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Postural role of lateral axial muscles in developing bottlenose dolphins (*Tursiops truncatus*)

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Foetal dolphins (*Tursiops truncatus*) are bent ventrolaterally, such that the tailflukes and lower jaw are juxtaposed. The lateral flexibility required *en utero* may compromise the efficiency of the dorsoventral oscillations required of the swimming neonate. The *m. intertransversarius caudae dorsalis* (IT) is the most laterally placed epaxial muscle. Bilateral contractions of the IT could limit lateral deformations of the flexible tailstock of the early neonate. We test the hypothesis that the IT is functioning as a postural muscle in neonates by examining its morphological, histological and biochemical properties. The neonatal IT has a relatively large cross-sectional area and bending moment, as well as a large proportion of slow-twitch fibres and elevated myoglobin concentrations. Our results demonstrate that the IT is functionally capable of performing this specific postural function in neonatal dolphins. In later life-history stages, when postural control is no longer needed, the IT serves to fine-tune the position of the tailstock during locomotion. The changing function of the adult IT is concomitant with changes in morphology and biochemistry, and most notably, with an increase in the proportion of fast-twitch fibres. We suggest that these changes reflect strong selective pressure to improve locomotor abilities by limiting lateral deformations during this critical life-history stage.

Keywords: myoglobin; posture; fibre types; development

1. INTRODUCTION

En utero, the position of foetal dolphins differs markedly from that of other mammals. Rather than the dorsoventral curvature that typifies the mammalian 'foetal position', dolphins are bent laterally and ventrally, such that the tail flukes and throat are juxtaposed (figure 1; Slijper 1966; Cockcroft & Ross 1990). The dolphin foetal position requires extreme lateral flexibility in the axial skeleton, associated locomotor muscles and connective tissues when compared with that of an adult dolphin. This extreme posture may be associated with the large size of the foetus, which, in bottlenose dolphins (*Tursiops truncatus*), can be fully half the length of the mother (Mead & Potter 1990). Upon birth, the neonatal dolphin must be able to swim to the surface to breathe using dorsoventral oscillations of its tailstock. Thus, the dolphin must immediately transition from its laterally bent posture *en utero* to the dorsoventral bending pattern of a freely swimming animal in response to changing functional demands.

Although neonatal dolphins are precocial locomotors (Dearolf *et al.* 2000), they are initially rather uncoordinated swimmers (Cockcroft & Ross 1990). Anecdotally, young dolphins begin to swim more competently a few weeks after birth (Cockcroft & Ross 1990). This early lack of coordination may be associated with a neonate's inability to stabilize lateral deformations of the tailstock. In terrestrial mammals, body excursions that do not contribute to locomotion decrease energetic efficiency (Gàl 1993). Similarly for dolphins, the dorsoventral oscillations required to swim may be compromised by the lateral flexibility necessitated by their *en utero* position. Mechanisms that stabilize the neonatal tailstock against lateral deformations may increase locomotor efficiency during this critical life-history stage.

Both passive (connective tissue and bone) and active (muscle) elements may enhance lateral stability of the neonatal tailstock. Because foetal dolphins are bent immediately before birth, we suggest that passive elements could not be used to stabilize lateral motions until after a period of postnatal development. By contrast, active muscular elements could limit lateral deformations immediately upon birth.

This study investigates active elements that may contribute to lateral stability in neonatal dolphins. The tailstock of cetaceans moves in response to the contraction of muscles whose actions are determined in large part by their position relative to the axial skeleton (figure 2). The dorsoventral oscillations used to swim are powered by muscles positioned dorsal and ventral to the transverse processes of the vertebrae. Laterally positioned muscles, namely the *m. intertransversarius caudae dorsalis* (IT), contribute more to side-to-side movements (reviewed by Pabst 1990). We hypothesize that the IT can also serve a postural role in neonatal dolphins and contribute to lateral stability of the tailstock by contracting bilaterally. We test this hypothesis by comparing the features of the *m. intertransversarius caudae dorsalis*, a lateral-flexing muscle, with those of the *m. extensor caudae lateralis* (ECL), a dorsal-flexing locomotor muscle, across ontogeny.

If the neonatal IT is acting as a postural muscle, we expect it to display a specific suite of physical characteristics.

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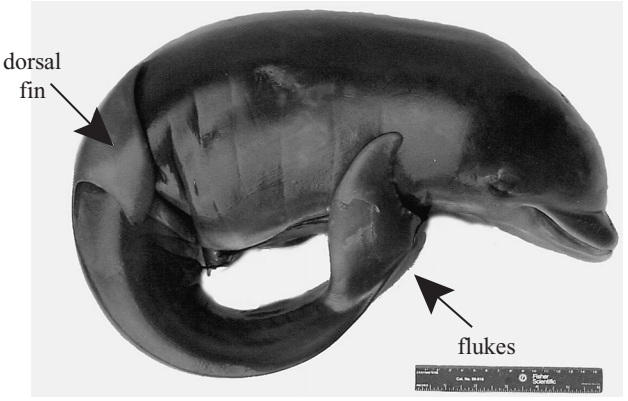

Figure 1. *En utero* posture of a foetal dolphin (PTM 114f, total length 81 cm). The tail flukes are juxtaposed to the chin. Note that the dorsal fin and flukes are floppy in foetal dolphins. The scale bar is 15.8 mm in length.

First, we predict that the neonatal IT will have a relatively larger cross-sectional area, and, thus, relatively larger force production, than the adult IT. Cross-sectional area is correlated with force production in muscles (reviewed in Schmidt-Nielsen 1997). The proportion of that force available for lateral and dorsoventral movements will be determined by calculating the moments for both the IT and ECL (*sensu* Arkowitz & Rommel 1985). Second, we predict that the neonatal IT will have a higher proportion of slow-twitch fibres than the neonatal ECL. Slow-twitch fibres are slow contracting, fatigue-resistant muscle fibres associated with postural or endurance muscles (Brooke & Kaiser 1970). Finally, we predict that the neonatal IT will have a greater concentration of myoglobin (Mb) than the neonatal ECL, because increased Mb concentrations are associated with the high oxygen demands of continuously acting muscles (Moore *et al.* 2002). Previous studies have demonstrated that Mb concentrations in dolphin locomotor muscles increase during the transition from neonate to adult (Dolar *et al.* 1999; Noren *et al.* 2001). This study is the first to extend these results into foetal animals. In this study, we examine physical characteristics of the IT and ECL in foetal, neonatal and adult dolphins to explore the critical transition from a laterally bent foetus to an adult that swims with dorsoventral oscillations of the tailstock.

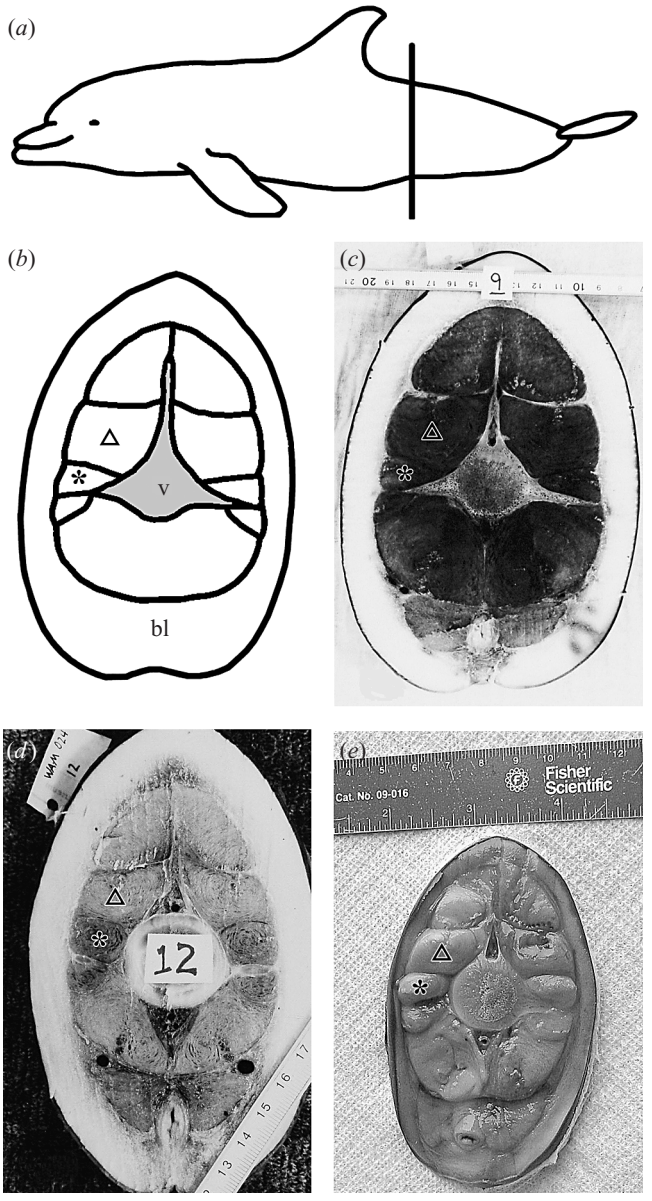

Figure 2. Schematic diagram of sampling location and a cross-section at the anus identifying muscles used in this study. (*a*) Samples were taken at the level of the anus during necropsy. (*b*) The IT (asterisk) and ECL (triangle) were sampled for histology and Mb concentrations. Abbreviations: bl, blubber; v, vertebra. Cross-sections of (*c*) a juvenile, (*d*) a neonate and (*e*) a foetus were used for analysis of muscle cross-sectional area and bending moments.

2. MATERIAL AND METHODS

(a) *Samples*

Muscle samples were taken from freshly stranded carcasses collected in collaboration with the Northeast and Southeast Regional Stranding Network, under a Letter of Authorization from the National Marine Fisheries Service (table 1). All animals were determined to be in good body condition based upon both external and internal exams (Geraci & Lounsbury 1993; McLellan *et al.* 2002). Animals were dissected within 3–5 h of recovery or frozen intact for up to several months before thawing and dissecting.

Specimens were placed into three life-history stages: foetuses, neonates and adults. Foetuses were collected *en utero* and ranged from 82 to 96.5 cm in total length. Neonates were classified based upon a suite of characters, as defined by Dearolf *et al.* (2000). All neonates had floppy dorsal fins and tailflukes and ranged between 105 and 119 cm in total length. Adults were defined as animals greater than 200 cm in total length. Although the adults in this study represented both sexually mature and immature animals (Mead & Potter 1990; Read *et al.* 1993), they were considered to exhibit mature muscle characteristics.

The IT and ECL are long epaxial muscles that were sampled at a single position at the level of the anus (figure 2). This position was chosen because it exhibits the largest range of both flexion and extension during normal swimming (Pabst 1993, 1996; Long *et al.* 1997) and because it allowed comparison with a previously published study (Dearolf *et al.* 2000).

Table 1. Specimens of bottlenose dolphins (*Tursiops truncatus*) used in the histological, biochemical and morphological studies.

identification number	total length (cm)	sex	life-history stage	histology	biochemistry	morphology
ASF033f	82	M	foetus	IT: ECL	IT: ECL	
WJW007f	86.5	M	foetus	IT: ECL	IT: ECL	
WAM545f	92	F	foetus	IT: ECL	IT: ECL	
WAM535f	96.5	F	foetus	IT: ECL	IT: ECL	
WAM024	105	M	neonate			IT: ECL
VMSM961035	106	M	neonate	IT		
EMM010	106	M	neonate		IT: ECL	
VMSM20011080	110	M	neonate		IT: ECL	
NC98086	111	M	neonate	IT		
VMSM20021042	111.5	M	neonate		IT: ECL	
CALO96-23	119.2	F	neonate	IT	IT: ECL	
ASF044	126	M	neonate	IT		
no number[a]	170.9	F	juvenile			IT: ECL
VMSM20001049	207	M	adult	IT		
NEFSC5332	228	M	adult		IT: ECL	
MMB002	235	M	adult		IT: ECL	
WAM559	239	F	adult	IT	IT: ECL	
WAM560	245	F	adult	IT	IT: ECL	
WAM533	261	F	adult	IT		

[a] Animal classified as a juvenile based on total length.

(b) *Muscle area and bending moments*

A juvenile and neonatal dolphin (table 1) were serially sectioned with a bow saw at 3–5 cm intervals along their length (Pabst 1990). The larger animal could not be classified as an adult based upon our chosen criteria, thus, the reported measures probably underestimate both muscle area and bending moments in an adult dolphin. Cross-sections were originally photographed with a known-length metric in the image. For each animal, a digital image (Sony MVC-FD95 digital still camera) was taken of the original photograph of the cross-section at the level of the anus (figure 2). A foetal dolphin (table 1) was cut in cross-section at the level of the anus with a large knife and photographed with the same digital camera. For each cross-section, the areas of the following muscles or muscle groups were measured using Image Pro Plus (Media Cybernetics, Silver Spring, MD, USA) software (figure 3): IT, ECL, epaxial muscle (*m. extensor caudae medialis*, ECL and IT), and total axial locomotor muscle (*m. extensor caudae medialis*, ECL, IT and *m. hypaxialis lumborum*). The precision of these measures, based upon five replicate measures of one muscle, was ± 1.3%. The area of each muscle or muscle group was measured in triplicate and averaged. These values were used to calculate the area of the IT and ECL relative to total axial locomotor muscle area and relative to epaxial muscle area at the level of the anus.

Bending moments were calculated based upon the assumption that muscle forces are parallel to the vertebral column and that bending moments are applied about an axis of rotation through the centre of the vertebra (Arkowitz & Rommel 1985). The bending moment produced is the product of muscle force and the perpendicular distance from the axis of rotation, as measured from the centroid (centre of mass) of the vertebra to the centroid of each muscle cross-section. All measures were taken using Image Pro Plus. We used cross-sectional area as a proxy for force; thus, calculated bending moments are an index of true bending moments. Bending moments were calculated for dorsoventral and lateral planes of motion for both the IT and ECL.

(c) *Histology*

Muscle samples of the IT were taken at the level of the anus in foetal ($n = 4$), neonatal ($n = 4$) and adult ($n = 4$) bottlenose dolphins. The ECL was sampled at the level of the anus in the same four foetal dolphins. Values for the ECL in the neonatal and adult dolphins were obtained from Dearolf *et al.* (2000). Samples were wrapped in Saran Wrap, placed in plastic bags and frozen flat at −20 °C.

For histochemical assays, tissue blocks *ca.* 5 mm^3 were subsampled from the centre of each muscle. Previous studies have shown that the central portion of the ECL does not differ statistically from other regions within the muscle cross-section (Dearolf *et al.* 2000). Thawed tissue blocks were mounted on cork blocks using 5% gum tragacanth, and frozen in isopentane chilled to −160 °C with liquid nitrogen (Hermanson & Hurley 1990). Tissue blocks were serially sectioned (10 μm thickness) at −19 °C with a cryostat (Leica Cryocut 1800) and mounted on glass slides.

Muscle sections were stained for myosin ATPase in acidic (pH 4.3, 4.4, 4.5) and basic media (pH 10.3) following the procedure of Hermanson & Hurley (1990). Following the scheme of Brooke & Kaiser (1970), individual muscle fibres were classified as slow twitch (type I) or fast twitch (type IIa and IIb) based upon reactivity to both basic and acidic protocols. Further histochemical assays were not pursued because preliminary results, as well as published reports (White *et al.* 1978; Dearolf *et al.* 2000), indicate their ineffectiveness for foetal and neonatal muscles.

(i) *Fibre-type profiles*

Serial images of muscle fibres were captured by using an Olympus BH-2 light microscope coupled with a Spot Camera (RT Colour Diagnostic Instruments, Inc.). Slides were viewed at a magnification, ×20 (adult tissue) or ×40 (neonatal and foetal tissue). Using printed images from slides incubated at pH 4.4, dark (slow-twitch) and light (fast-twitch) fibres were identified until at least 500 fibres were counted (typically four fields of

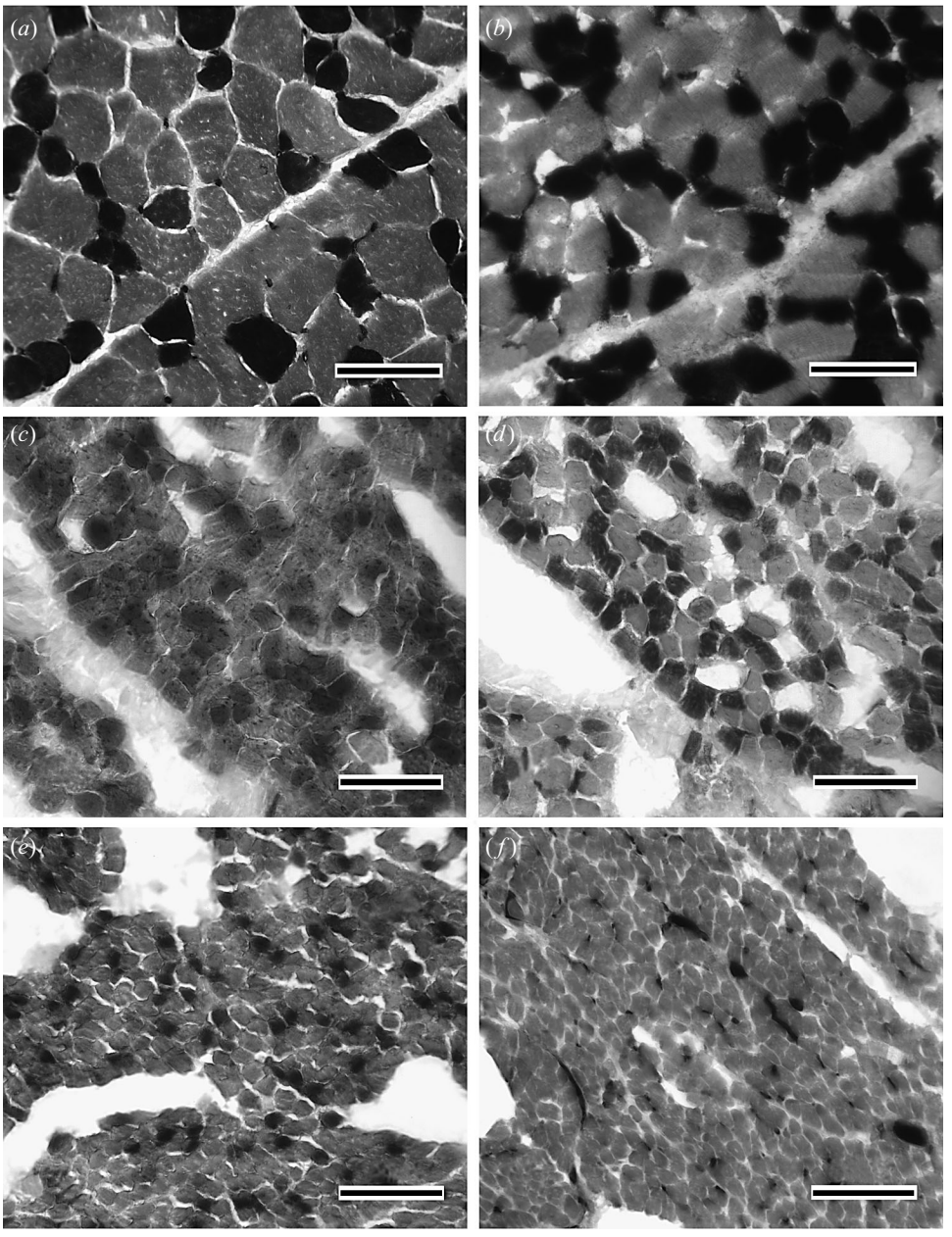

Figure 3. Representative cross-sections of IT and ECL from bottlenose dolphins (*Tursiops truncatus*) after histochemical staining. (*a*) Adult dolphin IT muscle (WAM560) after acidic pre-incubation. (*b*) Neonatal dolphin IT muscle (NC98086) after acidic pre-incubation. (*c*,*d*) Foetal dolphin IT muscle (WAM545f) after acidic and basic pre-incubation, respectively. (*e*,*f*) Foetal dolphin ECL muscle (WAM545f) after acidic and basic pre-incubation, respectively. Slow-twitch muscles appear dark in acid pre-incubation (*a*,*b*,*c*,*e*), whereas fast-twitch fibres appear dark in basic pre-incubation (*d*,*f*). Note that there is no differentiation under basic pre-incubation in the foetal ECL, compared with distinct differentiation in the foetal IT. Scale bars: (*a*) 100 μm; (*b*,*c*,*d*,*e*,*f*) 50 μm.

view). If the fibre type could not be determined based upon information from both acidic and alkaline pre-incubation protocols, the fibre was counted as an 'unknown' fibre. This designation was used primarily in the foetal samples. The number of each fibre type was divided by the total number of fibres to obtain the percentage of each fibre type by count.

The developmental states of the IT and ECL were compared by using a muscle developmental index (percentage of slow-twitch fibres in foetal or neonatal muscle/percentage of slow-twitch fibres in adult muscle) based upon the percentage of each fibre type by count (Cobb *et al.* 1994; Dearolf *et al.* 2000).

(ii) *Fibre area*

A Mertz-curvilinear grid system (Russ 1986) was used to determine the percentage of area occupied by slow-twitch and fast-twitch fibres on sections incubated at pH 4.4. The grid was placed over each image and the numbers of points residing in slow-twitch, fast-twitch fibres or white space were counted. A minimum of 288 points (typically four fields of view) was counted for each specimen. If fibre type could not be determined, it was considered an unknown fibre. We then calculated the percentage area of each fibre type (Russ 1986; Dearolf *et al.* 2000) and corrected for slide sections of finite thickness (Russ 1986).

(iii) *Fibre diameters*

A single image from each individual was analysed to determine the average diameter of slow-twitch and fast-twitch fibres as seen on sections incubated at pH 4.4. The fibres arbitrarily selected for analysis were relatively circular in cross-section and appeared qualitatively similar to other fibres within the image. Image Pro Plus was used to measure the diameter of 20 fibres of each type. This software measures the diameter of the fibre at 5° intervals around the centroid of each fibre and averages these values.

To test the precision of this technique, we measured the diameter of muscle fibres from one image three separate times. For each trial, we measured 10 fast-twitch and 10 slow-twitch fibres that were chosen arbitrarily. A one-way analysis of variance did not detect a significant difference between trials in either fast-twitch ($F = 0.4292$, $p = 0.65$) or slow-twitch ($F = 0.5114$, $p = 0.60$) diameters.

(d) *Myoglobin concentrations*

Muscle samples from the centre of the IT and ECL were taken at the level of the anus in foetal ($n = 4$), neonatal ($n = 4$) and adult ($n = 4$) dolphins. Muscle samples were collected at necropsy in 5 ml polypropylene freezer vials and frozen (−20 °C) before testing. The technique used was based upon Reynafarje (1963). Partly thawed tissue was cleaned of fat and connective tissue, accurately weighed (*ca*. 0.5 g) and placed in a glass homogenizer tube placed within an ice bath. We performed three replicate assays for each muscle. Phosphate buffer (0.04 M at pH 6.6) was added at a dilution factor of 39.25 ml g^{-1} for adult tissue and 19.25 ml g^{-1} for neonatal and foetal tissue. Tissue was homogenized, sonicated and then centrifuged following the protocol outlined by Reynafarje (1963). Five millilitres of supernatant was bubbled with carbon monoxide (CO) for 8 min. Simultaneously, 5 ml of phosphate buffer was also bubbled for 8 min as a control. After 8 min, 0.03–0.04 g of sodium hydrosulphite was added to each tube and bubbled with CO for another 2 min.

To calculate the concentration of Mb within the tissue, we measured the difference in absorbance at 568 (A_{568}) and 538 (A_{538}) nm with a spectrophotometer (Pharmacia Biotech UltraSpec 3000). Mb concentration is calculated as

$$\frac{\text{mass of Mb (g)}}{\text{100 g muscle}} = 5.865(A_{568} - A_{538}) \times \text{dilution factor}.$$

Replicate values for each muscle were averaged for analysis. A Mb developmental index was calculated for each muscle (Mb concentration in foetal or neonatal muscle/Mb concentration in adult muscle).

(e) *Statistical methods*

All statistical analyses were calculated using Jmp In v. 4.0.3 (SAS Institute, Cary, NC, USA) statistical software. Histological samples did not fit a fully paired design, because values for the adult and neonatal ECL came from previously published results. Mb concentrations were a fully paired design with samples for both the IT and ECL from each individual animal.

3. RESULTS

(a) *Muscle area and bending moments*

Total axial locomotor muscle area increased from the foetus (23.1 cm^2) to the neonate (31.4 cm^2) to the juvenile (110.6 cm^2), as did the cross-sectional area of the IT and the ECL (table 2). The IT was smaller in cross-sectional area than the ECL (figure 2; table 2) in all three life-history stages. In relative terms (expressed as a percentage of total axial locomotor and total epaxial muscle area), the IT represented a larger percentage of the locomotor and epaxial cross-sectional area in the neonate than in either the foetus or the juvenile. By contrast, the ECL was relatively smaller in area in the neonate compared with either the foetus or the juvenile (table 2).

The IT and ECL both contribute to lateral and dorsoventral bending moments, but the amount varies in different life-history stages. The IT contributes up to 45% of the combined bending moments available for lateral movements in neonates, compared with only 39% in foetuses and 27% in juveniles.

(b) *Histology*

Qualitatively, foetal and most neonatal samples were soft and difficult to cut and orient, as has been described in previous work on other mammalian muscles (White *et al.* 1978; Hermanson & Hurley 1990). Life-history stage and histochemical staining quality were inversely related. Adult samples exhibited well-defined cell margins and clear differentiation of fibre types, whereas foetal samples exhibited poorly defined cell margins and poor or ambiguous differentiation of fibre types (figure 3). Foetal samples were run concurrently with adult samples that stained well, suggesting that foetal results were unlikely to be due to protocol error.

Fibres from all life-history stages were grouped into slow twitch (type I) and fast twitch (type IIa and IIb). A third group, defined as 'unknown' fibres, was seen almost exclusively in the foetal samples and did not show differentiation under any of the pH treatments. This result suggests that myosin ATPase was not present in these fibres or that it was labile under all tested pH values. Note that these 'unknown' fibres do not correspond to previously reported 'undifferentiated fibres'. These fibres, otherwise described as IIc foetal fibres (Dearolf *et al.* 2000), are stable at all tested pH values.

In the foetal samples (figure 4), which represented paired data from the same individuals, there was no detectable difference between muscles in the percentage of slow-twitch fibres by count (IT, 31.9%; ECL, 24.7%; $p = 0.170$). Over 75% of fibres in the foetal ECL were classified as unknown, compared with only 39% in the foetal IT (figure 4). These unknown fibres virtually disappeared in the neonatal samples. The samples from neonatal and adult muscles were not paired, and, thus, had to be analysed separately. A two-way ANOVA comparing slow-twitch fibre percentages in the IT and ECL across the neonatal and adult life-history stages detected a significant interaction between muscle type and life-history stage ($p = 0.013$). Life-history stage influenced slow-twitch percentages in neonates, but not in adults. The neonatal IT had a higher percentage of slow-twitch fibres than the adult IT, whereas these two values were the same in the ECL (figure 4).

The calculated developmental indices varied between muscles and between life-history stages. The foetal ECL displayed 61.4% of the adult profile, while the neonatal ECL had 95% of the adult fibre type profile of this

Table 2. Absolute and relative cross-sectional areas of the IT and ECL in different life-history stages, as well as their bending moments.
(Bending moments are based on cross-sectional area and, thus, represent an index of the true moments in units of cm × cm^2. Values for the muscles on the left and right sides have been summed in each column.)

	muscle and life-history stage					
	IT			ECL		
	foetus	neonate	juvenile	foetus	neonate	juvenile
area (cm^2)	2.3	3.6	7.4	5.3	6.6	27.6
total axial locomotor muscle (%)	9.8	11.4	6.7	23.0	21.0	25.0
epaxial muscle (%)	17.7	19.4	11.8	41.4	35.9	44.3
dorsoventral bending moment (cm × cm^2)	0.5	1.6	1.5	6.8	12.3	63.4
lateral bending moment (cm × cm^2)	4.2	7.8	25.5	6.7	9.6	70.7

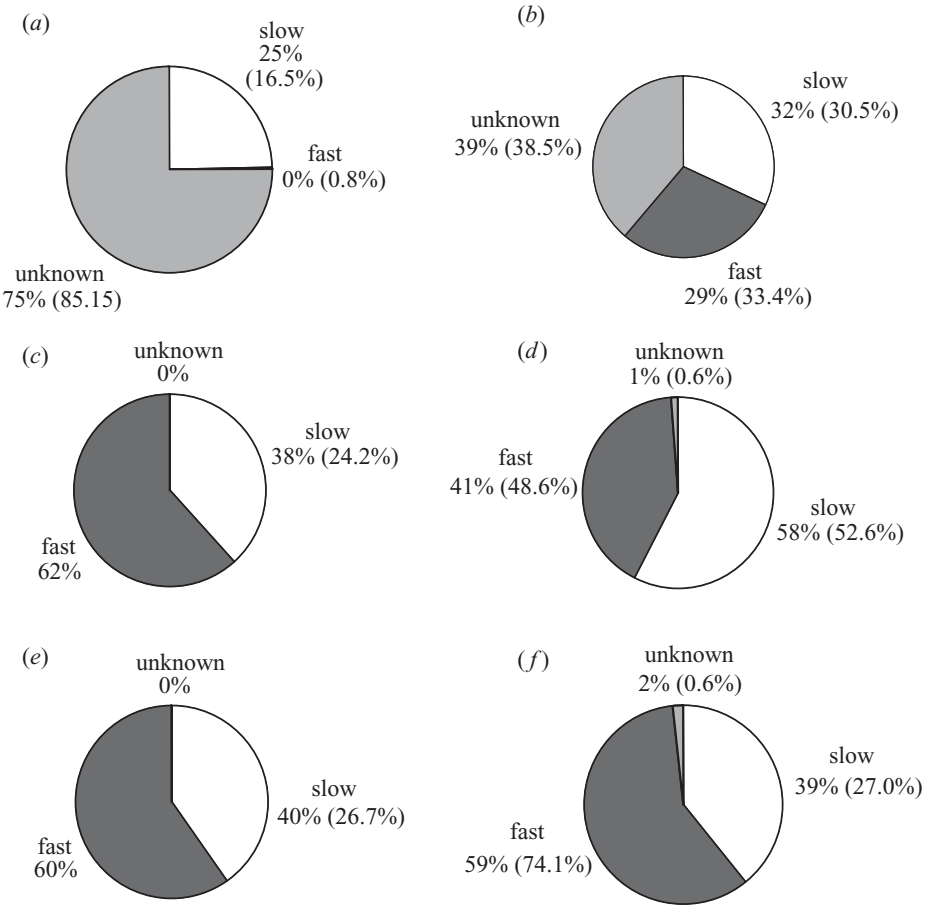

Figure 4. Percentages of each fibre type, by count, for the IT and ECL in all three life-history categories. Area percentages, when available, are shown in parentheses. The area values differ from the fibre counts owing to variation in the diameter of each fibre type. (*a*) Foetal ECL, (*b*) foetal IT, (*c*) neonatal ECL (data from Dearolf *et al.* (2000)), (*d*) neonatal IT, (*e*) adult ECL (data from Dearolf *et al.* (2000)) and (*f*) adult IT.

locomotor muscle (Dearolf *et al.* 2000). By contrast, the foetal IT had 81.4% of the adult complement, whereas the neonatal IT had 146.6% (figure 5). Thus, the percentage of slow-twitch fibres in the neonatal IT exceeded that of an adult. Similar patterns are observed when values were analysed based upon percentage of total area covered by each fibre type (figure 4). The percentages differ slightly owing to differences in fibre diameters (see figure 4), both for life-history stage and fibre type.

Fibre diameters increased across life-history stage. Slow-twitch fibres in the ECL averaged 9.5, 12.7 and 37.6 μm in diameter, in foetal, neonatal and adult samples, respectively. Fast-twitch fibres averaged 16.1 and 54.5 μm in diameter in the neonatal and adult samples, respectively. There were no fast-twitch fibres in the foetal ECL. Muscle fibres in the IT were consistently larger than those of the ECL, regardless of fibre type or life-history stage. Slow-twitch fibres in the IT were 11.3, 19.9 and

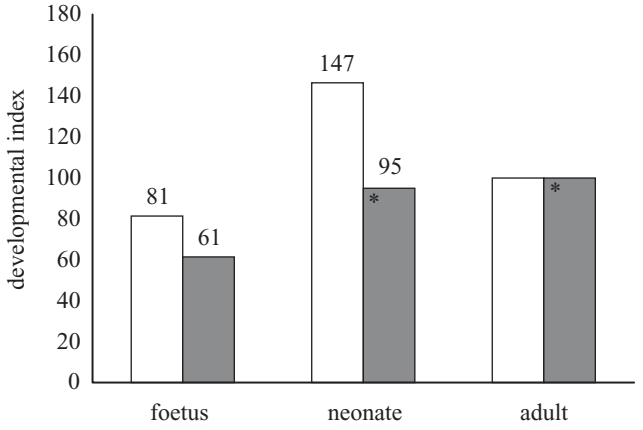

Figure 5. Developmental index of the IT (open bars) and ECL (filled bars). The developmental index is calculated as [(slow-twitch fibres in foetus or neonate/slow-twitch fibres in adult) × 100.] (Asterisks indicate data from Dearolf *et al.* (2000).)

42.0 μm in diameter, in foetal, neonatal and adult samples, whereas fast-twitch fibres were 10.7, 23.8 and 56.3 μm in diameter, respectively.

(c) *Myoglobin concentrations*

Mb concentrations increased significantly (IT: $F = 112.19$, $p = 0.001$; ECL: $F = 454.93$, $p = 0.001$) across all life-history stages (table 3). Mb concentrations were very low in foetuses (IT: 0.203 g Mb per 100 g muscle; ECL: 0.249 g Mb per 100 g muscle), increasing by three to four times immediately following birth. Concentrations were an order of magnitude higher in adults, with 2.8–2.9 g Mb per 100 g muscle. In the IT, the Mb developmental index was 7.0% for foetal muscle and 27.5% in neonates, whereas the index for the ECL was 8.9% and 24.7%, respectively.

A paired analysis of the IT and ECL in the different life-history stages detected a significant difference between Mb concentrations in these two muscles ($F = 4.5291$, $p = 0.0436$). A Tukey–Kramer honestly significant difference test for multiple comparisons detected a significant difference between neonatal and foetal samples ($p = 0.05$). The mean difference between the concentration of Mb in the IT and the ECL ($Mb_{IT} - Mb_{ECL}$) was -0.046 g Mb per 100 g muscle in foetal samples (i.e. on average the ECL had a greater concentration of Mb compared with the IT), 0.102 g Mb per 100 g muscle in the neonates and 0.088 g Mb per 100 g muscle in the adults (figure 6). In all neonates sampled, the Mb concentration in the IT was greater than that in the ECL, whereas this pattern was more variable in both foetuses and adults (figure 6).

4. DISCUSSION

Bottlenose dolphins must make the transition from their laterally bent foetal position to a straightened, dorsoventrally bending swimmer at the moment of birth. We hypothesized that the laterally placed, axial locomotor muscle *m. intertransversarius caudae dorsalis* could contribute to lateral stability by acting as a postural muscle. The results of this study support this functional hypothesis.

Both the IT and ECL contribute to dorsoventral and lateral movements, but their relative contributions to each depend upon their size and position with respect to the axis of rotation (table 2). The relatively large cross-sectional area of the neonatal IT allows it to contribute 45% of the lateral bending moment in the neonatal tailstock, whereas it contributes 39% in foetuses and only 25% in the juvenile tailstock. This result suggests that the function of the IT as a lateral postural muscle may be critical in neonatal dolphins. The dorsoventral bending moments of the ECL increase almost 10-fold during this same transition, suggestive of its increasingly important locomotor role in adult dolphins.

The foetal IT and ECL each exhibit a large percentage of slow-twitch fibres, with few or no fast-twitch fibres (figure 4). This finding contradicts the general model for the development of mammalian muscle, in which fast-twitch fibres appear relatively early in development and slow-twitch fibres appear later (Dubowitz 1965; Rubenstein & Kelly 1978; White *et al.* 1978; Bechtel & Kline 1987; Wigston & English 1992; Umezu *et al.* 1992; Cobb *et al.* 1994; Dearolf *et al.* 2000). The appearance of 'unknown' fibres in foetal samples suggests that the protocol employed here cannot be used to identify certain foetal muscle fibres. There were considerably more unknown fibres in the foetal ECL, suggesting that the ECL is less developed than the IT.

Neonatal muscles are considered well developed if they exhibit a fibre-type profile that shares a 75% or greater similarity with that of the adult (i.e. developmental index as defined by Grand (1992) and Dearolf *et al.* (2000)). By this criterion, both the neonatal IT and ECL are well developed, as is the foetal IT (figure 5). The neonatal IT, with a developmental index of 146.6%, greatly exceeds previously reported developmental indices for mammalian muscle (Dubowitz 1965; Rubenstein & Kelly 1978; White *et al.* 1978; Bechtel & Kline 1987; Dearolf 2003). The high percentage of slow-twitch fibres in the neonatal IT permits this muscle to function in a postural role. By contrast, the fibre-type profile of the adult IT and ECL are very similar, suggesting that the IT functions as a typical locomotor muscle in the adult life-history stage (figure 4).

We detected a significantly greater concentration of Mb in the neonatal IT compared with the ECL (table 3). Visual inspection of the neonatal cross-section reveals that the IT is darker in appearance than the ECL (figure 2). The higher concentration of Mb in the IT may provide more oxygen to support sustained postural function. Thus, both quantitative and qualitative results suggest that the concentration of Mb in the IT across different life-history stages may be worthy of further investigation. Mb concentrations determined for neonates and adults are similar, although higher than previously published values for *T. truncatus* (Dolar *et al.* 1999; Noren *et al.* 2001).

(a) *Developmental trajectories*

Neonatal animals must exist in the same environment, with the same predators, as adult animals. Yet, owing to their small size and limited locomotor and aerobic abilities, there may be strong selective pressure for improved locomotor performance in young animals (*sensu* Carrier 1996). As animals change size and shape and become more adept locomotors, the selective pressures may also

Table 3. Mb concentrations in foetuses, neonates and adults.

		mass of Mb per 100 g muscle (g)		
life-history stage	specimen	IT	ECL	IT − ECL
foetus	ASF033f	0.121	0.180	−0.059
	WJW007f	0.192	0.328	−0.137
	WAM545f	0.239	0.223	0.016
	WAM535f	0.262	0.266	−0.004
	mean	0.203	0.249	−0.046
neonate	EMM010	0.911	0.813	0.098
	VMSM20011080	0.809	0.645	0.164
	VMSM20021042	0.899	0.805	0.094
	CALO96-23	0.570	0.517	0.053
	mean	0.797	0.695	0.102
adult	MMB002	2.620	2.700	−0.080
	NEFSC5332	2.590	2.697	−0.107
	WAM559	2.883	2.827	0.057
	WAM560	3.520	3.037	0.483
	mean	2.903	2.815	0.088

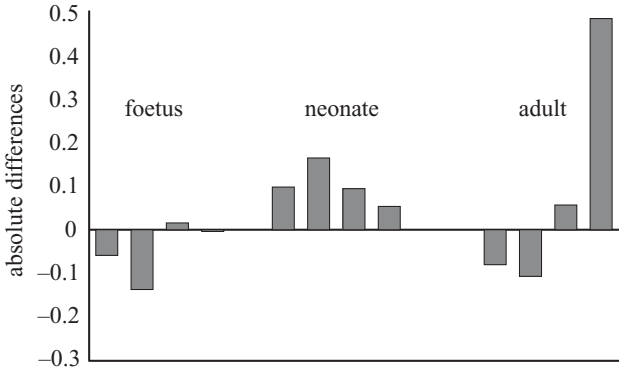

Figure 6. Absolute differences in Mb concentration (mass of Mb per 100 g muscle (g)) between the IT and ECL. Animals increase in total length from left to right. Absolute differences represent the Mb concentration of the IT minus the concentration of the ECL. Note that the Mb concentration in the IT is always higher than the ECL in the neonatal life-history stage.

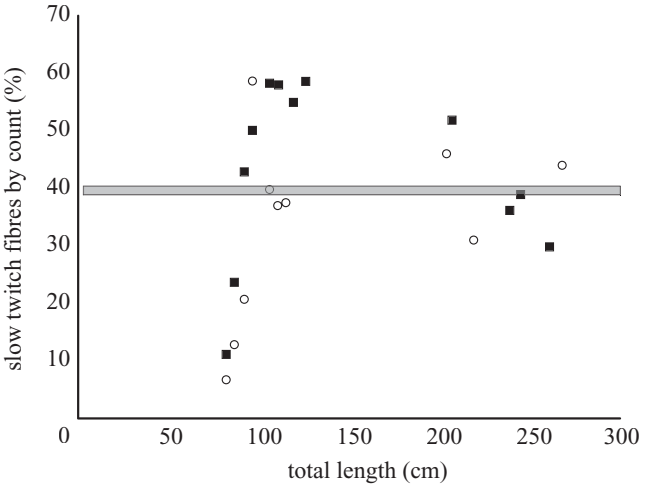

Figure 7. Developmental trajectories, based on percentage of slow-twitch fibres, for the IT (filled squares) and ECL (open circles). The grey box represents the range of average adult values for the two muscles (IT: 39%; ECL: 40%).

vary, permitting the functional role of individual components to change in different life-history stages. As the functional demands on the dolphin tailstock change during the transition from foetus to neonate to adult, the function of the individual elements also change. The early postural role of the IT as a lateral stabilizer may reflect an adaptation to increase locomotor efficiency during this critical developmental stage (Gàl 1993).

The functional role of the IT appears to change across life-history stages and these changes may be correlated with morphological changes in the dolphin tailstock. We hypothesize that lateral stability is actively controlled by bilateral contraction of the IT in neonatal dolphins, whereas adult animals have enhanced lateral stability through passive mechanisms, such as connective tissues. Thus, the proposed neonatal stabilizing function of the IT may be superceded by other structures in adult dolphins. Research by Etnier *et al.* (2003) suggests that the stability of the backbone itself changes soon after birth, mitigating the need to actively control lateral deformations. In adults, the IT is hypothesized to control fine adjustments in the position of the tailstock (Pabst 1990), rather than serving a postural function.

The developmental trajectories of these two muscles (figure 7) illustrate the differing roles of the IT and ECL through developmental time. The fibre-type profile of the ECL is relatively constant after birth, suggesting that its locomotor function is relatively constant throughout postnatal life. By contrast, the variable fibre-type profile of the IT is indicative of its changing functional role in different life-history stages.

We suggest that the functional role of Mb also changes across life-history stages in bottlenose dolphins, from a diffusion facilitator to an oxygen storage molecule. The Mb concentrations of foetal and neonatal dolphins are

similar to that of typical terrestrial vertebrates (Reynafarje 1963), suggesting that Mb may be more important as a diffusion facilitator, rather than an oxygen storage system in young dolphins. Most marine mammals reach adult Mb concentrations when they begin to forage independently (Castellini & Somero 1981; Thorson & Le Boeuf 1994; Noren & Williams 2000; Noren *et al.* 2001), which can take up to 2 years in bottlenose dolphins (Noren *et al.* 2001). It is at this point that the oxygen storage role of Mb becomes critical to a diving animal. In marine mammals, Mb is typically considered with respect to its oxygen storage abilities, and there is no doubt that the high Mb concentrations in adult marine mammals are critical to their diving abilities. The low Mb concentrations in the foetal and neonatal samples suggest that muscle oxygen stores are not yet critical in these life-history stages. Rather, in neonatal animals, Mb serves a different function by increasing the diffusion rate of oxygen into the muscles, particularly the IT.

The *en utero* position of bottlenose dolphins, as well as other cetaceans, potentially compromises the locomotor capabilities of neonates. The IT actively counteracts initial lateral flexibility in the tailstock until passive mechanisms develop postnatally, at which point the IT assumes a minor locomotor role. Temporal changes in the functional role of the IT may reflect the varying selective pressures at play during different life-history stages of dolphins. Similar changes may be found in other organisms that face dramatically different functional demands across life-history stages.

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