1 # A digital dissection of two teleost fishes: comparative

2 # functional anatomy of the cranial musculoskeletal

3 # system in pike (*Esox lucius*) and eel (*Anguilla anguilla*)

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Running header: Digital Dissection of *Esox* and *Anguilla*

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Abstract

Advances in X-ray computed tomography (CT) have led to a rise in the use of non-destructive imaging methods in comparative anatomy. Among these is contrast-enhanced CT scanning, which employs chemical stains to visualize soft tissues. Specimens may then be "digitally dissected", producing detailed, three-dimensional digital reconstructions of the soft- and hard-tissue anatomy, allowing examination of anatomical structures in situ and making accurate measurements (lengths, volumes, etc.). Here we apply this technique to two species of teleost fish, providing the one of the first comprehensive three-dimensional description of teleost cranial soft tissue and quantifying differences in muscle anatomy that may be related to differences in feeding ecology. Two species with different feeding ecologies were stained, scanned and imaged to create digital 3D musculoskeletal reconstructions: *Esox lucius* (Northern Pike), predominantly a suction feeder, and *Anguilla anguilla* (Eruopean eel), which captures prey predominantly by biting. Muscle cross sectional areas were calculated and compared between taxa, focusing on muscles which serve important roles in feeding. The adductor mandibulae complex – used in biting - was larger in *Esox* than *Anguilla* relative to head size. However, the overall architecture of the adductor mandibulae was also very different between the two species, with that of *Anguilla* better optimised for delivering forceful bites. Levator arcus palatini and sternohyoideus – which are used in suction feeding - are larger in *Esox*, whereas the levator operculi is larger in *Anguilla*. Therefore, differences in the size of functionally important muscles do not necessarily correlate neatly with presumed differences in feeding mode.

Keywords

Introduction

Teleost fishes make up approximately half of vertebrate diversity (Nelson, 2006) with over 31,000 living species and new species being described every year (Eschmeyer and Fong, 2013); they occupy a huge variety of ecological niches and exhibit a wide range of morphological and behavioural adaptations (Helfman et al., 2009). A major source of variation in teleosts is the diverse array of feeding modes employed by different species. The majority of teleosts (like most aquatic animals) capture their prey using suction feeding (Wainwright et al., 2015), in which expansion of the buccal cavity through elevation of the neurocranium, depression of the hyoid and lateral expansion of the suspensorium creates a negative pressure inside the mouth. The resulting pressure gradient draws prey into the mouth and through the oral cavity for swallowing (Day et al., 2015; Lauder, 1985; Westneat, 2006).

Suction feeding is believed to be the ancestral feeding mode of teleosts, and some groups (e.g. Cypriniformes, Acanthomorpha) have become highly specialised for suction feeding (Wainwright et al., 2015). Another major feeding mode within teleosts is biting, capturing prey items by grasping them firmly in the oral jaws (Alfaro et al., 2001; Mehta and Wainwright, 2007a). Biting behaviours frequently involve removing pieces of prey items, circumventing the constraints that maximum gape size places on prey size in suction feeders (Alfaro et al., 2001).

Biting and suction feeding are often presented as a dichotomy, but this is misleading as the use of biting does not preclude suction generation (Alfaro et al., 2001). Some biting taxa use suction to position prey and almost all biters retain suction capacity for intra-oral transport (with a few derived exceptions e.g. moray eels (Mehta and Wainwright, 2007b)). Biting and suction feeding place differing functional demands on the skull and jaws, which are predicted to strongly influence the form of the cranial musculoskeletal system (Barel, 1982; Liem, 1990; Wainwright et al., 2004; but see Van Wassenbergh et al., 2007).

63   Biting is powered by the action of the adductor mandibulae complex (Alfaro et al., 2001)

64   which is responsible for jaw closure. Suction feeding is powered by the axial musculature, with the

65   epaxial and the hypaxial muscles driving expansion of the buccal cavity through multiple

66   musculoskeletal linkages (Camp et al., 2015; Camp and Brainerd, 2014). Whilst previous models of

67   suction feeding posited major roles for the cranial and hyoid muscles - the levator arcus palatini,

68   levator opercula and sternohyoideus - in the linkages responsible for lateral and ventral cranial

69   expansion (Liem, 1980a; Lauder, 1982, 1985; Westneat, 2006), new measurements have shown that

70   most (if not all) of the power comes from the axial musculature (Camp et al., 2015). Nevertheless,

71   the cranial and hyoid muscles are likely to still have an important role over the precise control of

72   suction feeding kinematics, which will affect prey capture efficiency (Camp and Brainerd, 2015).

73   There have been several broad surveys of teleost musculoskeletal anatomy (Gregory, 1933;

74   Greenwood, 1971; Winterbottom, 1973; Datovo and Vari, 2013, 2014) as well as detailed descriptive

75   (Geerinckx and Adriaens, 2007; Huysentruyt et al., 2007; Leysen et al., 2011) and functional studies

76   (Herrel et al., 2002; Van Wassenbergh et al., 2005; Goulet et al., 2016) focusing on specific taxa.

77   However, these data are almost exclusively presented in two dimensions as either photographs or

78   line drawings, and so are limited in the amount of information they can subsequently convey on 3D

79   muscle orientation and topology. This, in turn, limits the accuracy of functional inferences that can

80   be drawn (e.g. bite force estimates) (Lautenschlager, 2013).

81   Contrast-enhanced computed tomography (CT) scanning is a technique which has become

82   increasingly popular in comparative anatomy, allowing non-destructive imaging of both hard and soft

83   tissues and has been shown to produce excellent resolution in both small and large vertebrate

84   specimens (Jeffery et al., 2011; Gignac et al., 2016)(Fig. 1). Specimens are stained with contrast

85   enhancing agents prior to scanning which are taken up differentially by soft tissues, improving soft

86   tissue resolution and allowing differentiation of tissue types (Descamps et al., 2014; Gignac et al.,

87   2016). As a result, contrast-enhanced CT scans have been used to "digitally dissect" specimens,

88   producing interactive three dimensional virtual anatomical atlases (Holliday et al., 2013;

89   Lautenschlager et al., 2014; Porro and Richards, 2017) and provide useful functional data on soft

90   tissue, e.g., muscle volumes (Cox and Faulkes, 2014; Sharp and Trusler, 2015).

91   To date, this method has mostly been applied to tetrapods (Cox and Jeffery, 2011; Holliday et

92   al., 2013; Lautenschlager et al., 2014; Sharp and Trusler, 2015; Porro and Richards, 2017), but there

93   are examples of contrast enhanced CT and "digital dissection" of teleosts (De Meyer et al., 2018a).

94   Although several studies have presented 3D reconstructions of teleost anatomy both graphically

95   (Anker, 1974; Adriaens and Verraes, 1996a, 1996b) and digitally (Leysen et al., 2011; Bouilliart et al.,

96   2015), these are almost all based on destructively sampled histological data; the non-destructive

97   nature of contrast-enhanced CT makes it an attractive option, and the digital anatomical models can

98   be readily used in biomechanical modelling applications (Cox et al., 2011; Orsbon et al., 2018).

99   In order to better understand the link between skull form and function in teleosts, specimens

100   of two taxa with different feeding modes were subjected to contrast-enhanced CT scanning - the

101   Northern Pike (*Esox lucius* Linnaeus 1758), predominantly a suction feeder, and the European eel

102   (*Anguilla anguilla* Linnaeus 1758) which predominantly uses biting. Both taxa are piscivorous and

103   adapted to taking fairly large prey. In addition, neither possesses extreme morphological

104   specialisations for their preferred feeding mode, e.g., the protrusible upper jaws of suction feeding

105   Cypriniformes and Acanthomorpha (Wainwright et al., 2015), or the raptorial pharyngeal jaws of

106   biting Moringuids (Mehta and Wainwright, 2007b), making them more directly comparable.

107   The musculoskeletal anatomy of the skull and hyoid is reconstructed in 3D to produce digital

108   dissections of these two teleosts (Fig. 2-7). In order to perform quantitative comparisons between

109   species with different feeding mechanisms, muscle volume and cross-sectional area was measured

110   for functionally important muscle groups and compared between *Esox* and *Anguilla.* Given the

111   differences in predominant feeding mode between these two taxa, it is hypothesised that the jaw

112   adductor muscles which power biting would be larger in *Anguilla,* and the suspensorial, opercular

113 and hyoid muscles which control suction feeding would be larger in *Esox*. In addition to testing these

114 specific hypotheses, the detailed digital dissections provide additional data on musculoskeletal

115 architecture which may be of functional relevance.

116 ## Methods

117 A sub-adult *Esox* (head length (HL) measured from the anterior edge of the premaxilla to the

118 posterior edge of the epiotic = 60mm) and an adult *Anguilla* (HL = 26mm) were used in this study.

119 *Anguilla anguilla* exhibits a broad vs narrow head shape dimorphism which is related to diet (Ide et

120 al., 2011; De Meyer et al., 2018a, 2018c); the specimen used in this study was broad headed

121 individual, which are more piscivorous. The *Esox* specimen was micro-computed tomography (μCT)

122 scanned at the Imaging and Analysis Centre at the Natural History Museum (London, UK) on a X-Tek

123 HMX-ST μCT 255 scanner (Nikon Metrology, Tring, UK) at 180 kV and 120 μA with a 0.5 mm copper

124 filter producing 1999 TIFF images with a resolution of 0.043mm/voxel. Afterwards, the *Esox*

125 specimen was fixed in 4% buffered paraformaldehyde solution and stained using a 10% I_2KI solution

126 for 7 days. Following staining, the *Esox* head was briefly rinsed with 90% ethanol (to wash excess

127 stain from the skin) and scanned at 185 kV and 180 μA with a 0.1 mm copper filter producing 1999

128 TIFF images with a resolution of 0.043mm/voxel. The *Anguilla* specimen was preserved in 4%

129 buffered formalin solution and scanned at the UGCT Department at the University of Ghent

130 (Belgium) with the HECTOR μCT scanner at 120 kV and 117 μA, producing 1170 TIFF images at a

131 resolution of 0.07 mm/voxel. The head was then stained in a 5% solution of phosphomolybdic acid

132 (PMA) for 29 days, then scanned again at 120 kV and 260 μA, producing 668 TIFF images at a

133 resolution of 0.07 mm/voxel.

134   All CT data were imported into Avizo 7.0 - 8.0 (FEI Visualization Science Group, Oregon, USA).

135 Bones were separated from soft tissues in the unstained datasets using automatic thresholding,

136 although some manual segmentation was necessary to separate individual bones from each other.

137 Segmentation of soft-tissue structures in the stained dataset was performed manually. For each

138 taxon, the two datasets (unstained and stained) were aligned and merged to create single

139 musculoskeletal models containing all hard and soft tissues using landmarks visible in both data sets.

140   For the quantitative comparison across the two taxa, muscle volumes were computed using

141 the "Material Statistics" module in Avizo. Muscle volumes were divided by muscle length to obtain

142 estimates for cross sectional area (CSA) for the adductor mandibulae muscles. Total muscle length

143 was used here as an approximation for muscle fibre length, measured using the standard Measure

144 tools in Avizo, as pennation angle (and hence physiological cross sectional area) could not be readily

145 determined from the stained CT scan data. To account for differences in specimen size, the results for

146 *Anguilla* were scaled to those of a specimen with the same cranial volume (defined as the volume

147 enclosed by the head for all slices anterior to the occipital condyle) as the *Esox* specimen. Finally, a

148 correction factor was applied to the *Esox* muscle measurements as I_2KI is known to cause soft tissue

149 shrinkage (Vickerton et al., 2013). This correction factor was derived through comparison of total

150 tissue volume and cross-sectional area in the un- and stained *Esox* datasets. Hard tissue volume was

151 computed from the unstained dataset, and total cranial volume for both the stained and unstained

152 datasets using the "Material Statistics" module in Avizo. Soft tissue volumes pre- and post-staining

153 were then calculated by subtracting hard tissue volume from these totals. Soft tissue volume

154 decreased 35% as a result of staining, and cross-sectional area decreased by 25%. No correction was

155 necessary for the *Anguilla* data, as PMA does not cause noticeable soft tissue shrinkage (Descamps

156 et al., 2014). Cross-sectional area (proportional to muscle force output) was compared for the

157 adductor mandibulae complex, and muscle volumes (proportional to muscle power output) were

158 compared for the adductor mandibulae, as well as the suspensorial, opercular and hyoid muscles.

159 Three muscles of particular interest are the levator arcus palatini, the levator operculi, and the

160 sternohyoideus, all of which are hypothesised to play roles in suction feeding.

161 ## Results

Osteology

The osteology of the skull in teleosts has been described extensively (Gregory, 1933). For a brief illustrated summary of the various skull bones of *Esox* and *Anguilla*, which are referenced during the descriptions of the cranial muscles, see Fig. 2.

Cranial Musculature

Adductor Mandibulae

Following the nomenclature of Datovo and Vari, (2013) the adductor mandibulae is divided into two segments: a facial segment, the segmentum facialis (Fig. 3), and a mandibular segment, the segmentum mandibularis (Fig. 4).

Segmentum Facialis

In teleosts, the segmentum facialis consists of three parts: the pars malaris, pars rictalis and pars stegalis (Fig. 3). The pars rictalis forms the ventro-lateral portion, the pars malaris forms the dorso-lateral portion, and pars stegalis forms the medial portion. In some taxa, including *Esox*, the rictalis and malaris forms a compound facial segment, the ricto-malaris (Datovo and Vari, 2014). In *Anguilla*, the rictalis and malaris segments share a common origin, but are divided anteriorly into distinct parts (Datovo and Vari, 2014), and so are discussed separately here. Additionally, the pars stegalis may be differentiated into a dorso-lateral epistegalis portion and a medio-ventral substegalis portion. This division is present in *Anguilla*, but in *Esox* there is only a single undivided stegalis section (Datovo and Vari, 2014).

Pars Rictalis & Malaris

The ricto-malaris in *Esox* originates ventral to the levator operculi, adductor operculi and dilator operculi muscles, lateral to the stegalis. The origin site covers multiple bones of the suspensorium, including ventral and lateral portions of the hyomandibula, the medial side of the anterodorsal

185 portion of the preopercula, and the lateral side of the quadrate. A group of fibres originating from

186 the posterior process of the hyomandibula pass through the ovoid fenestra bounded by the

187 hyomandibula and preopercula. The remaining fibres, which form the main body of the muscle, are

188 directed anteriorly, lateral to the stegalis and palatal muscles. In *Esox,* the ricto-malaris inserts onto

189 the medial side of the dentary and angulo-articular via the mandibular tendon and the mandibular

190 raphe, at the level of the coronoid process.

191   In *Anguilla*, the origin of the rictalis covers the dorsal portions of the preopercula, the lateral

192 side of the posterior portion of the hyomandibular body, and the lateral surface of the pterotic. The

193 muscle fibres are directed anteroventrally, ventral to the malaris and lateral to the dilator operculi

194 (posteriorly) and the stegalis (anteriorly). The fibres of the rictalis insert onto the lower jaw via the

195 intersegmental aponeurosis and a large tendinous sheet, attaching to the coronoid process of the

196 dentary. The malaris muscle in *Anguilla* is subdivided into a retromalaris and promalaris (Datovo and

197 Vari, 2014). Both subdivisions share the epaxialis and supra-cranial fasciae as part of their origin

198 sites, but additionally, the promalaris originates from the frontals, parietals and the mid-sagittal

199 tendinous raphe, whereas the retromalaris originates from the pterotic (Datovo and Vari, 2014). The

200 whole malaris section inserts onto the lower jaw via the mandibular tendon.

201 **Pars Stegalis**

202   The stegalis segment of *Esox* originates from the metapterygoid, and the anterior portions of

203 the hyomandibula, medial to the rictomalaris, travelling along the lateral side of the anteriorly

204 projecting hyomandibular arm. The fibres of the stegalis are directed anteriorly, and the stegalis

205 inserts onto the lower jaw via the Meckelian tendon, which attaches to the coronomeckelian bone.

206   In *Anguilla*, the epistegalis has its origin on the lateral surface of the pterotic and anterior

207 face of the sphenotic. The substegalis originates from the lateral side of the hyomandibula (within

208 the "fork" of the two processes that contact the quadrate). The fibres of both the epistegalis and

209 substegalis travel anteroventrally, but whilst the epistegalis inserts directly onto the medial surface of

210 the dentary and articular, the substegalis inserts onto the coronomeckelian bones via the Meckelian

211 tendon.

212 **Segmentum Mandibularis**

213 The segmentum mandibularis is made up of two parts: the pars coronalis and the pars mentalis (Fig.

214 4). In *Esox*, this entire section originates from the mandibular raphe (Datovo and Vari, 2014), a band

215 of connective tissue which separates the mandibular and facial segments of the adductor

216 mandibulae. In *Anguilla*, the segmentum mandibularis is completely absent.

217 **Pars coronalis**

218 The pars coronalis forms the dorsal part of the segmentum mandibularis in *Esox*. It attaches to the

219 dorsal surface of Meckel's cartilage, as well as the dorsomedial aspect of the dentary.

220 **Pars Mentalis**

221 The pars mentalis forms the ventral part of the segmentum mandibularis in *Esox*. It attaches to the

222 ventro-medial aspect of the dentary and Meckel's cartilage.

223 **Suspensorial Musculature**

224 The three major muscles are the levator arcus palatini, the adductor arcus palatini and the adductor

225 hyomandibulae (Fig. 5).

226 **Levator Arcus Palatini**

227 The levator arcus palatini serves to laterally expand the buccal cavity through abduction of the

228 suspensorium. In both *Esox* and *Anguilla* this muscle originates from the ventral surface of the

229 sphenotic, posterior to the eyeball. In *Esox*, the muscle fibres are directed posteriorly, inserting on

230 the dorsomedial side of the anterior arm of the hyomandibula, and on the anterior portions of the

231 hyomandibular body.

232   In *Anguilla*, the fibres of the levator arcus palatini "fan out" from their origin on the ventral

233   sphenotic, and insert posteriorly onto the lateral side of the hyomandibular body, mid-way onto the

234   anterior arm of the hyomandibular, and anteriorly onto pterygoids.

235   **Adductor Arcus Palatini**

236   The adductor arcus palatini is responsible for suspensorial adduction. The muscle originates on the

237   postero-ventral portion of the parasphenoid and ventro-lateral sides of the braincase in *Esox*, ventral

238   to the eyeball. The muscle is directed posteriorly, and terminates ventral to adductor operculi,

239   inserting onto the medial side of the hyomandibular body and the dorsal portion of metapterygoid.

240   The origin site is similar in *Anguilla*; the ventral margins of the braincase and lateral edges of

241   the postero-ventral body of the parasphenoid. The muscle inserts on the medial side of the

242   hyomandibula (attaching to the anteriorly projecting "arm").

243   **Adductor Hyomandibulae**

244   In *Esox*, the adductor hyomandibulae origin lies posterior to that of the adductor arcus palatini, on

245   the posterior ventral surface of the parasphenoid and ventrolateral parts of the braincase. The

246   adductor hyomandibulae travels posterolaterally towards its insertion on the medial side of the

247   hyomandibula and metapterygoid, at the contact between the two bones. This muscle is absent in

248   *Anguilla* (De Schepper et al., 2007).

249   **Opercular Muscles**

250   The three major muscle are the dilator operculi, the adductor operculi and the levator operculi (Fig.

251   6).

252   **Dilator Operculi**

253   The dilator operculi is responsible for opercular abduction. In *Esox*, this muscle originates from the

254   ventrolateral surface of the pterotic (dorsal to the levator arcus palatini). The fibres of the dilator

255   operculi then run for a short distance posteriorly to insert on the articular head of the opercular

256   bone.

257   In *Anguilla*, the dilator operculi origin site covers the posterolateral portions of the pterotic

258   and sphenotic, dorsal to the origination sites of the pars substegalis and levator arcus palatini. The

259   dilator operculi muscle then passes posteriorly, medial to the rictalis, ventral to the malaris,

260   dorsolateral to the adductor operculi. The dilator operculi then inserts onto the dorsolateral part of

261   the anterior process of the opercular that contacts the hyomandibula.

262   **Adductor Operculi**

263   The adductor operculi is responsible for opercular adduction. In *Esox*, this muscle has its origins on

264   the posterior parts of the neurocranium and the posterior half of the medial side of the

265   hyomandibular body and posterior arm. The muscle fibres course posteriorly, dorsomedial to the

266   rictalis, medial to the dilator operculi, and ventromedial (then ventral) to the levator operculi. It then

267   inserts onto the antero-medial faces of the main body of the opercular bone.

268   The adductor operculi originates from the ventral surface of the pterotic in *Anguilla*, dorsal

269   to the adductor arcus palatini and ventromedial to the origins of the levator arcus palatini. The

270   muscle fibres run posteriorly and flares laterally, medial to the levator operculi. The muscle inserts

271   onto the medial side of the hyomandibular body and the medial part of the anterior process of the

272   opercular.

273   **Levator Operculi**

274   The levator operculi of *Esox* originates from the posterior edge of the hyomandibular body and the

275   posteroventral edges of the pterotic, dorsolateral to the adductor operculi, and dorsomedial to the

276   dilator operculi. The muscle is directed posteriorly, dorsolateral to adductor operculi and inserts

277   along the dorsolateral edge of the opercular bone - the insertion site of the levator operculi

278   continues further posteriorly than that of the adductor operculi.

279   The levator operculi in *Anguilla* originates from the ventrolateral margins of the pterotic and

280   the postero-lateral faces of the hyomandibular body, ventral to the rictalis. The muscle then passes

281   posteriorly, flares dorso-ventrally lateral to the opercular, and inserts onto the lateral face of the

282   main opercular body.

283   **Hyoid Muscles**

284   The major hyoid muscles include the intermandibularis, protractor hyoidei, inferior hyohyoideus,

285   adductor hyohyoideus and sternohyoideus (Fig. 7).

286   **Intermandibularis**

287   This muscle in *Esox* connects the left and right dentaries, stretching transversely between the two

288   halves of the lower jaw. It is absent in Anguilla.

289   **Protractor Hyoidei**

290   In *Esox*, this muscle originates posterior to the intermandibularis from the medial surface of the

291   dentary. The fibres then travel posteriorly to insert onto the lateral and ventrolateral faces of the

292   anterior ceratohyal. In *Anguilla*, the protractor hyoidei also originates from the medial surface of the

293   dentary, just posterior to mandibular the symphysis and inserts onto the lateral face of the posterior

294   ceratohyal.

295   **Inferior Hyohyoideus**

296   In *Esox*, this muscle originates from the midline and ventrolateral aspect of the urohyal. The fibres

297   run dorsolaterally and insert onto the ventrolateral face of the anterior ceratohyal. It is absent in

298   *Anguilla*.

299

300   **Abductor and Adductor Hyohyoidei**

301 The abductor and adductor hyohyoidei surround the gill chamber. The abductor runs from the

302 midline to the first branchiostegal ray, and the adductor then runs between all subsequent

303 branchiostegal rays. These muscles are much larger in *Anguilla* than in *Esox* due to the greatly

304 expanded branchiostegal rays.

305 **Sternohyoideus**

306 The sternohyoideus is a large muscle, which connects the pectoral girdle to the hyoid apparatus in

307 both *Esox* and *Anguilla*. It is composed of left and right halves, which originate from the left and right

308 cleithra respractively and inserts anteriorly onto the urohyal via a well-developed tendon. In

309 *Anguilla*, this muscle is partially covered by the expanded adductor hyhyoidei.

310 **Muscle Functional Comparison**

311 After correcting for head size differences and soft-tissue shrinkage caused by the staining process,

312 both the total CSA and volume of the adductor mandibulae complex is greater in *Esox*. However, if

313 only the segmentum facialis is considered, then muscle volume and CSA are larger in *Anguilla* (Table

314 1, Fig. 8). In both taxa, the stegalis section is the smallest part of the adductor mandibulae, making

315 up less than 20% of jaw adductor muscle CSA (Table 1, Fig. 8). In *Anguilla*, all of the remainder (81%)

316 is made up of the pars rictalis and subdivided pars malaris. In *Esox*, the presence of the segmentum

317 mandibularis makes a significant contribution (Fig. 8), accounting for 33% of the CSA of the adductor

318 mandibulae, with the ricto-malaris section making up the remainder (50%) (Table 1). Similar patterns

319 are seen when muscle size is measured in terms of volume (Table 1). In terms of muscle division,

320 although *Esox* possesses the additional segmentum mandibularis, the segmentum facialis has far

321 more divisions in *Anguilla* than *Esox*.

322 All of the suspensorial muscles are larger in *Esox* than in *Aguilla*, but in particular the levator

323 arcus palatini, which is involved in suction feeding (Table 2, Fig. 9). Also, when comparing the relative

324 sizes of the suspensorial abductors – the levator arcus palatini – and adductors – the adductor arcus

325   palatini and adductor hyomandibulae – the abductors are significantly larger in *Esox*, but in *Anguilla*

326   the two muscle groups are of roughly equal size (Table 2).

327   The opercular muscles, by contrast, are larger in *Anguilla* than in *Esox* overall (Table 2). This is

328   due entirely to the greatly enlarged levator operculi, which is responsible for dorsal rotation of the

329   operculars (Table 2, Fig. 9). Both the adductor operculi and dilator operculi - which adduct and

330   abduct the operculars respectively - are larger in *Esox* than in *Anguilla* (Table 2, Fig. 9).

331   *Anguilla* has a greater volume of hyoid musculature overall than *Esox*, although this is due to

332   the greatly expanded adductor hyohyoidei associated with the enlarged branchiostegal rays (Table 2,

333   Fig. 7, 9). The most important muscle for suction feeding is the sternohyoideus; this muscle is larger

334   in *Esox* than in *Anguilla* (Table 2). The protractor hyoidei, which is involved in generating suction for

335   respiration (Osse, 1968), is also larger in *Esox* (Table 2). The hyoid musculature of *Esox* is also more

336   complex, with several additional muscles – the intermandibularis and the hyohyoideus inferior – that

337   are not present in *Anguilla* (Fig. 7, 9).

338   ## Discussion

339   The results presented here demonstrate some interesting – and in some cases unexpected – patterns

340   in the anatomy of the cranial musculature with respect to feeding mode. As the two taxa here are

341   not radically specialised for solely biting or suction feeding, it would be premature to try and

342   generalise these results to all biting and suction-feeding fishes; more studies are needed on a greater

343   range of taxa, and the division between "biters" and "suction-feeders" is somewhat arbitrary,

344   although relative specialisations certainly do exist (Ferry et al., 2015). Still, this demonstrates how

345   the two taxa involved in our study have both solved the problem of piscivory in very different ways –

346   one mainly relying on biting, the other mainly on suction – and how this has influenced the evolution

347   of the cranial musculoskeletal system.

348   **Differences in Size and Arrangement of the Adductor Mandibulae**

349   Our hypothesis that the biting taxon *Anguilla* would have larger jaw closing muscles than the suction

350   feeding taxon *Esox* does not seem to be supported by the data presented here. Both the volume and

351   cross sectional area of the adductor mandibulae is greater in *Esox,* which utilises suction feeding to a

352   greater extent than *Anguilla*. It has been reported that the adductor mandibulae is typically larger in

353   taxa that capture prey primarily by biting rather than suction (Alfaro et al., 2001; Turingan and

354   Wainwright, 1993), and so the observed pattern may seem surprising. However, differences in

355   specific feeding styles (and their functional demands) used by each taxon could be explained by

356   more subtle differences in anatomy than overall adductor muscle size. Eels use biting as well as

357   suction (Mehta and Wainwright, 2007) for capturing small prey, but rely on the production of high

358   bite forces in order to remove pieces from large prey items and break into armoured small prey

359   (Proman and Reynolds, 2000; De Meyer et al., 2018c, 2018b). Pike use suction to capture small prey

360   (Rand and Lauder, 1981); large prey are initially drawn into the mouth by suction, then bitten into

361   and grasped with the teeth, before being moved through the oral cavity and swallowed using

362   subsequent suction events, resulting in a ratcheting effect   (LB Porro, A Herrel, personal

363   observations).

364   Bite force and jaw closing velocity are dependent on muscle cross sectional area as well as

365   the orientation of a muscle's line of action (Herrel et al., 2002; Van Wassenbergh et al., 2005). The

366   larger adductor mandibulae in *Esox* is due to the presence of an additional segment, the segmentum

367   mandibularis, which has an almost horizontal orientation and makes up ~40% of the adductor

368   mandibulae CSA. This segment is absent in *Anguilla*. In contrast, both the volume and CSA of the

369   other portion of adductor mandibulae, the more vertically oriented segmentum facialis, are greater

370   in *Anguilla* than in *Esox*. The segmentum facialis also has finer subdivisions in *Anguilla* than *Esox*,

371   perhaps suggesting the entire muscle needs to be more functionally flexible, or that finer control is

372   needed during biting or processing. An additional consideration is that these results do not account

373   for muscle pennation, as this could not be reliably determined from the CT data. The muscles in

374   *Anguilla* are pennate with short fibres whereas *Esox* has long fibres and low pennation angles. As

375   a result, we would expect *Anguilla* to be capable of producing higher bite forces than *Esox,*

376   particularly at low gape angles. This is in agreement with bite force data collected *in vivo* (Dutel et al.,

377   2015; De Meyer et al., 2018b). By contrast, the longer fibres of *Esox* should permit a larger range of

378   movement and wider gape angles.

379   As *Esox* utilises suction feeding to a greater extent we might expect the feeding system to be

380   more adapted to rapid opening and closing of the jaws to quickly capture prey (Barel, 1982). The

381   substantial mass of the segmentum mandibularis of *Esox* likely functions to facilitate fast jaw closing

382   at high gape angles during suction feeding on large prey, when the muscle fibres are vertically

383   oriented. The increased posteriorly-directed force generated by the segmentum mandibularis at

384   lower gape in *Esox* may also be advantageous, resisting anteriorly-directed forces produced when

385   grasping struggling prey. Additionally, *Esox* has proportionally longer jaws than does *Anguilla*, which

386   decreases the mechanical advantage of the jaw adductor muscles but also increases jaw closing

387   velocity (more characteristic of suction feeders (Wainwright et al., 2004; Westneat, 2004)).

388   A final difference in the organisation of the jaw musculature between these two taxa is the

389   connection between the adductor mandibulae and the epaxial musculature found in *Anguilla*. In

390   addition to "standard" biting behaviour, *Anguilla* is known to engage in rotational feeding, or

391   "death-roll" style behaviour, whereby the jaws clamp firmly onto a prey item and the eel then spins

392   its body via contraction of the axial musculature in order to tear pieces off when scavenging (De

393   Schepper et al., 2005; Helfman and Clark, 1986). During such behaviour, the linkage between the

394   axial muscles and the jaw adductors may be highly advantageous, increasing force transmission to

395   the lower jaws and hence, bite forces, although this hypothesis still requires further testing.

396   **Differences in size and arrangement of the "Suction" Muscles**

397   Suction feeding is a complex process, involving multiple musculoskeletal linkages, including the

398   cranial muscles, but also the axial musculature (Lauder, 1985; Westneat, 2006; Camp et al., 2015;

399   Day et al., 2015). Therefore, it is difficult to make inferences about suction feeding performance

400 based on cranial musculature alone. Classic models of suction feeding mechanics proposed

401 important roles in suction generation for three cranial muscles: the levator arcus palatini, levator

402 operculi and sternohyoideus (Liem, 1980a; Lauder, 1982, 1985; Westneat, 2006). Even though it is

403 now known that these muscles do not contribute strongly to generating the power for suction

404 feeding (Camp et al., 2015), they still play an important role in kinematic control.

405   The levator arcus palatini, which controls lateral flaring of the suspensorium and expansion

406 of the buccal cavity (Lauder, 1985; Westneat, 2006), is larger in *Esox* than in *Anguilla*. This may

407 permit greater control over suction strike kinematics, which will impact feeding performance (Day et

408 al., 2015). Control over the lateral aspect of buccal cavity expansion though contraction of the

409 levator arcus palatini could be particularly useful during asymmetric strikes (Liem, 1980b) when

410 attempting to catch more elusive prey. Alternatively, it may help *Esox* to manipulate and position

411 larger prey items which cannot be fully ingested in one suction event.

412   The levator operculi controls mouth opening through the opercular four-bar mechanism -

413 dorsal rotation of the operculars retracts the interopercular bone and the ligament connecting this

414 bone to the lower jaw (Lauder, 1980; Van Wassenbergh, 2005). Despite being thought to be

415 important for suction feeding, this muscle is larger in *Anguilla*. This may be due to the fact that these

416 muscles are also active during ventilation, and the large jaw adductor muscles of the segmentum

417 facialis in *Anguilla* stiffens the suspensorium, increasing the load the levator operculi needs to work

418 against. Whilst it is possible the levator operculi is enlarged in *Anguilla* due this role in gill

419 ventilation, the other opercular muscles do not follow the same pattern; the dilator operculi is

420 similarly sized in both taxa, and the adductor operculi is smaller in *Anguilla* than in *Esox*.

421   An additional complication in *Anguilla* is that the epaxial muscles are mechanically coupled

422 to the adductor mandibulae (presumably an adaptation to torsional feeding (Liem, 1980a; De

423 Schepper et al., 2005), contacting the pars malaris via a tendinous sheet. This means that the power

424 generated through contraction of the epaxial muscles, rather than solely driving neurocranial

425   elevation as in other teleosts, is being transmitted to the mandible to elevate the lower jaws and

426   assist during biting. Therefore, whilst this mechanism may increase bite force in *Anguilla*, the epaxial

427   muscles may also be contributing less than they otherwise would towards neurocranial elevation and

428   lower jaw depression; however, the precise mechanics of this linkage require further investigation.

429   One possible explanation for the large levator operculi in *Anguilla* may be that there is an increased

430   reliance on this muscle to power jaw opening; an alternative is that it may serve to stabilize the

431   operculars against the action of the epaxial musculature, as well as preventing damage from

432   rotational movements during rotational feeding.

433   Comparing the hyoid muscles, the protractor hyoidei is of equivalent size in the two taxa,

434   and the abductor and adductor hyohyoidei are greatly expanded in *Anguilla*, as they sheath the

435   lengthened branchiostegal rays. The ventral cranial musculature of *Esox* includes two additional (but

436   small) muscles not present in *Anguilla*; the intermandibularis and the hyohyoideus inferior. The

437   functional roles for these muscles remain unclear. The presence of the intermandibularis in *Esox*

438   may assist in resisting the forces produced by struggling prey and resisting stress at the mandibular

439   symphysis. The inferior hyohyoidei is involved in the adduction of the ceratohyals, and so it may be

440   linked to either control of hyoid kinematics during suction feeding, or during prey processing.

441   The sternohyoideus has the biggest role in suction feeding of the hyoid muscles, as it

442   controls ventral expansion of the buccal cavity through hyoid depression and drives lower jaw

443   rotation via the mandibulohyoid ligament (Lauder, 1980; Aerts, 1991). However, it has been shown

444   that this muscle in fact lengthens during the expansive phase of suction feeding, and is mainly acting

445   as ligament to transmit power generated by the hypaxial musculature (Van Wassenbergh et al.,

446   2007; Camp and Brainerd, 2015). Therefore, power generated by the active shortening of the

447   sternohyoideus must be involved in other behaviours such as prey processing and transport. The

448   sternohyoideus has been shown to exhibit asymmetric activity during prey processing in bony fishes

449   (Lauder and Norton, 1980), and this may be particularly important for *Esox* to manipulate and

450   position large prey which requires multiple suction events to fully ingest.

451   The cranial and hyoid musculature involved in suction feeding plays an important role in

452   force and power transmission from the axial muscles, and the control of suction feeding kinematics

453   (Camp et al., 2015). However, because these muscles are not actually generating power, it's possible

454   that muscle volume is not the most relevant functional metric. Cross-sectional area may be more

455   relevant as this relates to the muscle's force transmission ability, especially if the muscle in question

456   is transmitting force via eccentric or isometric contraction, as is the case for the sternohyoideus (Van

457   Wassenbergh et al., 2007; Camp and Brainerd, 2015). If cross-sectional area for the cranial muscles

458   involved in suction feeding can predict the amount of force they're transmitting, then this could

459   potentially be used to estimate cranial expansion and suction power. However, this hypothesis

460   would require further testing, as well as more detailed investigations into the axial muscles

461   themselves and how the power they generate is transmitted through the cranial and hyoid linkages.

Conclusions

462

463   Contrast-enhanced CT scanning and "digital dissection" are promising methods in

464   comparative anatomy, and are here applied to teleosts, the most diverse group of living

465   vertebrates. In addition to permitting the highly detailed description of the soft-tissue

466   anatomy in these taxa in situ, such digital approaches lend themselves to quantification,

467   allowing easy, non-destructive (and hence repeatable) measurements of the musculoskeletal

468   system, which can then be used in further computational biomechanical analyses. Iodine has

469   received the greatest attention as a tool for contrast-enhanced CT; however, if precise soft

470   tissue quantification is desired then alternative staining agents, such as PMA, should be

471   considered, as iodine staining protocols are still being optimised to reduce potential tissue

472   shrinkage.

473   *Esox,* which feeds primarily using suction, has a larger adductor mandibulae complex,

474   despite *Anguilla* primarily using biting as its main feeding mode. However, biting

475   performance is not determined by the size of the jaw adductors alone, and in *Anguilla* the

476   more vertical orientation and greater structural complexity of the muscles likely improves

477   biting performance compared to *Esox*. The increased size of the levator arcus palatini in *Esox*

478   may serve a role during breathing. Alternatively, along with the enlarged sternohyoideus, it

479   could be related to suction-feeding, but its role (if any) is more likely to be fine kinematic

480   control rather than power production which is provided by the axial musculature. The larger

481   levator operculi in *Anguilla* may also serve a respiratory role, but other possible functions

482   include stabilization of the operculum during rotational feeding, or assisting in mouth

483   opening. Whilst these are interesting possibilities, further functional studies are required in

484   order to fully understand the implications of these observed anatomical differences.

493   **Author Contributions**

494   LBP and EJR conceived of and designed the project. AH provided specimens of *Esox*. LBP stained and

495   CT scanned the specimen of *Esox*. DA provided, stained and CT scanned specimens of *Anguilla*. RJB

496   and LBP processed the CT scan data. RJB produced the 3D reconstructions and the anatomical

497   descriptions. RJB made quantitative muscle measurements. RJB wrote the paper. All authors read

498   and provided feedback on the manuscript.

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679

680 <u>Tables</u>

			Muscle CSA (mm^2)		Muscle Volume (mm^3)	
			Anguilla	*Esox*	*Anguilla*	*Esox*
Segmentum Facialis	Rictomalaris Section	Pars Rictalis	40.0 (37%)	67.1 (50%)	942 (39%)	1500 (50%)
		Pars Promalaris	30.8 (28%)		791 (32%)	
		Pars Retromalaris	17.3 (16%)		415 (17%)	
	Stegalis Section	Pars Epistegalis	13.6 (12%)	23.4 (17%)	194 (8%)	307 (10%)
		Pars Substegalis	7.3 (7%)		108 (4%)	
Segmentum Mandibularis	Pars Coronalis		NA	13.1 (10%)	NA	397 (13%)
	Pars Mentalis		NA	31.1 (23%)	NA	808 (27%)

681

682 Table 1. Muscle cross sectional area and volume data for the adductor mandibulae complex of *Anguilla* and *Esox*. Data for
683 *Anguilla* has been scaled to account for head size differences with the *Esox* specimen. *Esox* data has been corrected for
684 shrinkage caused by the iodine staining. N/A indicates that that particular muscle is either absent or could not be
685 distinguished from another muscle. Values reported to 3 significant figures.

		Esox	*Anguilla*
Suspensorial Muscles	Adductor Arcus Palatini	451	229
	Adductor Hyomandibulae	72.3	N/A
	Levator Arcus Palatini	618	244
Opercular Muscles	Dilator Operculi	167	145
	Adductor Operculi	158	109
	Levator Operculi	155	360
Hyoid Muscles	Intermadibularis	53.4	N/A
	Protractor Hyoidei	321	280
	Hyohyoid Inferior	57.4	N/A
	Adductor Hyohyoideus	384	2540
	Sternhyoideus	1450	1190

686

687 Table 2. Muscle volume data (in mm^3) for the suspensorial, opercular and hyoid muscles of *Anguilla* and *Esox*. Data for
688 *Anguilla* has been scaled to account for head size differences with the *Esox* specimen. *Esox* data has been corrected for
689 shrinkage caused by the iodine staining. N/A indicates that that particular muscle is either absent or could not be
690 distinguished from another muscle. Values reported to 3 significant figures.

691

Figure Captions

692   <u>Figure Captions</u>

693   Figure 1. Comparison of coronal CT slices showing transverse sections of *Anguilla anguilla* (A, B) and

694   *Esox lucius* (C, D) and specimens before (A, C) and after (B, D) contrast-enhancement staining. Images

695   not to scale.

696   Figure 2. Cranial, mandibular and hyoid osteology of *Esox* (A, B, C, D, E) and *Anguilla* (F, G, H,I, J).

697   Skulls in lateral (A, F), dorsal (B, G) and ventral (C, H) views with lower jaw removed. Lower jaws (D, I)

698   and hyoids (E, J) in medial view. All scale bars =5mm. Abbreviations: an, angular; ar, articular; an-ar,

699   angulo-articular; bb, basibranchial; bh, basihyal; cha, anterior ceratohyal; chp, posterior ceratohyal;

700   cm, corono-meckelian; co, circumorbital series; d, dentary; de, dermethmoid; de/v, fused

701   dermethmoid and vomer ep, epitotic; ept, ectopterygoid; fr, frontal; hy, hyomandibular; iop, inter-

702   opercular; la, lacrimal; mc, Meckel's cartilage; mpt, metapterygoid; mx, maxilla; na, nasal; ne,

703   neurocranium; op, opercular; p, parietal; pa, parasphenoid; pe, proethmoid; pfr, pre-frontal; pl,

704   palatal; pmx, pre-maxilla; pmx/v, fused pre-maxilla and vomer; pop, pre-opercular; pr, pterotic; pt,

705   pterygoid; q, quadrate; s, symphysis; so, super-occipital; sp, sphenotic; sop, sub-opercular; smx,

706   supra-maxilla; uh, urohyal.

707   Figure 3. Individual muscles of the adductor mandibulae segmentum facialis in *Esox* (A, D) and

708   *Anguilla* (B, C, E, F), in oblique views. *Esox* ricto-malaris section, sRM (A) and stegalis section, sS, (D).

709   *Anguilla* pars rictalis, pR, (B); pars malaris, pM, with division into pro- and retro-malaris indicated by

710   the dotted line (C); pars epistegalis, pES, (E); pars substegalis, pSS, (F). Images not to scale.

711   Figure 4. Individual muscles of the adductor mandibulae segmentum mandibularis (AMSM) in *Esox*

712   shown in oblique view. Pars coronalis, pC, (A); pars mentalis, pM, (B).

713   Figure 5. Individual suspensorial muscles of *Esox* (A, C, E) and *Anguilla* (B, D), in oblique view. Levator

714   arcus palatini, LAP, (A, B); adductor arcus palatini, AAP, (C, D); adductor hyomandibulae, AH (E).

715   Images not to scale.

716   Figure 6. Individual opercular muscles of *Esox* (A, C, E) and *Anguilla* (B, D, F), in oblique view. Dilator

717   operculi, DOP, (A, B); adductor operculi, AOP, (C, D); levator operculi, LOP, (E, F). Images not to scale.

718   Figure 7. 3D visualizations of the hyoid msuculature of *Esox* (top) and *Anguilla* (bottom).

719   Abbreviations: IM, intermandibularis; PHH, protractor hyoidei; IHH, inferior hyohyoidei; AHH,

720   abductor and adductor hyohyoidei; SH, sternohyoideus. Images not to scale.

721   Figure 8. Graphs showing the cross-sectional area and volume for the adductor mandibulae complex

722   of *Anguilla* and *Esox,* following corrections for differences in head size and possible soft tissue

723   shrinkage. Hatching indicates compound sections. Individual muscles are colour coded as in the 3D

724   reconstructions.

725   Figure 9. Graphs showing the volume for the suspensorial, opercular and hyoid muscles of *Anguilla*

726   and *Esox,* following corrections for differences in head size and possible soft tissue shrinkage.

727   Individual muscles are colour coded as in the 3D reconstructions.