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Ontogenetic changes in the structural stiffness of the tailstock of bottlenose dolphins (*Tursiops truncatus*)

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SUMMARY

Late-term fetal bottlenose dolphins (*Tursiops truncatus*) are bent ventrolaterally *en utero*, requiring extreme flexibility of the axial skeleton and associated soft tissues. At birth, neonatal dolphins must immediately swim to the surface to breath, yet the dorsoventral oscillations used during locomotion may be compromised by the lateral flexibility evident in the fetus. The unique fetal position of dolphins, coupled with their need to swim at birth, places conflicting mechanical demands on the tailstock. Our previous research demonstrated that neonatal dolphins possess laterally placed, axial muscles that are functionally specialized to actively maintain the straightened posture of the tailstock. Here, we investigated the development of passive lateral stability in the tailstock of bottlenose dolphins by performing whole-body bending tests on an ontogenetic series of stranded dolphin specimens (N=15), including fetuses, neonates and juveniles (total length 58–171 cm). Structural stiffness increased, while overall body curvature decreased, with increasing body length. Scaling analyses suggest that increased structural stiffness is due to increases in size and probably changes in the passive material properties of the tailstock through ontogeny. The neutral zone was approximately constant with increasing size, while the relative neutral zone (neutral zone/total length) decreased. The lateral stability of the tailstock appears to be controlled by a combination of active and passive systems and the role of these systems varies through ontogeny. While neonates use active, muscular mechanisms to limit lateral deformations of the tailstock, the stability of the maturing tailstock is due primarily to its passive tissue properties.

Key words: cetacean, dolphin, backbone, structural stiffness, ontogeny, mechanics, *Tursiops truncatus*.

INTRODUCTION

Late-term fetal bottlenose dolphins (*Tursiops truncatus*) exhibit extreme levels of lateral flexion prior to birth (Fig. 1). *En utero*, the fetus is bent laterally and ventrally such that the tail flukes are juxtaposed to the throat region (Slijper, 1966; Cockcroft and Ross, 1990). Evidence of this extreme posture can be seen in the fetal folds (Fig. 1), which can persist for several weeks following birth (Cockcroft and Ross, 1990). The folded posture may be related to the extreme length of the fetus, which can be fully half the length of the mother (Mead and Potter, 1990). Immediately following birth, the neonatal dolphin must swim to the surface to breathe using dorsoventral oscillations of its tailstock. Thus, the functional demands on the tailstock change dramatically as the dolphin transitions from its laterally bent posture *en utero* to a freely swimming animal.

Although neonatal bottlenose dolphins are precocial locomotors (reviewed in Dearolf et al., 2000), they are initially rather uncoordinated swimmers, only gaining swimming competency a few weeks following birth (Cockcroft and Ross, 1990; Noren et al., 2006). This lack of coordination may be associated with the neonate's early inability to stabilize lateral deformations of the tailstock.

Histological, biochemical and morphological data suggest that neonatal dolphins actively limit lateral deformations, thus increasing lateral stability (Etnier et al., 2004). In neonates, the laterally positioned m. intertransversarius caudae dorsalis (ICD) has a significantly higher proportion of slow twitch muscle fibers, elevated myoglobin concentrations, and a relatively large cross-sectional area compared with other axial locomotor muscles. As a dolphin matures, the proportion of slow twitch fibers in the ICD decreases, its muscle fiber profile becomes similar to other locomotor muscles, and its relative cross-sectional area becomes smaller. These results suggest that the laterally positioned ICD functions in a postural role to actively control lateral deformations in neonates, but is unlikely to play this role in adult dolphins (Etnier et al., 2004). We hypothesized that older dolphins may use passive mechanisms (i.e. not associated with muscular contractions but, rather, due to the underlying morphology and mechanical behavior of the backbone and connective tissues) to stabilize lateral deformations and that these passive mechanisms may vary throughout ontogeny.

In this study, we investigated the passive mechanical properties of the caudal tailstock in an ontogenetic series of bottlenose dolphins, including fetuses, neonates and juveniles. The tailstock includes the terminal lumbar and caudal vertebrae, axial locomotor muscles and tendons, subdermal connective tissue sheath and blubber (Pabst, 1990; Rommel, 1990; Pabst, 1993; Pabst, 1996; Pabst et al., 1999; Pabst, 2000; Dearolf et al., 2000; Etnier et al., 2004). Previous studies on common dolphins (*Delphinus delphis*) have shown that the stiffness of the isolated axial skeleton differs in dorsal and ventral bending, and also exhibits regional differences along its length (Long et al., 1997). Studies on other aquatic vertebrates have demonstrated the importance of the integrated

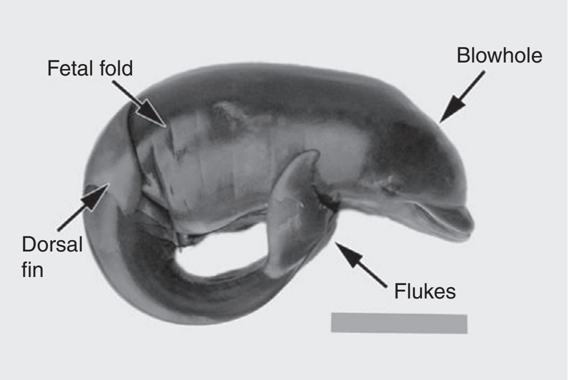

Fig. 1. *En utero* position of a fetal dolphin (PTM 114f, total length 81 cm). The dorsal fin and tail flukes are still floppy, with the flukes juxtaposed to the chin. Note the presence of prominent fetal folds. The scale bar is 15.8 cm in length.

contributions of multiple tissues to the overall mechanical properties of locomotor structures (Long et al., 1996). Thus, we examined the mechanics of the intact caudal tailstock with respect to both regional and directional bending in an ontogenetic series of dolphins. Specifically, we tested the hypothesis that tailstock stiffness increases through ontogeny as a passive mechanism to stabilize lateral deformations.

In this paper, we modeled the caudal tailstock of a dolphin as a bending beam, thus the deformations we are concerned with are the deflections of the tailstock from the central axis. Using terms borrowed from engineering practice, flexural stiffness quantifies the resistance of a structure to deformation under a given load (Roark, 1943). Flexural stiffness is a composite variable whose magnitude is influenced by both material and structural properties (Wainwright et al., 1976). The material properties are described by the elastic modulus (E in $\mathrm{N\,m^{-2}}$), while the structural properties are described by the second moment of area (I in m^4). The second moment of area reflects the geometry of a cross-section of the beam and its value is proportional to the radius to the fourth power, thus radius is a very strong determinant of stiffness (Roark, 1943; Wainwright et al., 1976; Vogel, 1988). While biological beams are commonly modeled using engineering beam theory (Gàl, 1993; Long et al., 1997; Etnier and Vogel, 2000), many of the assumptions of classical beam theory are violated (Roark, 1943). These include, but are not limited to, the requirement that the beam does not vary in size or shape along its length and that it is composed of a single material. Thus, we will refer to measured values of EI as structural stiffness, to delineate our reported values from true engineering stiffness.

Because structural stiffness is a composite variable, the stiffness of a beam can be altered by changing either its material makeup or the distribution of that material. We used a simple scaling analysis to determine how these two factors may be influencing the passive stiffness of the developing dolphin tailstock. If the dolphin grows isometrically, then the radius (r in m) of the tailstock is proportional to body length (total length, TL in m). Therefore:

$$R \propto \mathrm{TL}\,. \qquad (1)$$

Since I is proportional to radius to the fourth power, a simple substitution yields:

$$I \propto \mathrm{TL}^4\,. \qquad (2)$$

Thus, if the material properties remain constant with growth, structural stiffness will be proportional to body length to the fourth power. If structural stiffness increases more rapidly than predicted by this geometric model then we can conclude that the material properties of the tailstock are changing throughout ontogeny. Note that this simple model assumes an average material property for the entire tailstock, rather than considering the properties of individual elements. Additionally, this model assumes that the relative positions of these elements remain constant through ontogeny.

For deflections of the tailstock that are less than 10% of total body length, stiffness can be calculated using the basic formula for end-loaded cantilever beams:

$$EI = \frac{Fl^3}{3y}\,, \qquad (3)$$

where F is the force applied (in N), l is the length of the beam (in m) and y is the deformation (in m) of the tip of the beam (Roark, 1943). Eqn 3 is limited to beams that deflect no more than 10% of their total length. For larger deflections, the stiffness of a beam can be described using a proportionality constant. The stiffness constant (k in $\mathrm{Nm^2}$) is the instantaneous slope of the line relating the applied moment (M in Nm) to the resulting curvature (C in $\mathrm{m^{-1}}$) of the dolphin tailstock (Wainwright et al., 1976), where curvature is the inverse of the radius of curvature. Thus:

$$k = \frac{\mathrm{d}M}{\mathrm{d}C}\,. \qquad (4)$$

Using Eqns 3 and 4, we quantitatively describe tailstock stiffness for both small and large deflections in an ontogenetic series of dolphin carcasses salvaged along the North Carolina and Virginia coasts. We also used a scaling analysis to determine whether ontogenetic changes in stiffness are due to changes in material and/or structural properties.

MATERIALS AND METHODS

Samples

Stranded bottlenose dolphin carcasses (*Tursiops truncatus*, Montagu 1821) were collected in collaboration with the Northeast and Southeast Regional Stranding Network, and under a Letter of Authorization to UNCW from the National Marine Fisheries Service (Table 1). All animals were determined to be in good body condition based upon both external and internal examinations (Cox et al., 1998; McLellan et al., 2002). Although the exact time of death could only be determined for one euthanized animal, all carcasses were fresh (Smithsonian Institution Code 1 or 2) (Geraci and Lounsbury, 1993)

Table 1. Specimens of bottlenose dolphins (*Tursiops truncatus*) used in this study

Identification number	Total length (cm)	Sex	Life-history stage
PTM 109f	58	F	Fetus
WAM 560f	63.5	F	Fetus
ASF 033f	82	M	Fetus
WJW 007f	85.5	M	Fetus
WAM 545f	92	F	Fetus
EMM 010	106	M	Neonate
VMSM20011080	110	M	Neonate
MMB 003	111	M	Neonate
VMSM20021042	112	M	Neonate
WAM 584	117	F	Neonate
VMSM20011087	130	M	Juvenile
ASF 042	146	F	Juvenile
VMSM20021089	148	M	Juvenile
VMSM20021085	157	F	Juvenile
DAP 034	171	F	Juvenile

and none were in *rigor mortis* upon retrieval or during preliminary examination. To avoid potential complications with the onset of *rigor mortis*, all carcasses were subsequently frozen intact and remained frozen for up to several months before thawing and testing. Previous researchers have shown that the mechanical properties of human intervertebral joints (Smeathers and Joanes, 1988) and tendinous connective tissues (Boon-ho and Siaw-Meng, 2003) are not affected by freezing. Specimens were placed into three life-history stages: fetuses (*N*=5), neonates (*N*=5) and juveniles (*N*=5). Fetuses were collected *en utero* and ranged from 58 to 92 cm total length (TL). Neonates and juveniles were classified based on a suite of characters, as defined by Dearolf and colleagues (Dearolf et al., 2000) and elaborated by Struntz and colleagues (Struntz et al., 2004). All neonates had floppy dorsal fins and tail flukes, and ranged between 106 and 117 cm TL, while juveniles ranged between 130 and 171 cm TL. We chose to focus on these particular life history stages because the mechanical changes in question presumably occur during the first year of life. In addition, juvenile bottlenose dolphins exhibit muscle morphology, histology and locomotor behaviors very similar to those of adults (Cockroft and Ross, 1990; Dearolf et al., 2000).

Mechanical tests

Mechanical values were measured on freshly thawed dolphin carcasses by applying a load to the caudal tailstock and measuring the resultant deformation. We designed an apparatus consisting of a sheet of standard plywood reinforced with wooden joists (Fig. 2A). The plywood was marked with scale bars at multiple sites and the central axis of the apparatus was marked with a thick dark line for orienting the animal. Narrow slits were cut into the plywood to allow through passage of tie-downs. On either side of the plywood, laterally placed joists (35 mm×85 mm×2.4 m) supported long metal guides [Harken 5 ft MidRange travelers salvaged from a Hoby Cat catamaran (Harken, Pewaukee, WI, USA)]. Swivel joints (Harken Traveler Cars) were fitted into these tracts, which were lubricated with Aquaseal silicon spray (McNett, Bellingham, WA, USA). Prior to testing, the surface between the apparatus and the specimen was coated with Omni ultrasound transmission gel (iMed.com, New York, NY, USA) to reduce frictional forces.

To measure lateral deformation, specimens were placed ventrally supine on the apparatus, with their mid-sagittal axis aligned along the center of the plywood (Fig. 2B). Two ratchet tie-downs positioned just cranial and caudal to the pectoral flippers were used to immobilize the thorax of the animal. We chose to grip the thoracic region of the animal because previous studies have shown that deformations occur predominantly caudal to the dorsal fin in freely swimming dolphins (Fish, 1993; Pabst, 1993). The central axis of the animal, as well as the position of the center of the dorsal fin, the anus and the midpoint between anus and fluke insertion, was marked with zinc oxide ointment. These marks were used to reliably locate these positions in digital images. A rope was tied around the fluke insertion and then passed through the swivel joint running in the laterally positioned metal track. The rope was connected in series with a 20 kg Pesola spring scale (Forestry Suppliers, Jackson, MI, USA) held manually by a researcher. The scale had a resolution of 0.2 kg. The precision of the scale, based on five repeated measures of a known load, was 1% with a coefficient of variation of less than 0.5. Prior to testing, the caudal tailstock was deformed three times to both the left and right to eliminate any directional bias. The direction (left or right) of the first test was determined randomly with a coin toss.

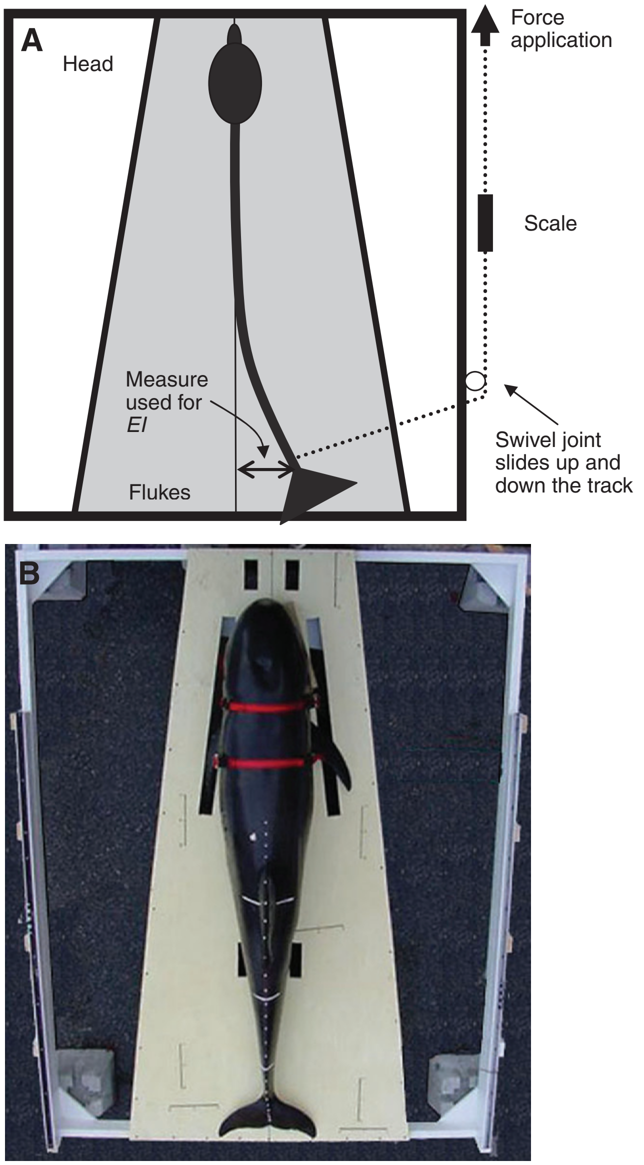

Fig. 2. (A) Schematic drawing of the bending apparatus used for determining structural stiffness (*EI*, Nm^2). The thick gray line represents the central axis of the animal and the triangle at the bottom of the image represents the flukes. (B) Actual image of the bending apparatus as seen from above. The specimen shown here is a *Grampus griseus* (WAM 570, total length 215 cm), positioned for lateral deformations. The animal is immobilized with two ratchet tie-downs cranial and caudal to the pectoral fin. The central axis of the animal is marked with zinc oxide dots, while the center of the dorsal fin, the anus and the mid-peduncle are marked with horizontal white lines.

In practice, a load was applied at the fluke insertion, causing the caudal tailstock to deform from the center line of the apparatus. The neutral zone was operationally defined as the amount of deformation permissible with no measurable applied load. Loads were applied manually, over approximately 3 s, and then that position was held while a digital image was taken to record the deformation of the specimen. Digital images were taken from a height of approximately 2 m above the testing apparatus. These digital images were used for subsequent analyses, as described below. Following the initial deformation, two to three greater loads were applied, with additional images taken for each load. Thus, loads were applied incrementally, ranging from 0.1 to 5 kg, depending on the size of the specimen. The test was concluded when an increased load caused no further deformation or caused unnatural distortions of the animal. Because the load was applied through a moveable swivel joint mounted on the lubricated metal guide, the load remained perpendicular to the axis of the animal during deformation.

After completing lateral deformation tests, the right fluke blade was removed just lateral to the mid-sagittal plane such that the vertebral elements remained intact. While previous work has shown that movements of the tailstock passively affect the shape of the flukes (Fish et al., 2006), there is no evidence documenting that movements (or removal) of the fluke influence the shape of the tailstock. The animal was placed on its left side and positioned longitudinally such that its rostrum and the caudal vertebrae of the tailstock were on the center line of the apparatus. Because of the natural curvature of the mammalian spine, the thoracic vertebrae tended to be positioned dorsal to this line. Dorsal and ventral deformations were tested as described above for lateral deformations.

Mechanical analyses

Digital images of specimens subjected to a series of applied loads were analyzed using Image Pro Plus imaging software (Media Cybernetics, Silver Spring, MD, USA). Using the zinc oxide markers applied during testing, we established nine positions, numbered 0–8, on the central axis of each animal. These positions were (0) halfway between the caudal tie-down and the leading edge of the dorsal fin (mid-thoracic region), (1) leading edge of the dorsal fin, (2) middle of the dorsal fin, (4) anus, (6) halfway between anus and fluke insertion, and (8) fluke insertion. Positions 3, 5 and 7 were halfway between the two neighboring points. Positions 2, 4, 6 and 8 on the central axis of the animal were used to create line drawings (Microsoft Powerpoint) illustrating the deformation of the axial skeleton under different loads (Fig. 3). These line drawings allowed qualitative comparisons to be made between different loading regimes and different animals.

The shape of the entire tailstock can be described quantitatively using curvature (C in m^{-1}), which is the inverse of the radius of curvature. A high curvature value corresponds to a tightly bowed shape. In contrast, a straight line would have a curvature approaching zero. Curvature for the caudal tailstock at maximum deformation was calculated with Image Pro Plus software based on five defined points along the length of the animal.

Overall curvature, which describes the shape of the entire caudal tailstock, was calculated using positions 2, 4, 6, 7 and 8, as defined above. Reported curvature values are means based on three repeated measurements. To test our procedure, seven circles with radii ranging from 15 to 100 cm (which approximates observed values) were positioned on the testing apparatus, photographed and measured following this same procedure. The coefficient of variation was 4.6 for all calibration circles.

Overall curvature provides a measure for the shape of the entire tailstock. Our observations suggested that the tailstock of a dolphin could deform in a smooth, arc-shape (so that the curvature would be similar along the entire length), or could be bent tightly at one specific point while remaining relatively straight elsewhere. To differentiate between these two patterns, we examined regional curvature at seven points along the length of the tailstock, using the same technique as above. In this case, the points used to establish curvature were equally spaced around the region of interest. For example, to calculate regional curvature centered about the anus, we chose five points evenly spaced between positions 3 and 5. Thus, our regional curvature measurements allowed us to more precisely describe where and how tailstock deformation occurred.

The final descriptive measure of deformation in the tailstock was the angle of deformation, which describes the position of the fluke insertion relative to the long axis of the animal when loaded maximally. Thus, this measure doesn't describe the shape of the tailstock; rather, it is a measure of how far the flukes shift from the central axis of the animal during bending. Image Pro Plus was used to draw a straight line between the point marking the center of the dorsal fin and the fluke insertion. The angle formed between this drawn line and the central axis of the apparatus (which marks the central axis of the animal in its unloaded position) is the angle of deformation. Thus, an animal in the neutral position has an angle of 0 deg., while an animal with its flukes at its throat would have an angle approaching 180 deg.

Allometric growth equations were used to examine the relationship between total body length and the height and width of the tailstock. Equations of the form $\log Y=\alpha+\beta\log X$ were generated, where Y was the height or width of the caudal peduncle, α was the y-intercept and β was the slope of the line fitted to the data (Reiss, 1989; Schmidt-Nielsen, 1984). If we detected a significant relationship between total length and the tailstock parameters, slopes were tested to determine whether they were significantly different from 1.0. If the slope was not significantly different from 1, then the tailstock was presumed to grow isometrically. The tailstock parameters used in this analysis were the height and width of the tailstock at the anus. These values were obtained from digital images using Image Pro Plus software.

We calculated structural stiffness with Eqn 3 using F as the force applied, l as the distance from the restraining straps to the point of load application, and y as the deformation of the fluke insertion as measured perpendicularly from the original position. We calculated the mean for bending to both the left and the right, resulting in a single value for lateral stiffness. Results from all tests in which the animal deformed less than 10% of total body length were averaged for calculation of structural stiffness. For fetuses and neonates, this typically only occurred at the smallest loads, thus reported values represent a single data point in many cases.

For larger deflections, we calculated the stiffness constant (k) using Eqn 4. The moment was determined by multiplying the applied force by the moment arm, which was defined as the distance between the fluke insertion (where load was applied) and the dorsal fin, and was in a line perpendicular to force application. The stiffness constant is the instantaneous slope of the line relating the calculated moment to the resulting overall curvature.

The smallest three fetuses posed a challenge for both methods of assessing tailstock stiffness. These animals were so flexible that they could be moved into an extreme folded position (see Fig. 1) with no measurable load recorded on our scale (which had a resolution of 0.2 kg). Fetal deformations thus easily exceeded the 10% criterion required for EI calculations, with angles of deformation as high as 176 deg. in the lateral direction, 143 deg. in the ventral direction and 43 deg. in the dorsal direction. In addition, because we could not measure multiple points as additional forces were applied, we could not calculate the stiffness constant, k. To provide comparative information for these smallest of fetuses, we assumed a standard load of 0.1 kg (0.98 N) and report only structural stiffness, acknowledging that these values should be considered estimates.

The neutral zone is the deformation that occurs with no measurable applied load. Low friction joints tend to have a large neutral zone (Gàl, 1993). To determine the neutral zone, the

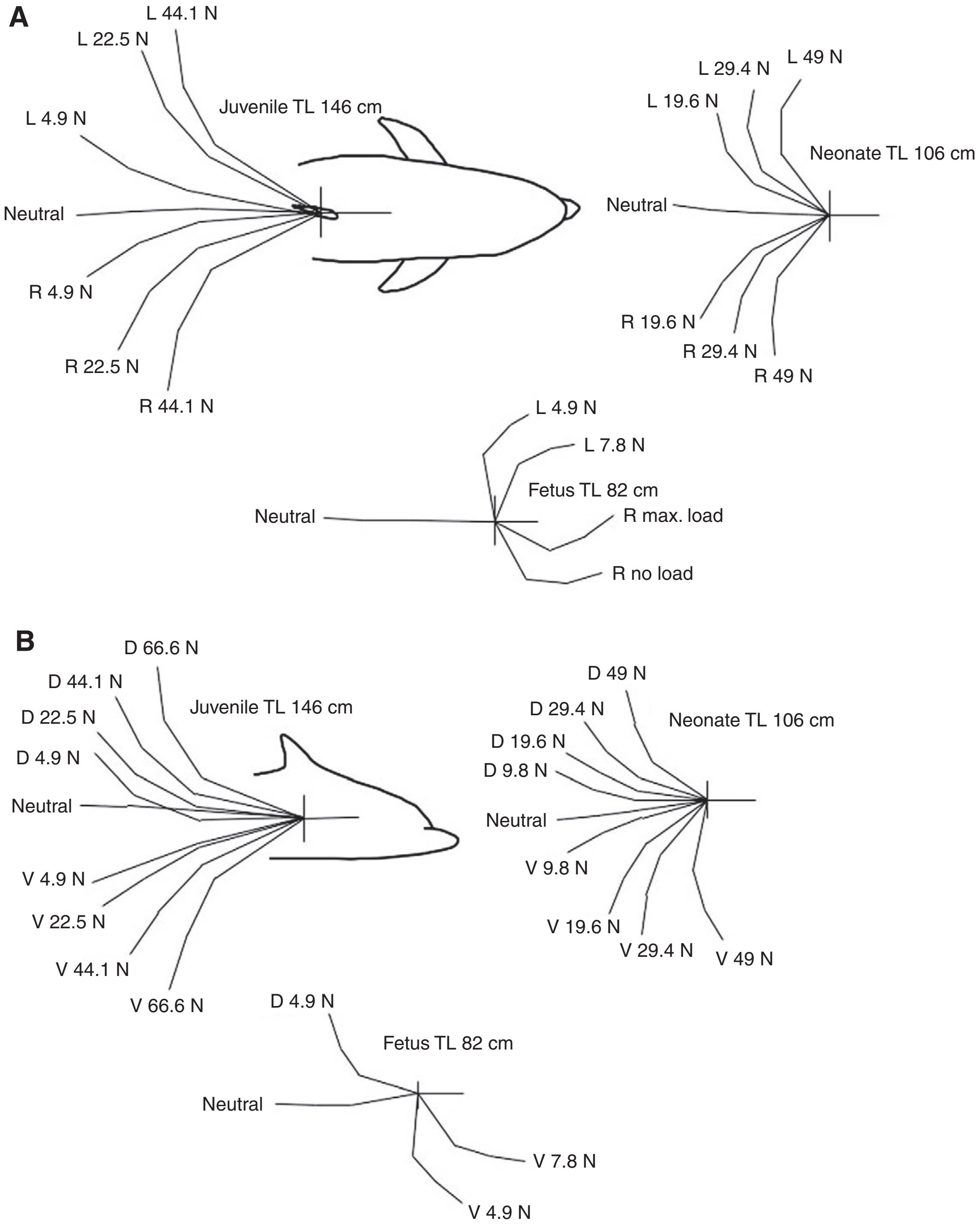

Fig. 3. Representative diagrams showing dorsoventral and lateral deformations of the caudal tailstock of one individual from each of the different life-history stages under various loads. The vertical line represents the center of the dorsal fin and the head is pointing to the right in all images. The lines showing deformation of the tailstock depict the central axis of the animal, as determined by positions 2, 4, 6 and 8 as described in Materials and methods. The direction and applied load are listed next to the resultant deformation, while the neutral line shows the posture of the animal with no applied load. (A) Dorsal view of lateral deformation for loads ranging from 0 to 49 N. In the fetus, bending to the right occurred freely, with no applied load (R no load), while maximum deformation (R max. load) occurred with the application of a load too small to be quantified by our apparatus. (B) Lateral view of dorsoventral deformations for loads ranging from 0 to 66.6 N. Note that the images are not to scale. L, left; R, right; D, dorsal; V, ventral; TL, total body length (cm).

researcher would pull on the spring scale, causing the tailstock to deform. The researcher would continue this process until a measurable force could be recorded on the scale. The neutral zone was measured as the straight line distance from the fluke insertion to the central axis of the apparatus. Dorsal and ventral neutral zones were added together to determine the dorsoventral neutral zone, while the lateral neutral zone was the sum of values for deformations to the left and right. In addition, we also calculated the relative neutral zone (neutral zone/total length), which normalizes the neutral zone with respect to the size of the individual.

Table 2. Position of maximal regional curvature

Life-history stage	Dorsal bending	Lateral bending	Ventral bending
Fetus	4.3	4.1	4.4
Neonate	4.2	5.3	4.2
Juvenile	5.5	6.1	4.0

The mean position of maximal regional curvature in different life-history stages of dolphins. Position 4 represents the anus, position 6 is halfway between the anus and fluke insertion, and position 8 represents the fluke insertion.

Statistical analyses

All statistical analyses were calculated using SAS version 8.2 (SAS Institute, Cary, NC, USA) statistical software. Statistical differences with respect to length and direction of deformation were analyzed using a general linear analysis of covariance (ANCOVA), with direction as a qualitative factor and length as a quantitative factor. Structural stiffness and the stiffness constant were log-transformed for this analysis. Linear regression was used to investigate the relationship between log-transformed measurements of tailstock height and width and total body length.

RESULTS

The results of the experiments can be described both qualitatively and quantitatively. As loads were applied, the animal deformed according to the passive properties of the tailstock. In many cases, the deformation achieved experimentally greatly exceeded that seen in nature, thus these results are over-estimates of deformations expected in a living, actively moving dolphin.

The line drawings illustrate deformation patterns for fetal, neonatal and juvenile dolphins in both lateral and dorsoventral directions (Fig. 3A,B). Note that the amount of load needed to achieve maximum deformation differed greatly across age classes. When deformed laterally, both juvenile and neonatal dolphins exhibited rather symmetric deformations (Fig. 3A). In contrast, fetal dolphins showed marked ‘handedness’, in which the animal deformed farther in one direction than another. Dorsal and ventral deformations were asymmetric in all life-history stages, although the largest differences were still evident in fetal dolphins (Fig. 3B).

Log-curvature at maximum deformation decreased significantly with increasing length ($F_{1,39}$=206.27, P<0.0001, R^2=0.845), indicating that the tailstock of longer individuals remained relatively straight compared with the tailstock of a fetus (Fig. 4). Curvature also varied significantly with respect to the direction of bending, with significantly lower curvature values for bending in the ventral direction ($F_{2,39}$=8.99, P<0.0006). Note that this overall curvature describes the shape of the entire caudal tailstock, and thus is an estimate of overall shape.

Regional curvature values provided a more detailed examination of curvature along the length of the tailstock (Table 2). Regardless of life-history stage or direction of bending, the position of maximum curvature was always at or caudal to the anus. In the ventral direction, maximum curvature was always at the anus (position 4). In both lateral and dorsal bending, the position of maximum curvature moved caudally during ontogeny. Fetuses always bent maximally at the anus, while maximal curvature occurred in the caudal tailstock in juveniles.

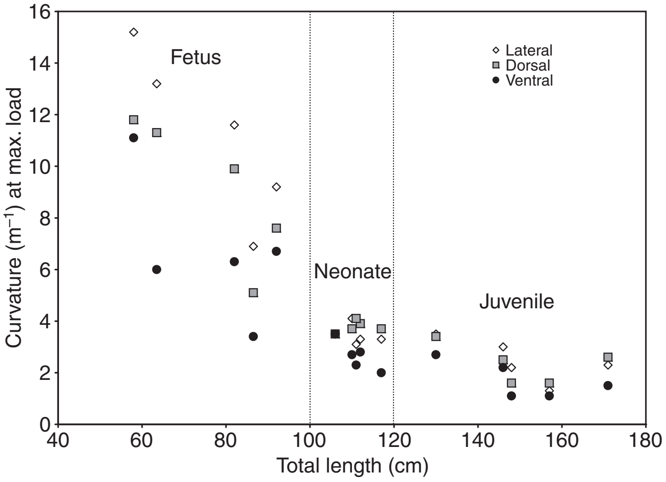

Fig. 4. Overall curvature (m^{-1}) for an ontogenetic series of dolphins when deformed with a maximal load. Values are shown for deformation in the lateral (mean value for both left and right), dorsal and ventral directions. The maximal load is the point at which any increased load resulted in no further deformation or caused unnatural distortion of the body. Vertical dotted lines distinguish different life-history stages.

The relationship between angle of deformation and total body length was investigated by ANCOVA with length and direction of deformation as factors. The angle of deformation at maximum load did not change significantly with respect to increasing size in the dorsal direction (t=0.00, P=0.9982). In contrast, both lateral and ventral angles of deformation decreased significantly with increasing size (t=–5.63, P<0.0001, t=–3.60, P<0.0009, respectively). Table 3 summarizes the average deformation values for different life-history stages.

For deformations less than 10% of total body length, structural stiffness increased significantly (Fig. 5A) with increasing body length ($F_{1,39}$=215.59, P<0.0001, R^2=0.847). Stiffness values for the smallest fetuses were near zero, reflecting the fact that they could be deformed without any measurable applied load. We did not detect a significant difference in structural stiffness values for bending in different directions. For larger deflections, the stiffness constant, k, also increased significantly with increasing body length (Fig. 5B; $F_{1,25}$=59.60, P<0.0001, R^2=0.706). Again, we did not detect a significant difference in the stiffness constant for bending in different directions.

Table 3. Mean angle of deformation when deformed maximally

Life-history stage	Dorsal bending (deg.)	Lateral bending (deg.)	Ventral bending (deg.)
Fetus	41.8 (22.3)	137.9 (20.3)	94.0 (34.3)
Neonate	46.1 (5.0)	77.5 (6.2)	82.8 (10.2)
Juvenile	40.3 (17.1)	57.4 (14.7)	47.5 (10.2)

Mean angle of deformation across life-history stages for bending in the dorsal, lateral and ventral direction. The angle of deformation describes the position of the fluke insertion relative to the long axis of the animal at maximal deformation. The standard deviation for each value is given in parentheses. Note that analysis of these data was based on an ANCOVA (linear model), rather than a comparison of means.

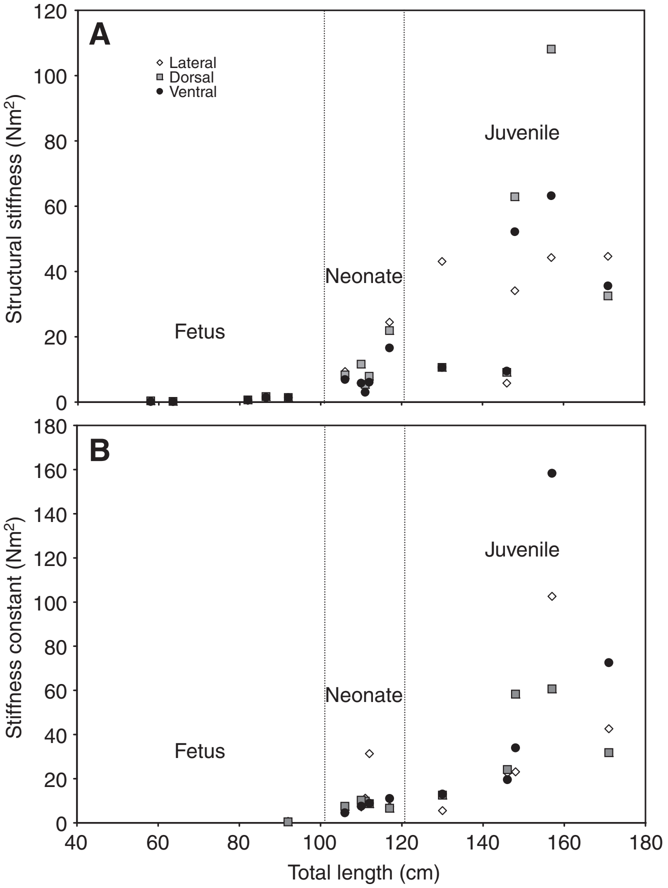

Fig. 5. Structural stiffness and stiffness constants for an ontogenetic series of dolphins. Values are shown for deformation in the lateral (mean value for both left and right), dorsal and ventral directions. Vertical dotted lines distinguish different life-history stages. (A) Structural stiffness was calculated for deformations that were less than 10% of total body length and thus often represent single data points. For dorsal and ventral deformations, the average standard deviation was 7.5 and 0.8, respectively. For lateral deformations, the average standard deviation was 9.8. Fetal deformations typically exceeded the stated criterion, even with loads that were too small to be measured with our apparatus. For these animals, we assumed a load of 0.98 N and used the resultant deformations. Thus, these values should be considered approximations. (B) Stiffness constant (*k*) for an ontogenetic series of dolphins. Values were calculated for all deformations that exceeded 10% of total body length.

The growth rates (β) for both tailstock height and width were not significantly different from a slope of 1 [β=1.21, 95% confidence interval (CI)=0.96–1.46, t_{13}=1.796, P=0.0957, R^2=0.8919; β=1.20, 95% CI=0.90–1.51, t_{13}=1.432, P=0.1758, R^2=0.8459, respectively]. If the material properties of the tailstock remain constant with growth, structural stiffness will be proportional to body length to the fourth power. We found that structural stiffness increased more rapidly than predicted in the dorsal (β=5.44, 95% CI=4.17–6.71, t_{13}=2.450, P=0.0292, R^2=0.8680), ventral (β=5.76, 95% CI=4.78–6.73, t_{13}=3.896, P=0.0018, R^2=0.8657), and lateral (β=5.72, 95% CI=4.37–7.08, t_{13}=2.758, P=0.0163, R^2=0.9260) directions.

The neutral zone did not differ significantly across life-history stages in either the lateral or the dorsoventral direction (Fig. 6A). The neutral zone was highly variable in both directions, with a mean value of 32±14 cm in the lateral direction and 29±15 cm in the dorsoventral direction. Thus, a 58 cm fetus and a 157 cm juvenile dolphin bent laterally approximately the same distance from the long axis of the body with no applied load. In contrast, the relative neutral zone (neutral zone/total length) decreased significantly with length (Fig. 6B) in both the lateral (t_{13}=–4.80, P<0.0003, R^2=0.6398) and the dorsoventral direction (t_{13}=–4.39, P<0.0007, R^2=0.5968). In small animals, the relative neutral zone was almost 50% of the total length, while the relative neutral zone was closer to 20% in the largest animals in both directions (Fig. 6B).

DISCUSSION

At birth, bottlenose dolphins immediately transition from a laterally bent fetal position to a dorsoventrally bending swimmer. We hypothesized that the structural stiffness of the caudal tailstock increases due to passive mechanisms during postnatal growth. The results of this study support this functional hypothesis, but also indicate that the stiffness of the tailstock increases in all directions, rather than simply in the lateral direction.

In both the juvenile and neonatal line drawings (Fig. 3A,B), lateral deformations were relatively symmetric, although the amount of deformation was greater in the neonate. In contrast, lateral deformations were markedly asymmetric in the fetus, which may reflect its late-term posture within the mother's uterus. Fetal dolphins investigated here appeared to have a 'handedness' with respect to their lateral flexibility that was apparent in both the line drawings and stiffness measurements (Figs 3 and 5). Three out of five of our fetal specimens appeared to have been curved to the right *en utero*, based on the mechanical properties.

Ventral deformations exceeded dorsal deformations, particularly in smaller animals, which may reflect the underlying morphology of the vertebral column. These results support previous work indicating that isolated vertebral joints are stiffer in dorsal extension than in ventral flexion (Long et al., 1997).

Curvature

Early in development, the fetus is small enough that its posture can be relatively straight or only slightly curved into a smooth arc, resulting in a large curvature. At small sizes, it is likely that the fetus can still move easily within the uterus (Stone et al., 1999) and the direction of curvature can change as the animal shifts. As the fetus gets larger, its position probably becomes more constrained until there is very little possible movement of the fetus within the uterus. At these larger body sizes, the fetus is literally folded in half within the uterus (Fig. 1). When in this folded posture, the deformation is localized to a very small region of the body near the anus and the tailstock extends cranially in a straight manner. Thus, the fetus includes both highly curved and relatively straight regions, which lowers the overall measurement of curvature.

The laterally bent posture of the cetacean fetus is remarkable because it differs so dramatically from the dorsoventral curvature seen in the typical mammalian fetus. Lateral curvature is often associated with fetal or maternal death during birth in terrestrial mammals (Jackson, 1995). Our results, though, suggest that maximum curvature always occurs at or caudal to the anus, indicating that the deformations seen in a dolphin are occurring in the post-anal tail, rather than in the thoracolumbar region. In general, mammalian tails are flexible and easily demarcated from the body. What is remarkable about a dolphin tail is that it is so highly modified into a locomotor structure, with a fusiform shape and no externally distinct vertebral regions. Thus, the dramatic position of the fetal dolphin *en utero* (Fig. 1) represents predominantly deformation of the tail, rather than of the body. In fetuses, the extreme lateral flexibility of the tail may be a requirement for dolphins and porpoises to achieve their large fetal body size, but this flexibility is lost as locomotor functions mature.

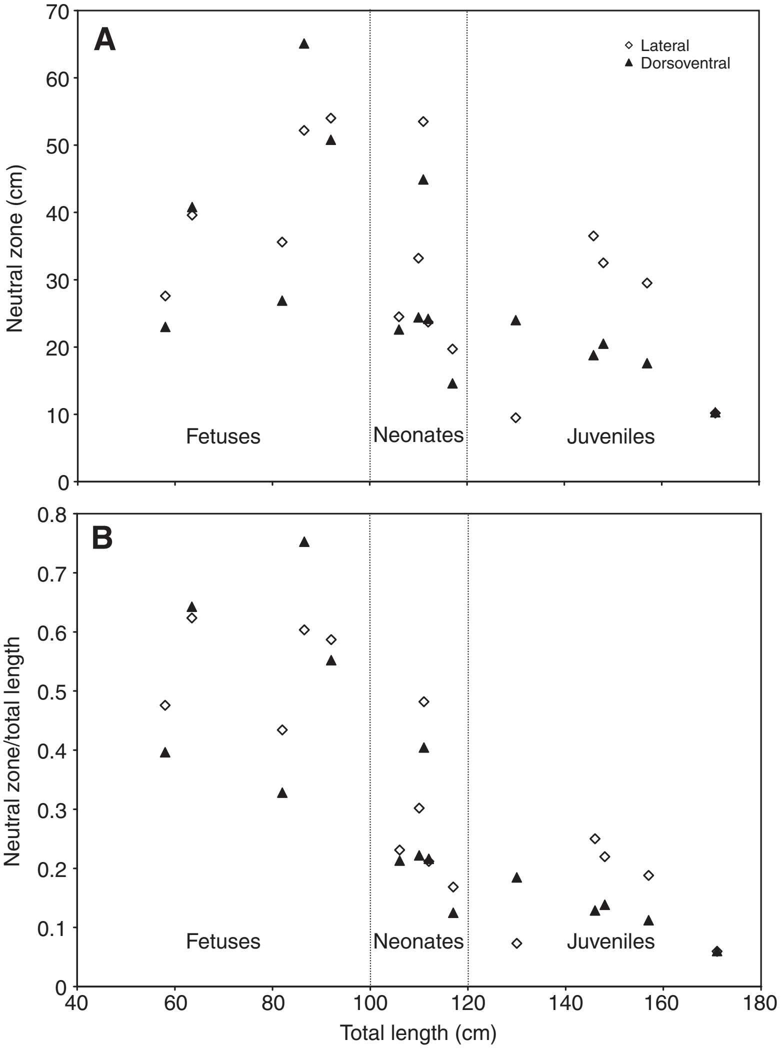

Fig. 6. (A) The neutral zone for an ontogenetic series of dolphins. The neutral zone is defined as the deformation exhibited with no measurable applied load. (B) The relative neutral zone (neutral zone/total length) for the same specimens. Vertical dotted lines distinguish different life-history stages.

During mechanical tests, we also discovered that the *en utero* position of a fetal bottlenose dolphin is not due to pure lateral bending. Rather, it is a combination of lateral and ventral bending, coupled with slight axial rotation in the caudal tailstock. The ventral bending is similar to that seen in a typical mammalian fetus, while the lateral bending and axial rotation may be unique to the dolphin fetal position. While we predicted increases in lateral stiffness through ontogeny to offset fetal flexibility, the concomitant increases in dorsoventral stiffness may also be a consequence of the dolphin fetal position.

In fetuses, lateral curvature when loaded maximally was consistently greater than either dorsal or ventral curvature (Fig. 4). This pattern, which was not evident in neonates and juveniles, supports our previous findings (Etnier et al., 2004), which suggested that fetuses use active control mechanisms to control curvature in the lateral direction. In adults, deformation in all directions is controlled in large part by passive mechanisms, presumably associated with changes in the vertebral column and associated connective tissues.

Neutral zone

The neutral zone reflects the amount of deformation possible when no load is applied, and thus indicates the free movement that is possible without activating either passive or active control mechanisms. While the neutral zone is a common and useful variable for comparisons between different species (e.g. Gàl, 1993), its constancy in this study suggests that the relative neutral zone (i.e. neutral zone/total length) may be a more useful variable in ontogenetic studies. The marked decreases in the relative neutral zone suggest that the passive mechanical properties of the tailstock are changing significantly across life history stages.

Stiffness

Neonatal dolphins are described as uncoordinated swimmers (Cockcroft and Ross, 1990), potentially due to their inability to stabilize body deformations. Our results, as well as anecdotal evidence on neonatal swimming behavior (Cockroft and Ross, 1990), suggest that important mechanical changes may take place as early as 3 weeks following birth. Lateral, dorsal and ventral stiffness were all found to increase at a similar rate with increasing body length (Fig. 5), thus animals quickly become stiffer following birth. An unanswered question is whether the observed increases in structural stiffness are a result of changes in material properties (E), or are simply due to an increase in tailstock size (I). The scaling analysis demonstrated that tailstock height and width grew at rates that were not significantly different from 1. Because structural stiffness increased at a significantly higher rate than would be expected based upon increases in tailstock size alone (specifically, with a slope greater than 4), our results suggest that the passive material properties of the tailstock may be changing through ontogeny.

While our results suggest that changes in the material properties are contributing to the changing mechanical properties of the tailstock, the small sample size limits the precision in estimating tailstock growth and stiffness values, and thus the exact role of changes in material properties and/or size cannot yet be fully determined. In addition, we cannot state specifically whether the changing material properties are due to some or all of the various elements making up the tailstock of the dolphin.

Future studies should more fully investigate the growth patterns of the dolphin tailstock, not only at the anus but also along its entire length. In addition, detailed studies of the growth patterns and mechanical properties of individual components of the tailstock are needed to fully understand the observed ontogenetic changes in this structure. We suggest that the complex connective tissues of the tailstock, including the subdermal connective tissue sheath, tendons and the backbone itself, are probably contributing to the increasing stiffness of the tailstock, although the exact contribution of each of these materials remains to be determined.

Interestingly, even our largest individuals continued to have the ability to deform in the lateral direction. While some research suggests that body excursions that do not contribute to locomotion decrease energetic efficiency in terrestrial animals (Gàl, 1993), others have argued that lateral forces may enhance both passive stability and active maneuverability during locomotion (Dickinson et al., 2000; Fish, 2002). Thus, while the extreme lateral flexibility required *en* *utero* may compromise locomotor abilities in neonates, the moderate lateral flexibility observed in older animals is likely to be functionally important for both stability and maneuverability. Cetaceans laterally flex and twist the tail flukes during turning, in response to active muscular control (Fish, 2002). We suggest that the increased axial stiffness observed in older dolphins may be associated with increased locomotor abilities during steady swimming, while the ability to actively flex the tail may be important for maneuverability, although this hypothesis has not been tested.

Conclusion

The neonatal period is an extremely vulnerable time in the life history of any mammal, due in part to physical limitations on performance (Carrier, 1995). The neonatal dolphin is faced with the remarkable challenge of accommodating extreme changes in posture with the immediate mechanical demands of locomotion. Thus, there may be very different selective pressures at different life-history stages, and these pressures may change over very short time periods, such as at birth. The tailstock must bend laterally *en utero*, while such movements are potentially detrimental in a freely swimming neonate. Neonatal dolphins appear to actively control the stability of the caudal tailstock using laterally positioned axial muscles (Etnier et al., 2004). While neonates limit lateral deformations of the tailstock using active, muscular mechanisms, the results of this study suggest that the stability of the maturing tailstock is due to changes in both structural and material properties. Thus, during postnatal development, dolphins use different mechanisms to limit lateral stability, moving from active mechanisms in young animals to passive mechanisms in older animals. We suggest that the mechanical changes occurring during this time period are essential for the successful transition from fetus to neonate in bottlenose dolphins and other cetaceans. Similar mechanical and morphological changes may occur in other organisms through ontogeny when the functional demands on a given life-history stage vary dramatically.

LIST OF ABBREVIATIONS

C	curvature (m^{-1})
E	elastic modulus ($N\,m^{-2}$)
EI	structural stiffness (Nm^2)
F	applied load (N)
I	second moment of area (m^4)
ICD	m. intertransversarious caudae dorsalis
k	stiffness constant (Nm^2)
l	beam length (m)
M	moment (Nm)
r	radius (m)
TL	total length of dolphin (cm)
y	deformation of beam tip (m)

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