Functional diversity in biters: the evolutionary morphology of the oral jaw system in pacus, piranhas and relatives (Teleostei: Serrasalmidae)

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Serrasalmid fishes form a highly specialized group of biters that show a large trophic diversity, ranging from pacus able to crush seeds to piranhas capable of cutting flesh. Their oral jaw system has been hypothesized to be forceful, but variation in bite performance and morphology with respect to diet has not previously been investigated. We tested whether herbivorous species have higher bite forces, larger jaw muscles and more robust jaws than carnivorous species. We measured in vivo and theoretical bite forces in 27 serrasalmid species. We compared the size of the adductor mandibulae muscle, the jaw mechanical advantages, the type of jaw occlusion, and the size and shape of the lower jaw. We also examined the association between bite performance and functional morphological traits of the oral jaw system. Contrary to our predictions, carnivorous piranhas deliver stronger bites than their herbivorous counterparts. The size of the adductor mandibulae muscle varies with bite force and muscles are larger in carnivorous species. Our study highlights an underestimated level of functional morphological diversity in a fish group of exclusive biters. We provide evidence that the trophic specialization towards carnivory in piranhas results from changes in the configuration of the adductor mandibulae muscle and the lower jaw shape, which have major effects on bite performance and bite strategy.


INTRODUCTION

Feeding performance is a determining factor for the survival and fitness of animals (Wainwright, 1988). In the animal kingdom, bite force (i.e. the ability of an animal to generate force with its jaws) is an important metric of whole-organism performance because of the relationship between jaw morphology and the feeding ecology of an animal (Anderson et al., 2008). This trait of feeding performance has received increasing interest in recent decades and has been measured in a wide variety of vertebrate groups: sharks (e.g. Huber et al., 2005, 2006; Ferrara et al., 2011; Rice et al., 2016), teleosts (e.g. Hernandez & Motta, 1997; De Schepper et al., 2008; Habegger et al., 2017), amphibians (e.g. Deban & Richardson, 2017; Lappin et al., 2017), lizards (e.g. Herrel et al., 1999, 2001, 2004, 2014; Meyers et al., 2018), turtles (e.g. Herrel et al., 2002, 2017; Pfaller et al., 2010), birds (e.g. van Der Meij & Bout, 2004; Herrel et al., 2005; Rao et al., 2018) and mammals (e.g. Aguirre et al., 2002; Dumont & Herrel, 2003; Thomas et al., 2015; Ginot et al., 2018). Previous studies have demonstrated that bite force is usually related to body size (e.g. Erickson et al., 2003; Huber et al., 2006; Herrel et al., 2014), cranial morphology (e.g. Herrel et al., 2001; Lappin et al., 2006; Da Silva et al., 2016;
Dufour et al., 2018), jaw muscle size (e.g. Raadsheer et al., 1999; Herrel et al., 2002; van der Meij & Bout, 2004) and mandible properties (e.g. Greaves, 2002; Nogueira et al., 2009; Dollion et al., 2017). Bite force usually varies with diet (e.g. Mehta, 2008; Nogueira et al., 2009; Dollion et al., 2017), prey type (e.g. Huber et al., 2006; Santana et al., 2010; Rao et al., 2018) and food hardness (e.g. Aguirre et al., 2003; van der Meij & Bout, 2006; Herrel & Holanova, 2008).

Ecomorphological studies devoted to studying variations in the feeding apparatus (i.e. oral and pharyngeal jaws, head and digestive tract morphology) in teleost fishes are plentiful. Cichlids are probably the best studied fish taxa regarding their diversity of trophic morphology (Liem, 1993; Albertson & Kocher, 2006; Cooper et al., 2011) but several other families such as Labridae (Wainwright et al., 2004), Apogonidae (Barnett et al., 2006), Pomacanthidae (Konow & Bellwood, 2005, 2011), Cyprinidae (Hernandez & Staab, 2015), Chaetodontidae (Konow et al., 2017) and Pomacentridae (Frédérich et al., 2008a, b) have also been explored. Compared with other vertebrates, teleosts vary largely in their feeding modes with three generally recognized methods of prey capture (Liem, 1993): suction-feeding, ram-feeding and biting. Most studies have illustrated functional morphological variation associated with diet specialization in fish taxa including various feeding modes. For example, the disparate trophic groups of wrasses (e.g. Wainwright et al., 2004) and cichlids (e.g. Cooper et al., 2011) include biters, suction-feeders and ram-feeders and a large amount of functional morphological disparity is explained by evolutionary shifts between feeding modes. Surprisingly, few studies (but see Konow & Bellwood, 2011) have explored the functional morphological variation in fish clades showing a large diversity of diet despite being based on a single feeding mode.

Serrasalmidae, a monophyletic family of South American freshwater fishes, is atypical among Neotropical characiforms because of its trophic diversification (Correa et al., 2007) and its exclusively ‘biting’ feeding mode on diverse prey items (e.g. fishes, other vertebrates, fins, scales, crustaceans, molluscs, insects, aquatic plants, flowers, fruits, seeds, algae). According to recent molecular phylogenies, Serrasalmidae (~98 species) is divided into three major subclades (Supporting Information, Fig. S1): (1) the ‘pacus-clade’ including the herbivorous genera Colossoma, Mylossoma and Piaractus; (2) the ‘myleus-clade’ with the herbivorous genera Mylesinus, Myleus, Myloplus, Ossubitus, Tometes and Utiaritichthy; and (3) the ‘piranhas-clade’ with the lepidophagous genus Catoprin, the herbivorous genus Metynnis and the carnivorous genera Pristobrycon, Pygocentrus, Pygopristis and Serrasalmus (Thompson et al., 2014).

The herbivorous genus Acnodon may be considered as the sister-group of the ‘myleus-clade’ (Ortí et al., 1996, 2008; Calcagnotto et al., 2005; Thompson et al., 2014). Interestingly, serrasalmid species diversified into various feeding habits that are all dependent on biting and oral manipulation, suggesting the ability to generate bite force as a basal trait. The different trophic guilds of serrasalmids range from the pacus, capable of crushing hard-shelled fruits and seeds, to the piranhas capable of slicing pieces of fleshy prey (Correa et al., 2007) but also include the wimple piranha, Catoprin mento, which specializes on extracting scales (Janovetz, 2005). This fish family offers us the opportunity to test in a phylogenetic context (1) how bite performance varies within a group of highly specialized biters and (2) which morphological and functional traits of the oral jaw system vary in accordance with bite performance and diet specialization.

The black piranha, Serrasalmus rhombeus, may hold the record for relative bite force among extant vertebrate taxa (Grubich et al., 2012). The force of its jaws is, proportionally to body size, up to three times stronger than the bite force for a great white shark (Wroe et al., 2008; Ferrara et al., 2011) or an adult alligator (Erickson et al., 2003). In addition to an extreme jaw force, the black piranha has a single row of highly specialized sharp and triangular teeth on each jaw allowing it to cut small pieces of flesh from larger animals (Shellis & Berkovitz, 1976; Jégu, 2003). In contrast, the herbivorous pacus, Colossoma macropomum and Piaractus brachypomus, have one or two rows of specialized multicuspid incisiform-to-molariform teeth on the lower and upper jaws respectively, which are adapted for crushing fruits and hard-shelled seeds (Goulding, 1980; Goulding & Carvalho, 1982; Jégu, 2003). Differences in digestive tract length have also been highlighted between carnivorous and herbivorous serrasalmids (Pelster et al., 2015). Other possible morphological and performance changes in jaws and associated muscles have, however, never been investigated and compared between dietary groups. So, how do bite performance and buccal morphological traits vary between carnivorous piranhas and their herbivorous counterparts?

In this study, we aim to conduct a comparative functional morphological analysis of the oral jaw system in the Neotropical family Serrasalmidae. We hypothesize that herbivorous species that mainly feed by crushing hard plant items (fruits and seeds) should have higher bite forces than carnivorous species that feed on soft animal prey (meat and flesh). In addition, species with a more durophagous diet should have more strongly developed muscles and more robust jaws than carnivorous species. We here seek to (1) determine bite forces empirically and theoretically

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in serrasalmid species with varied feeding habits (carnivorous and herbivorous), (2) investigate the association between bite performance and diet, (3) compare the morphology and functional properties of the oral jaw system between dietary groups and (4) explore whether the studied functional morphological traits may explain bite performance in serrasalmids.

MATERIAL AND METHODS

FISH SAMPLING AND DIET INFORMATION

We studied a total of 647 specimens from 27 serrasalmid species (Table 1). Additional information about sampling, provenance and maintenance of serrasalmid species are given in the Supporting Information (Text S1). We used live specimens from 20 species from nine different genera to record in vivo bite forces (BF in vivo). Specimens from 22 species, including seven additional species and two other genera, were euthanized to estimate theoretical bite forces (BF theoretical) from the adductor mandibulae muscle (which closes the lower jaw and is therefore responsible for the bite). Specimens used for this last approach were euthanized by an overdose of ethyl 3-aminobenzoate methanesulfonate 98% (MS-222, Sigma Aldrich) dissolved in water. Several of these specimens also came from two other studies in our laboratory (Mélotte et al., 2016, 2018). Specimens from 15 species studied via the above two methods were used to assess the validity of our bite force estimates. In addition, euthanized specimens were used for dissections, calculations of jaw mechanical advantages and type of jaw occlusion, and landmark-based geometric morphometric analyses.

In serrasalmid fishes, tooth shape and dentition patterns can be used as a reliable proxy of diet (e.g. Géry, 1972; Goulding, 1980; Nico & Taphorn, 1988; Jégu, 2003; Supporting Information, Table S1). We thus grouped species into two main dietary categories based on their dentition (Fig. S2): carnivorous and herbivorous species (Table 1). Catoprion mento, a specialist lepidophagous species, formed a third category but we did not include it in comparative statistical analyses.

BITE FORCES

In vivo bite forces

We recorded in vivo bite forces in 543 live specimens of 20 serrasalmid species (Table 1) using a piezo-electric and isometric Kistler force transducer (type 9203, range ± 500 N, Kistler Inc.; see Herrel et al., 1999). For each bite session, we caught fish in their aquarium or in their natural environment using fishing nets or hook-and-lines. Fishes were then held by hand and we measured in vivo bite forces by introducing bite plates between the oral jaws. For each specimen, we recorded three to five consecutive ‘aggressive’ or ‘crushing’ bites depending on diet. The point of application of the bite force was standardized by the fixed length of the bite plates. We retained the highest recorded bite force value as the maximal BF in vivo value for the animal (Herrel et al., 1999) although this value may in some cases reflect a maximum effort and not a physiological limit for the specimen (Astley et al., 2013). We corrected these maximal BF in vivo values by the lever arms of the set-up (i.e. the distance from the bite point to the fulcrum) before using them in further analyses.

For each specimen, we also directly measured the standard length (SL) using a calliper or photographs where the specimen was positioned on graph paper for scale. We obtained body mass (BM) using a normal balance (max = 3 kg; Silvercrest) or a spring scale (max = 100, 2500, 5000 or 10 000 g; PESOLA).

All procedures and methods applied in this study were approved by the Animal Care and Use Committee of the University of Liège, Belgium (ethics case 1835). All experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals.

Theoretical bite forces

We isolated the right adductor mandibulae muscle and lower jaw in 158 euthanized specimens of 22 serrasalmid species (Table 1) under a binocular microscope (Leica Wild M10). From the adductor mandibulae muscle, we estimated BF theoretical using standard equations of (1) muscle physiological cross-sectional area (PCSA, Powell et al., 1984), (2) maximum isometric muscle force (F max, e.g. Huber et al., 2006) and (3) output force (F out, e.g. Moran & Ferry, 2014) for each muscle subdivision of the adductor mandibulae muscle (Supporting Information, Text S2). For each specimen, we added up the output forces of each muscle subdivision and then duplicated the total jaw force to account for the bilateral bite to determine a value for BF theoretical.

FUNCTIONAL MORPHOLOGICAL TRAITS OF THE ORAL JAW SYSTEM

Adductor mandibulae muscle

In the same 158 specimens (Table 1), we recorded the origin sites of the adductor mandibulae muscle on the skull and suspensorium. We then completely removed the right ‘adductor-mandibulae–lower-jaw’ complex and recorded the insertion sites of the different muscle subdivisions. We also photographed the muscle subdivisions using a camera (Canon EOS 6D digital, Canon Inc.) with graph paper in view for scale. The
Table 1. Summary of diet, fish sampling, body size, and in vivo and theoretical bite forces in 27 serrasalmid species included in this comparative study

<table>
<thead>
<tr>
<th>Diet</th>
<th>Genus</th>
<th>Species name</th>
<th>N total</th>
<th>Live specimens</th>
<th>Euthanized specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>N</td>
<td>BM (g)</td>
<td>SL (mm)</td>
</tr>
<tr>
<td>Carnivorous</td>
<td>Pristobrycon</td>
<td>Pristobrycon striolatus</td>
<td>7</td>
<td>50 ± 22</td>
<td>113 ± 15</td>
</tr>
<tr>
<td></td>
<td>Pygocentrus</td>
<td>Pygocentrus cariba</td>
<td>2</td>
<td>15 ± 3</td>
<td>70 ± 5</td>
</tr>
<tr>
<td></td>
<td>Pygocentrus nattereri</td>
<td></td>
<td>218</td>
<td>79 ± 161</td>
<td>103 ± 42</td>
</tr>
<tr>
<td></td>
<td>Pygocentrus piraya</td>
<td></td>
<td>17</td>
<td>49 ± 26</td>
<td>104 ± 15</td>
</tr>
<tr>
<td></td>
<td>Pygopristis</td>
<td>Pygopristis denticulata</td>
<td>14</td>
<td>25 ± 30</td>
<td>77 ± 33</td>
</tr>
<tr>
<td></td>
<td>Serrasalmus</td>
<td>Serrasalmus brandii</td>
<td>35</td>
<td>230 ± 140</td>
<td>186 ± 42</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus compressus</td>
<td>5</td>
<td>361 ± 183</td>
<td>209 ± 44</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus elongatus</td>
<td>10</td>
<td>109 ± 7</td>
<td>8 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus humeralis</td>
<td>5</td>
<td>23 ± 3</td>
<td>84 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus maculatus</td>
<td>40</td>
<td>120 ± 41</td>
<td>169 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus manueli</td>
<td>41</td>
<td>56 ± 9</td>
<td>124 ± 6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus marginatus</td>
<td>61</td>
<td>120 ± 41</td>
<td>169 ± 17</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus rhombeus</td>
<td>3</td>
<td>19 ± 15</td>
<td>84 ± 12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Serrasalmus spilopleura</td>
<td>7</td>
<td>25 ± 6</td>
<td>84 ± 12</td>
</tr>
<tr>
<td>Herbivorous</td>
<td>Acnodon</td>
<td>Acnodon oligacanthus</td>
<td>3</td>
<td>37 ± 6</td>
<td>115 ± 7</td>
</tr>
<tr>
<td></td>
<td>Colossoma</td>
<td>Colossoma macropomum</td>
<td>5</td>
<td>70 ± 72</td>
<td>120 ± 50</td>
</tr>
<tr>
<td></td>
<td>Metynnis</td>
<td>Metynnis hypsauchen</td>
<td>11</td>
<td>11 ± 9</td>
<td>55 ± 28</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metynnis lippincottianus</td>
<td>9</td>
<td>4 ± 4</td>
<td>103 ± 3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Metynnis maculatus</td>
<td>78</td>
<td>104 ± 34</td>
<td>136 ± 15</td>
</tr>
<tr>
<td></td>
<td>Myleus</td>
<td>Myleus micans</td>
<td>4</td>
<td>2525 ± 613</td>
<td>405 ± 40</td>
</tr>
<tr>
<td></td>
<td>Myloplus</td>
<td>Myloplus rhomboidalis</td>
<td>2</td>
<td>32 ± 1</td>
<td>94 ± 1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myloplus rubripinnis</td>
<td>12</td>
<td>50 ± 19</td>
<td>120 ± 27</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myloplus schomburgkii</td>
<td>18</td>
<td>22 ± 6</td>
<td>82 ± 8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Myloplus ternetzi</td>
<td>24</td>
<td>129 ± 100</td>
<td>138 ± 37</td>
</tr>
<tr>
<td></td>
<td>Piaractus</td>
<td>Piaractus brachypomus</td>
<td>34</td>
<td>574 ± 1165</td>
<td>164 ± 131</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Piaractus mesopotamicas</td>
<td>13</td>
<td>3581 ± 605</td>
<td>502 ± 25</td>
</tr>
</tbody>
</table>

Additional information and references regarding serrasalmid diet are given in Table S1. In some cases, the total fish sampling is not consistent with the sum of sample sizes for in vivo and theoretical bite forces because the sample size of theoretical bite forces includes euthanized specimens from live specimens of the present study as well as preserved specimens from other studies (Mélotte et al., 2016, 2018). Body size and bite force entries are means ± SD. The lepidophagous species Catoprion mento was not included in comparative statistical analyses. Abbreviations: BF <sub>in vivo</sub>, in vivo bite force; BF <sub>theoretical</sub>, theoretical bite force; BM, body mass; N, sample size; N total, total fish sampling; SL, standard length.
terminology of Datovo & Vari (2013) was used to describe the subdivisions of the adductor mandibulae muscle. For each muscle subdivision, we obtained muscle mass using a Cobos precision balance (±0.001 g, type M-150-SX). In addition, for each specimen, we calculated the percentage of the adductor mandibulae muscle relative to body mass and the percentage of each muscle subdivision relative to the total adductor mandibulae muscle mass.

**Jaw mechanical advantages**

The force transmission of the adductor mandibulae muscle to the lower jaw can be determined by the mechanical advantage, which is the ratio of the in-lever arm ($L_{in}$, distance between the jaw joint and the muscle insertion onto the lower jaw) and the out-lever arm ($L_{out}$, distance between the jaw joint and the application point of bite force) (Westneat, 2004) (Fig. 1A). For each muscle subdivision, we calculated anterior and posterior mechanical advantages (AMA and PMA, respectively) according to Anderson (2009) to examine whether muscle subdivisions have a different force transmission. We measured the out-lever arm for AMA from the jaw joint to the anterior tip of the lower jaw. We then measured the out-lever arm for PMA from the jaw joint to the most posterior tip of the tooth row on the lower jaw. Lever arm ratios are dimensionless.

**Type of jaw occlusion**

To determine whether bite strategy differs between carnivorous and herbivorous serrasalmid species, we measured the quadrate offset distance of the lower jaw following the method of Anderson (2009). The quadrate offset distance is a metric that can be used to estimate the type of jaw occlusion. This metric represents the orthogonal distance between the quadrat–anguloarticular joint of the lower jaw and the dentition. The shorter the distance, the more imperfect is the occlusion between upper and lower jaws as in a ‘scissor-like’ system. The higher the distance, the more perfect is the jaw occlusion as in a ‘vice-like’ system. For each specimen, we photographed the right lower jaw in lateral view using a camera (Canon EOS 6D digital) with the jaw positioned on graph paper for scale. We then drew a line tangential to the tooth row passing by the jaw joint on each photograph. We measured the direct distance between this line and the jaw joint as the quadrate offset distance (Fig. 1B). Finally, we divided this value by the overall length of the lower jaw to make the value dimensionless.

**Size and shape of the lower jaw**

We used 12 homologous landmarks to capture shape variation of the lower jaw (Fig. 1C). We digitized the x- and y-coordinates of each landmark from photographs of the lateral view of the right lower jaw using the morphometric software TPSDig v.1.40 (F. J. Rohlf, 2004, http://life.bio.sunysb.edu/morph/; accessed 30 March 2019). We then conducted a Generalized Procrustes Analysis (GPA) to align specimens to a common coordinate system and remove variation in their position, orientation and size (Rohlf & Slice, 1990). Next, we determined the lower jaw size for each specimen from the landmark configurations as centroid size, i.e. the square root of summed squared distances of landmarks from the centroid (Bookstein,
BITE FORCE AND ORAL MORPHOLOGY IN SERRASALMIDAE

The Procrustes tangent coordinates were used as shape variables for all specimens (Adams et al., 2013). Finally, we calculated mean configurations for each species from size and shape variables.

STATISTICAL ANALYSES

Bite force, body mass and jaw muscle mass data were log10-transformed before statistical analyses to meet assumptions of normality and homoscedasticity. Jaw mechanical advantages, quadrate offset distance and lower jaw shape data were not transformed because they are dimensionless. For each variable, we calculated a mean value per species. To take into account the variation in body mass in serrasalmid fishes (Azevedo, 2010), we regressed the log10-transformed BF \(_{in\,vivo}\), BF \(_{theoretical}\) and adductor mandibulae muscle mass against the log10-transformed body mass (Habegger et al., 2017). In addition, we regressed the log10-transformed muscle mass of each jaw muscle subdivision against the log10-transformed adductor mandibulae muscle mass. Residuals from these regressions were used in statistical analyses.

First, we tested for divergence in relative bite performance (BF \(_{in\,vivo}\) and BF \(_{theoretical}\)) between carnivorous and herbivorous species. A divergence in bite performance between dietary groups may result from a difference in slope and/or intercept of the bite force vs. body mass relationship (van der Meij & Bout, 2004). We performed an analysis of covariance (ANCOVA) with diet as factor and body mass as covariate to test for the homogeneity of slopes and intercept divergence for the two groups. We also determined whether BF \(_{theoretical}\) is a good predictor of BF \(_{in\,vivo}\) using a linear regression of BF \(_{in\,vivo}\) and BF \(_{theoretical}\) mean values (Santana et al., 2010).

Next, we tested for morphological variation of the adductor mandibulae muscle and its muscle subdivisions between carnivorous and herbivorous species. We compared slopes and intercepts of the linear regressions on log10-transformed jaw muscle mass data using ANCOVAs (van der Meij & Bout, 2004). We also determined for each species the mean percentage of the adductor mandibulae muscle relative to body mass as well as the mean percentage of the mass of each muscle subdivision relative to the total adductor mandibulae muscle mass. We then tested for differences in these mean percentages between dietary groups using the non-parametric Mann–Whitney \(U\) test. Similarly, we evaluated the functional variation of the oral jaw system between carnivorous and herbivorous species using non-parametric Mann–Whitney \(U\) tests on jaw mechanical advantages and quadrate offset distance.

To compare independently the size and shape of the lower jaw between dietary groups, we tested for differences in lower jaw size (i.e. centroid size) and shape between carnivorous and herbivorous species using a Procrustes ANOVA with diet as factor (Collyer et al., 2015). We also carried out a principal components analysis (PCA) on shape variables to explore lower jaw shape variation among species and dietary groups. Deformation grids were used to describe the trends of the lower jaw shape variation along PC axes.

Next, we tested for the hypothetical association between the size of the adductor mandibulae muscle and bite force (BF \(_{in\,vivo}\)) using a linear regression. Similarly, we explored hypothetical relationships between BF \(_{in\,vivo}\) and the other functional morphological traits of the oral jaw system, i.e. jaw mechanical advantages (AMAs and PMAs), type of jaw occlusion (quadrate offset distance) and lower jaw shape (PC1 axis, which explains 85% of the total shape variation – see Results). Finally, to identify which functional morphological traits best explain the variability in BF \(_{in\,vivo}\) we conducted a stepwise multiple linear regression with BF \(_{in\,vivo}\) as the response variable and adductor mandibulae muscle mass, mean AMA, mean PMA, quadrate offset distance and PC1 as predictor variables and we kept the final model (Herrel et al., 2014).

As a first step, we conducted regular statistical analyses but we also used phylogenetically corrected statistical methods (Revell, 2009) such as phylogenetic generalized least squares (PGLS) regressions and phylmorphospace analyses because species are not phylogenetically independent (Felsenstein, 1985). The regular statistics allowed the study of all species, while the phylogenetically corrected analyses excluded nine species from our study (Metyynis lipincottianus, Metyynis maculatus, Myleus micans, Myloplus ternetzi, Piaractus mesopotamicus, Pygocentrus cariba, Serrasalmus brandtii, Serrasalmus elongatus and Serrasalmus maculatus) because no phylogenetic information is available for these species. For this reason, we first described results from statistics without phylogenetic correction.

All statistical analyses were conducted in the R environment (R Core Team, 2017), except slope homogeneity tests which were implemented in PAST 3.13 (Hammer et al., 2001). All analyses of shape variation were performed using the R package ‘geomorph’ (Adams & Otárola-Castillo, 2013; Adams et al., 2017) and phylogenetic comparative analyses using the R packages ‘geiger’ (Harmon et al., 2007) and ‘phytools’ (Revell, 2012).

RESULTS

BITE FORCES

Absolute mean values of BF \(_{in\,vivo}\) (Table 1) range widely between the herbivorous Piaractus mesopotamicus with the highest value (93 ± 13 N,
mean ± SD), the carnivorous Pygocentrus nattereri (84 ± 68 N), the herbivorous Myloplus schomburgkii (9 ± 4 N) and the lepidophagous Catoprion mento with the lowest value (2 N). Body size (i.e. body mass) positively and significantly influences in vivo bite force in serrasalmid fishes (all species: $r = 0.70$; carnivorous: $r = 0.91$; herbivorous: $r = 0.79$; all $P < 0.01$). Slopes of this linear model do not vary between carnivorous and herbivorous species (slope homogeneity test: $F = 0.13$, $P = 0.73$) but the intercepts vary significantly (ANCOVA: $F_{1,16} = 17.61$, $P < 0.001$, $\eta^2_p = 0.69$). Carnivorous species show higher relative BF$_{in\,vivo}$ than herbivorous species (Fig. 2A). Phylogenetic ANCOVA is, however, not significant (Table 2), indicating that diet does not explain divergence in BF$_{in\,vivo}$ considering the phylogenetic configuration used in this study (Supporting Information, Fig. S1). Additionally, note that the in vivo bite force data presented here can be influenced by several factors such as sample size, animal motivation, age, captive state and physiological conditions of the bite.

Similarly, slopes of the linear model of BF$_{theoretical}$ against body mass do not vary between carnivorous and herbivorous species (slope homogeneity test: $F = 2.25$, $P = 0.15$) but the intercepts vary significantly (ANCOVA: $F_{1,18} = 9.79$, $P < 0.05$, $\eta^2_p = 0.80$). Carnivorous species show higher relative BF$_{theoretical}$ than herbivorous species (Fig. 2B) but this variation is not supported by phylogenetic ANCOVA (Table 2). Moreover, BF$_{theoretical}$ from the adductor mandibulae muscle is a reasonably good predictor of BF$_{in\,vivo}$ in the 15 serrasalmid species for which we have both estimates ($R^2 = 0.63$, $P < 0.001$; Fig. 2C). This linear model can thus be used to approximate bite performance of serrasalmid species for which BF$_{in\,vivo}$ is not available.

ANATOMICAL DESCRIPTION OF THE ADDUCTOR MANDIBULAE MUSCLE

The adductor mandibulae muscle is divided into a segmentum facialis and a segmentum mandibularis. Both muscle segments are connected via a strong

Figure 2. Linear regressions of: (A) log$_{10}$-transformed in vivo bite force (log$_{10}$ BF$_{in\,vivo}$) against log$_{10}$-transformed body mass (log$_{10}$ BM) in 20 species; (B) log$_{10}$-transformed theoretical bite force (log$_{10}$ BF$_{theoretical}$) against log$_{10}$-transformed body mass (log$_{10}$ BM) in 22 species; and (C) residual in vivo bite force (BF$_{in\,vivo}$) and residual theoretical bite force (BF$_{theoretical}$) in 15 serrasalmid species with different diet. Key in A applies also to B and C. Catoprion mento was not included in comparative statistical analyses.

In A, regression line equations are log$_{10}$ BF$_{in\,vivo}$ = 0.54 log$_{10}$ BM + 0.31 ($R^2 = 0.84$) for carnivorous species and log$_{10}$ BF$_{in\,vivo}$ = 0.47 log$_{10}$ BM − 0.08 ($R^2 = 0.62$) for herbivorous species. In B, these equations are log$_{10}$ BF$_{theoretical}$ = 0.95 log$_{10}$ BM − 0.35 ($R^2 = 0.90$) for carnivorous species and log$_{10}$ BF$_{theoretical}$ = 0.58 log$_{10}$ BM − 0.20 ($R^2 = 0.45$) for herbivorous species. In C, each point represents the species residual mean of two bite force measurements, and the equation of the regression line is residual BF$_{in\,vivo}$ = 0.84 residual BF$_{theoretical}$ + 0.12 ($R^2 = 0.63$). Abbreviations of species names are given in Table S1.
tendinous complex termed the intersegmental aponeurosis. The segment of the cheek, the segmentum facialis, is subdivided into three subdivisions (Fig. 3).

- The pars rictalis attaches onto the lower jaw on the coronoid process of the dentary and on the anguloarticular bone (Fig. 3C, D). This subdivision may have different sites of origin on the suspensorium depending on diet: (1) the horizontal and vertical arms of the preoperculum as well as the quadrate bone in all herbivorous species and (2) only the horizontal arm of the preoperculum and the quadrate bone in the lepidophagous Catoprion mento and most carnivorous species (Fig. 3A, B). However, we recorded that the pars rictalis of the carnivorous Pygopristis denticulata and Pristobrycon striolatus also originates on the vertical arm of the preoperculum (Table S2).

- The pars malaris is strongly attached to the lower jaw mainly via the intersegmental aponeurosis tendinous complex that divides into a Meckelian tendon inserting onto the coronomeckelian bone and a mandibular tendon inserting onto the dentary (Fig. 3C, D). The main sites of origin of the pars malaris are on the skull and suspensorium (i.e. the neurocranium, the hyomandibula, the vertical arm of the preoperculum, the metapterygoid and the quadrate–metapterygoid window). However, variations may also be present depending on diet: the muscle fibres (1) only attach on the lateral surface of the hyomandibula in herbivorous species such as Metynnis hypsauchen, Metynnis lippincottianus and Myloplus ternetzi, (2) attach onto the lateral surface of the hyomandibula and on a small portion of the hyomandibula–neurocranium joint in other herbivorous species and the lepidophagous Catoprion mento, and (3) attach both onto the lateral and medial surfaces of the hyomandibula and on a large portion of the hyomandibula–neurocranium joint in carnivorous species (Table S2).

- The pars stegalis attaches onto the lower jaw via a short tendon joining the intersegmental aponeurosis tendinous complex at the level of the mandibular tendon (Fig. 3C, D). This subdivision may have divergent sites of origin depending on diet: the muscle fibres (1) only attach on the metapterygoid in all herbivorous species and the lepidophagous Catoprion mento, and (2) attach on the metapterygoid and the sphenoid bone of the neurocranium in all carnivorous species, except for Pygopristis denticulata in which it more closely resembles that in herbivorous species (Table S2).

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Table 2. Results of phylogenetic comparative methods used in this study

<table>
<thead>
<tr>
<th>Phylogenetic regressions (PGLS)</th>
<th>df</th>
<th>Intercept ± SE</th>
<th>Slope ± SE</th>
<th>t</th>
<th>P</th>
</tr>
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<tbody>
<tr>
<td>log₁₀ adductor mandibulae muscle mass ~ log₁₀ BM</td>
<td>17</td>
<td>2.89 ± 0.19</td>
<td>-0.34 ± 0.12</td>
<td>24.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log₁₀ pars rictalis ~ log₁₀ adductor mandibulae muscle mass</td>
<td>17</td>
<td>1.18 ± 0.05</td>
<td>0.38 ± 0.04</td>
<td>24.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log₁₀ pars malaris ~ log₁₀ adductor mandibulae muscle mass</td>
<td>17</td>
<td>1.12 ± 0.05</td>
<td>0.28 ± 0.04</td>
<td>24.93</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>log₁₀ pars stegalis ~ log₁₀ adductor mandibulae muscle mass</td>
<td>17</td>
<td>1.16 ± 0.14</td>
<td>0.12 ± 0.06</td>
<td>24.93</td>
<td>&lt;0.001</td>
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<table>
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<tr>
<th>Phylogenetic ANCOVAs</th>
<th>df</th>
<th>SS</th>
<th>MS</th>
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<th>P</th>
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<tbody>
<tr>
<td>log₁₀ BF in vivo ~ diet + log₁₀ BM</td>
<td>9</td>
<td>1.53</td>
<td>0.17</td>
<td>2.36</td>
<td>0.17</td>
</tr>
<tr>
<td>log₁₀ BF theoretical ~ diet + log₁₀ BM</td>
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<td>2.75</td>
<td>0.18</td>
<td>4.48</td>
<td>0.05</td>
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<tr>
<td>log₁₀ adductor mandibulae muscle mass ~ diet + log₁₀ BM</td>
<td>15</td>
<td>3.49</td>
<td>0.23</td>
<td>10.33</td>
<td>0.005</td>
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<tr>
<td>log₁₀ pars rictalis mass ~ diet + log₁₀ adductor mandibulae muscle mass</td>
<td>15</td>
<td>2.63</td>
<td>0.21</td>
<td>0.44</td>
<td>0.52</td>
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<tr>
<td>log₁₀ pars malaris mass ~ diet + log₁₀ adductor mandibulae muscle mass</td>
<td>15</td>
<td>3.77</td>
<td>0.25</td>
<td>3.44</td>
<td>0.08</td>
</tr>
<tr>
<td>log₁₀ pars stegalis mass ~ diet + log₁₀ adductor mandibulae muscle mass</td>
<td>15</td>
<td>3.77</td>
<td>0.25</td>
<td>3.44</td>
<td>0.08</td>
</tr>
</tbody>
</table>

The table first presents the main results of the phylogenetic regressions (PGLS) and then phylogenetic ANCOVAs. Abbreviations: BF in vivo, in vivo bite force; BF theoretical, theoretical bite force; BM, body mass; df, degrees of freedom; F, value of the F-statistic; P, probability of significant difference between groups. Significant values are given in bold type.
size of the adductor mandibulae muscle and its subdivisions

The adductor mandibulae muscle is four times heavier with respect to body mass in carnivorous (1.6 ± 0.4%, mean ± SD) than in herbivorous species (0.4 ± 0.2%; Figs 4A, S3A; Table 3). The jaw muscle of the lepidophagous Catoprion mento is even smaller (0.2 ± 0.1%). In addition, the mass of the three main muscle subdivisions of the adductor mandibulae muscle differ between dietary groups. The pars rictalis is four times larger in herbivorous (43.1 ± 4.2%) than in carnivorous species (10.5 ± 4.9%; Figs 4B, S3B; Table 3). The pars malaris is almost twice as large in carnivorous (83 ± 5.9%) than in herbivorous species (50.6 ± 5.9%; Figs 4C, S3C; Table 3). The pars stegalis is equivalent in carnivorous (6.5 ± 2.1%) and herbivorous species (6.2 ± 0.3%; Figs 4D, S3D; Table 3). Phylogenetic ANCOVAs describe the same results between carnivorous and herbivorous species except for the pars rictalis and the pars stegalis (Table 2).

jaw mechanical advantages

The AMA of the pars rictalis ranges from 0.32 ± 0.07 to 0.63 ± 0.06 (mean ± SD), of the pars malaris ranges from 0.49 ± 0.03 to 0.54 ± 0.03 and of the pars stegalis ranges from 0.35 ± 0.03 to 0.58 ± 0.04 in carnivorous and herbivorous species, respectively (Fig. 5A). The mean AMA of each muscle subdivision of the adductor mandibulae muscle is significantly greater in herbivorous than in carnivorous species (Table 3), indicating that the adductor mandibulae muscle and its subdivisions transmit relatively more force to the front of the lower jaw in herbivorous species. By contrast, the PMA of the pars rictalis ranges from 0.87 ± 0.06 to 0.92 ± 0.09, of the pars malaris ranges from 0.74 ± 0.08 to 1.47 ± 0.32 and of the pars stegalis ranges from 0.79 ± 0.06 to 0.97 ± 0.21 in herbivorous and carnivorous species, respectively (Fig. 5B). Mean PMA values of the pars malaris and pars stegalis are significantly greater in carnivorous than in herbivorous species (Table 3), suggesting that the adductor mandibulae muscle and mainly both pars malaris and pars stegalis subdivisions transmit relatively more force to the back of the lower jaw in carnivorous species relative to herbivorous species. The AMA and PMA of the lepidophagous Catoprion mento are close to those of carnivorous species except for the pars malaris, which transmits relatively little force to the lower jaw.

type of jaw occlusion

The quadrate offset distance in carnivorous species ranges from 0.09 in Serrasalmus elongatus to 0.41 in Pygopristis denticulata (Fig. 6). In herbivorous species, the quadrate offset distance ranges from 0.37 in Acnodon oligacanthus to 0.63 in Myloplus rhomboidalis. The specialist lepidophagous Catoprion mento has a quadrate offset distance of 0.30. Herbivorous species have a significantly higher mean quadrate offset distance (0.56 ± 0.08,
mean ± SD) than carnivorous species (0.26 ± 0.11; Table 3). The distance between the quadrate–anguloarticular joint of the lower jaw and the dentition is therefore significantly greater in herbivorous species and their jaw occlusion resembles a ‘vice-like’ bite (Fig. 6). Conversely, this distance is lower in carnivorous species and their jaw occlusion resembles a ‘scissor-like’ bite.

LOWER JAW SHAPE

The centroid size of the lower jaw does not differ between carnivorous and herbivorous species (Procrustes ANOVA: $F_{1,19} = 1.79, P = 0.20$) but the two dietary groups show significant variation in lower jaw shape (Procrustes ANOVA: $F_{1,19} = 56.79, P = 0.001$; phylogenetic Procrustes ANOVA: $F_{1,16} = 6.32, P = 0.001$). The first three principal components (PC1 = 85%, PC2 = 7% and PC3 = 3%) account for 95% of the total shape variation in the lower jaw. Along PC1 in the phylomorphospace (Fig. 7), positive scores are associated with herbivorous species such as the pacu Piaractus brachypomus for which lower jaws have an anteriorly contracted tooth row, a low dentary, a high coronoid process and a large anguloarticular bone. Negative scores are associated with carnivorous species such as the piranhas Pygocentrus nattereri or Serrasalmus manueli for which lower jaws have an expanded tooth row, an elongated dentary, a low coronoid process and a reduced anguloarticular bone. The piranha Pygopristis denticulata has an intermediate lower jaw shape between the species with negative and positive PC1 scores. Shape variation along PC2 (Fig. 7) is related to the height of the lower jaw. Positive scores are associated with shallow lower jaws whereas negative scores are associated with tall lower jaws. Both herbivorous and carnivorous species show variation in lower jaw height.
Bite Force and Functional Morphological Traits of the Oral Jaw System

The adductor mandibulae muscle mass and the PMA of the *pars malaris* are the only functional morphological traits of the oral jaw system showing a linear relationship with BF<sub>in vivo</sub>. In addition, these traits together provide the best multiple linear model explaining the variability in BF<sub>in vivo</sub> (stepwise multiple linear regression, final model: $R^2 = 0.68$, $P < 0.001$). However, using phylogenetically corrected methods, these results are no longer significant [phylogenetic linear regression BF<sub>in vivo</sub> ~ adductor mandibulae muscle mass: Akaike information criterion (AIC) = 10.11, $P = 0.89$; phylogenetic linear

Table 3. Results of Mann–Whitney U tests and ANCOVAs for the functional morphological traits of the oral jaw system

<table>
<thead>
<tr>
<th>Jaw muscle size</th>
<th>Mann–Whitney U tests</th>
<th>ANCOVAs</th>
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<tbody>
<tr>
<td></td>
<td>U</td>
<td>P</td>
</tr>
<tr>
<td>Adductor mandibulae muscle</td>
<td>106</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Pars rictalis subdivision</td>
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<td>&lt;0.001</td>
</tr>
<tr>
<td><em>Pars malaris</em> subdivision</td>
<td>108</td>
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</tr>
<tr>
<td>Pars stegalis subdivision</td>
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<td>0.7</td>
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<table>
<thead>
<tr>
<th>Jaw mechanical advantage</th>
<th>Mann–Whitney U tests</th>
<th>ANCOVAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMA pars rictalis</td>
<td>108</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AMA <em>pars malaris</em></td>
<td>95</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>AMA pars stegalis</td>
<td>108</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PMA pars rictalis</td>
<td>71.5</td>
<td>0.23</td>
</tr>
<tr>
<td>PMA <em>pars malaris</em></td>
<td>108</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>PMA pars stegalis</td>
<td>81.5</td>
<td>0.05</td>
</tr>
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</table>

<table>
<thead>
<tr>
<th>Type of jaw occlusion</th>
<th>Mann–Whitney U tests</th>
<th>ANCOVAs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quadrato offset distance</td>
<td>108</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Abbreviations: AMA, anterior mechanical advantage; PMA, posterior mechanical advantage; df, degrees of freedom; $F$, value of the $F$-statistic; MS, mean sum of squares; $P$, probability of significant difference between groups; SS, sum of squares; $U$, value of the non-parametric $U$-statistic. Significant values are given in bold type.

Figure 5. Bar graphs of anterior (A) and posterior (B) jaw mechanical advantages for the three subdivisions of the adductor mandibulae muscle (pars rictalis, *pars malaris* and pars stegalis) in serrasalmid species with different diet. *Catoprion mento* was not included in comparative statistical analyses. Error bars are the standard deviations and bars marked with an asterisk are significantly different (*$P \leq 0.05$; **$P \leq 0.01$; ***$P \leq 0.001$).
DISCUSSION

The present comparative study demonstrates that bite performance varies considerably within the highly specialized group of serrasalmid biters and that these variations may be related to diet. In addition, this study illustrates that morphological and functional traits of the oral jaw system vary in accordance with bite performance, diet specialization and evolutionary history within this Neotropical fish family.

Bite performance and diet

In contrast to our predictions, herbivorous species that mainly feed by crushing hard plant items (fruits and seeds) do not have higher bite forces than carnivorous species that feed on soft animal prey (meat and flesh). Most studies linking diet to bite performance, however, have demonstrated that species with a more durophagous diet have higher bite forces (Huber et al., 2005; Mehta, 2008; Herrel & Holanova, 2008; Herrel et al., 2017). Independent of the variation in body size, our data show that in vivo and theoretical bite forces are significantly higher in carnivorous than in herbivorous species, irrespective of the kind of 'herbivory' (Fig. 2A, B). This observation suggests that diet is probably the major driving force explaining this variation in bite performance. However, our results with phylogenetic correction provided only partial support for this hypothesis (Table 2). Consequently, we cannot reject the hypothesis that other factors may explain the observed divergence among trophic groups. Alternatively, the strong clustering of diet with clades may simply prevent us from detecting significant differences when taking phylogeny into account.

Furthermore, this observation also supports the suggestion that the carnivorous diet of piranhas requires powerful bite forces to capture and process prey (Grubich et al., 2012), which are likely to be highly specific. The highest bite forces observed here were for Pygocentrus nattereri and Pygocentrus piraya, piranha species which usually attack in numbers live prey larger than themselves (Goulding, 1980; Jégu,
The size of the prey relative to consumer body size as well as the type of feeding behaviour might be the drivers of bite performance within carnivorous piranhas and herbivorous serrasalmid fishes. The piranha species with lower bite forces, *Serrasalmus compressus* and *Serrasalmus elongatus*, are reported to generally consume small prey items such as fins or scales of other fish (Goulding, 1980; Jégu, 2003). The specialized wimple-piranha *Catoprion mento* also eats mainly scales (Goulding, 1980; Jégu, 2003; Janovetz, 2005) and has the lowest bite force of any taxon in our study. This observation is consistent with previous studies on bite performance and prey size in other vertebrate groups. In lizards, higher bite forces are also associated with the consumption of large prey relative to body size (Herrel et al., 2001; Verwaijen et al., 2002). Similar observations have been made for carnivorous mammals for which highest estimated bite forces were recorded in animals that usually kill and consume large prey (Wroe et al., 2005; Christiansen & Wroe, 2007).

In herbivorous species, the issue of prey size might be compensated for by an increase in overall body size to improve bite performance, as suggested by Habegger et al. (2012) for bull sharks. The pacu species, *Colossoma macropomum*, *Piaractus mesopotamicus* and *Piaractus brachypomus*, are known to be among the largest fish of the Amazon basin (Goulding & Carvalho, 1982) and they consume large fruits and seeds (Correa et al., 2007). Being larger, these herbivorous species would thus have access to larger vegetal prey (e.g. Brazil nuts) than smaller herbivorous species such as *Metynnis hypsauchen*, *Metynnis lippincottianus*, *Myloplus rubripinnis* or *Myloplus ternetzi*. In addition, the high variability in BF in vivo among herbivorous species (Fig. 2A) suggests this guild might be separated into two clusters: species feeding on hard plant items (fruits and seeds) with higher BF in vivo and species feeding on soft plant items (algae, aquatic plants and flowers) with lower BF in vivo. However, more detailed analyses of stomach contents and the relationship between food hardness and bite force are needed to reach clearer conclusions.

**Bite performance and functional morphological diversity**

Variation in bite performance between carnivorous and herbivorous serrasalmid species is supported by differences in the anatomy of the oral jaw system. The adductor mandibulae muscle is proportionally larger (Fig. 4A) with more sites origin of on the skull and
BITE FORCE AND ORAL MORPHOLOGY IN SERRASALMIDAE

adductor muscles (Herrel et al., 2002). The size of the adductor mandibulae muscle and the insertion site onto the lower jaw (Fig. 3C, D) are the main morphological traits of the oral jaw system that best predict bite force in serrasalmids. The largest adductor mandibulae muscle combined with the most anterior insertion of this muscle on the lower jaw provide the strongest bite force.

The morphological differences between carnivorous and herbivorous serrasalmid species also suggest different biomechanical functions and thus divergent bite strategies. Regarding the jaw muscle arrangement, the pars rictalis and pars malaris are the largest subdivisions of the adductor mandibulae muscle. They contribute more significantly to force transmission to the lower jaw than the pars stegalis and show more variation between dietary groups. The pars rictalis is larger in herbivorous (~40% of the total jaw muscle mass) than in carnivorous species (~10%), and, the pars malaris is more massive in carnivorous (~80%) than in herbivorous species (~50%). Moreover, these subdivisions have relatively ventral and dorsal origin sites on the suspensorium, respectively (Table S2).

Force transmission is considered to be maximal when the muscle subdivisions are perpendicular to the axis passing by their insertion on the lower jaw and the jaw joint during mouth closing (Barel, 1983; Turingan, 1994). From this biomechanical point of view, the relatively vertical position of the pars malaris with respect to the lower jaw implies a better position for firmly grasping prey during mouth closing. Conversely, the relatively horizontal disposition of the pars rictalis suggests a better fit for speed. Carnivorous species might thus be more adapted to deliver powerful bite forces while firmly holding prey in the mouth. Herbivorous species might, by contrast, be capable of generating faster bite forces; however, it must be borne in mind that real differences between the two diets represent trade-offs among different constraints (Parmentier et al., 2000). In parallel, differences in physiological traits of the adductor mandibulae muscle (e.g. deep red, slow oxidative vs. intermediate vs. fast twitch muscle fibres) might also be a factor driving differences in bite abilities between dietary groups.

Furthermore, the arrangement of the pars rictalis and pars malaris is consistent with the lower jaw shape. In herbivorous species, the anteriorly shortened tooth row in association with larger coronoid process and anguloarticular bone provide a wider anterior attachment site for the jaw muscles and mainly the pars rictalis (Fig. 3D). This ‘herbivorous configuration’ improves the in-lever arms and therefore gives better anterior mechanical advantages to the jaw system (Fig. 5A) to transmit powerful bite forces mainly at the front. In carnivorous species, the dentary is longer and possesses a greater number of larger, laterally oriented teeth. The coronoid process is reduced and the anguloarticular bone provides a limited attachment site for all jaw muscles (Fig. 3C). This ‘carnivorous configuration’ improves the posterior mechanical advantages of the jaw muscles (Fig. 5B) to generate more powerful bite forces at the back. In serrasalmid fishes, the mechanical advantage of the various muscle subdivisions can thus differ by either shifts in muscle insertion (L_m) or length change in the lower jaw (L_out). Note, however, that because mechanical advantages are dimensionless, these ratios can still end up being the same despite different anatomies.

Additionally, lower jaw shape may also be closely related to the bite strategy and diet specialization because the quadratic offset distance of herbivorous species is closer to ‘1’ compared with carnivorous species (Fig. 6). Quadratic offset values approximating ‘1’ have been shown to correspond to a near-perfect jaw occlusion and a ‘vice-like’ bite strategy in which all teeth occlude simultaneously in arthrodirid species (Anderson, 2009). By contrast, quadratic offset values approximating ‘0’ have been shown to correspond to an imperfect jaw occlusion and a ‘scissor-like’ bite strategy in which two sides of the dentition occlude progressively from posterior to anterior (Anderson, 2009). Our data support that distinctive bite strategies may be found within a highly specialized fish group of biters. The acquisition of food in herbivorous species is mainly done using a close-to-vice-like bite strategy to crush fruits and seeds or to shear pieces of plants. Feeding in carnivorous species is primarily done using a close-to-scissor-like bite strategy to cut or slice pieces of fleshy prey. In addition to tooth shape differences between dietary groups, a scissor-like bite provides greater bite stresses due to the progression of the bite point across the occlusive surface. Conversely, a vice-like bite increases the bite surface area and therefore reduces bite stresses. Variation in the type of jaw occlusion and bite strategy has also been highlighted in sharks that crush hard-shelled molluscs compared to piscivorous sharks (Ramsay & Wilga, 2007). These authors further proposed that the shorter distance between the jaw joint and the dentition (i.e. imperfect jaw occlusion) may serve to maximize mouth gape to perform larger bites, as should certainly be the case for carnivorous piranhas as well.

The major differences in the oral jaw system of carnivorous and herbivorous serrasalmid species may represent an interesting functional parallelism...
with mammals. In mammals, jaw closing is achieved by both masseter and temporalis muscles (Herring & Scapino, 1973; Herring et al., 2001). The masseter muscle attaches on the zygomatic arch and on the lateral side of the mandible. It is mainly used to crush food at low gape angles. The temporalis muscle originates on the temporal bone of the skull and attaches on the coronoid process of the mandible. It allows the lower jaw to close at high speed (Schwenk, 2000) and provides optimal bite force at high gape. All mammals possess both muscles but the masseter muscle is usually larger and stronger in herbivorous than in carnivorous mammals whereas the temporalis muscle is larger in carnivorous species (Turnbull, 1970; Schwenk, 2000). Interestingly, these differences in the proportion of muscles are also related to lower jaw shape. In herbivorous mammals, the angular process is more expanded for the insertion of the masseter muscle whereas the coronoid process is proportionally more developed in carnivorous mammals in relation to the development of the temporal muscle (Schwenk, 2000). However, the increase in size of the coronoid process in carnivorous mammals, an adaptation for generating bite force at large gape, is not observed in carnivorous serrasalmid fishes where the coronoid process is reduced. Differences in jaw occlusion related to lower jaw shape have been highlighted in mammals as well (Turnbull, 1970; Herring & Herring, 1974; Greaves, 1982; Herring, 1993). In herbivorous mammals, the entire tooth rows of the upper and lower jaw contact the food simultaneously. Conversely, in carnivorous mammals the jaw joint is positioned low on the skull, providing these animals with a ‘scissor-like’ bite strategy.

**EVOLUTIONARY HISTORY AND INTERMEDIATE MORPHOLOGY**

In Serrasalmidae, carnivory has been hypothesized to be a derived condition from the herbivorous or omnivorous diet of the common ancestor to modern serrasalmids based on maximum-likelihood ancestral character reconstructions of diet (Correa et al., 2007). Previous studies on serrasalmid fishes (e.g. Gosline, 1951) also hypothesized that the single row of sharp teeth in carnivorous piranhas was derived from the two rows of large incisiform-to-molariform teeth in herbivorous species. However, we do not have information about the tempo of functional morphological evolution linked to this evolutionary shift in diet. The most likely hypothesis is that of a gradual morphological evolution requiring intermediate morphs as indicated by the serrasalmid fossil record and the intermediate zig-zag pattern of dentition of the presumed bone-crushing Megapiranha paranensis (Gayet & Meunier, 1998; Dahdul, 2004; Cione et al., 2009; Grubich et al., 2012).

Similarly, the extant *Pygopristis denticulata* has a single row of dentition with pentacuspid teeth and an intermediate oral morphology between carnivorous and herbivorous species, supporting such a hypothesis. First, the size of the adductor mandibulae muscle of *Pygopristis denticulata* relative to its body mass is close to that of herbivorous species (Fig. S3A). Its pars rictalis subdivision is also more developed than in the other carnivorous species (Fig. 4B). The sites of origin of its pars rictalis are on both horizontal and vertical arms of the preoperculum as in herbivorous species (Table S2). Its pars malaris subdivision, however, is more developed than in herbivorous species (Fig. 4C). Its lower jaw thus shows an intermediate shape between the two dietary groups with an elongated dentary similar to carnivorous species but a large coronoid process and anguloarticular bone similar to herbivorous species (Fig. 7). *Pygopristis denticulata* might thus be an extant intermediate morph between two specialized serrasalmid groups. However, the fact that this species could also have an omnivorous diet explaining its intermediate oral morphology cannot be discarded because precise information about its diet are unavailable.

Finally, the serrasalmid family has undoubtedly diversified into various feeding habits that are all dependent on biting (herbivory, granivory, frugivory, carnivory, piscivory, lepidophagy, etc.). The extinct species *Megapiranha paranensis* is even considered to have been capable of piercing and crushing bones (i.e. osteophagy) following the hypothetical reconstruction of the jaw system from *Serrasalmus rhombeus* and estimations of bite performance (Grubich et al., 2012). Along with our results, it might be hypothesized that the ability to generate strong bite forces is a derived biomechanical trait in the ‘piranhas-clade’ that evolved in response to their specific diet constraints.

**CONCLUSION**

To the best of our knowledge, this study represents the first time that such a large diversity in bite force and functional morphology of oral jaw system has been demonstrated in a fish group relying on a unique ‘biting’ feeding mode. In addition to the variation in dentition, our results highlight that the diet specialization towards carnivory in serrasalmid fishes has been accompanied by important changes in the size of the adductor mandibulae muscle (mainly the pars rictalis and pars malaris subdivisions) and lower jaw shape with major functional effects on bite performance and bite strategy. The increase in size...
of the pars malaris subdivision at the expense of a decrease in size of the pars rictalis, in combination with an elongation of oral jaws and a greater number of highly specialized teeth, probably helped to increase bite force in an ancestor to modern serrasalmids. Furthermore, the decrease in size of the anguloarticular bone of the lower jaw, which shortens the quadrate offset distance, probably led to changes in the type of jaw occlusion in carnivorous species to closely match a ‘scissor-like’ bite.

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REFERENCES


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**SUPPORTING INFORMATION**

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Figure S1. Phylogenetic relationships and diet in 40 serrasalmid species based on molecular data from Thompson et al. (2014). The original tree was pruned to include only examined species in our study (in black) and to exclude species that were not studied (in grey). Nine additional species investigated here (*Metynnis lippincottianus*, *Metynnis maculatus*, *Myleus micans*, *Myloplus ternetzi*, *Piaractus mesopotamicus*, *Pygocentrus cariba*, *Serrasalmus brandtii*, *Serrasalmus elongatus* and *Serrasalmus maculatus*) were not studied in the original phylogeny and were thus not included in the phylogenetic comparative analyses. The diet of carnivorous species is represented by a red dot, that of herbivorous species by a green dot and that of the lepidophagous species *Catoprion mento* by a black dot.

**Figure S2.** Photographs of the dentition in (A) the carnivorous *Pygocentrus nattereri* and (B) the herbivorous *Piaractus brachypomus*.

**Figure S3.** Linear regressions of (A) log$_{10}$-transformed adductor mandibulae muscle mass against log$_{10}$-transformed body mass (log$_{10}$ BM) and (B) log$_{10}$-transformed pars rictalis mass, (C) log$_{10}$-transformed *pars malaris* mass and (D) log$_{10}$-transformed pars stegalis mass against log$_{10}$-transformed adductor mandibulae muscle mass in 22 serrasalmid species with different diet. Key in A applies also to B–D. *Catoprion mento* was not included in comparative statistical analyses. In A, regression line equations are log$_{10}$ adductor mandibulae muscle mass = 0.95 log$_{10}$ BM − 1.77 ($R^2 = 0.87$) for carnivorous species and log$_{10}$ adductor mandibulae muscle mass = 0.84 log$_{10}$ BM − 2.77 ($R^2 = 0.61$) for herbivorous species. In B, these equations are log$_{10}$ pars rictalis mass = 1.22 log$_{10}$ adductor mandibulae muscle mass − 0.9 ($R^2 = 0.91$) for carnivorous species and log$_{10}$ pars rictalis mass = 1.0 log$_{10}$ adductor mandibulae muscle mass − 0.36 ($R^2 = 0.98$) for herbivorous species. In C, these equations are log$_{10}$ *pars malaris* mass = 0.98 log$_{10}$ adductor mandibulae muscle mass − 0.1 ($R^2 = 0.99$) for carnivorous species and log$_{10}$ *pars malaris* mass = 0.95 log$_{10}$ adductor mandibulae muscle mass − 0.34 ($R^2 = 0.97$) for herbivorous species. In D, these equations are log$_{10}$ pars stegalis mass = 1.1 log$_{10}$ adductor mandibulae muscle mass − 1.15 ($R^2 = 0.91$) for carnivorous species and log$_{10}$ pars stegalis mass = 1.4 log$_{10}$ adductor mandibulae muscle mass − 0.91 ($R^2 = 0.69$) for herbivorous species. Abbreviations of species names are given in Table S1.

**Table S1.** List of the abbreviations of species names used in this comparative study and additional references on serrasalmid diet.

**Table S2.** Summary of the sites of origin of the three subdivisions of the adductor mandibulae muscle (pars rictalis, *pars malaris* and pars stegalis) in 22 serrasalmid species with different diet.

**Text S1.** Additional information about sampling, provenance and maintenance of serrasalmid species.

**Text S2.** Calculation of theoretical bite forces.