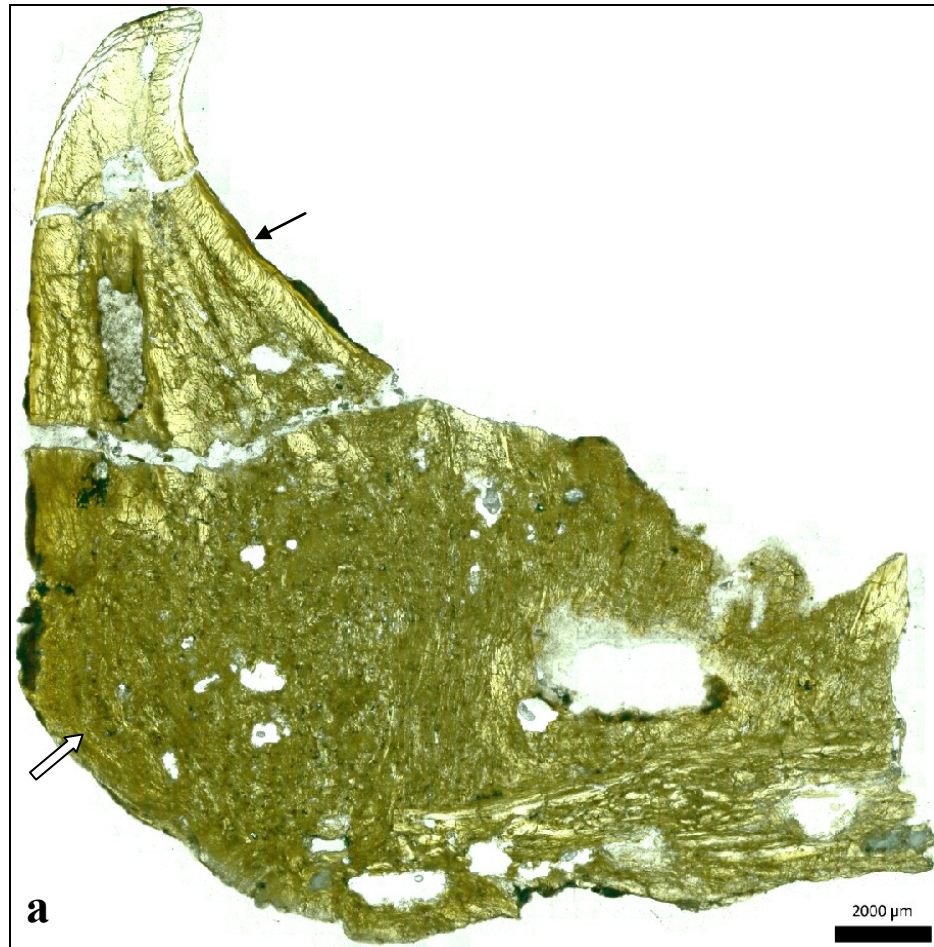
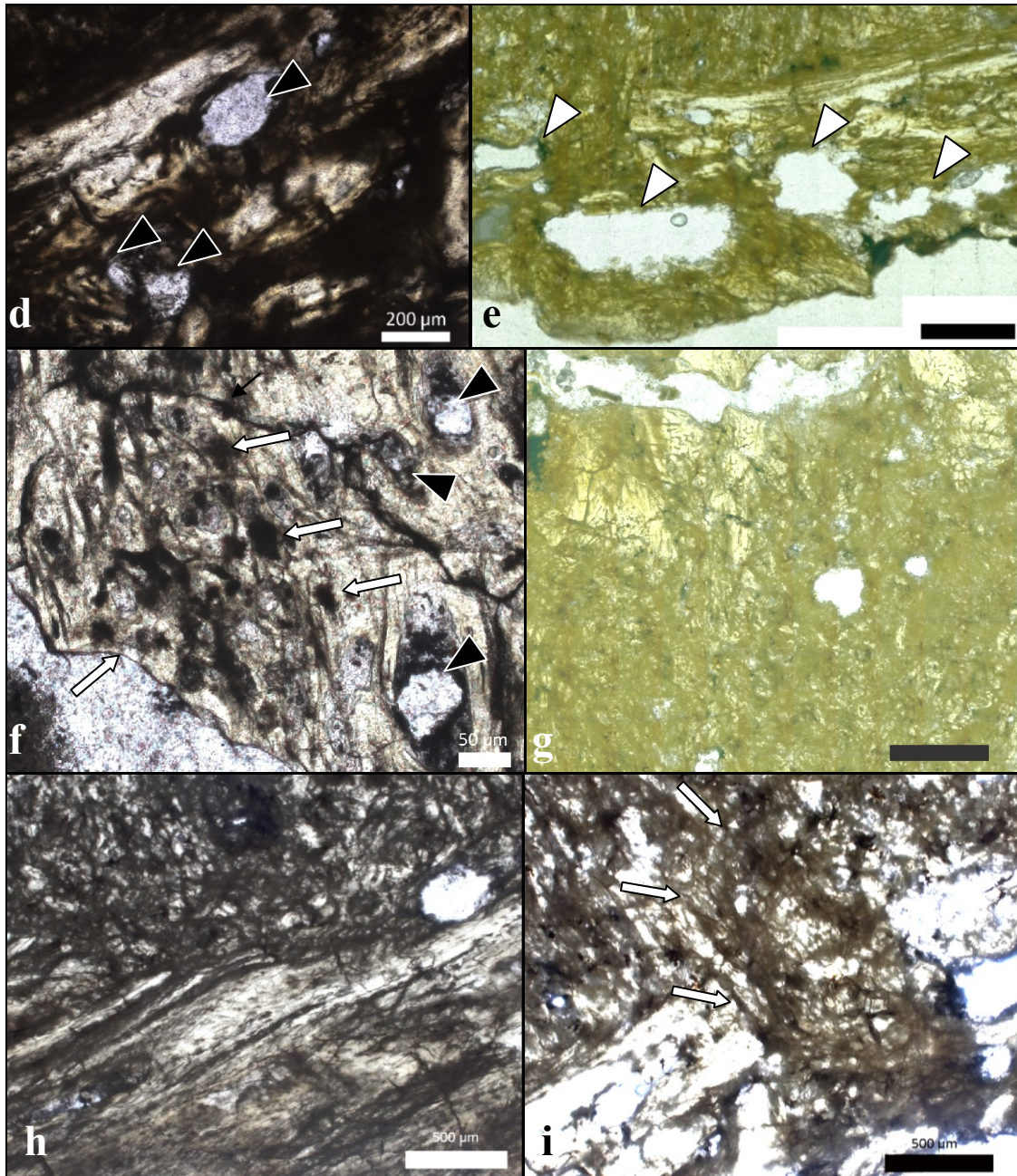


Figure 5.3 – Pliocene *Oncorhynchus nerka* (UWBM-87112, Pliocene, Skokomish Valley, Washington) tissues of interest. (a) An enormous spongy tooth base (white arrow) supports an equally enormous anterior breeding tooth (black arrow). The tooth base is well-infiltrated by (b) channels for blood vessels (black arrow heads) and (c) spaces for fat accumulations (white arrow heads).



**Figure 5.3 – Continued.** (d) Spaces for blood vessels (black arrow heads) and (e) fat accumulations (white arrow heads), several of which are extremely large, are numerous. (f) Also within the tooth base are several aggregations of chondrocyte-like elements (black arrows), although these features are difficult to distinguish and may be artifacts. These chondrocyte-like cells are surrounded by many small blood vessels (black arrow heads). (g) The transition between the spongy bone of the tooth base and the tooth itself is difficult to distinguish. (h) Contrarily, the transition between the tooth base and the lamellar bone located more ventrally and posteriorly within this specimen is relatively distinct. However, (i) near the lateral margin of this specimen, this transition is blurred by the presence of fibrous, needle-like extensions that penetrate deep into the tissues on either side of this transition zone (some examples indicated by white arrows).

Another feature of this specimen, and in particular of its tooth base, is the

presence of aggregations of cell-like features that are round and lack cell processes (Fig. 5.3 f, black arrows). These features may be chondrocytes, but do not group closely together, lack secreting halos, and are somewhat indistinct. These chondrocyte-like cells are surrounded by many small blood vessels (black arrow heads). None of these chondrocyte-like elements are found in the laminar bone region.

The transition between the spongy tooth base and the tooth itself is difficult to distinguish (Fig. 5.3 g). Striations similar to those found on the tooth may be identified deep within the tooth base, and there is no clear distinction between the material of which the tooth is composed and that of the tooth base using light microscopy alone.

As with the other specimens examined in this study, there is a distinct transition between the anterior, spongy tissue of the tooth base and the laminar bone of the posterior and ventral margin (Fig. 5.3 h). However, near the lateral margin of this specimen, several fibrous extensions proliferate between the two tissue types, somewhat blurring the margin (Fig. 5.3 i, white arrows). These elements are needle-like and extend deep into both the ventral laminar bone and the dorsal, spongy tooth base, though they appear to lack any associated chondrocytes.

The combination of these needle-like extensions, a large breeding tooth and tooth base, and some evidence of chondroid bone within the tissues of this specimen suggests that not only was this specimen in its spawn-run at the time of its death, but that it was kype-bearing as well. However, as is the case with living *Oncorhynchus* specimens, skeletal spurs and extensions from the dentary are minimal and it is instead the breeding tooth and its supportive base that are the main components of the kype in this specimen.

#### ***Oncorhynchus (Smilodonichthys) rastrosus***

As in the other specimens examined, there is some variation in the structure and organization of bone depending on the location within a given specimen (Fig. 5.4 a). A posterior, and somewhat ventral, region of laminar bone (Fig. 5.4 a, white arrow) is dominated by many blood vessels (Fig. 5.4 b, black arrow heads), as well as small and large fat accumulations (white arrow heads).

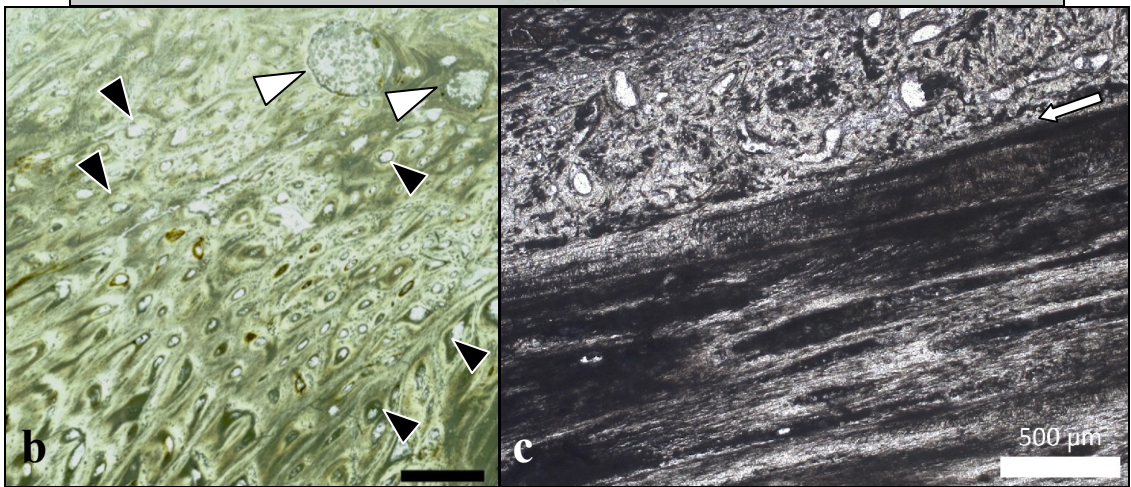
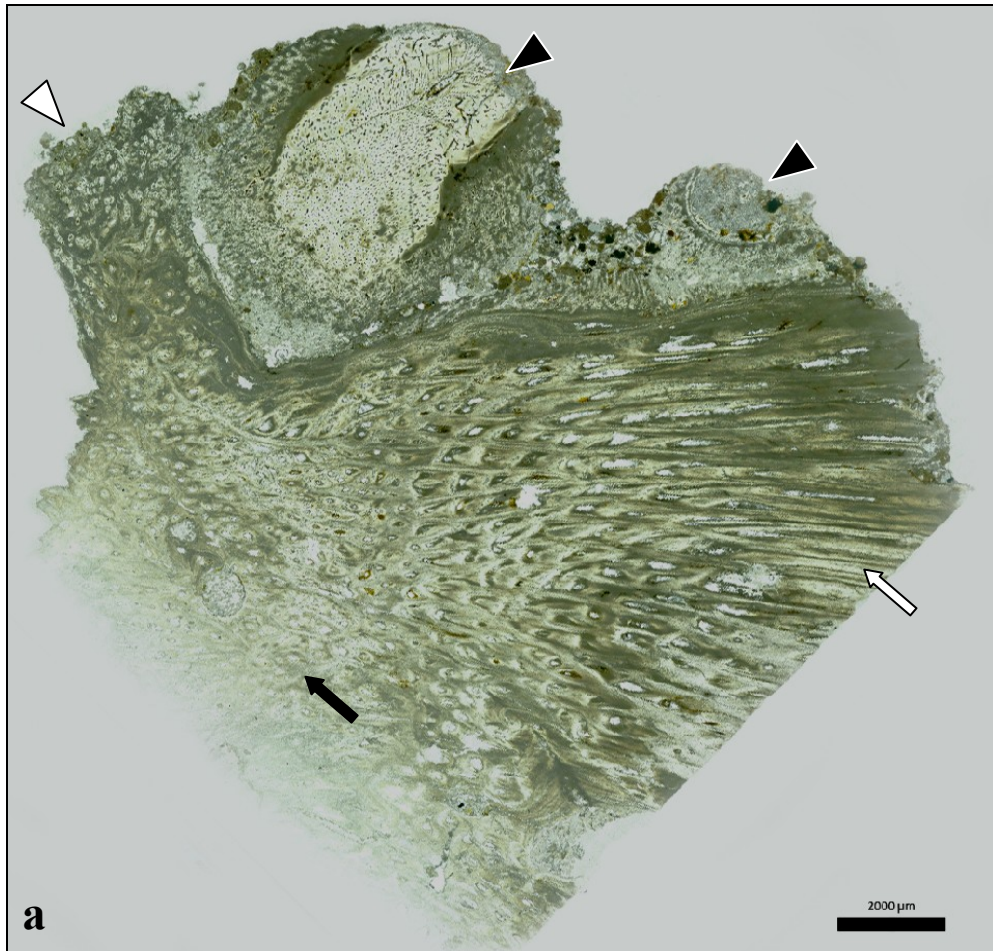
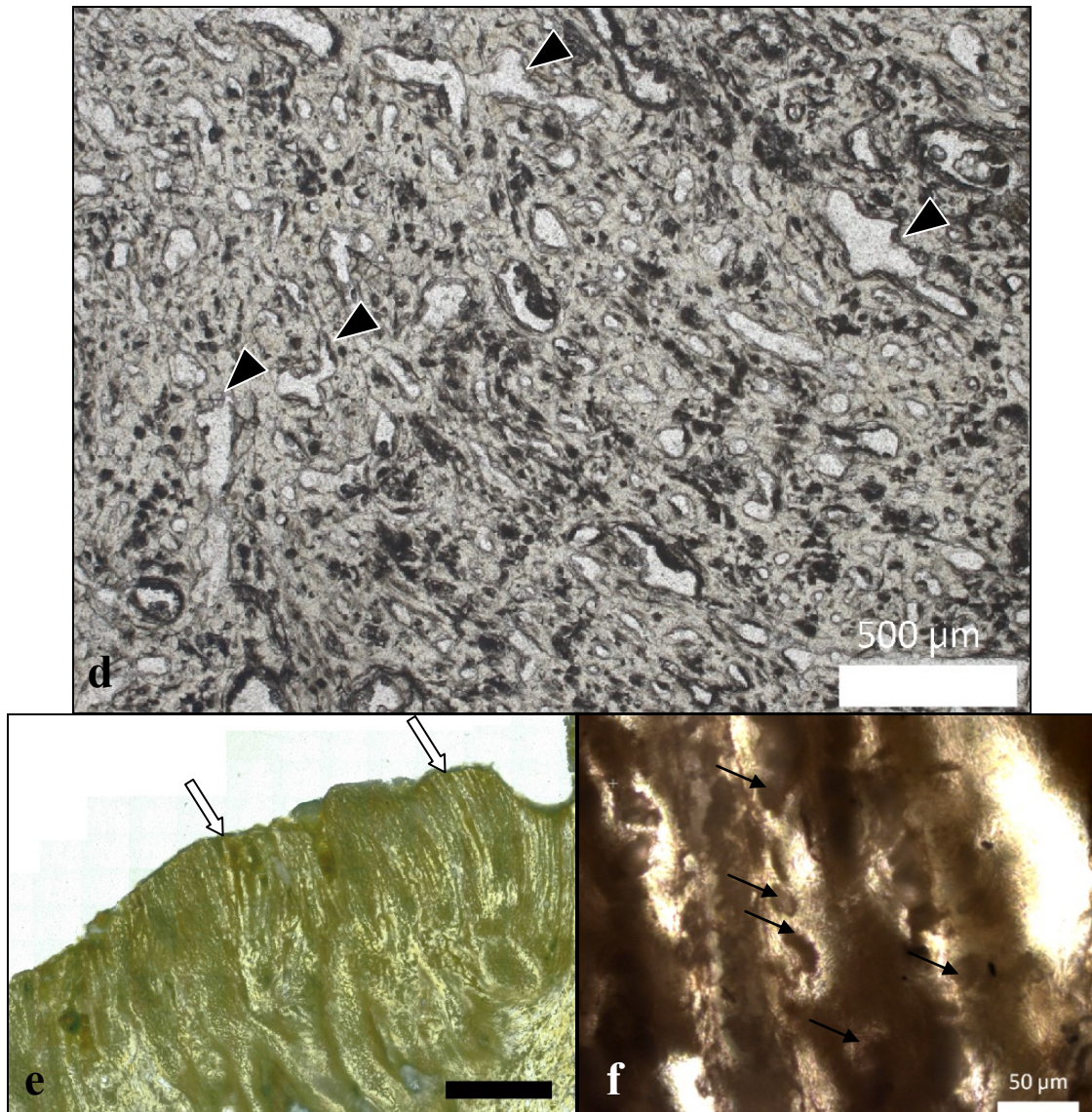


Figure 5.4 – Pliocene *Oncorhynchus rastrosus* tissues of interest. (a) Several distinct regions of tissues are apparent within this specimen including well-organized laminar bone (white arrow), less organized, spongier bone within the tooth base (black arrow), teeth (black arrowheads), and anterior compact bone heavily modified by its association with the tooth base (white arrow head; UWBM-50816, Arlington, Oregon). (b) Within the laminar bone, many blood vessels (some examples indicated by black arrow heads) and fat accumulations (white arrow heads) persist (UWBM-50816). (c) This laminar bone transitions abruptly to the less organized tissues of the tooth base (white arrow; UWBM-71908a, Ringold Formation).



**Figure 5.4 – Continued.** (d) As with the laminar bone, the tooth base also displays an extensive network of spaces for many small blood vessels (some examples indicated by black arrow heads; UWBM-71908a). (e) The internal structure of the surficial textures first identified in physical examination and Micro-CT scans of the UWBM-71908 specimens is also evident. Within this tissue region, many needle-like extensions are arranged near the margin of the bone and penetrate the laminar bone of the compact dentary (white arrows; UWBM-71908a). (f) These needle-like elements are also associated with numerous chondrocyte-like cells arranged in strings (black arrows), very similar to what is seen in living, kype-bearing individuals. Unless otherwise indicated, scale bars are 1000 $\mu$ m.

Anteriorly, this laminar bone transitions abruptly into a spongy, less organized region where a large tooth base dominates (Fig. 5.4 c). The tooth base appears to be built on the pre-existing compact bone of the dentary and greatly affects the entire anterior region of the bone, yielding a region of tissue near the anterior tip of the dentary that resembles the tooth base more than it does the laminar bone (Fig. 5.4 a, white

arrowheads). Within this tooth base many small blood vessels (Fig. 5.4 d, black arrow heads) form an intricate network similar to those seen in living *Oncorhynchus* individuals. The tooth base is also the site of teeth not visible in either physical examination or Micro-CT assessment. For instance, in UWBM-50816, two large teeth that had not yet erupted at the time of this individual's death are in evidence in paleohistological section (Fig. 5.4 a, black arrowheads).

While the tooth base of the fossil *O. nerka* specimen, above, greatly resembled the alveolar bone found in modern specimens, the tooth base of *O. rastrosus* is somewhat different. Lacking the loops and whorls found in the alveolar bone of these other animals, the tooth base of *O. rastrosus* is instead rather compact, with many small blood vessels closely associated with one another (Fig. 5.4 c). While not as well organized as in laminar bone, the *O. rastrosus* tooth base does display a moderate degree of patterning, with the largest blood vessels being located at the centre of the region, near the actual teeth. The *O. rastrosus* tooth base is also rooted much deeper within the bone of the compact dentary than in other specimens. While the tooth base of *O. nerka* (UWBM-87121) appeared to merely rest upon the surface of the dentary (Fig. 5.3 a), that of *O. rastrosus* penetrates nearly three quarters of the way through the compact bone it rests on.

The final tissue of interest to be seen in the *O. rastrosus* paleohistology is the internal structure of the nodular, kype-like, surficial textures seen in the Micro-CT scans and physical examinations of UWBM-71908 a and b. The structure of these textures is markedly different than that of the tissues surrounding it (Fig. 5.4 e, white arrows). Within this tissue region, numerous striations composed of needle-like fibres extend into the laminar bone of the compact ventral dentary. These needles represent the anterior boundary of the breeding tooth base, much as is seen in living spawning *Oncorhynchus* individuals. Furthermore, these needles are associated with and divided by strings of round, chondrocyte-like cells (Fig. 5.4 f, black arrows), much like the chondrocyte strings found in association with similar needle-like fibres in the kype skeleton of living specimens.

## **Discussion**

The structure and composition of bone and cartilage, once fossilized, can be preserved for hundreds of millions of years (Bailleul et al., 2012; Chinsamy-Turan, 2005). As a result, paleohistology is of great use in gathering information as to the growth, physiology, and behaviour of fossil animals. A technique that has been used for centuries to study the microstructure of fossil vertebrates (Padian and Lamm, 2013), paleohistology is a means of gaining insight into the lives and experiences of animals known only from their fossilized remains.

Paleohistological analysis can be used, in much the same way as histology in living specimens, to determine if the tissue composition of the lower jaws of fossil salmonids reflects evidence of the presence of a kype. Paleohistology can be used to determine whether co-occurring chondroid bone and Sharpey-fibre bone are present in the jaw even when evidence of the structure of the kype is not. This technique is particularly useful in assessing the presence of a very subtle, enigmatic kype, the presence of which cannot be confirmed by Micro-CT analysis or physical examination, as may be the case in *O. rastrosus*. Paleohistological assessments can also be used to assess the composition of the lower jaw in general, especially in regards to the other tissues of interest in this study, namely teeth, blood vessels, and fat accumulations. Finally, paleohistology can supplement data from living specimens in determining when and how the evolution of kype growth as a secondary feature occurred in the salmonids.

### ***Evidence of a presence of a kype***

Paleohistology not only confirmed the data gathered from the physical examination and Micro-CT assessment of specimens in the current study, but it also revealed new information as to the composition and organization of tissues in these fish. Just as histological analysis of living material in Chapter 3 permitted the identification of enigmatic kype in *O. kisutch* (UW 015091) and determined an absence of kype in *O. clarki* (UW 028776), the addition of data from paleohistological analysis makes it possible to properly identify enigmatic structures in fossil specimens at the cellular level. In particular, the internal structures of the kype-like textures on the anterior lateral dentary surfaces in the *Salvelinus* and several *Oncorhynchus* specimens and that of the

*Oncorhynchus* breeding teeth are revealed, as is the complexity of blood vessel and fat accumulation networks at the interior of several specimens.

First identified in the physical examinations and Micro-CT assessments of these specimens, the nodular, kype-like textures on the anterior lateral margins of *Salvelinus* (UOF-26785, UOF-26786, and UOF-26787), *Oncorhynchus nerka* (UWBM-87121), and *O. rastrosus* (UWBM-71908 a and b) are much in evidence in paleohistological section, as well. Where sectioned sagittally, the nodular and fibrous features of which these textures are composed appear to display the high to low-density tissue gradient first seen in Micro-CT reconstruction. However, while osteocytes are identified in high numbers within the posterior and ventral laminar bone in these specimens (Fig. 5.1 b), these bone-forming cells are virtually absent in high tissue density regions (Fig. 5.1 c). Thus, while, as shown in Chapters 2 and 3, high density in modern specimens is correlated to a high degree of mineralization, it would seem that the high density of tissues located at the peripheral margin of these nodular elements is an artifact of replacement during fossilization.

Rather than being associated with a large number of osteocytes, these nodes and spurs are often the sites of needle-like extensions into surrounding tissues (Fig. 5.1 g) as well as aggregations of chondrocyte-like cells (Fig. 5.1 e and f). While it is not possible to identify the halos of newly secreted material seen in living chondrocytes, these cells are rounded, lack cell processes, and may occur in pairs of newly divided cells. These chondrocyte-like cells may be closely associated with the needle-like elements extending from nodes and spurs into the surrounding dentary (Fig. 5.1 g, Fig. 5.3 f, Fig. 5.4 e), forming strings adjacent to them (Fig. 5.4 f). However, these chondrocyte-like cells may also form clusters within the nodular features of this region, particularly when these features are in cross-section (Fig. 5.1 f).

These nodular textures, identified in *Salvelinus* and *Oncorhynchus* specimens believed to be in their spawn-run, exhibit many kype-like characteristics. Their location on the anterior lateral margin of the dentary and the structure and composition of the fibrous, spur- and needle-like elements of which they are comprised is suggestive of the kype skeleton of living eusalmonines. The combination of Sharpey-fibre-like needles extending from the periosteum of compact dentary bone and the chondrocyte-like cells



aggregating in and around these needles and the spurs extending from the dentary are similar to the elements of the kype skeletons of living specimen shown in Chapters 2 and 3. This similarity in composition and organization of these tissues supports my hypothesis that Sharpey-fibre bone and chondroid bone, present in the kype and in the lower jaw tissues of living specimens, are also found in those of fossil salmonids. It is, however, important to point out that chondrocytes and secondary cartilage may be difficult to identify in paleohistological section (Bailleul et al., 2012). That being said, there is precedence for their identification in the fossil record (Chinsamy-Turan, 2005; Scheyer, 2007) and they share many of the characteristics of modern chondrocytes, as noted above.

In paleohistological section of *O. rastrosus* UWBM-71908 a and b, these nodular features display many needle-like protrusions that extend from the tooth base and penetrate the laminar bone of the compact dentary (Fig. 5.4 e). Many of these needles are often associated with strings of chondrocyte-like cells (Fig. 5.4 f). Similar nodular features and the tissues of which they were composed in the *Salvelinus* specimens were suggestive of the presence of a kype, making the presence of similar structures in *O. rastrosus* curious. In combination with the structures and features found in these specimens as discussed in Chapter 4, it seems possible that an enigmatic kype may be present in at least two of the *O. rastrosus* specimens examined in this study.

### ***Composition of other tissues of interest in the lower jaw***

Other tissues of interest evident in paleohistological sections of ancient salmonid jaws include teeth, and the breeding teeth of *Oncorhynchus* in particular; blood vessels; and fat accumulations.

#### *Teeth*

Much like the kype skeleton, another site of chondrocyte-like cell activity is near the anterior margins of the bone (Fig. 5.1 e). These cells appear to be particularly active when in association with anterior breeding teeth and the bases to which they are attached, as in *O. nerka* (Fig. 5.3 f). In *O. nerka* in particular, these tooth bases, composed of spongiöse alveolar bone, are often very difficult to distinguish from the breeding teeth that they bear (Fig. 5.3 g). Tchernavin (1938) noted that it is not possible to physically separate the

breeding teeth of *Oncorhynchus* specimens from their respective bases without risking damage to one or both elements.

These breeding teeth are major components of the dentaries in these specimens, dominating and manipulating the tissues upon which they rest and with which they are associated (Fig. 5.4 a, white arrowheads). These teeth and their tooth bases are also the site of many blood vessels and channels (Fig. 5.4 d, black arrowheads).

#### *Blood vessels*

Hardly restricted to breeding teeth and tooth bases, blood vessels are found in high numbers in each of the specimens examined in this study (Fig. 5.1 h; Fig. 5.3 b and d; Fig. 5.4 b and d, black arrowheads). Often secondarily in-filled with a mineral that differs from the dentary within which they lie, these channels are very numerous in each specimen examined, but occur in very high numbers in the *Salvelinus*, *O. nerka*, and, particularly, the *O. rastrosus* material. As such, just as was the case in living fish, it would appear that dentary blood vessels occur in the highest numbers in spawning individuals, where a ready supply of the resources for growth and maintenance of the kype and other associated tissues is required.

#### *Fat accumulations*

Also found in very high numbers in the spawning individuals were fat accumulations. In the non-spawning specimens, namely the unspecified *Oncorhynchus* fossils, and in particular UOF-31389, which appears to be a juvenile, fat accumulations were not identified. In spawners, on the other hand, these accumulations of fat formed a series of networks in both the laminar bone of the compact dentary (Fig. 5.1 j; Fig. 5.3 e; Fig. 5.4 b, white arrowheads) and, to a lesser degree, the tooth bases as well (Fig. 5.3 c, white arrowhead). As in living animals, and as discussed previously, fat stores would provide the resources needed during the long period of starvation and migration that is the spawn-run, as well as serve to lighten the skeletal load.

#### ***Determining when and how the kype evolved***

As I discussed in Chapters 2 and 3, spawning salmonids experience a variety of

modifications in the tissues of their lower jaws. These modifications include an increase in the thickness of the epithelium and, likewise, an increase in the number of mucosal goblet cells in that epithelium. Furthermore, the number, size, and extent of blood vessels and vascular sinuses increase, as do accumulations of fat, particularly in anadromous and migrating species. While, as I've discussed throughout my thesis, variation in the extent of these modifications persist across the Salmonidae, these modifications occur to some extent in each spawning individual. However, while many salmonids experience spawning related modification to the tissues of their lower jaw, only eusalmonines experience kype growth.

The extent of the kype, and of some the tissues associated with it, varies from genus to genus (see also Behnke, 2002; Scott and Crossman, 1973). Variation in the size, extent, and structure of kype growth within genera and even within species exists as well and may depend on resource availability and life history mode. When comparing fossil and living material, as was discussed in Chapter 4, some variation resulting from evolutionary transitions from arguably more basal to more derived conditions also persists. While differences in kype growth and spawning-related modification to the tissues of the lower jaw exist at the family, genus, species, and even population level, several factors in regards to the combination and organization of tissues in the kype skeleton remains constant.

As was previously found in Atlantic salmon (Witten and Hall, 2002, 2003), the kype of *Oncorhynchus* and *Salvelinus* are also composed of a combination of Sharpey-fibre bone and chondroid bone. While some modifications co-occurring with the kype specific to a particular lineage may exist, such as the breeding teeth of *Oncorhynchus*, Sharpey-fibre bone and chondroid bone are present in the kype of each kype-bearing genus. However, Sharpey-fibre bone and chondroid bone are present to some degree in the tissues of nearly every specimen examined in this study. Rather than being a quick and easy identifier for the presence of a kype, chondroid bone and Sharpey-fibre bone have a much more complex relationship with the kype than previously thought. For instance, the presence of a combination of chondroid bone and Sharpey-fibre bone in salmonid anterior lower jaw may be a pre-adaptation for the kype. However, while even non-kype bearing genera display chondroid bone and Sharpey-fibre bone in their tissues,

it is the combination of other tissues present in the lower jaw and the specific organization of chondroid bone and Sharpey-fibre bone that result in a kype.

The specific organization of these tissues yields a supportive meshwork of skeletal spurs that extends from the compact dentary to support an anterior build-up of connective tissue and epithelium. These spurs and spongy tissues that form the framework of the kype skeleton serve as compartments containing accumulations of fats. Thus, while each of these tissues (chondroid bone, Sharpey-fibre bone, fat accumulations, epithelium, and connective tissue) is present in non-kype-bearing individuals, it is their specific organization, found only in *Salvelinus*, *Oncorhynchus*, and *Salmo*, that results in a kype.

The presence of fat, epithelium, and connective tissue in non-kype-bearing salmonids is not surprising given that they are tissues found in virtually all vertebrates. However, the presence of chondroid and Sharpey-fibre bone in these fish, particularly in combination, is unexpected. While Sharpey-fibres have been identified in the dentaries of a variety of animals, including rats (Johnson, 2005; Kuroiwa et al., 1994), and while secondary cartilage, a tissue with a similar developmental pathway to chondroid bone, has been identified in juvenile Atlantic salmon (Gillis et al., 2006), chondroid bone in the lower jaw of fish has previously only been found in the Atlantic salmon kype (Witten and Hall, 2002).

Evidence of chondroid bone and Sharpey-fibre bone in the tissues of non-kype-bearing species would suggest that the kype evolved when tissues already present in the lower jaw were incorporated into a new structure. If this incorporation of pre-existing tissues is the case, then, while the kype is specific to the eusalmonines, the presence of a combination of Sharpey-fibre bone and chondroid bone in the lower jaw is much older. While further research that considered the histology of other Salmoniformes would be required to establish when these tissues first co-occurred in the lower jaw, one possibility that would explain their co-option to form a kype lies in starvation-related deformation of tissues.

As previously discussed in Chapters 3 and 4, many lipids and fats are stored in the bone and muscle of salmonids. In addition to serving to maximizing strength of the skeleton while limiting its mass (Parfitt, 1988), these fat accumulations are used as resources during the starvation period of the spawn-run (Cavender and Miller, 1972;

Jobling et al., 1998; Jørgensen et al., 1997; Phleger et al., 1989; Phleger et al., 1995). These resources, as well as others such as calcium phosphate in the bones, drop significantly over the course of the starvation period (Phleger et al., 1989; Phleger et al., 1995). The loss of these resources can result in transformation of bone to cartilage, which in turn yields deformation of the skeleton. Perhaps loss of resources, metamorphosis of bone to cartilage, and deformation of the skeleton in the dentary initiated the process of kype evolution, a process that incorporated the rapidly growing Sharpey-fibres and chondroid bone already present in the jaw.

While starvation-related deformation may explain the overall structure of the kype, it does not explain the genus-specific variation in the kypes of *Salvelinus*, *Oncorhynchus*, and *Salmo*. However, it may be possible to pinpoint environmental factors that would influence the evolution of the kype and of modification to the tissues of the lower jaw during spawning. Several researchers have argued that paleogeography greatly influenced salmonid evolution (Smith et al., 1981; Waples et al., 2008). Given that the differences in environments experienced by these fish likely affected the evolution of anadromous, migratory lifestyles in many eusalmonines, there is every reason to suspect that kype evolution was influenced as well.

Using North American species as an example, Atlantic salmon and trout inhabit the east coast of North America and large portions of western Eurasia (Aas et al., 2011; Behnke, 2002). These regions are marked by relatively stable, dormant coastlines with low continental gradients (Waples et al., 2008). The fish themselves exhibit little diversity, in large part because few species of *Salmo* survived the ice age conditions of the Holocene. Pacific salmon and trout, on the other hand reside on the west coast of North America and within eastern Asia (Behnke, 2002; Groot and Margolis, 1991). In these regions, geologic activity and high mountain ranges resulting in large continental gradients and high water energy dominate (Waples et al., 2008).

Given the demanding environmental conditions prevalent in the home range of *Oncorhynchus*, the long, delicate skeletal needles of the Atlantic salmon kype skeleton would be a hindrance, costly and easily damaged. Instead, Pacific salmon have minimized the extent of the skeletal needles in their kype skeleton, favouring a smaller array of shorter, squatter needles combined with several large, robust breeding teeth. As a result,

while the kype of *Oncorhynchus* is functionally similar to that of *Salmo*, it is much better suited for the environmental conditions this genus faces. It would, however, be very interesting to test this hypothesis of environmental influence on kype evolution. Flow and hydrodynamic studies on representatives of the three kype-bearing genera of Eusalmoninae might reveal differences in the physical properties of different kype as they travel through the water column.

Using a combination of fossil and living material, it is possible to determine where on the phylogenetic tree the kype first evolved and how tissues already present in the jaw might have been modified to form a new structure (as shown in Fig. 3.18). Furthermore, starvation-related deformation may have acted as the catalyst that initiated formation of this structure. Finally, a hypothesis explaining variation in the structure of the kype in different genera can be formulated by considering the paleoenvironment of the home regions of each genus. This hypothesis can also be tested by using flow and hydrodynamic studies that test the capacity and properties of the kype in living individuals.

### ***Sources of Error***

Loss of portions of individuals is not the only time information is lost during fossilization. Incomplete preservation or diagenetic alteration of tissues may result in artifacts within the tissues. Modification to tissues or structures during replacement of organic material or during mineral growth may render them unrecognizable. The introduction of material to the specimen from the outside, such as fungi, algae, mold, mud and clay, or other foreign objects, may make analysis of fossil tissues even more difficult. However, I believe that by comparing fossil tissues to modern material analyzed previously, I was able to minimize confusion over the structures and cells present within the study specimens, thereby limiting error.

Finally, the production of paleohistological slides resulted in the loss of much of the specimens being examined. The sawing, grinding, and polishing necessary to produce slides led to a loss of approximately nine tenths of each fossil. Due to this loss of material, while I was able to create multiple, sequential sections from the same specimens, these sections do not fairly represent a complete series, as was the case with

living specimens (Chapter 3), where loss of material was minimal. This being said, as mentioned previously, this study represents one of, if not the first, paleohistological analysis of fossil salmonids that I am aware of. That this analysis includes series of multiple sections from the same specimen makes it even more novel, as serial sectioning is not a standard procedure in paleohistology. Being the first such analysis of fossil salmonid material, this study brings to light a great deal of information as to the composition and organization of tissues in ancient representatives of an important group of fish.

### ***Summary***

- This study represents the first use of paleohistological analysis to assess the characteristics of composition and structure of the lower jaw in a number of ancient salmonids. The use of multiple sections from the same specimen in that analysis allows researchers to assess the composition and organization of tissues throughout the lower jaws of that specimen.
- As predicted, paleohistology has been used to establish compositional elements such as skeletal needles and aggregations of chondrocytes that are reminiscent of the tissues of living kype-bearers.
- As shown in Chapter 3, the tissues previously associated with kype growth in living Atlantic salmon (Sharpey-fibre bone and chondroid bone) are present in animals even when a kype is not.
- The kype is a synapomorphy of the Eusalmoninae. However, Sharpey-fibre bone and chondroid bone evolved much earlier and were later incorporated into a new structure: the kype..
- Differences in the structure of the kype of different genera may have resulted from modifications of the basal kype skeleton in response to environmental conditions specific to the home ranges of and environments inhabited by each genus.

As with Chapter 4, the results of the current chapter supplement the findings of previous research in regards to presence and absence of kype. However, while this previous research considers only structural evidence of kype growth, the current study also

incorporates compositional evidence of kype presence and of other associated, spawning-related tissues of interest. In Chapter 6, this evidence, and the data gathered from the other chapters of this thesis, will be summarized and discussed as a whole.



## Chapter 6 – Conclusion

The kype, a spawning-related elongation and modification of the lower jaw, has long been known to develop in a number of salmonid genera (Day, 1887, Tchernavin, 1938). Previous work on the kype in Atlantic salmon found that it was a structure with specific types and organization of tissues (Witten and Hall, 2002, 2003). This organization is such that a large anterior connective tissue is supported by a framework of spongiöse skeletal needles extending from the compact bone of the dentary. These needles are composed of an unusual combination of tissues not found elsewhere in the skeleton: chondroid bone and Sharpey-fibre bone. These previous assessments also identified that the kype shares a number of characteristics with other fast-growing, secondarily developing structures, including the antlers of deer and their relatives. Both the kype and antlers exhibit unusual patterns of growth, developing periosteally at a specific site on the dermal skeleton (Hall, 2005; Witten and Hall 2002). Metaplastic conversion from cartilaginous tissue to bone occurs in both structures, thereby resulting in rapid growth with minimal energetic and resource cost.

This previous research provides general knowledge as to the presence of a kype in some salmonids and of the structure and composition of the kype in one genus, *Salmo*. However, little was known concerning presence or absence of a kype and the genus-specific characteristics of the kype across the salmonid tree; the composition and structure of the kype and of the lower jaw in general, particularly in regards to the characteristics of the kype and of associated structures and tissues of different genera or of individuals of different life history modes and stages; and the evolution of the kype. The current study sought to answer these questions using physical examinations, Micro-CT assessments, and histology and paleohistology of living and fossil specimens, as well as literature surveys. Additionally, this research reveals several other areas of interest to further research in regards to the evolution of the kype and of the other tissues of interest in the lower jaw.

***The presence and absence of a kype on the salmonid phylogenetic tree***

This study confirms that the kype is a structure found exclusively within the eusalmonines with the exception of *Hucho*. In fact, in light of the trends in presence and absence of a kype in living and fossil specimens, the lack of a kype in *Hucho* supports research suggesting that *Hucho* does not belong within the Eusalmoninae (Holčík et al., 1988; Nordern, 1961; Oleinik and Skurikhina, 2008). Furthermore, trends in the growth and modification of tissues of interest in the lower jaw such as breeding teeth, epithelium, and fat accumulations, fit most parsimoniously to a salmonid phylogenetic tree that separates *Hucho* from the other eusalmonine genera (Fig. 3.18, reproduced below). A tree that keeps *Hucho* within the eusalmonines is much less parsimonious, further supporting the hypothesis that a relocation of *Hucho* is required.

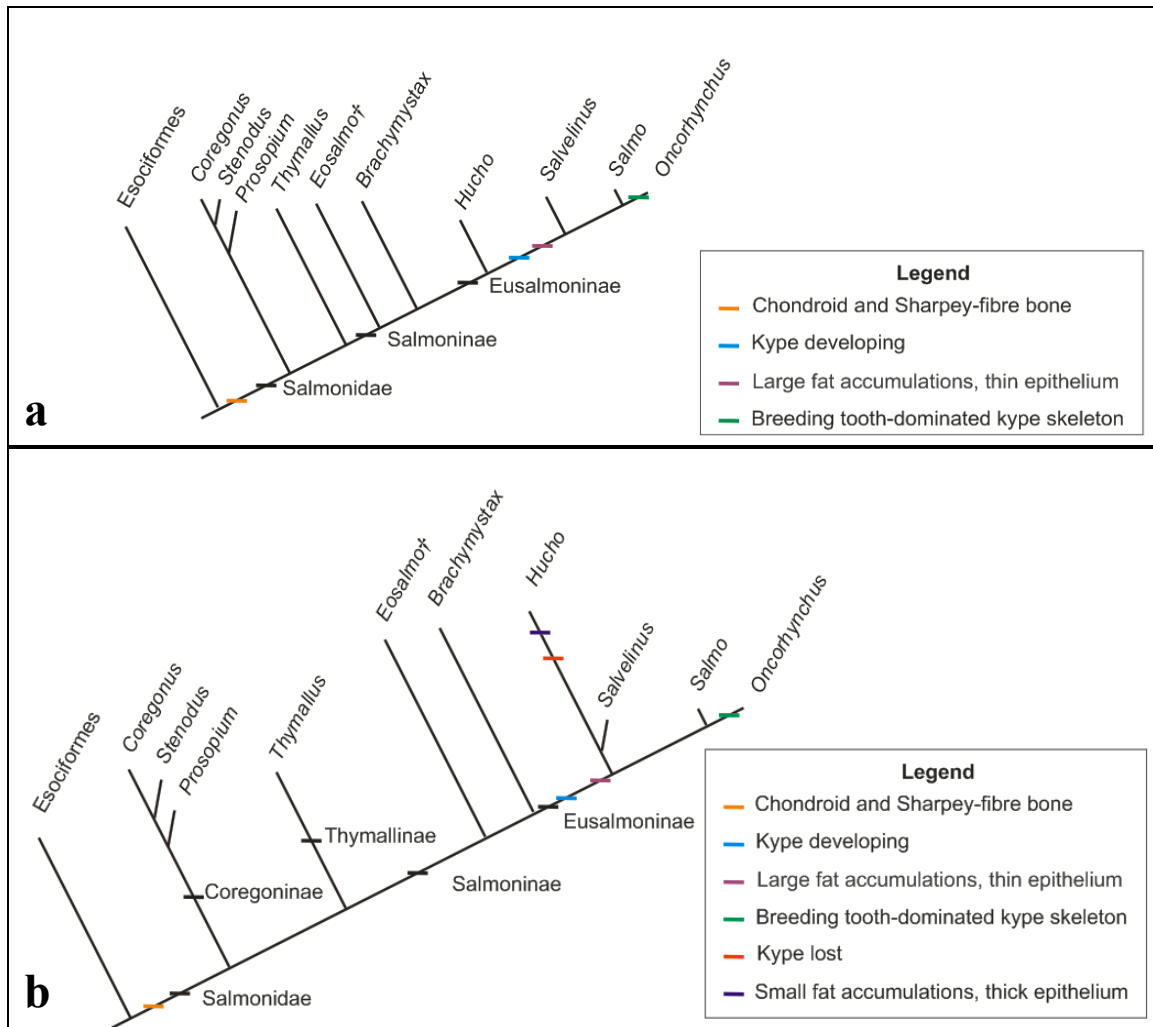


Figure 3.18 – Summary phylogenetic tree showing trends in tissues of the lower jaw. These possible trees place *Hucho* (a) with (modified from Stearley and Smith, 1993) and (b) outside of (modified from Oleinik and Skurikhina, 2008) the other eusalmonines. On each tree, chondroid bone and Sharpey-fibre bone is found in combination in all salmonids to some extent. Kype is only found in Eusalmonines, but is absent in *Hucho*. While *Salvelinus* and *Salmo* kype skeletons are dominated by skeletal needles, that of *Oncorhynchus* is dominated by large breeding teeth and their associated bases. Breeding teeth are not found in any other salmonid lineage. Large fat accumulations are found in eusalmonines in particular, though this may be result of a more resource rich life history mode than of a phylogenetic cue. Thick epithelium is found in basal salmonids and in *Hucho*. The addition of other tissues of interest to these trees support the hypothesis expressed in Chapter 2 (See Fig. 2.58) that a tree that separates *Hucho* from *Salvelinus*, *Salmo*, and *Oncorhynchus* is more parsimonious.

In addition to a relocation, the *Hucho* genus may require a more thorough reassessment. While it was not possible to confirm in this study evidence of the presence of a kype in *Hucho perryi*, if *H. perryi* does grow a kype, this would support the hypothesis expressed by several researchers that *Hucho* is not monophyletic and should instead be divided into *Hucho* (*H. hucho* and *H. taimen*) and *Parahucho* (*Parahucho perryi*) (see Hendry and Stearns, 2004; Holčík et al., 1988; and Nelson, 2006 for

discussion). If this is the case, *Parahucho perryi* may need to be repositioned within the salmonid phylogenetic tree such that it is an outgroup of the *Salmo-Oncorhynchus* sister group (Fig. 2.59, reproduced below).

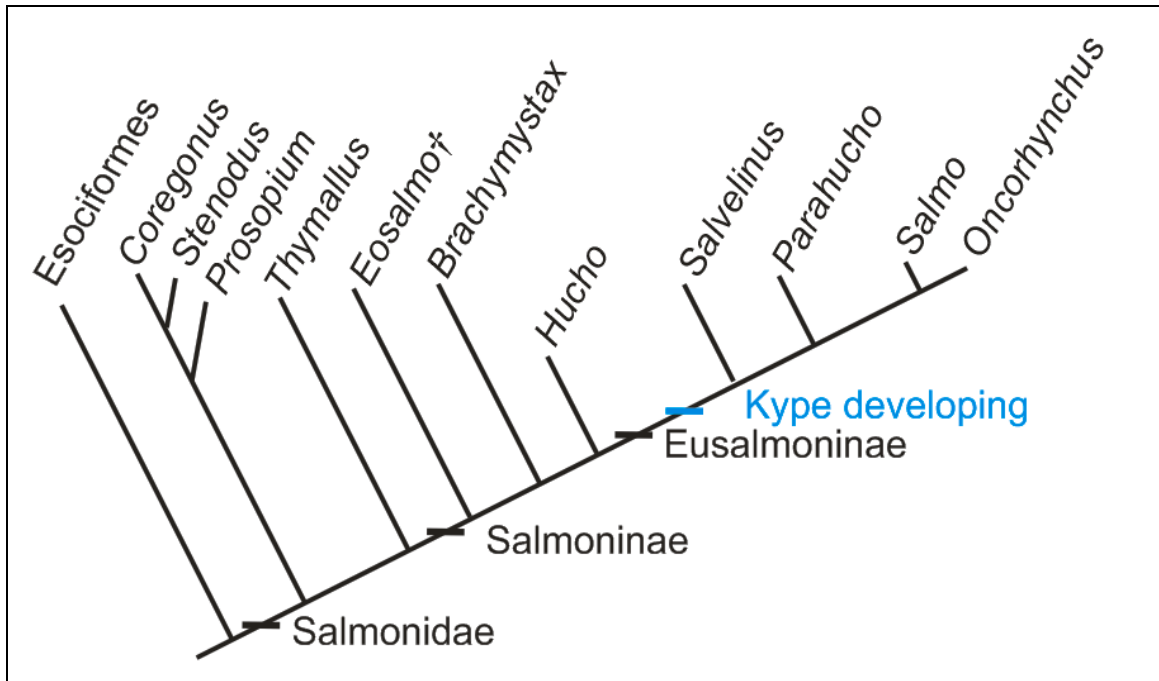


Figure 2.59 – “Kype developing” character mapped on a salmonine supertree built using a compilation of morphological, molecular, and behavioural data showing *Parahucho perryi* clustered with *Salmo* and *Oncorhynchus*. Modified from Wilson and Williams (2010).

### ***The composition and structure of the kype and of other tissues of interest in the lower jaw***

While the organization and structure of the kype is unique to the eusalmonines, chondroid bone and Sharpey-fibre bone, formerly believed to be specific to the kype (Witten and Hall, 2002, 2003), are found even when evidence of a kype is not. Rather than being associated only with the kype, these tissues are found in combination in living and fossilized individuals from non-kype bearing genera and non-spawning specimens from kype-bearing lineages. The presence of kype-associated tissues in non-kype-bearing salmonids, in both living and fossil specimens, is consistent with my hypothesis that the kype evolved using pre-existing structures and tissues already present in the lower jaw that were co-opted to form the kype.

*The kype and associated structures of different genera*

Kype structure is somewhat different in *Salmo*, *Salvelinus*, and *Oncorhynchus*. While in each case the dentary changes morphology, this change is much more dramatic in *Salmo* and *Salvelinus*, where an extensive needle-like array encompasses a large portion of the dentary (Fig. 6.1 a). In *Oncorhynchus*, these needle-like extensions are small and appear to be localized anterior to several large breeding teeth (Fig. 6.1 b). However, while the skeletal changes in the kype are somewhat smaller in *Oncorhynchus* than in either *Salmo* or *Salvelinus*, the combination of connective tissue build-up, breeding teeth development, and skeletal modifications result in a kype that is, overall, much larger and more prominent (as has been described by Behnke (2002), Fleming and Gross (1994), and Scott and Crossman (1973)). Thus, although the same tissues and structures are present in the kype of each kype-bearing genus, dimensions and number of these elements vary: the dominant character of the kype of *Oncorhynchus* is breeding teeth, while the kype in both *Salmo* and *Salvelinus* are dominated by a large number of slender skeletal needles (Fig. 6.1). Differences in the structure of the kype of different genera may have resulted from modifications on the basal kype in response to environmental conditions specific to the home ranges and migration routes of each genus (Aas et al., 2011; Behnke, 2002; Groot and Margolis, 1991; Smith et al., 1981; Waples et al., 2008).

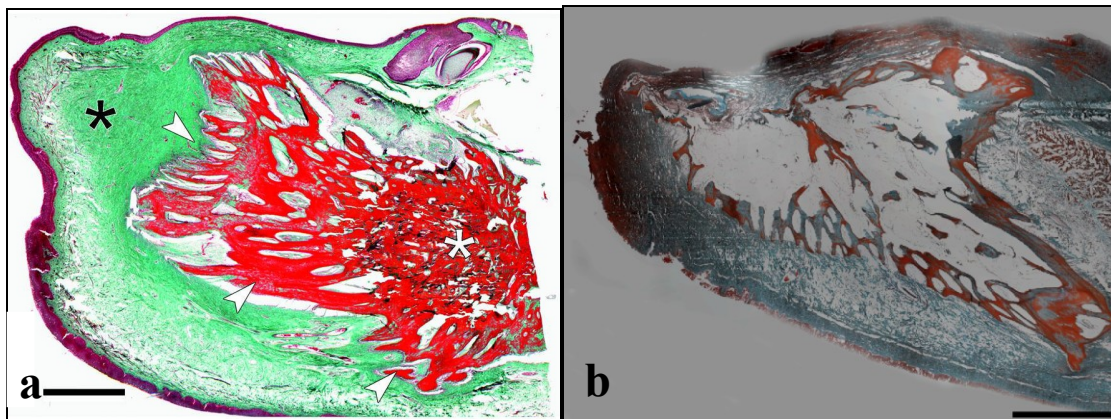
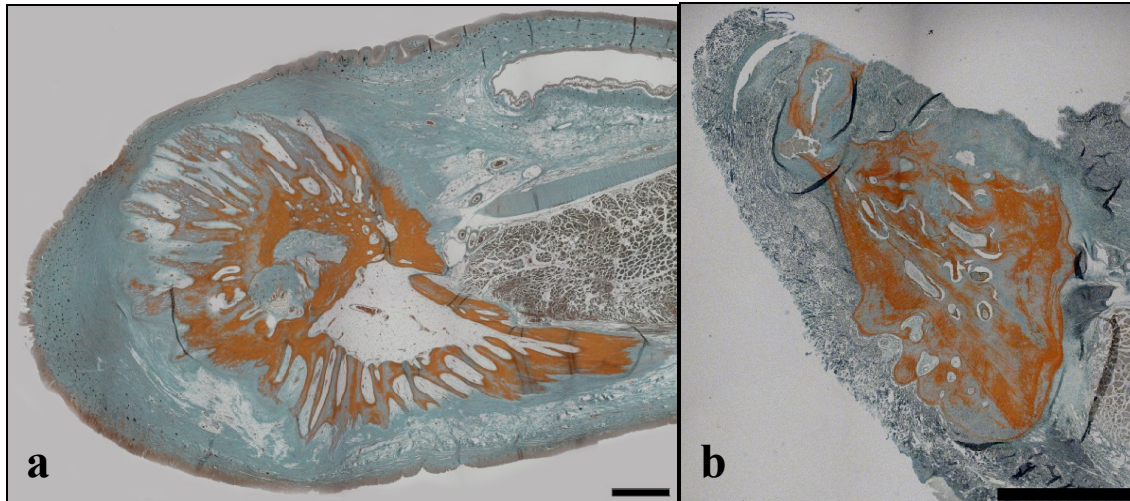


Figure 6.1 – Comparison of the organization of kype tissues between genera. (a) *Salmo* (*Salmo salar* shown), as with the *Salvelinus*, favours an extensive array of long, delicate skeletal needles in its kype skeleton (Witten and Hall, 2003). (b) *Oncorhynchus* (*O. kisutch*) has minimized that array in favour of very large breeding teeth. Scale bars are 1000  $\mu\text{m}$ .

Another major difference in kype development lies in the other craniofacial modifications during the spawn-run, one of which compensates for the development of kype, ostensibly to allow kype-bearing animals to maintain the seal that allows them to push water over the gills (Scott and Crossman, 1973). This compensatory feature, found on the upper jaw, is different in each genus. While *Salmo* develops a hole that the kype slips into, *Salvelinus* develops a notch formed when the premaxillae and maxillae separate slightly. The majority of *Oncorhynchus* species, on the other hand, build up soft tissue at the tip of upper jaw and a slight curve in the maxillae. The combination of soft tissue and curved maxillae results in a snout with which the kype dovetails.

#### *The kype as relates to life history mode and stage*

Trends in the structure and organization of kype-related tissues are not specific to phylogenetic position. Trends in the size and complexity of kype growth can also be correlated to life history mode (Fleming and Gross, 1989; Stolz and Schnell, 1991; Willson, 1997; Scott and Crossman, 1973). The largest and most dramatic kype is found within species and populations that are anadromous. This trend is particularly true in populations that are semelparous or, at the very least, have low to minimal post-spawning survivorship. Thus, the most dramatic spawning-related craniofacial changes are found in anadromous, semelparous or near-semelparous species, in particular *O. kisutch*, *O. gorbuscha*, *O. keta*, and *O. nerka*. These species are the “type 1” Pacific salmon, as defined by Stearley and Smith (1993). Semelparous animals experience more extensive remodelling of the lower jaw than iteroparous animals. Migratory (anadromous), semelparous individuals (Fig. 6.2 a) tend to grow larger and more extensive kype than non-migratory or first-time spawning, iteroparous individuals (Fig. 6.2 b).



**Figure 6.2 – Comparison of the organization of kype tissues between life history modes. (a) Anadromous, semelparous individuals (*O. gorbuscha* shown) grow a large and more extensive kype than (b) non-anadromous, iteroparous individuals (*O. mykiss aguabonita* shown). Scale bars are 1000  $\mu\text{m}$ ).**

The growth of a more substantial kype and the greater degree of craniofacial modification in these animals may be due to a combination of factors. First, there is higher resource availability in oceans, meaning that anadromous animals have more resources at their disposal that they can invest in kype growth (Gross et al., 1988; McDowall, 1997, 2001; Willson, 1997). Secondly, semelparous individuals have skewed resource allotment, spending nearly all of their available resources on the development of primary and secondary sexual characteristics and keeping little or no resources in reserve for post-spawn survival (Fleming, 1998; Willson, 1997).

While the kype in a given individual may be prominent, it may also be subtle, as was shown by Stearley and Smith (1993) when they used kype presence and absence as well as extent of kype development to assess the phylogenetic implications of kype growth. Evidence of a kype may not be apparent except in histological examination. Furthermore, many other tissues relating to the growth of a kype, as well as to the survival and behaviour of a spawning salmonid, are apparent in histological and paleohistological section. These tissues included breeding teeth, fat in muscle and bone, epithelium thickness, and the presence of blood vessels and sinuses. Just as trends exist in the structure of the kype in animals of different life history stage and life history mode, the tissues of the lower jaw and of the kype are also variable. More mature, migratory

individuals have thicker epithelium and higher fat content than less mature and resident animals (Fig. 6.3).

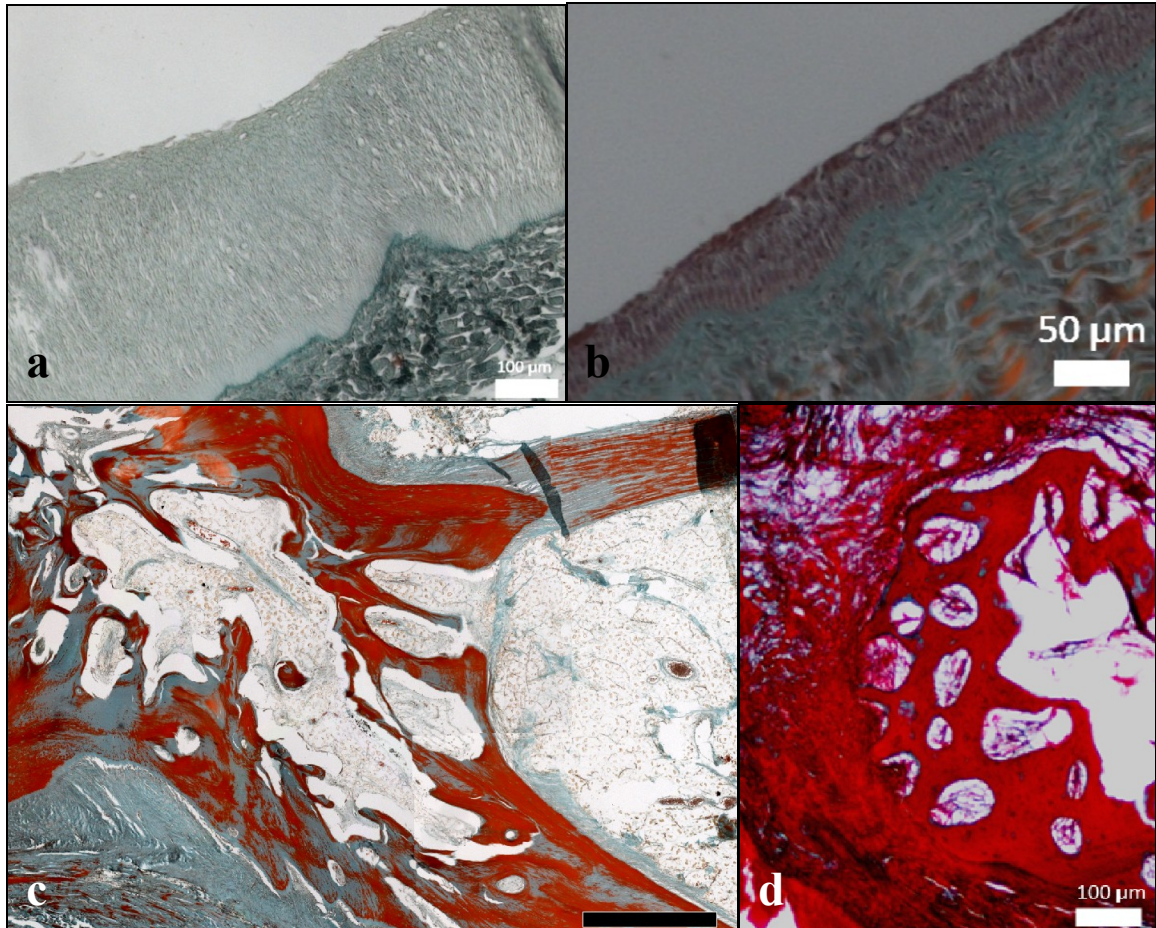


Figure 6.3 – Comparisons of other tissues of interest between life history modes and stages. Epithelium thickness is greater in spawning, resident *O. mykiss aguabonita* than in (b) non-spawning, resident *O. mykiss*. Fat accumulations are larger in (c) spawning, anadromous *Salvelinus alpinus* than in (d) non-spawning, non-anadromous *S. alpinus*. Unless otherwise indicated, scale bars are 1000µm.

The trends in the structure, composition, and organization of the kype and of other tissues of interest in the lower jaw of living specimens are also visible in fossil specimens. This study represents the first use of paleohistological analysis to assess the conditions across a number of ancient salmonids. The use of multiple sections from the same specimen in that analysis allows for the assessment of the composition and organization of tissues throughout the lower jaw of that specimen. Paleohistology was used to establish the presence of compositional elements such as skeletal needles and aggregations of chondrocytes that are very reminiscent of the tissues of living kype-bearers. Additionally,



large fat stores, many blood vessels, and enormous breeding teeth are very much in evidence in fossil spawners. Furthermore, transitions from basal salmonids to more derived salmonines, and, indeed, eusalmonines, can be identified in *Eosalmo* (Wilson, 1977, 1996; Wilson and Li, 1999). Transitions from basal to more derived character states within the same genus or species can also be distinguished, as shown in *Salvelinus* and *Oncorhynchus*, particularly in *O. nerka* and *O. rastrosus* (as discussed in Chapter 4).

### ***The evolution of the kype***

Several of the trends identified in living and fossil specimens are useful in understanding the evolution of the kype and of the tissues associated with it. For instance, a better understanding of the other tissues in the lower jaw may validate the hypothesis that the evolution of the kype was catalyzed by starvation-related deformation of these other tissues of interest. As was shown by Phleger et al. (1989) and Phleger et al. (1995), loss of calcium phosphate in the dentary during starvation results in transformation from bone to cartilage. This transformation, in turns, yields deformation of the mandible, a deformation that may have catalyzed the evolution of the rapidly growing kype. Furthermore, the trends and relationships between life history stage and mode, environment, and phylogenetic position may also be of use in validating alternative salmonid phylogenetic trees, such as those discussed above.

### ***Further research***

Several avenues of further research would expand on this study of the tissues of the salmonids and of the kype, as well as improve our understanding of the phylogenetic relationships of the salmonids. These avenues include further comparison of the kype in each of the three kype-bearing genera, comparisons of the structure and composition of the kype in repeat spawners versus those of first-time spawners, assessments of the characteristics of the species within *Hucho*, and further consideration of the arguments for the redefining and relocating of those species, and an establishment of the origin of the combination of Sharpey-fibre and chondroid bone in the dentary.

While I found in the current study that it was possible to use physical examinations, Micro-CT assessments, and histological analysis of several representatives

of each kype-bearing genera to establish that the structure of the kypes of *Oncorhynchus* species differ from those of *Salmo* and *Salvelinus*, my sample size was very small. An additional study that used a larger number of known kype-bearing individuals is required in order to confirm that the kype of *Oncorhynchus* differs in structure and organization from those of *Salmo* and *Salvelinus*. Furthermore, if this study used flow and hydrodynamic tests on representatives of these three kype-bearing genera, it may reveal that differences in the morphology of the kype in fact represent adaptation to the respective environment of each genus.

In this study, I was able to establish that relationships between the extent and size of the kype and the phylogenetic position, life history mode, and environment of a given individual exist. However, as I did not have known repeat spawners available for this study, further research that compared the structure and composition of the kype in known repeat spawners versus those of semelparous, one-time spawners is required in order to further distinguish trends in kype growth and complexity. The study of the conditions of the kype in repeat spawners would improve our knowledge as to how the maintenance of portions of the kype, and of modifications to tissues of interest, between spawning events affects its structure and composition. Further work is also required to assess the implications of these modifications to the various tissues of interest within the lower jaw (the kype, the compact dentary, fat accumulations, breeding teeth, and epithelium)

Much in the same way that my analysis of factors that influence that size and the extent of the kype was limited by the specimens I had available to me, my analysis of the characters present in species of *Hucho* was limited by my minimal access to representatives of that genus. Further research that assesses the characteristics of the species within *Hucho*, and of *H. perryi* in particular, is needed to advocate for a relocation of *Hucho* on the salmonid phylogenetic tree. Furthermore, this research may aid in validating the creation of a separate *Parahucho* genus, as well as in verifying where on the salmonid phylogenetic tree that genus belongs.

Finally, while the kype is only present in the eusalmonines, the tissues of which it is composed are found in all salmonids. Sharpey-fibre bone and chondroid bone are found, in combination, in virtually all of the salmonid species examined in this study. In order to establish the evolution of this unusual combination of tissues, paleohistology on

ancestral Salmoniformes fish such as *Helgolandichthys* (Taverne, 1981) or histology on other Protacanthopterygians such as the Esociformes (pikes and mudminnows) or Osmeriformes (galaxiids and smelts) is required.

### ***Final conclusions***

Improving our understanding of how the kype and the tissues associated with it influence one another and evolved will help us to better determine the evolution of one of our most socially, economically, and culturally important groups of fishes, the salmonids. Salmonids and eusalmonines display a variety of tissues and tissue modifications, some of which, such as secondary cartilage, are found in few other teleosts (Benjamin, 1988, 1989a, 1989b; Hall, 2005). Of particular interest are the Sharpey-fibre bone and chondroid bone found in the kype of many spawning eusalmonines and non-spawning salmonids alike. The combination of these two skeletal elements in a single tissue is highly unusual and, in addition to the variety of other tissues within the salmonid skeletal network, makes salmonids a model system for the study of these tissue types. In studying the nature of the relationships between skeletal tissues such as chondroid and Sharpey-fibre bone or skeletal elements such as the kype and the other tissues of the lower jaw like fat accumulations, epithelium, blood vessels, and breeding teeth, this project expands our knowledge as to the importance and implications of secondarily developing tissues and structures in teleosts and vertebrates in general.

Genus and species	Specimen ID	Condition	Date collected	Collection site	SL (mm)	Sex	Life history stage
<i>Coregonus</i>							
- <i>C. clupeaformis</i>	UW 05587	Fleshed	December 1947	Great Slave Lake, Northwest Territories, Canada	440	Male?	Spawning
<i>Stenodus</i>							
- <i>S. leucichthys</i>	CMNFI-Z004302	Defleshed	July 1995	Anderson River, Northwest Territories, Canada	660	Male	Pre-spawning
<i>Prosopium</i>							
- <i>P. williamsoni</i>	UW 005260	Fleshed	November 1945	Columbia River, Washington, USA	370	Male	Pre-spawning
- <i>P. williamsoni</i>	CMFI-1969-0203.1	Defleshed	June 1969	Cultus Lake, Chilliwack, British Columbia, Canada	254	Unsexed	Post-spawning
<i>Thymallus</i>							
- <i>T. arcticus</i>	CMNFI-1977-ID undesignated	Defleshed	July 1977	Mouth of Johnson Creek, Old Crow River, Yukon, Canada	390	Unsexed	Pre-spawning
<i>Brachymystax</i>							
- <i>B. lenok</i>	UW 022131	Fleshed	June 1990	Junction of Sharlan and Hongui Rivers, Origin of Eroo River, Selenge Province, Mongolia	126	Unsexed	Immature

Genus and species	Specimen ID	Condition	Date collected	Collection site	SL (mm)	Sex	Life history stage
<i>Hucho</i>							
- <i>H. hucho</i>	UW 022130	Fleshed	June 1990	Junction of Sharlan and Hongui Rivers, Origin of Eroo River, Selenge Province, Mongolia	473	Male	Post-spawning
- <i>H. hucho</i> *	ZIN 7872	Fleshed	Unknown	Lake Tranvensis, Russia	358	Unsexed	Mature
- <i>H. perryi</i> *	ZIN 13160	Fleshed	March 1901	Lutoga River, Sakhalin, Russia	395	Unsexed	Spawning
- <i>H. perryi</i> *	ZIN (ID unknown)	Defleshed	Unknown	Sakhalin, Russia	598	Unsexed	Mature
<i>Salvelinus</i>							
- <i>S. fontinalis</i>	UW 28888	Fleshed	October 1935	Unplaced	285	Unsexed	Mature
- <i>S. fontinalis</i>	CMFI-1973-0255.1	Defleshed	July 1973	Ontario, Canada	215	Unsexed	Pre-spawning
- <i>S. namaycush</i>	ZP 2013-07-01(A)	Fleshed	July 2013	Kogaluk River system, northern Labrador, Canada	880	Unsexed	Mature, pre-spawning?
- <i>S. namaycush</i>	ZP 2013-07-01(B)	Fleshed	July 2013	Kogaluk River system, northern Labrador, Canada	Unrecorded (670-880)	Unsexed	Mature, pre-spawning?
- <i>S. namaycush</i>	ZP 2013-07-01(C)	Fleshed	July 2013	Kogaluk River system, northern Labrador, Canada	Unrecorded (670-880)	Unsexed	Mature, pre-spawning?
- <i>S. namaycush</i>	ZP 2013-07-01(D)	Fleshed	July 2013	Kogaluk River system, northern Labrador, Canada	670	Unsexed	Mature, pre-spawning?
- <i>S. namaycush</i>	ZP 2013-07-01(E)	Fleshed	July 2013	Kogaluk River system, northern Labrador, Canada	Unrecorded (670-880)	Unsexed	Mature, pre-spawning?

Genus and species	Specimen ID	Condition	Date collected	Collection site	SL (mm)	Sex	Life history stage
<i>Salvelinus</i>							
- <i>S. namaycush</i>	ZP 2013-07-01(F)	Fleshed	July 2013	Kogaluk River system, northern Labrador, Canada	Unrecorded (670-880)	Unsexed	Mature, pre-spawning?
- <i>S. namaycush</i>	UW 020761	Fleshed	Unknown	Lake Michigan, Port Washington, Wisconsin, USA	Unrecorded	Unsexed	Pre-spawning
- <i>S. namaycush</i>	CMNFI-1982-0385	Fleshed	August 1979	Nain Bay, east of Tessioak Lake, Labrador, Canada	400	Unsexed	Mature
- <i>S. namaycush</i>	CMNFI-1958-0100A.1	Defleshed	July 1915	Lake at Bernard Harbour, Nunavut, Canada	Unrecorded, large	Male	Post spawning
- <i>S. namaycush</i> x <i>fontinalis</i> hybrid ("splake")	CMNFI-1976-0187.1	Defleshed	April 1975	Algonquin Park, Ontario, Canada	342	Unsexed	Immature
- <i>S. leucomaenis</i>	UW 042437	Fleshed	August 1998	Northern Iturup; Okhotsk Sea Side, inland from Slavnaya Bay; Glush River, Kuril Archipelago, Russia	216	Unsexed	Pre-spawning
- <i>S. confluentus</i>	UW 20760	Fleshed	October 1956	Athabasca River, Alberta, Canada	500	Male	Pre-spawning
- <i>S. alpinus</i>	UW 041192	Fleshed	June 1953	Lake Nerka, Alaska, USA	410	Unsexed	Immature (nearly mature?)
- <i>S. alpinus</i>	CMNFI-1961-0229	Fleshed	Sept 1961	Eskimo Point, Western Hudson Bay, Canada	470	Male	Pre-spawning, spawning
- <i>S. alpinus</i>	CMNFI-1968-1262.1	Fleshed	February 1962	St. Lawrence Island beach, Bering Sea Alaska, USA	380	Male	Spawning

Genus and species	Specimen ID	Condition	Date collected	Collection site	SL (mm)	Sex	Life history stage
<i>Salvelinus</i>							
- <i>S. alpinus</i>	CMNFI-1977-0348.1	Defleshed	Summer 1977	Pauline Cove, Herschel Island, Yukon Territory, Canada	Unrecorded, large	Unsexed	Mature
- <i>S. alpinus</i>	CMNFI-1979-1001.1	Defleshed	May 1972	Baffin Island, Northwest Territories, Canada	397	Male	Immature
- <i>S. alpinus aureolus</i>	UW 020740	Fleshed	November 1972	Floods Pond, Maine, USA	~300	Unsexed	Immature
<i>Salmo</i>							
- <i>S. trutta</i>	UW 005350	Fleshed	July 1940	Angeline Lake, Washington, USA	290	Male	Pre-spawning
- <i>S. salar</i>	UW 019807	Fleshed	Unknown	St. Johns River, Nova Scotia, Canada	250	Unsexed	Mature
- <i>S. salar</i>	CMFI-1980-0181.1	Defleshed	Fall 1979	St. Andrews International Atlantic Salmon Foundation hatchery	610	Male	Spawning
<i>Oncorhynchus</i>							
- <i>O. clarki</i>	UW 028776	Fleshed	August 1952	Wolf Creek, Montana, USA	242	Unsexed	Pre-spawning
- <i>O. clarki</i>	CMNFI-1973-0393.1	Fleshed	October 1960	Kennedy Lake, West Coast of Vancouver Island, British Columbia, Canada	221	Unsexed	Pre-spawning
- <i>O. mykiss</i>	UW 016073	Fleshed	May 1961	Warden Lake, Washington, USA	~200	Unsexed	Immature

Genus and species	Specimen ID	Condition	Date collected	Collection site	SL (mm)	Sex	Life history stage
<i>Oncorhynchus</i>							
- <i>O. mykiss aguabonita</i>	UW 015967	Fleshed	December 1961	Goldendale Hatchery, Columbia River Basin, Washington, USA	310	Female	Spawning
- <i>O. kisutch</i>	UW 015091	Fleshed	September 1952	Unknown	Unrecorded, large	Female?	Pre-spawning
- <i>O. kisutch</i>	CMNFI-Z000804	Defleshed	Unknown	Unknown	Unrecorded, large	Male	Spawning
- <i>O. tshawytscha</i>	UW 015893	Fleshed	August 1952	Puget Sound, Possession Point, South Whidbey Island, Washington, USA	330	Unsexed	Pre-spawning
- <i>O. keta</i>	UW 13664	Fleshed	July 1957	Aleutian Islands, False Pass, Alaska, USA	Unrecorded, large	Male	Pre-spawning
- <i>O. gorbuscha</i>	UW 16027	Fleshed	August 1961	Chukchi Sea, Alaska	430	Male	Pre-spawning, Spawning
- <i>O. gorbuscha</i>	CMNFI-1984-0169.1	Fleshed	August 1983	Norton Sound, Bering Sea, Alaska, USA	235	Unsexed	Mature, pre-spawning?
- <i>O. nerka</i>	UW 005624	Fleshed	1939, month unknown	Alaska Peninsula, Shumagin Island, South East Alaska, USA	367	Unsexed	Mature
- <i>O. nerka</i>	CMNFI-1977-0277.1	Defleshed	November 1976	Qualicum Beach, Vancouver Island, British Columbia, Canada	Unrecorded, large	Male	Spawning

\*Received photographs and/or X-Ray images of specimens, but did not have physical access to them



**Appendix B – Numbers of fleshed and defleshed specimens examined**

<b>Genus and species</b>	<b>Fleshed</b>	<b>Defleshed</b>	<b>Total</b>
<b>Coregoninae</b>	<b>2</b>	<b>2</b>	<b>4</b>
- <i>Coregonus clupeaformis</i>	1		1
- <i>Stenodus leucichthys</i>		1	1
- <i>Prospium williamsoni</i>	1	1	2
<i>Thymallus arcticus</i>		<b>1</b>	<b>1</b>
<i>Brachymystax lenok</i>	<b>1</b>		<b>1</b>
<b>Hucho</b>	<b>3</b>	<b>1</b>	<b>4</b>
- <i>H. hucho</i> *	2		<b>2</b>
- <i>H. perryi</i> *	1	1	<b>2</b>
<b>Salvelinus</b>	<b>15</b>	<b>5</b>	<b>20</b>
- <i>S. fontinalis</i>	1	1	2
- <i>S. namaycush</i>	8	1	9
- <i>S. namaycush</i> / <i>S. fontinalis</i> hybrid (“splake”)		1	1
- <i>S. leucomaenis</i>	1		1
- <i>S. confluentus</i>	1		1
- <i>S. alpinus</i> **	4	2	6
<b>Salmo</b>	<b>2</b>	<b>1</b>	<b>3</b>
- <i>S. trutta</i>	1		1
- <i>S. salar</i>	1	1	2
<b>Oncorhynchus</b>	<b>10</b>	<b>2</b>	<b>12</b>
- <i>O. clarki</i>	2		2
- <i>O. aguabonita</i>	1		1
- <i>O. mykiss</i>	1		1
- <i>O. kisutch</i>	1	1	2
- <i>O. tshawytscha</i>	1		1
- <i>O. keta</i>	1		1
- <i>O. gorbuscha</i>	2		2
- <i>O. nerka</i>	1	1	2
<b>TOTAL</b> 23 species	<b>33</b>	<b>12</b>	<b>45</b>

\* Received photographs and/or X-Ray images of specimens, but did not have physical access to them

\*\*Includes a specimen identified as *S. alpinus aureolus*

Genus and species	Specimen No.	Age	Collection site	Details	Life history stage
<i>Stenodus</i>					
- <i>S. leucichthys</i>	CMNFV-48886	Quaternary	11A Old Crow River, Yukon, Canada	Left dentary	Unknown
<i>Eosalmo</i>					
- <i>E. driftwoodensis</i>	RBCM.EH 2011.006.00 01	Eocene	Driftwood Canyon, Smithers, British Columbia, Canada	Partial skeleton	Unknown
- <i>E. driftwoodensis</i>	UWBM-82489	Eocene	C0658 Corner Lot, Klondike Formation, Republic, Washington, USA	Partial skeleton	Unknown
<i>Salvelinus</i>					
- sp.	UOF-26785	Miocene	UO2227 Worden, Oregon, USA	Right dentary	Spawning?
- sp.	UOF-26786	Miocene	UO2227 Worden, Oregon, USA	Right dentary	Spawning?
- sp.	UOF-26787	Miocene	UO2227 Worden, Oregon, USA	Left dentary	Spawning?
<i>Oncorhynchus</i>					
- sp.	UOF-26773	Miocene	UO2227 Worden, Oregon, USA	Left dentary	Unknown
- sp.	UOF-26782	Miocene	UO2404 Jackass Butte, Oregon, USA	Right dentary	Unknown
- sp.	UOF-31389	Miocene	UO2404 Jackass Butte, Oregon, USA	Left dentary	Unknown
- <i>O. nerka</i>	UWBM-87112	Pliocene	C0759 South Fork, Skokomish Valley, Washington, USA	Partial skeleton	Spawning
- <i>O. nerka</i>	UWBM-87121	Early Pleistocene (1Ma)	Skokomish Valley, Washington, USA	Right ventral breeding tooth	Spawning
- <i>O. nerka</i>	UWBM-87122	Pliocene	Skokomish Valley, Washington, USA	Partial skeleton	Spawning

Genus and species	Specimen No.	Age	Collection site	Details	Life history stage
<i>Oncorhynchus</i>					
- <i>O. nerka</i>	UWBM-95842	Pleistocene	C0759 South Fork, Skokomish Valley, Washington, USA	Left dentary, part of left premaxillary (with tooth)	Spawning
- ( <i>O. Smilodonichthys</i> ) <i>rastrosus</i>	UOF-3335	Late Miocene	UO 2250 Gateway Locality, Madras Formation, Oregon, USA	Partial skeleton (left dentary of interest)	Spawning
- <i>O. (Smilodonichthys) rastrosus</i>	UOF-26799	Late Miocene	UO 2250 Gateway Locality, Madras Formation, Oregon, USA	Partial skeleton (left dentary of interest)	Spawning
- <i>O. (Smilodonichthys) rastrosus</i>	UWBM-50816	Middle Pliocene	B1532 Arlington 3, Oregon, USA	Left dentary	Unknown
- <i>O. (Smilodonichthys) rastrosus</i>	UWBM-71908a	Pliocene	A204 Ringold Formation, Washington, USA	Two left dentaries	Spawning?
- <i>O. (Smilodonichthys) rastrosus</i>	UWBM-71908b	Pliocene	A204 Ringold Formation, Washington, USA	Two left dentaries	Spawning?

**Appendix D – Numbers of fossil specimens examined**

<b>Genus and species</b>	<b>Fossils</b>
Coregoninae	<b>1</b>
- <i>Stenodus leucichthys</i>	1
<i>Eosalmo</i>	<b>2</b>
- <i>E. driftwoodensis</i>	<b>2</b>
<i>Salvelinus</i>	<b>3</b>
- sp.	3
<b><i>Oncorhynchus</i></b>	<b>12</b>
- <i>O. nerka</i>	4
- <i>O. (Smilodonichthys) rastrosus</i>	5
- sp.	3
<b>TOTAL</b> 4 identified species	<b>20</b>

## **Appendix E – Standard paleo-slide production procedures**

With the exception of UWBM 87121, prior to embedding, small portions of tissues of interest were removed from the fossil using the table saw feature of an Isomet® 1000 precision saw. UWBM 87121 had a pre-existing break that divided the tissues of interest from the remainder of the dentary. This break had been mended using an unknown consolidant, which was dissolved using acetone, thereby dividing the tissue of interest from the main without causing any further damage to the fossil. In the case of some of the weaker specimens, namely UOF-26773 and UOF-26782, tissues were reinforced using Technovit® 5071, a fast curing resin easily dissolved in acetone.

While dissected specimens were left to dry, embedding moulds were prepared. Moulds of an appropriate size for each specimen were reinforced and built up with duct tape and then coated with a polyvinyl alcohol separator to ensure that the cured resin block containing the embedded specimen could be easily removed. Next, an initial pour of 0.5 cm of Castolite® AP, a polyester resin used in specimen embedding, and its catalyst, Eager Hardener, would ensure that specimens “float” in their embedding medium and do not rest on the bottoming of the embedding mould. Before the resin of the initial pour could harden, moulds were placed in a vacuum chamber for 5 minutes to remove as many gas bubbles as possible.

After 24 hours, the initial pour of embedding moulds were cured and specimens were dry. Specimens were placed in moulds and submerged in Castolite® resin. Working quickly, submerged specimens were oriented into the desired position and moulds were placed in a vacuum chamber for approximately 25 minutes. Once as many gas bubble as possible were removed, moulds were set aside for 24 hours to cure.

Following resin curing, resin blocks were removed from moulds and cutting orientation was denoted on each block. One at a time, blocks were set in the chuck of the gliding arm of the Isomet® 1000 saw such that the blade at 180 rpm was in line with the desired cutting orientation. The weighted gliding arm used gravity to slowly pull the block onto the rotating blade, stressing the embedded specimen as little as possible. The cut surfaces of the specimen were then hand polished and were left to dry.

While polished specimens were left to dry, glass slides were prepared by frosting with a Hillquist thin section machine hand grinder. Slides, cleaned with ethanol, were wetted and fixed to the rotating arm of the Hillquist machine using surface tension. The slide was slowly moved into contact with the grinding surface of the machine and was ground down micrometers at a time to a wide of approximately 10  $\mu\text{m}$ . Once appropriately frosted, slides were hand polished and left to dry.

When both the cut surface of the specimen and the frosted surface of the slide were dry, the slide was greased using a Bohle alcohol-based slide cleaner, and the polished surface of the embedding block was glued to the slide using 3M<sup>TM</sup> Scotch-Weld CA40 cyanoacrylate adhesive. A small amount of adhesive was poured onto exposed bone, following which the slide was slowly laid onto the block, avoiding bubbles as much as possible. Scraping off excess glue, the adhesive was left to dry.

Approximately three hours later, the slide could be placed into the slide holder of the gliding arm of the Isomet® 1000. The position of the gliding arm was then zeroed with the blade of the saw resting against the slide, following which the gliding arm was moved approximately 5 mm from the blade. Gently resting the block onto the moving blade, the slide was slowly pulled onto the blade by gravity and cut.

Once cut, both the block, containing the remainder of the specimen, and the slide were polished down. The block was polished by hand alone, and the slide was initially thinned using the thin sectioning blade of the Hillquist thin section machine and then, once the specimen was of an appropriate thickness to view under the microscope, the surface of the slide was hand polished to remove scratching from the cutting and grinding blades. The standard process of slide preparation, gluing block to slide, specimen cutting, and subsequent polishing could then be repeated to produce multiple, consecutive paleo-sections for each specimen.

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