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Precocial Development of Axial Locomotor Muscle in Bottlenose Dolphins (*Tursiops truncatus*)

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ABSTRACT At birth, the locomotor muscles of precocial, terrestrial mammals are similar to those of adults in both mass, as a percent of total body mass, and fiber-type composition. It is hypothesized that bottlenose dolphins (*Tursiops truncatus*), marine mammals that swim from the instant of birth, will also exhibit precocial development of locomotor muscles. Body mass data from neonatal and adult dolphins are used to calculate Grand's (1992) Neural and Muscular Indices of Development. Using these indices, the bottlenose dolphin is a Condition "3.5" neonate, where Condition 4 is the documented extreme of precocial development in terrestrial mammals. Moreover, myosin ATPase (alkaline preincubation) analyses of the epaxial locomotor *m. extensor caudae lateralis* show that neonatal dolphins have fiber-type profiles very similar to those of adults. Thus, based on mass and myosin ATPase activity, muscle development in dolphins is precocial. However, succinic dehydrogenase and Nile red histochemistry demonstrate that neonatal dolphin muscle has mitochondrial and lipid distributions different from those found in adults. These data suggest that neonates have a lower aerobic capacity than adults. Dolphin neonates may compensate for an apparent lack of aerobic stamina in two ways: 1) by being positively buoyant, with a relatively increased investment of their total body mass in blubber, and 2) by "free-riding" off their mothers. This study investigates quantitatively the development of a dolphin locomotor muscle and offers suggestions about adaptations required for a completely aquatic existence. J. Morphol. 244:203–215, 2000.

KEY WORDS: development; muscle; histochemistry; swimming; dolphin

Mammals can be placed into two broad categories based on the developmental condition in which they are born (Ewer, 1973). Altricial neonates are relatively helpless at birth, while precocial neonates are more fully developed than altricial neonates and are "miniaturized versions" of adults (Ewer, 1973). However, this simple classification system does not account for the diversity in developmental states found across species and even within a single individual. For example, neonates of both golden lion tamarins (*Leontopithecus rosalia*) and white-bearded wildebeests (*Connochaetes taurinus*) are considered precocial, but there are clear and measurable differences in their developmental states (Grand, 1992). The tamarin has a highly developed brain, but a poorly developed locomotor system at birth, whereas the wildebeest has highly developed brain and muscle (Grand, 1992). The little brown bat (*Myotis lucifugus*) is considered altricial because it cannot fly at birth (Powers et al., 1991; Schutt et al., 1994), but this bat has precocially developed leg muscles that allow it to cling to the substrate of the roost (Powers et al., 1991).

The locomotor capabilities of precocial mammals soon after birth are also highly variable. Some precocial animals, as defined by Eisenberg (1981), remain in burrows or cling to their mothers for days to months before independently negotiating their environment (Fig. 1A). Conversely, many precocial ungulates can locomote minutes to hours after birth (Fig. 1A) (Eisenberg, 1981; Nowak and Paradiso, 1983; Kingdon, 1989).

The broad range of both developmental states and locomotor abilities observed at birth reveals the need for more precise and quantitative definitions of the terms altricial and precocial. Grand (1992) used body mass data to create indices of neural and muscular development to quantitatively compare neonates and adults (Fig. 1B). Cobb et al. (1994) quantitatively compared neonatal and adult muscle fiber-

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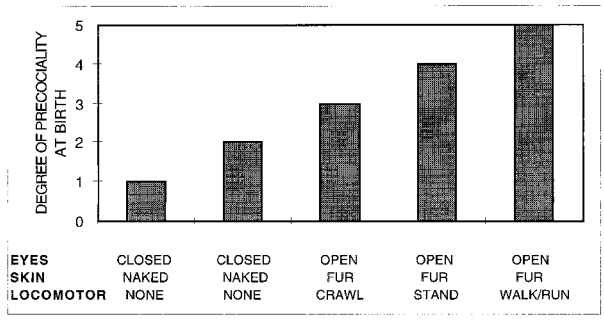

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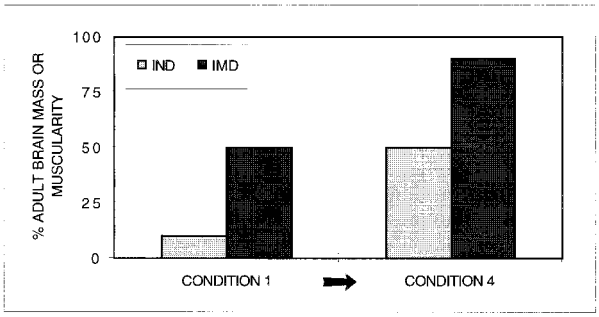

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Fig. 1. Two models of development in mammals. **A**: In Eisenberg's (1981) model, observable, morphological characters and locomotor abilities are used to score neonates on a scale of 1–5 as to their degree of precociality at birth. **B**: In Grand's (1992) model, the x-axis spans from the extremely altricial, Condition 1 developer, to the extremely precocial, Condition 4 developer. The gray bars represent the index of neural development (IND), calculated by dividing the brain mass of the neonate by the brain mass of the same sex adult. The black bars represent the index of muscular development (IMD), calculated by dividing the muscularity of the neonate (total muscle mass/total body mass) by the muscularity of the same sex adult.

type profiles to define altricial and precocial muscle development (Fig. 2) (see also Dubowitz, 1965; Rubinstein and Kelly, 1978; White et al., 1978; Bechtel and Kline, 1987; Wigston and English, 1992; Umezu et al., 1992). These studies demonstrated that muscles of terrestrial, precocial locomotors were similar to those of adults both in mass (as a percentage of total body mass) (Grand, 1992) and in fiber-type profile, as demonstrated by the myosin ATPase assay (Dubowitz, 1965; Gutmann et al., 1974; White et al., 1978; Bechtel and Kline, 1987; Walker and Luff, 1995).

To date, quantitative analyses of precocial locomotion and muscle development have focused on terrestrial vertebrates. However, cetaceans (whales, dolphins, and porpoises) define an extreme in precocial locomotion in that neonates must be effective swimmers at the instant of birth. Although the adult fiber-type profiles of the axial locomotor muscles from two delphinid species (*Tursiops truncatus* and *Delphinus delphis*) have been described to be mixed (Tulsi, 1975; Suzuki et al., 1983; Bello et al., 1985), thus far no study has investigated the histochemical properties of developing dolphin muscle.

The goal of this study is to investigate the developmental state of the locomotor muscles of cetacean neonates. To test the hypothesis that cetacean muscle development is precocial, locomotor and neural tissue mass data will be used to determine the developmental state of bottlenose dolphins (*Tursiops truncatus*) based on criteria published for terrestrial mammals (sensu Grand, 1992). In addition, muscle histochemical data for dolphins will be used to compare the fiber-type profiles of neonates with those of adults.

MATERIALS AND METHODS

Specimens

Fresh carcasses of adult and neonatal bottlenose dolphins (*Tursiops truncatus* Montagu) were collected by the Northeast and Southeast regional marine mammal stranding networks and the National Marine Fisheries Service (NMFS) (Table 1). Sixteen animals were frozen within hours of their stranding

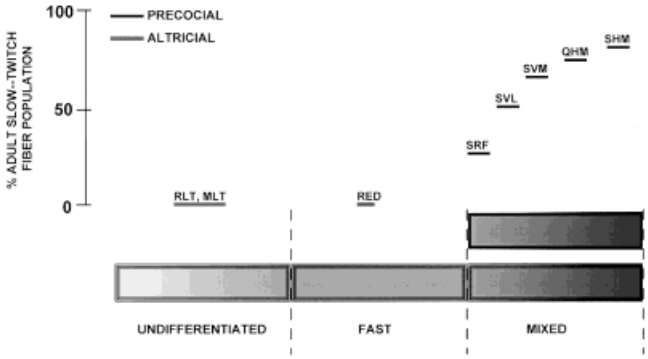

Fig. 2. Development model of a mixed muscle based on fiber-type profile as demonstrated by the myosin ATPase assay. The x-axis is a continuum, representing the sequential developmental states of a muscle as it matures to the adult profile. The y-axis is percent of the adult slow-twitch fiber population exhibited by the neonate. Muscles begin in an undifferentiated state; as they differentiate, all fibers exhibit the histochemical reaction of fast-twitch fibers. Those fibers destined to become slow fibers subsequently change their myosin ATPase reaction to that of slow-twitch fibers. The individual lines on the graph represent 11 muscles from four different species from those studies that quantitatively compare the neonatal and adult profiles. The muscles of altricial and precocial developers differ in where they are along this continuum at birth. In the muscles investigated quantitatively to date, altricial neonates can exhibit an undifferentiated or fast profile (although qualitative descriptions suggest they can also be mixed) (Dubowitz, 1965). Precocial neonates apparently exhibit only a mixed profile. Percentages reported here were calculated as percent slow-twitch fibers by count exhibited by the neonate/percent slow-twitch fibers exhibited by the adult as reported in the literature. MLT, mouse limb and trunk; QHM, quarterhorse middle gluteal; RED, rat extensor digitorum longus; RLT, rat limb and trunk; SHM, standardbred horse middle gluteal; SRF, sheep rectus femoris; SVL, sheep vastus lateralis; SVM, sheep vastus medius (Dubowitz, 1965; Rubinstein and Kelly, 1978; White et al., 1978; Bechtel and Kline, 1987).

TABLE 1. Bottlenose dolphin (Tursiops truncatus) *specimens utilized in this study**

Identification number	Total length (cm)	Sex	Muscle age category[a]
VMSM 961035[b,c]	106.0	M	Neonate
VMSM 961028[b,c]	110.5	M	Neonate
VMSM 971053[b]	112.0	F	Neonate
VMSM 93146[b]	115.0	M	Neonate
VMSM 961053[b,c]	115.0	F	Neonate
VMSM 921016[b]	127.0	M	Neonate
MMSC 92-11[b]	202.2	M	Adult
KMT 013[b,c]	204.0	F	Adult
NEFC 016[b]	208.5	F	Adult
VGT 087[b]	209.8	F	Adult
VGT 155[b]	216.0	M	Adult
KMT 100[c]	219.0	F	Adult
KR 001[b]	223.0	M	Adult
KMT 023[b]	223.5	F	Adult
VMSM 951045[b]	239.0	F	Adult
VGT 168[b]	247.0	F	Adult
VGT 049[b]	253.0	F	Adult
VMSM 921006[b]	265.0	F	Adult
VMSM 951035[b]	267.0	F	Adult
WAM 530[c]	268.0	M	Adult
VMSM 951051[b]	278.0	M	Adult

[a]Neonate category (defined in Table 2); adult category (animals > 200 cm in length).
[b]Specimen used in mass analyses.
[c]Specimen used in histochemical analyses.
*Specimens are identified by their collector's field number.

and remained frozen for up to several months before thawing and dissection. The remaining animals were either dissected within 3–5 h of their stranding, or placed in ice baths and dissected within 24 h.

Neonatal dolphins, all less than 132 cm in length (Mead and Potter, 1990; Read et al., 1993), were aged using a suite of neonatal characteristics derived from the literature (Table 2). All neonates utilized in this study had floppy dorsal fins and tailflukes, suggesting that they were under 2 weeks of age (Mcbride and Kritzler, 1951). Dolphins greater than 200 cm were considered to exhibit mature muscle characteristics; however, these specimens represented both sexually mature and immature animals (Mead and Potter, 1990; Read et al., 1993).

Body Mass

All dolphins (n = 19) were dissected following a body mass compartmentalization protocol (Rommel et al., 1991; McLellan et al., 1995). The subsets of data used for this study were total body mass, brain mass, and total axial locomotor muscle mass (epaxial and hypaxial muscles as defined by Pabst, 1990) (Table 3). The index of neural development (IND) was calculated by dividing the mean brain mass of neonates by the mean brain mass of adults. The index of muscular development (IMD) for dolphins was calculated by dividing the mean muscularity (total axial locomotor muscle mass / total body mass) of neonates by the mean muscularity of adults. In this study, males and females were combined, rather than doing same sex comparisons (Grand, 1992), because of the small sample size of neonates (n = 6). Further, only axial locomotor muscle mass was used because of the reproducibility of our dissection protocol. To remove the effects of blubber mass from the locomotor muscle analysis, values of lean IMD were calculated using dolphin lean body mass (total body mass - total blubber mass).

Muscle Histochemistry

Muscle samples were collected from six dolphins: two adult females, one adult male, one neonatal female, and two neonatal males (Table 1). Approximately 4.0-cm thick cross-sections of the caudal extension of the *m. longissimus*, the *m. extensor caudae lateralis* (ECL) were removed at the level of the anus (Fig. 3A). This body site was chosen because it undergoes the largest range of flexion and extension during swimming (Pabst, 1993, 1996; Long et al., 1997). In addition, the ECL and the *m. extensor caudae medialis* (ECM) at this site separate into two discrete muscle masses (Pabst, 1990, 1993) (Fig.

TABLE 2. Neonatal characters used to age bottlenose dolphin (Tursiops truncatus) *specimens*

Neonatal characteristic	Adult characteristic	Age at which neonatal character is lost	Reference
Unhealed umbilicus	Healed umbilicus	day 22	Cockcroft and Ross (1990)
	small scar	day 44	
Rostral hairs	No rostral hairs	day 22	Cockcroft and Ross (1990)
		<1 month	Mcbride and Kritzler (1950)
Fetal folds	Lightly pigmented lines	day 22	Cockcroft and Ross (1990)
	no fetal folds	day 62	
No teeth in either jaw	First maxillary teeth	day 91	Cockcroft and Ross (1990)
	teeth in both jaws	day 163	
Prominent 'neck'	No 'neck'	day 30	Cockcroft and Ross (1990)
Floppy dorsal fin	Stiff dorsal fin	2 weeks	Mcbride and Kritzler (1951)
		few hours	Tavolga and Essapian (1957)
Floppy tailflukes	Stiff tailflukes	2 weeks	Mcbride and Kritzler (1951)

TABLE 3. Mass data for bottlenose dolphins (Tursiops truncatus)

Specimen	Body mass (g)	Locomotor muscle mass (g)	Brain mass (g)	Blubber mass (g)
Neonate category*				
VMSM 961035	14,300	2,190	647	4,233
VMSM 961028	20,500	3,160	672	4,850
VMSM 971053	18,600	3,610	582	4,517
VMSM 931046	20,410	3,519	N/E	5,216
VMSM 961053	18,864	3,586	N/E	N/E
VMSM 921016	26,600	5,510	597	6,625
Adult category*				
MMSC 92-11	75,200	22,160	1,547	17,073
KMT 013	98,200	24,344	1,260	25,640
NEFC 016	114,300	40,213	N/E	18,725
VGT 087	122,727	33,117	1,585	25,428
VGT 155	186,363	38,220	1,510	32,630
KR 001	149,000	41,405	1,667	27,555
KMT 023	150,400	43,805	1,383	34,363
VMSM 951045	126,400	29,156	1,330	24,387
VGT 168	164,653	34,467	N/E	38,577
VGT 049	254,545	55,500	1,540	56,250
VMSM 921006	210,455	49,504	1,787	40,200
VMSM 951035	199,000	53,400	N/E	44,780
VMSM 951051	196,000	46,005	1,480	34,029

*Neonate category (defined in Table 2); adult category (animals > 200 cm in length).

3B). This separation permitted definitive sampling of the ECL in both neonates and adults. The cross-sections of the ECL were placed into plastic bags, frozen flat at -20°C, and stored at either -20°C or -80°C.

The entire cross-section was partitioned into approximately 1.0 cm × 1.0 cm blocks, yielding 8–9 sample sites for adults and 3–4 sites for neonates (Fig. 3C). To investigate regional variation in fiber-type profile, one adult muscle (KMT 013) was sampled across the entire cross-section (Fig. 3C, Protocol A). A comparison of the distributions of percent slow-twitch fibers (by area) for the entire cross-section vs. a grid that sampled the height of the central section of the muscle demonstrated that the mean percent values were statistically similar. Thus, the central section of the muscle was considered to be representative of the entire cross-section, and the remaining adults were sampled using this protocol (Fig. 3C, Protocol B). All neonatal muscles were sampled across the entire cross-section. Tissue blocks from each site were serially sectioned (10.0 μm thickness) in a cryostat (Leica Cryocut 1800) at -19°C and mounted on glass slides.

Muscle sections were stained for myosin ATPase (Guth and Samaha, 1970; Riley et al., 1992). For adult tissue, one series of serial sections was fixed for 5 min at 4°C in 5% paraformaldehyde, 68 mM $CaCl_2$, 130 mM sodium cacodylate, 340 mM sucrose (pH 7.6) and then preincubated in an alkaline medium for 15 min (18 mM $CaCl_2$, 100 mM 2-amino-2-methyl-1-propanol, pH 10.3) at 25°C. Alternate serial sections were preincubated in an acidic medium for 1 min (50 mM KAc, 18 mM $CaCl_2$, pH range 4.15–4.35). Neonatal tissue required some modification of the alkaline protocol: unfixed tissue was used and the preincubation time in alkaline buffer was reduced to 10 min. No modification of the acid preincubation protocol was required. All samples were then incubated for 30 min in a freshly prepared ATP solution (18 mM $CaCl_2$, 100 mM 2-amino-2-methyl-1-propanol, 50 mM KCl, 3 mM ATP, pH 9.4) at 37°C. Additional serial sections from each site were stained for succinic dehydrogenase (SDH) (Nachlas et al., 1957) to assess oxidative potential and with Nile red to quantify lipids (Fowler and Greenspan, 1985; Santilli et al., 1989).

Fibers were classified as type I (slow-twitch) or type II (fast-twitch) on the basis of acidic and basic preincubation protocols for myosin ATPase following the classification scheme of Brooke and Kaiser (1970). Fibers were also identified as type I or type II by their SDH activity (Peter et al., 1972).

Immunocytochemistry

To verify the accuracy of the histochemical results for neonates, immunocytochemistry on additional serial sections at one site per cross-section was performed using a primary antibody to fast myosin. Peroxo-Block (Zymed Laboratories, San Francisco, CA) was applied to the sections to quench endogenous peroxidase activity before Serum Blocking Solution (Zymed) was introduced. The sections were then incubated with the primary antibody (MY-32, Zymed) in a humidity chamber at 25°C for 60 min. Secondary antibody labeling and staining were conducted subsequently using standard protocols (Zymed Histostain™-Plus Kit).

Data Collection and Statistical Methods

Images of the sections were projected onto a Sony Trinitron color video monitor using an Olympus

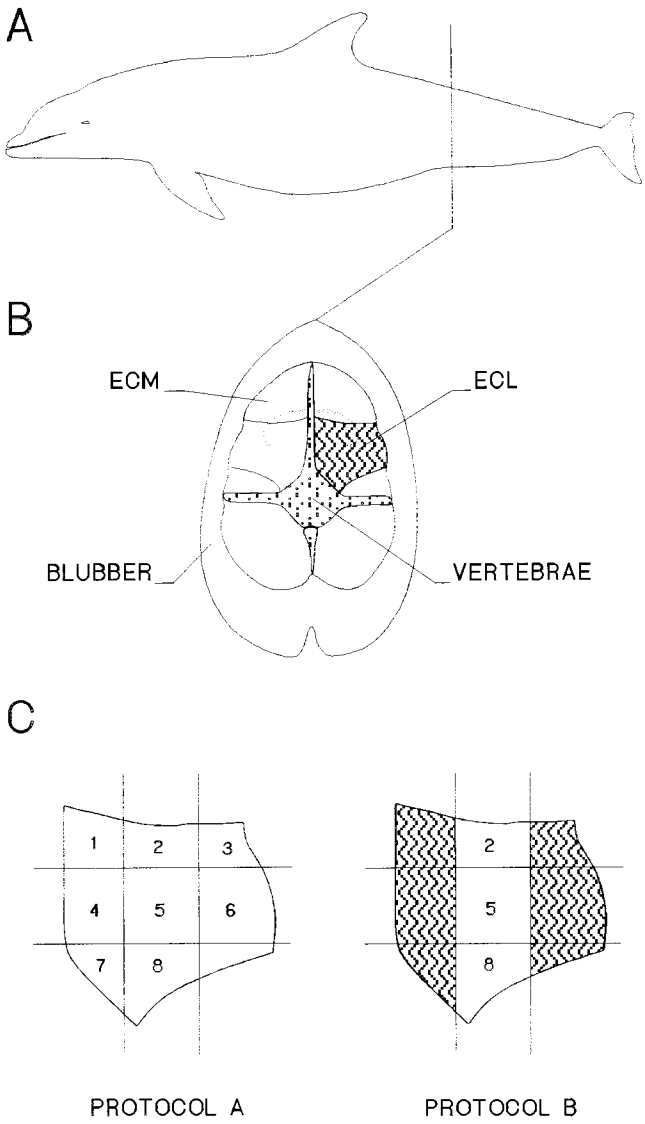

Fig. 3. Schematic diagram of muscle sampling location and cross-section sampling protocol in a bottlenose dolphin (*Tursiops truncatus*). A cross-section of the axial muscle mass was taken at the level of the anus (**A**), and the *m. extensor caudae lateralis* (ECL) was separated from the other axial muscles (**B**). The cross-section of the ECL was partitioned into approximately 1.0 × 1.0 cm sites (**C**). For one adult (KMT 013) and all neonates, the entire cross-section was sampled (Protocol A). This protocol resulted in 9 sites for KMT 013 (pictured here) and 3–4 sites in the smaller neonates. The muscles of the other adults were subsampled along a central 1.0-cm wide strip from the dorsal to the ventral surface of the ECL (represented as sites 2, 5, and 8 in Protocol B). This subsample included 3–5 sites, depending on the size of the entire cross-section. (ECM, *m. extensor caudae medialis*). Drawing produced using EasyCAD (Evolution Computing, Tempe, AZ).

BH-2 light microscope and a Hamamatsu C2400 video camera system. Sections from adults were viewed at a final magnification of 66×, while neonates were viewed at 132×. To determine the percentage of area occupied by type I and II fibers, a Mertz-curvilinear test system was placed onto the video screen and the points residing in each fiber type (P_I and P_{II}) and in the white space (P_S) were counted (Russ, 1986). This process was repeated for each cross-section (usually n = 4) at each site until the least represented fiber-type was counted 100 times. To calculate the percentage area occupied by each fiber-type, P_S was subtracted from the total number of points counted, P_T. The resulting value calculated was equal to the number of points residing in the muscle tissue, P_M. The points residing in each fiber-type were then divided by P_M and multiplied by 100%, resulting in the percent area for each fiber-type, where $P_P = A_A$ (P_P - points per unit point [point fraction]; A_A - area per unit area [area fraction] and $A_A \times 100$ = area percentage [Russ, 1986]). For example: $P_T - P_S = P_M$ and $P_I/P_M \times 100\%$ = area percentage of type I fibers. These percentages of the area were corrected for errors caused by sections of finite thickness using the following formula: $A_{A'} = A_A + S_V \cdot t/4$ where $S_V = 2\ P_L$ (points per line) and t is the section thickness (10.0 μm) (Russ, 1986). All the corrected area values from each cross-section for each site were then averaged for the site to obtain the percent areas used for analyses. These percentages were obtained for all treatments (acid and alkaline preincubation, and SDH) (Table 4).

Alkaline preincubated (adult and neonate) slides from each site in the subsampling protocol (Fig. 3C, Protocol B) were examined to obtain the number of fibers of each type on one cross-section by placing a 10.0 × 10.0 cm grid onto the video screen. The number of each type of fiber within the square was counted, and this process was repeated on each section until 150 fibers were counted. The numbers of fibers of each type were then summed, divided by the total number of fibers, and multiplied by 100% to express fiber number as a percentage of the total number of fibers.

Images of one cross-section from each site from neonates (alkaline slides) and the subsampled sites of adults (SDH and alkaline slides) were digitized (Image-1/Metamorph Imaging System, Universal Imaging Corp., West Chester, PA) to measure fiber diameters (NIH Image, National Institutes of Health, Bethesda, MD). The fibers chosen for analysis had relatively circular cross-sections, and their size was not qualitatively different from other fibers of the same type in the area. For each fiber, the diameter was calculated as the mean of four measurements taken as diagonals across the cross-section. Ten fibers of each type were measured and their diameters were averaged for analysis.

Histochemical datasets were statistically analyzed using SAS release 6.11 and JMP version 3.2 (SAS Institute, Cary, NC). Muscle data from one adult animal (KMT 013) were graphically analyzed to assess the feasibility of using the subsampling protocol (Fig. 3C, Protocol B), rather than the entire cross-section for the adult muscles. Adult (subsampling protocol) and neonatal (entire cross-section) slow-twitch percent areas (alkaline preincubation)

TABLE 4. Summary of the number of observations obtained by animal, site, and treatment

Specimen	# of Sites	Treatment			Totals
		Acid	Alkaline	SDH	
Neonate category*					
VMSM 961028	3	1	11	N/E	12
VMSM 961035	4	16	17	N/E	33
VMSM 961053	4	20	16	N/E	36
Totals	11	37	44	N/E	81
Adult category*					
KMT 013	8	33	32	47	112
KMT 100	5	8	11	11	30
WAM 530	7	6	23	23	52
Totals	20	47	66	81	194

*Neonate category (defined in Table 2); adult category (animals > 200 cm in length).

were then compared. Analyses were performed to compare the acid and alkaline preincubation profiles of all six muscle samples and the alkaline and SDH profiles of the three adults.

RESULTS

Body Mass

The mean values calculated for IND and IMD for neonatal dolphins were 41% and 72%, respectively (Fig. 4). The dolphin's IND of 41% is similar to those calculated from a compilation of published bottlenose dolphin brain masses, which ranged from 41–44% IND (Ridgway et al., 1966, 1987; Ridgway and Brownson, 1979; Ridgway, 1990; Tarpley and Ridgway, 1994). Removing blubber from the total mass increased the mean IMD from 72 to 78% (Fig. 4). The calculated IND and IMD values placed the bottlenose dolphin between the tamarin (*Leontopithecus rosalia*), a Condition 3 developer, and the wildebeest (*Connochaetes taurinus*), the extremely precocial, Condition 4 developer (Grand, 1992). Thus, based on body mass data the dolphin can be classified as a Condition "3.5" developer.

Muscle Histochemistry

There was regional variation in fiber-type profile across the cross-section of the adult (KMT 013) (Table 5). The largest differences were found at the medial boundary of the section, nearest to the vertebrae (Sites 1 and 7) (See Fig. 3C, Protocol A). The distributions of percent slow-twitch fibers (by area) for the entire cross-section and the subsample were found to be statistically similar, and the variability in the subsample values was much less (Fig. 5). Thus, the subsample data from the muscles of animals in the adult category were used in subsequent analyses.

Percentages of fiber-type areas of adult and neonatal dolphins, as demonstrated by the alkaline preincubation protocol of the myosin ATPase assay (n = 79 measurements) (see Fig. 6), overlapped and had similar variation (Table 6, Fig. 7A,B). Neonates show about a 2% lower mean percentage of slow-twitch fibers than the adult mean, but this difference is not statistically significant (Wilcoxon rank sum test $n_{adults} = n_{neonates} = 3$, $P = 4.0$).

If the data from the acid and alkaline preincubation protocols are paired for each animal, the difference between the two treatments is not significant (Wilcoxon signed-rank test: n = 6, $P = 0.69$). Similarly, the alkaline preincubation protocol and the SDH assay were not significantly different for the three adults (Wilcoxon signed-rank test: n = 6, $P = 0.50$).

One neonate (VMSM 961035) showed a fiber population (approx. 13% of the area) that stained darkly with both acid and alkaline preincubation protocols (Table 7). These dark fibers were classified as type IIc (Brooke and Kaiser, 1970). Fibers of neonatal dolphin muscle also were not differentially stained by the SDH assay (Fig. 6F). The staining intensity of

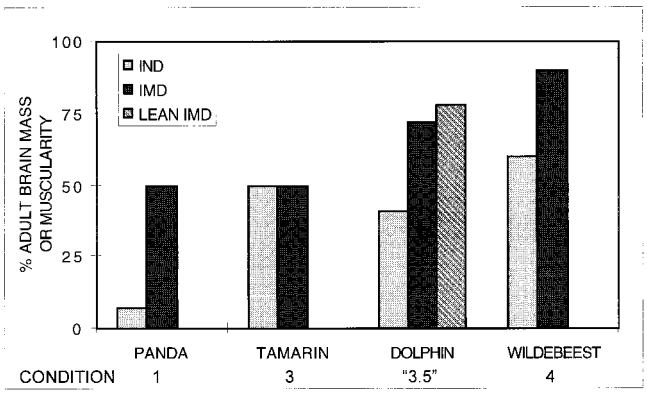

Fig. 4. The developmental state of the bottlenose dolphin (*Tursiops truncatus*) in comparison to the developmental states of the terrestrial mammals investigated by Grand (1992). The x-axis spans from the extremely altricial, Condition 1 red panda (*Ailurus fulgens*) to the extremely precocial wildebeest (*Connochaetes taurinus*). Based on percent muscularity, the dolphin is placed between the wildebeest and the tamarin (*Leontopithecus rosalia*). Thus, the dolphin's developmental state is termed Condition "3.5." IND, index of neural development; IMD, index of muscular development; lean IMD, lean (total body mass - blubber mass) index of muscular development.

TABLE 5. Fiber-type composition by area across the cross-section of the m. extensor caudae lateralis *at the level of the anus of a bottlenose dolphin* (Tursiops truncatus) *in the adult muscle category (KMT 013)*[a]

Site[b]	% Type I (slow-twitch) fibers[c]	S.D. (n)[d]	% Type II (fast-twitch) fibers[c]
1	14.0	1.4 (2)	86.0
2[e]	24.0	4.0 (3)	76.0
3	21.0	3.6 (3)	79.0
4	24.7	1.5 (3)	75.3
5[e]	28.3	1.6 (6)	71.7
6	27.6	2.9 (5)	72.4
7	35.8	2.6 (4)	64.2
8[e]	27.7	2.0 (6)	72.3
Total cross-section	26.7	5.6 (32)	73.3
Subsampling protocol	27.2	2.7 (15)	72.8

[a]Myosin ATPase alkaline preincubation results reported.
[b]Site locations indentified in Figure 3C (Protocol A).
[c]Muscle fiber-type expressed as mean.
[d]Standard deviation and number of cross-sections per site used in calculations.
[e]Sites included in subsampling protocol (Fig. 3C, Protocol B).

the neonatal muscle was qualitatively less than the staining of either the adult fast- or slow-twitch fibers (Fig. 6E).

Lipid was found predominantly in the slow-twitch fibers of adults (Fig. 8A). However, a comparison of Nile red stained sections with ATPase stained-sections showed that there were also some fast-twitch fibers that contained high concentrations of lipid droplets. Lipid was also found in similar densities in both fiber-types in neonatal muscle (Fig. 8B).

The muscle fiber diameters of neonates were smaller than those of adults (see Fig. 6). Neonatal type I fibers averaged 12.7 μm in diameter and type II fibers averaged 16.1 μm. Adult type I and type II fibers were 37.6 and 54.5 μm in diameter, respectively. The difference in size between type I and type II fibers is smaller in the neonate than in the adult (Table 6). The small size of the neonatal fibers resulted in a slight difference between neonates and adults in the percentage of each fiber-type by count.

Immunocytochemistry

Immunocytochemical reactions showed two populations of fibers in neonatal and adult muscle, which supports the results of the myosin ATPase assay. Stereology and fiber-count data collection were hindered by the loss of tissue integrity during the immunocytochemical assay. This procedure resulted in sections that were three-dimensional when viewed at the magnifications required for stereological analyses and fiber-count data collection (adults: 66×; neonates: 132×). Therefore, no quantitative data were collected from these sections.

DISCUSSION

The locomotor muscles of the bottlenose dolphin, based on both relative mass and myosin ATPase fiber-type profile, are precocially developed. Mass data show the dolphin to be a Condition "3.5" developer, with an IMD of 72%. Additionally, their mean fiber-type profile is 24.2% slow-twitch by area, which is similar to that of adults (Fig. 7). To place dolphins within the muscle fiber-type development model (see Fig. 2), the mean percent slow-twitch fibers by count was calculated (Table 6). This value (38.2%) was 95% of the adult profile (Fig. 9). Thus, the ECL of neonatal dolphins is the closest in fiber-type profile to that of the adult of any mixed muscle investigated quantitatively to date (Fig. 9) (Dubowitz, 1965; Rubinstein and Kelly, 1978; White et al., 1978; Bechtel and Kline, 1987). However, quantitative differences

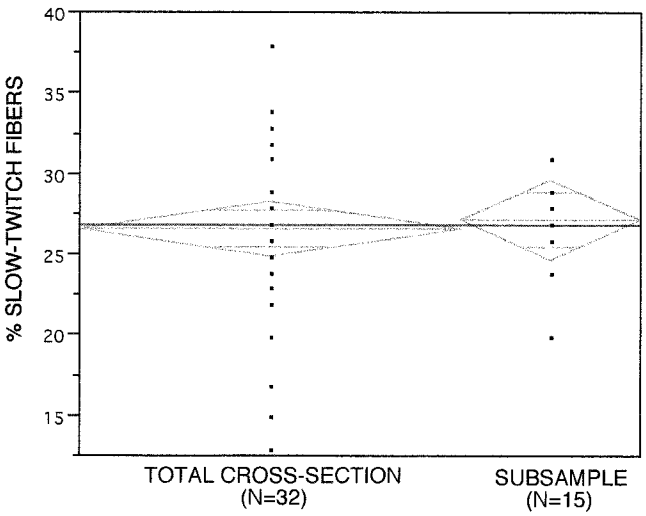

Fig. 5. Comparison of percent slow-twitch fibers by area of total cross-section vs. subsample of the *m. extensor caudae lateralis* of a bottlenose dolphin (*Tursiops truncatus*) in the adult muscle category (KMT 013). Results were based on the alkaline preincubation protocol of the myosin ATPase assay. Note that these datasets have similar means and that the variability in the subsample values is much less. Thus, histochemical data for adults used in subsequent analyses were derived from the subsampling protocol.

TABLE 6. Mean fiber-type percentages (± SD) and mean ratio of type II (fast-twitch) to type I (slow-twitch) fiber diameters in the m. extensor caudae lateralis *of bottlenose dolphins* (Tursiops truncatus)[a]

Specimen	% Type I fibers by area (n)[b]	% Type I fibers by count	Dia II:I
Neonate category[c,d]			
VMSM 961035	23.3 ± 2.2 (17)	39.8 ± 4.1	1.30
VMSM 961028	25.2 ± 1.9 (11)	37.0 ± 2.0	1.30
VMSM 961053	24.4 ± 4.2 (16)	37.5 ± 3.1	1.20
Mean	24.2 ± 3.1 (44)	38.2 ± 3.2	1.30
Adult category[c,e]			
KMT 013	27.2 ± 2.7 (15)	46.0 ± 8.1	1.80
KMT 100	23.6 ± 3.9 (5)	31.0 ± 5.0	1.60
WAM 530	27.3 ± 2.5 (15)	44.0 ± 5.7	1.50
Mean	26.7 ± 3.0 (35)	40.2 ± 9.1	1.50

[a]Myosin ATPase alkaline preincubation results reported.
[b]Total number of cross-sections for all sites.
[c]Neonate category (defined in Table 2); adult category (animals > 200 cm in length).
[d]Means calculated from entire cross-section protocol.
[e]Means calculated from subsampling protocol (Fig. 3C, Protocol B).

exist between the developmental indices of dolphins and those of Grand's (1992) precocial, terrestrial mammals, and between the muscle fiber-type profiles of neonates and those of adults. Functional interpretations of these differences are discussed below.

Body Mass

Although the dolphin must be able to swim from the moment of birth, it seems to invest less of its total body mass in brain and muscle than the extremely precocial wildebeest (Grand, 1992) (Fig. 4). However, the brain mass of neonatal dolphins falls within the range predicted for precocial animals when compared to other animals with similar-sized mothers (Pagel and Harvey, 1990). Thus, although the dolphin's IND is less than that of the tamarin and wildebeest (Fig. 4), it is within the range predicted for precocial mammals (Pagel and Harvey, 1990).

The differences in muscularity between wildebeests and dolphins (Fig. 4) may reflect differences in their environments. The muscles of neonatal wildebeests must function both to create locomotor movements and to do work against gravitational forces. On the other hand, dolphins live in an environment where the buoyant force of water supports their body mass. Because of their environment, neonatal dolphins may not require as large an investment in muscularity as terrestrial, precocial neonates.

There is also anecdotal evidence that neonatal dolphins are positively buoyant. They tend to "pop up" at the surface instead of exhibiting the smooth, surfacing behavior of adults (Cockcroft and Ross, 1990). Interestingly, there is morphological evidence to support this hypothesis, as more of the neonatal dolphin's body mass is invested in blubber in comparison to the adult (26% vs. 20%) (Table 3). This large investment in blubber may make neonates positively buoyant and reduce their locomotor costs to remain at or near the surface.

Dolphins may also take advantage of fluid flow regimes that could further decrease their cost of locomotion. Neonatal dolphins apparently have the ability to "free-ride" (Lang, 1966) off their mother. This behavior has been documented in both captive (Mcbride and Kritzler, 1951; Tavolga and Essapian, 1957; Cockcroft and Ross, 1990) and free-ranging dolphin populations (Smolker et al., 1993). During "free-riding" behavior, the neonatal dolphin moves very close to the lateral flank of its mother or another dolphin, and noticeably reduces its tailbeat frequency while moving forward with the other animal (Norris and Prescott, 1961). In this position, the dolphin apparently does not need to provide all of its own locomotory forces (Prescott, 1977). Thus, neonatal dolphins may not require the high muscularity found in precocially locomoting, terrestrial mammals, such as the wildebeest, because of their buoyancy and the hydrodynamic benefits of "free-riding."

Muscle Histochemistry

The myosin ATPase assay demonstrates that there are two populations of fibers within neonatal muscle and that the fiber-type profile of neonates is similar to that found in adults (Table 6, Fig. 7). However, muscle fibers of neonatal dolphins differ from those of adults in the distribution of mitochondria and lipid content (Figs. 6, 8). These results suggest that neonates have a lower aerobic capacity than adults. In terrestrial mammals, a lower aerobic capacity relates to decreased locomotor stamina. Thus, precocial, terrestrial neonates cannot locomote at high speeds for long periods of time (Estes, 1991; Carrier, 1994/1995).

In dolphins, a decreased aerobic capacity affects both locomotor stamina and the ability to breath-hold dive. "Free-riding" behavior may compensate for the apparent decreased locomotor stamina of neonatal dolphins. Although no study of the diving

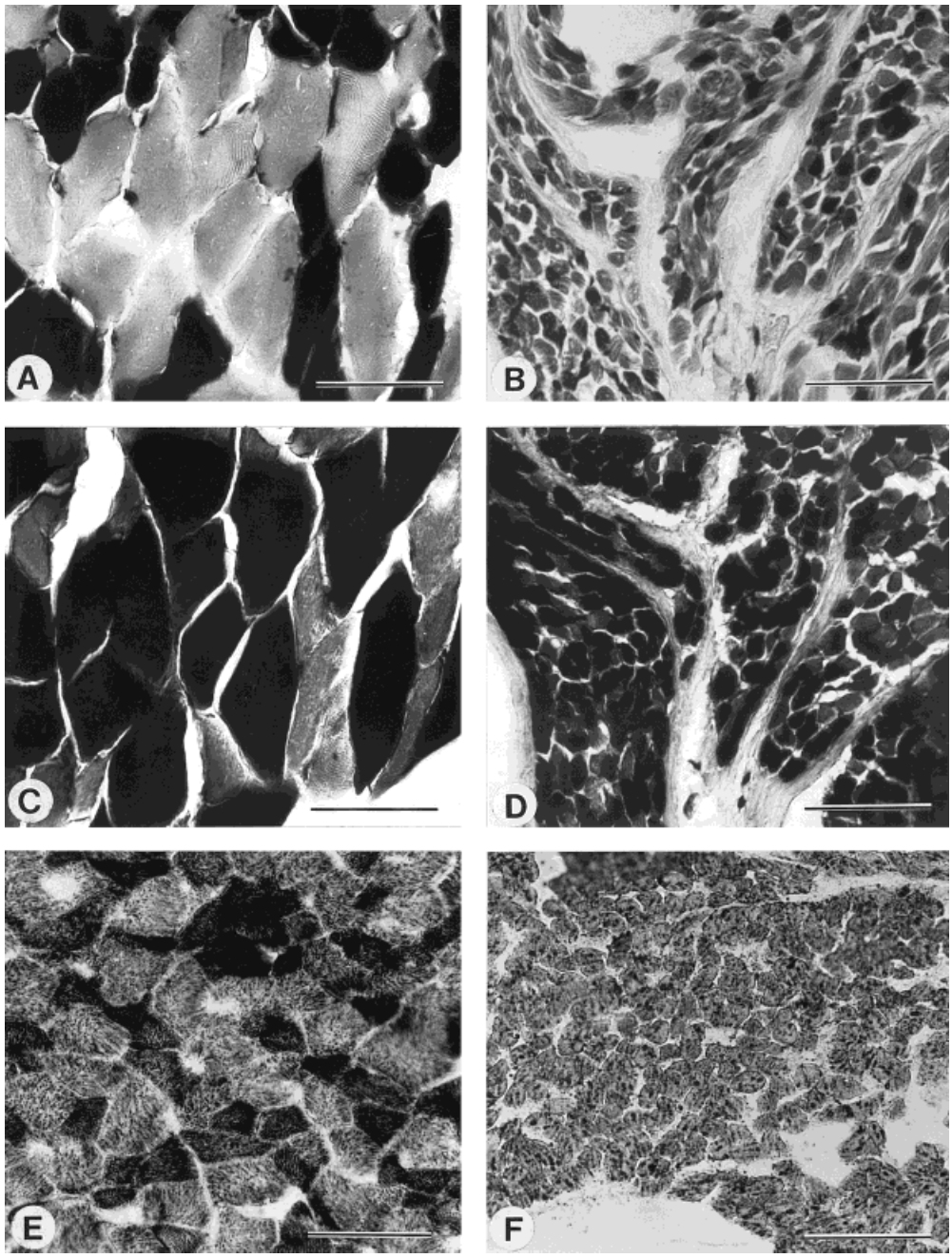

Fig. 6. Representative cross-sections of *m. extensor caudae lateralis* (ECL) from bottlenose dolphins (*Tursiops truncatus*) after histochemical staining. **A,C**, and **E**: Adult muscle (WAM 530, **A**,**C**; KMT 013, **E**). **B,D**, and **F**: Neonatal muscle (VMSM 961035). The ECL was stained for myosin ATPase activity after acidic (**A,B**) and alkaline preincubation (pH 10.3) (**C,D**) and for its succinic dehydrogenase activity (**E,F**). In adult muscle, type I (slow-twitch) fibers appear dark in **A** and **E**. Type I fibers of neonatal muscles are dark in **B**, but there is no difference in staining intensity between type I and type II (fast-twitch) fibers when the muscles are assayed for SDH activity (**F**). Scale bar = 100 μm.

behavior of neonatal dolphins was found, there is circumstantial evidence that neonates are limited in their diving abilities. In the Eastern Tropical Pacific (ETP), lactating female spotted dolphins (*Stenella attenuata*) have a diet that differs from that of pregnant females (Bernard and Hohn, 1989). Pregnant females feed on squid, which is the main prey of spotted dolphins in the ETP. To feed on these animals, dolphins are required to dive to moderate depths. However, lactating females feed on flying fish. Flying fish have a higher caloric density than squid and may be a preferential food source during the energetically demanding lactation period. An alternative hypothesis posed by Bernard and Hohn (1989) is that lactating females feed on flying fish because they need to remain at the surface with their dependent calves. This hypothesis implies that a calf is not able to dive with its mother to the depths that would be required for her to feed on squid and supports the findings that neonates have a lower aerobic capacity than adults.

Characterizing Muscle Development

To adequately characterize muscle development, five properties of neonatal muscle should be inves-

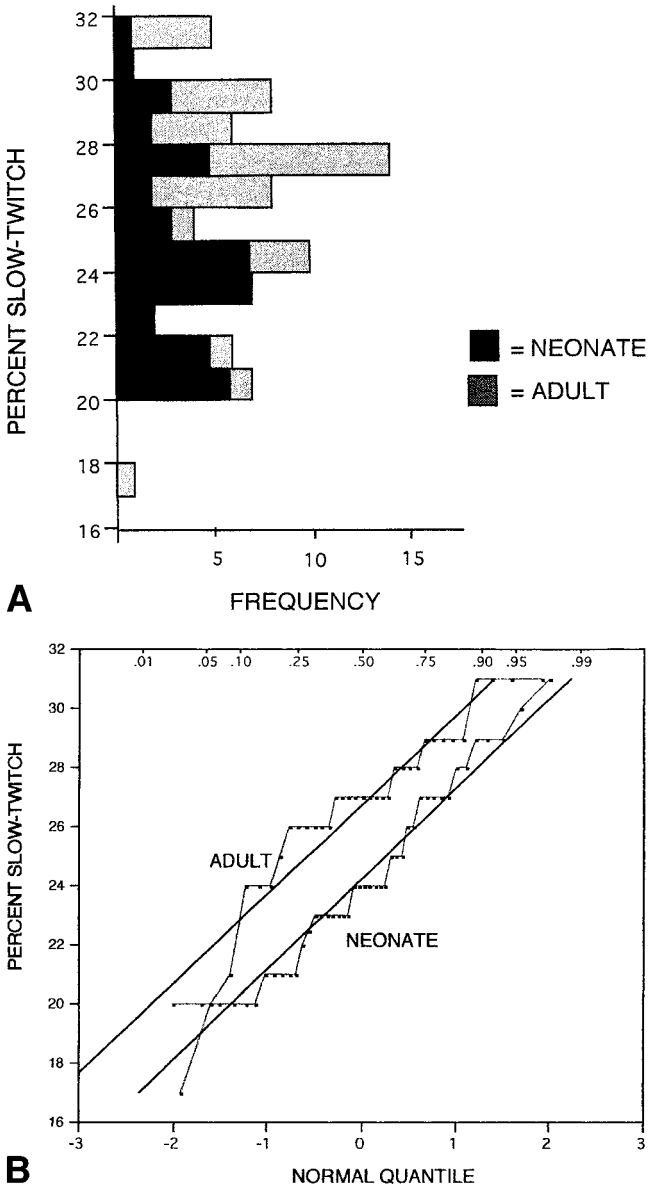

Fig. 7. Comparison of percent slow-twitch fibers by area in the *m. extensor caudae lateralis* of adult (animals >200 cm in length) vs. neonatal (defined in Table 2) bottlenose dolphins (*Tursiops truncatus*). Results were based on the alkaline preincubation protocol of the myosin ATPase assay (n = 79) **A**: The distributions of adult and neonatal slow-twitch area percentages overlap. Note that in neonatal muscle, there is higher frequency of lower percentage values. **B**: Note the similar variability in adult and neonatal distributions and that the average values for neonates are approximately two percentage points less than adults.

tigated: 1) relative mass (e.g., Grand, 1992), 2) histochemistry, 3) immunocytochemistry, 4) biochemistry (e.g., Cobb et al., 1994), and 5) contractile properties (e.g., Reiser et al., 1988; Walker and Luff, 1995). This study examined three of these five characteristics of neonatal muscle. Although the fiber-type profiles of neonates and adults were remarkably similar (Table 6, Fig. 7), the findings below suggest that the functional significance of these results should be interpreted with caution.

The myosin ATPase assay used here does not discriminate between 1) adult fast-oxidative-glycolytic (FOG) and fast-glycolytic (FG) fibers, or 2) fetal, neonatal, or adult myosins (Cobb et al., 1994; Picquet et al., 1997). The mean percent area of slow-twitch fibers for these three adults was calculated to be 32.0% by the SDH assay in comparison to the 26.7% resulting from the myosin ATPase assay (alkaline preincubation) (Table 6). This result suggests that a small percentage of the fast-twitch fibers by area have high densities of mitochondria and are probably FOG fibers. Discrimination between oxidative and glycolytic fast-twitch fibers was not possible in the neonatal muscle because distinct differential staining of the fibers for SDH did not occur.

In the muscle of one neonate (VMSM 961035), the acid and alkaline preincubation protocols revealed two different fiber-type profiles (Table 7), suggesting the presence of either a neonatal or fetal form of myosin. These profiles were apparently the result of a population of cross-reacting (IIc) fibers (Brooke and Kaiser, 1970). Previous research has documented that IIc fibers have fetal and/or neonatal forms of myosin (Cobb et al., 1994; Picquet et al., 1997). The lack of cross-reactivity in the ECL of the other neonates suggests that their muscle fibers contained adult myosins.

Immunocytochemistry attempted to confirm the identity of the myosin isoforms found in neonatal dolphin muscle fibers. Although the application of this method to the neonatal muscle demonstrated two fiber populations, the MY-32 antibody used cross-reacts with all age-classes of myosins (Schutt et al., 1994). Thus, neither of the methods utilized in this study

TABLE 7. Comparison of alkaline and acid data from m. extensor caudae lateralis *of bottlenose dolphins* (Tursiops truncatus)

	% Type I fibers by area[a]	
Specimens	Alkaline	Acid
Neonate category[b,c]		
VMSM 961035	23.3 ± 2.2 (17)	36.0 ± 2.5 (16)
VMSM 961028	25.2 ± 1.9 (11)	24.0 ± . . . (1)
VMSM 961053	24.4 ± 4.2 (16)	25.4 ± 2.7 (20)
Mean	24.2 ± 3.1 (44)	30.0 ± 5.9 (37)
Mean[d]	24.7 ± 3.4 (27)	25.4 ± 2.7 (21)
Adult category[b,e]		
KMT 013	27.2 ± 2.7 (15)	26.8 ± 5.1 (10)
KMT 100	23.6 ± 3.9 (5)	22.0 ± 5.1 (4)
WAM 530	27.3 ± 2.5 (15)	26.0 ± 1.4 (2)
Mean	26.7 ± 3.0 (35)	25.5 ± 5.0 (16)

[a]Muscle fiber-type expressed as the mean % ±SD (number of observations).
[b]Neonate category (defined in Table 2); Adult category (animals > 200 cm in length).
[c]Means calculated from entire cross-section protocol.
[d]Mean with VMSM 961035 data removed.
[e]Means calculated from subsampling protocol (Fig. 3C, Protocol B).

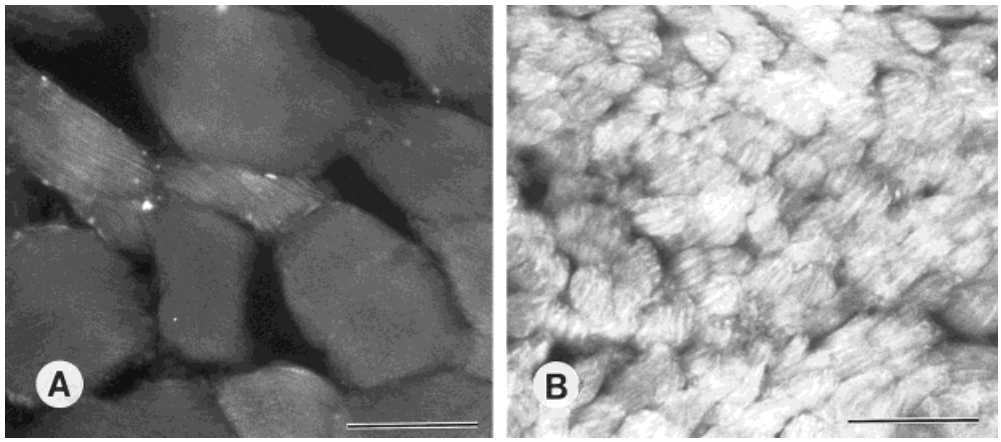

Fig. 8. Representative cross-sections of *m. extensor caudae lateralis* from bottlenose dolphins (*Tursiops truncatus*) after staining with Nile red. In adult muscle (WAM 530) (**A**), lipid was found primarily in the smaller, type I (slow-twitch) fibers (stained brightly). However, in neonatal muscle (VMSM 961035) (**B**), lipid was found in both fiber-types in similar densities. Scale bar = 100 μm.

allows for a definitive characterization of the myosin isozymes in dolphin neonatal muscle fibers.

The neonatal fiber-type profile described here may not reflect the contraction speeds of the muscle (Gutmann et al., 1974; Kelly and Rubinstein, 1980; Reiser et al., 1988; Walker and Luff, 1995). Neonatal muscles with a fiber-type profile dominated by slow-twitch fibers can have contraction times that are faster than in the adult (Kelly and Rubinstein, 1980; Reiser et al., 1988; Walker and Luff, 1995). Conversely, neonatal fast-twitch muscle contraction times are slower than in adult muscles (Walker and Luff, 1995). Thus, although the precocial, neonatal dolphin has a fiber-type profile that is similar to that of the adult, neonatal muscle fibers may not have developed contraction times similar to adults.

CONCLUSION

This study offers insights into the adaptations required for a completely aquatic lifestyle. Cetaceans and sirenians, the only completely aquatic marine mammals, have large, precocially locomoting young. This shared developmental strategy suggests that having a precocially developed locomotor system at birth is required for a mammal to be fully aquatic. In this study, data on muscle mass and histochemistry in bottlenose dolphins (*Tursiops truncatus*) show a precocially developed locomotor system. Furthermore, the fiber-type profile of the ECL of neonatal dolphins is closest to that of the adult as compared with all the mixed muscles investigated quantitatively to date (Fig. 9). However, the muscles of neonatal dolphins are still different from those of adults in aerobic capacity and lipid distribution. It appears that newborn dolphins are able to capitalize on some of the physical properties of their marine environment to decrease the metabolic costs of locomotion. Their body mass is supported by water, and they are able to "free-ride" off their mothers, a behavior that is not available to terrestrial mammals. Blubber may also be used by these animals to increase their buoyancy and thereby reduce the need for both an extremely large muscle mass and a high aerobic capacity at birth. Thus, the muscles of dolphin neonates permit them to be functional, precocial locomotors at the instant of birth.

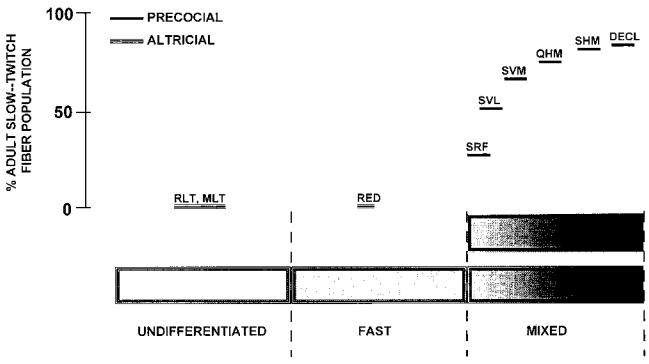

Fig. 9. The developmental state of the bottlenose dolphin (*Tursiops truncatus*) based on fiber-type profile. The dolphin *m. extensor caudae lateralis* (DECL) exhibits the largest percentage (95%) of the adult profile of any mixed muscle investigated quantitatively to date. Refer to Figure 2 for full explanation of the developmental model, identification of other muscles, and procedure used to calculate percent of adult profile.

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