DOES THE NOTOCHORD MATTER?
BENDING MECHANICS OF HAGFISH (*MYXINE GLUTINOSA*)

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The craniate notochord appears to have several important mechanical functions when it bends: during frog embryogenesis, it helps straighten the bent embryo (Adams, D.S. et al., *Development* 110: 115-130, 1990); during steady swimming in adult sturgeon, it may resist bending and transmit forces (Long, J.H., Jr. *Env. Biol. Fishes* 44: 199-211, 1995). The magnitude of the force for straightening, resisting, or transmitting is determined by the notochord's bending stiffness — the ratio of the bending moment (Nm) required to cause a given amount of bending (measured as °, radians, strain, or curvature). While dynamic bending tests have yet to be conducted on the notochord of hagfish, *Myxine glutinosa*, quasi-static tests show that tensile stiffness of the hagfish notochord is determined, in part, by the physiochemical properties of the macromolecules and the resulting ionic conditions in its core (Kielstein, J.T. et al., *Bulletin MDIBL* 35: 105-107, 1996). The notochordal core is composed of vacuolated cells (Tretjakoff, D. *Z. f. Zellforschung u. mikr. Anatomie* 4: 266-311, 1924) that generate a hydrostatic pressure in sturgeon (Long, J.H., Jr. *Env. Biol. Fishes* 44: 199-211, 1995), and presumably hagfish, by swelling against the encircling fibrous sheath (Koob, T.J. et al., *Bulletin MDIBL* 33: 5-8, 1994). Given the notochord's biochemical complexity and potential mechanical function in swimming, it is surprising that little is known about the bending properties of the notochord *relative* to those of the intact body. Is the notochord stiff enough to play a mechanical role in bending of the whole body? To address this question, we measured, over a range of bending frequencies (Hz) and amplitudes (°), the flexural stiffness (Nm^2) and the net work to bend per unit curvature (Jm) of an intact body section compared to that of an isolated notochord. Our preliminary results, reported herein, suggest that the notochord influences total body flexibility and the mechanical work to bend the body.

Since the stiffness of vertebrate axial structures varies with strain and strain rate (Long, J.H., Jr. *J. exp. Biol.* 162: 131-155, 1992; Long, J.H., Jr. et al., *J. exp. Biol.* 199: 2139-2151, 1996), a dynamic analysis is required in order to accurately model swimming mechanics (Long & Nipper, *Am. Zool.* 36(6): 678-694, 1996). To this end, we used the Newtonian equation of motion for a single-degree-of-freedom system (Den Hartog, J.P. *Mechanical Vibrations*. 4th edn. McGraw-Hill, 1956) to calculate, at each bending frequency and amplitude, the dynamic angular stiffness, k ($Nm\ rad^{-1}$), which is the proportionality constant between an applied external bending moment, M (Nm) and the resulting angular displacement, θ (radians or °), at the time when angular velocity is zero. The calculations, measurements, and bending machine are described in detail elsewhere (Long, J.H., Jr. *J. exp. Biol.* 162: 131-155, 1992; Long, J.H., Jr. et al. *J. exp. Biol.* 200 (1): 65-81, 1997). In order to measure the net work, W (J), required to bend the test section over one complete cycle, the phase lag, δ (radians or °), between the bending moment and the angular displacement was measured. W is the product of π, the maximum M, the maximum θ, and sin δ (Den Hartog, J.P. *Mechanical Vibrations.* 4th edn. McGraw-Hill, 1956). To normalize both k and W for the differences in size between the notochord and body, we calculated the flexural stiffness, EI (Nm^2) the product of k and L, and the work per unit curvature, W' (Jm, where curvature is in units of m^{-1} and is inversely proportional to L), the product of W and L, where L is the length (m) of the test section (0.0042 m for the isolated notochord and 0.010 m for the intact body). The isolated notochord and intact body section were bent at frequencies (0.5, 1.0, 2.0, 3.0 Hz) and amplitudes, θ ($\pm$ 7.2 and 9.4 °) that preliminary video analysis of swimming hagfish showed were within the physiological range.

Live, wild-caught hagfish (*Myxine glutinosa*) were purchased from Huntsman Marine Laboratory in New Brunswick, Canada, and were kept for two weeks in running seawater at temperatures varying from 13 to 15° C. Two individuals were used: (1) one of 37.7 cm total length and 39.2 g total weight for the intact body test; (2) one of 42.5 cm total length and 64.5 g total weight for the isolated noto-chord test. Please note that we did not use isolated notochords from specimens used in the intact bending tests in order to reduce artifacts caused by the deterioration of the tissues after death. Prior to testing, hagfish were killed with an overdose of propylene phenoxetal. Notochords were iso-lated from the excised vertical septum, which held the notochord medially. The ends of the 33.5 cm long isolated notochord were ligated with 00-silk suture. The section of the body and the sec-tion of the isolated notochord bent were at 37% and 38% total length from the tip of the rostrum, respectively. Once mounted in the bending machine, and during the 30 minutes required for test-ing, the body or isolated notochord was bathed in a 13 to 16° C hagfish Ringer solution (Riegel, J.A. *J. exp. Biol.* 73: 261-277, 1978).

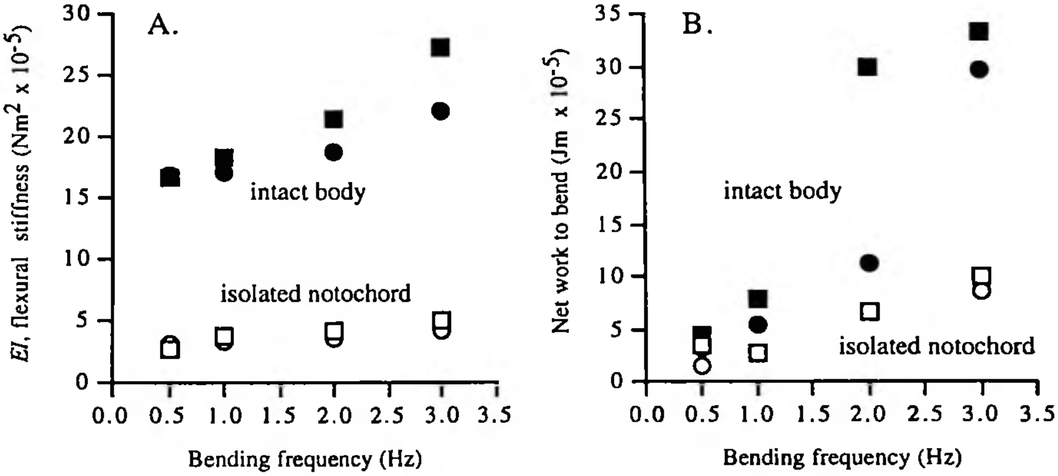

Figure 1. Dynamic mechanical properties of the body (filled symbols) and the isolated notochord (open symbols). Squares and circles indicate a bending amplitude of ± 9.4° and 7.2°, respectively.

While one should interpret these results cautiously at this preliminary stage, the result of bending one isolated notochord and one intact body section show clearly that the flexural stiffness is greater in the intact body (Fig. 1A). This is not surprising since *EI*, while independent of section length, is dependent on the radius to the fourth power in structures with circular cross-sections; that radius is much larger in the intact body than in the notochord. Both the body and the notochord increase in *EI* with increasing bending frequency; both also increase with increasing bending amplitude. Thus, we predict that the body of a hagfish will become stiffer as it increases its tailbeat frequency or bending amplitude, both of which are modulated in order to change swimming speed and to maneuver in other species Bainbridge, R. *J. exp. Biol.* 35: 109-133, 1958; Hunter, J.R. & Zweifel, J.R. *Fish. Bull.* 69: 253-266, 1971). The notochord accounts for ap-proximately 20% of the total passive (non-muscular) body stiffness (Fig. 2) at all frequencies and amplitudes. Thus, we predict that the notochord plays a significant role in determining the bending stiffness of the body during undulatory swimming.

Given the large differences in the flexural stiffness of the body and the notochord, it is surprising to see that at low bending frequencies the values of curvature-specific work to bend the body and the notochord are almost equal (Fig. 1B). This suggests that the muscles and skin must be highly elastic, storing and releasing mechanical work as strain energy without dissipating much as heat. This result puts the notochord in the curious position of functioning as an energy sink during dynamic bending, requiring up to 75% of the total net work to bend the body over a complete tailbeat cycle (Fig. 2). Thus the notochord, relative to the body, behaves mechanically as a viscous damper. What are the functional consequences of this mechanical behavior?

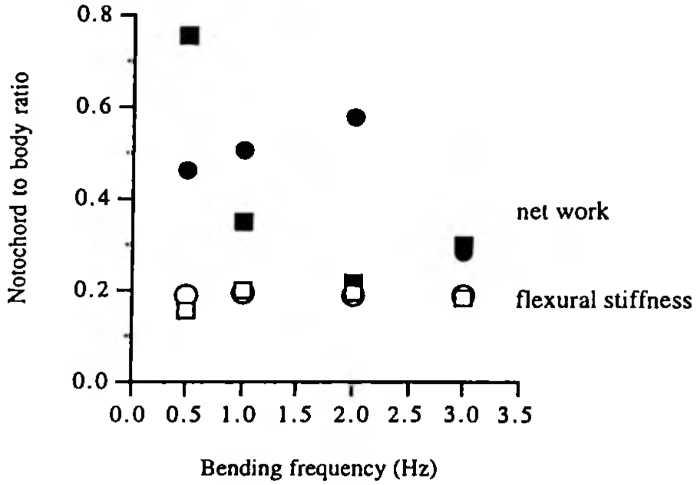

Figure 2. The relative influence of the notochord on the bending mechanics of the intact body, as indicated by the notochord/body ratio of bending work (filled symbols) and *EI*, flexural stiffness (open symbols). Squares and circles indicate a bending amplitude of ± 9.4° and 7.2°, respectively.

Dampening is a mechanical behavior that is important in vibrating structures, helping to prevent break-away oscillations as the bending or driving frequency approaches the system's natural frequency of oscillation (Den Hartog, J.P. *Mechanical Vibrations.* 4th edn. McGraw-Hill, 1956). Thus we predict that the hagfish's notochord functions as a vibration dampener during swimming, stabilizing the body's bending and permitting smooth control and coordination of the large-amplitude curvatures that characterize the swimming and feeding of this species.

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