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RESEARCH ARTICLE

Muscle biochemistry of a pelagic delphinid (*Stenella longirostris longirostris*): insight into fishery-induced separation of mothers and calves

Shawn R. Noren[1,*] and Kristi West[2,*]

ABSTRACT

The length of time required for postnatal maturation of the locomotor muscle (longissimus dorsi) biochemistry [myoglobin (Mb) content and buffering capacity] in marine mammals typically varies with nursing duration, but it can be accelerated by species-specific behavioral demands, such as deep-diving and sub-ice transit. We examined how the swimming demands of a pelagic lifestyle influence postnatal maturation of Mb and buffering capacity in spinner dolphins (*Stenella longirostris longirostris*). Mb content of newborn (1.16±0.07 g Mb per 100 g wet muscle mass, *n*=6) and juvenile (2.77±0.22 g per 100 g, *n*=4) spinner dolphins were only 19% and 46% of adult levels (6.00±0.74 g per 100 g, *n*=6), respectively. At birth, buffering capacity was 52.70±4.48 slykes (*n*=6) and increased to 78.53±1.91 slykes (*n*=6) once a body length of 141 cm was achieved, representing 1.6- to 2.0-year-old dolphins. Based on the age of weaning (1.3–1.6 years post-partum), muscle maturation occurred just after weaning as described for coastal bottlenose dolphins (*Tursiops truncatus*). Thus, a pelagic lifestyle does not promote rapid maturation of muscle biochemistry. Rather, it promotes enhanced muscle biochemistry: newborn and adult spinner dolphins had four- and two-times greater Mb contents than newborn and adult bottlenose dolphins, respectively. Indeed, adult levels rivaled those of deep-diving cetaceans. Nonetheless, the relatively underdeveloped muscle biochemistry of calves likely contributes to documented mother–calf separations for spinner dolphins chased by the tuna purse-seine fishery.

KEY WORDS: Myoglobin, Muscle acid buffering capacity, Marine mammal, Cetacean, Swim performance, Tuna purse-seine fishery

INTRODUCTION

It is well known that aquatic birds and mammals have high myoglobin contents and increased muscle buffering capacities compared with terrestrial birds and mammals (for a review, see Castellini and Somero, 1981). However, recent research on pigeon guillemot (*Cepphus columba*; Haggblom et al., 1988), penguins (Weber et al., 1974; Ponganis et al., 1999; Noren et al., 2001), sea otter (*Enhydra lutris nereis*; Thometz et al., 2015), pinnipeds (seals, sea lions and walruses; P. H. Thorson, Development of diving in the northern elephant seal, PhD thesis, University of California, Santa Cruz, 1993; Noren et al., 2005, 2015; Burns et al., 2005, 2007; Richmond et al., 2006; Fowler et al., 2007; Weise and Costa, 2007; Kanatous et al., 2008; Lestyk et al., 2009; Verrier et al., 2011) and cetaceans (whales and dolphins; Dolar et al., 1999; Etnier et al., 2004; Noren, 2004; Noren et al., 2001, 2014; Cartwright et al., 2016; Noren and Suydam, 2016; B. P. Velten, A comparative study of the locomotor muscle of extreme deep-diving cetaceans, MSc thesis, University of North Carolina, Wilmington, 2012) have shown that a period of postnatal development is required in order to achieve mature muscle myoglobin content and muscle buffering capacity after birth. It was anticipated that the immediate demands of hypoxia should promote rapid muscle maturation in cetaceans since they are born directly into the ocean, compared with pinnipeds that are born on land and typically spend several months to years on land before foraging on their own (for reviews, see Noren et al., 2005, 2015). Yet it was discovered that cetaceans can take approximately 1–3 years after birth to achieve mature myoglobin content (Dolar et al., 1999; Noren et al., 2001, 2014; Noren and Suydam, 2016) and buffering capacity (Noren, 2004; Noren et al., 2014; Noren and Suydam, 2016) in the muscle.

The prolonged muscle maturation of cetaceans may be associated with the protracted maternal dependency periods of cetaceans, as variation in the duration required for muscle maturation in pinnipeds is correlated with variation in maternal dependency periods (for reviews, see Noren et al., 2014, 2015). Interestingly, recent investigations on Arctic marine mammal species [Pacific walruses (*Odobenus rosmarus divergens*; Noren et al., 2015) and beluga whales (*Delphinapterus leucas*; Noren and Suydam, 2016)] suggested that habitat influences the postnatal development of muscle biochemistry. Walruses and belugas achieved mature muscle properties well before the age of weaning. The disconnect between the age of nutritional independence and age of muscle maturation in these species was attributed to the requisite need of walrus and beluga calves to transit amongst sea ice, where breathing holes are patchily distributed and ephemeral (Noren et al., 2015; Noren and Suydam, 2016).

Compared with pinnipeds, investigations of the postnatal maturation of the muscle biochemistry of the major locomotor muscle that supports swimming and diving in cetaceans is limited. Adequate sample sizes to provide an estimate of the age in which the muscle biochemistry is fully matured is only available for three species, a delphinid (Noren et al., 2001; Noren, 2004), phocoenid (Noren et al., 2014) and monodontid (Noren and Suydam, 2016). Yet there are 88 cetacean species recognized by the International Union for Conservation of Nature that live in very diverse habitats (e.g. coastal to pelagic and tropical to polar). The paucity of data from cetaceans limits our ability to discern the synergistic influences of life history patterns and habitat on the postnatal maturation of muscle biochemistry within this taxonomic group. We wondered if,

[1]Institute of Marine Science, University of California, Santa Cruz, Center for Ocean Health, 100 Shaffer Road, Santa Cruz, CA 95060, USA. [2]College of Natural and Computational Sciences, Hawaii Pacific University, 45-045 Kamehameha Hwy., Kaneohe, HI 96744, USA.

*Authors for correspondence (snoren@ucsc.edu; kwest@hpu.edu)

S.R.N., 0000-0003-3122-8289

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like the Arctic environment, pelagic (offshore) living promotes rapid postnatal maturation of muscle biochemistry. Thus, we examined the ontogeny of the aerobic (myoglobin content) and anaerobic (acid buffering capacity) properties of the locomotor muscle (longissimis dorsi) that supports swimming and diving in a pelagic delphinid, the spinner dolphin (*Stenella longirostris longirostris*), and compared it with the postnatal development of the locomotor muscle of a coastal delphinid, the bottlenose dolphin (*Tursiops truncatus*), which was previously described by Noren et al. (2001) and Noren (2004).

An examination of the postnatal development of the muscle biochemistry that supports the swimming performance of spinner dolphins is timely because spinner dolphin species are chased and captured during fishery operations for yellowfin tuna (*Thunnus albacares*) in the eastern tropical Pacific Ocean (ETP). Historically, captured dolphins were killed, and as a result of this, eastern spinner dolphins (*Stenella longirostris orientalis*) are only at 29% of their pre-1959 abundance levels when the tuna purse-seine fishery initiated setting on dolphin schools (Wade et al., 2007). Currently, reported fishery-related mortality is less than 0.1% of the estimated 450,000 eastern spinner dolphins in the ETP because procedures are now used to release captured dolphins (Gerrodette and Forcada, 2005). However, despite the reduced observed dolphin mortality associated with this fishery, dolphin populations are not recovering at growth rates (4% year^{-1}) consistent with the level of depletion (Gerrodette and Forcada, 2005). Several studies have suggested that unobserved calf mortality could affect recovery of dolphin populations in the ETP (Archer et al., 2001; Archer et al., 2004). Examination of the age composition of dolphins historically killed in the purse-seine nets demonstrated that fewer 0- to 1-year-old eastern spinner dolphins (Chivers, 2002) were present than expected, as calves did not accompany 75–95% of the killed lactating females (Archer et al., 2004). These findings imply that dolphin calves become separated from their mothers during tuna purse-seine activities, as indicated by a series of photographs depicting an ETP dolphin calf falling behind its mother during chase (Weihs, 2004). An examination of the postnatal development of the muscle biochemistry that supports swimming provides insight into the age range in which spinner dolphin calves are vulnerable to being separated from the pod during fishery-induced chase.

MATERIALS AND METHODS

Specimen and muscle collection

Freshly stranded spinner dolphins (*Stenella longirostris longirostris*) were collected in the state of Hawaii (USA) via a stranding program authorized by the National Marine Fisheries Service (NMFS) at Hawaii Pacific University. Upon collection, sex, developmental state and body length were recorded. With the exception of two code 3 (moderately decomposed) carcasses, all specimens included in this study were of code 1 or 2 condition (code 1: recovered fresh dead but initially stranded live and so time of death was known; code 2: recovered fresh dead) (Geraci and Lounsbury, 2005). All code 1 and 2 carcasses were necropsied immediately or within 12 h of carcass recovery; for cases where necropsies were not conducted immediately, the carcass was covered with ice or refrigerated. The code 3 carcasses were frozen at −20°C for approximately 2 months prior to thawing and necropsy. During necropsy, a minimum of 10 g of muscle were obtained from the midbelly of the major swimming muscle (longissimus dorsi) directly anterior of the dorsal fin, which is in accordance with Noren et al. (2001).

Muscle samples were wrapped in foil and sealed in a Ziploc bag, or placed directly into a sterile sample bag (Whirlpack) that was vacuum sealed at the time of collection. Sealed samples were then stored in a −80°C freezer for ∼5 days to 12 months until shipped frozen on dry ice to University of California, Santa Cruz (UCSC). Once at UCSC, the samples were stored in a −80°C freezer until biochemical analyses were performed within 6 months. Sample transfer to UCSC was approved by NMFS and US Fish and Wildlife Permit to obtain, import and export marine mammal specimens through permit number 960-1528-01/MA017891, on which S.R.N. is listed as a collaborator. Obtaining muscle samples from stranded dolphin carcasses for the purpose of analyzing muscle biochemistry was approved by UCSC IACUC under NORES1306_A1.

A total of 17 individuals were sampled, representing a range of developmental states, from fetal through to sexually mature. The specimens were assigned to a life history class (fetus, neonate, juvenile and adult) according to body length and observed developmental state (see Table 1).

Muscle biochemistry

To examine the oxygen storage capacity in the muscle, myoglobin content (Mb; reported in g Mb per 100 g wet muscle) was determined using the procedure of Reynafarje (1963), which was adapted for marine mammals by Noren and Williams (2000). Approximately 0.5 g thawed muscle was extracted from deep within the muscle sample to avoid sampling from exterior regions of the sample that may have become slightly dehydrated during storage. The muscle was minced in a low ionic strength buffer (40 mmol l^{-1} phosphate, pH=6.6) and then sonicated (Sonifier Cell Disrupter Model 450, Branson Ultrasonics Corporations, Danbury, Connecticut, USA) for 2–3 min on ice. We used a higher buffer-to-tissue ratio (39.25 ml buffer per g wet tissue) compared with that used previously (19.25 ml buffer per g wet tissue; Noren and Williams, 2000); the higher buffer-to-tissue ratio ensured the complete extraction of myoglobin from the tissue since muscles obtained from adult specimens were expected to have very high myoglobin levels based on their dark black red color. This higher buffer-to-tissue ratio was recently used in a study on beluga whales, and it was recommended that subsequent investigations on the myoglobin of marine mammals use this approach (Noren and Suydam, 2016). Nonetheless, to validate this approach, we analyzed the muscles (pale pink color) from neonatal specimens using both buffer-to-tissue ratios; the values were identical. Therefore, we also recommend that the myoglobin content of the locomotor muscle of marine mammals be analyzed using the higher buffer-to-tissue ratio. The samples were then centrifuged at −4°C and 28,000 ***g*** for 50 min (Sorvall RC-5C Plus superspeed refrigerated centrifuge, DuPont Instruments). The clear supernatant was extracted and then bubbled at room temperature with pure CO for approximately 8 min.

Table 1. Life history class delineations for the specimens in this study based on morphological characteristics and body length

Life history class	Life history class characteristics	Female lengths (cm)	Male lengths (cm)
Fetus*	Full-term	99	
Neonate	Unhealed or partially healed umbilicus	88, 88	75, 86, 87, 89
Juvenile	Still nursing and/or sexually immature	96	114, 132, 140
Adult	Sexually mature	185, 196	170, 184, 193, 213

*Recovered from a female that likely died due to complications associated with delivering an extraordinarily large calf.

Journal of Experimental Biology (2017) 220, 1490-1496 doi:10.1242/jeb.153668

To ensure a complete reduction, 0.02 g of sodium dithionite was added. The absorbance of each sample was read at room temperature at 538 and 568 nm on a spectrophotometer (UV-160, Shimadzu Corporation, Kyoto, Japan). All samples were run in triplicate alongside a muscle sample obtained from a harbor porpoise (*Phocoena phocoena*), which served as a control because the myoglobin content of this specimen was determined previously (Noren et al., 2014).

To examine a component of the anaerobic capacity of the muscle, we explored the ability of the muscle to buffer against lactic acid. The muscle buffering capacity (β) due to non-bicarbonate buffers was determined using procedures of Castellini and Somero (1981) and adapted by Noren (2004). Briefly, ∼0.5 g thawed muscle was extracted from deep within the muscle sample to avoid sampling from exterior regions of the sample that may have become slightly dehydrated during storage. The sample was minced in 10 ml normal saline solution (0.9% NaCl), and sonicated (Sonifier Cell Disrupter Model 450, Branson Ultrasonics Corporations, Danbury, CT, USA) for 3 min on ice. Samples were maintained at 37°C by immersion of the test flask in a warm water bath and titrated with 0.2 N NaOH. Buffering capacity was measured in slykes (μmoles of base required to raise the pH of 1 g wet muscle mass by one pH unit, over pH range 6.0–7.0). Changes in pH were measured using an Accumet basic pH/mV/°C meter (AB15+, Fisher Scientific) with an Accumet liquid-filled, glass body single-junction combination pH Ag/AgCl Electrode (13-620-285, Fisher Scientific) and separate ATC probe (13-620-19, Fisher Scientific). All samples were run in triplicate alongside a muscle sample obtained from a harbor porpoise (*P. phocoena*), which served as a control because the acid buffering capacity of this specimen was determined previously (Noren et al., 2014).

Statistics

Owing to limited sample sizes, sex-specific differences in muscle biochemistry were not examined. This is typical of studies on cetaceans because obtaining samples from cetaceans is extremely difficult (for reviews, see Noren et al., 2014; Noren and Suydam, 2016). Thus, as in our previous studies, data from both sexes were combined for analyses. The relationships between body length (x) and muscle biochemistry (y; either myoglobin content or buffering capacity) were examined using segmented regression analysis (SEGREG, www.waterlog.info) to identify the breakpoint in these relationships. The breakpoint represents the body length at which muscle biochemistry plateaus and has matured. The selection of the best breakpoint and function type was based on maximizing the statistical coefficient of explanation, and performing tests of significance across seven types of functions (www.waterlog.info/pdf/regtxt.pdf). With knowledge of the body length at which muscle biochemistry matured, we could estimate the age at which muscle biochemistry matured from parameter value estimates for length-at-age relationships for eastern spinner dolphins (*S. l. orientalis*; table 4 in Larese and Chivers, 2009). We used a length-at-age relationship for a different subspecies of spinner dolphin because a length-at-age relationship has not been determined for *S. l. longirostris*.

Life history analyses enabled us to compare our results with previous studies. Therefore, myoglobin content and buffering capacity were compared across neonate, juvenile and adult by a one-way analysis of variance in combination with an all pairwise multiple comparison procedure (Holm–Šídák method) with a significance level of $P<0.05$. A sample size of one excluded the fetus from all life history comparisons. Comparisons across species (pelagic spinner versus coastal bottlenose dolphins) were then done according to life history class (neonate, juvenile and adult) using a one-tailed t-test, as we assumed a pelagic lifestyle would promote greater myoglobin content and buffering capacity in the muscle. The data for bottlenose dolphins used in these analyses are from Noren et al., 2001 and Noren 2004. Values are reported as means±1 s.e. Statistics were performed by Sigma Stat 4.0 (Jandel Scientific).

RESULTS

Muscle biochemistry

Spinner dolphins are not born with mature biochemical properties in the major locomotor muscle (longissimus dorsi; Fig. 1, Table 2). Only one function (positive linear regression) best represented the relationship for body length and myoglobin content; we were unable

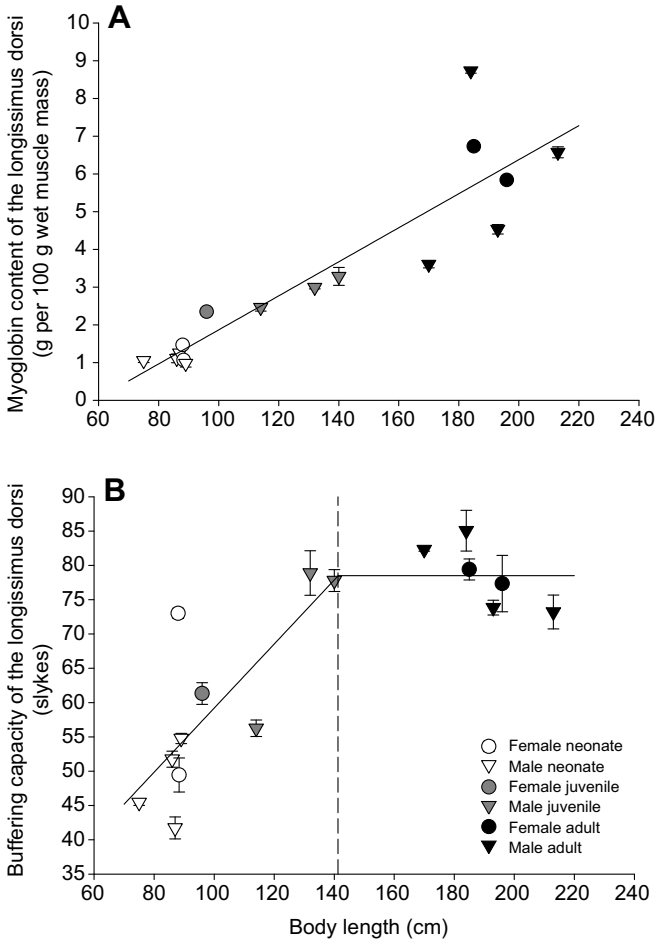

Fig. 1. Muscle myoglobin content and buffering capacity of spinner dolphins throughout postnatal development. Each dot represents the mean±s.e. myoglobin content (A) and buffering capacity (B) for an individual specimen that was analyzed in triplicate. The solid lines represent the linear increases in muscle myoglobin content and buffering capacity with body length (our index of age), until an asymptote for mature muscle biochemistry was achieved. The relationship for body length versus myoglobin (Mb) content was best described by only one relationship, a positive linear regression increased according to [Mb]=0.05 length–2.64 ($F_{1,\ 14}$=63.27, P<0.001, r^2=0.82). The relationship for body length versus buffering capacity was best described by two relationships, a positive linear regression followed by a horizontal line. Buffering capacity increased according to: buffering capacity=0.47 length+12.04 ($F_{1,\ 9}$=11.44, P=0.01, r^2=0.59), until a body length of 141 cm was obtained. The details on the segmented regression analysis are described in the Materials and Methods.

Journal of Experimental Biology (2017) 220, 1490-1496 doi:10.1242/jeb.153668

Table 2. Muscle myoglobin content and buffering capacity of cetaceans according to species and life history class

Suborder/ family	Species	Life history class (*N*)	% Adult length	Mb (g per 100 g wet muscle)	% Adult [Mb]	Buffering capacity (slykes)	% Adult buffering capacity
Odontoceti							
Phocoenidae	Harbor porpoise (*Phocoena phocoena*)[a]	F (2)	28%	0.26	10%	28.8	37%
		N (10)	55%	1.30	51%	53.8	69%
		C (6)	65%	2.50	98%	60.5	77%
		J (5)	78%	2.95	115%	69.9	89%
		A (7)	–	2.56	–	78.5	–
Delphinidae	Bottlenose dolphin (*Tursiops truncatus*)[b,c]	N (3)	49%	0.27	10%	44.8	65%
		J (4)	72%	1.58	57%	67.4	97%
		A (5)	–	2.76	–	69.1	–
	Pacific white-sided dolphin (*Lagenorhynchus obliquidens*)[b,c]	F (1)	34%	0.15	4%	34.8	55%
		J (1)	90%	2.93	85%	58.9	93%
		A (2)	–	3.45	–	63.7	–
	Common dolphin (*Delphinus capensis*)[b,c]	N (2)	45%	0.70	20%	53.9	62%
		A (3)	–	3.58	–	86.5	–
	Striped dolphin (*Stenella coeruleoalba*)[b,c]	J (1)	66%	3.94	68%	75.4	100%
		A (1)	–	5.78	–	74.9	–
	Spinner dolphin (*Stenella longirostris longirostris*)[d]	F (1)		0.46	8%	64.30	46%
		N (6)	45%	1.16±0.07	19%	52.70±4.48	58%
		J (4)	64%	2.77±0.22	46%	68.57±5.74	100%
		A (6)	–	6.00±0.74	–	78.53±1.91	–
	Fraser's dolphin (*Lagenodelphis hosei*)[e]	C (2)	48%	3.00	42%	–	–
		J (2)	69%	5.15	73%	–	–
		A (6)	–	7.10	–	–	–
	Short-finned pilot whale (*Globicephala macrorhynchus*)[f]	C (1)	52%	3.35	49%	73.4	104%
		J (1)	63%	6.88	100%	77.3	109%
		A (6)	–	6.82	–	70.7	–
Monodontidae	Beluga whale (*Delphinapterus leucas*)[g]	F (1)	40%	0.70	10%	39.08	46%
		N (2)	43%	1.56	23%	48.88	58%
		C (5)	62%	7.05	102%	80.44	95%
		J (6)	84%	7.22	104%	84.51	100%
		A (9)	–	6.91	–	84.31	–
Ziphiidae	Gervais' beaked whale (*Mesoplodon europaeus*)[f]	N (1)	50%	6.55	88%	92.3	93%
		J (1)	86%	7.42	100%	105.7	107%
		A (2)	–	7.41	–	98.8	–
Mysticeti							
	Humpback whale (*Megaptera novaeangliae*)[h]	N (1)	–	0.10	8%	–	–
		C (5)	–	0.30	24%	–	–
		MC (1)	–	0.46	37%	–	–
		J (1)	–	0.72	57%	–	–
		A (2)	–	1.26	–	–	–
	Gray whale (*Eschrichtius robustus*)[h]	N (2)	–	0.17	13%	–	–
		C (1)	–	0.22	17%	–	–
		MC (1)	–	0.32	25%	–	–
		J (1)	–	0.36	28%	–	–
		A (2)	–	1.27	–	–	–
	Minke whale (*Balaenoptera acutorostrata*)[h]	N (1)	–	0.42	17%	–	–
		J (1)	–	1.46	60%	–	–
		A (2)	–	2.42	–	–	–

Fetus (F), neonate (N), calf (C), migrating calf (MC), juvenile (J) and adult (A). [a]Noren et al., 2014; [b]Noren et al., 2001; [c]Noren, 2004; [d]This study; Life history class means calculated from data published in [e]Dolar et al., 1999; [f]B. P. Velten, A comparative study of the locomotor muscle of extreme deep-diving cetaceans, MSc thesis, University of North Carolina, Wilmington, 2012; [g]Noren and Suydam, 2016; [h]Cartwrtight et al., 2016.

to identify the breakpoint where myoglobin content achieved a plateau. Thus, we are not able to report the body length at which myoglobin content attains mature levels (see Fig. 1A for statistics). Meanwhile, two functions (positive linear regression followed by a horizontal line) best represented the relationship for body length and buffering capacity. The breakpoint (141 cm) between the two different relationships represented the body length at which spinner dolphins achieve mature buffering capacity (see Fig. 1B for statistics). Based on a published age-at-length curve for eastern spinner dolphins (Larese and Chivers, 2009), the estimated age of a 141-cm-long spinner dolphin is ∼1.6–2.0 years. Spinner dolphins are weaned at 1.3–1.6 years postpartum (Table 3). Based on this, it appears as though muscle maturation occurs just after weaning.

The variation in muscle biochemistry across life history classes of spinner dolphins was significant for both myoglobin content ($F_{2,\ 13}$= 27.718, P<0.001) and buffering capacity ($F_{2,\ 13}$=11.921, P=0.001). The myoglobin levels across all life history classes were significantly different from each other for myoglobin (P<0.05). For buffering capacity, the value for the neonatal life history class was significantly lower than all other life history classes, while the juvenile and adult life history classes were not statistically different from each other (P<0.05).

There were differences in muscle biochemistry between pelagic and coastal delphinids (Table 2). Indeed, neonatal, juvenile and adult spinner dolphins had significantly higher myoglobin contents than neonatal, juvenile, and adult bottlenose dolphins, respectively (neonate: t=8.274, d.f.=7, P<0.001; juvenile: t=2.924, d.f.=6,

Journal of Experimental Biology (2017) 220, 1490-1496 doi:10.1242/jeb.153668

Table 3. Life history characteristics of cetacean species that have descriptions of the full ontogeny of their muscle biochemistry

Species	Habitat	Gestation interval (years)	Age at weaning (years)	Calving interval (years)	Age at sexual maturity (years)	Estimated age at myoglobin maturity (years)	Age at buffering capacity maturity (years)
Harbor porpoise (*Phocoena phocoena*)[c]	Coastal	0.92	0.67	1–2	M: 4[a] F: 3[a]	0.75–0.83	2–3
Bottlenose dolphin (*Tursiops truncatus*)[d]	Coastal	1	1.58	2–3	M: 12 F: 11	1.5–3.4	1.5–3.4
Spinner dolphin (*Stenella longirostris*)[e]	Pelagic	0.79–0.89	1.25–1.58	2.9–3.3	M: 6–9 F: 5	?	1.6–2
Fraser's dolphin (*Lagenodelphis hosei*)[f]	Pelagic	1.04[b]	?	2[b]	M: 7–10[b] F: 5–8[b]	1–4	?
Beluga whale (*Delphinapterus leucas*)[g]	Arctic	1.21	1.67–2	3	M: 8–9 F: 4–7	1.17	1.17

Gestation, age at weaning, and calving interval from table 8.5 and sexual maturity from table 8.6 in Evans (1987), except data from [a]Gearin et al. (1994) and [b]Amano et al. (1996). Gearin et al. (1994) was used because the porpoises were from the same population as those in the muscle study. Amano et al. (1996) was used because data for Fraser's dolphin not provided in Evans (1987). Habitat based on where the specimen for the muscle study was from and the age at myoglobin maturation was estimated from the minimum body length at which the animals achieved mature myoglobin, using species-specific age-at-length curve; details provided in original manuscripts: [c]Noren et al., 2014; [d]Noren et al., 2001 and Noren, 2004; [e]Present study; [f]Dolar et al., 1999; [g]Noren and Suydam, 2016.

P=0.013; adult: t=3.938, d.f.=9, P=0.002). Meanwhile, only the adult life history class revealed differences in buffering capacity between the two species. The buffering capacity of the muscle of adult spinner dolphins was higher than that of adult bottlenose dolphins (t=2.085, d.f.=9, P=0.033) despite the similarities in the buffering capacities of the neonatal muscles (t=1.164, d.f.=7, P=0.141) and juvenile muscles (t=0.177, d.f.=6, P=0.433) of these two species.

DISCUSSION

An important factor that supports the swimming and diving performance of marine mammals is the metabolic support at the level of the working skeletal muscle (Hochachka, 1986; Castellini and Somero, 1981). When blood perfusion to muscle tissue is decreased, oxygen depletion of that area is retarded by the release of myoglobin-bound oxygen (Salathe and Chen, 1993). When oxygen depletion in an area of the body is prolonged, glycogenolytic pathways may become increasingly important (Hochachka and Storey, 1975; Kooyman et al., 1980); high buffering capacity in the muscle can counteract changes in pH associated with lactic acid accumulation from these anaerobic processes (Castellini and Somero, 1981). Although elevated myoglobin contents and elevated acid buffering capacities in the longissimis dorsi are important to support the swimming and diving performance of marine mammals, we found that, similar to other cetaceans (Table 2), spinner dolphins require postnatal development to achieve mature levels (Fig. 1).

Surprisingly, a pelagic lifestyle did not promote rapid postnatal maturation of the muscle acid buffering capacity or myoglobin content in spinner dolphins. Our analyses indicated that the buffering capacity of the muscle was mature once spinner dolphins achieved a body length of 141 cm (Fig. 1B). We were unable to statistically identify the breakpoint (body length) at which myoglobin content matured in spinner dolphins. It appears that myoglobin maturation occurred after the maturation of muscle buffering capacity (Fig. 1), which is similar to the pattern observed for another delphinid species, bottlenose dolphins (Noren et al., 2001). Nonetheless, a body length of 141 cm corresponds to spinner dolphins that are ∼1.6–2.0 years old [age estimate based on a length-at-age relationship for spinner dolphins; Larese and Chivers, 2009] and since spinner dolphins are weaned at 1.3–1.6 years postpartum (Evans, 1987), maturation of the muscle biochemistry examined in this study occurs after the age of weaning. Likewise, the maturation of the muscle buffering capacity and myoglobin content of bottlenose dolphins occurs after the age of weaning (Table 3). Thus, the postnatal developmental trajectory of this pelagic species is not expedited compared with a coastal species.

In contrast, it appears that a pelagic lifestyle does promote elevated muscle biochemistry. The myoglobin levels of neonatal spinner dolphins are higher than myoglobin levels measured in coastal cetacean neonates, including the harbor porpoise (*P. phocoena*) and bottlenose dolphin (Table 2). Indeed, comparisons between the pelagic spinner dolphin and coastal bottlenose dolphin indicated that myoglobin contents of the pelagic species were consistently higher than the corresponding age class of the coastal species (Table 2). Furthermore, the myoglobin levels (range from 6.00 to 7.10 g Mb per 100 g wet muscle mass) of adult pelagic delphinids [spinner (present study) and Fraser's dolphin, *Lagenodelphis hosei* (Dolar et al., 1999)] fall within the range (6.82–7.41 g Mb per 100 g wet muscle mass) measured for deep-diving cetaceans, such as short-finned pilot whales (*Globicephala macrorhynchus*; Velten et al. 2013) and Gervais' beaked whales (*Mesoplodon europaeus*; Velten et al. 2013). Myoglobin levels of adult pelagic delphinids also fall within the range (6.91–7.87 g Mb per 100 g wet muscle mass) reported for adult Arctic dwelling cetaceans, such as the narwhal (*Monodon Monoceros*; Williams et al., 2011) and beluga whale (Noren and Suydam, 2016), that must transit under sea ice. Interestingly, the behavioral demands of pelagic swimming, deep-diving and sub-ice transit necessitated the need to retard oxygen depletion in the muscle, resulting in convergent evolution favoring elevated myoglobin contents.

Given the importance of this muscle biochemistry in supporting swim performance, an additional aim of this study was to define the age of vulnerability for spinner dolphin calf separations from the dolphin pod during high-speed chase induced by tuna purse-seine fisheries in the eastern tropical Pacific (ETP; NRC, 1992). Previously, in an effort to assess the potential for calf separation, numerous studies were undertaken to quantify how swim performance changes with age in dolphins. The bottlenose dolphin (*T. truncatus*) was used

Journal of Experimental Biology

as a model because its accessibility in human care afforded controlled experimentation (Noren et al., 2006; Noren et al., 2008; Noren and Edwards, 2011), and the routine speeds (2.1 m s^{-1}) of bottlenose dolphins (Williams et al., 1993) and dolphins in the ETP (Scott and Chivers, 2009) are similar. Moreover, at the time of those studies, a review of the physiological and behavioral development of delphinids suggested that the postnatal development of the muscle biochemistry that supports swimming was similar across cetacean species (Noren and Edwards, 2007). However, based on recent research on harbor porpoise and beluga whales (Table 3), we now know that the rate of postnatal development for the musculature that supports swim performance varies across cetacean species according to age of weaning and habitat. This raised the question as to whether or not the bottlenose dolphin was an appropriate model for ascertaining how swim performance of spinner dolphins might change throughout ontogeny. The present study demonstrates that the developmental trajectory of the muscle biochemistry that supports swimming is similar across bottlenose and spinner dolphins, validating the bottlenose dolphin as an adequate model.

According to Noren et al. (2006), the range of average (1.43–3.29 m s^{-1}) and maximum (2.91–4.51 m s^{-1}) swim speeds of solitary swimming calves <12 months old are lower than the range of average (3.88–4.39 m s^{-1}) and maximum (5.00–5.61 m s^{-1}) swim speeds of juveniles and adults. Under normal circumstances, calves mediate limitations to swim performance by swimming in echelon position alongside their mothers; in this position the calves draft off of their mothers so that their swim performance is increased while their swim effort is decreased (Noren et al., 2008). However, at fishery-induced chase speeds, dolphins porpoise (jump out of the water), and this breaching effectively breaks up the drafting interaction (Weihs, 2004). Nonetheless, this formation can be quickly re-established as long as the dolphin pair leave and re-enter the water synchronously with similar speed and splash formation (Weihs, 2004). However, neonates and young calves lack physical coordination, making them less adept at porpoising such that they either breach or return at non-optimal penetration angles, which increases their drag and causes a speed differential between the mother and calf (Weihs, 2004). In addition, if the calf does not emerge from the water at the right angle, its aerial trajectory will be shorter and the calf will end up further behind the mother (Weihs, 2004). The underdeveloped aerobic and anaerobic capacities of the locomotor muscle of calves <1.6 years old (Fig. 1, Table 2) limits swim performance, making it difficult for nursing calves to re-establish echelon position.

Although the follower response of immature dolphins is strong (Mann and Smuts, 1998), once calves become separated from their mothers, the calves' physiological limitations preclude them from sustaining adult swim speeds (Noren et al., 2006). Compared with natural predatory events, fishery interactions are prolonged and occur at high speeds. Chase can occur for 20 min and is often followed by 100 min of escape response (Myrick and Perkins, 1995), during which dolphins elevate routine speeds to chase and burst speeds of 2–4 m s^{-1} and 5–8 m s^{-1}, respectively (Au and Perryman, 1982; Au et al., 1988; Chivers and Scott, 2002). Assuming the dolphin pod swims away from the purse-seine fishery boats at 4 m s^{-1} for 20 min of chase prior to being captured in the nets, neonates, 3-month-olds and 6-month-olds that are separated from their mothers at the initiation of the chase will be 1.3–3.1 km, 1.4–2.6 km and 0.3–1.4 km away from the captured pod, respectively (assuming calves vary their speed between average and maximum speeds; speeds from table 1 in Noren et al., 2006). This supports the observation of Archer et al. (2004), that there is a historical deficit of dolphin calves caught in the nets, suggesting that calves become separated from the pod during fishery-induced chase. If calves do not reunite with their mothers prior to the pod being released from the net, neonates, 3-month-olds and 6-month-olds become separated from their mothers by an additional 6.5–15.4 km, 7.0–12.8 km and 1.6–6.9 km, respectively, after 100 min of chase. Indeed, photographs of an ETP dolphin school evading a vessel revealed that a mother dolphin did not change her trajectory during chase, despite her calf falling behind (Weihs, 2004). Without their mothers, calves have an increased risk of mortality as a result of starvation and predation (Noren and Edwards, 2007).

In summary, we found that the muscle biochemistry that supports swimming and diving varies within delphinids according to habitat, with adult pelagic dolphins having elevated myoglobin contents and buffering capacities compared with adult coastal dolphins. In contrast, disparate lifestyles (pelagic versus coastal) did not influence the life history stage at which maturation occurred, since both spinner and bottlenose dolphins achieved mature muscle acid-buffering capacity and myoglobin levels after the age of weaning. Prior to this maturation, underdeveloped muscle biochemistry limits swim speed and stamina. This study illuminated the age of vulnerability for spinner dolphin calves to become separated from the pod during high-speed long duration chases induced by the tuna purse-seine fishery in the ETP. Quantifying the physiological capacities of animals improves our ability to determine the range of environmental conditions under which an animal can persist (Wikelski and Cooke, 2006) and provides a valuable tool to predict demographic consequences of anthropogenic perturbations.

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Competing interests
The authors declare no competing or financial interests.

Author contributions
S.R.N. secured funding for the study, developed the approach, performed the laboratory and data analyses, and prepared the manuscript. K.W. secured funding for this study, collected and performed necropsies on the specimens to obtain the muscle samples, and also provided edits and revisions of the manuscript.

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