Regional variation in the mechanical properties of the vertebral column during lateral bending in *Morone saxatilis*

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Unlike mammalian, disc-shaped intervertebral joints (IVJs), the IVJs in fishes are biconid structures, filled with fluid and thought to act as hydrostatic hinge joints during swimming. However, it remains unclear which IVJ structures are dominant in mechanical resistance to forces in fishes, and whether variation in these tissues might impact the function of the vertebral column along its length. Here, we measured the dynamic mechanical behaviour of IVJs from striped bass, *Morone saxatilis*. During lateral bending, angular stiffness was significantly lower in the caudal and cervical regions, relative to the abdominal region. The neutral zone, defined as the range of motion (ROM) at bending moments less than 0.001 Nm, was longer in the caudal relative to the abdominal IVJs. Hysteresis was 30–40% in all regions, suggesting that IVJs may play a role in energy dissipation during swimming. Cutting the vertical septum had no statistically significant effect, but cutting the encapsulating tissues caused a sharp decline in angular stiffness and a substantial increase in ROM and hysteresis. We conclude that stiffness decreases and ROM increases from cranial to caudal in striped bass, and that the encapsulating tissues play a prominent role in mechanical variation along the length of the vertebral column.

Keywords: biomechanics; vertebral column; intervertebral joints; striped bass; *Morone saxatilis*

1. INTRODUCTION

The vertebral column plays a dichotomous role during locomotion across vertebrate taxa, providing both the stiffness and flexibility required for locomotion [1–10]. To some extent, different mechanical properties in different regions of the vertebral column can meet the conflicting demands of stiffness and flexibility. For example, in humans, the lumbar region has a smaller range of motion (ROM) and a greater stiffness than the more flexible cervical region [11–13]. Ultimately, the structure of the individual vertebra and its interaction with the adjacent vertebra via the intervertebral joint (IVJ) determine the mechanics of not only individual segments but also across entire regions, and along the full length of the column as well [3,5–8,14–16].

The IVJs of fish and mammals are superficially similar, with a gelatinous matrix of notochordal remnants in the centre, surrounded by stiff ligaments. However, mammalian vertebrae are acoelous, with flat ends that result in disc-shaped IVJs, whereas the vertebrae of the vast majority of bony and cartilaginous fishes are amphicoelous, with hourglass-shaped centra and biconid IVJs (figure 1a–d). Thus, in fishes, the IVJ structures not only lie between the two adjacent vertebrae but are also housed within their hollow cones (figure 1d) [9,10,17].

These soft tissues of the IVJs in fishes include fluid-filled extracellular lacunae, a mass of notochordal cells with varied degrees of vacuolization and a complex of three encapsulating tissues that bind the rims of the centra adjacent to one another (figure 1d) [9,10,18,19]. A sheet of connective tissue, the vertical septum, spans the space between adjacent neural and haemal spines (figure 1a,c). This combination of fluid-filled structures and tensile elements has led some researchers to hypothesize that the amphicoelous IVJ acts as a hydrostatic hinge, resisting compressive loads but allowing the lateral bending associated with swimming in fishes [10,19].

One of the goals of the present study was to determine the relative roles of these complex IVJ tissues in resisting the forces, and allowing the lateral bending, associated with locomotion in fishes. In mammalian spine studies, selectively cutting various ligaments in isolated cadaveric material has shown that the annular ligaments of the intervertebral disc resist the vast majority of sagittal flexion and extension, while the external ligaments and bony processes tend to resist...
lateral bending and axial rotation [2–6,11,12,20]. Here, we emulate these lesion studies by cutting first the vertical septum and then the encapsulating tissues, and measuring changes in ROM, angular stiffness and hysteresis in lateral bending in striped bass, *Morone saxatilis*. We expect that this sequential lesioning will yield an insight into the relative roles of the vertical septum, the encapsulating tissues and the notochordal cell mass in the mechanics of amphicoelous IVJs.

A second goal of the present study was to determine the effect of bending frequency on ROM, angular stiffness and hysteresis across the three lesion treatments in striped bass. The viscoelastic behaviour of biological soft tissues suggests that the frequency of loading should have a noticeable impact on the mechanical properties of these tissues [21]. However, it is unclear whether these viscoelastic properties manifest in the bending mechanics of IVJs. While frequency did not alter the characteristics of stiffness and hysteresis in mammalian joints [5], a study of the mechanics of shark vertebral columns reported an increase in joint stiffness with an increase in frequency [7]. In contrast, blue marlin IVJs suggest a possible decrease in angular stiffness with an increase in bending frequency [22]. Here, we test whether bending frequency affects IVJ stiffness in the relatively non-specialized striped bass.

The third goal of this study was to measure the variations in ROM, angular stiffness and hysteresis along the length of the vertebral column in striped bass. In previous studies on regional variations in fishes, Hebrank [23] reported uniform angular stiffness along the vertebral column of a relatively non-specialized, sub-carangiform swimmer, the Norfolk spot (*Leiostomus xanthurus*), but the vertebral column of the skipjack tuna (*Katsuwonus pelamis*) was found to be far stiffer than the Norfolk spot and more regionalized. The more anterior joints of the tuna were uniformly stiff, while the caudal peduncle increased greatly in joint stiffness followed by a decrease in stiffness of the joints within the caudal fin complex. In addition, investigations of the vertebral column of blue marlin (*Makaira nigricans*) revealed an increase in angular stiffness from cranial to caudal that seemed to be correlated with vertebral centrum length [22,24].

In contrast to these findings of constant or increasing IVJ stiffness from cranial to caudal, a recent morphological study predicted decreasing flexural stiffness and increasing ROM from cranial to caudal in striped bass, *M. saxatilis* [19]. The study found a longitudinal variation in the soft tissues of the IVJ; the volume of the notochordal cell mass and the cross-sectional area of the helically wound fibrous sheath both decreased from cranial to caudal, and regional variation was found in the number of fibre populations and the insertion angle of those populations in the vertical septum. The width (mediolateral diameter) of the vertebral centra decreased.

Figure 1. Morphology of amphicoelous fish vertebrae. (a) Schematic of vertebral column from cranial to caudal. Red box indicates the two adjacent vertebrae that are magnified in (c) and (d). (b) Oblique view of one centrum. (c) Left lateral view of the two boxed caudal centra in (a), rostral to the left and caudal to the right. (d) Sagittal section of the two boxed caudal centra in (a), showing the intervertebral joint tissues between and within the hollow cones, rostral to the left and caudal to the right. a, amphicoel; c, centrum; ec, encapsulating tissues; ee, elastica externa; eil, external intervertebral ligament; fs, fibrous sheath; ha, haemal arch; hs, haemal spine; lac, extracellular lacunae; na, neural arch; ncm, notochordal cell mass; ns, neural spine; vs, vertical septum.
from cranial to caudal, but the length of the IVJs did not, suggesting a greater ROM for lateral bending in the caudal joints. Estimates of the second moment of area for the IVJs along the length of the fish predicted a lower stiffness in the most rostral and caudal regions, relative to the abdominal (middle) joints. Thus, on the basis of the soft-tissue morphology and geometry of vertebrae and IVJs, this study predicted an increase in ROM from cranial to caudal and a lower stiffness in the caudal than in the abdominal IVJs [19].

This morphological study of *M. saxatilis* also proposed, based on vertebral and IVJ morphology, the division of the striped bass vertebral column into three regions: a cervical region containing vertebrae 1–4, an abdominal region containing vertebrae 5–12 and a caudal region containing vertebrae 12–24 [19]. Vertebrae were shown to vary in size, overall shape and position of bony projections between the cervical and abdominal regions and between the abdominal and caudal regions. The striped bass was selected for the previous morphological and current mechanical study because it is a relatively non-specialized, sub-carangiform swimmer and has a low vertebral number, indicating potentially large deformations per IVJ (because the overall lateral bending is spread across the IVJs, a smaller number of joints should strain more [16,25]). In the present study, we aim to combine the mechanical investigation of striped bass with this previously reported regional variation in morphology to investigate the form–function relationships of the striped bass vertebral column.

2. METHODS

2.1. Experimental protocol and animals

Five adult striped bass, *M. saxatilis* (Walbaum), were obtained from the Susquehanna Aquaculture, Inc. fish hatchery (York Haven, PA, USA) and housed in a 300 gallon circular aquarium at 19–22°C. The total length (TL) and body mass of each individual were 31.75, 32.64, 34.29, 36.07, 38.10 cm, and 0.55, 0.51, 0.60, 0.56, 0.70 kg, respectively. Fish were killed on the day of specimen preparation by an overdose of buffered tricaine methanesulphonate (MS-222, 0.25 g l⁻¹). Specimens were stored in Ringer’s solution and refrigerated for the previous morphological and current mechanical study because it is a relatively non-specialized, sub-carangiform swimmer and has a low vertebral number, indicating potentially large deformations per IVJ (because the overall lateral bending is spread across the IVJs, a smaller number of joints should strain more [16,25]). In the present study, we aim to combine the mechanical investigation of striped bass with this previously reported regional variation in morphology to investigate the form–function relationships of the striped bass vertebral column.

2.2. Specimen preparation

Test specimens consisted of six vertebrae spanning five IVJs. Three test specimens were excised from each fish: vertebrae 1–6 for testing the cervical region (IVJ between vertebrae 1 and 2), vertebrae 7–12 for testing the abdominal region (IVJ between vertebrae 9 and 10) and vertebrae 18–23 for testing the caudal region (IVJ between vertebrae 20 and 21). All ribs and muscle tissues were removed from these specimens such that only the vertebrae and IVJ tissues, including the vertical septum, were intact (figure 2). Neural and haemal spines were removed from most of the vertebrae, leaving only the spines and vertical septa of the joint of interest intact. Both ends of the specimens were potted with Loctite professional heavy duty 5 min epoxy in ¼ inch (15.9 mm) outer diameter, ½ inch (12.7 mm) inner diameter polyvinylchloride (PVC) tubing such that two IVJs on each end were immobilized, and bending was restricted to one joint of interest in the centre of the specimen (figure 2). Epoxy was allowed to cure for 20 min during which the unrestricted joint and tissues were kept damp with the application of teleost Ringer’s solution (111.00 mM NaCl, 5.37 mM KCl, 1.00 mM CaCl₂, 0.60 mM MgSO₄ and 5 mM hydroxyethylpipera-zinemethanesulphonic acid (HEPES)). Once the epoxy had set, three bone markers were implanted onto the lateral surface of the exposed vertebrae to facilitate angular displacement calculations (figure 2). Bone marker implantation was performed with care so as not to puncture through the bone into the joint cavity. Specimens were then stored in Ringer’s solution and refrigerated overnight. Specimens were never frozen.

2.3. Specimen mounting

Specimens were stored in Ringer’s solution at room temperature at all times throughout the day when not mounted in the testing apparatus. The order of specimen testing was randomized in an attempt to control for any order effect. When mounted, each specimen was clamped into the testing apparatus such that one end of the potted vertebrae was securely fastened with the ventral aspect of the exposed joint facing the camera (figure 3a). The servomotor was positioned above the specimen and connected to the specimen by two aluminium beams. Beam 1 was oriented horizontally and was attached to the servomotor arm at one end and to the second beam at the opposite end with two pin joints. It should be noted that these two pin joints are likely to be subject to frictional forces that may have influenced our measurements. Given that the same set-up was used for all of our tests, we believe that
these forces will influence measurements similarly across all trials. Beam 2 was oriented vertically and was clamped to the cantilevered, potted vertebrae (figure 3a). In this way, as the servomotor arm moved left, the specimen is bent laterally about the unrestricted joint, with a resultant reaction force vector (\( f_r \)). Red dots indicate bone markers, markers about each joint of the apparatus and centre of mass (com) of each segment. These marker positions were measured from high-speed video for inverse dynamics calculations. Note that the vertebral specimen is mounted on its lateral side such that the ventral aspect of the joint is facing the camera.

2.4. Dynamic testing

Pilot testing was performed to determine the maximum force that could be applied without causing damage to the IVJs. Pilot specimens were tested to failure, and a force value of 4 N was determined to be the maximum bending force that could be applied to this apparatus without the danger of causing plastic deformation in any of the specimens.

Specimens were preconditioned with 30 cycles of bending at 2 Hz. Linear displacements of the distal tip of the servomotor actuator arm were then determined for each specimen incrementally until the load reached approximately 4 N. Cyclic dynamic testing was then performed up to that value of maximum displacement of the servomotor arm at 2, 5 and 7 Hz. These frequencies lie within the biologically relevant range of steady swimming and burst swimming for striped bass \([26,27]\). Data were recorded at a sampling frequency of 1000 Hz using a 16-bit A/D converter (National Instruments, Austin, TX, USA). Control of the servomotor and data collection was performed using Icor Pro software (Wavemetrics, Tigard, OR, USA). Raw data were filtered using a Butterworth filter, with a cut-off frequency of 25 Hz.

2.5. Tissue disruption treatments

To investigate the role of the vertical septum and encapsulating tissues during lateral bending, each specimen was subjected to three tissue disruption treatment conditions. First, the joint was tested intact (IT). Second, the vertical septum was cut (VC) while the specimen was mounted to avoid variation owing to remounting, and dynamic tests were performed again at all frequencies. Finally, the encapsulating complex was cut (EC) by carefully cutting the tissues of the joint capsule around the perimeter of the joints, while the specimen remained mounted to avoid variation owing to remounting. This final treatment leaves just the notochordal cell mass and fluid-filled lacunae within the joint cavity intact. We did not observe any loss of fluid as a result of this final treatment, and, therefore, are confident that the fluid-filled lacunae were not punctured. All tests were then performed a third time, after cutting the encapsulating complex.

2.6. Angle calculation

Each mechanical test was filmed with a Photron Fastcam 1024 PCI model 100 k high-speed video camera (Photron USA, Inc., San Diego, CA, USA) at 1000 frames per second. Video and mechanical testing were synchronized with a shared trigger switch. The bone markers were digitized with DLTdataviewer software [28]. Raw \( x \)- and \( y \)-coordinates of each marker were filtered using a Butterworth filter with a 25 Hz cut-off frequency. Intervertebral joint angles were then calculated using the slope of the two lines that ran through the three digitized points. Line 1 is taken as the line segment passing through markers 1 and 2 in the first frame of each sequence. Line 2 is taken as the line segment passing through markers 2 and 3 in each frame. Calculations were performed using MATLAB (Mathworks, Inc., Natick, MA, USA).

2.7. Moment calculation

Inverse dynamics analysis was used to calculate the total net moment at the unrestricted IVJ. The experimental set-up was broken down into a link segment model with the reaction force being measured by a force transducer...
integrated into the servomotor system and calibrated to the distal tip of the servomotor lever arm (figure 3). The masses of each beam were recorded prior to experimentation. The mass of just the cantilevered portion of the specimen was measured after experimentation and added to the mass of beam 2. This protocol assumes that the clamped attachment of beam 2 to the cantilevered vertebrae is rigid.

Markers were placed about each pin joint of the apparatus and also at the centre of mass of each segment prior to testing (figure 3). These points were digitized, and centre of mass accelerations and angles about each pin joint were calculated.

Given the masses of the beams, the centre of mass kinematics of each beam and the measured reaction force at the servomotor lever arm, the following equations were solved for each segment and used to calculate the moment about the unsupported IVJ [29]:

\[ F_{x,y} = m a_{x,y} \]

and

\[ M_j = I_j a_j, \]

where \( F_{x,y} \) is the external force in the horizontal and vertical directions (including gravity and frictional forces at the pin joints), \( m \) is the mass of the segment, \( a_{x,y} \) is the linear acceleration of the centre of mass in each direction, \( M_j \) is the moment generated by \( F_{x,y} \) about the centre of mass, \( I_j \) is the moment of inertia about the centre of mass and \( a_j \) is the angular acceleration. The moment of inertia for beam 1 was calculated as a rod rotating about its centre of mass. Similarly, the moment of inertia of the cantilevered potted vertebral specimen was calculated as a rod rotating about the centre of mass. The much smaller mass of the second aluminium beam was added as a point mass to the cantilevered specimen. In this experiment, the joint was bent laterally to one side only; thus, the resulting moments were all positive.

Calculations were performed using MATLAB.

It is important to note that the present methodology assumes that deformation is concentrated in the relatively compliant soft tissues, and that the bony vertebrae of striped bass do not undergo measurable strain during lateral bending. Previous research has revealed that both chondrichthyan cartilage and teleost bone fall within the same range of stiffness as mammalian bone [30–35]. Thus, it is reasonable to assume that the strain associated with lateral bending in striped bass will be focused on the tissues of the exposed IVJ and resisted by the stiff bony vertebrae.

2.8. Data analysis

Moment–angle curves were generated for each test, and neutral zone range, angular stiffness and hysteresis were calculated (figure 4). The neutral zone of bending has been described as the range over which a spinal segment moves freely and without measurable resistance to applied loading [36]. The border of this zone is therefore defined as the point at which the moment signal is large enough to be discriminated from zero [37]. For the current study, this neutral zone border was defined at 0.001 Nm of moment about the joint. Using this definition, the range of the neutral zone was measured from 0° of lateral bending to the angle at which the moment about the joint exceeded 0.001 Nm (figure 4).

Angular stiffness was calculated at 10° and 15° for each specimen. On the basis of the kinematics data for striped bass during the startle response [38], these angles are within the biologically relevant ROM for striped bass. During pilot testing, specimens from all three regions were found to be capable of bending up to 15° without plastic deformation. While some joints were capable of bending to greater angular displacements, 15° displacements were within the elastic range of all the joints, and thus could be compared across all specimens. At angles less than 10°, the moments generated about the IVJ were not greater than 0.001 Nm and therefore we did not focus our analysis of angular stiffness at smaller angles. For each specimen, angular stiffness was calculated as the moment at the specified angle divided by the tangent of the moment–angle curve at the specified angle (in radians) [24].

Finally, to investigate the elastic efficiency of the system, we calculated a hysteresis value for each dynamic test. Hysteresis is defined as the ratio of the energy lost in the system per energy expended during a load–unload cycle. This is equivalent to the difference between the area underneath the load curve and the area underneath the unload curve divided by the area underneath the load curve [39]. Graphically, the result is the area within the load–unload curves (figure 4). To calculate this, we integrated the load and unload curves for a complete cycle, and took the difference between the two values and divided them by the integral of the load curve. Resilience, i.e. the proportional energy recovered by the system, is equal to 1−hysteresis [40].

2.9. Statistical analysis

Regional variation (cervical, abdominal and caudal) of IVJ mechanics was tested using a one-way nested
ANOVA with individual nested within region. We tested the effect of region on the neutral zone range, angular stiffness and hysteresis. Analyses of the various treatments and frequencies were conducted separately. Differences between each pair of regions were then tested post hoc using Tukey HSD multiple comparisons based on least-square means.

The effect of each treatment on IVJ mechanics was tested using a repeated measures ANOVA analysis. For each specimen, the data gathered for each preparation (IT, VC and EC) were treated as a repeated measure. We then compared each pair of treatments with one another post hoc using paired Student’s t-tests at a Bonferroni-corrected significance level of 0.0033.

Finally, the effect of frequency on IVJ mechanics was tested using a repeated measures ANOVA analysis. For each specimen, the data obtained at each frequency were treated as the repeated measure. We tested for differences between each pair of frequencies using paired Student’s t-tests at a Bonferroni-corrected significance level of 0.0033. Tests were performed on the neutral zone, angular stiffness and hysteresis.

All statistical analyses were performed using JMP software (v. 8.0.1, SAS Institute, Cary, NC, USA).

3. RESULTS

The moment–angle curves for IT specimens from the cervical, abdominal and caudal regions all showed long neutral zones and some hysteresis (figure 5a). Qualitatively, cutting the vertical septum did not seem to have much effect, but cutting the encapsulating complex changed the shape and position of the curve (figure 5b). Also, qualitatively, varying the bending frequency from two to five to seven cycles per second did not have a large effect on the curve shape or position, but some quantitative differences are reported below (figure 5c).

3.1. Regional effects on neutral zone, angular stiffness and hysteresis

We tested the effect of vertebral region on the range of the neutral zone and found a significant difference among regions for each treatment and frequency (table 1). At all three treatments and frequencies, the range of the neutral zone was the greatest in the caudal region of the fish (table 1 and figure 6a). For all three treatments, we found that the abdominal region exhibits the lowest neutral zone range followed by the cervical and caudal regions, respectively. The post hoc analysis indicated a significantly larger neutral zone in the caudal IVJs relative to the abdominal and cervical IVJs (table 1, superscripts). While the neutral zone of the cervical joints is consistently larger than that of the abdominal joints, the post hoc analysis indicates that these differences are not statistically significant (p = 0.2004, p = 0.0980, p = 0.4887, for IT, VC and EC, respectively).

We tested the effects of vertebral region on the angular stiffness of the joints at both 10° (table 2) and 15° (table 3) of lateral bending for each treatment at each frequency. All tests performed on IT and VC joints resulted in significant differences between the cervical and abdominal regions and the abdominal and caudal regions, such that the abdominal region emerged as the most mechanically stiff region of the vertebral column (tables 2 and 3; figure 6b at 2 Hz and 15°). For both the IT (black symbols) and VC (blue symbols) joints, the abdominal region is significantly more stiff than the cervical or caudal joints. However, when we cut the encapsulating tissues, the effect of region on mechanical stiffness at both 10° and 15° is no longer significant (tables 2 and 3; figure 6b, red symbols).
Finally, we found no effect of region on hysteresis for any of the treatments and frequencies tested (table 4). Similar values of energy loss in the system were revealed along the length of the vertebral column of approximately 37 per cent for the IT joints, 41 per cent for the VC joints and 53 per cent for the EC joints. This trend is consistent across the three different frequencies tested (table 4 and figure 6c at 2 Hz).

### Table 1. Regional effects on mean neutral zone range (+1 s.d.; degrees).

<table>
<thead>
<tr>
<th>Region</th>
<th>CV</th>
<th>AB</th>
<th>CD</th>
<th>Mean (±1 s.d.)</th>
<th>p-value</th>
<th>p-value</th>
<th>p-value</th>
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</thead>
<tbody>
<tr>
<td>IT 2 Hz</td>
<td>11.9 ± 3.32°</td>
<td>15.2 ± 4.27°</td>
<td>16.4 ± 4.76°</td>
<td>12.7 ± 2.21°</td>
<td>0.00053</td>
<td>0.00047</td>
<td>0.00001</td>
</tr>
<tr>
<td>VC 2 Hz</td>
<td>9.1 ± 1.57°</td>
<td>9.9 ± 1.48°</td>
<td>15.6 ± 1.92°</td>
<td>13.2 ± 1.61°</td>
<td>0.00047</td>
<td>0.00001</td>
<td>0.00001</td>
</tr>
<tr>
<td>EC 2 Hz</td>
<td>21.0 ± 5.82°</td>
<td>18.7 ± 5.15°</td>
<td>28.0 ± 5.57°</td>
<td>26.5 ± 2.92°</td>
<td>0.00001</td>
<td>0.00001</td>
<td>0.00001</td>
</tr>
</tbody>
</table>

3.2. Treatment effects on neutral zone, angular stiffness and hysteresis

There was a significant effect of tissue disruption (i.e. ligament cutting) on the neutral zone at all three frequencies (p = 0.0002, p = 0.0003, p = 0.0005, for 2, 5
Table 2. Regional effects on mean angular stiffness at 10° of lateral bending (± 1 s.d.; Nm rad⁻¹). CV, cervical; AB, abdominal; CD, caudal; IT, intact; VC, vertical septum cut; EC, encapsulating tissues cut. *p*-values are for overall effect of region within separate treatment and frequency tests; bold indicates a significant effect. Superscripts indicate *post hoc* pairwise comparisons among regions. Means with the same superscripts are not significantly different.

<table>
<thead>
<tr>
<th></th>
<th>2 Hz</th>
<th>5 Hz</th>
<th>7 Hz</th>
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<tbody>
<tr>
<td></td>
<td>CV</td>
<td>AB</td>
<td>CD</td>
</tr>
<tr>
<td>IT</td>
<td>0.05 ± 0.01a</td>
<td>0.11 ± 0.03b</td>
<td>0.02 ± 0.006c</td>
</tr>
<tr>
<td>CV</td>
<td>0.05 ± 0.02d</td>
<td>0.13 ± 0.06e</td>
<td>0.02 ± 0.007e</td>
</tr>
<tr>
<td>EC</td>
<td>0.02 ± 0.003</td>
<td>0.01 ± 0.004</td>
<td>0.01 ± 0.004</td>
</tr>
</tbody>
</table>

Table 3. Regional effects on mean angular stiffness at 15° of lateral bending (± 1 s.d.; Nm rad⁻¹). CV, cervical; AB, abdominal; CD, caudal; IT, intact; VC, vertical septum cut; EC, encapsulating tissues cut. *p*-values are for overall effect of region within separate treatment and frequency tests; bold indicates a significant effect. Superscripts indicate *post hoc* pairwise comparisons among regions. Means with the same superscripts are not significantly different.

<table>
<thead>
<tr>
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<th>2 Hz</th>
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<tbody>
<tr>
<td></td>
<td>CV</td>
<td>AB</td>
<td>CD</td>
</tr>
<tr>
<td>IT</td>
<td>0.16 ± 0.07a</td>
<td>0.28 ± 0.075b</td>
<td>0.08 ± 0.036c</td>
</tr>
<tr>
<td>VC</td>
<td>0.13 ± 0.05j</td>
<td>0.25 ± 0.073k</td>
<td>0.06 ± 0.028l</td>
</tr>
<tr>
<td>EC</td>
<td>0.03 ± 0.01</td>
<td>0.02 ± 0.01</td>
<td>0.01 ± 0.001</td>
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Table 4. Regional effects on mean hysteresis (± 1 s.d.; % energy loss). CV, cervical; AB, abdominal; CD, caudal; IT, intact; VC, vertical septum cut; EC, encapsulating tissues cut. *p*-values are for overall effect of region within separate treatment and frequency tests; there were no significant effects of region on hysteresis.

<table>
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<tr>
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<th>2 Hz</th>
<th>5 Hz</th>
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<tr>
<td></td>
<td>CV</td>
<td>AB</td>
<td>CD</td>
</tr>
<tr>
<td>IT</td>
<td>36 ± 6</td>
<td>37 ± 3</td>
<td>38 ± 7</td>
</tr>
<tr>
<td>VC</td>
<td>44 ± 7</td>
<td>41 ± 6</td>
<td>39 ± 5</td>
</tr>
<tr>
<td>EC</td>
<td>57 ± 3</td>
<td>53 ± 9</td>
<td>55 ± 5</td>
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A significant effect of treatment occurred at both 10° (p = 0.0063, p = 0.0015, p = 0.0034, for 2, 5 and 7 Hz, respectively) and 15° (p = 0.0046, p = 0.0036, p = 0.0097, for 2, 5 and 7 Hz, respectively). Post hoc analyses revealed no impact of cutting the vertical septum on angular stiffness at both 10° (p = 0.3533, p = 0.1451, p = 0.1122, for 2, 5 and 7 Hz, respectively) and 15° (p = 0.2248, p = 0.1659, p = 0.2662, for 2, 5 and 7 Hz, respectively). However, the angular stiffness of the joint dropped precipitously when the encapsulating complex was cut at both angles (p < 0.0001 for each frequency at both 10° and 15°; figures 5b and 6b).

Finally, there was a significant effect of treatment on hysteresis at the three frequencies (p = 0.0018, p = 0.0026, p = 0 < 0.0001, for 2, 5 and 7 Hz, respectively). Again, post hoc analyses revealed the greatest differences between the EC joints and the IT joints and between the EC joints and the VC joints (p < 0.0001 for all frequencies and pairs). There are no significant differences between the IT and VC joints (p = 0.0653, p = 0.1322, p = 0.0999, for 2, 5 and 7 Hz, respectively; figures 6c and 7c).

### 3.3. Frequency effects on neutral zone, angular stiffness and hysteresis

We did not find an effect of frequency on the neutral zone of the IT joints, the VC joints or the EC joints (table 5 and figure 7a). This pattern held true for all regions. In addition, there were no interaction effects between frequency, region and treatment (table 5).

For the IT and VC joints, there was no statistically significant effect of frequency on the angular stiffness of the joints at 10° or 15° (table 5 and figure 7b). While there appears to be a slight decline in angular stiffness at 7 Hz, the decrease in stiffness was not significant (figure 7b). Again, this trend was consistent across all regions. In addition, we did not see a significant interaction between frequency, treatment and region (table 5).

At both 10° and 15°, there was an effect of frequency once the encapsulating tissues were cut (table 5). However, post hoc analyses revealed that, at 15°, only the comparison between the 2 and 5 Hz EC tests was significant (p = 0.0006). Given the Bonferroni-corrected significance level, there was no difference between the 2 and 7 Hz EC tests (p = 0.0035) or the 5 and 7 Hz EC tests (p = 0.1944). This is apparent in the similar values of angular stiffness found at 15° across frequencies of the EC joints (figure 7b, red symbols). Additionally, at 10°, the differences between the three frequencies were not significant (p = 0.0214, p = 0.1583 and p = 0.1351, for comparisons between the 2 and 7 Hz, 2 and 5 Hz and 5 and 7 Hz, respectively).

Finally, there was a significant effect of frequency on hysteresis for the IT joints, such that the higher frequencies resulted in lower values of hysteresis (table 5 and figure 7c). For example, in the cervical region, we see the hysteresis values drop to approximately 20 per cent at 7 Hz for the IT treatment (figure 7c, black symbols). This trend was consistent across regions as well. Post hoc analyses on these IT joints showed that the 7 Hz testing repeatedly resulted in lower hysteresis values than either the 5 Hz tests or the 2 Hz tests (p < 0.0001 for all pairs). However, differences between the 2 and 5 Hz tests were not significant (p = 0.0453). In addition, the interactions between variables were not significant (table 5). When the encapsulating complex was cut, the hysteresis values were not significantly different from one another across frequencies (table 5 and figure 7c).
4. DISCUSSION

Contrary to prior results from other bony fish species [22–24], but in agreement with our predictions from morphology [19], striped bass shows a substantial decrease in stiffness and increase in ROM of the vertebral column from cranial to caudal. In agreement with predictions from vertebral morphology and second moment of area of IVJs [19], the caudal region exhibits a lower stiffness than the abdominal region, with a longer neutral zone and a lower angular stiffness at both 10° and 15° of lateral bending. The angular stiffness of the cervical IVJs is also significantly lower than the abdominal IVJs, supporting the recognition of a separate cervical region in M. saxatilis (as proposed by Nowroozi et al. [19]). In addition, the vertical septum does not significantly impact lateral bending mechanics, while the encapsulating complex plays a prominent role. Upon cutting the encapsulating complex, joint stiffness decreases significantly and hysteresis increases significantly.

4.1. The effect of vertebral region on intervertebral joint mechanics in striped bass

In mammalian vertebral columns, the cervical, thoracic and lumbar regions exhibit substantial differences in stiffness and ROM [1–4,11–14,20,41,42]. In striped bass, we also found a substantial variation in the length of the neutral zone and angular stiffness among the cervical, abdominal and caudal regions. Neutral zone results show that the cervical and abdominal joints offer measurable resistance to lateral bending at smaller angular displacements, approximately 10–12°, compared with the 16–18° exhibited by the caudal joints (table 1 and figure 6a). We interpret these results to indicate a greater potential ROM of the caudal joints relative to abdominal and cervical joints. In addition, angular stiffness at 10° and 15° of lateral bending is lower in the caudal than in the abdominal region (tables 2 and 3; figure 6b). The cervical and abdominal joints show similar neutral zone ranges, but, once the IVJ tissues begin to resist bending, the abdominal joints are significantly more difficult to bend. Thus, it takes less force to bend the cervical joints beyond their neutral zones in comparison with the abdominal joints.

In contrast to our findings here that stiffness is lower in the caudal IVJs, previous studies on blue marlin and skipjack tuna found higher angular stiffness in the caudal region, although the tuna did show a compliant zone of bending within the specialized caudal fin complex [22,23]. Further, in contrast to all of these findings of regional variation in stiffness, the Norfolk spot showed uniform stiffness along the length of the fish [23]. Some of this interspecific variation could be related to swimming style. The two species with increased stiffness in the caudal region are thunniform swimmers with relatively specialized musculoskeletal anatomy. However, the Norfolk spot and the striped bass are both sub-carangiform swimmers, yet still show different patterns. It is possible that methodological differences might have contributed to these differences, because the vertebral columns of spot were tested quasi-statically, but we did not find strong effects of bending frequency within the range of frequencies tested here (figure 7).

A few studies have measured regional variation in midline curvature during steady swimming and startle responses in various fishes, and all found the greatest curvature in the caudal region [43–47]. These results are congruent with our finding of greater compliance in the caudal region of striped bass. However, in vivo kinematics from this species is necessary to determine the realized (as opposed to potential) ROM for the three regions and whether larger IVJ deformations occur in the caudal region.

4.2. The vertical septum

In striped bass, we found that cutting the vertical septum did not substantially impact the mechanical properties of the IVJ during pure lateral bending. There is no statistically significant effect of cutting the vertical septum on neutral zone range, angular stiffness at either 10° or 15° or on hysteresis (figure 6). This result contradicts a previous model that suggests the vertical septum plays a role in resisting lateral bending and possibly contributing to elastic energy storage [48]. The trends are always towards slightly lower stiffness and slightly greater hysteresis after the vertical septum was cut, suggesting that there may be a small effect that we could not detect statistically. Since IT tests were always conducted before VC runs, some of the reduced stiffness could also have been induced by degradation of the tissues (figures 5–7).

The morphology of the vertical septum in striped bass shows substantial regional variation, with a complex set of four overlapping fibre populations in the cervical and abdominal regions but just one in the caudal region [19]. It is possible that this variation in
vertical septum morphology may produce regional variation in IVJ stabilization against axial rotation, dorsoventral bending or shear. Further mechanical testing in these other dimensions would be necessary to determine whether the vertical septum plays a role in resisting these other deformations, while offering little or no resistance to lateral bending.

4.3. The encapsulating complex

The significant impact of cutting the encapsulating tissues shown in the present study suggests that these IVJ tissues play a prominent mechanical role in the lateral bending of the IVJs (figures 5b, 6 and 7). The large increase in the range of the neutral zone after cutting the encapsulating tissues (figures 5b, 6a and 7a) and the large drop in angular stiffness at both 10° and 15° (tables 2 and 3; figures 6b and 7b) indicate that these tissues are the primary source of joint stiffness in lateral bending.

Our previous investigation of morphological variation of the IVJ structures of the striped bass [19] revealed a significant decline in the notochordal cell mass within the joint cavity and in the fibrous sheath of the encapsulating complex from cranial to caudal. In the present experiment, once the encapsulating tissues have been cut, the notochordal cell mass and the fluid-filled lacunae within the joint cavity are the primary structures resisting bending. Upon cutting the encapsulating tissues, the differences in angular stiffness across regions disappear (tables 2 and 3; figure 6b). Thus, the results suggest that morphological variation in the encapsulating complex may be important in the mechanical regionalization of the vertebral column. Further, the result indicates that the remaining notochordal cell mass alone does not contribute to the mechanical variation among regions of the vertebral column. However, we cannot rule out the possibility that mechanical interactions between the encapsulating tissues and the notochordal remnants, perhaps behaving together as a hydrostat, may contribute to regional variation in the intact joints.

4.4. Hysteresis and elastic energy storage in intervertebral joints

There is a significant increase in the proportion of cyclical energy loss upon cutting the encapsulating tissues (table 4; figures 6c and 7c), suggesting that any energy storage the joint is capable of appears to lie in the encapsulating complex. This result is not surprising given that the only elastin-containing tissue of the IVJ of many fishes, including the striped bass, is a small tissue sandwiched within the encapsulating complex known as the elastica externa [9,10,19,49]. It is possible that this small tissue and the helically wound collagenous fibrous sheath just deep to it are providing the small degree of resilience that is afforded to the joint.

It is important to note that hysteresis values throughout the experiment do not indicate large degrees of energy storage and energy return. In comparison with highly resilient biomaterials such as collagen (90% resilience), elastin (90% resilience) and resilin (92% resilience) [50], the IVJs of striped bass have a relatively low resilience (60–70% resilience). From this point of view, striped bass IVJs seem to fall between highly resilient materials and materials that are effective in energy dissipation, such as proximal mussel byssal (53% resilience) [50,51] and spider silk (35% resilience) [40]. A potential role of energy dissipation by IVJs may be quite common across vertebrate taxa. When compared with the IVJs of mammals, the resilience of striped bass joints is quite similar to the 75 per cent resilience in monkeys, 69 per cent resilience in wallabies, 67 per cent resilience in tigers and 48 per cent resilience in seals [5]. To accurately distinguish whether energy is dissipated or returned to the system in a swimming fish, we must determine where the in vivo angles of bending occur along the moment–displacement curve for each region.

4.5. Frequency

The effect of frequency on the stiffness of the IVJs of striped bass is unclear. There was no effect of frequency on the range of the neutral zone (table 5 and figure 7a). Similarly, there was no effect of frequency on the angular stiffness of the IT and VC joints at either 10° or 15° (table 5 and figure 7b). This result is similar to that seen in mammalian IVJs [5], but it is still unusual given that we think of these joints as viscoelastic fluid-filled structures [7,9,10]. In a previous study, increased frequency resulted in an increase in joint stiffness in both blacktip sharks and bonnethead sharks [7]. We would expect this increase in angular stiffness as the frequency of bending increases [7,21]; however, once the encapsulating tissues are cut in the present study, there is a significant decrease in angular stiffness between 2 and 5 Hz at 15° (table 5 and figure 7b). This result suggests that the fluids and structures within the IVJ cavity may be imparting shear-thinning properties to the IVJs of striped bass. This negative response in joint stiffness to increased frequency has been shown in the IVJs of blue marlin as well [22]. It is important to note that this result was not significant between 2 and 7 Hz at 15° or for any of the comparisons at 10° of lateral bending. The present study focused on a range of frequencies that included steady swimming at 2 and 5 Hz as well as the lower range of burst swimming at 7 Hz. It is possible that focusing on the lower frequencies relevant to much slower steady swimming and station holding, or the much higher frequencies associated with the startle response, may result in a greater viscoelastic effect. Thus, further investigation is required, possibly including lower and higher frequencies of bending, to confirm that frequency does not impact joint stiffness in striped bass.

Finally, there is an expected decrease in hysteresis with an increase in frequency in both the IT and VC joints (figure 7c, black circles and blue diamonds). This effect is no longer significant once the encapsulating tissues are cut (figure 7c, red squares). This again suggests that the primary tissues involved in energy storage in the system are those of the encapsulating complex.

4.6. Concluding remarks

The results of the present study reveal substantial variation in the stiffness and ROM of the vertebral column.
from cranial to caudal. This regional variation in mechanical stiffness of IVJs can, to some degree, be explained by the morphological variation previously reported [19]. Yet, the potential impact of this variation on whole body stiffness during locomotion is still unclear. It seems likely that, in pure lateral bending, the vertebral column is functioning primarily to allow flexibility and to dissipate energy. The present data, combined with an in vivo investigation of lateral bending angles, will be essential to determining vertebral column function during locomotion. Knowledge of where on the moment–angle curves the in vivo lateral bending angles occur will provide the necessary information to investigate the mechanical and energetic roles of the vertebral column during locomotion.

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